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Abstracts of the 10th Congress of the European Academy of Andrology

11 - 13 October 2018

Budapest, Hungary

The merged journal of the American Society of Andrology and the European Academy of Andrology, now including the former *International Journal of Andrology* and *Journal of Andrology*

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- Letters to the Editor
- Letters to the Editor Responses reply to a Letter to Editor
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Original studies should be clearly divided into the following sections: introduction; materials and methods; results; discussion with conclusions and references. Please remember to include acknowledgements funding information disclosures, and author's contributions between the main text and reference list.

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ANDROLOGY

WELCOME NOTE

MESSAGE FROM THE EAA PRESIDENT

Csilla Krausz
EAA President

ECA2018 represents the most relevant scientific and educational biennial event of the European Academy of Andrology (EAA). This year it will be a special edition because it coincides with the 25-year history of the EAA. Academicians and Affiliated members are going to celebrate this anniversary together with hundreds of andrologists and basic scientists coming from all over the world. As for the previous nine editions, it will be an excellent forum for presenting cutting-edge basic/translational and clinical research in all areas of Andrology and for discussing about clinical practice related to male infertility and sexual dysfunction. ECA2018 will also allow reinforcing our relationship with the European Society of Endocrinology (ESE), the American Society of Andrology (ASA) and the European Society of Andrological Urology (ESAU). It will be the place for teaching our young fellows, updating our senior members, and exchanging ideas for promoting andrology in Europe and outside the old continent. I thank the Local Organizing Committee and the Scientific Program Organizing Committee for having organized such a wonderful program and for giving us the opportunity to pursue our mandate for excellence in Andrology.

On behalf of the European Academy of Andrology, I warmly welcome you in Budapest,

MESSAGE FROM THE CHAIR OF THE PROGRAM ORGANIZING COMMITTEE

Dimitrios G. Goulis
Program Organizing Committee, Chair

The Program Organizing Committee (POC) has made every possible effort to compile an attractive, informative, updated, and educational program for the 10th European Congress of Andrology, the leading scientific activity of the European Academy of Andrology (EAA) Program highlights include:

- The well-established pre-congress course that this year will provide a 'systematic approach of the infertile male' through 10 educational lectures.
- Seven state-of-the-art lectures by prominent scientists on cutting-edge research issues.
- Presentation of the EAA scientific work through the announcement of the first three EAA clinical guidelines (management of oligo-astheno-teratozoospermia, bone health, gynecomastia) and the evolving EAA ultrasound project.
- Nine symposia that cover the whole spectrum of basic and clinical Andrology (genetics, transitional Andrology, late-onset hypogonadism, testis physiology, oncology, general male health, seminology).
- Three joint sessions that highlight the very successful collaboration between the EAA and the European Society of Andrological Urology (ESAU) section of the European Association of Urology (EAU).
- Presentation of the impressive scientific work conducted by the young colleagues through the Network of Young Researchers in Andrology (NYRA) session, 'golden communications', 'selected oral communications', and 'selected posters'.
- Finally, announcement of the current research activities in the field of Andrology through a large number of poster presentations.

The POC does hope that you will enjoy three days full of Andrology and you will return home fully updated on all the essential aspects of the male health.

MESSAGE FROM THE CHAIR OF THE LOCAL ORGANIZING COMMITTEE

Zsolt Kopa
Local Organizing
Committee Chair

The Local Organizing Committee (LOC) and the Program Organizing Committee (POC) of the 10th European Congress of Andrology have completed the final scientific program of the conference, which will be held in Budapest, Hungary.

The Hungarian state was founded in 895 and became a Christian Kingdom in 1000 by the crowning of St. Stephan, recognized by the Pope. During and following the Arpad Dynasty medieval Hungary was mainly flourishing, as an idol for European countries Mathias Corvinus made Hungary a Central European renaissance cultural center. In the history before and also after the Turkish occupation, the Kingdom of Hungary and later the Austro-Hungarian Monarchy served as a bulwark for the continent. The former agricultural country became a well-established industrial economy. Budapest as Capital city evolved to be one of the leading European cities with a unique scope and many novel investments, for example, building the first underground in Europe.
After the World Wars, Hungary lost two-thirds of its former territory but Budapest remained and developed again to one of the world's most popular tourist destination offering unforgettable experiences to its visitors. Beside the beauties of the city, our Capital emerges to be known also as a host for several scientific events.

We are proud and privileged to welcome you at the 10th European Congress of Andrology, an event of the European Academy of Andrology with joint sessions of the Section of Andrological Urology of the European Association of Urology.

Through plenary and keynote lectures, selected oral presentations, poster talks, poster sessions, and industry symposia, the ambition of the meeting is to report on the most advanced research progresses and results in Andrology and presents the way for future scientific and practical challenges.

2018 ECA will offer a lively and convivial share platform to senior and younger researchers of Andrology.

Local Organizing Committee

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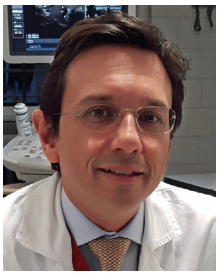

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ANDROLOGY

PROGRAM

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10th EUROPEAN CONGRESS OF ANDROLOGY

SCIENTIFIC PROGRAM

Thursday, 11 October 2018 – Ballroom A

09.00–15.00	**Pre-congress course**	**Systematic approach of the infertile male** *Chairpersons: Rafael Oliva (ESP), Sabine Kliesch (GER)*
09.00–09.30	PC01	**Semen characteristics and male pathology** Roger Mieusset (FRA)
09.30–10.00	PC02	**Endocrine evaluation** Dirk Vanderschueren (BEL)
10.00– 11.00	PC03	**Imaging of the male reproductive system** Andrea Isidori (ITA)
11.00–11.30	PC04	**Perspectives of genetics in the Andrology Clinic** Maris Laan (EST)
11.00–12.00	PC05	**Global health assessment of the infertile men** Alberto Ferlin (ITA)
12.00–12.30	PC06	**Clinical value of proteomic biomarkers** Rafael Oliva (ESP)
12.30–13.30	**Lunch**	
13.30–14.00	PC07	**Sexual transmitted disease and fertility** Alvaro Vives (ESP)
14.00–14.30	PC08	**Medical therapy of the infertile men** Giovanni Corona (ITA)
14.00–14.30	PC09	**Surgical treatment** Sabine Kliesch (GER)
14.30–15.00	PC10	**Assisted Reproduction Techniques** Dimitrios G. Goulis (GRE)
15.00–15.30	**Opening Ceremony**	
15.30–17:00	**Golden oral communications 1**	*Chairpersons: Andrea M. Isidori (ITA), Jolanta SŁowikowska-Hilczer (POL)*
15.30–15.45	OC01	**Risk of prostate cancer in men undergoing assisted reproduction** Yahia Al-Jebari (SWE)
15.45–16.00	OC02	**Impact of cancer therapy on risk of congenital malformations in children fathered by men treated for germ cell testicular cancer** Yahia Al-Jebari (SWE)
16.00–16.15	OC03	**In vitro culture of Klinefelter spermatogonial stem cells: from mouse to human** Guillermo Galdon (USA)
16.15–16.30	OC04	**Mouse in-vitro spermatogenesis on alginate-based 3D bioprinted scaffolds** Yoni Baert (BEL)
16.30–16.45	OC05	**Metabolic endotoxaemia as a mechanism for reduced serum testosterone following a high fat meal** Kelton Tremellen (AUS)
16.45–17.00	OC06	**Comparative analyses of AZF microdeletions in leukocytes and testis tissue of TESE patients reveal genetic mosaicism in germ line impairing sperm prognosis** Peter H. Vogt (GER)
17.00–18.00	**State of the art lectures**	*Chairpersons: Aleksander Giwercman (SWE), Roger Mieusset (FRA)*
17.00–17.15	L01pros	**Endocrine disruptors and reproductive health: PROS** Jorma Toppari (FIN)
17.15–17.30	L01cons	**Endocrine disruptors and reproductive health: CONS** Jens Peter Bonde (DEN)
17.30–18.00	L02	**EAA ultrasound project** Francesco Lotti (ITA)
18.00–19.30	**Poster session**	*Chairpersons: Osama Kamal Zaki Shaeer (EGY), Istvan Kiraly (HUN)*
19.45–21.00	**Welcome reception**	

Friday, 12 October 2018 – Ballroom A

08.30–10.00	**Symposium 1**	**Genetics and Omics in male infertility** *Chairpersons: Maris Laan (EST), Francesco Lombardo (ITA)*
08.30–08.50	RT01-1	**Genetic causes of meiotic arrest** Frank Tüttelmann (GER)
08.50–09.10	RT01-2	**Sperm chromatin remodeling: revisiting histone-to-protamine exchange** Sophie Rousseaux (FRA)

(continued)

09.10–09.30	RT01-3	**Towards an understanding of sperm metabolomics** Joao Ramalho Santos (POR)
09.30–09.45	OC07	**Long-term effect of testicular germ cell tumor treatments on sperm DNA fragmentation** Elena Casamonti (ITA)
09.45–10.00	OC08	**TEKT5 is a new candidate gene for male infertility** Corinna Friedrich (GER)
10.00–11.00	**Symposium 2**	**Late-onset hypogonadism: is its time over?** *Chairpersons: Dirk Vanderschueren (BEL), Giovanni Corona (ITA)*
10.00–10.20	RT02-1	**LOH is dead – is it?** Eberhardt Nieschlag (GER)
10.20–10.40	RT02-2	**LOH is still alive** Mario Maggi (ITA)
10.40–11.00	RT02-3	**LOH to be alive is not enough** Ilpo Huhtaniemi (FIN)
11.00–11.30	**Coffee break**	
11.30–12.00	**ASA-EAA exchange lecture**	*Chairperson: Csilla Krausz (ITA)*
	L03	**The GEMINI study: fine-mapping the genetic architecture of severe male infertility** Don F. Conrad (USA)
12.00–12.30	**ESE-EAA exchange lecture**	*Chairperson: Hermann M. Behre (GER)*
	L04	**Gonadotrophins in male fertility** Manuela Simoni (ITA)
12.30–13.30	**Lunch**	
13.00–14.30	**Industry symposium 1**	**Hypogonadism Therapy - Sprint or Marathon - Bayer Symposium Update on testosterone therapy for the treatment of hypogonadal men** *Chairperson: Stephan van Haehling (GER)*
13.00–13.15	IS01	**Testosterone Therapy in men with type 2 diabetes, Quality of Life and Mortality** Michael Zitzmann (GER)
13.15–13.30	IS02	**Hypogonadism therapy the Urologists view** Alexander Pastuszak (USA)
13.30–13.45	IS03	**What do we know about long-term effects of testosterone therapy?** Farid Saad (GER)
13.45–14.00	IS04	**Practical management of hypogonadal men with testosterone therapy** Janine David (GBR)
14.30–15.30	**Symposium 4**	**EAA guidelines** *Chairpersons: Giovanni Corona (ITA), Dimitrios G. Goulis (GRE)*
14.30–14.50	RT04-1	**Management of bone health in the andrological outpatient clinic** Vincenzo Rochira (ITA)
14.50–15.10	RT04-2	**Management of Oligo-Astheno-Teratozoospermia** Giovanni Colpi (ITA)
15.10–15.30	RT04-3	**Gynecomastia evaluation and management** Niels Jørgensen (DEN)
15.30–16.00	**Coffee break**	
16.00–17.00	**Symposium 5**	**Onco-Andrology** *Chairpersons: Sandro La Vignera (ITA), Ahmed Mahmoud (BEL)*
16.00–16.20	RT05-1	**Andrological care in adults after childhood cancer** Christine Wyns (BEL)
16.20–16.40	RT05-2	**Late effects of oncological treatment in prostate cancer** Nikolaos Sofikitis (GRE)
16.40–17.00	RT05-3	**Infertility and cancer: a genetic link?** Csilla Krausz (ITA)
17.00–18.00	**Symposium 6**	**From andrological diseases to general health** *Chairpersons: Alberto Ferlin (ITA), Mustafa F. Usta (TUR)*
17.00–17.20	RT06-1	**Erectile dysfunction and risk factors of socially significant diseases** Stepan Krasnyak (RUS)
17.20–17.40	RT06-2	**Long-term health in ICSI children** Herman Tournaye (BEL)
17.40–18.00	RT06-3	**Semen quality as a marker of general health** Niels Jørgensen (DEN)
18.00–19.30	**EAA General assembly**	
19.30–20.00	Transport	
20.00–23.00	**Gala Dinner**	

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Friday, 12 October 2018 – Ballroom B

08.30–10.00	**ESAU-EAA session 1**	**Debate and State of The Art Lectures** *Chairpersons: Gert Dohle (NET), Zsolt Kopa (HUN)*
08.30–08.45	ESAU01-1	**Debate in Andrology: Sperm DNA integrity testing: Just one more test?** Thorsten Diemer (GER)
08.45–09.00	ESAU01-2	**Debate in Andrology: A test with a major role in the prediction of sperm fertilizing potential** Jolanta SŁowikowska-Hilczer (POL)
09.00–09.15	ESAU01-3	**State of The Art Lecture: Hypogonadism in young men treated for cancer** Aleksander Giwercman (SWE)
09.15–09.30	ESAU01-4	**State of The Art Lecture: Effects of primary testicular damage on early embryonic development** Nikolaos Sofikitis (GRE)
10.00–11.00	**ESAU session 2**	**Surgery of male infertility** *Chairpersons: Sabine Kliesch (GER), Wolfgang Aulitzky (AUT)*
10.00–10.15	ESAU02-1	**Surgery for sperm recovery from a testis with malignant disease: sperm recovery rate and consequences in testicular function** Marij Dinkelman-Smit (NET)
10.15–10.30	ESAU02-2	**Do the effects of varicocelectomy on Leydig cellular secretory function, sperm DNA integrity, and sperm functional assays raise the need to revisit the indications for varicocelectomy?** Suks Minhas (GBR)
10.30–10.45	ESAU02-3	**Surgery for testicular torsion-detorsion: is there a place for potential adjunct pharmacological treatment?** Sabine Kliesch (GER)
10.45–11.00	ESAU02-4	**Sperm recovery for cryopreservation from young boys with oncological disease: which is the best approach?** Zsolt Kopa (HUN)
11.00–11.30	**Coffee break**	
12.30–13.30	**Lunch**	
13.30–14.30	**Symposium 3**	**Testis Endocrinology and Biology** *Chairpersons: Stefan Arver (SWE), Giancarlo Balercia (ITA)*
13.30–13.50	RT03-1	**Leydig cell function beyond testosterone** Luca De Toni (ITA)
13.50–14.10	RT03-2	**Testicular macrophages: how hormones determines their function?** Andreas Meinhardt (GER)
14.10–14.30	RT03-3	**Sertoli cell function beyond spermatogenesis** Davor Jezek (CRO)
14.30–15.30	**ESAU session 3**	**Erectile dysfunction** *Chairpersons: Eduard Ruiz Castane (ESP), Ates Kadioglu (TUR)*
14.30–14.45	ESAU03-1	**Indications for ectopic reservoir placement during inflatable penile implant surgery** Eduard Ruiz Castane (ESP)
14.45–15.00	ESAU03-2	**Is there a role for non-invasive approaches in the treatment of iatrogenic priapism?** Antoine Faix (FRA)
15.00–15.15	ESAU03-3	**Parameters associated with the degree of penile deformity in Peyronie disease** Ates Kadioglu (TUR)
15.15–15.30	ESAU03-4	**Cardiovascular risk factors associated with erectile dysfunction** Oleg Apolikhin (RUS)
15.30–16.00	**Coffee break**	
16.00–17.00	**Selected oral communications 2**	*Chairpersons: Osvaldo Rajmil (ESP), Matyas Benyo (HUN)*
16.00–16.15	OC09	**Metabolomic profiling by 1H-NMR of human seminal plasma and database-driven analysis reveal new features for glycerophosphocholine** Marie Walschaerts (FRA)
16.15–16.30	OC10	**Presentation, clinical features, and long term follow up of Leydig cell tumors (LCTs) of the testis: a single centre experience** Carlotta Pozza (ITA)
16.30–16.45	OC11	**Establishing a SNP-panel on single nucleotide polymorphisms associated with FSH action: an approach for personalized FSH treatment in men with unexplained infertility** Maria Schubert (GER)
16.45–17.00	OC12	**A chromosomal scan of single sperm cell by combining: Fluorescence-activated cell sorting and Next-generation sequencing** Ty Tran Quoc (EST)
17.00–18.00	**Selected oral communications 3**	*Chairpersons: Kamal Zaki Mahmoud Shaeer (EGY), Felice Francavilla (ITA)*

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17.00–17.15	OC13	**Testicular ultrasound inhomogeneity is more informative than testicular volume in fertility evaluation** Giorgia Spaggiari (ITA)
17.15–17.30	OC14	**Antisperm-antibodies prevalence and relationship of autoimunisation degree with semen parameters and post-coital test outcome: A retrospective analysis on over 10,000 men** Arcangelo Barbonetti (ITA)
17.30–17.45	OC15	**Testosterone replacement therapy is able to reduce prostate inflammation in men with BPH, metabolic syndrome and hypogonadism: preliminary results from a randomized placebo-controlled clinical trial** Giulia Rastrelli (ITA)
17.45–18.00	OC16	**Effects of different follicle-stimulating hormone preparations on pre-pubertal porcine Sertoli cell cultures: preliminary results** Giovanni Luca (ITA)
18.00–19.30	**National symposium (Russian Federation)**	**Serenoa Repens medicines in prostate diseases treatment**
18.00–18.30	NS01	**Treatment and prevention of relapse of chronic prostatitis as a factor in the BPH pathogenesis?** Andrey Z. Vinarov (RUS)
18.30–19.00	NS02	**Prevention of BPH progression – myth or reality?** Leonid G. Spivak (RUS)
19.00–19.10	NS03	**10-year experience of continuous Serenoa Repens usage in the treatment of BPH patients** Andrey Z. Vinarov (RUS)
19.10–19.20	NS04	**15-year experience of continuous Serenoa Repens usage in the treatment of BPH patients** Leonid G. Spivak (RUS)
19.20–19.30		Discussion

Saturday, 13 October 2018 – Ballroom A

09.00–09.30	**State of the art lecture**	*Chairperson: Raffael Oliva (ESP)*
	L05	**Future options for fertility preservation** Stefan Schlatt (GER)
09.30–11.00	**Young researchers in Andrology**	*Chairpersons: Yoni Baert (BEL), Thomas Darde (FRA)*
09.30–10.00	YR01	**The effect of exposure to endocrine disruptors on development of the human fetal testis** Rod Mitchell (GBR)
10.00–10.15	OC17	**Molecular and functional characterization of a unique genotype in a man affected by congenital hypogonadotropic hypogonadism** Francesca Cioppi (ITA)
10.15–10.30	OC18	**X-chromosome exome sequencing in highly selected idiopathic azoospermic patients: identification of novel and recurrent genetic factors for early spermatogenic failure** Antoni Riera-Escamilla (ESP)
10.30–10.45	OC19	**Top-down proteomic approach to study the protamine post-translational modifications profile in the human spermatozoa** Ada Soler-Ventura (ESP)
10.45–11.00	OC20	**Functional characterization of Binder of SPerm homolog 1 in sperm-egg interaction and fertilization** Hamed Heidari-Vala (CAN)
11.00–11.30	**Coffee break**	
11.30–12.30	**Symposium 7**	**Transitional Andrology: from adolescence to adult** *Chairpersons: Jorma Toppari (FIN), Margus Punab (EST)*
11.30–11.50	RT07pros	**Early endocrine treatment for Klinefelter syndrome? – PROS** Anders Juul (DEN)
11.50–12.10	RT07cons	**Early endocrine treatment for Klinefelter syndrome? – CONS** Julia Rohayem (GER)
12.10–12.30	RT07-3	**Optimal management of disorders of sex development** Martine Cools (BEL)
12.30–13.00	**Closing ceremony/Awards**	
13:00–14:00	**Lunch**	

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Saturday, 13 October 2018 – Ballroom B		
09.30–11.00	**Industry symposium 2**	**Berlin-Chemie Menarini Symposium: Andrological disease** *Chairperson: Zsolt Kopa (HUN)*
09.30–09.50	IS06	**Premature ejaculation** Emmanuele A. Jannini (ITA)
09.50–10.10	IS07	**Medical management of erectile dysfunction** Maarten Albersen (BEL)
10.10–10.30	IS08	**Lower Urinary Tract Symptoms and erectile dysfunction: is there a causal relationship?** Gert Dohle (NET)
10.35–11.00	**Industry symposium 3**	**Ferring mini-symposium: Testosterone replacement**
	IS09	**Testosterone replacement therapy: patient-focused care** Zsolt Kopa (HUN)
11.00–11.30	**Coffee break**	
11.30–12.30	**Symposium 8**	**Beyond standard semen analysis** *Chairpersons: Hermann M. Behre (GER), Gerhard Haidl (GER)*
11.30–11.50	RT08-1	**WHO Manual: Status Report and WHO perspectives** Elisabetta Baldi (ITA)
11.50–12.10	RT08-2	**Sperm RNA and male fertility** Meritxell Jodar (ESP)
12.10–12.30	RT08-3	**How many sperm function tests do we need?** Verena Nordhoff (GER)

ANDROLOGY

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INVITED LECTURES
PRE-CONGRESS COURSE: SYSTEMATIC APPROACH OF THE INFERTILE MALE

PC01

Semen characteristics and male pathology

R. MIEUSSET
Université Toulouse III-Paul Sabatier, Human Fertility Research Group, Toulouse; Andrologie-Médecine de la Reproduction, Hôpital Paule de Viguier, CHU de Toulouse, Toulouse

No text available.

PC02

Endocrine evaluation

D. VANDERSCHUEREN
Department of Endocrinology, University Hospital, KU Leuven, Belgium

Male infertility may be the first presentation of pretesticular problems secondary to pituitary failure, hyperprolactinemia, hemochromatosis or even anabolic androgenic steroid abuse. Also testicular problems such as spermatogenic as well Leydig cell failure, Leydig cell tumors and Klinefelter syndrome may be diagnosed during male fertility evaluation. Therefore, such evaluation should include not only clinical but also endocrine work-up in every patient with azoospermia, cryptozoospermia or severe oligoasthenoteratozoospermia in order to fully exclude these potential underlying conditions.

Both Leydig cell as well as Sertoli cell function and their hypothalamic-pituitary feedback are evaluated by respectively measurement of testosterone /LH and inhibin B/ FSH.

Increase of FSH is the most sensitive early marker of spermatogenic failure. It however is important to use narrow reference limits for FSH of a fertile male population. Inhibin B is inversely related to FSH. Spermatogenic arrest – in contrast with other forms of spermatogenic failure – may not be reflected by a rise of FSH. Leydig cell failure may be present in patients with male subfertility and may be confirmed by low morning fasting serum testosterone relative to high LH concentrations. Hypogonadotropic hypogonadism (either functional or organic) is - in contrast- characterized by low serum testosterone in combination with low or inappropriate low normal gonadotrophins. The diagnosis of hypogonadotropic hypogonadism may require additional investigations such as determination of serum prolactin, iron saturation as well as measurement of other pituitary hormones. Magnetic resonance imaging of pituitary is indicated when testosterone concentrations are very low and /or other signs or pituitary dysfunction or mass are present. Functional hypogonadotropic secondary to co-morbid conditions, drugs or obesity may be a reversible condition. Fertility related to hypogonadal hypogonadism (organic as well as functional) may also be successfully treated with gonadotrophins. Finally, endocrine evaluation also often allows the differentiation between obstructive and non-obstructive azoospermia in most cases. However, the success of testicular sperm retrieval is not always predicted by endocrine evaluation. In conclusion, endocrine evaluation is an important part of the diagnostic work-up of every men suffering from infertility.

PC03

Imaging of the male reproductive system

A. M. ISIDORI

No text available.

PC04

Perspectives of genetics in the Andrology Clinic

M. LAAN
Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia

Male infertility affects up to 7% of men worldwide. Current clinical practice enables to assign a definite causative factor for only 40% of men with impaired sperm counts (Punab et al 2017, Hum Reprod). There is a growing understanding that a substantial proportion of idiopathic male infertility cases could result from rare genetic defects affecting the complex process of spermatogenesis and/or by more common genetic variants jointly modulating reproductive physiology. However, today's knowledge of the mutational landscape behind male infertility is lagging behind several other fields in medical genetics. Recommendations for genetic tests in the andrology clinic are limited to cytogenetic profiling, and analyzing either Y-chromosomal deletions or mutations in the *CTFR* gene for selected patients. These tests explain around 25% of azoospermia, but only 1–2% of oligozoospermia cases. It has been challenging to find proper methodological approaches to identify genetic contribution to impaired spermatogenesis and reproductive physiology due to high number of potentially involved genes and genetic variants. In addition, the specific clinical consequence of impaired reproduction hinders traditional family-based genetic studies.

During last 10 years, novel tools have been introduced to basic and translational research in medical genetics. The lecture covers currently applicable approaches for genetic research and molecular diagnostics in andrology, and provides an overview about recent novel insights as well as challenges in the field. Potential implications of the accumulated knowledge at the level of an individual patient will be envisioned. Perspectives to broaden the palette of genetic tests, improve the current clinical guidelines and reach a higher diagnostic yield in the andrology practice will be discussed.

PC05

Global health assessment of the infertile man

A. FERLIN
Endocrinology Unit, University of Brescia, Department of Clinical and Experimental Sciences, Brescia, Italy

Definition of an etiological diagnosis of male infertility is fundamental for proper management of the couple, treatment decision and to have prognostic information. Full diagnostic work up of the male partner of an infertile couple is necessary when significant risk factors for infertility, spermatogenesis and reproductive tract disorders are present, as well as when semen analysis highlighted quantitative or qualitative sperm defects and/or semen fluid alterations. In these cases, appropriate diagnostic procedures are recommended and might include endocrine assessment, imaging of testes and seminal tract, microbiological evaluation, genetic tests, cytology/histology of the testes and second step sperm analysis as indicated.

However, fertility evaluation gives men the unique opportunity for general health assessment and disease prevention. Men of infertile couples should be not only correctly diagnosed for definition of the fertility potential, but also should be globally evaluated and followed because they could have an increased chance of morbidity and mortality. In fact, increasing evidence suggested that infertile men are at increased risk for hypogonadism, metabolic derangements and osteoporosis, and have higher long-term morbidity and mortality than controls.

We tested whether semen quality and reproductive function could represent a marker of general male health, by prospectively recruiting 5177 males of infertile couples who underwent full fertility evaluation (semen analysis and culture, reproductive hormones, testis ultrasound, karyotype, Y chromosome microdeletions, CFTR gene mutations when indicated) and biochemical determinations for glucose and lipid metabolism. Subjects with hypogonadism underwent also dual-energy x-ray absorptiometry for bone mineral density (BMD). In very summary, we found that men with low sperm have increased risk of having hypogonadism, higher BMI, waist circumference, systolic pressure, LDL-cholesterol, triglycerides, and HOMA-index, and lower HDL-cholesterol, and in general they have higher prevalence of metabolic syndrome. All data were worse in men with hypogonadism, but interestingly, low sperm count per sé was associated with poor metabolic parameter. Men with hypogonadism had lower BMD and 51% prevalence of osteoporosis/osteopenia.

Therefore, sperm quality is a mirror of the general male health, as low sperm count is associated with poorer metabolic, cardiovascular and bone health. Clinicians should be aware that global health assessment in infertile patients is recommended and patients should correctly followed up even after a pregnancy is obtained.

PC06

Clinical value of proteomic biomarkers

R. OLIVA
Molecular Biology of Reproduction and Development Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clinic from Barcelona, Faculty of Medicine, University of Barcelona, Casanova 143, Barcelona, Spain, roliva@ub.edu

Conventional semen analysis still remains as the tool of choice in the routine evaluation of male factor fertility. Although the basic seminal assessment provides valuable information, its capacity to predict reproductive potential is limited. Currently, other additional screening tools, such as sperm DNA damage and oxidative stress, as well as epigenomic and proteomic markers are being discovered and evaluated in their potential to predict reproductive outcomes in a more accurate way (Carrell DT, et al., Cell Tissue Res. 2016;363:295–312). So far, mass spectrometry-based proteomics has led to the identification of 6871 proteins in human ejaculated spermatozoa, 2064 in seminal fluid, and 6612 the human testis (Jodar M, et al. J Proteomics 2017;162:125–134). Knowledge of this proteomic composition provides an essential first step to understand the molecular basis of the reproductive functions. Most of the sperm proteins are related to spermatogenesis and sperm function, but less abundant protein groups with potential post-fertilization roles have also been detected. 103 human sperm proteins are functionally related to the process of fertilization and 93 to preimplantation embryo development (Castillo J, et al. Hum Reprod Update 2018, May 23). Additionally, 560 sperm proteins have been found to be involved in modulating gene expression by the regulation of transcription, DNA methylation, histone post-translational modifications and non-coding RNA biogenesis and function. Some of these sperm proteins may be critical for gene expression regulation after embryo genome activation, and therefore, may be potentially involved in epigenetic transmission of altered phenotypes. The characterization of specific sperm protein alterations in different infertility phenotypes is currently leading to the identification of novel specific biomarkers that may help in the diagnosis, prognosis and treatment of male infertility (Codina C, et al. Expert Rev Proteom 2015;12:255–77). While the recent advances have not yet reached the widespread routine application in the clinic, case-control studies are now feasible to determine which of the proteins so far detected as deregulated in infertile patients have a predictive value of male fertility.

PC07

Sexual transmitted disease and fertility

A. VIVES

No text available.

PC08

Medical therapy of the infertile men

G. CORONA
Endocrinology Unit, Maggiore-Bellaria Hospital, Bologna, Italy

Background: Infertility affects around 15% of couples trying to conceive. A male factor is involved in about 50% of cases. Unfortunately, in the vast majority of the cases it is not possible to identify a specific etiological factor; in the latter cases only empirical therapy is possible.
Methods: A comprehensive review of available data will be performed providing the best evidence based information published so far.
Results: Lifestyle modification including weight loss and smoking cessation should be the first step in all obese or overweight infertile subjects. Hypogonadotropic hypogonadism (HHG) is one of the few causes of male infertility that can benefit from medical therapy. In this case the combined use of gonadotropins can allow sperm production in about 50% of cases. Antibiotic therapy can be useful in patients with symptomatic male genitalia tract infections whereas its role in asymptomatic leukocytospermia is conflicting. The use of follicular stimulating hormone (FSH) alone has been demonstrated to improve sperm parameters and pregnancy outcomes even in idiopathic oligo-astheno-teratozoospermia. In the latter case FSH receptor (FSHR) genotype can modulate the final response. A beneficial effect of nutraceuticals in the treatment of idiopathic male infertility has also been described. However, despite their large use in clinical setting, evidence supporting the use of antioxidants for the treatment of male infertility is of low quality. Similar considerations can be drawn for the use of steroids in autoimmune infertility.
Conclusion: Medical therapy plays an important role in the management of male of infertile couple. A correct diagnosis and specific therapy can improve fertility outcome independent of the use of assisted reproductive techniques.

PC09

Surgical treatment

S. KLIESCH

No text available.

PC10

Assisted reproduction techniques

D. G. GOULIS
Unit of Reproductive Endocrinology, 1st Department of Obstetrics and Gynecology, Medical School, Aristotle University of Thessaloniki, Greece.

Definition: Assisted Reproduction Techniques (ART) can be defined as procedures that involve *in vitro* handling of human oocytes, sperm or embryos for achieving a pregnancy. ART include: a) Intra-uterine insemination (IUI), with woman's partner (AIH) or donor sperm (AID), b) *in vitro* fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI), c) testicular sperm extraction (TESE) or microsurgical testicular sperm extraction (micro-TESE) and d) semen cryopreservation.
General considerations: ART are purely symptomatic measures, which do not cure the underlying cause of infertility. Therefore, before their appliance, every attempt must be made to establish the diagnosis of male infertility, as the latter may have severe implications for the infertile man. In general, ART are applied to improve the chance of achieving pregnancy, in case other treatment options [lifestyle modifications, gonadotrophins, selective estrogen receptor modulators (SERMs), antioxidants] are not available or not efficient.
Indications for IUI: Unexplained infertility, mild male factor infertility, disability (physical or psychological) preventing vaginal sexual intercourse, conditions that require specific consideration in relation to methods of conception, as part of donor insemination (AID), mild endometriosis, fertility preservation. Also, IUI in stimulated cycles may be considered while waiting for IVF, or when in women with patent tubes IVF is not affordable. For IUI to be successful, typically 5 million motile sperm must be present in the ejaculate.
Indications for cryopreservation: Sperm may be for IUI, IVF or ICSI, in cases of a) severe oligo-astheno-teratozoospermia (OAT) or intermittent presence of sperm (cryptozoospermia), b) treatment of infertility that may not persist, such as gonadotrophin treatment for hypogonadotropic hypogonadism, c) special situations, such as assisted ejaculation for patients with spinal cord injury or sperm from retrograde ejaculation in urine and d) men who are unable to provide fresh semen on the day of an ART procedure.

STATE OF THE ART LECTURES

L01pros

Endocrine disruptors and reproductive health: PROS

J. TOPPARI
Institute of Biomedicine, Centre for Integrative Physiology and Pharmacology, University of Turku, Turku, Finland

Male reproductive health has deteriorated. Semen quality of young men is globally bad, while the incidence of testicular cancer has multiplied and the incidence of cryptorchidism and hypospadias is high. Reasons for poor reproductive health are not known, but the trends point to environmental factors. Genetic susceptibility is always the other important contributor to any complex disease. There are some hints from the genetics that guide us in search for causes of reproductive problems. Any genetic defect affecting androgen production or action can cause the problems listed above. Similarly, any chemical agent affecting androgen action has the same capacity. Currently we know hundreds of chemicals that are antiandrogenic. However, we are usually not exposed to any of them in high enough doses to be harmed. Troubles appear when we are exposed to high enough cocktail dose of these chemicals, which has been shown in several experimental models. Furthermore, other signaling routes matter, too. Dioxins and estrogenic compounds act in the same direction as antiandrogens, adding load in the whole exposome.

Individual chemicals can be recognized as reproductive toxicants usually only in occupational settings (e.g., di-bromo chlor-propane) or in chemical accidents (e.g., Seveso). Male reproductive toxicity of diethylstilbestrol was recognized years after placebo-controlled randomized clinical trial where men had been exposed in utero. We hope that we would not need occupational or environmental accidents any longer to recognize compounds that are risky for reproductive health but could consider all available scientific evidence to prevent any reproductive harm in the future.

L01cons

Endocrine disruptors and reproductive health: CONS

J. P. BONDE

No text available.

L02

EAA ultrasound project

F. LOTTI[1], G. BALERCIA[2], A. BARBONETTI[3], H. M. BEHRE[4], A. E. CALOGERO[5], J. -F. CREMERS[6], F. FRANCAVILLA[3], G. SALVIO[2], A. M. ISIDORI[7], S. KLIESCH[6], S. LA VIGNERA[5], A. LENZI[7], M. MARCOU[4], A. PILATZ[8], O. POOLAMETS[9], M. PUNAB[9], M. F. PERAZA GODOY[10], O. RAJMIL[10], O. SHAEER[11], W. WEIDNER[8] AND M. MAGGI[1]

[1]Sexual Medicine and Andrology Unit, Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy; [2]Division of Endocrinology, Department of Clinical and Molecular Sciences, Polytechnic University of Marche, Ancona, Italy; [3]Andrology Unit, Department of Life, Health and Environment Sciences, University of L'Aquila, L'Aquila, Italy; [4]Center for Reproductive Medicine and Andrology, Martin Luther University Halle-Wittenberg, Halle, Germany; [5]Section of Endocrinology, Andrology and Internal Medicine, Department of Medical and Pediatric Sciences, University of Catania, Catania, Italy; [6]Department of Clinical and Surgical Andrology, Center of Reproductive Medicine and Andrology, University Hospital of Münster, Münster, Germany; [7]Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy; [8]Department of Urology, Pediatric Urology and Andrology, Justus Liebig University, Giessen, Germany; [9]Andrology Unit, Tartu University Hospital, Tartu, Estonia; [10]Andrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, Instituto de Investigaciones Biomédicas Sant Pau (IIB-Sant Pau), Barcelona, Spain; [11]Department of Andrology, Kasr El Aini Faculty of Medicine, Cairo University, Cairo, Egypt

The EAA ultrasound project was aimed at investigating the colour-Doppler ultrasound (CDUS) features of the male genital tract (MGT) in healthy, fertile men, to obtain "normative" parameters for both scrotal and transrectal CDUS. The project was developed in Florence (practical investigator meeting) and during the Berlin ECA Congress (protocol discussion) in 2012. The characteristics of the study are available at http://www.andrologyacademy.net/studies. The primary aim was to evaluate the CDUS features of the scrotal and prostate-vesicular organs in healthy, fertile men. The secondary aim was to correlate the CDUS findings with clinical, seminal and biochemical parameters evaluated during the same CDUS session. The study was designed as a cohort, multicentric, international, observational study. Eleven EAA Centers joined the project (Ancona, Italy; Barcelona, Spain; Cairo, Egypt; Catania, Italy; Florence, Italy; Giessen, Germany; Halle-Saale, Germany; L'Aquila, Italy; Muenster, Germany; Rome, Italy; Tartu, Estonia). The Florence EAA Center was the coordinating center. The main inclusion criteria were: men aged $\geq$ 18 years, without serious organic diseases, partners of a pregnant woman in the second or third trimesters of pregnancy or who fathered a child during the last year, following natural conception. The study protocol included the following procedures, performed during the same day: 1) personal and medical history; 2)physical examination; 3) blood samples for determination of hormonal and glycometabolic parameters, evaluated in the Florence Central Lab (including gas chromatography/mass spectrometry for steroid hormones); 4)scrotal and transrectal CDUS evaluated before and after ejaculation; 5)semen analysis (according to the WHO, 2010). We enrolled 247 healthy, fertile men. Data on 247 scrotal and 187 transrectal ultrasound evaluations are available. The results of the study will be presented at the Budapest ECA Congress 2018. New knowledge generated by our multicenter research consortium will define the impact of MGT-CDUS on reproductive medicine. Standardization of MGT-CDUS parameters in a healthy, fertile population will lead to an improvement of our diagnostic skills in identifying etiological factors of male infertility.

L05

Future options for fertility preservation

S. SCHLATT

Center for Reproductive Medicine and Andrology, Westfälische-Wilhelms University Münster, Münster, Germany

Cryopreservation of sperm is the standardized fertility preservation method in adult patients facing gonadotoxic oncological therapy. However, this strategy cannot be applied in prepubertal boys. Breakthroughs in spermatogonial research encourage andrologists to explore novel options for ex vivo generation of sperm. These techniques may be applicable for a wide range of applications in andrology but may be most relevant to improve fertility preservation in boys. Cancer remains the primary, but not the only entity of pediatric patients which may benefit. The testis contains a population of spermatogonial stem cells (SSCs). These cells provide the seminiferous epithelium with a high regenerative potential as they harbor an enormous expansion capacity. The cells are present from birth to adulthood. The testicular stem cells are located in niches whose definition and function in mammals is still not fully resolved but renders a sub-fraction of spermatogonia mitotically quiescent. The differentiating spermatogonia are known to be highly chemo- and radiosensitive.

Even small toxic insults lead to a spermatogenic block at premeiotic stages of spermatogenesis. The depleted spermatogonia are replenished from spermatogonial stem cells which recolonize the basement membrane of the seminiferous tubules. The new cohorts of germ cells are highly selected for DNA integrity through a thus far unknown mechanism. Due to the cellular features of spermatogonial stem cells, germ cell transplantation is considered a promising strategy for fertility preservation. While this strategy has become a widely used research tool in rodents, the transfer into a clinical application must still be regarded as experimental. However, its successful application in non-human primates revealed that it may offer a clinical perspective. Another alternative is to use fragments from immature testes containing stem cells. When these tissues are grafted or cultivated under optimal conditions, full spermatogenesis can be achieved. The generation of sperm in xeno- or autografts as well as the in vitro generation of sperm has recently evoked enormous scientific interest. Focusing on in vitro spermatogenesis, it became obvious that isolated spermatogonial stem cells can be expanded in vitro but that these cells do not enter gametogenesis when cultured as homogenous populations. Spermatogonial stem cell-like cells, however, can be generated in vitro by stepwise application of specified culture conditions from pluripotent precursors. To create conditions that the spermatogonial cells differentiate and enter male or female gametogenesis in vitro, the cells have to be co-cultured or intermingled with immature gonadal tissues. Such strategy leads to formation of gonadal organoids prompting the germ cells by an adequate gonadal microenvironment to enter gametogenesis finally leading to mature and fertilization-competent gametes. This talk presents an update on testicular stem cells and an outlook on spermatogonia-based technologies which could enter into clinical scenarios. Challenges and limitations are also discussed.

YR01 (Network of Young Researchers in Andrology)

The effect of exposure to endocrine disruptors on development of the human fetal testis

R. T. MITCHELL
MRC Centre for Reproductive Health, University of Edinburgh; Royal Hospital for Sick Children, Edinburgh

The most common male reproductive disorders (cryptorchidism, hypospadias, testicular cancer and infertility) have been increasing in incidence over recent decades. These associated disorders are often referred to as Testicular Dysgenesis Syndrome (TDS). The development of TDS may be influenced by genetic factors or environmental insults that occur during fetal life. In particular, in-utero exposure to Endocrine Disrupting Chemicals (EDCs) has been associated with effects on testosterone production and germ cell development in rodents which may manifest as male reproductive disorders in postnatal life. However, evidence for the effect of EDCs on the human fetal testis is less well described.

We have validated in-vitro (hanging-drop) and ex-vivo (xenograft) systems using human fetal testis that can model in-utero development and function of the human testis. Using these systems we have investigated the effect of exposure to a variety of proposed EDCs including environmental (phthalates and bisphenols) and pharmaceuticals (analgesics and chemotherapeutics) using human-relevant exposures and regimens. We have demonstrated important differences between rodent and human fetal testis in terms of effects of environmental exposures (e.g. di-n-butyl phthalate, bisphenol A) on testosterone production. We have also shown that exposure to analgesics (paracetamol and ibuprofen), using human-relevant therapeutic regimens, results in a reduction in testosterone and loss of germ cells in human fetal testes. I will present a comprehensive description on the evidence for and against testicular effects of EDC exposure in human fetal testis development and function. In addition I will discuss the advantages and limitations of the experimental systems used to assess the effects of EDC exposure on the developing human testis.

ASA-EAA EXCHANGE LECTURE

L03

The GEMINI study: fine-mapping the genetic architecture of severe male infertility

L. NAGIRNAJA[1], J. RUSCH[1], A. LOPES[2], K. ALMSTRUP[3], B. HOUSTON[4], E. RAJPERT-DE MEYTS[3], C. KRAUSZ[5,6], M. LAAN[7], M. O'BRYAN[4], K. I. ASTON[8], D. F. CONRAD[1,9]
AND ON BEHALF OF THE GEMINI CONSORTIUM
[1]Department of Genetics, Washington University in St. Louis, USA; [2]IPATIMUP, University of Porto, Portugal; [3]Rigshospitalet, University of Copenhagen, Denmark; [4]Monash Biomedicine Discovery Institute, Monash University, Australia; [5]Andrology Department, Universitat Autònoma de Barcelona, Barcelona, Spain; [6]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Italy; [7]Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia; [8]Department of Surgery, University of Utah, USA; [9]Oregon National Primate Research Center, Oregon Health & Science University, USA

Introduction: Male infertility due to spermatogenic failure is a common disorder found in 30 million men worldwide. Genetic factors are known to contribute to the manifestation of the disease, however the properties of the underlying genetic variation have largely remained elusive. It is feasible that rare patient-specific mutations across a multitude of genes essential for sperm development may lead to the disease. An international network of andrologists have established a multi-center study Genetics of Male INfertility Initiative (GEMINI) to fine-map the genetic landscape of idiopathic severe male infertility.

Objectives: We aimed to perform whole exome sequencing and case-by-case mutation discovery in a large cohort of non-obstructive azoospermia (NOA) cases to identify rare variants of large effect as the likely disease cause.

Methods: Whole exome sequencing data was generated on 890 well phenotyped NOA cases from 6 countries. Exome libraries were sequenced on Illumina HiSeq 2500/3000 and analyzed using bwa, GATK and XHMM, producing a map of SNVs, indels and CNVs. Case-by-case variant

prioritization and annotation was performed using a statistical analysis framework developed to assess "$n = 1$" cases of genetic disease. Genes of interest were functionally validated by experimental manipulation in model organisms, mainly Drosophila melanogaster.

Results: Putative rare disease-causing genetic variants were identified in 44% of patients. The affected genes were largely case-specific (92%) and previously undescribed in NOA. The largest fraction of cases (45%) were characterized by autosomal dominant mutations, half of which were loss-of-function (LoF) variants. Most interestingly, 5 new human biallelic knockouts were identified in genes with no previous reports of knockouts in the literature or large databases. *In silico* functional annotation of NOA candidate genes indicated that rare mutations were more likely to occur in genes previously studied in testis or sperm function, and genes specific to Apale spermatogonia critical for the expansion of male germ cell pool. Functional validation confirmed that disruption of many candidate genes led to gonadal dysfunction in model organisms, with an enrichment at least 3-fold above random chance.

ESE-EAA EXCHANGE LECTURE

L04

Gonadotropins in male fertility

M. SIMONI, L. CASARINI AND D. SANTI
Unit of Endocrinology, University of Modena & Reggio Emilia, Italy

Background: FSH and LH are fundamental for spermatogenesis, testosterone production and fertility. Given their central role, several studies evaluated whether gonadotropin supplementation to idiopathic infertile men are able to improve sperm output and fertility. FSH action is mediated by the FSHR, which exist in polymorphic variants affecting FSH activity *in vitro* and *in vivo*. In addition, a polymorphism in the *FSHB* gene promoter influences serum FSH levels. The scope of this presentation is to review the most recent data on the potential for FSH therapy in male idiopathic infertility.

Methods: The following evidence data will be discussed: pharmacogenetics of FSH action; interindividual variability of response to FSH (responders vs. non-responders), effects of FSH on pregnancy rate (from meta-analyses), pharmacodynamics markers of FSH action, pharmacogenetic trials.

Results: The combination of *FSHB* and *FSHR* polymorphisms gives rise to haplotypes related to better/worst response to FSH. However, the pharmacogenetic trials conducted so far did not consider the *FSHB-FSHR* haplotype in a prospective, controlled design. Infertile men carriers of the *FSHR* p.A680S homozygous A genotype responded to FSH treatment with a significant improvement of sperm DNA fragmentation index (DFI). Existing meta-analyses show that FSH treatment of unselected patients improves significantly pregnancy rate. In addition, DFI is significantly higher in infertile vs. control men and improves after FSH treatment. The dosage of FSH and the duration of treatment employed so far are largely empirical and, most probably, insufficient in most men to stimulate spermatogenesis.

Conclusion: FSH treatment of idiopathic infertile men is variably effective. There is urgent need to standardize treatment, and stratify the patients, identifying the responders beforehand. New gonadotropin preparations recently available on the market should be tested in future trials.

SYMPOSIUM 1: GENETICS AND OMICS IN MALE INFERTILITY

RT01-1

Genetic causes of meiotic arrest

F. TÜTTELMANN

No text available.

RT01-2

Sperm chromatin remodeling: revisiting histone-to-protamine exchange

S. ROUSSEAUX, A. VARGAS, S. BARRAL AND S. KHOCHBIN
Epigenetics and cell signaling, Institute for Advanced Biosciences, INSERM U1209, CNRS UMR 5309, Université Grenoble Alpes, France

Post-meiotic haploid male germ cells, spermatids, undergo genome-wide re-organization and compaction of their chromatin, associated with a nearly complete replacement of histones, major protein components of the somatic-type chromatin, by sperm specific DNA-associated proteins, protamines (Prms).

Although this unique process has been described for many years and has been recognized as a crucial step for fertility in many species, including Mammals, the molecular mechanisms driving it have only recently been investigated. In particular, for many years, textbooks have described a two-step process, where transition proteins (TPs) first replace histones, and are then removed and replaced by Prms.

Thorough investigations of the molecular mechanisms at the basis of the histone-to protamine exchange has recently revealed that the incorporation of testis-specific histone variants and specific post-translational histone modifications of histones are guiding these events, by driving stage-specific gene expression programs as well as a direct effect on chromatin. Additionally, these studies demonstrate that Prms are the major histone displacers and TPs, expressed and incorporated at the same time as Prms, are elements that buffer Prms actions and hence fine-tune the Prm-mediated histone replacement. Based on these data, we propose a revised consideration of textbooks dogma on the stepwise replacement of histones.

RT01-3

Towards an understanding of sperm metabolomics

J. RAMALHO-SANTOS
Center for Neuroscience and Cell Biology and Department of Life Sciences, University of Coimbra, Portugal

The mechanisms controlling sperm metabolism, and notably energy metabolism, could provide crucial clues into the function of the male gamete and how it may be manipulated to either select better cells for Assisted Reproduction, or understand certain cases of male infertility. Research into human sperm metabolomics is not an easy task, given both the characteristics of the male gamete (limited cytoplasm, and often difficulty in extracting metabolites) and the heterogeneity of samples available for research, often requiring the use of pooled material. In fact, most available data on sperm metabolism has been extrapolated from functional studies on sperm mitochondria, and on proteomics projects. The latter have revealed the presence of both well-known metabolic pathways and novel candidates, since validated, such as the beta-oxidation of fatty acids. More recently techniques such as NMR and Mass-Spectrometry have been applied to the study of sperm metabolomics. Unsurprisingly these distinct techniques have revealed themselves to be complementary, each being able to identify different types of metabolites. In addition, novel techniques, such as Raman Spectroscopy are being applied to sperm, with the ultimate goal of providing non-invasive and non-destructive analysis of the metabolic status of sperm. These issues will be critically discussed in the talk.

SYMPOSIUM 2: LATE-ONSET HYPOGONADISM: IS ITS TIME OVER?

RT02-1

LOH is dead – is it?

E. NIESCHLAG
Center of Reproductive Medicine and Andrology, University of Münster, Münster, Germany

Late-onset hypogonadism (LOH) was defined first in the ISA, ISSAM, EAU, EAA and ASA endorsed Recommendations for Investigation, Treatment and Monitoring of LOH (2005 and 2008) as "A clinical *and* biochemical syndrome associated with advancing age, characterized by symptoms *and* a deficiency in serum testosterone (T)". LOH was classified as a combined primary and secondary hypogonadism since the endocrine capacity of the testes as well as the pituitary are impaired. Symptoms of LOH were listed as: loss of libido, erectile dysfunction, loss of muscle mass, increased body fat, anemia, osteoporosis, depressed mood, decreased vitality, sweating and hot flushes. Since these symptoms may also have other origins than LOH, exclusion of other disease entities and subnormal serum T levels were considered a prerequisite for the diagnosis and possible treatment of LOH. However, during following years these guidelines were often neglected and, especially in the USA, "low T-clinics" sprung up and indiscriminate prescribing of T was widely practised so that the US FDA became alarmed and warned against such irresponsible behaviour. In Europe T prescribing remained largely restricted to LOH as defined above. Nevertheless, a discussion started whether LOH really exists or is only a consequence of age-related comorbidities. Numerous studies have helped to clarify the situation, in particular, the results of the European Male Aging Study (EMAS) and of the US-initiated *7 T trials*. Consequently the most recent US Endocrine Society Practice Guideline on T treatment (2018) states: "We suggest against routinely prescribing T therapy to all men 65 years or older with low T concentrations. In men >65 years who have symptoms or conditions suggestive of T deficiency (such as low libido or anemia) and consistently and unequivocally low morning T concentrations, we suggest that clinicians offer T therapy on an individualised basis after explicit discussion of the potential risks and benefits." This sounds rather similar to the original LOH recommendations and what a responsible physician/endocrinologist would do.

RT02-2

LOH is still alive

M. MAGGI
Sexual Medicine and Andrology, University of Florence, Florence, Italy

Background: Late onset hypogonadism (LOH) is a form of testosterone deficiency (TD) apparent during adulthood and more prevalent during senescence. Beside TD, LOH is characterized by vague symptoms not different from those typical of the physiological aging process. Sexual problems represent the main complaints referred by the patients, whereas increased waist circumference, decreased muscle mass and osteoporosis are the main clinical correlates. LOH is often considered as a functional disorder, associated with metabolic derangements (glucose intolerance, obesity, dyslipidemia clustered in the metabolic syndrome construct) which hampers GnRH and gonadotropin secretion. According to its *functiona*l origin, treating the underlying condition is often considered the main treatment, whereas testosterone supplementation (TS) is reserved to the *organic* alterations of the hypothalamus-pituitary-testis axis. In fact, the effect of TS in LOH is considered not clinically meaningful and potentially dangerous for cardiovascular and prostate health.
Methods: To evaluate the effect of TS in LOH, we meta-analyzed results obtained in randomized controlled trials (RCTs) performed in population primarily affected by the so-called *functional* hypogonadism. Effect of TS on sexual function, body composition, prostate health and cardiovascular diseases (CVD) were scrutinized and meta-analyzed. Considering that some pharmaco-epidemiological studies indicate unwanted effect of TS on CVD a separate analysis was also performed including results of these studies.
Results: In RCTs, TS was associated to a significant improvement in sexual symptoms, including sexual desire, orgasm, erection and overall satisfaction, even when only studies using IIEF were selected. However, considering erectile dysfunction, results with testosterone were modest (2–3 points IIEF) and definitively lower than those described with PDE5 inhibitors. TS was associated with a

significant modification of fat and fat-free mass, which decreases and increases, respectively, to the same extent. Glucose metabolism was also improved. No major cardiovascular or prostate problems were detected, even considering results from pharmaco-epidemiological studies.
Conclusion: Our data indicate that TS is a valuable option, beside lifestyle modifications, to treat LOH.

RT02-3

LOH to be alive is not enough

I. T. HUHTANIEMI
Institute of Reproductive and Developmental Biology, Department of Surgery & Cancer, Hammersmith Campus, Imperial College London, London W212 0NN, UK

The term late-onset hypogonadism (LOH) is a misnomer, and the condition is more appropriate to be called functional hypogonadism (FH). It occurs mainly due to other causes than chronological aging, mainly overweight and aging-related comorbidities. Recent studies have clarified its diagnostic criteria, defining the critical testosterone concentrations and the associated hypogonadal symptoms. Unlike organic hypogonadism FH can be reversible, and elimination of its causes are generally considered the first line of treatment, including weight loss, lifestyle modification and optimization of treatment of comorbidities. Although low T can be considered the causative factor, the bulk of good-quality information indicates that low T is rather a risk marker that risk factor of FH-associated ill health, albeit the relationship can also function bidirectionally. This downplays the importance of testosterone administration in the treatment of FH. Nevertheless, information in mounting, still mainly from uncontrolled observational studies, but also from proper RCTs, that some FH symptoms respond favorably to testosterone treatment. Most importantly, however, the long-term benefits and risks of testosterone therapy still remain largely unknown. The aim of this presentation is to present an opinion about the current state and future needs of testosterone treatment of FH.

SYMPOSIUM 3: TESTIS ENDOCRINOLOGY AND BIOLOGY

RT03-1

Leydig cell function beyond testosterone

L. DE TONI
Department of Medicine and Unit of Andrology and Reproductive Medicine, University of Padova, Padova, Italy

Increasing evidence locate the testis in a novel multi-organ endocrine circuit, involving skeletal muscle, adipose tissue, bone and pancreas, of high penetrance in both energy metabolism and male fertility. Testosterone (T) has been classically considered the main steroid hormone connecting testis function and skeletal muscle, as well as other target tissues such as bone. However, the endocrine activity of the testis has been recently widened to other non-steroidal hormones that are produced by Leydig cells and that elicit anabolic action, such as Insulin like-peptide 3 (INSL3). INSL3 is a male-specific hormone produced by the Leydig cells with a fundamental role on testicular descent during fetal life. Recent evidence suggests that both T and INSL3 exert interesting and largely overlapping roles in the musculoskeletal system. In addition, Leydig cells are recently recognized to maximally express CYP2R1, the main enzyme responsible for the activation of vitamin D (VitD) into 25-hydroxy Vitamin D (25OH VitD), a key mediator of bone mass accrual and maintenance.
On the other hand, bone tissue has been shown to take part in this crosstalk through undercarboxylated osteocalcin (ucOC), a bone derived protein exerting systemic effects on tissues expressing the metabotropic receptor GPRC6A. The recognized effects of ucOC are the improvement of insulin secretion from the pancreas, the amelioration of systemic insulin sensitivity, in particular in skeletal muscle and adipose tissue, and the stimulation of the global endocrine activity of the Leydig cell, including vitamin D 25-hydroxylation and T production. The supporting evidence of this endocrine circuit in both animal and human models will be discussed.

RT03-2

Testicular macrophages: how hormones determines their function?

A. MEINHARDT
Institute of Anatomy and Cell Biology, Justus-Liebig-University of Giessen, Giessen, Germany

Testicular macrophages (TM), which represent the largest pool of immune cells in the testis, are intricately linked to normal testicular functions such as regulating spermatogonial numbers and Leydig cell steroidogenesis. Conventionally, macrophages are classified into a classical inflammatory-activated (M1) state and an alternatively activated immunosuppressive (M2) state. Accumulating evidence suggests that local micro-environmental factors contribute to the specialization of tissue-specific macrophage phenotypes and functions. TM may also acquire an M2 macrophage phenotype via an irreversible differentiation program dependent on unique master regulators and transcription factors, controlled by the testicular microenvironment through the consistent exposure to the interstitial fluid (IF). Although the immunosuppressive properties of IF have been known for considerable time now, the molecules involved have not been fully characterized. Moreover, whether and how chronic exposure to IF polarizes macrophages to the M2 phenotype has not been investigated. Data presented in this talk support the hypothesis that the testicular microenvironment establishes and sustain the alternative immunosuppressive M2 macrophage phenotype of TM. Our study provides evidence that PGE_2, PGI_2, testosterone and corticosterone are important immunoregulatory molecules present in the IF with corticosterone playing a dominant role in shaping the phenotype and function of TM. Moreover, corticosterone was found at very high levels in IF, exceeding those in serum by a high magnitude. Cell isolation studies imply TM as a local source of glucocorticoid production in the testis and thus imply TM as a

previously unrecognized steroid hormone producing cell in the male gonad.

RT03-3

Sertoli cells function beyond spermatogenesis

D. JEZEK
University of Zagreb, School of Medicine, University Hospital "Zagreb", Depts. of Histology and Embryology & Transfusion Medicine and Transplantation Biology, Zagreb, Croatia

The seminiferous epithelium consists of supporting somatic Sertoli cells and spermatogenic cells. Sertoli cells are large columnar cells extending from the basement membrane up to the lumen of seminiferous tubules. The unique feature of the neighbouring Sertoli cells is tight junctions positioned at the end of their basolateral cell processes, thus dividing seminiferous epithelium into two compartments: basal (with spermatogonia) and apical (adluminal or meiotic, with haploid spermatogenic cells predestined to became spermatozoa). Sertoli cells have a receptor for FSH, produce inhibin, activin, as well as androgen-binding protein (ABP). Together with myoid or peritubular cells, they build a common basement membrane of the seminiferous epithelium. Sertoli cells secrete an array of paracrine factors that enable a complex crosstalk with spermatogenic, peritubular and Leydig cells, and most probably other interstitial cells, like macrophages. During the development of the indifferent gonad, under the influence of Sry, the vast majority of pre-Sertoli cells are thought to migrate inside the mesenchyme of the genital ridge from the surface coelomic epithelium. Namely, the coelomic subpopulations of cells that express steroidogenic factor-1 (Sf-1) are picked by delaminating signals of the underlying mesenchyme of the genital ridge. They differentiate into pre-Sertoli cells (that express fibroblast growth factor 9 /Fgf9/ and Sox 9 /Sry-related HMG box-9/) and become the chief organizers of sex cords. The expression of Sf1 is maintained in early Sertoli cells and Leydig cells. Under the influence of Sf1, Sertoli cells produce anti-Müllerian hormone (Amh), whereas Leydig cells produce testosterone. Amh induces a regression of the paramesonephric (Müllerian) duct, whereas testosterone strongly promotes the development of mesonephric (Wolffian) duct and certain parts of the male reproductive system. Thus, Sertoli cells are crucial for the normal function of the adult testis and the development of the "male" genital ridge.

SYMPOSIUM 4: EAA GUIDELINES

RT04-1

EAA clinical guideline on management of bone health in the andrological outpatient clinic

V. ROCHIRA[1,2], L. ANTONIO[3] AND D. VANDERSCHUEREN[3,4,5]
[1]*Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Italy;* [2]*Azienda Ospedaliero-Universitaria di Modena, Ospedale Civile di Baggiovara, Modena, Italy Address: Unit of Endocrinology, Ospedale Civile di Baggiovara, Via P. Giardini 1355 41126 Modena, Italy;* [3]*Department of Endocrinology, University Hospitals Leuven, Leuven, Belgium;* [4]*Department of Clinical and Experimental Medicine, Laboratory of Clinical and Experimental Endocrinology, KU Leuven, Leuven, Belgium;* [5]*Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium*

Background: Male osteoporosis is now a well-recognized medical disorder with established clinical guidelines for both diagnosis and management. Prevention as well as management of osteoporosis in men consulting the andrological outpatient clinic because of low testosterone, however, is not well established. This gap of knowledge is -at least partly- explained by the controversy with respect to the threshold of testosterone needed for skeletal maintenance. However, testosterone deficiency may be clearly associated with bone loss as well frailty in men. The aim of the guideline was to provide andrologists with the most updated, evidence based advices on the management of bone disease in men and to make them aware of the potential silent presence of osteoporosis in hypogonadal men.

Methods: The GRADE (Grading of Recommendations, Assessment, Development, and Evaluation) system for grading the quality of evidence and the strength of recommendations was used to grade recommendations.

Results: A total of 32 recommendations were provided concerning clinical evaluation, diagnosis and therapy of male osteoporosis in the andrological patient.

Conclusion: Therefore, the management of patients with potential hypogonadism should include a complete bone health assessment, besides clinical and biochemical evaluation of gonadal status. Such bone health assessment should include specific items in medical history and physical examination related to fracture risk. Furthermore, dual-energy absorptiometry is indicated to evaluate fracture risk in men with confirmed clinical hypogonadism.

Regarding treatment, besides general measures to prevent or manage male osteoporosis testosterone replacement can be initiated (as described in guidelines for hypogonadism), but data on its efficacy in preventing fractures are lacking. Thus, additional anti-osteoporotic may be needed, especially in men with very low testosterone who are at high risk for bone loss and/or in men not able to receive testosterone replacement.

RT04-2

Management of Oligo-Astheno-Teratozoospermia

G. M. COLPI[1], S. FRANCAVILLA[1], G. HAIDL[1], K. LINK[2], H. M. BEHRE[3], D. G. GOULIS[4], C. KRAUSZ[5] AND A. GIWERCMAN[6]

[1]Friedrich Wilhelm University, Dermatology/Andrology unit; [2]Skåne University Hospital Malmö, Reproductive Medicine Center; [3]Center for Reproductive Medicine and Andrology, UKH / University of Halle; [4]Unit of Reproductive Endocrinology, First Department of Obstetrics and Gynecology Medical School, Aristotle University of Thessaloniki, Greece; [5]University of Florence, Florence, Italy; [6]Lunds Universitet, Translational Medicine

Oligo-astheno-teratozoospermia (OAT) is frequently reported in men from infertile couples. Its etiology remains, in the majority of cases, unknown with a variety of factors to contribute to its pathogenesis. The aim of this European Academy of Andrology (EAA) guideline is to provide an overview of these factors and to discuss available management options.

PubMed was searched for papers in English for articles with search terms: male infertility and oligo-astheno-teratozoospermia. For evidence-based recommendations, the GRADE system was applied. Issues related to urogenital infections / inflammations have not been included in this document as they will be covered by separate guidelines.

For men with OAT, the EAA recommends:

- A general physical examination to assess signs of hypogonadism.
- A scrotal physical examination to assess i) the testes and epididymes for volume and consistency, ii) deferent ducts for total or partial absence and iii) occurrence of varicocele.
- Performing two (2) semen analyses, according to World Health Organization (WHO) guidelines to define an OAT.
- An endocrine evaluation.
- A scrotal ultrasound (US) as part of routine investigation.
- Karyotype analysis and assessment of Yq microdeletions in infertile men with a sperm concentration ≤ 5 x 106/mL.
- Cystic fibrosis transmembrane conductance regulator (CFTR) gene evaluation in case of suspicion for incomplete congenital obstruction of the genital tract.
- Against quitting physical activity in order to improve the chance of achieving pregnancy.
- Against androgen replacement therapy in order to improve the chance of achieving pregnancy.
- Assisted Reproduction Techniques (ART) in order to improve the chance of achieving pregnancy, in case other treatment options are not available or not efficient.
- Androgen replacement therapy in patients with biochemical and clinical signs of hypogonadism, after completion of the fertility treatment.

RT04-3

Gynecomastia evaluation and management

G. A. KANAKIS[1,*], L. NORDKAP[2,*], A. KIRSTINE BANG[2], A. E. CALOGERO[3], G. BARTFAI[4], D. G. GOULIS[1,*] AND **N. JØRGENSEN**[2,*]

[1]Unit of Reproductive Endocrinology, First Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Greece; [2]University Department of Growth and Reproduction, and International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), Rigshospitalet, Copenhagen, Denmark; [3]Department of Clinical and Experimental Medicine, University of Catania, Italy; [4]Department of Obstetrics, Gynecology, and Andrology, Albert Szent-Györgyi Medical University, Szeged, Hungary.

Gynecomastia (GM) is a benign proliferation of glandular tissue of the breast in men. It is a frequent condition with a prevalence of 32–65%, depending on the age and the criteria used for definition. The occurrence of GM shows three discrete peaks throughout a man's lifespan: 1) during infancy, 2) during puberty, and 3) in middle-aged and elderly men. GM of infancy is a common, benign condition that usually resolves spontaneously, typically within the first year of life. GM of puberty is also common, affecting approximately 50% of mid-pubertal boys and in the majority of cases resolves spontaneously within 24 months. GM of adulthood is more prevalent among the elderly and proper investigation may reveal an underlying pathology in 45–50% of cases. The purpose of GM assessment should be the detection of underlying pathological conditions, the administration/abuse of aggravating substances, and the discrimination from other breast lumps that mimic GM, particularly breast cancer. This assessment should comprise a thorough medical history and physical examination including the breast and genitalia (complete with testicular ultrasound). The evaluation may be integrated by a "first-line" package of laboratory investigations: testosterone (T), estradiol (E_2), sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicular stimulating hormone (FSH), thyroid stimulating hormone (TSH), prolactin, human chorionic gonadotropin (hCG), alpha-fetal protein (AFP), liver and renal function tests, and breast imaging, whenever a clinical examination is equivocal. However, in suspicious breast lesions, core needle biopsy should be sought directly. Watchful waiting is recommended after treatment of an underlying pathology or discontinuation of the administration/abuse of substances associated with GM. T treatment should be offered only to men with proven testosterone deficiency. The use of selective estrogen receptor modulators (SERMs), aromatase inhibitors (AIs) and non-aromatizable androgens is not justified, except for prevention of GM after androgen deprivation therapy for prostate cancer, and, in rare cases, the treatment of painful GM of recent onset. Surgical treatment is the therapy of choice for patients with long-lasting GM.*Shared first authors, ** Shared last authors

SYMPOSIUM 5: ONCO-ANDROLOGY

RT05-1

Andrology care in adults after childhood cancer

C. WYNS
Department of Gynecology-Andrology, Cliniques universitaires Saint-Luc, 1200 Brussels, Belgium; Institut de Recherche Expérimentale et Clinique (IREC), 1200 Brussels, Belgium

A major long-term effect of childhood cancer treatments is infertility as chemotherapy and radiotherapy may induce spermatogonial stem cell (SSC) depletion resulting in azoospermia in up to 25% of adult survivors, with an even higher risk of up to 85% when preconditioning chemotherapy before bone marrow transplantation or total body irradiation have been administered. As there is no absolute predictive factor for gonadal dysfunction and as fertility is considered an important quality of life issue, fertility preservation measures must be proposed to these patients. For adolescents, cryopreservation of spermatozoa before gonadotoxic therapy is the priority to allow medically assisted reproduction when parenthood is considered. For children, as the prepubertal state does not protect against cytotoxic damages to the testes, cryopreservation of SSCs in the form of testicular tissue or cell suspensions is the only possibility so far. Substantial advances have been achieved regarding immature testicular tissue (ITT) cryopreservation and fertility restoration options using either cryopreserved cells/tissue transplantation or in vitro maturation. Indeed, preclinical studies on the feasibility and risk assessment of re-transplantation of SSCs as well as achievements in non-human primates with SSCs and ITT transplantation now point to the potential of a near future clinical application.

For patients who did not benefit from cryostorage of mature or immature gametes prior to their cancer treatment, repeated semen sample analyses after cure may be proposed as sperm recovery may take several years. In case of persistent azoospermia, surgical sperm retrieval with TESE has also been successfully used to obtain sperm in close to 50% of these patients.

Furthermore, besides the risk of spermatogenic impairment due to a direct effect to the seminiferous tubules, damage to Leydig cells with reduced testosterone secretion and central effects on the hypothalamus and/or pituitary may also occur both justifying hormonal follow-up of these patients.

RT05-2

Late effects of oncological treatment in prostate cancer

N. SOFIKITIS

No text available.

RT05-3

Infertility and cancer: a genetic link?

C. KRAUSZ
Department of Biomedical, Experimental and Clinical Sciences "Mario Serio", University of Florence, Florence, Italy

A growing number of epidemiological studies indicate an overall increased risk for chronic disease (including cancers) in infertile men. The strongest association is with testicular cancer but also other malignancies (melanoma, thyroid cancer, leukemia, lymphoma, prostate cancer) have been linked to infertility. The biological mechanisms are still undefined but a number of possible factors have been proposed: genetic, hormonal, environmental, lifestyle or in utero factors. As approximately 10% of the human genome is involved in male reproduction, it is plausible that defects in any of these genes may also be tied to the development of cancers of the reproductive system as well as other organ systems. In favor of a genetic link are: i) the observation of an elevated risks of cancer seen in the family members of infertile men; ii) database searches identified candidate genes and gene classes shared between cancer and reproductive function impairment; iii) mutations in mismatch repair genes (for instance the *FANCA* gene links SCOS with Fanconi Anemia, a cancer prone disease); *FANCM* mutations cause both infertility and solid cancers (breast cancer); iv) significant higher deletion load (genomic instability) in infertile men in respect to normozoospermic men (Krausz et al 2012; Lopes et al 2013; Tuettelmann et al 2012). The above genetic anomalies are present in a subset of patients. For instance, *FANCA* mutation should be suspected in case of SCOS with mild alterations of specific blood parameters (Krausz, Riera-Escamilla et al 2018). An early diagnosis of "occult" FA allows family screening and preventive measures by onco-hematologists. In fact, the diagnosis of a common genetic link has relevance not only for the patient itself but also for his family members and his descendants. Ongoing exome and specific gene panel analyses in well-characterized patients are likely to discover novel shared genetic causes in the near future.

SYMPOSIUM 6: FROM ANDROLOGICAL DISEASES TO GENERAL HEALTH

RT06-1

Erectile dysfunctions and risk factors of socially significant diseases

O. I. APOLIKHIN[1], E. A. EFREMOV[1], I. A. SHADERKIN[1], O. V. ZOLOTUHIN[2], Y. Y. MADYKIN[2], M. M. ZELENSKY[1] AND S. S. KRASNYAK[1]
[1]*N. Lopatkin Scientific Research Institute of Urology & Interventional Radiology - branch of the federal state budgetary institution "National Medical Research Center of Radiology" of the Ministry of health care of Russia, Moscow, Russia;;* [2]*Voronezh State Medical University named after N.N. Burdenko, Voronezh, Russia*

Background: Cardiovascular diseases (CVD) are the leading cause of death worldwide. According to literature, CVDs have a very similar profile of risk factors with such a common reproductive disorder as erectile dysfunction (ED). At the same time, there are different opinions about the prevalence of ED and lower urinary tract symptoms (LUTS) in the Russian population and their relationship to physical illnesses.
Methods: We present data from evaluation of 1261 men aged 40–65 years living in the Voronezh and Kaluga regions. Patients filled the IPSS and IIEF-5 questionnaires. Anamnestic data, the results of anthropometry (height, weight, body mass index (BMI), waist circumference), and laboratory research methods: plasma cholesterol and glucose levels were recorded. The statistical analysis was carried out using IBM SPSS Modeler™ 18 platform. In addition to conventional statistics we used methods of predictive analytics (CHAID algorithm, neural network).
Results: Prevalence of ED was 51.04%. In this case, a mild ED is observed in 38% of men, moderate and severe ED - in 10.5% and 2.5%, respectively. After applying the algorithm of CHAID decision tree, we found that the most significant risk factor for ED occurrence is the severity of LUTS (an importance factor of 0.64), then with a significant lag follow waist circumference (0.08), cholesterol level (0.05), hypertension (0.05). Accuracy of the prediction of the trained neural network was 81.9%. Comparison of the predictions of both models showed the agreement coefficient between the models at 84.87%. Predictors of arterial hypertension development were pulse frequency, severity of LUTS age and body weight. The use of predictive analytics methods allowed to generate an algorithm for predicting the development of cardiovascular diseases with an accuracy of 80.3%.
Conclusion: Medical science continues to accumulate information on the possible mechanisms of connections between ED and diseases of the cardiovascular system. However, already at the present time, using erectile dysfunction as an early marker of cardiovascular diseases, urologist can not only improve the quality of life of a patient with erectile dysfunction, but also save his life.

RT06-2

Long-term health of ICSI children

H. TOURNAYE, F. BELVA AND M. BONDUELLE
Centre for Reproductive Medicine and Centre for Medical Genetics, University Hospital, Dutch-speaking Brussels Free University (Vrije Universiteit Brussel), Laarbeeklaan 101, B-1090 Brussels, Belgium

Background: Since we introduced intracytoplasmic sperm injection (ICSI) in 1991, worldwide more than 5 million ICSI-children were born. From the start, monitoring the health of these children was part of the ICSI project. Today we can look back at a follow-up of more than 25 years.
Methods: All couples were asked to give their consent for a prospective follow-up at the start of their ICSI treatment. Pregnancy and perinatal data were gathered from questionnaires sent to both parents and gynecologists. Parents were invited for detailed physical examination of their child 2 months after giving birth and at older ages.
Results: while there were no differences between IVF- or ICSI-conceived pregnancies/children, compared to spontaneous conception, we observed a higher risk of preterm birth, small-for-gestational age and congenital malformations. The genital development in boys at 8 and 14 years old was found comparable to spontaneously-conceived controls including their hormonal profiles. However, young ICSI men had a nearly 2-fold lower sperm concentration than spontaneously conceived controls and were 4 times more likely to have sperm counts below 39 Million. There was no correlation of sperm parameters between fathers and their ICSI-offspring.
Conclusion: These findings on the long-term reproductive health in this (small) cohort of young male adults indicates that a continued follow-up till older age is needed in a larger study population

RT06-3

Semen quality as a marker of general health

N. JØRGENSEN
University Department of Growth and Reproduction, Rigshospitalet, Blegdamsvej 9, section 5064, DK-2100 Copenhagen, Denmark

15–20% of all couples suffer of infertility. Thus, a significant proportion of a relatively young population (average age 30–35 years) is in need for medical advice because of childlessness. Recently, it was shown that infertile men may be at risks of shorter life expectancy and increased long-term morbidity. Whereas, these studies also point to increased frequencies of comorbidities at the time of infertility diagnosis, the majority of infertile men are without any obvious conditions that may explain a long-term impairment of their health.
The mechanisms explaining the association between impaired semen quality/infertility and long-term health is not understood. However, reduced semen quality is a marker which has been linked to mortality in a dose-dependent manner and recently also to morbidity. E.g. men with a sperm concentration of 195–200 million/mL were, on average, hospitalized for the first time 7 years later than men with a sperm concentration of 0–5 million/mL. Impaired semen quality has also been linked to a higher risk of testicular cancer in men in the years following an infertility evaluation, and some studies have suggested a link to later development of prostate cancer, although this has not been confirmed by all studies. However, these diseases do not account for any major part of the association between semen quality and mortality. Although, it is still not understood which late appearing diseases are associated with impaired semen quality, it seems that fertility and especially impaired semen quality may represent a universal biomarker of later health and survival. Thus, impaired semen quality should be regarded as the "Canary in the coal mine" regarding long term health, and not merely as a "current" fertility problem.

SYMPOSIUM 7: TRANSITIONAL ANDROLOGY: FROM ADOLESCENCE TO ADULT

RT07pros

Early endocrine treatment for Klinefelter syndrome? - PROS

A. JUUL

No text available.

RT07cons

Early endocrine treatment for Klinefelter syndrome? CONS

J. ROHAYEM
Center for Reproductive Medicine and Andrology, Clinical and Operative Andrology, University of Münster, Germany

Background: Availability of testicular sperm extraction (mTESE) and intra-cytoplasmic sperm injection (ICSI) nowadays enables fatherhood in otherwise infertile men. Patients with Klinefelter syndrome experience progressive testicular degeneration, resulting in impaired endocrine function and azoospermia. What proportion of adolescents develop testosterone deficiency during puberty and how early testosterone replacement should be initiated, is a matter of debate.

It is meanwhile established that up to 50% of young Klinefelter patients have islets of full spermatogenesis in their testicles, allowing spermatozoa to be harvested by mTESE and that some have spermatozoa in semen. A prerequisite for meiotic entry of spermatogonia is gonadotropin-driven puberty. Any testosterone replacement performed before or during this delicate period of gonadal maturation would suppress the hypothalamo-pituitary-gonadal axis and thus impair spermatogenesis.

Methods: We assessed HPG axis function in 281 patients with non-mosaic Klinefelter's syndrome aged 10–25 years and testosterone effects in target tissues. In late pubertal adolescents, semen analyses were performed. Data were compared to those of 233 age-matched controls.

Results: Serum T levels ≥10 nmol/L were reached in 62% of KFS patients and in 85% of controls at ages 15–25 (T_{KFS}: 12.2 ± 5.4 vs. T_C: 16.6 ± 7.2 nmol/L). LH_{KFS} levels were elevated >10 U/L in 84%, and normal in all controls (LH_{KFS}: 18.6 ± 12.2 vs. LHC: 3.5 ± 1.6 U/L). In nine of 130 (7%) KFS adolescents, spermatozoa (oligozoospermia) were found in semen; all had T levels >7 nmol/L and eight of nine had LH levels ≤18 U/L. Controls had normal sperm concentrations in 73% (46/63).

Conclusion: Hypergonadotropic hypogonadism in KFS adolescents remains compensated in over 60% during ages 15–25, with sufficient testosterone secretion for spontaneous accomplishment of pubertal development. Spermatozoa in semen are rare and associated with T levels >7 nmol/L. Hormone replacement in young patients should preferably not be initiated before paternity prospects have been addressed.

RT07-3

Optimal management of disorders of sex development

M. COOLS
Ghent University Hospital, Pediatric Endocrinology Unit and Ghent University, Department of Internal Medicine and Pediatrics, Ghent, Belgium

Background: Disorders (or Differences) of Sex Development (DSDs) have long been considered a pediatric topic. Adult endocrinologists used to be rarely exposed to these patients, due to the rarity of the individual conditions and dispersion of patients among decentrally organized health care systems. In recent years, the emphasis has been increasingly on the organization of lifelong holistic care by multidisciplinary teams. The transition period is pivotal in this process. Here we will focus on a subset of themes related to endocrine and andrological care, ranging from gonadal function and fertility to hormone replacement therapy and gonadal cancer risk.

Methods: Literature review.

Results: **N**eeds of patients are different according to the specific condition. Participation in international standardized protocols for clinical revision can greatly improve the quality of care and patient outcome, and can on a longer term entail major advancement in clinical research. In CAH, optimisation of metabolic control is crucial. Men who have 45,X/46,XY or 46,XY DSD require expert follow-up of gonads and sometimes HRT, the latter is also of relevance for men who have 46,XX testicular DSD. In conditions with an increased gonadal cancer risk, the risk is inversely related to gonadal differentiation and function. Fertility has been described for some conditions such as partial androgen insensitivity and *NR5A1* mutations; early sperm cryopreservation and TESE procedures may be beneficial in individual cases. Having hypospadias at birth may be a sign of later testicular insufficiency in specific subgroups. Recently, a magnitude of adult outcome data have emerged from the DSD-Life and Danish cohort studies. In general, the role of peer support cannot be underestimated in adjusting one's life to the condition.

Conclusion: Optimization of adult care for individuals who have a DSD is currently in the focus of attention. Management should be individualized and tailored to the specific condition and needs of the patient.

SYMPOSIUM 8: BEYOND STANDARD SEMEN ANALYSIS

RT08-1

WHO manual: status report and WHO perspectives

E. BALDI AND M. P. FESTIN*
*Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy, *World Health Organization, Genève, Switzerland*

Background: The fifth edition of the WHO laboratory manual for the examination and processing of human semen dates back 2010. This manual includes information on sperm preparation for clinical use or specialized assays and on cryo-preservation, an expanded section on quality

control in the semen analysis laboratory and evidence-based reference ranges and reference limits for various semen characteristics. Since the time the manual was edited, new developments in terms of processes for analyses of semen have been developed, and the standards need to be reviewed and updated as necessary.
Methods: The editorial team will be convened by WHO Development and Research Training in Human Reproduction. An international body of experts on andrology and semen analyses have agreed to participate in the revision of the Manual constituting the editorial team which has the task of considering topics to be included, modified, or removed from the present manual. Each topic that needs to be updated and all the additional procedures to be included will be object of systematic reviews that are currently ongoing.
Results: For the first part of the manual (semen analysis) revision and update will be considered for sperm count, management of samples with low or no spermatozoa, sperm morphology and motility evaluation and assessment of semen leukocytes. The chapter on computer-aided sperm analysis will be updated and likely extended according to recent development of these instruments. Updates are foresee for sperm preparation techniques including for virus infected semen samples, and for sperm cryopreservation techniques. Obsolete tests (such as post-coital, sperm-oocyte or sperm-zona interaction and sperm-zona free hamster oocyte tests) are likely to be removed from the next edition of the manual.
Conclusions: The editorial team is working to build an updated WHO laboratory manual for the examination and processing of human semen, which, likely, will be easier and fluent to follow and will contain new optional and advanced procedures useful for male fertility diagnosis.

RT08-2

Sperm RNA and male fertility

M. JODAR
Molecular Biology of Reproduction and Development Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Fundació Clínic per a la Recerca Biomèdica, Faculty of Medicine, University of Barcelona, Casanova 143, Barcelona, Spain

RNAs retained in mature sperm reflect past spermatogenic events and could resolve some pathogenic mechanisms contributing to male factor infertility, as well as to identify potential biomarkers clinically useful for the conduction of assisted reproduction treatments (ART). For instance, our study of human sperm RNAs by Next Generation Sequencing (NGS) have enabled the stratification of idiopathic infertile couples based on the presence or absence of a complete set of 648 sperm RNA elements (SRE). These SRE seem to be able to discern which idiopathic infertile patients have a high likelihood to achieve pregnancy using less invasive ART, such as intrauterine insemination (IUI), while advising others to directly undergo *in vitro* ART. However, specific retained sperm RNAs are not only merely remnants of spermatogenesis, but also could display their function in early embryogenesis. A single spermatozoon contains approximately 50 fg of long RNA and 0.3 fg of small non-coding (snc) RNA, being approximately 200 times less than the quantity of RNA contained in the oocyte. Despite of this low RNA amount, sperm delivers a complex population of transcripts including coding RNAs, linear non-coding RNAs and circular RNAs to the oocyte at the time of fertilization that likely influences this process, as well as the early embryo development and the phenotype of the offspring and next generations. Particularly, growing evidence points to sperm sncRNAs the cause of phenotypic variations reflective of the father's life experience in the progeny. In addition to those sncRNAs, our recent integrative analysis of the human sperm, oocyte and embryo proteomes and transcriptomes has revealed a set of sperm coding RNAs that could be translated using the oocyte translational machinery. More interestingly, the resultant paternally derived embryo proteins include transcript factors and chromatin modifiers which could be also able to modify the epigenetics of the embryo.

RT08-3

How many sperm function tests do we need?

V. NORDHOFF
Centre of Reproductive Medicine and Andrology, University Hospital of Münster, Münster, Germany

The analysis of an ejaculate for the evaluation of male fertility using the procedures and parameters described in World Health Organization (WHO) laboratory manual for the examination of human semen is - despite some controversy - the gold standard for clinical sperm analysis. The main parameters are spermatozoa concentration, motility and morphology. However, further functional characterization of spermatozoa beyond standard semen analysis has been developed in recent years. New methods for the selection or detection of the best sperm have been developed of which some have been tested for and been applied in clinical routine. Among those are tests for acrosome integrity, binding assays for detection of sperm maturity and sperm DNA fragmentation assays. However, two categories have to be considered when using the new tests: Some tests diagnose spermatozoa as a population, e.g. the analysis of DNA-fragmentation. This has the drawback, that post analysis all spermatozoa are unusable for fertilization; on the other side methods exists identifying single spermatozoa. While in principle individual sperm can be selected it has to be established that sperm selection is safe and will improve the outcome of assisted reproduction. This talk will give an overview on current techniques for sperm analysis and selection and will discuss how many sperm function tests beyond standard semen analysis are eventually necessary.

ESAU-EAA SESSION 1: DEBATE AND STATE OF THE ART LECTURES

ESAU01-1

Debate in Andrology: Sperm DNA integrity testing: Just one more test?

T. DIEMER
Department of Urology, Pediatric Urology and Andrology, UKGM GmbH, Campus Giessen, Justus-Liebig-University Giessen, Germany

Background: DNA-fragmentation of spermatozoa has been debated for decades as hazard for male fertility. DNA damage in spermatozoa appears to be associated with impaired birth rates considering spontaneous conception and live births following artificial reproductive technologies (ART). Different test for DNA damage in spermatozoa have been developed over time, however, accuracy and validity of respective tests are not unanimously accepted. DNA damage has also been linked to specific andrological diseases such as varicocele and might explain the effects of surgical treatment aside from spermatological alterations.
Methods: DNA integrity test systems as well as pathophysiolgical concepts are reviewed. The clinical value is debated and an overview is provided for further use of these test in clinical andrology
Results: Guideline recommendation for the use of DNA integrity tests in andrological patients are missing due to conflicting data and lack of randomized prospective trials indicating a proven diagnostic value. However, certain concepts do exist and DNA integrity testing might in fact provide a useful addition to conventional sperm analysis resulting in clinical decisions.
Conclusion: DNA integrity testing might provide a useful hint, particularly in cases where conventional sperm analysis fails to explain male infertility or failed success in ART.

ESAU01-2

Debate in Andrology: A test with a major role in the prediction of sperm fertilizing potential

J. J. SŁOWIKOWSKA-HILCZER
Department of Andrology and Reproductive Endocrinology, Medical University of Lodz, Lodz, Poland

Semen analysis is the first step to identify male infertility. Sperm total number, concentration, progressive motility and morphology are discriminative for male infertility or subfertility. However, normal semen parameters are not one hundred percent proof for male fertility because none of these parameters addresses sperm function and their clinical value in predicting fertility is questionable. For this reason in addition to the routine semen analysis many sperm functional tests are used to determine sperm ability to fertilize oocyte. Many advanced sperm function tests have been developed and introduced to clinical practice, such as sperm nuclear and mitochondrial DNA quality tests, sperm motion parameters, sperm penetration assay, sperm-zona pellucida binding test (hemizona assay), acrosomal reaction test, hyaluronan binding test, hypo-osmotic swelling test, magnetic activated cell sorting, oxidative stress tests and others. They enable to diagnose reasons of unexplained infertility, recurrent pregnancy loss, intrauterine insemination failure and failed assisted reproduction in some cases and undoubtedly help clinicians in optimizing male factor infertility treatment. There is currently evidence supporting the use of these tests in specific clinical groups. However, they are expensive and not used in routine diagnostics. Moreover, there is criticism that the formation, maturation and physiological workings of the normal and dysfunctional spermatozoa are not fully elucidated and the functional tests do not have confirmed clinical significance. An urgent need of high quality long-term funding to support studies on reproductive medicine, and male infertility in particular, arises.

ESAU01-3

State of the Art Lecture: Hypogonadism in young men treated for cancer

A. GIWERCMAN
Department of Translational Medicine and Reproductive Medicine Centre, Lund University, Malmö, Sweden

Thanks to the improvements of cancer therapy, the survival rates for some of the most common malignant diseases of young age have increased significantly exceeding 95% for testicular germ cell cancer (TC) patients and 80% for childhood cancer (CC). Thus, the issue of life quality of cancer survivors is an important aspect of management of this growing patient group.
The reproductive system seems to be rather sensitive to the adverse effects of cancer and its treatment. Initially, the research on adverse reproductive effects of cancer therapy focused on the issue of fertility of cancer survivors. However, during the last years, more attention has been given to endocrine disturbances.
There is now substantial knowledge showing that that both TC and CC survivors are at increased risk of long term morbidity and mortality. The panorama of diseases threatening the well-being and lives of these patients include increased risk of metabolic syndrome, cardiovascular diseases and osteoporosis. The magnitude of this risk is linked to the treatment modality and intensity, but our and others research point to hypogonadism – testosterone deficiency – as possible pathological link between cancer treatment and subsequent metabolic, cardiovascular and skeletal disturbances.
Levels of testosterone below the lower normal range and/or increased LH levels have been found in approximately 30% of male survivors of CC and in 40% of those who have been treated for TC. Even the risk of hypogonadism is associated with the type and intensity of the cancer treatment given. Low testosterone has – in older men – been identified as a marker of increased premature mortality and it also seems to be true for younger subjects (under 50 years of age).
However, although low testosterone and/or high LH possibly can be used for identifying male cancer survivors which are at increased risk of long term morbidity and decreased life expectancy, there are now studies showing that testosterone replacement therapy may prevent such sequelae. Therefore, randomized placebo controlled

studies aiming to elucidate if sex hormone treatment has any preventive effect, are needed. So far, well established preventive measures – including dietary and lifestyle related recommendations – should be applied.

ESAU01-4

State of the Art Lecture: Effects of primary testicular damage on early embryonic development
N. SOFIKITIS
No text available.

ESAU SESSION 2: SURGERY OF MALE INFERTILITY

ESAU02-1

Surgery for sperm recovery from testis with malignant disease: sperm recovery rate and consequences in testicular function
M. DINKELMAN-SMIT
Erasmus MC, University Medical Center Rotterdam, the Netherlands

Background: The gold standard for fertility preservation in men is sperm banking prior to gonadotoxic treatment. In men with testicular cancer it is recommended to cryopreserve sperm before radical orchiectomy. The majority of patients will present with stage I Testicular Germ Cell Tumors and will be indicated for adjuvant gonadotoxic treatment. Despite the recommendation for the timing of sperm cryopreservation, supported by the argument that semen quality in men with testicular cancer seems to be superior before ablative surgery, this treatment sequence is not standard of care for many general urologists. A knowledge gap about the prevalence of azoospermia in patients that present with testicular cancer, outcome of Testicular Sperm Extraction from testis with malignant disease (oncoTESE) and fear of introducing time delay are barriers that maintain inadequate clinical practice.
Methods: Semen analysis in 588 men with TGCT who presented for a sperm cryopreservation attempt at our institution yielded azoospermia in 6.6% of cases. Cryptozoospermia was observed in 8% of TGCT patients. Overall sperm quality in TGCT patients was significantly lower than in patients with hematological malignancies referred for fertility preservation. Since 2014 oncoTESE was systemically offered to patients with absence of sperm, immotile sperm only or sperm concentration below 100.000/ml at semen cryopreservation attempt.
Results: Between 2011–2017 40 patients with non-obstructive azoospermia and TGCT diagnosis were treated with oncoTESE. Sperm Retrieval Rate (SRR) was 64%. Median Testosterone levels were 11 nmol/l. No major adverse events or complications were observed. Updates on outcome of TESE-ICSI pregnancy rates will be presented at the meeting.
Conclusion: Semen cryopreservation before radical orchiectomy is mandatory to ensure identification of men with azoospermia at the time of TGCT diagnosis. In our patient population, nearly 15% of patients with TGCT referred for sperm cryopreservation have indication for oncoTESE. OncoTESE is successful in 64% of patients and will enable them to father biological offspring.

ESAU02-2

Do the effects of varicocelectomy on Leydig cellular secretory function, sperm DNA integrity, and sperm functional assays raise the need to revisit the indications for varicocelectomy?
S. MINHAS
No text available.

ESAU02-3

Surgery for testicular torsion-detorsion: is there a place for potential adjunct pharmacological treatment?
S. KLIESCH
No text available.

ESAU02-4

Sperm recovery for cryopreservation from young boys with oncological disease: which is the best approach?
Z. KOPA
No text available.

ESAU SESSION 3: ERECTILE DYSFUNCTION

ESAU03-1

Indications for ectopic reservoir placement during inflatable penile implant surgery
E. R. CASTANE
No text available.

ESAU03-2

Is there a role for non-invasive approaches in the treatment of iatrogenic priapism?
A. FAIX
No text available.

ESAU03-3

Parameters associated with the degree of penile deformity in Peyronie disease
A. KADIOGLU
No text available.

ESAU03-4

Cardiovascular risk factors associated with erectile dysfunction
O. I. APOLIKHIN
No text available.

INDUSTRY SYMPOSIUM 1 - BAYER SYMPOSIUM: UPDATE ON TESTOSTERONE THERAPY FOR THE TREATMENT OF HYPOGONADAL MEN

IS01

No text available.

IS02

No text available.

IS03

No text available.

INDUSTRY SYMPOSIUM 2 - BERLIN-CHEMIE MENARINI SYMPOSIUM: ANDROLOGICAL DISEASE

IS04

Premature Ejaculation
E. A. JANNINI
No text available.

IS05

Medical management of erectile dysfunction
M. ALBERSEN
No text available.

IS06

Lower urinary tract symptoms and erectile dysfunction: is there a causal relationship?
G. R. DOHLE
Center for Reproductive Medicine and Andrology, Erasmus MC, Rotterdam, The Netherlands

Background: lower urinary tract symptoms (LUTS) and erectile dysfunction (ED) are both common conditions in ageing men. We have searched the literature for a causal relationship between both disorders and for the best treatment for men with LUTS and ED.
Methods: We selected recent literature on ED and LUTS in Pubmed and Embase and reviewed the evidence for a common explanation of both conditions. In addition treatment options for ED associated with LUTS were evaluated.
Results: LUTS can be caused by different conditions, including benign prostatic hyperplasia (BPH), bladder neck obstruction, urethral strictures, pelvic floor dysfunction, overactive bladder syndrome and urinary tract infections. In ageing men BPH and OAB are common, causing difficulty with voiding and bladder emptying (obstructive voiding), frequent voiding and nocturnia due to a diminished functional bladder capacity (storage symptoms). OAB occurs both in men and woman and increases with age. In most cases OAB is idiopathic, but urinary tract infections and obstructive voiding are predisposing factors.
Erectile dysfunction is common in ageing men and related to vascular diseases, diabetes, neurological diseases, hypogonadism, depression, anxiety and chronic stress.
Different community-based studies have confirmed the association between LUTS and ED, indicating that men with ED more often suffer from severe forms of LUTS. Also, men with hypertension and diabetes and MetS are frequently bothered by both conditions.
Several research groups have investigated potential common biological pathways for both LUTS and ED, including the activity of nitric oxide synthase (NOS) and Rho-kinase, resulting in a decreased oxygenation of the cavernosal bodies and decreased smooth muscle relaxation. Furthermore, a high sympathetic tone, as seen in men with chronic stress and in hypertensive patients can explain OAB and ED symptoms.
A targeted therapy for both LUTS and ED are PDE5-inhibitors. Randomized studies with Sildenafil and Tadalafil have shown that both LUTS and ED improve with daily use of a low dose of a PDE5-inhibitor.
Conclusion: LUTS and ED often occur simultaneously in ageing men, suggesting a common etiology. Low NOS and Rho-kinase activity and increased sympathetic tone could explain the association between both conditions. PDE5-inhibitors are a successful targeted therapy for ageing men with LUTS and ED.
Key reference: De Nunzio C, Roehrborn CG, Andersson K-E, et al. Erectile dysfunction and lower urinary tract symptoms. Eur Urol Focus. 2017;3: 352–63.

INDUSTRY SYMPOSIUM 3 – FERRING MINI-SYMPOSIUM: TESTOSTERONE REPLACEMENT

IS07

Testosterone replacement therapy: patient-focused care
Z. KOPA
No text available.

ANDROLOGY

ABSTRACTS

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ORAL COMMUNICATIONS
GOLDEN COMMUNICATIONS

OC01

Risk of prostate cancer in men undergoing assisted reproduction

Y. AL-JEBARI[1], A. ELENKOV[2], A. GIWERCMAN[1] AND Y. LUNDBERG GIWERCMAN[1]

[1]Lund University, Department of Translational Medicine, Lund, Sweden; [2]Medical University Sofia, Department of Urology, Sofia, Bulgaria

Background: Register-studies have reported lower risk of incident prostate cancer for childless men than biological fathers. Other studies have indicated that men with impaired fertility are at higher risk for prostate cancer than fertile men. In order to investigate if this discrepancy is due to the hormonal influence on spermatogenesis, men undergoing intracytoplasmic sperm injection (ICSI) treatment, often due to severely impaired spermatogenesis were compared with those treated with in vitro fertilization (IVF) and fathers by natural conception. The former having insufficient spermatogenesis to fertilize an oocyte ex vivo; therefore their subfertility is likely related to hypogonadism.

Methods: This register-based study sourced data from the Medical Birth Register, the Cancer Registry, and the Quality Register for Assisted Reproduction. All fathers and their first child born 1994–2014 were identified. ICSI fathers were compared to those who had become fathers by natural conception (controls) and in vitro fertilization (IVF) fathers regarding incident prostate cancer during a follow up until 2016, in total 51990101 person-years). Sensitivity analysis stratified upon age at diagnosis of prostate cancer.

Among all fathers (n = 1181490), 20618 and 14882 had undergone IVF and ICSI, respectively; and 3211 were diagnosed with prostate cancer. Associations between mode of conception (ICSI/IVF/natural) and subsequent prostate cancer were investigated using Cox regression models, adjusted for age and education level. Early and late-onset prostate cancer was defined according to age at diagnosis: ≤50 and >50 years.

Results: Fathers who had undergone ICSI had a higher risk of prostate cancer (at any age) as compared to controls (HR = 1.47, CI 95% 1.15–1.89; p = 0.002). Conversely, IVF-men did not have an increase in prostate cancer risk when compared to controls (HR = 1.14, CI 95% 0.91–1.43; p = 0.25). When stratified into age groups at cancer, the fathers who had conceived through ICSI had higher risk for early-onset prostate cancer (HR = 2.94, 95% CI = 1.84–4.71; $p < 0.001$) i.e. diagnosed before 50 years of age. However, ICSI-men did not have an increased risk for late-onset prostate cancer compared to controls. No increased risk of early onset PCa was detected for IVF-fathers (HR = 1.06, 95% CI = 0.57–1.98; p = 0.86).

Conclusion: The results show immense risk for early-onset prostate cancer, generally considered more aggressive, in men referred for ICSI. These men may already have a latent tumor at the time of ICSI, why the possible benefits of targeted screening could be considered.

OC02

Impact of cancer therapy on risk of congenital malformations in children fathered by men treated for germ cell testicular cancer

Y. AL-JEBARI[1], I. GLIMELIUS[2], C. B. NORD[3], G. COHN-CEDERMARK[3], O. STÅHL[4], L. RYLANDER[5] AND A. GIWERCMAN[1]

[1]Molecular Reproductive Medicine, Department of Translational Medicine, Lund University, Malmoe, Sweden; [2]Clinical Epidemiology Unit, Karolinska Institute, Departments of Immunology, Genetics and Pathology and Oncology-Pathology, Uppsala University, Uppsala, Sweden; [3]Karolinska Institute and Karolinska University Hospital, Stockholm, Sweden; [4]Department of Oncology, Skåne University Hospital, Lund, Sweden; [5]The Unit for Environmental Epidemiology, Division of Occupational and Environmental Medicine, Lund University, Lund, Sweden

Background: There is concern for increased risk of congenital malformations among children of fathers treated for cancer, due to the potential mutagenic effects of chemo- and radiotherapy, as indicated in animal studies. Register studies indicate a slight excess of malformations for children conceived after paternal cancer diagnosis, but these registries lack information on given anti-cancer treatment. Furthermore, we recently reported increased malformation risk in children born prior to paternal germ cell testicular cancer (TC) diagnosis.

Objective: The aim of this study was, with detailed treatment data from the Swedish Norwegian Testicular Cancer Group (SWENOTECA), investigate whether anti-neoplastic treatment implies additional malformation risk.

Methods: All children born in Sweden 1994–2014 (n = 2027997) were included. Paternal TC diagnoses (n = 2380) and treatment data were gathered from SWENOTECA. Offspring malformation diagnoses were sourced from the Swedish Medical Birth Register. Among children born to fathers with TC (n = 4337), 122 had a major malformation. These children were grouped according to the paternal treatment regimen: chemotherapy, or radiotherapy; and according to if the child was conceived pre- (n = 2770) or post-treatment (n = 1437). Logistic regression was applied to calculate odds ratio (OR) for congenital malformation. Adjustments for parental age, maternal smoking and body mass index were made.

Results: Children to fathers with TC had a higher risk for major malformations as compared to children born to fathers without TC (OR = 1.36, 95% CI = 1.24 to 1.49, $p < 0.001$, 2.9% vs. 2.2) However, when comparing children conceived prior to and after treatment, no risk increase associated with chemotherapy (OR = 0.93, 95% CI = 0.57 to 1.51, p = 0.77, 3.0% vs. 3.1%) or with radiotherapy (OR = 1.21, 95% CI = 0.23 to 6.33, p = 0.82, 2.4% vs. 2.0%) could be detected. With all malformations as end point, the risk estimates were similar to those for major malformations.

Conclusion: No statistically significantly increased risk of congenital malformations was seen in children of TC men treated with radio- or chemotherapy. However, paternal TC was associated with a higher risk for malformations.

OC03

In vitro culture of Klinefelter Spermatogonial stem cells: from mouse to human

G. GALDON[1], N. P. ZARANDI[1], M. J. PETTENATI[2], S. HOWARDS[3], W. G. KEARNS[4], S. J. KOGAN[1,3], Y. LUE[5], R. S. SWERDLOFF[5], A. ATALA[1,3] AND H. SADRI-ARDEKANI[1,3]

[1]Wake Forest Institute for Regenerative Medicine (WFIRM); [2]Section of Medical Genetics, Department of Pathology; [3]Department of Urology; Wake Forest School of Medicine, Winston-Salem, NC, USA; [4]AdvaGenix and John Hopkins Medicine, Rockville and Baltimore, MD, USA; [5]Division of Endocrinology, Department of Medicine, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Torrance, CA, USA

Background: Klinefelter Syndrome (KS) is characterized by masculine phenotype and supernumerary sex chromosomes (47 XXY). Starting at the onset of puberty, KS patients develop progressive testicular fibrosis and impaired fertility due to loss of spermatogonial stem cells (SSC). It's been hypothesized that early SSC cryopreservation could be an option for future fertility treatments in these patients including SSC transplantation or in vitro spermatogenesis. However, in order to do so it is critically important to adapt current in vitro SSC propagation systems for KS patients. Initial proof of concept was conducted on 3 day old KS mouse model testicular tissue and then the system was translated into KS human tissue.

Methods: 3 day old KS mouse testicular tissue was used from the model that has been developed and characterized by UCLA researchers. KS human testicular tissue was donated by three patients ages 13, 15 and 17 years old enrolled in experimental testicular tissue bank at Wake Forest Baptist Health. All three selected patients were non-mosaic 47, XXY according to peripheral blood karyotype. Testicular cells were isolated from cryopreserved tissue and propagated in long term culture adapting our previously established method on normal human testes. Propagated testicular cells were characterized using q-PCR, digital PCR, Flow Cytometry and Magnetic Activated Cell Sorting, next generation sequencing (NGS) based molecular karyotype and X/Y chromosome FISH staining.

Results: Both mouse and human KS cells were successfully isolated and propagated in culture for 80 days or longer. Cell specific gene expression confirmed the presence of all 4 main cell types expected in testes: Spermatogonia, Sertoli, Leydig and Peritubular cells. A population of ZBTB16 + undifferentiated spermatogonia was identified all along culture using digital PCR. Flow Cytometry analysis detected a HLA-/CD9 + /CD49f+ population suggesting a stem cell subpopulation all along culture. NGS testing showed all cells being 47, XXY up to 90% confidence interval. FISH staining for chromosomes X and Y showed most of cells in culture containing XXY combination but also small population of XY and XX cells. Both XY and XX populations were enriched by MACS sorting for CD as SSC enriched marker.

Conclusion: To the best of our knowledge this is the first study showing successful isolation and propagation of testicular cells from mouse and human KS testes. We believe these findings have the potential to impact fertility therapeutic options for azoospermia KS patients either in vitro or in vivo.

OC04

Mouse in-vitro spermatogenesis on alginate-based 3D bioprinted scaffolds

Y. BAERT[1], S. GÜNDOGAN[1], H. MARGARYAN[2], K. DVORAKOVA-HORTOVA[2,3] AND E. GOOSSENS[1]

[1]Biology of the Testis, Research Laboratory for Reproduction, Genetics and Regenerative Medicine, Vrije Universiteit Brussel (VUB), Brussels, Belgium; [2]Group of Reproductive Biology, Institute of Biotechnology CAS, v.v.i., BIOCEV, Vestec, Czech Republic; [3]Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

Background: In-vitro spermatogenesis has already been successfully achieved in rodents by an organ culture system and a soft agarose culture system. However, the former has proven difficult to extrapolate to the human and the latter irreversibly encapsulates all cells limiting its translation and application in the clinic.

Objective: We aim to explore a new culture system in a mouse model using 3D bioprinting which gives control over cell deposition and scaffold design. Specifically, the goals were: Firstly, to culture testicular cells in easy-to-access macropores of printed cell-free scaffolds. Secondly, to culture tubular cells in the pores of printed interstitial cell-laden scaffolds.

Methods: Testicular tissue from prepubertal (max 6 dpp) C57BL/6NCrl (Acr3-EGFP: green acrosome) mice and adult (21 dpp) SV129/C57BL mice were used. Dissociation of the prepubertal tissues into single cells by enzymatic digestion was followed by separation of the tubular and interstitial cells through Magnetic-Activated Cell Sorting (MACS) for CD49f. Per cell-free scaffold, 2.7x105 CD49f+ and 0.2x105 CD49f- cells were consecutively seeded with 2 h interval in the macropores and cultured at the gas-liquid phase (= testicular organoids). In order to print cell-laden scaffolds, the bioink was first diluted 1/10 with adult interstitial cells (± 107/mL). These cells were obtained by separating the seminiferous tubules and digesting the interstitium of adult tissues using collagenase IA. Per cell-laden scaffold, 3.08x105 CD49f+ cells were seeded and cultured (= testicular constructs). Organ culture of prepubertal tissue served as positive control for IVS. The organ culture, organoids and testicular constructs were cultured for 40, 48 and 41 days, respectively. Periodic Acid-Schiff (PAS)/hematoxylin staining, selective peanut agglutinin (PNA) staining of the acrosome and double immunofluorescence (EGFP/CREM) were used to detect postmeiotic cells.

Results: PAS/hematoxylin staining indicated the presence of postmeiotic cells in the organ cultures, organoids and testicular constructs. Immunofluorescence confirmed round spermatids with EGFP/CREM expression and PNA binding to the acrosome in nearly all cultured testicular tissues (n = 4/5). Occasionally, even elongating spermatids with EGFP+ and PNA+ acrosome were observed. Organoid formation was observed in the weeks following cell seeding on cell-free scaffolds. Immunofluorescence showed CREM+ round spermatids in the Acr3-EGFP- organoids of one scaffold (n = 1/3), while two repeats with Acr3-EGFP+ organoids (n = 2/3) even showed elongated spermatids with PNA binding to the acrosome. Round spermatids with EGFP/CREM expression were also

observed in printed testicular constructs (n = 2/3), of which one testicular construct showed elongating spermatids with PNA staining.
Conclusion: We confirmed previous reports by showing differentiation towards postmeiotic germ cells in the organ culture system. In addition, we showed germ cell differentiation in testicular organoids on cell-free printed scaffolds and in testicular constructs using cell-laden scaffolds. So far, this is the first report applying a 3D bioprinting approach for in-vitro spermatogenesis. It remains to be tested whether the germ cells generated on the alginate-based scaffolds are functional.

OC05

Withdrawn.

OC06

Comparative analyses of AZF microdeletions in leukocytes and testis tissue of TESE patients reveal genetic mosaicism in germ line impairing sperm prognosis

P. H. VOGT[1], U. BENDER[1], J. ZIMMER[1], J. DIETRICH[2] AND T. STROWITZKI[3]
[1]Reproduction Genetics Unit, Department of Gynecological Endocrinology and Infertility disorders, University of Heidelberg, Germany; [2]IVF lab, Department of Gynecological Endocrinology and Infertility Disorders, University of Heidelberg, Germany; [3]Department of Gynecological Endocrinology and Infertility disorders, University of Heidelberg, Germany

Background: Three distinct microdeletions in the Azoospermia Factor (AZF) locus of the human Y chromosome cause male infertility with distinct testicular pathologies; they were therefore designated AZFa, AZFb, and AZFc, respectively. Their analysis is frequently used when patients suffering from non-obstructive azoospermia decided for testicular sperm extraction (TESE) before application of ICSI for reproduction in the IVF clinic. It is believed that complete AZFa or AZFb deletion in their leukocyte Y-DNA suggests, that no mature sperms are present in their testicular tubules, whereas patients with complete AZFc deletion do have a very good prognosis for presence of sperms in at least some of their testis tubules. Partial AZF deletions including deletions of only single AZF genes can cause the same testicular pathology as the corresponding complete deletion (e.g. DDX3Y gene deletions in AZFa), or might not be associated with male infertility at all (e.g. some DAZ gene deletions in AZFc). One assumes thereby that rate and extension of the AZF deletion identified in the patient leukocyte Y-DNA corresponds to that present in the patient's germ cells in the testis tissue. However, experimental proof of this prediction is still lacking.
Methods: We therefore collected a number of patients diagnosed with nonobstructive azoospermia and presence of a classical AZFb or AZFc deletion in their leukocyte Y-DNA which decided for TESE, to analyse for comparison the rate and extension of these AZFb/AZFc deletion in genomic Y-DNA samples extracted from their left and right testis tissue. For this purpose we performed PCR multiplex assays according to Vogt & Bender (in: Meth. Mol. Biol. vol. 927: 187–204, 2012.). This protocol is able to distinguish classical and partial AZFa, AZFb, AZFc deletions including deletions of only one of the 14 protein coding AZF genes. It can be certified as robust standard protocol because it includes the basic principles of quality control essential for each molecular genetic diagnostic deletion assay according to the strict guidelines of the "European Molecular Genetics Quality Network" (EMQN: www.emqn.org).
Results: We found seven samples with some heterogeneity in the genomic extensions of their AZFb/AZFc deletion in leukocytes and testis tissue. One patient presents a partial AZFb + complete AZFc deletion but only in leukocytes; 6 patients with complete AZFc deletions in leukocytes displayed only partial AZFc deletions in testis tissue. We conclude that the divergent AZF Y deletions observed in the testis samples of these patients suggest some genetic mosaicism of these AZF deletions in the patients germ line. It points to dynamic rearrangement(s) of the long Y arm in these patients testis DNA, probably due to the high frequency of recombination hot spots found in the repetitive sequence blocks concentrated in the distal AZFb and all AZFc amplicons.
Conclusion: Our results can probably help to explain the large heterogeneity of testicular pathologies found in patients with complete AZFb/AZFc deletions in their leukocytes and are important for clinical counselling when they ask for their sperm prognosis before performing testis biopsy.

SELECTED ORAL COMMUNICATIONS

OC07

Long-term effect of testicular germ cell tumor treatments on sperm DNA fragmentation

E. CASAMONTI[1], M. G. FINO[1], F. CIOPPI[1], S. VINCI[1], A. RIERA-ESCAMILLA[1,2], L. TAMBURRINO[1], A. MAGINI[1], M. MURATORI[1] AND C. KRAUSZ[1]
[1]Department of Biomedical, Experimental and Clinical Sciences "Mario Serio", University of Florence, Florence, Italy; [2]Andrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, Instituto de Investigaciones Biomédicas Sant Pau (IIB-Sant Pau), Barcelona, Catalonia, Spain

Background: Testicular Germ Cell Tumor (TGCT) is the most frequent malignant disease in young men. In the large majority of cases, spermatogenesis is recovered after cancer-treatment hence the future welfare of children conceived by a father treated with cytotoxic therapy is of concern. Information are scarce, regarding the long-term effect of cytotoxic treatments on sperm DNA, with a maximum observation period of up to 2 years. In some studies, high DNA fragmentation and sperm aneuploidy rate were reported in a proportion of patient after 24 months, raising questions about the right timing for natural conception.

Objective: To evaluate the effect of cytotoxic therapies on Sperm DNA Fragmentation (SDF) after 2 (T2) and 3 years (T3) from the end of treatment in TGCT patients.
Methods: Cross-sectional study. Total and brighter SDF (SDFtot and SDFbr, respectively) were analyzed in 2 TGCT-patients groups: 2 years post-therapy (T2 group, $n = 76$) and 3 years post-therapy (T3 group, $n = 54$). The analysis based on TUNEL-assay, was performed on 10x10^6 sperm cells. In each group: (i) data were compared with those of 58 healthy men (control group); (ii) the proportion of patients with %SDFbr>75th percentile of "normality" (i.e. >25%) was calculated.
Results: Patients were divided according to the type of treatment for each time-category (T2 and T3): Carboplatine ($n = 22$ and 14, respectively), PEB with ≤2 cycles ($n = 18$ and 14, respectively) or with ≥3 cycles ($n = 20$ and 11, respectively), radiotherapy ($n = 14$ and 12, respectively) and chemotherapy+radiotherapy ($n = 2$ and 3, respectively). The mean %SDFtot and %SDFbr of the control group were 29.11% ± 11.11% and 19.53% ± 9.48%, respectively.
i) T2 group: both %SDFtot and %SDFbr were significantly higher in patients vs fertile men in the following treatment's groups: (i) PEB with ≥3 cycles: %SDFtot = 39.98% ± 15.32%, $p = 0.001$; %SDFbr = 25.61%±10.37%, $p < 0.05$; (ii) radiotherapy: %SDFtot = 43.60%±17.35%, $p < 0.001$; %SDFbr = 32.75%±16.54%, $p < 0.001$; (iii) chemotherapy+radiotherapy: %SDFtot = 68.72% ± 38.13%, $p < 0.001$; %SDFbr = 39.88% ± 30.33%, $p < 0.01$. in the Carboplatine therapy group only %SDFtot was significantly higher compared to controls (36.34% ± 16.86%, $p < 0.05$). T3 group: both %SDFtot and %SDFbr were significantly higher in subjects treated with chemotherapy+radiotherapy vs fertile controls (% SDFtot = 54.45% ± 24.01%, $p = 0.001$; %SDFbr = 37.80% ± 14.15%, $p < 0.01$). in PEB with ≥3 cycles group only SDFtot was significantly higher compared to controls (38.64% ± 19.86%, $p < 0.05$).
ii) The proportion of patients with clearly pathological SDFbr value (i.e. >75th percentile) was above 30% in the T2 group for all type of treatments. In T3 time-category, the proportion was lower than this percentage in the less aggressive treatments-groups (i.e. Carboplatine therapy, PEB with ≤2 cycles) whereas it was above 30% in the most aggressive ones (i.e. PEB with ≥3 cycles, radiotherapy, chemotherapy+radiotherapy).
Conclusion: Currently, spontaneous pregnancy is not recommended during the first two years after cytotoxic treatments; in light of our finding of a relatively high incidence of patients with pathological SDF values after 3 years post-therapy, it would be advisable to re-evaluate the waiting period beyond this time-limit. In order to provide greater certainty to cancer patients (regarding most appropriate timing for the search of a natural conception), sperm DNA fragmentation study could be introduced in the clinical practice to monitor the genomic damage.

OC08

TEKT5 is a new candidate gene for male infertility

C. FRIEDRICH[1], A. RÖPKE[1], B. WESTERNSTRÖER[2], D. GROßE-KATHÖFER[1], L. HANKAMP[1], M. HOFFMANN[1], S. BURKHARDT[1], S. SCHLATT[2], S. KLIESCH[2], A. MÜLLER[3], S. PERREY[4] AND F. TÜTTELMANN[1]
[1]Institute of Human Genetics, University of Münster, Münster, Germany; [2]Centre of Reproductive Medicine and Andrology, University Hospital Münster, Münster, Germany; [3]Center of Pediatric Pathology and Pathology, University Hospital Bonn, Bonn, Germany; [4]Institute for Bioinformatics and Chemoinformatics, Westphalian University of Applied Sciences, Campus Recklinghausen, Germany

Background: Diagnostics of male infertility includes semen and hormone analyses which often result in descriptive classifications but do not explain underlying causes. Genetic causes of male infertility include chromosomal aberrations, AZF deletions, CBAVD and CHH but overall only explain 4% of cases. The large majority of ~70% remains unresolved (1). Here we report on a new candidate gene for male infertility, namely TEKT5. TEKT5 is the youngest member of the Tektin protein family. Tektins are filament-forming proteins in the male germ cell-lineage present in centrioles, basal bodies and within ciliary and flagellar doublet microtubules. TEKT5 was initially identified in the rat. It is present in sperm flagella, plays an important role in flagella formation during spermatogenesis, and has also been implicated in sperm motility. The expression of TEKT5 mRNA is restricted to the testis in normal adult tissues (2, 3).
Methods: First, a specifically selected group of 36 men with complete bilateral Sertoli-Cell-Only syndrome (SCOS) were analysed by whole-exome sequencing (WES). In the next step, a larger cohort of well characterised infertile men ($n = 250$) with different phenotypes (SCOS $n = 109$, meiotic arrest $n = 24$, mixed atrophy $n = 22$, non-obstructive azoospermia [no biopsy] $n = 62$, severe oligozoospermia $n = 33$) was analysed by WES. Variants with MAF<1% in public genome databases (dbSNP, ESP, gnomAd, ExAc) were assessed by in silico algorithms (PolyPhen-2, SIFT, MutationTaster) and were modelled using Phyre-2 homology prediction and visualized by Pymol. IHC staining of TEKT5 was performed in fetal and adult testicular tissue from mouse and human and from patients with TEKT5 alterations. BeWo cells, which endogenously express TEKT5, where stained by immunofluorescence.
Results: In the initial patient cohort ($n = 36$) we prioritized TEKT5 as highest ranked candidate gene due to the identification of three heterozygous variants (1 stop-gained, 2 missense variants) and described testicular expression. In the entire patient cohort ($n = 286$) we identified 19 novel or rare (MAF<1%) heterozygous variants (2 stop-gained, 1 frameshift, 16 missense variants) and one deletion of the complete TEKT5 gene from our previous array-CGH analyses (4). IHC staining of TEKT5 was similar in adult mouse and human testes (late spermatocytes, round spermatids and spermatids). In addition, in fetal mouse and fetal human testes, TEKT5 is also expressed in germ cells. No TEKT5 expression could be detected by IHC in testicular biopsies of the patient showing a complete deletion.

TEKT5 immunofluorescence staining of BeWo cells showed a signal within the nucleus until confluency, implicating a so far undescribed nuclear role of TEKT5.
Conclusion: We propose for the first time that the testicular expressed TEKT5 gene is a new candidate gene for male infertility. We showed the expression of TEKT5 in germ cells and suspect a to date unknown nuclear role.
This work was carried out within the frame of the DFG Clinical Research Unit 'Male Germ Cells: from Genes to Function' (CRU 326).
References:
1. Tüttelmann et al. Medizinische Genetik. 2018;30:12–20
2. Steffen et al. Proc Natl Acad Sci USA. 1988;85:2643–2647
3. Cao et al. J Androl 2011;32:10.2164
4. Tüttelmann et al. PLoS One. 2011;6:e19426

OC09

Metabolomic profiling by 1H-NMR of human seminal plasma and database-driven analysis reveal new features for glycerophosphocholine

M. WALSCHAERTS[1], S. ARNAULT[1], E. OBEUF[1], C. CANLET[2], L. DEBRAUWER[2], F. ISUS[1], M. DAUDIN[1], S. DEJEAN[3], N. SAVY[3] AND S. HAMDI[1]
[1]Groupe de Recherche en Fertilité Humaine, EA3694, Université Paul-Sabatier, CHU de Toulouse, Toulouse, France; [2]UMR 1331 TOXALIM, Plateau Metatoul AXIOM, Université Paul-Sabatier, INRA, INP, Toulouse, France; [3]Equipe Statistique et Probabilités, Institut de Mathématiques de Toulouse, Université Paul-Sabatier, Toulouse, France

Background: Seminal plasma (SP) is a complex mixture that dilutes and transports spermatozoa and is recognized as an anatomical and functional reflect of the male genital tract. Therefore, SP exploration is informative for the etiological diagnosis of male infertility. Recently, 1H NMR studies of human biofluids have attracted much attention as a powerful tool to understand pathophysiological processes at metabolites level.
Objective: To perform and to compare untargeted metabolomic profiling by 1H-NMR of seminal plasma from normozoospermic (NZ) and azoospermic (AZ) patients.
Methods: SP samples from 47 consecutive NZ (according to WHO 2010 criteria) and 38 consecutive AZ were stored after routine investigations and analyzed by batches on a Bruker DRX 600 MHz NMR Spectrometer. A manual signal assignment of metabolites was performed and spectrum-shaped metabolomics data from NZ and AZ patients were transformed by the wavelet method before a Sparse-PLS-DA-based comparison. For further statistical analysis, data of 993 NZ patients and 461 AZ patients referred to our Andrological Center between 2005 and 2015 were extracted from the database.
Results: In preliminary experiments, 1H- and 13C–RMN spectra (1D, 2D COSY, 2D HSQC) were obtained and manual signal assignment retrieved 30 unambiguous metabolites. Sparse PLS-DA on wavelets-transformed spectra identified several intervals of chemical shift with significant differences between NZ and AZ. In the 2,9–3,9 ppm interval, 3 metabolites could be identified : choline, fructose and glycerophosphocholine (GPC). Enzymatic assays confirmed a difference for total seminal GPC between NZ and AZ (respectively 6.89 ± 4.06 and 3.11 ± 3.32 μmol, $p < 0.0001$) but not for total choline or fructose. Comparing NZ and AZ from the center database confirmed this difference in total GPC (respectively 7.9 ± 7.6 and 3.1 ± 3.0 μmol, $p < 0.001$). A detailed analysis of the NZ dataset revealed that total GPC is negatively correlated to patients' age ($p = 0.0003$) and BMI ($p = 0.003$), positively correlated to spermatozoa concentration ($p < 0.0001$) but not to sperm motility or viability.
Conclusion: Metabolomic profiling by 1H-NMR of SP and statistical analysis of 1454 patients shed light on GPC and revealed its link with spermatozoa concentration, opening new research perspectives.

OC10

Presentation, clinical features, and long term follow up of Leydig cell tumors (LCTs) of the testis: a single centre experience

C. POZZA, M. TENUTA, M. G. TARSITANO, D. GIANFRILLI, M. MINNETTI, G. PULIANI, V. HASENMAJER, A. LENZI AND A. M. ISIDORI
Department of Experimental Medicine, Sapienza University, Rome, Italy

Background: With the improved imaging techniques, Leydig cell tumors (LCTs) are frequently found. Natural history of LCTs is relatively unknown, because of the small size and heterogeneity of available studies. Long-term follow-up is missing.
Objective: Our aim was to report the experience with a large cohort of prospectively collected LCTs.
Methods: Patients with LCTs were enrolled from 2005 to 2017. Clinical and biochemical features of LCTs were compared with two cohorts: patients with seminomas and patients without testicular lesions (NoL) randomly selected among patients referred in the same period.
Results: 83 patients had LCTs, 90 had seminomas, and 2683 had no lesion (NoL). Testicular volumes ($p = 0.001$), sperm concentration ($p = 0.001$) and morphology ($p < 0.001$) were lower in LCTs compared to NoL; gonadotropins ($p < 0.001$) and SHBG ($p < 0.001$) were higher and testosterone/LH ratio was lower ($p < 0.001$) in LCTs vs NoL. No differences were found in gonadal steroid after hCG test, between groups.
When compared to seminomas, LCTs did not show differences except for higher SHBG levels ($p = 0.001$), lower sperm concentration ($p = 0.029$) and motility ($p = 0.001$). Cryptorchidism (chi square=28.272, $p < 0.01$), gynaecomastia (chi square = 54.223, $p < 0.001$) and low testicular volume (chi square = 11.133, $p = 0.001$) were associated with a higher risk of LCTs. After a median follow-up of 66 months, no metastases have been detected.
Conclusion: LCTs have good prognosis when correctly recognized. Based on the largest existing series, we showed that infertility, gynecomastia, low testicular volume, and cryptorchidism are frequently associated with LCts, supporting the hypothesis that testicular dysgenesis syndrome could play a role. Active surveillance appears to be a safe option, but monitoring of Leydig cell failure remains necessary.

OC11

Establishing a SNP-panel (on single nucleotide polymorphisms) associated with FSH action – an approach for personalized FSH treatment in men with unexplained infertility

M. SCHUBERT[1], L. P. LANUZA[2], M. WÖSTE[3], F. TÜTTELMANN[4], S. KLIESCH[1] AND J. GROMOLL[2]
[1]*Department of Clinical and Surgical Andrology, Centre of Reproductive Medicine and Andrology;* [2]*Centre of Reproductive Medicine and Andrology;* [3] *Institute of Medical Informatics, University Hospital Münster, Germany;* [4]*Institute of Human Genetics, University Hospital Münster, Germany*

Supported by the German Research Foundation (CRU 326 – Male Germ Cells: from Genes to Function)

Background: 30–40% of infertile men suffer from unexplained infertility. Since there is no causal treatment, these patients are often referred to assisted reproductive techniques.

FSH is the key player in initiation and maintenance of spermatogenesis. General unclassified hormone treatment with FSH in infertile men only improves semen quality and pregnancy rates in an undefined subgroup. Previously it was shown that the single nucleotide polymorphism (SNP) within the FSHB gene (c.-211G>T) negatively impacts endocrine feedback, testicular size and spermatogenesis. However, polymorphisms in FSHB and FSHR only explain ~5% of FSH variation. Further polymorphisms, which have to be identified, will most probably contribute to FSH action. Taking the hypergonadotropic Klinefelter syndrome (KS) (47,XXY) as a model for FSH action we found the impact of the FSHB SNP on serum FSH levels in KS to be 23-fold increased compared to our infertile male cohort.

Methods: In a discovery study on 104 KS patients (no mosaicism, no prior treatment) SNP analysis was performed using HumanOmniExpressExome arrays. We used Illumina®GenomeStudio and plink (connection tool) to perform a quantitative association analysis based on FSH phenotype values. The SNPs were annotated using dbSNP, the Human Protein Atlas and expression data from GTEx Consortium datasets. Pathways related to the SNPs were analyzed using Ingenuity® Pathway Analysis and Reactome. SNPs were then manually prioritized based on the statistical and biological properties of the annotated dataset.

Results: Using a combination of statistical and biological properties (*p* value, expression in endocrine related genes/pathways, expression in hypothalamus/pituitary gland/testis) we identified $n = 252$ relevant SNPs for validation.

Conclusion: Here, the KS is used as model to identify further SNPs/genes affecting FSH action. In the array analysis, we found 252 candidate SNPs in testis, hypothalamus or pituitary genes showing associations with FSH levels. This presents the first step towards a targeted SNP panel, which will be validated in a large cohort of patients with unexplained infertility and be compared to fertile men. Further, functional analyses on the highest ranked genes will reveal further information on FSH signalling. Altogether, we aim to identify a subset of infertile men with specific SNPs, which render them eligible to FSH treatment.

OC12

A chromosomal scan of single sperm cell by combining. Fluorescence-activated cell sorting and Next-generation sequencing

T. T. QUOC[1,2,3], T. JATSENKO[1], O. TŠUIKO[1,2], D. LUBENETS[4], T. REIMAND[5], O. POOLAMETS[6], M. PUNAB[6], M. PETERS[1,7] AND A. SALUMETS[1,2,7,8]
[1]*Competence Centre on Health Technologies, Tartu, Estonia;* [2]*Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia;* [3]*Danang University of Medical Technology and Pharmacy, Danang, Vietnam;* [4]*Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia;* [5]*Chair of Human Genetics, University of Tartu, Tartu, Estonia;* [6]*Andrology Centre, Tartu University Hospital, Tartu, Estonia;* [7]*Department of Obstetrics and Gynaecology, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia;* [8]*Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland*

Background: Reciprocal translocations are the most frequent structural chromosomal rearrangements in humans that may produce unbalanced gametes by meiotic segregation.

Methods: In this study, we developed an experimental approach combining fluorescence-activated cell sorting (FACS), whole genome amplification, and next-generation sequencing that allows detecting the full aneuploidies and structural chromosomal alterations from single sperm cells.

Results: By applying this protocol, six sequenced single sperms from a normozoospermic man showed a normal 23-chromosome profile with two and four of the cells bearing X and Y chromosome, respectively. Furthermore, the analysis of 31 single sperms from one carrier of a reciprocal translocation 46, XY,t(7;13)(p10;q10) revealed that 35.6% of sperms had normal haploid chromosomal composition; and 64.5% of analyzed sperms showed several variants of aneuploidies. The sperms with partial or full aneuploidies of chromosomes 7 and 13 represented the meiotic products of adjacent I and II segregation and one sperm showed a gain of chromosome 9.

Conclusion: The application of this method enables comprehensive chromosomal aberration screening of a large number of sperms and provides an effective tool for studying the production of gametes from patients carrying chromosomal diseases. Moreover, it could be implemented in clinics to support the personalized family planning in several patients' groups, such as men with chromosomal aberrations and/or male infertility, and in couples with recurrent miscarriages.

OC13

Testicular ultrasound inhomogeneity is more informative than testicular volume in fertility evaluation

G. SPAGGIARI[1,2], D. SANTI[1,2], A. GRANATA[2] AND M. SIMONI[1,2]

[1]Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy; [2]Unit of Endocrinology, Department of Medical Specialties, Azienda Ospedaliero-Universitaria of Modena, OCSAE, Modena, Italy

Background: Testicular volume (TV) is proposed as a positive predictor of male fertility status considering its known relation to the seminiferous tubules content. Although the testicular size assessment remains in clinical practice the first approach to indirectly evaluate spermatogenesis, its real predictive significance remains not clearly detected.
Objective: To assess TV and ultrasound (US) characteristics of patients undergoing single operator-testis US, matching results with seminal and hormonal parameters.
Methods: All consequent out-patients undergoing testis US from March 2012 to March 2018 for any reason were considered eligible. TV was calculated using the ellipsoid formula with the 0.71-coefficient. Testis US inhomogeneity was defined as the absence of the uniform hypoechoic structure characterizing normal testicular parenchyma evaluable at ultrasound. Analyses were performed dividing patients according to clinical reason for attending. Patients examined for fertility workup were further subdivided according to the seminal status, whereas hypogonadal patients were subdivided considering the administration of replacement androgenic therapy. Correlations among TV and hormonal and semen parameters were evaluated.
Results: 302 men were enrolled (mean age 39.8 ± 15.2 years). Reasons for US evaluation were gynecomastia (12.3%, $n = 37$), hypogonadism (33.4%, $n = 101$), couple infertility (CI) (39.1%, $n = 118$) and sexual dysfunctions (15.2%, $n = 46$). CI patients presented normozoospermia in 25.8%, impaired semen quality (oligo- and/or astheno- and/or teratozoospermia) in 55.9% and azoospermia in 18.3% of cases. In CI group, the mean TV value was 14.96 ± 7.45 ml (right testis) and 13.83 ± 6.80 ml (left), significantly higher compared to hypogonadal patients ($p < 0.001$). A significant direct correlation between TV and testosterone levels was observed in not-treated hypogonadal patients ($R = 0.911$, $p < 0.001$), whereas this correlation was absent in CI group. Normozoospermic patients presented significantly higher TV (19.16 ± 8.51 ml and 18.24 ± 6.57 ml) compared to impaired semen quality (13.48 ± 5.69 ml and 12.69 ± 5.55 ml) ($p = 0.003$) and azoospermia (11.71 ± 6.67 ml and 9.15 ± 4.38 ml) ($p = 0.003$) groups. TV was directly related to sperm number only in normozoospermic patients ($R = 0.577$, $p = 0.005$). Testis US inhomogeneity was more frequent in patients with impaired sperm quality (55.0%) ($p = 0.007$), compared to azoospermic (40%) and normozoospermic (5%), whereas the US finding of microcalcifications did not differ ($p = 0.090$).
Conclusion: In our cohort, although testicular size is significantly higher in normozoospermic patients, TV does not appear informative of the fertility status. Indeed, TV correlates with sperm number in normozoospermic men, but not in patients with altered semen quality. Moreover, in our population, the direct correlation between TV and testosterone observed in hypogonadal not-treated patients suggests that testicular sizes could be related with the testosterone-secreting rather than the spermatogenic compartment. Conversely, since different distribution of testis US inhomogeneity is highlighted comparing normozoospermic and patients with poor seminal quality, the sonographic pattern could better relate with the fertility status. With this in mind, in the CI workup the US evaluation seems to be more informative rather than the simple TV assessment.

OC14

Antisperm-antibodies prevalence and relationship of autoimmunisation degree with semen parameters and post-coital test outcome. A retrospective analysis on over 10,000 men

A. BARBONETTI, C. CASTELLINI, S. D'ANDREA, M. TOTARO, A. MARTORELLA, E. MINALDI, G. CORDESCHI, S. FRANCAVILLA AND F. FRANCAVILLA

Medical Andrology Unit, Department of Life, Health and Environment Sciences, University of L'Aquila, 67100 L'Aquila, Italy

Background: Although the IgG-MAR test has been recommended by WHO as an integral part of semen analysis for the screening of anti-sperm antibodies (ASA), their prevalence and relationship with semen parameters, as well as the cut-off values of clinical relevance are controversial.
Methods: We retrospectively analyzed semen analysis from 12,296 consecutive men seeking semen evaluation, representing the largest series ever reported. Immunological screening was performed to all ejaculates by IgG-MAR-test. Positive samples (≥10%) were tested also for IgA-ASA. The prevalence of positive IgG-MAR tests and the relationship of both degree and isotype of sperm-autoimmunization with semen parameters were evaluated. Outcomes of post-coital test (PCT) performed in couples where the male partner exhibited a positive MAR-test were also analyzed.
Results: Excluding from analyses samples with not-executable MAR-test (2,271 out 12,296 samples), due to azoospermia or severe oligo- and/or astheno-zoospermia, the prevalence of MAR-test results ≥10%, ≥50% and 100% was 4.0%, 3.4% and 2.0%, respectively. Samples with 100% positive IgG-MAR-tests exhibited a significantly higher prevalence of both mixed positivity pattern and concomitant IgA-ASA and a significantly lower prevalence of tail-tip pattern. Both total sperm count (TSC) and progressive motility (PM) were significantly lower in samples with 100% positive IgG-MAR-test. At the bivariable regression models, TSC was independently associated to both IgG-MAR-test and IgA-MAR-test positivity, whereas, PM was independently associated with IgG-MAR-test but not with IgA-MAR-test positivity. Analyzing the PCT in 120 couples where the male partner exhibited a positive IgG-MAR-test, the number of forward-moving spermatozoa/HPF was negatively correlated with positivity % of both IgG-MAR-test ($r = -0.54$; $p < 0.0001$) and IgA-MAR-test ($r = -0.28$; $p = 0.001$).

However, at the bivariable regression analysis, an independent negative association was found for IgG-MAR-test (β: −1.7; $p < 0.0001$), but not for IgA-MAR-test positivity (β: −0.1; $p = 0.1$). The percentage of couples with negative PCT outcome significantly increased with the percentage of IgG-MAR-test positivity. At the multiple logistic regression analyses, in the case of 100% positive MAR-test, neither cervical mucus score nor seminal total motile sperm count significantly contributed to negative PCT outcome. Whereas, in the case of 50–99% positive MAR-test, total motile sperm count significantly contributed, besides the presence of ASA, to negative PCT outcome.

Conclusion: This study, the largest so far reported, provides a reliable estimate of ASA prevalence. Although 50% positive MAR-test represents the cut-off suggested by the WHO with possible clinical relevance, only 100% positive MAR-tests were significantly associated with a "mixed" pattern of link, concomitant occurrence of IgA-ASA, lower sperm quality and poor outcome of PCT, a surrogate infertility-related end-point, as the impairment of sperm penetration through the cervical mucus represents the primary mechanism of ASA interference with fertility.

OC15

Testosterone replacement therapy is able to reduce prostate inflammation in men with BPH, metabolic syndrome and hypogonadism: preliminary results from a randomized placebo-controlled clinical trial

G. RASTRELLI[1], S. CIPRIANI[1], F. LOTTI[1], I. CELLAI[1], P. COMEGLIO[1], V. BODDI[1], P. D. CAMERA[2], M. PALMA[1], M. GACCI[2], S. SERNI[2], M. MAGGI[1] AND L. VIGNOZZI[1]
[1]University of Florence, Deparment of Experimental Clinical and Biomedical Sciences – Sexual Medicine and Andrology Unit, Florence, Italy; [2]University of Florence, Dept. of Urology, Florence, Italy

Objective: BPH results from prostate tissue inflammation, which frequently occurs in men with metabolic syndrome (MetS). MetS is often associated with low testosterone (T). Recent evidence shows that low, rather than high, T levels are associated with BPH/lower urinary tract symptoms (LUTS). The aim of the study was to evaluate if T replacement therapy (TRT) for 6 months in BPH men with MetS and low T, is able to improve LUTS and prostate inflammation (as assessed by ultrasound and gene expression in prostate tissue).

Methods: 120 men in waiting list for BPH surgery and diagnosed with MetS were enrolled in the clinical trial. According to total T (TT) and calculated free T (cFT), they were categorized into eugonadal (TT≥12 nmol/L and cFT≥225 pmol/L; $n = 48$) and hypogonadal men (TT<12 nmol/L and/or cFT<225 pmol/L; $n = 72$). Hypogonadal men were randomly assigned to receive T gel 2% (5 g/daily) or placebo for 6 months. At baseline and follow-up visit (after 6 months), all men filled out the International Prostatic Symptoms Score (IPSS) and NIH-Chronic Prostatitis Symptom Index (NIH-CSPI) questionnaires and underwent a trans-rectal prostate ultrasound. After surgery, prostate tissue was collected.

Results: After adjusting for the baseline value, together with age, TT and waist circumference, NIH-CSPI total score significantly decreased in both the groups ($p < 0.001$ vs. baseline), whereas IPSS total score did not change in any of the groups. IPSS bother score significantly decreased only in T-treated ($p = 0.042$ vs. baseline value). Although a significant increase in total prostate and adenoma volume occurred in T-treated (both $p < 0.05$ vs. the baseline value), T arm was characterised by a significant decrease in ultrasound markers of prostate inflammation, including arterial velocity and acceleration (both $p < 0.01$ vs. baseline value). In a subset of patients (9 eugonadal, 11 placebo and 9 T-treated), the expression by prostate tissue of inflammatory markers was evaluated. COX2, MCP1 and RORC were found significantly decreased in T-treated as compared with placebo arm (all $p < 0.01$) and for COX2 and MCP1 even in comparison with eugonadal men (both $p < 0.05$).

Conclusion: Six-month treatment with T gel 2% in hypogonadal men with BPH and MetS is able to improve several clinical, ultrasound and molecular proxies of prostate inflammation. This results into a moderate improvement in symptoms, particularly prostatitis-like symptoms and bother for LUTS.

OC16

Effects of different follicle-stimulating hormone preparations on pre-pubertal porcine Sertoli cell cultures: preliminary results

G. LUCA[1,2], F. MANCUSO[1], I. ARATO[1], M. CALVITTI[1], M. C. AGLIETTI[3], G. CARPINELLI[2], C. BELLUCCI[1], C. LILLI[1], T. BARONI[1], M. BODO[1] AND R. CALAFIORE[2,3]
[1]Department of Experimental Medicine, University of Perugia, Perugia, Italy; [2]Division of Medical Andrology and Endocrinology of Reproduction, Saint Mary Hospital, Terni, Italy; [3]Department of Medicine, University of Perugia, Perugia, Italy

Background: At present, there are no "in vitro" studies on the effects of different preparations of follicle-stimulating hormone (FSH) on pre-pubertal Sertoli cells, which could provide important information in reproductive medicine. The different preparations of FSH available in the market, obtained both by recombinant technology (α and β follitropin) and post-menopausal urine (urofollitropin), are characterized by structural and functional heterogeneity lies on their different content in sialic acid C-terminus residue of the oligosaccharide chain. The unique testis receptor of FSH (FSH-r) is exclusively localized on Sertoli cells. The use of FSH in oligozoospermic males, a useful therapy in selected patients, is affected by conflictual findings.

Objective: The aim of our study was to assess the effects of different FSH preparations on ultrapure, viable and functional porcine pre-pubertal Sertoli cells (pSC) by evaluating modulation of their specific markers.

Methods: We have evaluated pSC, obtained from 15–20 days old neonatal porcine testes, in terms of purity by immunofluorescence and cytofluorimetric analysis. Subsequently, purified pSC culture were treated with:

- α-follitropin, β-follitropin and urofollitropin at the same molar concentration (100 nM) for 48 hours;

- Testosterone (T): 0.2 mg/ml;
- Combinations of different FSH preparations with T.

Both in basal and after 48 hours of FSH stimulation, we have performed:

a) Real Time PCR analysis of anti- Müllerian hormone (AMH), inhibin B and FSH-r;
b) Western blotting analysis (WB) of FSH-r, pospho-AKT, pospho-ERK1/2;
c) ELISA assay, both in cell extract and culture medium, for AMH and inhibin B.

Results: In our model, we observed, that:

- All three preparations of α-follitropin, β-follitropin and urofollitropin induced, as expected, a reduction of AMH in terms of mRNA, cell extract and secreted protein;
- All three preparations induced an increase of inhibin B in terms of mRNA and cell extract protein and, while interestingly, only α-follitropin induced an increase of inhibin B secreted in the culture medium;
- All three preparations induced, as expected, a reduction of FSH-r mRNA but only α-follitropin was associated with downregulation of FSH-r (WB).
- Only α-follitropin induced a downregulation of pospho-AKT (WB).
- All three preparations induced an increase of pospho-ERK1/2 (WB).

Conclusion: These results preliminarily showed, that the three FSH preparations were associated with different effects in terms of inhibin B secretion, arising the question if, in the treatment of the infertile male, should be preferred FSH preparations that increase inhibin B secretion or not. Our present study could help better understanding the effects of different FSH preparations, thus providing important information on both, the conflictual findings with regard to use of FSH in oligozoospermic males and, in general, reproductive medicine.

OC17

Molecular and functional characterization of a unique genotype in a man affected by congenital hypogonadotropic hypogonadism

F. CIOPPI[1], A. RIERA-ESCAMILLA[1,2], A. MANILLAL[3], E. GUARDUCCI[1], G. CORONA[4], C. A. FLANAGAN[3] AND C. KRAUSZ[1]

[1]Department of Biomedical, Experimental and Clinical Sciences "Mario Serio", University of Florence, Florence, Italy; [2]Andrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, Instituto de Investigaciones Biomédicas Sant Pau (IIB-Sant Pau), Barcelona, Catalonia, Spain; [3]School of Physiology, University of the Witwatersrand, Faculty of Health Sciences Johannesburg, South Africa; [4]Endocrinology Unit, Maggiore-Bellaria Hospital Bologna, Italy

Background: Congenital hypogonadotropic hypogonadism (cHH) is a rare endocrine disease (incidence of 1:8000 males), caused by the deficient production, secretion or action of gonadotropin-releasing hormone (GnRH). Its typical clinical manifestation is delayed puberty and azoospermia. Homozygous or compound heterozygous inactivating mutations in the GnRH receptor gene are among the most frequent causes of normosmic CHH (nCHH), accounting for about 10% of cases. The human GNRHR gene maps to chromosome 4 (4q13.2).

Methods: Molecular characterization of a novel homozygous mutation (p.Gly99Glu) in the exon 1 of the GnRHR through: i) gene dosage (qPCR); ii) SNP array; iii) functional studies consisting of competition binding assay and inositol phosphate (IP) signaling assay.

Results: A novel GnRHR mutation (p.Gly99Glu) was identified in a 20-year-old man with eunuchoid body shape, Tanner Stage 1 and bilateral cryptorchidism with low total testosterone, LH and FSH levels. The same mutation was found in heterozygosity in the mother, whereas the father was wild type. After confirming biological paternity, real-time PCR analyses (qPCR) showed two GnRHR copies. In order to investigate about the origin of homozygosity in the proband, SNP array (CytoScan® 750K Affymetrix) was performed. It revealed that the patient has inherited two copies of chromosome 4 from the mother (maternal heterodisomy; hUPD) with 2 regions showing loss of heterozygosity (maternal isodisomy; iUPD) on the long arm of chromosome 4 (4q12-q21.21 and 4q33-q35.2), one of which contains the mutated gene. Since our patient has no paternal contribution from the whole chromosome 4, we sought to explore the presence of maternally imprinted genes on chromosome 4. The NAP1L5 gene has monoallelic paternal expression. Our proband does not show any dysmorphic features or congenital malformations but he is of short stature, opening questions on the role of this gene in growth retardation. Functional studies revealed that cells transfected with the Gly99Glu mutant GnRH receptor showed no measurable radioligand binding. Appending a carboxy-terminal tail to the Gly99Glu mutant receptor did not recover radioligand binding, suggesting that any increased expression was not sufficient to allow binding of radioligand at the low concentration (~0.1 nM) used in competition binding assays. GnRH stimulated IP production in cells transfected with the Gly99Glu mutant GnRH receptor, suggesting that the mutant receptor protein was well-expressed. However, GnRH potency was three orders of magnitude lower (EC50, 899.3 nM). Appending the carboxy-terminal tail to the Gly99Glu mutant GnRH receptor had small effects on GnRH-stimulated Emax and GnRH potency. The above data suggested that the mutation severely decreases GnRH binding affinity. Thus, based on its functional consequences, this novel GnRHR variant can be classified as a severe partial loss of function (pLOF) mutation.

Conclusion: In conclusion, we have demonstrated both a novel causative missense mutation of GnRHR (p.Gly99Glu) described as severe pLOF and a maternal hUPD/iUPD of chromosome 4 causing ncHH. Similar chromosomal rearrangements of chromosome 4 have been described only in 4 cases, and it is the first case of a patient affected by nCHH due to this rare chromosomal rearrangement.

OC18

X-chromosome exome sequencing in highly selected idiopathic azoospermic patients: identification of novel and recurrent genetic factors for early spermatogenic failure

A. RIERA-ESCAMILLA[1,2], D. MORENO-MENDOZA[1], L. NAGIRNAJA[3], J. RUSCH[3], E. CASAMONTI[2], E. RUIZ-CASTAÑE[1], D. F. CONRAD[3] AND C. KRAUSZ[1,2]
[1]Andrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, IIB-Sant Pau, Barcelona, Catalonia Spain; [2]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", Centre of Excellence DeNothe, University of Florence, Florence, Italy; [3]Department of Genetics, Washington University School of Medicine, St. Louis, MO, USA

Background: The severest form of male factor infertility is non-obstructive azoospermia (NOA), which occurs in approximately 1% of all men in reproductive age and in the majority of patients the etiology remains unknown. Despite the well known enrichment of the X chromosome in genes specifically expressed in the testis, so far only two X-linked genes are diagnostic targets in NOA. The apparent paucity of X-linked NOA is likely to be the consequence of the lack of comprehensive, whole X chromosome targeting studies.
Methods: X-chromosome exome sequencing (a total of 836 protein-coding genes) in 50 idiopathic NOA patients with known testis histology. Variants were filtered and prioritized according to their minor allele frequency (MAF≤0.01), their predicted pathogenicity and their tissue expression profiling. Expression analyses was performed in human testis biopsies for the RBBP7 gene through RT-qPCR. RNA interference was used to determine the role of Caf1-55 (the human RBBP7 ortholog) in Drosophila spermatogenesis.
Results: We identified 74 rare and predicted as pathogenic variants in 38/50 NOA patients. 72 variants were private mutations whereas one was found in two unrelated patients. Nine genes (six of them with testis specific expression or overexpression in the testis) were recurrently mutated in 16 different patients. Two patients affected by spermatogonial arrest presented pathogenic mutations in the RBBP7 gene. Expression analysis in testis biopsies with different histology corroborates that RBBP7 is highly expressed in testis and shows an overexpression in spermatogonia Conditional Caf1-55 KO showed that male mutants had tiny testis, no spermatozoa and were sterile.
Conclusion: This is the first X chromosome exome analysis in highly selected NOA patients. Our approach was relatively successful in identifying candidate genes for the NOA phenotype. Up to now, we performed functional analysis only for the RBBP7 gene demonstrating that the protein is essential for Drosophila spermatogenesis hence we propose it as a novel genetic factor for early spermatogenic failure.
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OC19

Top-down proteomic approach to study the protamine post-translational modifications profile in the human spermatozoa

A. SOLER-VENTURA[1,2], M. JODAR[1,2], J. CASTILLO[1,2], M. GAY[3], M. VILANOVA[3], L. VILLARREAL[3], G. ARAUZ-GAROFALO[3], J. L. BALLESCÀ[4], M. VILASECA[3] AND R. OLIVA[1,2]
[1]Molecular Biology of Reproduction and Development Research Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Fundació Clínic per a la Recerca Biomèdica, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain; [2]Biochemistry and Molecular Genetics Service, Hospital Clínic, Barcelona, Spain; [3]Mass Spectrometry and Proteomics Core Facility, Institute for Research in Biomedicine (IRB Barcelona), BIST (Barcelona Institute of Science and Technology), Barcelona, Spain; [4]Clinic Institute of Gynecology, Obstetrics and Neonatology, Hospital Clínic, Barcelona, Spain

Background: The protamine 1 (P1) and protamine 2 family (P2) comprise the most abundant basic proteins in human spermatozoa. P1 is synthesized as a mature form, whereas the P2 family is generated from the proteolysis of a precursor, giving rise to the mature forms HP2, HP3, and HP4. Protamines pack approximately 85–95% of the paternal genome, while the other 5–15% remain attached to histones, which together with the histone post-translational modifications (PTMs) code contribute to the paternal epigenetic signature during the preimplantation embryogenesis. However, the epigenetic function of a potential protamine PTMs code has been poorly studied, although protamines are much more abundant in sperm chromatin. Protamines are utterly basic proteins with particular physical-chemical properties due to their amino acid sequences. Because of this, protamine identification by the well-established bottom-up mass spectrometry (MS) strategy on trypsinized proteins is not as straightforward as for other basic proteins. A top-down MS approach is therefore proposed to identify human protamines and their PTMs. The intact protamine-enriched fraction from one normozoospermic individual was isolated from purified spermatozoa after histone removal and disulfide bonds reduction. Protamine enrichment was verified through acid-urea gel electrophoresis. The protamine-enriched fraction was analyzed by nano-liquid chromatography coupled to tandem MS (nanoLC-MS/MS) using a chip-based Advion nanoelectrospray source and an Orbitrap Fusion Lumos (Thermo Scientific) mass spectrometer. The latter was operated in data-dependent acquisition (DDA) mode, and the most abundant ions were selected for fragmentation by Electron Transfer Dissociation (ETD). Data analysis was performed using Proteome Discoverer 2.1 with Prosight PD 4.0 and Sequest HT nodes and TopPIC software. In addition, RNA was isolated and purified from one individual to confirm the presence of a new potential P2 isoform at RNA level by direct sequencing. The top-down proteomic MS approach mainly allowed the identification of the intact naïve P1, while HP2 and HP3 were detected with minor peak intensities. In

contrast, HP4 was not detected, probably due to its physiological low abundance. A phosphorylation pattern in P1 and combinations of other PTMs among the different protamines were detected. Notably, hyperoxidation on cysteine residues irreversibly modified to sulfinic acid were found in almost 100% of HP2 and HP3 and in nearly 70% of HP1, most likely because of the presence of reactive oxygen species (ROS). However, the mass of the hyperoxidized HP2 totally matches with an alternative spliced variant of P2 identified at RNA level by our group and others. The incorporation of a middle-down MS strategy using a digestion with GluC would allow differentiating between the potential alternative spliced variant of P2 and the hyperoxidized HP2. The establishment of the normal protamine PTMs profile in fertile individuals and the identification of pattern alterations in different types of infertile patients, including those with abnormal elevated ROS, would provide insights into the role of protamine PTMs code to male fertility and its potential function as epigenetic mark during early stages of preimplantation embryogenesis.

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OC20

Functional characterization of Binder of SPerm homolog 1 in sperm-egg interaction and fertilization

H. HEIDARI-VALA[1,2], S. SABOUHI-ZARAFSHAN[1,3], B. PRUD'HOMME[1], A. ALNOMAN[1,2] AND P. MANJUNATH[1,2,3,4]

[1]Maisonneuve-Rosemont Hospital Research Centre, Montreal, Quebec, Canada H1T 2M4; [2]Department of Pharmacology and Physiology, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada H3C 3J7; [3]Department of Biochemistry and Molecular Medicine, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada H3C 3J7; [4]Department of Medicine, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada H3C 3J7

Background: In mice and humans, the Binder of SPerm homolog 1 (BSPH1) protein is exclusively expressed in the epididymis. BSPH1 proteins have been shown to be involved in the sperm membrane changes underlying capacitation. Findings from experiments with the recombinant mouse BSP homolog (rec-BSPH1) suggest that the protein initially resides on the surface of the sperm and then relocalizes over the head and mid-piece during capacitation, suggesting a potential role for BSPH1 in sperm-egg interaction.

Objective: In the current study, we investigated the role of mouse recombinant BSP homolog 1 (rec-BSPH1) in sperm-egg interaction using an in vitro fertilization (IVF) assay.

Methods: Mouse rec-BSPH1 was produced by transforming E. coli with a pET32a vector carrying BSPH1 cDNA and purified using immobilized metal (Ni2 +) affinity chromatography. Oocytes were pre-treated with rec-BSPH1, control proteins or media alone, and inseminated with capacitated sperm. In addition to IVF assay, the potential binding of rec-BSPH1 to the oocyte surface was investigated using immunofluorescence. Finally, sperm-bound native BSPH1 was immuno-neutralized by anti-rec-BSPH1 antibodies to indirectly verify implication of BSPH1 in sperm-egg interaction and fertilization.

Results: Our results showed that eggs pre-incubated with rec-BSPH1 protein exhibited a dose-dependent decrease in fertilization rate compared to those exposed to control proteins or media alone. Since BSPH1 binding sites were not identified on the egg, the observed inhibition in fertilization rate when eggs were pre-incubated with rec-BSPH1 suggested that an alternate mechanism was at play. Moreover, sperm immuno-neutralization with anti-rec-BSPH1 led to dramatic motility changes, followed by compromised fertilization.

Conclusion: Taken together, our results suggest that BSPH1 would be involved in the late sperm capacitation events. However, the mechanism through which egg pre-incubation with BSPH1 affects fertilization warrants further investigation. In view of these results, we conclude that BSPH1 could be a marker of sperm fertility and thus an eventual target for male contraceptive development. (Supported by the Canadian Institutes of Health Research)

POSTERS
SELECTED POSTERS (PRESENTED AS SHORT ORAL COMMUNICATIONS)

P001

Whole exome sequencing in non-obstructive azoospermia allows the identification of a high-risk subgroup of infertile men for undiagnosed Fanconi Anemia, a cancer-prone disease

A. RIERA-ESCAMILLA[1,2], C. CHIANESE[1,2], D. MORENO-MENDOZA[1], O. RAJMIL[1], E. CASAMONTI[2], E. RUIZ-CASTAÑE[1], J. SURRALLÉS[3] AND C. KRAUSZ[1,2]
[1]Andrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, IIB-Sant Pau, Barcelona, Catalonia, Spain; [2]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", Centre of Excellence DeNothe, University of Florence, Florence, Italy; [3]Genetics Department and Biomedical Research Institute, Hospital de Sant Pau, Center for Biomedical Research on Rare Diseases (CIBERER), and Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Barcelona, Catalonia, Spain

Background: The etiology of non-obstructive azoospermia (NOA) remains unknown in about 40% of cases and genetic factors are likely to be involved in a large proportion of them. Gene mutations involved in stem cell proliferation and DNA repair may cause isolated NOA or be responsible for syndromic diseases, such as Fanconi Anemia (FA). Although the most frequent presenting symptom in FA is bone marrow failure in childhood, in about 10% of cases the diagnosis is delayed until adulthood and in these late-onset cases the presenting syndrome is frequently a malignant tumor.
Methods: An idiopathic NOA patient (index case) with consanguineous parents was subjected to Whole-Exome Sequencing (WES) with the purpose to identify the etiology of NOA. In the second part of the study, two-steps Sanger sequencing of the Fanconi Anemia Complementation Group A gene (FANCA) in the brother of the index case and in 27 selected NOA patients was performed. DEB-induced chromosome breakage test was carried out to confirm the FA diagnosis.
Results: Through WES we identified a rare pathogenic homozygous FANCA variant (c.2639G>A) in the index case, affected by NOA due to Sertoli Cell only syndrome (SCOS). The patient's brother (also affected by NOA) has been found to be a homozygous carrier of the same mutation. The two brothers did not manifest overt anemia, though chromosomal breakage test revealed a reverse somatic mosaicism in the index case and a typical FA picture in the brother. Following this incidental finding of FA, we selected 27 NOA patients with similar testicular phenotype and borderline/mild hematological alterations. Sanger sequencing of the FANCA gene in this selected group of patients allowed the identification of one additional NOA patient with SCOS showing compound heterozygous variants (c.3788_3790delTCT and c.3913C>T). Following our investigation, the three subjects with FANCA mutations are now receiving specific medical attention including strict follow-up by oncohematologists.
Conclusion: Our study reports an unexpectedly high frequency of occult FA in a specific subgroup of NOA patients with mild or borderline hematological alterations (2/28; 7.1%). The screening for FANCA mutations in such patients may allow the identification of undiagnosed FA before the appearance of other severe clinical manifestations (cancer, bone marrow failure etc.) of the disease. Our finding highlights the importance to introduce the systematic evaluation of hematological parameters into the routine andrological workup in NOA patients. Moreover, corroborates previous epidemiological observations reporting a higher risk of morbidity (including cancer) and a lower life expectancy in infertile men in respect to fertile, normozoospermic men. Based on our data, we propose a novel genetic link between idiopathic NOA and a chronic, cancer-prone disease.
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P002

Protective effects of DA-9401 on Adriamycin-induced testicular oxidative stress, endoplasmic reticulum stress, and apoptosis

K. K. KARNA[1], B. R. CHOI[1], S. W. LEE[2], C. Y. KIM[3], Y. S. SHIN[1], H. K. KIM[4] AND J. K. PARK[1]
[1]Department of Urology, Chonbuk National University Medical School and Institute for Medical Sciences, Chonbuk National University and Biomedical Research Institute and Clinical Trial Center for Medical Devices of Chonbuk National University Hospital, Jeonju, Korea; [2]Department of Urology, Sungkyunkwan University Medical School, Seoul, Korea; [3]College of Pharmacy, Hanyang University, Ansan, Korea; [4]College of Pharmacy, Kyungsung University, Busan, Korea

Objective: To investigate a possible protective role of DA-9401 on Adriamycin (ADR)-induced testicular and spermatozoal toxicity associated with the oxidative stress, endoplasmic reticulum (ER) stress and apoptosis.
Methods: Fifty healthy 8-week-old male Sprague–Dawley rats were equally divided into five groups. The control group (CTR) received normal saline. Animal in DA-100 group received only (DA-9401 100 mg/kg/day per oral (p.o.). Animal in adriamycin group (ADR) received only adriamycin (2 mg/kg/once a week) intraperitoneally (i.p.), while the combination of ADR and DA-9401 was given to ADR + DA-100 group (2 mg/kg/once a week i.p. + DA-9401 100 mg/kg/day p.o.) and ADR + DA-200 group (2 mg/kg/once a week i.p. + 200 mg/kg/day p.o). At the end of the 8-week treatment period, reproductive organ weights, epididymal sperm parameters, serum testosterone, serum LH and FSH level, testicular tissue interleukin-6, TNF-α, and oxidative testicular toxicity were investigated. Histopathological changes in testis were observed by hematoxylin and eosin stain, and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining for spermatogenic cell density, Johnsen's score, and apoptosis. Testicular tissue was also used to evaluate endoplasmic reticulum (ER) stress response protein, star, CatSper and gsk expression levels by western blot.

Results: ADR administration was determined to cause significant decreases in reproductive organ weights, epididymal sperm count and motility, serum testosterone concentration, serum LH and FSH level, spermatogenic cell density and Johnsen's score, testicular SOD, catalase, GPx level, star, CatSper and gsk protein expression and significant increase in testicular interleukin-6, TNF-α, MDA level, ROS/RNS level, apoptosis index and ER-response protein expression levels when compared with the control group. DA-9401 administration to ADR-treated rats provided a significant decrease in interleukin-6, TNF-α, MDA level, ROS/RNS level, ER stress response protein levels and apoptosis index. Moreover, ADR-treated rats showed a significant increase in reproductive organ weights, Johnsen's score, spermatogenic cell density, sperm count and sperm motility, serum testosterone concentration, serum LH and FSH level, testicular SOD, catalase, GPx, star, CatSper and gsk protein level.
Conclusion: This study suggests that adriamycin treatment markedly impaired testicular function and DA-9401 exerts beneficial effects against oxidative stress, ER stress-induced cellular damage in testis tissue.

P003

DNA-demethylating drug acts as "time-bomb" in damaging mammalian embryonic germ cells

A. KATUSIC BOJANAC[1,2], T. MARIC[1,2], M. HIMELREICH[1,2], N. SINCIC[1,2], Z. HERCEG[3], M. VLAHOVIC[1,2], D. JEZEK[2,4] AND F. BULIC-JAKUS[1,2]
[1]Department of Medical Biology, School of Medicine, University of Zagreb, Zagreb, Croatia; [2]Research Unit for Biomedical Investigation of Reproduction and Development, Centre of Excellence on Reproduction and Regenerative Medicine, Zagreb, Croatia; [3]Epigenetics Group, International Agency for Research on Cancer, Lyon, France; [4]Department of Embryology and Histology, School of Medicine, University of Zagreb, Zagreb, Croatia

Background: DNA methylation is an important epigenetic mechanism involved in normal and disturbed spermatogenesis or carcinogenesis in adults (Jaiswal et al., 2015; Buljubasic et al., 2018). A DNA demethylating epigenetic drug 5-azacytidine (5-azaC), used for treatment of malignancies such as the myelodysplastic syndrome also negatively affected development of various tissues (Bulic-Jakus et al., 2016; Serman et al. 2009).
Objective: The aim of our present research was to investigate the stage-specific effect of DNA-demethylation on spermatogenesis in mammalian embryonic testis.
Methods: 5-azaC was intraperitoneally injected to Fisher dame rats on different days of gestation (day 13-day 16) and controls were sham treated by PBS. Embryonic testes were isolated prior to birth and analyzed by the light histology and TEM. Cleaved-caspase 3, and γH2AX were used to for detection and staging of apoptosis. Global DNA methylation levels at cytosines in CpG dinucleotides of repetitive elements (LINE-1 and Satellite regions) were assessed by pyrosequencing.
Results: More cells with middle to late apoptotic morphology were found in prospermatogonia of day 20 testes treated with 5-azaC in comparison to the controls. The apoptotic index in embryonic testes showed the correlation to the time of treatment, with the peak when treated with 5-azaC on day 15. To exclude a possible cytotoxic effect the apoptotic index was determined in several days after treatment and showed the rise of apoptotic cells at day 19. Epigenetic analysis has showed also a rise in global methylation at day 19 in normal testis. On the other hand in treated group, a global and satellite DNA hypomethylation was discovered, starting already from the day 18. We may conclude that day 15 of rat gestation seems to be the most sensitive stage to the DNA demethylation activity of 5-azaC. Because 5-aza-C replaces cytidine during DNA synthesis in cycling cells it is possible that its incorporation prevented de novo DNA methylation process that is necessary for epigenetic stability of prospermatogonia prior to birth. Therefore, this activity of 5-azacytidine may be compared to the "time-bomb".
Conclusion: This basic investigation done with a single dose of a DNA demethylating drug during the gestation might be of importance for further understanding of epigenetic disturbances important for the pathology of the testis.
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References: (1) Jaiswal et al. Association of the patterns of global DNA methylation and expression analysis of DNA methyltransferases in impaired spermatogenic patients. Asian Pacific Journal of Reproduction 4, 262–265.
(2) Buljubasic et al. (2018) Epigenetics and testicular germ cell tumors. Gene 661, 22–33.
(3) Bulic-Jakus et al. (2016) Wire's Dev Biol Teratoma: from spontaneous tumors to the pluripotency/malignancy assay 5, 186–209.
(4) Serman L et al. (2007) The Impact of 5-Azacytidine on Placental Weight, Glycoprotein Pattern and Proliferating Cell Nuclear Antigen Expression in Rat Placenta. Placenta. 28, 803–811.

P004

Non-obstructive azoospermia due to compound heterozygous TEX14 mutations

N. KÖCKERLING[1], C. KRALLMANN[2], L. HANKAMP[1], S. KLIESCH[2], F. TÜTTELMANN[1] AND A. RÖPKE[1]
[1]Institute of Human Genetics, University of Münster, Münster, Germany; [2]Centre of Reproductive Medicine and Andrology, Department of Clinical and Surgical Andrology, University Hospital Münster, Münster, Germany

Background: Male infertility is a clinically and genetically highly heterogeneous disease, mostly caused by spermatogenic failure, clinically noted as oligo- or azoospermia. Azoospermia can be subdivided into obstructive and non-obstructive azoospermia (NOA). NOA is often caused by genetic defects, such as chromosomal aberrations, Y-chromosomal microdeletions, or mutations in specific genes. One of these genes is TEX14 (Testis-Expressed 14; OMIM 605792). It has previously been shown that homozygous

mutations in this gene (one frameshift and one missense mutation) may cause NOA. In male mice, TEX14 plays a key role in the formation of intercellular bridges during meiosis.

Methods: The study cohort includes 264 NOA patients from the Center of Reproductive Medicine and Andrology, Münster, Germany (CeRA). Patients with chromosomal abnormalities or AZF deletions were excluded from this study. All 264 patients were analyzed by whole exome sequencing.

Results: We identified three men with compound heterozygous mutations in TEX14. Patient 1 had a testis biopsy demonstrating complete bilateral meiotic arrest. He carries a rare heterozygous missense mutation c.1508T>C (p.Leu503Pro) that was inherited from his mother and a frameshift mutation c.3763_3766del (p.Ser1255Metfs*4) inherited from his father. Patient 2 had a Sertoli-cell-only syndrome (SCOS). He carries a maternally inherited nonsense mutation c.1021C>T (p.Arg341*) and a paternal nonsense mutation c.3186C>A (p.Tyr1062*). Patient 3 also had SCOS and carries two heterozygous TEX14 missense mutations c.644C>A (p.Pro215His) and c.721G>A (p.Val241Met). The mutations were exclusively found on different reads of exon 7 supporting compound heterozygosity.

Conclusion: Based on our results, we propose that compound heterozygous mutations in TEX14 cause autosomal recessive male infertility due to NOA. Because these mutations were found in patients with meiotic arrest as well as in patients with SCOS the phenotypic spectrum needs further investigations.

This work was carried out within the frame of the DFG Clinical Research Unit ‚Male Germ Cells: from Genes to Function'(CRU 326).

References: (1) Fakhro et al. Genet Med. 2018; advance online publication (https://doi.org/10.1038/gim.2018.10).
(2) Gershoni et al. Genet Med. 2017;19:998–1006.
(3) Greenbaum et al. Proc Natl Acad Sci U S A. 2006;103: 4982–4987.

P005

The permeability of blood testis barrier to macromolecular substances under scrotal heat stress and pulsed unfocused ultrasound

Y. LI[1], X. WANG[1], X. DING[2] AND H. LI[1,3]
[1]Family Planning Research Institute/Center of Reproductive Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; [2]Center of Reproductive Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; [3]Wuhan Tongji Reproductive Medicine Hospital, Wuhan, China

Background: Blood-testis barrier (BTB) is largely formed by tight junctions between Sertoli cells, providing a stable microenvironment and an unique immune barrier for spermatogenesis. Meanwhile, the blood testis barrier blocks the access of drugs such as antiviral drugs, chemotherapeutical agents, antibiotics and antibodies which can clear pathogen and kill tumor cells, and prevents anti-sperm antibody from binding sperm specific antigen, thereby interfering with the immunocontraceptive effect.

Objective: This study aimed to investigate the effects of two physical approaches, that is, scrotal heat stress and pulsed unfocused ultrasound (PuFUS) on the permeability of the BTB in adult male Balb/c mice.

Methods: Mice received a single scrotal heat stress at 39, 41, or 43°C, for 30 min, or an PuFUS at 1.75w, 1.25w, 2.5w per square centimetre for 2, 5 and 10 min, respectively. The testicular interstitium of the mice were subjected to an injection of biotin, and macromolecular substances including IgG, IgM, exosome respectively after the scrotal heat stress or PuFUS.

Results: As detected by biotin tracer, both scrotal heat stress and PuFUS opened the BTB. After scrotal heat stress, BTB opening started from 2 days or later and lasting for 5 days or more; whereas after PuFUS, BTB opening started from 1.5 h and only lasting 24 h. Penetration of IgG, IgM, and exosome was observed 5 days after scrotal heat stress at 43°C, but was not observed at 39°C or PuFUS. The scrotal heat stress at 43°C showed a potential method for delivering macromolecular substances into seminiferous tubule; and PuFUS could be a novel quick and mild approach to open BTB.

Conclusion: This study providing a new strategy for drug-targeted delivery into testicular seminiferous tubules.

P006

Combined proteomic and miRNome analyses of mouse testis exposed to a mixture of endocrine disruptors chemicals reveal altered toxicological pathways involved in male

R. D. MORENO[1], J. BUÑAY[1], E. LARRIBA[2] AND J. D. MAZO[2]
[1]Department of Physiology, Pontificia Universidad Católica de Chile, Santiago, Chile; [2]Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas (CSIC), Madrid, Spain

Background: The increase of male idiopathic infertility has been associated with daily exposure of endocrine disruptors chemicals (EDCs) such as phthalates and alkylphenols.

Objective: We were interested in describing the consequence in the protein pattern along with the posttranscriptional control of transcripts encoding proteins by miRNAs in testis of male mice exposed to mixture of EDCs.

Methods: We combined proteomic and miRNome analyses of mouse testis chronically exposed to a low-doses of a define mixture of 0.3 mg/kg-bw/day of bis (2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP) and 0.05 mg/kg-bw/day of 4-nonylphenol (NP) and 4-tert-octylphenol (OP), administrated in the drinking water from conception until adulthood. In these mice we analyzed fertility parameters and global changes in the patterns of testis proteome by 2D-electrophoresis and mass spectrometry (MALDI-TOF), along with bioinformatic analyses of miRNA implicated in the control of deregulated proteins and their association with published data in human infertile patients.

Results: We detected exposed mice with a reduction in their potential fertility that were associated with changes in the expression of 18 proteins (10 up-regulated, 8 down-regulated), their functional analysis showed that most of them (89%) were involved in cell death. Furthermore, we found a group of 23 miRNAs/isomiRs (down-regulated) correlated with 6 up-regulated targets proteins (DIABLO, PGAM1, RTRAF, EIF4E, IVD and CNDP2) and we found that some of these miRNAs/proteins deregulations were reported in human testis with spermatogenic failures and subfertility or infertility. Overall, we suggest that the exposure to mixtures of EDCs could potentially lead to reproductive problems in men by some mechanisms that implicate changes in the interactions miRNAs/proteins involved in cell death.

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P007

Epigenetic regulation of the PIWI-LIKE 2 promoter in spermatogenesis

M. GIEBLER, T. GREITHER AND H. M. BEHRE
Center for Reproductive Medicine and Andrology, University Hospital, Martin Luther University Halle-Wittenberg, Halle, Germany

Background: Differentiation of human germ cells involves maintenance of pluripotency, but also controlled cell division, meiosis and terminal maturation. This tightly regulated cellular program is accompanied by repression of somatic genes as well as a unique epigenetic reprogramming. PIWI-LIKE 2, a member of the Argonaute protein family, is exclusively expressed in pre-pachytene and pachytene stages of spermatogenesis and acts in maintaining the stem cell character and furthermore guaranteeing the genomic integrity by repression of transposon elements. In the present study we investigated DNA methylation as potential mechanism for the regulation of human PIWI-LIKE 2 expression in cell lines related to spermatozoa precursor cells.

Methods: For epigenetic regulation studies TCam-2 (a seminoma cell line) and NT2D1 (a testicular embryonal carcinoma cell line) cells were used. We studied the expression of PIWI-LIKE 2 by quantitative real-time PCR and Western Blot after treatment with the DNA methylation inhibitor 5-Aza-2′-deoxycytidine (5AzadC). Analysis of the CpG methylation status of the PIWI-LIKE 2 promoter was assessed by bisulfite sequencing. PIWI-LIKE 2 promoter activity was analyzed by luciferase reporter gene assay.

Results: PIWI-LIKE 2 mRNA and protein was upregulated in TCam-2 cells after 5AzadC treatment, whereas NT2D1 showed no change in PIWI-LIKE 2 expression. Bioinformatics analysis identified 57 CpG dinucleotides in the promoter sequence from −300 to +600 bp around the transcription start site (TSS). Bisulfite sequencing of the CpG site demonstrated a different basal methylation level of PIWI-LIKE 2 in the cell lines. Treatment of the cells with 5-AzadC allows a partial demethylation of Piwil2 promoter in TCam-2 and NT2D1. Transfection of cells with different PIWI-LIKE 2 promoter constructs identified several regulatory regions, located in the region from −300 bp to the TSS, by an increase of the luciferase activity. Vice versa, in vitro methylation of selected fragments suppressed PIWI-LIKE 2 promoter activity.

Conclusion: Our data indicate that in humans DNA methylation is able to induce epigenetically silencing of PIWI-LIKE 2 expression and provide first hints for epigenetic alterations during spermatogenesis.

P008

A rare cause of male infertility: 46,XX, SRY negative males – summary of six cases

J. VARGHA AND Z. KOPA
Department of Urology, Andrology Centre, Semmelweis University, Budapest, Hungary

Background: Genetic failures are infrequent in the normal population (less than 1%) but more frequently can be found in infertile couples (~15%). De la Chapelle syndrome is a very rare genetic disease (1:20 000 males) characterized by 46, XX chromosomes in phenotypically masculine men. Components of testicular dysgenesis syndrome are commonly reported. In a recent PubMed and MEDLINE database search a total of 55 patients were diagnosed with a 46 XX karyotype during their fertility examinations: where the SRY gene was detected in 84% and was absent in 16% of the patients.

Methods: In our case series report azoospermic males with 46, XX karyotype are presented from couples seeking medical treatment for infertility from our Andrology Centre between 2009 and 2018. Genetic studies used peripheral blood leucocyte culture, karyotype analysis was performed with G-banding, the SRY gene locus and the AZF gene region detection were made by fluorescence in situ hybridization technique (FISH).

Results: Six 46,XX male patients were found, the mean age was 35.8 years (24–49). The patient's phenotype and the psychosexual behaviour were masculine. No major endocrinological symptoms could be observed, but lower testis volume could be fined by all of the 6 males, two of them had testicular maldescent in the medical history. Although endocrinological symptoms were not present, laboratory tests showed hypergonadotropic hypogonadism in five cases. Mean FSH was 25.46 IU/L, LH was 10.7 IU/L, total Testosterone was 9.97 nmol/L. Semen analysis revealed non obstructive azoospermia in all patients.

Karyotype analysis showed apparently 46,XX chromosomes. FISH analysis were performed in all cases: SRY translocation on the short arm of one of the X chromosomes was found in three cases, one patient had an SRY translocation to the autosome 9. Two patients had no detectable SRY region (46, XX, SRY negative males), in these cases SOX3 and 9 could explain the male differentiation.

Conclusion: 46, XX testicular disorders of sex development is a rare cause of male infertility. Most of the patients have normal phenotype, and just subclinical hormonal division. The first signs might be the testicular dysgenesis syndrome, when examining infertility. In SRY positive males

normal masculine development is explicable, but in SRY negative cases gene amplifications can be supposed to initiate male differentiation. Several gene region duplications or mutations are listed in the published reviews and case reports. In the interesting cases of transplantation chimerism with a female donor karyotyping means a diagnostic difficulty, lymphocytes and all blood cells represent donor's genetic status.

P009

Leukocytospermia and sperm parameters in men attending fertility clinics: a systematic review

C. CASTELLINI, S. D'ANDREA, A. BARBONETTI, A. MARTORELLA, E. MINALDI, M. TOTARO, S. NECOZIONE, F. FRANCAVILLA AND S. FRANCAVILLA
Andrology Unit, Department of Life, Health and Environmental Sciences

Objective: The association of leukocytospermia and male fertility in men asymptomatic for genital tract infection is still debated. Here we challenged the contention that the presence of leukocytospermia (≥1× 106 white blood cells/mL of semen according to the World Health Organization) in semen of men attending fertility clinics for couple subfertility is associated with a poor semen quality and a reduced pregnancy rate after ICSI.

Methods: Meta-analysis was conducted according to the Cochrane Collaboration and PRISMA statement. Data were combined using fixed effect models or random effect models, when appropriate. The Cochrane Chi-square X2 (Cochrane Q) statistic and the I^2 test were used to analyze heterogeneity. The publication bias was graphically explored through funnel plot. The risk of bias and quality of selected studies were assessed using the Cochrane algorithm and New-Castle-Ottawa scale. A complete literature search was carried out for English-language studies in MEDLINE, EMBASE, and the Cochrane Library.

Eligibility criteria for selecting studies. Case-control studies reporting mean ± standard deviation for seminal parameters, or Odds Ratio for pregnancy rate (PR) after ICSI in leukocytospermic (leuko) and non-leuko patients were analyzed.

Results: Six thousand five hundred and forty-three studies were retrieved from electronic. Five thousand four hundred forty-five studies were selected after duplicate removal, of which 5.212 were excluded as irrelevant based on titles and abstracts. A total of 73 trials were identified, but only 40 studies met the inclusion criteria for meta-analytic process. Leukocytospermia was associated to a reduced sperm concentration (Std mean difference IV, random: −0.32; 95% CI: −0.53, −0.11; $p = 0.01$, $I^2 = 88\%$, p for heterogeneity = <0.00001) and reduced forward sperm motility (Std mean difference IV, random: −0.74; 95% CI: −1.21, −0.27; $p = 0.0021$, $I^2 = 96\%$, p for heterogeneity = <0.00001). Sperm morphology and sperm vitality did not exhibit a significant difference between the two groups. A significant lower percentage of spermatozoa with DNA fragmentation was observed in non-leuko samples (Std mean difference IV, random: 0.87; 95% CI: 0.24, 1.50; $p = 0.007$, $I^2 = 88\%$, p for heterogeneity = <0.00001). The difference in sperm count and sperm motility between leuko and non-leuko samples disappeared when leukocytes were assessed by immunocytochemistry. No significant differences were observed for the pregnancy rate after ICSI in five studies (Risk Difference M-H, Fixed: 0.01; 95% CI: −0.08, 0.09; $p = 0.47$, $I^2 = 0\%$, p for heterogeneity = 0.84). A clear asymmetry in funnel plots indicated substantial publication bias in the overall analysis of all selected outcomes.

Conclusion: Leukocytospermia in men attending fertility clinics for couple subfertility was associated to impaired sperm quality albeit a high heterogeneity among the studies and publication bias suggest caution on conclusion. The use of immunocytochemistry, the most reliable method to assess semen leukocytes, failed to show differences in sperm count and motility between leukocytospermic and non-leukocytospermic samples. Leukocytospermia was not associated to a reduced pregnancy rate after ICSI.

P010

Comparison of testosterone measurement methods in predicting symptoms of male hypogonadism: the new 'gold standard' mass spectrometry is not superior to a traditional immunoassay

K. CZELOTH[1], J. BOBJER[2,3,4], K. BRONTON[2], O. CHRISTOFFERSON[2], S. A. HOLMBOE[1], A.-M. ANDERSSON[1], A. JUUL[1] AND A. GIWERCMAN[2,3]
[1]International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), University of Copenhagen, Copenhagen, Denmark; [2]Reproductive Medicine Centre, Skåne University Hospital, Sweden; [3]Molecular Reproductive Medicine, Department of Translational Medicine, Lund University, Sweden; [4]Department of Urology, Skåne University Hospital, Sweden

Background: Liquid tandem mass spectrometry (LC-MS/MS) was in 2007 defined as the new 'gold standard' for testosterone (T) measurement because of higher accuracy than traditional immunoassays (IA). The usefulness of T levels derived by the still widely used IA methodology has hence been questioned.

Objective: Comparison of T values from a traditional IA vs. LC-MS/MS regarding the diagnosis of male hypogonadism (MH) and the prediction of related symptoms.

Methods: Total Testosterone (TT) was measured by a two-step competitive IA with a luminometric technique and a Turbo Flow-LC-MS/MS method, respectively, in fasting blood samples from 287 men (161 subfertile men and 126 controls) participating in a study on hypogonadism in subfertile men. The two methods were compared regarding correlation, agreement, diagnosis of MH (T test-specific <9.5 and 10 nmol/L for IA and LC-MS/MS, respectively) and prediction of related symptoms (loss of libido, erectile dysfunction, metabolic syndrome and insulin resistance).

Results: A strong correlation ($r = 0.97$; $p < 0.001$) between the two methods were found albeit a bias was observed of approximately 20% lower T levels measured by the IA compared to the LC-MS/MS method. Hence 20 additional

men (7% vs. 14%) were characterized as hypogonadal using IA. The predictive value of measured T levels for symptoms of MH was overall low (AUC range: 0.61–0.78) irrespective of which method was used to measure T. Of the MH symptoms studied, the strongest prediction was seen in TT levels in relation to HOMA-IR with an AUC in ROC-curve analyses of 0.78 and 0.77 for TT measured by LC-MS/MS and IA, respectively. Furthermore, the similar AUC values indicate that none of the two methods was superior to the other in this clinical setting. TT was a slightly better predictor of MH symptoms than free testosterone (fT) in all tests.

Conclusion: Our results are in line with previously published data showing a strong correlation between IA and LC-MS/MS methods for measuring T in the range of adult male serum T levels. This study is the first to compare the predictive values of T levels acquired by IA and LC-MS/MS regarding MH related symptoms and shows no superiority of either analytical method over the other. The predictive value of T alone regarding MH symptoms is low. The diagnosis of MH is based on a combination of clinical symptoms in addition to low T levels. In this the clinical use in diagnosing MH and predicting associated symptoms T levels measured by traditional IAs are of similar value as T levels measured by LC-MS/MS.

P012

Study on the effect of non-aromatizable androgen Dihydrotestosterone (DHT) on the sexual behavior of ovariectomized female rats primed with estradiol

E. MASEROLI[1], M. MAGGI[1], J. G. PFAUS[2] AND L. VIGNOZZI[1]

[1]Sexual Medicine and Andrology Unit, Department of Experimental, Clinical and Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy; [2]Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montréal, Canada

Background: Hypoactive sexual desire disorder (HSDD) is the most common sexual dysfunction in women. Both in clinical studies and animal models, therapy with Testosterone (T) shows favorable effects on HSDD; however, it has not been elucidated whether these effects are exerted directly binding to androgen receptors (AR) in the brain or thanks to an increase in bioavailable estrogens. Dihydrotestosterone (DHT) is a non-aromatizable metabolite of T produced by its 5α-reduction and is 3-fold more active than T.

Objective: The aim of this study was to clarify the mechanisms of T facilitation of female sexual desire by assessing the effect of the AR stimulation through DHT using a validated animal model. Our main hypothesis was that DHT administration to ovariectomized (OVX) rats treated with estradiol benzoate (EB) would facilitate sexual behavior, in particular appetitive behaviors.

Methods: Forty-eight (n = 48) Long-Evans female rats were bilaterally ovariectomized and, after recovery, were hormonally primed with EB (10 μg) and progesterone (P) (500 μg), administered prior to each of 5 copulatory training sessions with experienced males. After a 2-week washout period, the OVX rats were randomly assigned to one of 4 conditions: oil (O) + O (n = 12), 10 μg EB + 500 μg P (n = 12), 10 μg EB + 500 μg DHT (n = 12), O + 500 DHT (n = 12) and tested at 8-day intervals. EB was administered 48 h, and P or DHT 4 h prior to sexual behavior sessions. Data on O + 10 μg E group were acquired from previous experiments performed in the same conditions. Frequencies of appetitive behaviors were considered as the main outcome measure and they were recorded by adding solicitations and hops and darts. As a secondary outcome measures, indexes of sexual receptivity were examined, specifically lordosis magnitudes. The frequency of lordosis magnitudes was scored on a 3-point scale according to Hardy and Debold and presented as a mean lordosis rating [LR = sum of points/(mounts + intromissions + ejaculations)]. Rejection responses were also coded.

Results: There was a statistically significant difference in appetitive behaviors (hops/darts and solicitations) and lordosis rating between the different regimens. Specifically, Long-Evans OVX rats treated with EB followed by DHT displayed significantly more appetitive behaviors and higher lordosis rating compared to all oil control groups, whereas no difference was observed between females treated with EB + DHT and EB + P. No statistically significant difference in rejection responses between the different regimens was found.

Conclusion: DHT acts synergistically with EB to facilitate appetitive behaviors and measures of receptivity in OVX female rats. Androgens seem to act directly and not through increase of E. Further studies are needed to confirm these data and investigate the molecular bases of facilitation of sexual desire by androgens.

P013

Study of the anti-inflammatory effects of dihydrotestosterone (DHT) in vaginal smooth muscle cells of rats

E. MASEROLI[1], C. CORNO[1], I. CELLAI[1], S. FILIPPI[2], M. ZIZZA[1], E. SARCHIELLI[3], P. COMEGLIO[1], A. MORELLI[3], G. B. VANNELLI[3], M. MAGGI[1] AND L. VIGNOZZI[1]

[1]Sexual Medicine and Andrology Unit, Department of Experimental and Clinical Biomedical Sciences Mario Serio, University of Florence, Florence, Italy; [2]Interdepartmental Laboratory of Functional and Cellular Pharmacology of Reproduction, Department of Neurosciences, Psychology, Drug Research and Child Health and Department of Experimental and Clinical Biomedical Sciences Mario Serio, University of Florence, Florence, Italy; [3]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

Background: Pathologies of the female reproductive system, characterized by chronic pain (including genital-pelvic and penetration pain, and the genito-urinary syndrome of menopause) are frequent, and it has been hypothesized that inflammation may play an essential role in their pathogenesis. Androgens, including dihydrotestosterone (DHT), have shown immunomodulatory and protective effects in models of chronic inflammation.

Objective: The aim of this study was the evaluation of the immunomodulatory and anti-inflammatory effects of DHT in a experimental model of rat vaginal smooth muscle cells (rSMCs).
Methods: The cells were isolated from distal vagina tissues of control female rats and characterized by the main markers of smooth muscle cells [α-smooth muscle actin (a-SMA) and myosin heavy chain 11 (MHCII)]. rSMCs were treated in vitro with a strong pro-inflammatory stimulus LPS (lipopolysaccharide; 100 ng/mL), in the presence or absence of DHT (30 nM) for 24 h; untreated cells were taken as control cells. In addition, immunofluorescence studies were performed in rSMCs to analyze the translocation of NF-kB, an important transcriptional mediator of the inflammatory response, after LPS stimulation with or without DHT co-treatment.
Results: The analysis of the RNA expression of the main TLRs (Toll-like Receptors), membrane receptors mediators of the inflammatory response, namely TLR-1, TLR-2, TLR-3, TLR-4, TLR-5, TLR-7, TLR-8, TLR-9 and TLR-10, suggests that rSMCs isolated from the distal vagina have a profile similar to APC (antigen presenting cells). We observed that LPS significantly increased the expression of the most important pro-inflammatory genes, such as IL-6 (interleukin-6), IL1-β (interleukin 1-β), CXCL-1 [chemokine (CXC motif) ligand], COX-2 (cyclooxygenase-2), CD4 (cluster of differentiation 4), RORC (RAR-related orphan receptor C) and MCP1 (monocyte chemotactic protein 1) and the expression of TLR-2 and TLR-4, compared to control cells. Co-treatment with DHT counteracted these effects.
In addition, the stimulation with LPS induced a significant nuclear translocation of NF-kB which was inhibited by DHT.
Conclusion: Our data show that, in this model of smooth muscle cell of rat distal vagina, DHT treatment inhibits the RNA expression of genes involved in the inflammatory response, as well as the NF-kB nuclear translocation induced by LPS stimulation. Our data therefore support the possible therapeutic role of androgens in chronic pain syndromes involving these tissues.

P014

The regulation of CIRBP by transforming growth factor beta during heat shock-induced testicular injury

D. KE[1], M. RAO[1,2], G. CHENG[1], S. HU[1], Y. WU[1], Y. WANG[1], F. ZHOU[1], H. LIU[1], C. ZHU[1,3] AND W. XIA[1,3]
[1]Family Planning Research Institute, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; [2]Department of Reproduction and Genetics, the First Affiliated Hospital of Kunming Medical University, Kunming, China; [3]Reproductive Medicine Center, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Background: Cold-inducible RNA-binding protein (CIRBP) was a protein closely related to cell stress, mainly expressed in the primary spermatocyte nucleus and the early round spermatozoa cytoplasm in the testis. As shown in our previous studies, scrotal hyperthermia could severely damage spermatogenesis, and downregulate the expression of CIRBP. In the fibroblasts and neuronal cells, transforming growth factor beta (TGF-β) could regulate the clock gene expression via downregulating CIRBP expression. However, whether TGF-β would mediate CIRBP in the testis remained unclear.
Objective: To investigate whether CIRBP was regulated by TGF-β during the process of the heat-induced testis damage.
Methods: Male adult ICR mice were exposed to scrotal hyperthermia (43°C) for 30 min, and treated with a local testicular injection of TGF-β antagonist (non-selective TGF-β I/II receptor inhibitor, 5 or 10 μg). TGF-β1, -β2, -β3 and CIRBP expression levels were analyzed using real-time PCR and western blotting, and testis morphology was observed by immunohistochemistry and hematoxylin and eosin (HE) staining. GC2-spd cells were cultured with different TGF-β isoforms (10 ng/mL), and CIRBP expression levels were examined 48 h later by PCR.
Results: In the testis, heat treatment significantly downregulated the relative CIBRP mRNA and protein expression ($p = 0.006$ and 0.011); and obviously upregulated three TGF-β isoforms expression, but only the increases in TGF-β2 and TGF-β3 were statistically significant ($p = 0.022$ and 0.04, for mRNA, and $p = 0.001$ for both protein levels). Testicular local injection of a TGF-β antagonist clearly attenuated this heat-induced CIRBP downregulation, and a significant increase in the CIRBP mRNA level in response to heat combined with high-dose ($p = 0.038$) but not low-dose TGF-β agonist therapy ($p = 0.36$). The testicular histology was severely damaged after heat stress, with seminiferous tubule degeneration, spermatocyte and spermatid abnormalities. However, this damage was clearly attenuated by treatment with a high dose of agonist. Furthermore, TGF-β2 and TGF-β3 significantly downregulated CIRBP mRNA expression in GC2-spd cells (all $p < 0.01$).
Conclusion: In summary, heat-induced CIRBP downregulation in the testes was mediated by an upregulation of TGF-β. Further studies are needed to clarify the molecular mechanisms underlying these processes.

P015

An update on recent trends in semen quality among young Finnish men: a comparison with Danish data

W. RODPRASERT[1], H. E. VIRTANEN[1], S. SADOV[1], A. PERHEENTUPA[1,2], N. E. SKAKKEBÆK[3], N. JØRGENSEN[3] AND J. TOPPARI[1,3,4]
[1]Research Centre for Integrative Physiology and Pharmacology, Institute of Biomedicine, University of Turku, Turku, Finland; [2]Department of Obstetrics and Gynecology, University of Turku and Turku University Hospital, Turku, Finland; [3]University Department of Growth and Reproduction and EDMaRC, Rigshospitalet, Copenhagen, Denmark; [4]Department of Pediatrics, Turku University Hospital, Turku, Finland

Background: Finnish men were reported to have higher semen quality and lower incidence of testicular cancer than Danish men. However, recent studies showed declining semen quality and increasing incidence of testicular cancer in Finland, but not in Denmark.

Objective: This study aimed to compare most recent data on semen quality of the young Finnish and Danish men.
Methods: In this cross-sectional study, 18- to 20-year-old men residing in Turku, Finland and Copenhagen, Denmark were invited to participate in 2008–2011. Each man filled in a questionnaire, provided one semen sample and underwent andrological examination. Semen samples were analyzed according to WHO. The external quality control for the assessment of sperm concentration was performed. Multi-way ANOVA was used to adjust semen variables for duration of sexual abstinence, age, (and time from ejaculation to the start of semen analysis for sperm motility) and to compare results between countries.
Results: Altogether 287 Finnish men and 873 Danish men participated in the study. Participation rate was 9% in Finland and 35% in Denmark. The adjusted median sperm concentrations were 49 and 47 million/mL for Finnish and Danish men, respectively ($p = 0.48$). The adjusted median total sperm counts were 148 million in Finland and 146 million in Denmark ($p = 0.87$). The adjusted median percentages of morphologically normal spermatozoa were 6.9% in Finland and 6.5% in Denmark, $p = 0.27$. Finnish men had higher adjusted median percentages of motile spermatozoa (A + B + C) than Danish men (80% vs. 69%, $p < 0.001$). The proportion of men who had low semen quality (sperm concentration, percentage of morphologically normal spermatozoa or percentage of progressively motile spermatozoa below WHO reference limits) was lower in Finland (25.4%) than in Denmark (34.6%), $p = 0.004$. Result of the subgroup analysis of the men who did not take any medications, had no andrological disease, or fertility problem gave similar results as in all men.
Conclusion: Our recent data showed that number of sperms and percentage of normal sperms were similar among young Finnish and Danish men. Significant proportion of men in both countries had low semen quality. The causes of the recent decline in semen quality in Finland are unclear. Our findings demonstrate the importance of continuing surveillance of semen quality.

POSTERS

P016

Epididymal more than testicular abnormalities are associated with the occurrence of antisperm antibodies as evaluated by the MAR test

F. LOTTI[1], E. BALDI[1], G. CORONA[1], F. LOMBARDO[2], E. MASEROLI[1], S. DEGL'INNOCENTI[1], L. BARTOLI[1] AND M. MAGGI[1]
[1]Sexual Medicine and Andrology Unit, University of Florence, Italy; [2]Department of Experimental Medicine, "Sapienza" University of Rome, Italy

Background: MAR test positivity has been more often reported in males of infertile couples than in fertile men. A positive MAR test has been detected in men with a history of testicular or post-testicular damage. No previous study has reported ultrasound (US) alterations related to MAR test positivity. The aim of this study was to systematically evaluate associations between a positive MAR test and clinical, seminal and US characteristics of the entire male genital tract in males of infertile and fertile couples.
Methods: Cross-sectional analysis of a consecutive series of 702 men with couple infertility. As a control group we evaluated 109 fertile men from a Florence spin-off of an ultrasound study on male fertility sponsored by the European Academy of Andrology. All subjects underwent a complete physical, biochemical, scrotal and transrectal US evaluation and semen analysis including seminal interleukin 8 (sIL-8, a surrogate marker of genital tract inflammation) within the same day. SpermMAR test IgG was performed when a sperm concentration of >107/mL and a forward progressive sperm motility of >10% were detected, as in a previous study. Of the 702 males of infertile couples, 181 (age 38.6 ± 6.6 years) had an assessable MAR test, whereas the test was assessable in all 109 fertile men (age 36.6 ± 5.2 years). The associations among MAR test positivity and the other studied parameters were investigated on a caseload of 290 men (patients + fertile men), and in the two cohorts of males of infertile and fertile couples.
Results: Of the 181 patients studied, 20 (11%) had a positive MAR test, of which 12 (6.6%) had a MAR test ≥50% (pathological threshold according to the WHO). Of the 109 fertile men, four (3.7%) had a positive MAR test, of which one (0.9%) had a MAR test ≥50%. MAR test positivity was found more often in men of infertile couples ($p < 0.05$). In the entire caseload ($n = 290$), and in both males of infertile and fertile couples, no correlations between MAR test positivity and seminal characteristics were observed. A positive MAR test was associated with epididymal US abnormalities, particularly with mean size of the epididymal body and tail (both $p < 0.0001$), and with an abnormal epididymal echotexture. In addition, subjects with positive MAR test more frequently showed a history of epididymitis and high sIL-8 levels. Considering hormonal parameters, only a positive correlation between MAR test positivity and LH levels was observed, even after adjusting for age and life-style (adj. $r = 0.232$, $p < 0.0001$), while no associations with testosterone and FSH levels were found.
Conclusion: MAR test positivity is associated with clinical, US and biochemical (sIL-8) signs suggestive of chronic epididymal inflammation, and with higher LH, but not testosterone and FSH levels, suggesting a partial damage of Leydig cells but not of the seminiferous tubules. Hence, when investigating a positive MAR test, epididymis, and not just the testis, should be evaluated. Furthermore, our data support a role of ASA in couple infertility, irrespective of conventional semen parameters.

P017

When is investigation on and treatment of midline prostatic cyst of clinical value in the work-up of males of infertile couples?

F. LOTTI[1], G. CORONA[1], A. COCCI[2], S. CIPRIANI[1], E. BALDI[1], S. DEGL'INNOCENTI[1], P. N. FRANCO[1], M. GACCI[2] AND M. MAGGI[1]
[1]Sexual Medicine and Andrology Unit, University of Florence, Italy; [2]Department of Urology, University of Florence, Italy

Background: Midline prostatic cysts (MPC) are relatively frequent in infertile men and are considered a

correctable cause of male infertility. However, they have been poorly investigated in an infertility setting. The aim of this study was to evaluate (i)the MPC prevalence and characteristics in males of infertile and fertile couples, (ii)a MPC volume predicting an unfavorable fertility status, (iii)the seminal and fertility outcome of MPC treatment and (iv) to create an algorithm to identify which subjects should undergo a prostate US to detect a MPC in an infertility setting.

Methods: Cross-sectional analysis of 648 men (37.1 ± 7.9 years) with couple infertility and without genetic abnormalities, and 103 fertile men (36.6 ± 5.0 years) from a Florence spin-off of an US study on male fertility sponsored by the European Academy of Andrology. All subjects underwent clinical, biochemical, scrotal and transrectal US evaluation and semen analysis within the same day. Furthermore, 11 infertile men with semen abnormalities and large MPC underwent Trans-Rectal Ultrasonically-guided Cyst Aspiration (TRUCA), semen analyses 1 and 3 months after TRUCA and follow-up 1 year after surgery to assess if a natural pregnancy occurred.

Results: A MPC was present in 66/648 (10.2%) males of infertile couples and in 6/103 (5.8%) fertile men. MPC occurrence and volume were higher in patients with severe oligo- or azoospermia than in fertile men (all $p < 0.05$). Infertile men with a MPC showed a lower seminal volume and sperm count and a higher prevalence of azoospermia than the rest of the infertile sample or fertile men, along with a higher frequency of US signs suggestive of ejaculatory duct obstruction. MPC volume was negatively associated with total sperm count. In fertile men, the highest MPC volume was 0.117 mL, suggesting it as a biological threshold not compromising fertility. In infertile men, using ROC curve analyses, a MPC volume >0.117 mL identified subjects with severe oligo- or azoospermia with an overall accuracy of ~75% (both $p < 0.005$). Eleven men with infertility, semen abnormalities and large MPC (>0.250 mL) underwent TRUCA, which led to sperm count improvement in all patients 1 month after surgery. Three months after TRUCA a lower sperm count and a higher MPC volume than 2 months before were observed, although both parameters were improved when compared to baseline. After TRUCA a natural pregnancy occurred in four couples. Finally, we propose an algorithm, based on semen parameters, useful in identifying a clinically significant MPC in males of infertile couples.

Conclusion: MPC are detectable in about one out of 10 infertile men. Their frequency and volume are higher in subjects with severe oligo- or azoospermia compared with fertile men. A MPC volume below 0.117 mL does not impair fertility, while a larger volume may be related to severe oligo- or azoospermia. Treatment of large MPC (>0.250 mL) with TRUCA leads to an improvement in semen parameters, and to natural pregnancy in almost half of cases. Finally, we originally propose an algorithm, based on semen parameters, useful to identify men who can benefit from a prostate US in the infertility work-up.

P018

More prevalent prescription of medicine for hypertension and metabolic syndrome in males from couples undergoing Intracytoplasmic Sperm Injection

A. ELENKOV[1,2,3], Y. AL-JEBARI[2] AND A. GIWERCMAN[1,2]
[1]Reproductive Medicine Center, Skåne University Hospital Malmö, Malmö, Sweden; [2]Department of Translational Medicine, Molecular Genetic Reproductive Medicine, Lund University, Malmö, Sweden.; [3]Department of Urology, Medical University Sofia, Sofia, Bulgaria

Background: Register-based studies have indicated that men with impaired fertility are at higher risk for developing various adult-onset diseases than fertile men. The majority of men undergoing ICSI treatment are sub-fertile and since they are in contact with the health care system, these men are well suited as target for preventive measures.

Objective: Our aim was to investigate what is the risk of being treated with medicines for different components of metabolic syndrome (MetS) among men who have undergone ICSI or IVF as compared to men conceiving neither by IVF nor ICSI (non IVF/ICSI).

Methods: Based on Swedish national registries we included all men (N = 898 284) that had one or more children born alive in Sweden between 2005 and 2016. The Swedish Medical Birth Register (MBR) and the Swedish National Quality Register for Assisted Reproduction (Q-IVF) were used for obtaining information regarding conception method and defining three groups of fathers – ICSI -treated, IVF – treated and non IVF/ICSI. We specifically searched for information regarding prescription and usage of at least one prescription for diabetes mellitus (DM), hypertension (HT) or dyslipidemia (DLE) among the study groups sourcing data form the Swedish Prescribed Drug Register (SPDR). If all three types of medicine were prescribed, the patient was considered as having MetS. Hazard ratios (HR) of filled prescriptions for DM, HT and DLE in patients treated for ICSI and IVF with corresponding confidence intervals (CI) were calculated using Cox proportional hazards models with the non IVF/ICSI treated male population as reference. Two analyses were performed – in the first, the start of the follow up was defined as starting at the time of the birth of the father, assuming common etiology behind MetS components and reduced male fertility, possibly arising early in life. In order to evaluate the significance of the risk of prescription following infertility treatment, a sub analysis was performed with the follow up starting from the time of child of conception and excluding fathers, who had been prescribed to medicines before the child was conceived.

Results: A total of 8514 men became fathers through ICSI; 10748 via IVF and 440504 succeeded to conceive without use of ICSI or IVF. Our analysis revealed ICSI treated males to have statistically significantly higher risk for HT medicine prescription (HR = 1.16; 95% CI: 1.08–1.24) and MetS (HR = 1.28; 95% CI: 1.03–1.58). When infertility treatment was used as start of follow up only HT prescription was statistically significant in the ICSI group (HR = 1.17; 95% CI: 1.06–1.30). Males in the IVF group showed decreased risk for DM (HR = 0.72; 95% CI: 0.63–0.84) when compared to non-IVF/ICSI in both analyses.

Conclusion: Our analysis revealed statistically significant increase in the risk of developing various metabolic disorders such as hypertension and MetS when using ICSI as proxy for male infertility. These men may be target for preventive measures aiming to decrease long term morbidity and increased mortality.

P019

Testosterone modulate accessory sexual gland function and seminal plasma composition in secondary hypogonadism – a quantitative proteomic approach

G. GRANDE[1,2], F. VINCENZONI[3], F. MANCINI[1], F. BARRACHINA[4,5], L. D. MARINIS[2], R. MARANA[1], A. URBANI[3], R. OLIVA[4,5], A. PONTECORVI[1,2] AND D. MILARDI[1,2]

[1]International Scientific Institute "Paul VI", Catholic University, Rome, Italy; [2]Division of Endocrinology, Fondazione Policlinico Universitario "Agostino Gemelli", Rome, Italy; [3]Institute of Chemistry and Clinical Biochemistry, Catholic University, Rome, Italy; [4]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Department of Biomedicine, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain; [5]Biochemistry and Molecular Genetics Service, Hospital Clinic, Barcelona, Spain

Background: Hypogonadotropic hypogonadism (HH) is characterized by low testosterone (T) plasma levels associated with normal/low FSH and LH levels. Total T levels <8 nmol/L highly support a diagnosis of hypogonadism whereas the grey zone between 8 and 12 nmol/L requires further evaluation and the usefulness of treatment is debated, so that novel markers of tissue action of androgens might be useful in orienting clinical actions. To better understand the effect of the hypothalamic pituitary-testicular axis on male accessory secretive function, we performed a quantitative proteomic analysis on seminal plasma of patients affected by secondary hypogonadism, before and after testosterone replacement therapy (TRT).
Methods: Ten male patients aged between 25 and 55 years with secondary postsurgical hypogonadism were consecutively enrolled for this study. Five patients were also evaluated after a 3-month of TRT with transdermal testosterone. Ten fertile men were enrolled as a control group in the protocol. An aliquot of seminal plasma from each individual, corresponding to 1 mg of total protein, was subjected to an in-solution digestion protocol and analyzed using an Ultimate 3000 RSLCnano HPLC apparatus coupled to a LTQ Orbitrap Elite mass spectrometer. The label-free quantitative analysis was performed via Precursor Ions Area Detector Node during the bioinformatic analysis. The relative protein level ratios between the groups were determined from the respective averages of protein abundances expressed in all samples. All the proteins detected with a ratio >1.5 (less abundant proteins in patients) or <0.67 (more abundant proteins in patients) between the studied groups have been considered for this study.
Results: Eleven proteins have been detected as reduced in hypogonadic patients vs. controls, mainly involved in hydrolase activity, and in protein binding activity. The comparison of the hypogonadic proteomes before and after TRT resulted in the detection of 6 differentially expressed proteins.
Conclusion: This is the first application of quantitative proteomics aimed to reveal an array of proteins reflecting an impairment of the epididymal and prostate epithelial cell secretory function in male secondary hypogonadism. This novel approach in the study of androgen action permitted us to identify clinical markers that might help in the follow-up of patients with different grades of male hypogonadism.

P020

General health and semen quality in night shift workers

D. MORENO-MENDOZA[1], A. RIERA-ESCAMILLA[1], G. D. FEDE[1], L. BASSAS[1], J. R. SANCHEZ-CURBELO[1], C. KRAUSZ[2] AND E. RUIZ-CASTAÑE[1]

[1]Andrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, IIB-Sant Pau, Barcelona, Spain; [2]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", Centre of Excellence DeNothe, University of Florence, Florence, Italy

Background: Night shift workers might be under the effect of an altered circadian rhythm, psychosocial stress and sleep deprivation which may influence their general health and semen quality. To date, association studies analyzing the relationship between night shift work and men's health suggest an increased risk of diabetes mellitus, hypertension, dyslipidemia and coronary heart disease in shift workers. On the other hand, the relationship between night shifts and semen quality remains debated.
Objective: To evaluate the effect of night shift work on routine sperm parameters and on general health in fertile men.
Methods: 469 fertile men prior vasectomy. All participants completed an interview about reproductive history, employment, occupational exposures, chronic diseases, medications, and lifestyle habits. Semen analysis was done according to WHO (2010 Manual). We divided the participants into two groups: 74 night shift workers (NW) and 395 Non NW workers (NNW).
Results: No differences in age, body mass index, number of children, number of abortion, alcohol consumption, tobacco consumption and certain occupational exposures were observed between the NW and NNW. Lipid and glucose metabolism: No statistically significant differences between NW and NNW concerning cholesterol HDL, LDL and triglycerides levels were observed. Night shift work is not associated with arterial hypertension neither (OR = 0.88; 95% CI: 0.30–2.62). On the contrary, glycemia was statistically significantly inferior ($p = 0.048$) in the NW (89.4 ± 8.9 mg/dL) vs. the NNW (92.3 ± 15.2 mg/dL).
Semen parameters: No differences concerning semen volume (mL) (3.2 ± 1. vs. 3.3 ± 1.6; $p = 0.871$), % of progressive sperm motility (43.7 ± 17.7 vs. 47.9 ± 16.4; $p = 0.66$) and % of normal sperm morphology (11.6 ± 8.6 vs. 12.7 ± 8.9; $p = 0.302$) were observed between NW and NNW, respectively. On the other hand, the total sperm number was significantly lower in the NW group vs. the NNW (175.6 ± 169.3 vs. 227.4 ± 188; $p = 0.02$). Similarly,

the total motile sperm count was significantly reduced in NW in respect to the NNW (85.4 ± 94 vs. 118.4 ± 107.8; $p = 0.008$). Finally, the percentage of non-normozoospermic men (those with at least one routine sperm parameter altered) were 48.6% in NW and 30.6% in NNW (OR = 2.14; 95% Cl: 1.30–3.55; $p = 0.003$).

Conclusion: NW does not significantly affect "fertility" status (number of children and time to pregnancy are the same) and general health (no relationship between night shifts and an increased risk of hypertension, diabetes or dyslipidemia was observed). Our is the first study reporting data on fertile men with known sperm count in relationship with NW. The significantly lower total sperm number/total motile sperm number in the NW group and the doubled likelihood of having at least one abnormal sperm parameter in respect to NNWs indicates a plausible biological effect on spermatogenesis. Although the exact mechanism by which the misregulation of circadian rhythms may alter spermatogenesis is not known, it has been proposed that it may affect chromatin remodeling of a number of genes involved in germ cell development.

P021

Semen quality and reproductive function as markers of general male health: a prospective cohort study on 5177 men

A. FERLIN[1], A. GAROLLA[2], M. GHEZZI[2], R. SELICE[2], P. PALEGO[2], N. CARETTA[2], A. D. MAMBRO[2], U. VALENTE[2], S. DIPRESA[2], L. SARTORI[2], M. PLEBANI[2] AND C. FORESTA[2]

[1]Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy; [2]Department of Medicine, University of Padova, Padova, Italy

Background: Evidence suggested that infertile men are at increased risk for hypogonadism, metabolic derangements and osteoporosis, have higher long-term morbidity and mortality than controls, but data are scarce and not conclusive. Here we tested whether semen quality and reproductive function could represent a marker of general male health.

Methods: This prospective cohort study included 5177 males of infertile couples. All subjects had semen analysis, reproductive hormones, testis ultrasound and biochemical determinations for glucose and lipid metabolism. Hypogonadism was defined as testosterone <10.5 nmol/L and/or LH > 9.4 IU/L. Subjects with total sperm count (TSC) <10 million had genetic testing (karyotype, Y chromosome microdeletions, CFTR gene mutations) and those with hypogonadism dual-energy x-ray absorptiometry for bone mineral density (BMD).

Results: Men with low sperm count (<39 million/ejaculate) have a 12-fold increased risk of having hypogonadism (OR 12.2, 95% CI 10.2–14.6) and the risk was highest in men with TSC <10 million, genetic causes, history of cryptorchidism and idiopathic forms. Men with low sperm count had higher BMI, waist circumference, systolic pressure, LDL-cholesterol, triglycerides, and HOMA-index, and lower HDL-cholesterol, and higher prevalence of metabolic syndrome (OR 1.246, 95 CI 1.005–1.545). All data were worse in men with low testosterone and milder, but significant, in men with isolated elevated LH. Low TSC per sé was associated with poor metabolic parameter. Men with hypogonadism had lower BMD and 51% prevalence of osteoporosis/osteopenia.

Conclusion: This large study suggests that low sperm count is associated with poorer metabolic, cardiovascular and bone health. Hypogonadism is mainly involved in this association, but low sperm count in itself is a marker of general health. Infertile patients have the great opportunity to benefit from the identification of accurate diagnostic and prognostic markers, and clinically important comorbidities and risk factors.

P022

Effect of endocrine disruptors on male reproductive function: a systematic review and meta-analysis

D. BLIATKA[1], S. LYMPERI[1], M. P. NIGDELIS[1], K. CHATZIMELETIOU[2] AND D. G. GOULIS[1]

[1]Unit of Reproductive Endocrinology, 1st Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Greece; [2]IVF Unit, 1st Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Greece

Background: Despite the conduction of many epidemiological studies, the impact of endocrine disruptors (EDs) on testicular function (both endogenous: testosterone production, and exogenous: sperm production) remains a controversial issue.

Objective: The aim of this study was to systematically review the literature and perform a quantitative synthesis of the best available evidence in order to evaluate the effect of EDs on testicular function.

Methods: A comprehensive search was conducted in PubMed, EMBASE and Cochrane Collaboration databases. Eligible for the systematic review and meta-analysis were studies in which there was a description of (i) a population that was exposed to EDs ("cases"), (ii) a population that was not exposed to EDs ("controls") and (iii) an outcome of interest [semen parameters: volume, sperm concentration, progressive motility, total motility, morphology; and/or reproductive hormone concentrations: follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, inhibin B]. Data from each of the studies included in the meta-analyses were expressed as standardized mean difference (SMD) with 95% confidence interval (CI) and were synthesized by the use of the random effects model.

Results: Through database searching, 648 records were identified. Of them, 13 studies, including 959 patients and 907 non patients in total, fulfilled the inclusion criteria. Exposure to ED [pesticides, Perfluorinated Chemicals (PFCs), Persistent Organic Pollutants (POPs), Bisphenol A (BPA) or phthalate metabolites] was associated with decreased LH ($n = 10$, 616 patients, SMD −0.17, 95% CI −0.33 to −0.02, $I^2 = 40.20\%$, $p = 0.030$), decreased progressive motility ($n = 3$, 133 patients, SMD −0.45, 95% CI −0.77 to −0.13, $I^2 = 38.36\%$, $p = 0.005$) and normal morphology ($n = 8$, 564 patients, SMD −0.50, 95% CI −0.85 to −0.14, $I^2 = 86.88\%$, $p = 0.005$). No association was observed between the exposure to EDs and the semen

volume (n = 9, 602 patients, SMD −0.18, 95% CI −0.46 to 0.10, I^2 = 81.33%, p = 0.218, sperm concentration (n = 9, 617 patients, SMD −0.29, 95% CI −0.62 to 0.04, I^2 = 86.03%, p = 0.087), total motility (n = 6, 408 patients SMD −0.67, 95% CI −1.46 to 0.11, I^2 = 96.26%, p = 0.092), FSH (n = 10, 616 patients, SMD −0.09, 95% CI −0.27 to 0.08, I^2 = 53.76%, p = 0.298), total testosterone (n = 7, 339 patients, SMD −0.12, 95% CI −0.42 to 0.18, I^2 = 73.50%, p = 0.445) or of inhibin B concentrations (n = 4, 322 patients, SMD −0.20, 95% CI −0.49 to 0.08, I^2 = 58.56%, p = 0.159).

Conclusion: This meta-analysis provided evidence that exposure to EDs is associated with decreased semen quality. Nevertheless, there is no evidence that the deterioration in semen quality is mediated by a disruption of hypothalamus – pituitary – testicular axis.

P023

Testosterone replacement therapy and cardiovascular risk: results from a meta-analysis of RCT and observational studies

G. CORONA[1], G. RASTRELLI[2], S. FILIPPI[3], G. D. PASQUALE[4], A. SFORZA[1], E. MANNUCCI[5] AND M. MAGGI[2]

[1]Endocrinology Unit, Medical Department, Azienda Usl Bologna Maggiore-Bellaria Hospital, Bologna, Italy; [2]Sexual Medicine and Andrology Unit, Department of Experimental Clinical and Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy; [3]Interdepartmental Laboratory of Functional and Cellular Pharmacology of Reproduction, Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence, Florence, Italy; [4]Cardiology Unit, Medical Department, Azienda Usl, Maggiore-Bellaria Hospital, Bologna, Italy; [5]Diabetology, University of Florence and Careggi Teaching Hospital, Florence, Italy

Objective: The relationship between testosterone (T) and cardiovascular (CV) risk in men is conflicting. By using a meta-analytic approach, we aimed at evaluating (i) the relationship between endogenous low T levels and incident CV events and (ii) the risk of incident CV diseases and CV mortality associated with T therapy.

Methods: We conducted a random effect meta-analysis considering all available data from prospective observational and pharmaco-epidemiological studies as well as randomized placebo-controlled trials (RCTs).

Results: After screening, 37 observational, 15 pharmaco-epidemiological and 93 RCT studies were considered. Low endogenous T at enrolment predicts overall and CV mortality, as well as CV morbidity when both unadjusted and fully adjusted models were considered. In addition, the analysis of pharmaco-epidemiological studies documented that T therapy (TTh) reduces overall mortality and CV morbidity. Conversely, in RCTs TTh had no clear effect, either beneficial or detrimental, on the incidence of CV events. However, a protective role of TTh on CV morbidity was observed when studies enrolling obese (BMI > 30 kg/m^2) patients were scrutinized [MH-OR = 0.51 (0.27;0.96); p = 0.04], although this association disappeared when only high quality RCTs were considered [MH-OR = 0.64 (0.22;1.88); p = 0.42]. Finally, an increased risk of CV diseases was observed in RCTs when T preparations were prescribed at dosages above those normally recommended, or when frail men were considered.

Conclusion: Low T is a biomarker of CV risk. Data from RCTs suggest that treatment with T is not effective in reducing this risk, however, when TTh is correctly applied, it is not associated with an increase in CV risk and it may have a beneficial effect in some sub-populations.

P024

Human testis cancer control by immune cells: potential role of tumor-infiltrating lymphocytes

D. PÜSCHL[1,2,3], L. BERTL[1], S. INDUMATHY[1,2,3], D. FIETZ[1], K. HARTMANN[1], S. KLIESCH[4], B. LOVELAND[5], F. WAGENLEHNER[6], M. P. HEDGER[2], K. L. LOVELAND[2,3], M. BERGMANN[1] AND H.-C. SCHUPPE[6]

[1]Department of Veterinary Anatomy, Histology and Embryology, Justus Liebig University, Giessen, Germany; [2]Centre for Reproductive Health, Hudson Institute for Medical Research, Clayton, Vic, Australia; [3]Department of Molecular and Translational Sciences, School of Clinical Sciences, Monash University, Clayton, Vic., Australia; [4]Centre of Reproductive Medicine and Andrology, University of Muenster, Muenster, Germany; [5]Burnet Institute, Melbourne, Vic, Australia; [6]Department of Urology, Pediatric Urology and Andrology, Justus Liebig University, Giessen, Germany

Background: In human testicular germ cell neoplasia, that is, seminoma and pre-invasive germ cell neoplasia in situ (GCNIS), infiltrating immune cells (IC) are frequently present. These IC have been identified as macrophages, dendritic cells and lymphocytes (T and B cells), with T cells representing a major component of the tumor-infiltrating lymphocyte (TIL) population. Recent studies indicate that functional polarization and the respective subtypes of TIL influence cancer development and prognosis. The implications for T cell subsets, including regulatory T cells (Treg), in the initiation and growth regulation of human testis cancer, however, are not well established to date.

Objective: Therefore, we aimed to identify and characterize subsets of T cells in seminoma and GCNIS, in comparison to non-neoplastic testes.

Methods: Human testis samples (seminoma, GCNIS ± lymphocytic infiltrates (ly), impaired spermatogenesis (hyp) + focal ly, and normal spermatogenesis (nsp); n = 10, each) were analyzed by immunohistochemistry and/or fluorescence. Markers detected were: CD3, CD4, CD8, CD20, CD68, CD11c, CD25, and FOXP3. Transcripts encoding cytokines (e.g. IL-10 and TGF-ß) were measured using quantitative RT-PCR from cryopreserved tissue samples.

Results: Our results confirm that both CD8+ and CD4+ immune cells accumulate in GCNIS+ly and seminoma. CD4+/FOXP3+ cells (notionally Treg) are located within focal lymphocytic infiltrates in hyp or GCNIS, with considerable interindividual variation in cell numbers. Similar to abundantly present macrophages, CD4+/FOXP3+ cells show a disseminated distribution pattern in seminoma,

which contrasts the previously described clustering of B cells. Whereas B cells are absent from normal testis, single T cells including Treg can be identified in the interstitial compartment in nsp samples. In addition, besides Th1 (CD4+/FOXP3−) driven immune responses by pro-inflammatory cytokine expression, increased levels of Treg-related anti-inflammatory cytokine transcripts, e.g. IL-10 and TGF-ß, were associated with testicular neoplasia.

Conclusion: Our data suggest that TIL in testicular neoplasia comprise a subset of CD4+/FOXP3+ Treg. Detailed functional characterization of TIL in testicular neoplasia will help to further elucidate the complex mechanisms of "immune editing" during testis cancer development.

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P025

Src is involved in the c-Met-mediated migrating and invading behavior of NT2D1 Embryonal Carcinoma cells

E. LEONETTI[1], L. GESUALDI[1], S. DINICOLA[2], M. G. MASIELLO[3], M. BIZZARRI[2], A. CUCINA[3], A. CATIZONE[1] AND G. RICCI[4]

[1]Department of Anatomy, Histology, Forensic-Medicine and Orthopaedics, "Sapienza" University of Rome, Italy; [2]Department of Experimental Medicine, Systems Biology Group Lab, Sapienza University of Rome, Italy; [3]Department of Surgery, "Pietro Valdoni", Systems Biology Group Laboratory, "Sapienza" University of Rome, Italy; [4]Department of Experimental Medicine, Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy

Background: The proto-oncogene c-Met is a tyrosine kinase receptor that binds, as unique ligand, the Hepatocyte Growth Factor (HGF). c-Met/HGF system is highly involved in the regulation of the embryonic development and, in the adult, of tissue homeostasis. It is well known that the deregulation of c-Met activation is responsible of the onset and progression of different human cancer types (KoeppenH. et al. J. Pathol. 2014, 232: 210–218). Notably, literature data of the last decades demonstrated that c-Met/HGF system is important for testicular physiology (Ricci G. and Catizone A., Frontiers in Endocrinology, 2014, 5:art.38). More recently, our group revealed that c-Met is expressed in both seminoma and non seminoma Testicular germ cell tumors (TGCTs), also demonstrating that NT2D1 cells, a non seminoma cell line with molecular features of Embryonal Carcinoma, respond to HGF activating their proliferative, migrating and invasive behavior (Corano Scheri K. et al. Oncotarget submitted).

Methods: In the present work, first of all, we provide definitive evidence that the HGF-dependent migrating and invading behavior of NT2D1 cells are mediated by c-Met receptor, using a c-Met inhibitor, called PF04217903, that counteracts specifically c-Met phosphorylation. This drug is not toxic for NT2D1 cells, but abrogates the HGF-dependent migration and invasiveness of NT2D1 cells, demonstrating that these responses are c-Met-mediated. Activated c-Met recruits several adaptor proteins such as PI3K/AKT, STAT3, MAPK, Src etc. that are responsible of the different signaling pathways triggered by c-Met (Baldanzi G. and Graziani A., Biomedicines, 2015, 3:1–31). In particular, Src adaptor is an oncogene with tyrosine kinase activity, that appears up-regulated in tumor progression and metastasis formation (Guarino M., J. Cell. Physiol., 2010, 223:14–26). In the present work we investigated the relevance of Src activation in the c-Met-mediated migrating and invading behavior of NT2D1 carcinoma cells. To this aim, we performed western blot analyses in order to evaluate Src protein both at baseline condition and after HGF administration. Notably, we observed an increase of Src protein 8 h after HGF administration compared with control samples. This finding suggests that Src is likely recruited during activation of the HGF-dependent c-Met related pathways. To deeper investigate the role of Src in the HGF-dependent migration and invasion of NT2D1 cells, we performed different migration and invasion experiments using the Src inhibitor-1, with or without HGF.

Results: Results demonstrated that both migration and invasion, triggered by HGF administration, are reverted by the co-administration of Src inhibitor-1, suggesting that Src plays an important role in the stimulation of HGF-dependent NT2D1 cell aggressive behavior. However, unexpectedly, the administration of Src inhibitor-1 alone stimulates cell invasion, and the response is similar to that obtained using the HGF alone.

Conclusion: This result indicates that Src is still required by opposite pathways in NT2D1 cells, promoting or alternatively inhibiting invasiveness, depending on the presence of HGF to clarify the complex role of Src activation in Embryonal Carcinoma onset and progression.

P026

Inhibin B: are modified ranges needed for testicular cancer orchiectomised patients?

A. PETROZZI, M. PELLONI, F. CARGNELUTTI, F. FAJA, A. RADICIONI, D. PAOLI, A. LENZI AND F. LOMBARDO

Laboratory of Seminology – "Loredana Gandini" Sperm Bank Department of Experimental Medicine – Medical Pathophysiology Section, "Sapienza" University of Rome, Italy

Background: Inhibin B is a gonadal hormone that down-regulates the pituitary production of FSH. In recent years, inhibin B has proved to be an excellent marker of spermatogenesis and even a predictive factor for the recovery of fertility in patients undergoing orchiectomy and anti-neoplastic treatments.

Objective: We propose to study inhibin B levels in testicular cancer orchiectomised patients, in order to identify a minimum value representative of normal semen quality.

Methods: This retrospective study evaluates hormonal and semen parameters of 290 normozoospermic patients, attending the Seminology Laboratory Sperm Bank for cryopreservation of seminal fluid following a diagnosis of testicular cancer (TC group) and of a control group of 117 healthy, normozoospermic men (CTR group). The percentile distribution of gonadotropin and inhibin B values in the TC and CTR group was analysed.

Results: There was a statistically significant difference between the two groups in the levels of all hormones ($p = <0.001$) and in all semen parameters: ($p < 0.05$).

About 20% of TC patients revealed inhibin B levels below 5th percentile of CTR group, despite normozoospermia and 31% had normal spermatogenesis in the presence of FSH values >95th percentile of CTR group. Orchiectomised patients for testicular cancer presented inhibin B levels lower than healthy patients, despite normozoospermia.

Conclusion: Our study revealed the poor sensitivity of the current inhibin B reference range when applied to monorchidic patients, suggesting the need to establish more representative ranges to enable more appropriate counselling in relation to the patient's new endocrine condition.

P027

PDEs analysis in Leydig cell tumor

F. BARBAGALLO[1], M. G. TARSITANO[1], F. CAMPOLO[1], C. POZZA[1], D. GIANFRILLI[1], M. TENUTA[1], F. NARO[2], A. LENZI[1] AND A. M. ISIDORI[1]

[1]Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy; [2]Department of Anatomical, Histological, Forensic and Orthopedic Sciences, Sapienza University of Rome, Rome, Italy

Background: Phosphodiesterases (PDEs) are enzymes that have the unique function of modulating cyclic nucleotide signaling by hydrolyzing cAMP and cGMP. PDEs play a pivotal role in the regulation of intracellular concentrations of these second messengers as well as of their signaling pathways and downstream biological effects. PDEs have been exploited pharmacologically for more than 50 years, becoming the target of some of the most successful drugs worldwide today. Mutations in PDEs genes have been identified as causative of certain human genetic diseases and functional variants of PDEs genes have been involved in the genesis of many tumor types. Leydig cell tumor (LCT) is the most frequent interstitial neoplasm of the testis, accounting for 0.8–3% of all testicular tumors and 4–9% of testis tumors in prepubertal males. The etiology of this tumor is not still fully understood. However, the disruption of the hypothalamic-pituitary-testicular axis seems to lead to an over-stimulation of LC by increased LH production. Several phosphodiesterases are expressed in the male gonad, and their relative concentration is modulated during testicular development. PDE1A, PDE1C, PDE3B, PDE5A, PDE8A, PDE10A and PDE11A are the most highly expressed in the testis. Even if the role of PDE11A in spermatozoa physiology and PDE8A in the regulation of hormone production have been still evaluated, their role in LTCs remain to be clarified.

Methods: Seventy testis biopsies from testicular cancer patients undergoing surgery were collected. Biopses from tumoral lesions were designed as "tumor" while biopsies from the distal part of the same testis were designed as "healthy controls". mRNA expression levels were analyzed using the QuantiGene 2.0 Plex Assay (Bio-Rad Laboratories) and confirmed by qPCR; PDEs protein expression levels were assayed by Western Blot analysis.

Results: mRNA and protein expression analysis reveal that PDE1A and PDE8B, but not PDE8A are specifically overexpressed in LCTs compared to healthy controls.

Conclusion: PDE8A and PDE8B are cAMP-specific phosphodiesterases that are highly expressed in LCs. PDE8A is largely associated with mitochondria, whereas PDE8B is broadly distributed in the cytosol. Our data demonstrate for the first time that PDE8B is selectively increased in LCT and may be therefore considered a valuable novel target for the treatment of Leydigiomas. Future studies on steroidogenesis and LCT are needed to elucidate the role of PDE8B in Leydig cell compartment damage. Calcium/calmodulin-dependent PDEs, PDE1A, PDE1B, and PDE1C, are abundant in testicular cells and in mature spermatozoa. More specifically, PDE1A mRNA is found in round to elongated spermatids while protein expression was found in the tails of elongated and maturing spermatids. In contrast, PDE1C mRNA accumulates during early meiotic prophase and throughout meiotic and postmeiotic stages. Up to date no data are available on PDE1A involvement in LCs and future investigations will be useful to understand its role in LCTs pathological mechanism. As well as PDE8B, PDE1C can become a new marker of hypofunctional Leydig cells.

P028

Clinical usefulness of a novel grading system for varicoceles using duplex Doppler ultrasound examination based upon postsurgical modifications of seminal parameters

G. CAVALLINI[1], F. I. SCROPPO[2] AND G. M. COLPI[3]

[1]Gynepro Medical Team, Andrological Section, Bologna, Italy; [2]Department of Urology and Andrology, Ospedale di Circolo e Fondazione Macchi, Varese, Italy; [3]Andrology and IVF Department, Clinica San Carlo, Paderno Dugnano (Milano), Italy

Background: The existing Doppler duplex classifications of varicoceles have poor predictive value on the effects of surgery on sperm count: we developed a new grading system.

Methods: A three-center and five step prospective research was done. The patients underwent medical history collection, objective examination, duplex Doppler ultrasound scrotal examination, hormonal profiles and two semen analyses before after surgery. All patients were operated with Colpi' s technique. 1 step. A population to be examined prospectively (i.e. dyspermic men having clinically detectable left varicoceles) was generated and how many patients should be examined to have a reliable chance of detecting the effect sought were calculated with power analysis using 0.90 as a standard for adequacy. 2 step. The differences before vs. after surgery (6 months) of standard sperm parameters were assessed with the Wilcoxon test. 3 step. Any classification needs of discrete variables, thus the reliability of the assessments of the subjective/discrete variables which are known to influence postoperative sperm count was assessed, computing the intra- and inter-operator variability with Wilcoxon test. There are two kinds of variables capable of influence postoperative standard sperm parameters: continuous/objective variables (i.e. standard seminal parameters, age, preoperative FSH, preoperative testicular volume) and discrete/subjective variables (i.e. extension of the venous

reflux and clinical grade of varicocele). 4 step. The relative weight of the variables studied on the improvement of the standard semen parameters after varicocele surgery was evaluated with Spearman's rank semiquantitative analysis. 5 step. The postoperative modifications of the sperm count were assessed according to the discrete to evaluate whether these variables might be clear-cut thresholds to predict postoperative sperm count modifications.

Results: Step 1: 173 patients were examined, median age 30, range 21–42. Step 2: All standard sperm parameters significantly increased after surgery: sperm concentration increased from [median, range (min–max)] 16 × 106/mL (range: 5–35) to 40 × 10^6/mL (range 30–50); progressive motility from 17% (range: 7–41%) to 50% (range: 45–60%) and morphology from 5% (range: 2–8%) to 8% (range 6–10%). Step 3: No significant inter/intra-operator variability emerged when clinical grading or when the reflux during Valsalva maneuver were assessed. However significant inter- and intra-operator variability emerged when the patients were categorized according to continuous reflux enhanced or not by Valsalva. Accordingly, the semiquantitative Spearman multivariate analysis (see step 4) used 2 semi-quantitative categorizations for venous reflux: venous reflux during Valsalva maneuver and continuous venous reflux. Step 4: List of the variables in decreasing order of weight capable of influencing postoperative standard sperm parameters: venous reflux extent, clinical grade, FSH, preoperative sperm count, testicular volume and age. Step 5: Sperm count significantly improved in clinical grade 2 and 3 varicoceles and in varicoceles with continuous reflux, but it does not improve in varicoceles with reflux limited to Valsalva.

Conclusion: The data in this study indicated that venous reflux extension was the most important variable capable of influencing the postoperative standard semen parameters which can be used to easily to distinguish varicoceles which may or may improve after surgery.

P029

The seminiferous tubule caliber pattern as evaluated at high magnification during microdissection testicular sperm extraction predicts sperm retrieval in patients with non-obstructive azoospermia

E. CAROPPO[1,2], E. M. COLPI[3], F. CASTIGLIONI[4], G. GAZZANO[5], L. VACCALLUZZO[3], E. PIATTI[3,4], G. D'AMATO[1,2] AND G. M. COLPI[3,4]

[1]Asl Bari, Department of Maternal and Child Health, Reproductive and IVF Unit, PTA "F Jaia", Conversano (Ba), Italy; [2]The Pelvis as a Mother Foundation, Bari, Italy; [3]Andrology and IVF Unit, Clinica San Carlo, Paderno Dugnano (MI), Italy; [4]Andrology Unit, Procrea Institute, Lugano, Switzerland; [5]Division of Anatomic Pathology, Istituto Auxologico Italiano IRCCS, Milano, Italy

Background: MicroTESE proved to be the gold standard surgical approach to patients with Non-Obstructive Azoospermia (NOA), but sperm retrieval rates vary considerably among centers. Few studies, most of them with small sample size, demonstrated that the finding of dilated tubules during MicroTESE was associated with a higher chance of successful sperm retrieval, but none provided diagnostic accuracy measures in support of these findings.

Objective: The present study sought to verify the diagnostic accuracy of the pattern of seminiferous tubules caliber in predicting the sperm retrieval in NOA patients.

Methods: The present cross-sectional study was conducted in the same location on 143 NOA patients undergoing unilateral (64) or bilateral (79) MicroTESE. Assessment of sperm retrieval was evaluated as per testis ($N = 222$). During MicroTESE, if present, dilated tubules (DT) were retrieved, otherwise any tubule whose caliber was greater than the surrounding ones at maximum magnification was taken (slightly dilated tubules – SDT). When no DT or SDT were found, non-dilated tubules (NDT) were excised according to a sort of mapping. A fragment of one or more of the tubules of the same diameter (DT, SDT or NDT) was fixed in Bouin's solution and sent for histological analysis.

Results: Sperm were retrieved in 83 out of 143 patients (58.0%), and in 95 out of 222 testes (42.8%). Successful sperm retrievals were found in 90% of DTs, in 47% of SDTs, and in only 7% of NDTs ($p < 0.0001$). The median number of sperm retrieved was significantly higher in DTs compared to SDTs and NDTs (5.000, IQ range 9.000–300.000, 1.000, IQ range 500–450.000, 500, IQ range 500 respectively; $p < 0.0001$). Stepwise binary logistic regression for SSR prediction excluded FSH, LH, testosterone and testicular volume from the model; on the other hand the tubules caliber pattern allowed the correct classification of 82.4% of testes [χ^2 133.731, $p < 0.0001$, intercept odds ratio (IOR) 0.84], while histology classified 72.7% of them (χ^2 63.329, $p < 0.0001$, IOR 38.9), with a good (AUC 0.89) and fair (AUC 0.70) diagnostic accuracy respectively, as demonstrated by ROC AUC estimate computed on the predictive probabilities. The combination of both variables, however, explained the results in terms of SR in 86.7% of cases (χ^2 156.749, $p < 0.0001$, IOR 28.4,) with an excellent diagnostic accuracy (0.93). Stepwise binary logistic regression for the prediction of seminiferous tubules pattern individuated testis histology as the only significant predictor (χ^2 33.843, $p < 0.0001$), although with a poor diagnostic accuracy (AUC 0.67). Limitations: The seminiferous tubules pattern was subjectively evaluated, but the skill and experience (more than 900 MicroTESE procedures performed to date) of the surgeon can account for the reliability of the results.

Conclusion: Our study provides reliable accuracy measures in support of the relationship between seminiferous tubules caliber pattern and SR in patients with NOA. The experience of the microsurgeon proves to be a crucial factor in being able to identify those tubules that have a caliber even slightly increased at the maximum magnification of the microscope (SDT).

P030

Human Papillomavirus is not an etiological factor in azoospermia

J. FEDDER[1], D. ØRNSKOV[2], B. ENGVAD[3], T. K. KRISTENSEN[3], N. MARCUSSEN[3] AND M. WALDSTRÖM[2]

[1]Centre of Andrology & Fertility Clinic, Odense University Hospital, Odense, Denmark; [2]Department of Pathology, Sygehus Lillebælt (Vejle), Vejle, Denmark; [3]Department of Clinical Pathology, Odense University Hospital, Odense, Denmark

Background: Azoospermia remains unexplained in more than 20% of azoospermic men (Fedder et al., 2004). HPV has been found in the testis of 6.5% of 185 men with non-obstructive azoospermia (Martorell et al., 2005). Additionally HPV has been found in the semen of infertile and healthy, fertile men in the range of 2–30% (Laprise et al., 2014).

Methods: Ninety-eight azoospermic, two cryptozoospermic, and 43 normal, healthy men undergoing vasectomy had a TruCut testis biopsy (Fedder, 2011). Biopsies from the azoospermic and cryptozoospermic men were divided into three pieces: one examined immediately for presence of sperm, one fixed in Bouin's fixative and examined by routine histological examination, and the final one used for HPV analysis. Thus, genital skin and a testis biopsy from each of the azoospermic men and genital skin, semen, Vas deferens and a testis biopsy from each of the healthy men undergoing vasectomy were analysed for the presence of HPV using the INNO-LiPA assay, which detects 32 of the most important HPV types. For the normal, healthy med, ejaculate volumes and sperm concentrations were read – using a Makler chamber.

Results: HPV was not detected in any testis tissue sample from normal, healthy or azoospermic men, although HPV was detected on the genital skin, in ejaculates and in Vas deferens of 28 (65%), 15 (35%), and 3 (7%) respectively, in the normal, healthy men. A very high concordance of the specific HPV genotypes on the genital skin and in the semen was found. Thus, in 13 of 15 men (87%) with HPV-positive semen samples, HPV was also detected on the skin swab, and in 11 men (73%) identical HPV types were found in the two locations. HPV was detected on the genital skin from 69% of the azoospermic men. For the normal, healthy men, neither sperm concentration nor total sperm count could be associated to the presence of HPV in the semen.

Conclusion: Testicular HPV infection should probably not be considered as an etiological factor in men with unexplained, non-obstructive azoospermia. Seminal HPV is due to contamination from the body surface. Sperm count is not associated to presence of HPV in the semen.

P031

Effects of physical exercise on metabolic syndrome-associated hypogonadotropic hypogonadism and erectile dysfunction

M. MAGGI[1], S. FILIPPI[1,2], P. COMEGLIO[1], I. CELLAI[1], E. SARCHIELLI[3], C. CORNO[1], M. ZIZZA[1], G. RASTRELLI[1], L. VIGNOZZI[1] AND A. MORELLI[3]

[1]Sexual Medicine and Andrology Unit, Department of Experimental and Clinical Biomedical Sciences Mario Serio, University of Florence, Florence, Italy; [2]Interdepartmental Laboratory of Functional and Cellular Pharmacology of Reproduction, Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA); [3]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

Background: Metabolic Syndrome (MetS) is a cluster of clinical conditions, associated to an increased cardiovascular (CV) and metabolic risk along with hypogonadism (HG) and erectile dysfunction (ED). Lifestyle modifications (including physical exercise, PhyEx) are well-known treatments for this condition. In particular, in clinical trials PhyEx was able to ameliorate ED and increase T levels. We previously established a rabbit model of MetS that recapitulates the human phenotype, including HG and ED. We now report studies on the effect of PhyEx on hypothalamus-pituitary-testis (HPT) axis and penile relaxation (as a readout of ED).

Methods: We used a non-genomic, high-fat diet (4% peanut oil and 0.5% cholesterol)-induced rabbit model of MetS (HFD rabbit). Rabbits fed a regular diet were used as controls (RD). RD and HFD rabbits were exercise-trained to run on a treadmill for 12 weeks (RD + PhyEx and HFD + PhyEx).

Results: HFD rabbits showed typical metabolic and CV features of MetS along with hypogonadotropic HG (reduced testosterone (T) and LH) and a reduction of androgen-dependent tissue weights (prostate and seminal vesicle). Immunostaining for GnRH was reduced in the HFD arcuate nucleus (ARC). In addition, genes related to inflammation (COX2, IL6, CD68), glucose metabolism (GLUT1, GLUT4, IRS-1), estrogen action (ERb, GPR30) were increased in HFD rabbits. Genes coding for inhibitory factors for GnRH were also increased, as NPY. Within the testis, HFD down-regulated LH receptor and all the steroidogenic enzymes leading to T synthesis, along with an increase in fibronectin expression. PhyEx completely restored T and LH plasma levels, prostate weight and GnRH immunostaining, doubling its gene expression in the ARC. All the aforementioned HDF-induced increase in genes related to inflammation, estrogen signaling and glucose metabolism in ARC were significantly reduced in HFD+ PhyEx, along with a decrease in MCP-1 and its receptor (CCR2), TNFα receptor and GLUT3, with the exception of IL6. In the ARC, PhyEx decreased orexigenic and GnRH-inhibiting factors (dynorphin and its receptors OPRD1 and OPRK1), whereas increased anorexigenic ones (POMC). Kiss1 receptor immunostaining, decreased by HFD, was restored by PhyEx. Within the testis, genes related to T formation (17βHSD3) and metabolism (5α-reductase 1) were increased by PhyEx, while fibronectin expression was normalized. Corpora cavernosa (CC) strips from HFD rabbits showed a hypo-responsiveness to

acetylcholine (Ach) and electrical field (EF) stimulation. In addition, sildenafil action on EF- or sodium nitroprusside-induced relaxation was also impaired in HFD CC. PhyEx reverted all these alterations. In CC extracts, several genes related to NO formation (DDAH1) and signaling (GCSa1, GCSb1, PDE5, PKG) were up-regulated by PhyEx, along with those involved in smooth muscle differentiation (SM22, αSMA) and androgen action (AR, STAMP2).
Conclusion: In this experimental model, endurance training (PhyEx) completely reverted MetS-induced hypogonadotropic hypogonadism and ED, having beneficial effects on the HPT axis and on the penis. In the hypothalamus PhysEX reduced HFD-induced inflammation, in the testis reduced fibrosis and in the penis allows better relaxation and response to sildenafil. Hence, aerobic exercise training can be considered an interesting strategy to combat MetS-associated hypogonadism and ED.

P032

Effects of testosterone replacement treatment in adipose tissue dysfunction and non-alcoholic hepatic steatosis (NAFLD) in obese patient candidate for bariatric surgery

E. MASEROLI[1], C. CORNO[1], P. COMEGLIO[1], I. CELLAI[1], S. FILIPPI[2], T. MELLO[1], A. GALLI[1], E. FACCHIANO[3], B. BELTRAME[3], M. LUCCHESE[3], M. MAGGI[1] AND L. ÌINDA VIGNOZZI[1]
[1]*Department of Experimental Biomedical Sciences and Clinics "Mario Serio", University of Florence, Italy;* [2]*Interdepartmental Laboratory of Functional and Cellular Pharmacology of Reproduction, University of Florence, Italy;* [3]*General Surgery, Bariatric, Metabolic, Santa Maria Nuova Hospital, Florence, Italy*

Background: In several clinical and experimental studies, testosterone replacement treatment of hypogonadism shows a beneficial effect on excessive visceral fat accumulation. Animal models also suggest that in vivo administration of testosterone (T) is able to restore the differentiation ability of preadipocytes, improving their sensitivity to insulin and reducing fat accumulation at the hepatic level, the process underlying the development of steatosis hepatopathy alcoholic (NAFLD).
Objective: To analyze the effects of testosterone replacement therapy in adipose tissue dysfunction and NAFLD in obese patients undergoing bariatric surgery.
Methods: Based on the presence of hypogonadism, with or without associated symptoms, obese patients candidates for bariatric surgery were divided into 3 groups: eugonadal (n = 8), untreated hypogonadal (n = 37) and symptomatic hypogonadal treated for 6–8 months with testosterone undecanoate 1000 mg i.m. every 12 weeks (n = 13). The treated patients underwent surgery immediately after the end of treatment. During bariatric surgery, samples of adipose tissue were taken, from which pre-adipocyte cell primary cultures (hPAD) were isolated and used to evaluate: (i) insulin sensitivity through glucose uptake; (ii) adipogenic potential through RT-PCR analysis of adipogenic genes mRNA; (iii) mitochondrial function by fluorescence microscopy analysis. NAFLD was evaluated by interleukin-10 (IL-10) mRNA analysis, analysis of mRNA ratio of M1/M2 macrophage markers and histological study of liver biopsies.
Results: Treatment with T significantly increases the insulin-sensitivity in the hPADs and the gene expression of STAMP2. It also significantly increases the expression of genes involved in brown adipogenesis, also compared to eugonadal samples. The mitochondria present in the hPADs of hypogonadal patients are dispersed, aggregated and fragmented. Treatment with T, instead, seems to re-establish a mitochondrial network in a continuous balance between fission and fusion. The T also significantly reduces the production of superoxide radicals. Finally, treatment with T induces a significant reduction of steatosis and inflammation in the liver, underlined by the increase in IL-10 mRNA and by the reduction in the ratio of macrophage markers M1/M2.
Conclusion: Our data suggest that treatment with T, in obese hypogonadic male patients, has a protective role on the progression of NAFLD, also inducing a metabolically healthier phenotype in hPADs.

P033

A fluorescence sensing approach to study the efficacy in vitro of PDE5i

L. CASARINI[1,2], L. RICCETTI[1], S. LIMONCELLA[1], S. SPERDUTI[1], C. LAZZARETTI[1], F. BARBAGALLO[3], I. MARTINELLI[4], M. SOLA[4], M. SIMONI[1,2,5] AND G. D. ROCCO[4]
[1]*Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy;* [2]*Center for Genomic Research, University of Modena and Reggio Emilia, Modena, Italy;* [3]*Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy;* [4]*Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy;* [5]*Azienda Ospedaliero-Universitaria di Modena, Modena, Italy*

Background: Phosphodiesterase 5 enzyme (PDE5) catalyzes the hydrolysis of cGMP mediating smooth muscle relaxation resulting in penile erection. PDE5 inhibitors (PDE5i), sildenafil, vardenafil and tadalafil, are used for treatment of male infertility and bind the PDE5 catalytic subunit, resulting in intracellular cGMP accumulation.
Objective: The aim of this study is to evaluate the efficacy of PDE5 inhibitors (PDE5i), tadalafil, sildenafil and vardenafil, evaluating their effect in experimentally-induced steroidogenesis through a new Forster Resonance Energy Transfer (FRET)-based method.
Methods: Steroidogenic mouse tumor Leydig cells mLTC1 and the HEK293 cell line were transiently transfected using the PDE5 catalytic subunit-expressing plasmid. This protein is fused with a 6-amino-acid motif CCPGCC (TC) at the C-terminus (PDE5C-TC) to be labeled with FlAsH-EDT2 administered to transfected cells. In order to evaluate the PDE5C-cGMP interaction, increasing concentrations (1–30 μM) of cGMP labeled with rhodamine (cGMPS-rhodamine) were added to cell media. FlAsH (Donor) was excited at 473 nm, resulting in a donor (D; λem = 528 nm) to acceptor (A; λem = 605 nm), energy transfer, due to the physical proximity of the two molecules at the intracellular level. The occurrence of FlAsH

(D)-to-rhodamine (A) FRET in PDE5C-TC-FlAsH/cGMPS-rhodamine complexes was demonstrated by the increase of the A/D ratio following a Hill trend for both cell lines. Control experiments were performed in cells transfected using plasmid generating untagged PDE5C-WT, which is unable to bind FlAsH. The FRET displacement for PDE5C-TC-FlAsH/cGMPS-rhodamine complex by PDE5i was then evaluated over 10 min by administration of increasing PDE5i concentrations to PDE5C-TC/WT-expressing cells, in the presence of fixed doses of FlAsH-EDT2 (0.5 μM) and cGMPS-rhodamine (9 μM). Final validation was performed by evaluation of cAMP production, extracellular-regulated kinase 1,2 and cAMP-responsive element binding-protein phosphorylation (pERK1/2; pCREB), as well as progesterone and testosterone synthesis in 1 pM–1 μM luteinizing hormone (LH)- and choriogonadotropin (hCG)-stimulated mLTC1 cells, in the presence of PDE5i.

Results: In transfected HEK293 cells, PDE5C-cGMP dissociation constant (Kd) was estimated as 6.0 μM ($n = 4$), while in mLTC1 cells was 15.0 μM ($n = 4$). Displacement experiments were performed using the lowest cGMPS-rhodamine concentration higher than the Kd (9.0 μM in HEK293; 18.0 μM in mLTC1 cells) in the presence of 0.5 μM sildenafil, vardenafil or tadalafil. All PDE5i displaced PDE5C from cGMP within 5 min except sildenafil, which resulted in longer action (10 min; two-way ANOVA; $p < 0.05$; $n = 4$). The dose-dependent analysis of the displacement of the PDE5C-cGMP complex by sildenafil allowed the determination of the binding affinity (Ki) of 8.0 nM ($n = 5$) in transfected HEK239. In mLTC1 cells, the presence of PDE5i is linked to similar cAMP increase, as well as pERK1/2 and pCREB activation, not depending on the presence of LH/hCG (two-way ANOVA; $p < 0.05$; $n = 5$). Progesterone production is PDE5i-dependent as well (two-way ANOVA; $p < 0.05$; $n = 5$). Treatment with 1 pM–1 μM LH/hCG induced progesterone-to-testosterone conversion in LH/hCG-treated cells, compared to gonadotropin-untreated mLTC1 (two-way ANOVA; $p < 0.05$; $n = 5$).

Conclusion: Results indicated that PDE5i are linked to different kinetics of PDE5C-cGMP displacement, however resulting in similar cAMP accumulation and steroid synthesis. We have developed a new FRET-based sensor for characterization of PDE5i efficacy in vitro.

P034

Isolation, culture and characterization of rat epididymal epithelial cells: an in vitro model to study epididymal functions

K. SANGEETA
University of Hyderabad, Hyderabad, Telangana, India

Background: The epididymis provides microenvironment for sperm maturation, storage and maintenance. The molecular mechanism that governs these events are poorly defined. Though animal models are available to study these functions, an in vitro model are lacking.

Objective: Hence we attempted to standardise the method for isolation and maintenance of rat epididymal primary cells.

Methods: Using standard digestion procedures epididymal cells were isolated from the caput epididymides of adult rats and primary cultures maintained.

Results: The isolated cells displayed adherent properties and proliferated in essential Eagle's medium. We successfully maintained them for more than 2 weeks without contamination. Further, the cells were subcultured and the new cultures were grown without contamination. The identity of the isolated cells was confirmed by analysis the expression of caput specific genes namely Cysteine Rich Secretory Protein (CRISP1), Androgen Receptor (AR) and Sperm Associated Antigen 11 E (SPAG11E) by RT PCR and western blotting.

Conclusion: Results of this study provide method to isolate epididymal epithelial cells which are an excellent in vitro model to study epididymal function.

P035

Are the cavernous tissue and serum levels of micro RNAs 200a and 206 elevated in patients with refractory veno-occlusive erectile dysfunction? A comparative study

S. F. G. ELDIN, L. A. RASHED, H. A. ALGHOBARY, L. T. TAWFIK AND M. S. ELSHEEMY
Cairo University, Egypt

Objective: To evaluate the association between miRNAs and veno-occlusive erectile dysfunction. Recently, this association between miRNAs and erectile dysfunction was extensively studied using animal models. Our aim was to explore the miRNAs expressions and functions in the development of erectile dysfunction, especially veno-occlusive dysfunction, using a human tissue.

Methods: We prospectively recruited 60 patients with erectile dysfunction and controls between July 2015 and July 2016. The 30 patients had refractory veno-occlusive erectile dysfunction that was proven by investigations. They were scheduled for penile implant. The 30 controls were scheduled for repair of their fracture. We measured miRNAs (200a and 206) and nitric oxide in cavernous tissue and serum of both patients with erectile dysfunction and controls.

Results: A significant association was found between the 2 mentioned miRNAs and erectile dysfunction ($p < 0.001$). Mean level of nitric oxide in cavernous tissue of the controls was significantly higher than that in the patients ($p < 0.001$). miRNA 200a showed a cutoff value of 1.135 with 95% sensitivity and 100% specificity, whereas miRNA 206 showed a cutoff value of 1.125 with 100% sensitivity and 100% specificity.

Conclusion: To the best of our knowledge, our study is the first report to measure the level of miRNAs in the cavernous tissue, using a human tissue. Furthermore, this study can be considered a good step of deploying miRNAs through a blood test to detect early negative changes that lead to erectile dysfunction. Finally, we recommend more studies.

P036

gr/gr deletion predisposes to Testis Cancer independently from altered spermatogenesis

D. MORENO-MENDOZA[1], E. CASAMONTI[2], A. RIERA-ESCAMILLA[1], S. DEGL'INNOCENTI[2], M. G. FINO[2], F. CIOPPI[2], E. RUIZ-CASTAÑE[1] AND C. KRAUSZ[2]

[1]Andrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, IIB-Sant Pau, Barcelona, Spain; [2]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", Centre of Excellence DeNothe, University of Florence, Florence, Italy

Background: The most common cancer in young men is Testicular germ cell tumor (TGCT). TGCT is considered a multifactorial pathology with a strong genetic predisposition. Only a few common genetic variants have been identified so far, accounting for about 20% of all expected genetic factors. A large multicenter study proposed the Y chromosome-linked gr/gr deletion as genetic risk factor for TCGT. This type of deletion removes half of the gene content of the AZFc region (including two copies of DAZ and one copy of CDY1, as well as several other transcription units) and it is considered also a genetic risk factor for impaired sperm production. Sperm parameters were not available in the large multicenter study hence it remains an open question whether the observed association between gr/gr deletion and TGCT is related to disturbed spermatogenesis.

Objective: To evaluate the role of AZFc gene dosage (deletions gr/gr) in patients affected by TGCT with known sperm count and full clinical characterization.

Methods: Five hundred patients with TGCT and 1335 controls (1055 normozoospermic and 941 infertile non –normozoospermic patients) visited at the Fundació Puigvert and the University Hospital of Careggi. Genetic testing was based on a multiple steps analysis: (i) PCR plus/minus analysis of sY1291 and sY1191 followed by confirmatory steps; (ii) gene dosage of CDY1 and DAZ by fluorescent semi quantitative analysis (Genescan software); (iii) RFLP analysis for the definition of the missing CDY/DAZ copies.

Results: 2.8% of the patients with TGCT presented gr/gr deletion vs. 0.95% of normozoospermic control group ($p = 0.009$). The presence of gr/gr deletion was significantly higher in patients with normozoospermic TGCT than in normozoospermic controls (OR 3.54; 95% CI 1.42–8.81; $p = 0.006$). gr/gr deletion frequency in patients with non-normozoospermic TGCT was similar to non-normozoospermic control patients (2.2% and 3.2%, respectively). The presence of gr/gr deletion was higher in patients with seminoma (3.2%) than in those affected by non-seminoma germ cell tumor (2.1%), without reaching statistical significance. We found statistically significant differences between the incidence of gr/gr deletion in patients with seminoma vs. normozoospermic controls ($p = 0.006$) with an OR 3.3; 95% CI 1.1–7.91.

Conclusion: Our data provide evidence that the gr/gr deletion is a risk factor for TGCT also in the Italian and Spanish populations and normozoospermic gr/gr deletion carriers have a 3 fold increased risk to develop TGCT. The observed significant association between gr/gr deletion and normozoospermic TGCT, suggests that gr/gr deletion is an independent risk factor from impaired spermatogenesis.

P038

Androgen receptor expression in dartos tissue of congenital penile malformations and controls

L. J. W. TACK[1], M. PRAET[2], J. V. DORPE[2], S. BUELENS[3], P. HOEBEKE[3], E. V. LAECKE[3], M. COOLS[1] AND A.-F. SPINOIT[3]

[1]Department of Pediatric Endocrinology and Diabetology, Ghent University Hospital, Ghent, Belgium; [2]Department of Pathology, Ghent University Hospital, Ghent, Belgium; [3]Department of Pediatric Urology, Ghent University Hospital, Ghent, Belgium

Background: Many mechanisms have been suggested to influence the development of congenital penile malformations (CPM) such as hypospadias and buried penis (BP). However, the role of androgen receptor (AR) expression remains controversial as both higher and lower levels have been reported as compared to controls.

Objective: To quantitatively assess AR expression in smooth muscle fibers of dartos tissue (DT) of a large cohort of patients with hypospadias, BP and circumcision as controls.

Methods: 409 foreskin samples were prospectively gathered: 211 CPM (180 hypospadias, 31 BP) and 198 circumcisions as controls. Immunohistochemical AR staining was quantitatively scored using a modified quick score (mQuicks) (0–6). This score assesses the intensity of AR staining (0 = negative, 1 = weak, 2 = moderate and 3 = strong) and the proportion of stained smooth muscle fibers (0 = none, 1 = <10%, 2 = 10–50%, 3 = >50%). mQuicks obtained in hypospadias and BP were compared with controls and proximal with distal hypospadias in different age groups (6–8 months; 8–24 months; 2–6 years; 6–11 years; >11 years) using a Mann–Whitney *U* test after testing for normality.

Results: AR expression shows a bimodal distribution in both CPM and controls. A first peak is seen between 6 and 12 months, with half of the samples showing positive AR staining (mQuicks ≥2). Since no circumcisions or hypospadias surgery were performed before the age of 6 months, no samples were available to assess if the first peak coincides with onset of minipuberty. Above 6 years, mQuicks increases to reach a maximum during puberty and remains stable thereafter. No significant difference in mQuicks were found between hypospadias, BP and controls or between proximal and distal hypospadias in all age groups.

Conclusion: The mQuicks is a straightforward and informative tool to assess AR expression in DT. AR expression shows a bimodal distribution, coinciding with physiological androgen production, i.e. (mini)puberty. These findings can explain conflicting results in previous studies assessing AR expression in DT of CPM and emphasizes the need for age-matched controls. No differences in AR expression were observed in hypospadias or BP and controls nor between proximal or distal hypospadias.

P039

Long–term testosterone replacement therapy in CHH patients maintains bone density but has only limited osteoanabolic effects

S. CAERELS[1], L. ANTONIO[1,2], E. DELAUNAY[3] AND D. VANDERSCHUEREN[1,2]
[1]*Department of Endocrinology, University Hospitals Leuven, Leuven, Belgium;* [2]*Department of Chronic Diseases, Metabolism and Ageing (CHROMETA), Laboratory of Clinical and Experimental Endocrinology, KU Leuven, Leuven, Belgium;* [3]*Department of Endocrinology, Heilig Hartziekenhuis Leuven, Leuven, Belgium*

Background: Congenital hypogonadotropic hypogonadism (CHH) is a rare condition characterized by complete sex steroid deficiency. Therefore, CHH is a unique human model to study the impact of long term testosterone replacement therapy (TRT). In this single-centre retrospective study, we assessed the short-term and long-term impact of TRT on femoral and lumbar bone mineral density (BMD) in adult CHH men.
Methods: Data from the medical records of 23 patients (age range: 12–41 years.) with CHH were included. Femoral and lumbar BMD was assessed by DEXA and expressed as a T-score.
In six patients (treatment naive group) BMD was measured before start of TRT. The other 17 (pretreated group) had received TRT on average for 8.29 years (median 6.0 years) before first BMD measurement. Men were followed up for a median duration of 20 years. Despite a small increase of lumbar BMD after treatment start, the majority (61%) of patients remained in the osteopenic/osteoporotic range. In spite of this initial increase TRT lumbar and femoral bone density remained stable during prolonged TRT with no further increase in BMD. However, in patients who ceased or interrupted TRT, bone density clearly decreased.
Conclusion: From these data we conclude that the majority of CHH patients that start TRT at an adult age are osteopenic. Despite some initial improvement after starting TRT, BMD remains in the osteoporotic range, but prolonged TRT can prevent further bone loss both at lumbar and femoral level.

P040

Effect of circulating testosterone levels on clinical exercise stress test parameters

B. REZANEZHAD[1], R. BORGQUIST[2], R. WILLENHEIMER[3] AND S. ELZANATY[4]
[1]*Department of Internal Medicine, Skåne University Hospital, Lund University, Lund, Sweden;* [2]*Department of Arrhythmia, Skåne University Hospital, Lund University, Lund, Sweden;* [3]*Heart Health Group, Malmö, Sweden;* [4]*Department of Translational Medicine, Skåne University Hospital, Lund University, Malmö, Sweden*

Objective: To investigate the effect of circulating testosterone levels on clinical exercise stress test parameters based on data from 119 middle-aged men from the general population.
Methods: All subjects underwent a clinical exercise stress test and data on baseline heart rate, maximal heart rate, dyspnea scale, aVF-ST deviations, and V5-ST deviations were collected. Serum testosterone levels were measured. Demographic data regarding age, BMI, waist circumference, smoking, alcohol consumption, and family history of CVD were also collected. Men were classified into two groups based on testosterone levels: hypogonadal (testosterone < 12 nmol/L), and eugonadal men (testosterone ≥12 nmol/L). Statistical analyses were performed using non-parametric Mann Whitney test, and adjusted multivariate regression analysis model.
Results: The subjects had a mean age of 55 years. Baseline heart rate and V5-ST deviations were significantly higher in hypogonadal compared to eugonadal men (75 bpm vs. 69 bpm; $p = 0.02$), (−0.14 mm vs. 0.30 mm; $p = 0.03$), respectively. On the other hand, maximal heart rate, dyspnea scale, and aVF-ST deviations did not differ significantly between groups. In a multivariate regression analysis model adjusted for the age of subjects, BMI, waist circumference, smoking, alcohol consumption, and family history of CVD, testosterone showed a negative significant association only with V5-ST deviations ($\beta = -0.50$, $p = 0.04$; 95% CI = −1.10, −0.02).
Conclusion: Our findings of an inverse effect of testosterone levels on V5-ST deviations suggesting low testosterone as a risk factor of cardiovascular diseases.

P042

Sexual behaviour: experimental German vs. persevering Hungarian medical students

K. M. HÖPPLER[1], O. TAKÁCS[2], M. KRON[1], H. SCHULWITZ[3], J. GSCHWEND[3], M. BENYÓ[4] AND K. HERKOMMER[3]
[1]*Institute of Epidemiology and Medical Biometry, University of Ulm, Ulm, Germany;* [2]*Faculty of General Medicine, University of Debrecen, Debrecen, Hungary;* [3]*Department of Urology, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany;* [4]*Department of Urology, University of Debrecen MHSC, Debrecen, Hungary*

Background: Gender specific differences in the sexual behaviour of medical students have been broadly studied. Differences within gender between countries have not been analysed yet. This study was aimed to investigate differences between medical students in Debrecen (Hungary) and Munich (Germany).
Methods: From January 2017 to January 2018 medical students in Debrecen (D) and Munich (M) completed a questionnaire about several items as demographic characteristics, sexual experiences, sexual activity, sexual health and consumption of pornography anonymously. Student subgroups were compared using *t*-test/Wilcoxon test or chi square test/Fisher's exact test as applicable.
Results: 408 students from Munich (female = 139, male = 116) and Debrecen (female = 91; male = 62) were included. For both genders the median age in D-students was 23 and 24 years in M-students. The median BMI was 23 in all subgroups. Female M-students had more life time sex partners (median D: 3.0; M: 5.0; $p = 0.002$). More than

one sex partner at the same time was noted by 1.3% in Debrecen and 17.7% in Munich. Five M-students (4.2%) had >2 sex partners at the same time ($p < 0.001$), but none in Debrecen. Furthermore, twice as many female M-students had experience with sex toys (D = 23.8%; M = 51.1%; $p < 0.001$) while there was a slight difference in porno consumption ($p = 0.03$). Experience with anal sex was slightly more common in M-students (D = 25%, M = 36.7%; $p = 0.07$). With 30 min the estimated duration of sexual intercourse was 5 min longer in D-students than in M-students ($p < 0.001$). In 80% of sexual intercourses female D-students reached an orgasm, in Munich 50% ($p < 0.001$). 81.2% of D-students were satisfied with their sex life and 73.0% of M-students ($p = 0.197$). The median number of vaginal intercourses in the last 4 weeks among D-students was 6.0 and 5.0 among M-students. Interestingly same trends could be found in male students but with less remarkable results. The median number of life time sex partners was 4.0 in D-students and 5.0 in M-students ($p = 0.512$). The maximal number of sex partners at the same time was higher in M-students. More than one sex partner at the same time was noted by 9.4% in Debrecen and 18.3% in Munich. Seven M-students (6.8%) reported >2 partners at the same time and two (3.8%) in Debrecen ($p = 0.180$). Experience with sex toys was not much different (D = 32.3%, M = 38.8%; $p = 0.388$). D-students estimated hours of porno consumption per week twice as high as M-students (2.0 vs. 1.0; $p < 0.001$). Anal sex was more common in M-students (37.1 vs. 29.0; $p = 0.282$). The estimated duration of sexual intercourse was with 30 min in D-students 10 min longer than in M-students ($p < 0.001$). D-students had more vaginal intercourses during the last 4 weeks (6 vs. 5) and a higher satisfaction with their sexual life (83.0% vs. 70.4%; $p = 0.098$). 21.6% of D-students demanded more sexual education at school in contrast to four times as many M-students (85.0%; $p < 0.001$).

Conclusion: Our findings showed interesting differences in German and Hungarian medical students. In general German students seemed to be more adventurous whereas Hungarian students' sex life was more active, steady and satisfying.

P043

Symptomatic androgen deficiency develops only when both total and free testosterone decline in obese men who may have incident biochemical secondary hypogonadism: prospective results from the EMAS

G. RASTRELLI[1], R. J. A. H. EENDEBAK[2], T. W. O'NEILL[3], T. AHERN[2], G. BARTFAI[4], F. F. CASANUEVA[5], G. FORTI[6], B. KEEVIL[7], A. GIWERCMAN[8], T. S. HAN[9], J. J. SŁOWIKOWSKA-HILCZER[10], M. E. J. LEAN[11], N. PENDLETON[12], M. PUNAB[13], L. ANTONIO[14], J. TOURNOY[15], D. VANDERSCHUEREN[14], M. MAGGI[1], I. T. HUHTANIEMI[16], F. C. W. WU[2] AND THE EMAS STUDY GROUP

[1]Sexual Medicine and Andrology Unit, Department of Experimental Clinical and Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy; [2]Andrology Research Unit, Division of Endocrinology, Diabetes & Gastroenterology, Domain of Cardiovascular, Metabolic and Nutritional Sciences, School of Medical Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, Old St Mary's Building, The University of Manchester, Manchester, UK; [3]Arthritis Research UK Centre for Epidemiology, Division of Musculoskeletal and Dermatological Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, University of Manchester and NIHR Manchester Musculoskeletal Biomedical Research Centre, Manchester University NHS Foundation Trust, Manchester, UK; [4]Department of Obstetrics, Gynaecology and Andrology, Albert Szent-György Medical University, Szeged, Hungary; [5]Department of Medicine, Santiago de Compostela University, Complejo Hospitalario Universitario de Santiago (CHUS); CIBER de Fisiopatología Obesidad y Nutricion (CB06/03), Instituto Salud Carlos III – University Hospital of Santiago de Compostela, Santiago de Compostela, Spain; [6]Endocrine Unit, Department of Experimental Clinical and Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy; [7]Department of Clinical Biochemistry, University Hospital of South Manchester, Manchester, UK; [8]Reproductive Medicine Centre, Malmö University Hospital, University of Lund, Malmö, Sweden; [9]Institute of Cardiovascular Research, Royal Holloway, University of London, Surrey, UK; [10]Department of Andrology and Reproductive Endocrinology, Medical University of Lodz, Lodz, Poland; [11]Department of Human Nutrition, University of Glasgow, Glasgow, UK; [12]Division of Neuroscience and Experimental Psychology, School of Biological Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre Hope Hospital, Salford, UK; [13]Andrology Unit, United Laboratories of Tartu University Clinics, Tartu, Estonia; [14]Laboratory of Clinical and Experimental Endocrinology, Department of Chronic Diseases, Metabolism and Ageing, Katholieke Universiteit Leuven, Leuven, Belgium; [15]Gerontology and Geriatrics, Department of Chronic diseases, Metabolism and Ageing, Katholieke Universiteit Leuven, Leuven, Belgium; [16]Department of Surgery and Cancer, Institute of Reproductive and Developmental Biology, Imperial College London, Hammersmith Campus, London, UK

Objective: Limited evidence supports the use of free testosterone (FT) for diagnosing hypogonadism when sex hormone binding globulin (SHBG) is altered. Low total testosterone (TT) is commonly encountered in obesity where SHBG is typically decreased. We aimed to assess the contribution of FT in improving the diagnosis of symptomatic secondary hypogonadism (SH), identified initially by low total testosterone (TT), and then further differentiated by normal FT (LNSH) or low FT (LLSH).

Methods: Prospective observational study with a median follow-up of 4.3 years. Patients: 3369 community-dwelling men aged 40–79 years from eight European centres. Subjects were categorised according to baseline and follow-up biochemical status into persistent eugonadal (referent group; $n = 1880$), incident LNSH (eugonadism to LNSH; $n = 101$) and incident LLSH (eugonadism to LLSH; $n = 38$). Predictors and clinical features associated with the transition from eugonadism to LNSH or LLSH were assessed.

Results: The cumulative incidence of LNSH and LLSH over 4.3 years was 4.9% and 1.9% respectively. Baseline

obesity predicted both LNSH and LLSH but the former occurred more frequently in younger men. LLSH, but not LNSH, was associated with new/worsened sexual symptoms, including low desire [OR = 2.67 (1.27–5.60)], erectile dysfunction [OR = 4.53 (2.05–10.01)] and infrequent morning erections [OR = 3.40 (1.48–7.84)].

Conclusion: These longitudinal data demonstrate the importance of FT in the diagnosis of hypogonadism in obese men with low TT and SHBG. The concurrent fall in TT and FT identifies the minority (27.3%) of men with hypogonadal symptoms, which were not present in the majority developing low TT with normal FT.

P044

Comparison of testosterone treatment effects in men with classical vs. functional hypogonadism: results from a 9-year-registry

M. ZITZMANN[1], J. CREMERS[1], M. SCHUBERT[1], A. TRAISH[2] AND S. KLIESCH[1]

[1]Centre of Reproductive Medicine and Andrology of the University, Muenster, Germany; [2]Urology and Biochemistry, Boston University School of Medicine, Boston, USA

Background: There are limited data on long-term effects of Testosterone (T) therapy in hypogonadal men and clinical value of this treatment in men with functional (so called "late-onset") hypogonadism remains hotly debated. A long-term registry comprising various groups of patients provides an efficient tool to approach this issue.

Methods: Registry data of max. 9 years comprising 650 patients with hypogonadism including 266 men with primary (mean age 34.0 ± 11.7 years), 196 with secondary (mean age 31.9 ± 12.0 years) and 188 with functional hypogonadism (mean age 42.3 ± 11.3 years) all receiving uniform treatment using intramuscular T undecanoate (1000 mg). Some patients receive this form of treatment since 20 years; these additional data are not part of this evaluation because of the low number of subjects.

Results: The registry contained 8358 time points with a subset of metabolic and safety parameters. Serum T concentrations increased from 5.7 ± 2.3 to 19.4 ± 2.8 nmol/L in men with classical hypogonadism and from 7.8 ± 2.4 to 19.2 ± 3.1 nmol/L in men with functional hypogonadism (difference in delta T: $p < 0.0001$).

There was an initial difference in the distribution of body mass index (BMI): 35.6% of men with classical hypogonadism and 51.6% of men with functional hypogonadism were obese (BMI > 30 kg/m^2, $p = 0.0006$). Changes over time using Kaplan-Meier models revealed fundamental differences in inter-individual effects: men with functional hypogonadism were more likely to lose 10% weight and 5% of waist circumference (WC) than men with classical hypogonadism (hazard ratio 1.3 (1.1–1.4), $p = 0.008$ and hazard ratio 1.4 (1.3–1.5), $p = 0.001$). There was no difference for increase in hematocrit. Changes in PSA levels were more likely to occur in functional hypogonadism [hazard ratio 1.3 (1.1–1.6), $p = 0.003$]. Significantly more pronounced effects of T therapy in functional hypogonadism could also be attributed to changes in parameters of lipid (levels of total cholesterol, triglycerides, LDL- and HDL-cholesterol) and glucose metabolism. Stepwise multiple Cox regression models could attribute these differences to the initial higher values in BMI, WC, lipids, glucose and age found in functional hypogonadism. The condition as such rather attenuated the effects of T therapy compared to those seen in classical hypogonadism, due to the lower increase of T concentrations during treatment.

Conclusion: This study provided major new findings regarding effects and safety of T therapy in different groups of hypogonadal men. Effects on factors influencing cardiovascular health are modulated by diagnosis and age. Patients with functional hypogonadism may experience greater benefits from T therapy; this being a function of their initially worse status in cardiovascular risk factors compared to men with classical forms of hypogonadism.

P045

"Healthy obesity" is a new risk factor for men with erectile dysfunction or couple infertility

G. CORONA, F. LOTTI, G. RASTRELLI, E. MASEROLI, S. CIPRIANI AND M. MAGGI

Sexual Medicine and Andrology Unit, University of Florence, Italy

Background: Obesity is a cause of erectile dysfunction (ED) whereas its relationship with male infertility is conflicting. The term "healthy obesity" (HO) has been used to describe an obese phenotype without the burden of any metabolic disorder.

Objective: The aim of this study was to analyze the contribution of HO in the pathogenesis of ED and its impact on male reproductive health, and to verify the value of HO in predicting major adverse cardiovascular events (MACE).

Methods: An unselected series of 4382 (51.4 ± 13.1 years) men with sexual dysfunction (SD) and a consecutive series of 231 (37.9 ± 9.1 years) males of infertile couples without genetic abnormalities were studied. A subset of men with SD ($n = 1687$) was enrolled in a longitudinal study. Several clinical and biochemical parameters were evaluated. Erectile function was assessed using the IIEF-15-erectile function domain in all subjects. In addition, prostatitis like-symptoms and benign prostatic hyperplasia (BPH)-related symptoms were assessed in men with couple infertility with the NIH-CPSI and the IPSS, respectively. All subjects underwent penile colour-Doppler ultrasound (PCDU) in flaccid conditions. Males of infertile couples underwent scrotal and transrectal ultrasound and semen analysis including interleukin 8 (sIL-8), a marker of genital tract inflammation. HO was defined as the presence of body mass index >30 kg/m^2, HDL > 40 mg/dL and absence of diabetes or hypertension. The rest of the obesity sample was defined as "complicated obesity" (CO). The characteristics of men with HO or CO were compared with those of healthy normal weight individuals (HNWI).

Results: Among the patients with SD, 723 (16.5%) were obese, 163 (3.7%) with HO and 560 (12.8%) with CO. Among males of infertile couples, 68 (28.4%) were obese, 19 (8.2%) with HO and 49 (21.2%) with CO. After adjusting for confounders, in both samples, either subjects with HO or CO showed lower total testosterone (TT) levels when

compared to HNWI, while no difference in TT levels was observed between HO and CO. In both samples, men with CO, but not those with HO, reported a worse erectile function when compared to HNWI. At PCDU, in both samples, peak systolic velocity was lower in both HO and CO when compared to HNWI. In the longitudinal study, both HO and CO were independently associated with a higher incidence of MACE [HR = 4.800 (1.265;18.214) and HR = 3.041 (1.078;8.573), respectively; both $p < 0.05$], when compared to the rest of the sample. Evaluating men with couple infertility, no differences in seminal parameters, prostatitis-like symptoms or BPH-related symptoms were observed among groups. However, when compared to HNWI, subjects with CO and HO showed a higher prostate volume, and men with CO also a higher risk of ultrasound and biochemical (sIL-8) features of prostatic inflammation.

Conclusion: HO and CO induce subclinical and clinical ED, respectively, irrespective of TT levels, and are associated with an increased cardiovascular risk. HO and CO are not associated with semen abnormalities, but they are both associated with prostate enlargement, and CO even with signs of prostate inflammation. Hence, HO represents an early risk factor for both sexual and prostatic health.

P046

Prediction of erectile dysfunction and cardiovascular diseases based on the risk factors profile

O. I. APOLIKHIN[1], E. A. EFREMOV[1], I. A. SHADERKIN[1], O. V. ZOLOTUHIN[2], Y. Y. MADYKIN[2], M. M. ZELENSKY[1] AND S. S. KRASNYAK[1]

[1]N.Lopatkin Scientific Research Institute of Urology & Interventional Radiology – Branch of the Federal State Budgetary Institution "National Medical Research Center of Radiology" of the Ministry of Health Care of Russia, Moscow, Russia; [2]Voronezh State Medical University named after N.N. Burdenko, Voronezh, Russia

Background: Cardiovascular diseases (CVD) are the leading cause of death worldwide. According to literature, CVDs have a very similar profile of risk factors with such a common reproductive disorder as erectile dysfunction (ED). At the same time, there are different opinions about the prevalence of ED and lower urinary tract symptoms (LUTS) in the Russian population and their relationship to physical illnesses.

Methods: We present data from evaluation of 1261 men aged 40–65 years living in the Voronezh and Kaluga regions. Doctors in words of the patients filled in the IPSS and IIEF-5 questionnaires. In addition to these questionnaires, anamnestic data, the results of anthropometry (height, weight, body mass index (BMI), waist circumference), as well as the results of laboratory research methods, a blood test for total cholesterol, and blood glucose were recorded in the electronic registration system. The statistical analysis was carried out using PS ClementinePro™ 1.8 software (Predictive Solution) based on the IBM SPSS Modeler™ 18 platform (IBM Corporation). In addition to conventional statistics we used methods of predictive analytics (CHAID algorithm, neural network).

Results: Prevalence of ED was 51.04%. In this case, a mild ED is observed in 38% of men, moderate and severe ED – in 10.5% and 2.5%, respectively. After applying the algorithm of CHAID decision tree, we found that the most significant risk factor for ED occurrence is the severity of LUTS (an importance factor of 0.64), then with a significant lag follow the waist circumference (0.08), cholesterol level (0.05), hypertension (0.05). The accuracy of the prediction of the trained neural network was 81.9%. Comparison of the predictions of both models showed the agreement coefficient between the models at 84.87%. Predictors of development of arterial hypertension were pulse frequency, severity of LUTS age and body weight.

Conclusion: The use of predictive analytics methods (algorithm CHAID, neural network) allowed to generate an algorithm for predicting the development of cardiovascular diseases with an accuracy of 80.3%. The use of predictive analytics methods allows predicting the development of CVD in patients with reproductive disorders.

P049

Seminal carnitine level and DNA fragmentation index, with high accuracy, impact progressive sperm motility in idiopathic oligoasthenozoospermic men treated with metabolic and essential nutrients

S. MICIC[1], N. LALIC[1], N. BOJANIC[2], U. KENIC[3], G. M. BUSETTO[4], A. VIRMANI[5] AND A. AGARWAL[6]

[1]Department of Andrology, Uromedica Polyclinic, Belgrade, Serbia; [2]Clinical Center of Serbia, Urologic Clinic, Belgrade, Serbia; [3]Bel Medic Hospital, Belgrade, Serbia; [4]Department of Urology and Gynecology, "La Sapianza" Universiti, Roma, Italy; [5]Research and Development department, Sigma tau Health Science, Utrecht, The Netherlands; [6]American Institute for Reproductive Medicine, Andrology Department, Cleveland, OH, USA

Background: Sperm DNA damage has been associated with adverse reproductive outcomes. Carnitines (L-acetyl carnitine, ALC, and L-carnitine, L-C) are essential compounds in cellular energy metabolism.

Objective: The goal of this randomized, double-blind, placebo-control study was to correlate DFI, seminal carnitine level and progressive sperm motility in a group of 175 oligoasthenozoospermic men treated with test formulation (Proxeed Plus-Sigma tau HealthScience, Utrecht).

Methods: The protocol was 2 wash-out and 6 months treatment (T-2, T0, T + 3, T + 6), with test formulation (125 patients) or placebo (50 patients). Analysis of ejaculate was done according to WHO 5th guideline. DFI was evaluated by Halosperm kit (Halotech DNA, S.L).

Results: The seminal plasma carnitine at T0 was 700.50 μmol/L (625.50 ± 800.00) and at T6 = 751.50 μmol/L (671.10 ± 896.80), and this difference was significant ($p = 0.014$, by Wilcoxon signed-rank test). DFI (%): T0 = 38.50 (32.00–48.75), T3 = 35.50 (25.50–44.00) and T6 = 31.00 (25.00–41.00) (Friedman test, $p < 0.001$). The Spearman's correlation test showed that, if DFI drops by more than 3%, after 6 months of therapy, it can be expected, with moderate accuracy, that men have sperm motility greater than 10% (AUC = 0.793; $p < 0.001$). DFI reduction (odds ratios = 1.106 with 95% confidence

intervals) independently of elevation carnitine, increases the likelihood that sperm motility is >10%. The correlation of seminal plasma carnitine and progressive sperm motility showed that an increase of seminal carnitine of 7.7%, after 6 months therapy, would impact progressive sperm motility >10% with moderate accuracy (AUC = 0.713). There was no significant difference in placebo group, in sperm motility, seminal carnitine and DFI, between T0 and T6.

Conclusion: This study showed that the change in DFI can be used in detection of men with better sperm motility after 6 months therapy. The increase of seminal carnitine positively impacted upon the patient progressive sperm motility.

P050

The involvement of the chemokine RANTES in regulating luminal acidification in the rat epididymis

Z. LI, B.-F. MA, X. FENG, P. CHENG, J.-H. WEI AND J. ZHAO

Department of Histology and Embryology, Fourth Military Medical University, Xi'an, Shaanxi, China

Background: A complex interplay between the different cell types present in the epithelium leads to activation of the luminal acidifying capacity of the epididymis, a process that is crucial for sperm maturation and storage. Basal cells sense the luminal angiotensin II (ANG II) and stimulate proton secretion in clear cells through nitric oxide (NO), which can theoretically be secreted by macrophages. Our previous study has shown the chemokine regulated upon activation normal T-cell expressed and secreted (RANTES) was mainly expressed in the macrophage of human epididymis.

Objective: To explore the involvement of RANTES in regulating the luminal acidification in the rat epididymis. This study first examined the expression of RANTES and its receptors in rat epididymis, and then established rat vasectomy to alter the luminal pH in the epididymis. RIA was used to measure the tissue homogenate ANG II concentration.

Methods: Real time-PCR and western blot were used to detect the expression levels of AGTR2, RANTES, CCR1, CCR5 and iNOs in rat epididymis. Moreover, the potential role of RANTES in the establishment and maintenance of the luminal acidification was explored by using in vivo perfusion.

Results: The results showed that RANTES was restricted to the macrophages of rat epididymal ducts and co-localized with its receptors CCR1 and CCR5. The concentration of the ANG II and the expression levels of AGTR2, RANTES, CCR1, CCR5 and iNOs were all significantly reduced in rat epididymis following vasectomy. In particular, the signal of iNOs was detected in the macrophage of rat epididymal ducts. Upon alkaline perfusion, the expression of RANTES was significantly increased and the apical accumulation of V-ATPase in the clear cells was induced. Both V-ATPase and iNOs were up-regulated by the addition of recombinant RANTES, while Met-RANTES perfusion led to a complete abrogation of the increased expression of V-ATPase and iNOS in the epididymal epithelium.

Conclusion: Our data suggest that activation of AGTR2 in basal cells by luminal ANG II may stimulate RANTES in macrophages to induce the production of NO, which increases proton secretion by adjacent clear cells. RANTES may act through its specific receptors CCR1 and CCR5. Thus, RANTES is possible to participate in the crosstalk between basal cells, macrophages and clear cells for the fine control of an optimum acidic luminal environment that is critical for male fertility.

P051

Effects of percutaneous varicocele repair on testicular volume: results from a 12-months follow-up

A. SANSONE[1], D. A. FEGATELLI[2], C. POZZA[1], G. FATTORINI[1], R. LAURETTA[3], M. MINNETTI[1], F. ROMANELLI[1], P. LUCATELLI[4], M. CORONA[4], M. BEZZI[4], F. LOMBARDO[1], A. LENZI[1], A. M. ISIDORI[1] AND D. GIANFRILLI[1]

[1]Department of Experimental Medicine, Section of Medical Pathophysiology, Food Science and Endocrinology, Sapienza – University of Rome, Rome, Italy; [2]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy; [3]Unit of Endocrinology, Regina Elena National Cancer Institute, Rome, Italy; [4]Vascular and Interventional Radiology Unit, Department of Radiological Oncological and Anatomo-pathological Sciences, Sapienza – University of Rome, Rome, Italy

Background: Varicocele is a common finding in men. Varicocele correction has been advocated for young patients with testicular hypotrophy, but morphofunctional follow-up data are lacking.

Objective: Assess whether percutaneous treatment of left varicocele is associated with testicular "catch-up growth" in the following 12-months.

Methods: We retrospectively reviewed data entered in an electronic database of 10 656 patients followed in our Clinic between 2006 and 2016. We selected all young adults (<35 years) with left varicocele who underwent percutaneous treatment, had a minimum US imaging follow-up of 12 months and had no other conditions affecting testicular volume.

Results: 114 men (mean age 22.8 ± 5.4 years) matched inclusion and exclusion criteria. Left testicular hypotrophy (LTH) – defined as a ≥20% difference between left and right testicular volume at baseline – was observed in 26 men (22.81%). Subjects with LTH (14.5 ± 2.7 mL) had lower baseline testicular volume compared to those without LTH (15.7 ± 3.8 mL; $p = 0.032$). Repeated measures mixed models showed significant interaction between LTH and time post-treatment when correcting for baseline left testicular volume (β = 0.113, IC 95% 0.018–0.209, $p = 0.021$), resulting in a catch-up growth of up to 1.36 mL/year (IC 95% 0.210–2.504). Age at intervention was also associated with reduced testicular volume (−0.074 mL/year, IC 95% −0.136 to −0.011; $p = 0.021$).

Conclusion: Percutaneous treatment of left varicocele in young adults with LTH can result in catch-up growth over 1 year of follow-up. Reproductive and psychological implications of these findings have to be confirmed in longer and larger prospective studies.

P052

Efficacy of adjuvant antioxidant therapy after microsurgical varicocelectomy in infertile men

R. OVCHINNIKOV, S. GAMIDOV AND A. POPOVA
Andrology and Urology Department, National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I.Kulakov, Moscow, Russia

Background: Infertility affects an estimated 15% of couples globally. Male factor infertility accounts for about a half of infertility cases. About 40% of infertile men have varicocele. The aim of this study was to investigate the efficacy and safety antioxidant complex of acetyl-L-carnitine, L-carnitine fumarate and alpha-lipoic acid (SpermActin; SA) for adjuvant antioxidant therapy after microsurgical varicocelectomy (MVE) in men with varicocele and assess its impact on the level of sperm DNA fragmentation.

Methods: This is an open, prospective, randomized trial comprising 114 men aged 25–45 (mean 34.1 ± 12.1) years who underwent MVE. The patients were allocated to receive either adjuvant SA (n = 38), SA in combination with a vitamin complex (VC) (n = 38) or no adjuvant therapy (n = 38). The efficacy was assessed at 3 months by testing standard semen parameters and the level of sperm DNA fragmentation. The statistical significance of the change in variables was calculated using the Wilcoxon test. Critical level $\alpha = 0.05$ was established for all criteria.

Results: MVE led to a 21.7% increase in the progressive sperm motility compared to the baseline level. In patients receiving SA, this was by 76.7% due to active sperm motility (category A) at 3 months. MVE with concurrent supplementation of SA resulted in a 22.3% decrease in the level of sperm DNA fragmentation at 3 months. When used in the postoperative period after MVE, SA and VC resulted in a 27% increase in the sperm concentration at 3 months. There were no side effects of pharmacotherapy.

Conclusion: Using antioxidant complex of acetyl-L-carnitine, L-carnitine fumarate and alpha-lipoic acid (SA) after MVE is an effective and safe adjuvant antioxidant therapy of male infertility in patients with varicocele which leads to an improvement in the basic sperm parameters (sperm concentration and motility) and a decrease in the level of sperm DNA fragmentation in the short term. Adjuvant antioxidant therapy of varicocele infertility potentiates the effects of monotherapy (both medical and surgical), leads to an increase in its effectiveness and shortens the time to pregnancy. Further studies in this field are needed to assess long-term outcomes of the treatment.

P053

The ReproGenomics Viewer 2.0: a major update integrating features for single-cell data

T. DARDE[1], E. LECLUZE[1], N. ALARY[1], A. LARDENOIS[1], O. COLLIN[2], S. NEF[3], A. D. ROLLAND[1] AND F. CHALMEL[1]
[1]Univ Rennes, Inserm, EHESP, Institut de Recherche en Santé, Environnement et Travail IRSET) – UMR_S1085, Rennes, France; [2]Institut de Recherche en Informatique et Systèmes Aléatoires (IRISA/INRIA) – GenOuest plat-form, Université de Rennes 1, Rennes, France; [3]Department of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland

Background: The ReproGenomics Viewer (1) is a cross-species genomic toolbox for the reproductive community. It aims to assist scientists in the analysis and the mining of a wide range of ultra high-throughput reprogenomics data. RGV allows hosting, visualization, and direct comparison of users' data to published genomics studies as well as to relevant genetic variations linked to reproductive disorders. RGV enables the direct comparison of datasets acquired in different organisms, which are currently related to reproduction including testis biology and spermatogenesis. The RGV is already accessible at http://rgv.genouest.org and the release (v2.0) will be available for fall 2018.

Methods: Briefly, raw data (fastq files) are downloaded directly from the NCBI GEO repository (2) and mapped on their corresponding genome sequences using STAR v2.0 (3). Finally a cross-species conversion to the human genome is performed using the CrossMap tool (4) with the chain files provided by the UCSC genome browser (5). This process allows the visualization of data from a given species to a selected reference genome (Human in the current version). Gene-level expression data are processed using the StringTie tool (6).

Results: In addition to the genome browser visualization, this new release will include several new features:
- A new user-friendly interface;
- Gene-level & Single-cell visualizations;
- A new study-/track-selector facilitating complex filtering.

More than 70 published studies covering:
- Several biological topics related to reproduction (including testis development and functions)
- 9 vertebrate species;
- 90 tissues or cell types associated with 587 experimental conditions (2465 samples);
- 12 distinct types of (gen-, epigen-, cistr- and transcript-) omic technologies;
- Genetic variants from ClinVar (7) associated with reproductive and fertility disorders.

Conclusion: In the next future we intend to keep improving RGV by:
- increasing the number of datasets, model organisms and biological topics related to reproduction;
- making the cross-species conversion available to rodent genomes;
- making RGV compatible with other technologies (such as bisulfite-seq).

The ReproGenomics Viewer is a webserver-based toolbox for our science community, so please, any suggestions and requests for additional genomes, datasets or features are welcome (frederic.chalmel@inserm.fr)!

References: (1) Darde TA, et al. Nucleic Acids Res. 2015 Jul 1;43(W1):W109–16.
(2) Barrett T, et al. Nucleic Acids Res. 2013 Jan;41:D991–5.
(3) Dobin A, et al. Curr Protoc Bioinformatics. 2015 Sep 3;51:11.14.1–19.
(4) Zhao H, et al. Bioinformatics. 2014 Apr 1;30(7):1006–7.
(5) Casper J, et al. Nucleic Acids Res. 2018 Jan 4;46(D1): D762–D769.
(6) Pertea M, et al. Nat Biotechnol. 2015 Mar;33(3):290–5.
(7) Landrum MJ, et al. Nucleic Acids Res. 2016 Jan 4;44 (D1):D862–8.

P054

How reliable is testicular ultrasound in predicting infertility? Proposal of a new grading system

M. G. TARSITANO[1], G. A. KANAKIS[2], M. MINNETTI[1], C. POZZA[1], M. TENUTA[1], F. SESTI[1], P. MAZZOTTA[1], D. GIANFRILLI[1], A. LENZI[1] AND A. M. ISIDORI[1]
[1]Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy; [2]Department of Endocrinology, Athens Naval & VA Hospital, Athens, Greece

Background: Male infertility is considered as the causative factor in half of infertile couples. In diagnostic process, ultrasound imaging is frequently used as a first level exam. However, few studies have evaluated the relationship between testicular US imaging and fertility status.
Methods: We retrospectively studied 2827 scrotal ultrasound examinations and semen analysis performed from March 2005 to May 2017 of patients referring to our Unit. Of this initial cohort, we excluded adolescents younger than 17 years old, men with known genetic disorders associated with testicular degeneration and men who had underwent previous orchiectomy. Thus, the final group study was of 714 patients. We integrated the b-mode US testicular characteristics in a new semi-quantitative scoring scheme including assessment of total testicular volume (TV) (0–2), presence and grade of testicular microlithiasis (0–2), homogeneity of testicular echo-structure (0–2), echogenicity of the parenchyma (0–1) and the presence of a lesion resembling as a tumor (0–2). Higher scores reflected greater morphological alterations. We plotted the relevant ROC curves that predicted the presence of oligospermia, asthenospermia and teratospermia according to the most recent WHO manual (2010) and compared to the area under the curve (AUC). Numeric values were expressed as median (25th–75th percentile).
Results: The median age of the participants was 32 years (22–38) and median TV was 27.5 mL (21.2–33.3). The median score of the cohort was 1 (0–3). The new scoring system correlated fairly with all basic semen parameters: sperm concentration (rho = −0.455; $p < 0.001$), total motility (rho = −0.437; $p < 0.001$) and typical forms (rho = −0.365 = ; $p < 0.001$). We further compared the performance of the in-house scoring scheme in its ability to predict the presence of aberrations in basic sperm parameters. Our ultrasound grading schemes performed satisfactory in predicting oligospermia (AUC 0.754), asthenospermia (AUC 0.710) and teratospermia (AUC 0.758).
Conclusion: Our results indicate that patients with high in-house score show poorer conventional sperm parameters. The study shows that testis ultrasound may contribute to the diagnostic work-up in infertile patients.

P055

Obesity markers and sperm parameters

M. KESZTHELYI[1], A. GYARMATHY[2], A. KAPOSI[3] AND Z. KOPA[1]
[1]Department of Urology, Andrology Centre, Semmelweis University, Budapest, Hungary; [2]Department of Health, Behavior and Society, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA; [3]Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary

Background: Lifestyle factors are known to reduce male fertility. While it is clearly accepted that increased body weight by females can cause decreased fecundity, this association is still not proved on the male side. A cross-sectional clinical study was performed to control the possible link between obesity and classical sperm parameters, results were compared to Body Mass Index (BMI) and waist to hip ratio (WHR) values.
Methods: A total of 1188 semen samples were analyzed. Chart review of body measurements and the results of WHO-V criteria semen analysis were evaluated. After statistical normalization of BMI and WHR values, Scatter plots were created with fitted linear regression lines in order to visualize the relationship between BMI/WHR and semen parameters. Univariate linear regression models were created to calculate the regression line slope coefficients and their 95% confidence intervals. For each semen parameter, min/max line plots were created comparing the slope coefficients of BMI vs. WHR for that particular parameter. Statistically significant differences between the slopes were considered when the point estimate of the WHR slope coefficient fell outside of the 95% confidence interval of the BMI slope coefficient [*ref]. SAS V9.4 software (SAS Institute Inc. Cary, NC) was used for data management and analysis, and data visualization.
Results: The mean age of the 1169 patients was 38.1 years (SD = 7.0; range: 17–67). The mean height and weight were 180.6 cm (SD = 7.5; range: 155–210) and 87.3 kg (SD = 15.9; range: 55–183), respectively – the mean BMI was 26.8 (SD = 4.5; range: 16.9–50). The mean waist and hip circumference were 100.9 cm (SD = 8.9, range: 56–149) and 94.8 cm (SD = 12.4, range: 59–165), respectively – the mean waist to hip ratio was 0.94 (SD = 0.07; range 0.63–1.22). The mean sperm concentration was 48.7 M/mL (SD = 55.7; range: 0–681.5). Mean values of progressive motility and normal morphology were 21.2% (SD 18.7; range: 0–80) and 4.8% (SD 4.6; range: 0–28), respectively. Both BMI and WHR were significantly correlated in all semen parameter regression models. When comparing the parameter estimates for BMI with those for WHR for each semen parameter, the parameter estimate for WHR was significantly lower (indicating a stronger negative association) than that for BMI for progressive motility, but not for normal morphology or concentration.
Conclusion: Obesity parameters have a strong link to sperm classical parameters. After standardization of our data regarding sperm parameters susceptibility to the changes in obesity markers, results show no difference comparing BMI vs. WHR in sperm concentration and normal morphology. In contrary analyzing sperm progressive motility data, WHR showed much higher sensitivity than BMI, it can be concluded that progressive motility was much more affected by WHR than BMI in our study. This recent observation raises a number of novel questions and indicates further researches. Our result highlights the importance of waist-hip ratio as a more relevant influencing factor for sperm progressive motility changes. Due to its significantly more sensitivity, WHR should be used to describe obesity effects on sperm progressive motility.

P056

Determinants of radiological vertebral fractures in HIV male patients under antiretroviral therapy

T. PORCELLI[1], F. MAFFEZZONI[2], A. DELBARBA[3] AND A. FERLIN[3]

[1]Unit of Endocrinology, Montichiari Hospital, Brescia, Italy; [2]Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; [3]Department of Clinical and Experimental Sciences, Unit of Endocrinology, University of Brescia, Brescia, Italy

Background: Over the recent years, there has been convincing evidence that HIV infection per se and antiretroviral therapy may cause skeletal fragility with an increased risk of fractures. However, available data on fractures are scanty and predictors of fractures in this clinical setting are still largely unknown. Indeed, the pathogenesis of osteoporosis and fractures in HIV-infected patients is multifactorial involving infection-related factors, highly active antiretroviral therapy (HAART), and traditional osteoporosis risk factors, such as smoking, alcohol intake, low body mass index (BMI), opiate use, and hypogonadism. Hypogonadism is common among HIV-infected men, although the true prevalence remains poorly defined and widely ranging from <10% to over 50% in several studies.

Objective: In this cross-sectional study, we aimed at investigating predictors of radiological vertebral fractures (VFS) in a cohort of HIV infected male patients.

Methods: HIV infected male patients under HAART were consecutively referred to our Endocrine Unit from local Infections Disease Unit. All patients underwent a complete clinical work-up, including medical and drug history and administration of questionnaires on their lifestyle including potential risk factors for osteoporosis. Bone mass density (BMD) of the lumbar spine, total hip and femoral neck was measured by dual- energy X-ray absorptiometry (DXA) (Hologic Inc., Waltham, MA, USA). Fractured vertebrae were excluded from the lumbar BMD analysis. BMD was expressed as *T*-score. A *T*-score ≤−2.5 SD at the hip or spine was defined as osteoporosis, whereas osteopenia was defined as a *T*-score between −1 and −2.5 SD. Morphometric study was performed on a thoracic-lumbar X-ray, using a translucent digitizer and a cursor and six points were marked on each vertebral body to describe vertebral shape (from T5 to L4). Circulating serum levels of calcium, phosphorus, 25-hydroxyvitamin D, intact PTH, total testosterone (TT), and 24-h urinary calcium were determined. Free testosterone (FT) levels were calculated after determination of sex-steroid biding globulin (SHBG) plasma levels.

Results: One hundred and thirty male HIV-infected patients were studied. Here we report data for those 37 with complete dataset and hormonal determination performed in the same central laboratory. The prevalence of VFs was 38% (14/37). There were no statistically significant differences for all clinical and biochemical parameters considered between subject with and without VFs. In particular, no difference was observed for age (median age 56 vs. 53 years), years of infection (median 16 vs. 11 years), duration of HART (median 11 vs. 9.5 years), 25-hydroxyvitamin D (34 vs. 34 ng/mL), TT (7.53 vs. 6.79 ng/mL), FT (100.0 vs. 92.4 pg/mL), SHBG (68.23 vs. 71.0 nmol/L), BMI (24 vs. 26). However, vertebral T-score was significantly lower in men with VFs with respect to those without (−2.40 vs. 1.05, $p < 0.05$).

Conclusion: HIV-infection has become a chronic disease, with an increased risk of osteoporosis. This study showed that prevalent vertebral fractures are frequent in males with HIV under HAART and are associated with low BMD. Testosterone, vitamin D and calcium-phosphorus metabolism seem to be less important in BMD determination and VF risk in this group of patients.

P057

Sperm proteins implicated in obesity-induced male infertility

B. SKOSANA, S. HATTINGH, I. WEBSTER, A. LOCHNER AND S. S. DU PLESSIS

Division of Medical Physiology, Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa

Background: Obesity has been implicated as a contributor to increased levels of male infertility. This increased incidence requires the need to understand the underlying mechanisms behind obesity-induced infertility. The modification and expression of human sperm proteins plays a critical role in sperm function. Differences in protein expression within the sperm population were investigated between control and diet sperm populations to ascertain possible changes induced by obesity on sperm proteins.

Methods: Male Wistar rats ($n = 40$) weighing 200–220 g were divided into control and diet groups and given standard rat chow or a high caloric diet for 60 weeks respectively. Spermatozoa were harvested and cryogenically frozen in liquid nitrogen. Sperm were homogenized, and protein concentrations determined by means of Bradford assays. Subsequent to a trypsin digest, samples were analysed using an Orbitrap Mass Spectrometer (LCMS/MS). Resulting data was subjected to a MSGF+ search against the RefSeq rat database and IDPicker produced spectral count tables. A minimum of 1.4 spectra on average for multiple replicate comparisons was required. After obtaining the "raw" *p*-value for each protein, the *p*-values were adjusted for multiple comparisons. BH (Benjamini & Hochberg, 1995) was used to control the false discovery rate (FDR), the expected proportion of false discoveries amongst the rejected hypotheses. FDR was controlled at 0.05. All the data process and analysis were carried out using R version 3.3.2 (2016-10-31) and p.adjust function in R is used for the adjusted *p*-values. Resultant proteins were subjected to database search using SwissProt for identification of proteins and analysed for differential protein expression between groups.

Results: Spectral counts of a total of 984 proteins were identified in each sample, with only 43 significant proteins with an adjusted *p*-value less than 0.05 being obtained.

Conclusion: The present study explored the molecular nature of sperm dysfunction. Majority of proteins identified were cytosolic and mitochondrial proteins involved in energy metabolism. Regulatory proteins involved in gene regulation, acrosome reaction and spermatogenesis were also identified, including heat shock proteins important for protein translation, folding, unfolding, and translocation. Structural proteins including one of the major outer

dense fiber proteins were identified. Defects in these fibers lead to abnormal sperm morphology and infertility. Proteins relating to energy metabolism, a major mechanism disrupted in obesity, were the predominant proteins identified. Proteins involved in sperm function and structure have also been identified. Final analysis will reveal how these proteins are differentially regulated between control and obese animals.

P058

Multilevel approach to male infertility using machine learning

D. SANTI[1,2], G. SPAGGIARI[1,2], M. MICHELANGELI[3], L. CASARINI[1], R. GRASSI[3], B. VECCHI[3], L. ROLI[4], M. C. D. SANTIS[4], E. BARALDI[4], M. SETTI[5], T. TRENTI[4] AND M. SIMONI[1,2]

[1]Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy; [2]Unit of Endocrinology, Department of Medical Specialties, Azienda Ospedaliero-Universitaria of Modena, OCSAE, Modena, Italy; [3]Hopenly S.r.l., Vignola, Modena, Italy; [4]Department of Laboratory Medicine and Anatomy Pathology, Azienda USL of Modena, Italy; [5]Service of Clinical Engineering, Azienda Ospedaliero-Universitaria of Modena, Modena, Italy

Background: Male infertility represents a complex condition requiring an accurate multilevel assessment burdened by diagnostic and therapeutical limitations. Conventional semen analyses are currently the only validated method in the male infertility workup, although they do not completely discriminate fertile from infertile men. Machine learning (ML) technology, combining large data series in nonlinear and highly interactive ways, could be innovatively applied to this medical multifactorial disorder.
Objective: To characterize male infertility, applying the ML approach for the first time.
Methods: Semen samples collected between January 2010 and March 2016 were considered in a big data analysis starting from a database containing 990 904 951 data. ML algorithms acted on dataset performing regression, multivariate and classification analyses, matching semen parameters with concomitant biochemical examinations, ambient temperature and air pollutants exposure. Several algorithms were consecutively applied to classify the dataset generated.
Results: The final database included 4994 patients, aggregating semen analyses, blood examinations and environmental parameters. No correlations among metabolic parameters and fertility status were found. Random forest regression recognized a cluster of subfertile patients with impaired sperm motility and morphology despite normal number. In classification analyses, k-nearest neighbour method discriminated oligozoospermic patients with 0.69 of accuracy, 0.78 of sensitivity and 0.41 of specificity. Stacked algorithm classified both teratozoospermia (accuracy: 0.65, sensitivity: 0.36, specificity: 0.83) and asthenozoospermia (accuracy: 0.62, sensitivity: 0.45, specificity: 0.75). Considering subfertility, the random forest method classified patients with 0.58 of accuracy, 0.58 of sensitivity and 0.57 of specificity. The tuning phase highlighted lymphocytes number, erythrocyte distribution and mean globular volume as most relevant variables in all categories.
Conclusion: This study represents the first ML application to male infertility generating a multilevel model that discriminates subfertile men with mild accuracy. Using this approach, a correlation between seminal and metabolic parameters was not found, suggesting that infertility is not able to predict the general male health status. Although no predictive factors were identified, the detection of hemochromocytometric variables in classification analyses allows to speculate about the existence of a possible hidden link between testicular and hematopoietic tissue, sharing similar proliferative properties. In general, this approach is able to identify several mathematical algorithms which classify patients according to their fertility status.

P059

Characterization of CCR6 and CCL20 in testis and ejaculates from men with chronic genital tract inflammation

Y.-G. DUAN[1,2], C. YU[2], H.-C. SCHUPPE[3], G. HAIDL[2] AND J.-P. ALLAM[2]

[1]Shenzhen Key Laboratory of Fertility Regulation, Center of Assisted Reproduction and Embryology, the University of Hong Kong – Shenzhen Hospital, Shenzhen, China; [2]Department of Dermatology/Andrology Unit, University of Bonn, Bonn, Germany; [3]Andrology Unit, Department of Urology, Pediatric Urology and Andrology, Justus-Liebig-University, Giessen, Germany

Objective: To investigate the expression characteristics of CCR6-CCL20 in human testis and sperm under various conditions, but also explore the possible relationship between sperm CCR6-CCL20 expression and semen parameters.
Methods: CCR6-CCL20 in testis was investigated using the immunohistochemical method. Sperm CCR6 was identified by confocal microscopy and flow cytometry. Seminal CCL20 was checked using ELISA. Setting: University hospital laboratory. Patients: 130 infertile men. Main Outcome Measure(s): Evaluation of the CCL20/CCR6 expression in testis and ejaculate. Correlation with semen parameters.
Results: Significant increase of CCL20/CCR6 expression in testis and ejaculate was associated with genital tract inflammation, and they were correlated with sperm motility, vitality and seminal IL-6, IL-17.
Conclusion: Under infections and inflammatory conditions, the abnormal increase of CCL20/CCR6 expression could disturb and impair both spermatogenesis and sperm functions such as motility, chemotaxis, apoptosis, etc.

P060

Long-term effect of lymphoma treatments on sperm DNA fragmentation

F. CIOPPI[1], M. G. FINO[1], E. CASAMONTI[1], S. VINCI[1], A. RIERA-ESCAMILLA[1,2], L. TAMBURRINO[1], A. MAGINI[1], M. MURATORI[1] AND C. KRAUSZ[1]

[1]Department of Biomedical, Experimental and Clinical Sciences "Mario Serio", University of Florence, Florence,

Italy; [2]Andrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, Instituto de Investigaciones Biomédicas Sant Pau (IIB-Sant Pau), Barcelona, Catalonia, Spain

Background: Hodgkin's and non-Hodgkin's lymphomas represent the most common hematological malignancies (HE) affecting men in reproductive age. Several protocols of cancer therapies have significantly improved survival rates of young patients, despite having a negative effect on testicular function, including damage or alterations to the sperm genome. The consequences of these treatments on sperm DNA represent a major concern since damage to the paternal genome may have detrimental effects on offspring. Data on post-therapy sperm DNA alterations are still controversial and limited to 2 years, thus the right timing for natural conception after treatment remains uncertain.

Objective: To evaluate the effect of cytotoxic therapies on the integrity of the sperm genome (Sperm DNA Fragmentation, SDF) after 2 (T2) and 3 years (T3) from the end of treatment in HM patients.

Methods: We analyzed the SDF of 2 HM patients groups, one evaluated 2 years post-therapy (T2 group, $n = 26$) and one evaluated 3 years post-therapy (T3 group, $n = 25$). The analysis, based on terminal-uridine nick and labelling end assay (TUNEL), was performed on 10×10^6 sperm cells. We evaluated total and brighter % SDF (SDFtot and SDFbr, respectively). The SDFbr is more strictly associated with sperm fertilizing potential and fertility outcome. Data of each group were compared with those of 58 healthy fertile men (control group) in a cross-sectional analysis.

Results: At each time points (T2 and T3) patients were divided according to the type of treatment: ABVD ($n = 11$ and 9, respectively), R-CHOP ($n = 2$ and 3, respectively) and mixed therapies (two or more different chemotherapies or chemo+radiotherapy) ($n = 13$ both for T2 and for T3). The mean %SDFtot and %SDFbr of the control group were 29.11 ± 11.11% and 19.53 ± 9.48%, respectively. (i) T2 group: both %SDFtot and %SDFbr in subjects treated with ABVD (39.06 ± 15.08%, 28.54 ± 14.28%) or with mixed therapies (43 16 ± 20.64%, 35.45 ± 19.62%) were significantly higher than those of fertile men ($p < 0.05$, $p \leq 0.01$ and $p = 0.001$, $p < 0.001$, respectively). (ii) T3 group: no significant differences were observed at T3 between patients and controls, with the exception of % SDFtot in the group that underwent mixed therapies (37.29 ± 15.16%, $p < 0.05$). (iii) Severe DNA damage expressed as % SDFbr >75th percentile of "normality" (>25%) was observed in 55% (6/11) and 62% (8/13) of patients treated with ABVD and mixed therapies, respectively, in the T2 group. In the T3 group, SDFbr >25% was observed in 33% of patients treated with either ABVD (3/9) or R-CHOP (1/3), and in 46% (6/13) of those treated with mixed therapies.

Conclusion: Our study indicates a long-term effect of cytotoxic therapies on DNA integrity in a relatively large proportion of patients. Pathological SDF values (above the 75th percentile of fertile controls) 3 years after treatment, were observed after all type of treatment with the highest percentage in patients treated with most aggressive therapies such as combined chemotherapies or chemo+ radiotherapy. DNA fragmentation analysis, particularly % SDFbr, should be proposed both to monitor the long-term effect of cytotoxic therapies and to help in decision making on the timing of natural pregnancy.

P061

Mutations in the ADGRG2 gene as a cause of CBAVD

K. STOUFFS[1], E. EKINCI[1], V. VLOEBERGHS[2], A. GHELDOF[1], E. DE WACHTER[3], H. TOURNAYE[2] AND S. SENECA[1]

[1]Center for Medical Genetics/Research Center Reproduction and Genetics; [2]Centre for Reproductive Medicine/Biology of the Testis; [3]CF Clinic, Vrije Universiteit Brussel (VUB), Universitair Ziekenhuis Brussel (UZ Brussel), Brussels, Belgium

Background: Congenital Bilateral Absence of the Vas Deference (CBAVD) is a common cause of obstructive azoospermia. Around 80% of these men have mutations in the CFTR gene. Part of the remaining 20% have a combined uro-genital problem. However, for the majority of the patients without CFTR alterations, the underlying (genetic) cause remains unknown. In 2016 Patat et al. for the first time described mutations in the X-linked ADGRG2 gene as causal of CBAVD. They analysed 26 CBAVD patients without kidney problems. In three patients, a truncating mutation was detected. Subsequently, Yang et al. (2017) described 2 more patients (out of 18) with missense mutations in the ADGRG2.

Methods: In the present study, we have investigated 20 CBAVD patients without mutations in the CFTR gene. Echography had already excluded kidney problems for the majority of these patients before their ADGRG2 gene (NM_001079858.2) was analysed by Sanger sequencing.

Results: We have identified a single patient with a truncating mutation in the ADGRG2 gene: c.920C>A, p.Ser307*. This nonsense variant has not been described before, and is located in exon 16 (from the 29 exons). Consequently, the majority of the protein (711 amino acids) is missing. Altogether, 64 patients (reported or included in our study) have been analysed for the presence of mutations in the ADGRG2 gene. PRESENTLY, around 9% of CFTR negative patients are hemizygous for an ADGRG2 gene mutation with nonsense mutations frequently seen.

Conclusion: It is worthwhile to include the analysis of the AGRGR2 gene in a routine diagnostic setting, after full investigation of the CFTR gene. Furthermore, since for a large group of patients, the underlying molecular mechanism IS STILL unknown, it is worthwhile to (re-)analyse THEM by genome-wide molecular analyses.

P062

Testosterone (T) and estradiol (E2) are poorly associated to the reduction of bone mineral density (BMD) in Young/Middle Aged Men with Human immunodeficiency virus (HIV)

S. D. VINCENTIS[1,2], M. C. DECAROLI[1,2], C. DIAZZI[1,2], F. MORINI[1], D. BERTANI[1], F. FANELLI[3,4], M. MEZZULLO[3,4], D. SANTI[1,2], U. PAGOTTO[3,4], G. GUARALDI[5] AND V. ROCHIRA[1,2]

[1]*Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Italy;* [2]*Unit of Endocrinology, Department of Medical Specialties, Azienda Ospedaliero-Universitaria of Modena, Italy;* [3]*Unit of Endocrinology, University of Bologna, Italy;* [4]*Center for Applied Biomedical Research, University of Bologna, Italy;* [5]*Multidisciplinary Metabolic Clinic, Unit of Infectious Diseases, University of Modena and Reggio Emilia, Italy*

Background: Osteopenia and osteoporosis, as well as hypogonadism, are common findings in men with HIV-infection and they occur at a younger age than healthy subjects. The reduction of BMD is due to both HIV-related and HIV-unrelated factors. Previous studies suggest that T deficiency is not or poorly associated with reduced BMD in HIV context. On the other hand, estrogens are considered more important than androgens for bone health in general population, but data about their role in HIV-infected men are still scanty.

Objective: To investigate the relationship between BMD and circulating sex steroids assessed by Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) in a cohort of young/middle aged HIV-infected men.

Methods: Prospective, cross-sectional, observational study on 233 consecutive HIV-infected male patients with ongoing Highly Active Antiretroviral Therapy (HAART), attending the Multidisciplinary Metabolic Clinic of Modena. Body composition and BMD at total body, lumbar spine (L1 to L4) and total hip were measured using a Hologic QDR-2000 densitometer (DXA). LC-MS/MS was used for hormonal assays. Statistical analysis: The nonparametric Mann–Whitney *U* test was used for group comparisons because variables were not normally distributed at the Kolmogorov-Smirnov test. Correlations were performed using linear regression models.

Results: Two hundred and thirty-three HIV-infected patients were enrolled (mean age 45.29 $\pm$ 5.33 years) with average duration of HIV-infection of 190.8 $\pm$ 102.8 months. Eight patients (3.4%) had hypogonadism, defined as total T serum levels below 300 ng/dL. Considering results at DXA examination, BMD was normal in 36.5% and reduced in 63.5% (55.8% osteopenia, 7.7% osteoporosis). Both total T and E2 did not significantly differ comparing patients with normal BMD to patients with reduced BMD. Body and lumbar BMD did not show any significant difference between eugonadal patients and patients with low T and/or low E2, while both femoral BMD and femoral T-score were significantly higher in patients with E2 above 20 pg/mL than in those with E2 below 20 pg/mL ($p = 0.043$ and $p = 0.033$, respectively). At linear and stepwise multiple regression analyses, BMD was positively associated with total lean mass ($R^2 = 0.154$, $p < 0.0001$); apart from it, neither T nor E2 correlated with BMD and T-score at any site.

Conclusion: Classical factors associated to BMD as E2 and T seem to be less relevant in this model of male osteoporosis. Other specific HIV-related factors, such as changes in body composition and consequent lipodystrophy, could be more deeply involved than sex steroids as potential mechanisms in bone loss in this setting. Finally, we confirm the high prevalence of reduced BMD in young/middle aged HIV-infected men, representing one of the clinical hallmarks of the premature aging process related to HIV infection.

P063

Endogenous testosterone supports spermatogenesis even in the absence of gonadotrophins: evidence from a case report

L. ANTONIO[1], M. ALBERSEN[2], J. BILLEN[3], G. MALEUX[4], A.-S. V. ROMPUY[5] AND D. VANDERSCHUEREN[1]

[1]*Department of Endocrinology, University Hospitals Leuven, Leuven, Belgium;* [2]*Department of Urology, University Hospitals Leuven, Leuven, Belgium;* [3]*Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium;* [4]*Department of Radiology, University Hospitals Leuven, Leuven, Belgium;* [5]*Department of Pathology, University Hospitals Leuven, Leuven, Belgium*

Background: In patients with testicular dysgenesis syndrome, reduced semen quality and testicular cancer are common. We report a case of a testicular tumour in a patient with a history of cryptorchidism and oligoasthenospermia. He had an unusual hormonal profile, which was not fully explained by the pathological findings.

Case report: A 31-year-old man was referred to our tertiary care andrology unit for primary infertility with a history of bilateral orchidopexy during childhood. Testes were small (12 cc). Gynaecomastia was absent. Semen analysis repeatedly showed oligoasthenospermia (2.4–7.1 million/mL, 85–92% immotile). Gonadotropins (LH and FSH < 0.1 U/L) were undetectable, but testosterone and estradiol were normal (850.7 ng/dL and 38.6 ng/L). Prolactin, other pituitary hormones, DHEAS, AFP, HCG and inhibin B were also normal. He denied using anabolic steroids. Suppressed gonadotrophins suggested a sex steroid producing testicular tumour. However, scrotal ultrasound only showed diffuse microcalcifications and three millimetric hypolucent lesions in the left testis, but no intratesticular mass. There were no suspicious lesions nor microcalcifications in the right testis. To further investigate the possibility of increased testicular sex steroid production, selective testicular venous sampling was performed. In the left spermatic vein, testosterone and estradiol levels were very high (3744 ng/dL and 378 ng/L), with a testis-to-periphery gradient of 4.4 and 9.0 respectively. There was no gradient in the right spermatic vein. These results confirmed increased sex steroid producing in the left testis. However, histopathological examination after orchidectomy revealed a multifocal seminoma (largest diameter 3 mm) and profuse germ cell neoplasia in situ. There were neither isolated syncytiotrophoblastic cells, nor choriocarcinoma. Leydig cell hyperplasia was present without Leydig cell tumour. HCG was remeasured with three different methods, all showing very low HCG between 0.6 and 1.1 IU/L. After orchidectomy gonadotrophin levels increased (LH 24.3 U/L, FSH 10.3 U/L), with normal total testosterone and estradiol, indicating recovery of suppression of the hypothalamic-pituitary-testis axis. Sperm concentration increased (10 million/mL.)

Conclusion: (i) Our case shows that endogenous testosterone may support spermatogenesis even without gonadotropins. (ii) In patients with suppressed gonadotropins, normal sex steroid levels and no testicular mass, selective testicular venous sampling can be useful in identifying the site of hormonal overproduction. (iii) Thus far, the

pathology findings cannot explain the hormonal profile. Further investigations are therefore ongoing.

P064

Multivarious clinical presentation of hypogonadotropic hypogonadism and reproductive challenge

D. PALANISAMY AND M. ASHRAF
Craft Hospital Kodungallur, Kerala, India

Objective: Hypogonadotropic hypogonadism in reproductive men is not uncommon. 32 cases of hypogonadotropic hypogonadism has been evaluated and their reproductive outcome in terms of etiology, normal conceptions, ivf/icsi by ejaculate, Tesa, Microtese use in sperm retrieval followed by icsi and medical management has been studied.

P066

Comparative analysis of apoptotic and proliferative activity in teratocarcinoma of the testis and the experimental mouse teratocarcinoma model

J. KRASIC[1,2], N. VUJNOVIC[1,2], R. TERLEVIC[3], A. K. BOJANAC[1,2], M. ULAMEC[4,5], F. BULIC-JAKUS[1,2], D. JEZEK[2,6] AND N. SINCIC[1,2]
[1]Department of Medical Biology, University of Zagreb School of Medicine, Zagreb, Croatia; [2]Centre of Excellence for Reproductive and Regenerative Medicine, University of Zagreb School of Medicine, Zagreb, Croatia; [3]Department of Pathology, General Hospital Pula, Pula, Croatia; [4]Ljudevit Jurak Clinical Department of Pathology and Cytology, University Clinical Hospital Center Sestre milosrdnice, Zagreb, Croatia; [5]Department of Pathology, University of Zagreb, School of Medicine, Zagreb, Croatia; [6]Department of Histology, University of Zagreb School of Medicine, Zagreb, Croatia

Background: Testicular Germ Cell Tumours (TGCT) are the most frequent malignancies in young male population and believed to be initiated by epimutations, i.e. aberrant epigenetics. Teratocarcinoma is a mixed TGCT usually merging teratoma and embryonal carcinoma in a single neoplastic entity. Teratoma is the most differentiated TGCT type encompassing all three germ layer derived tissues. EC cells constitute the pluripotent "core" of teratocarcinomas. EC cells are highly similar to embryonic stem cells and express the same pluripotent markers: OCT4, SOX2 and NANOG. EC cells may differentiate into teratoma tissue or retain their pluripotency. The well-known and established experimental mouse model of teratocarcinoma, at the Biology Department of Zagreb's School of Medicine, has been described based on a histological/histochemical basis and its molecular signature has not yet been comprehensively studied.
Objective: The aim of this study was to compare the rate of apoptotic and proliferative activity in the experimental mouse model to human teratocarcinomas.
Methods: Formalin-fixed paraffin-embedded tissue from 10 testicular teratocarcinoma from the Ljudevit Jurak Pathology and Cytology Department Archive and 10 animal model tumors from the Biology Department of Zagreb's School of Medicine was used for immunohistochemical detection of Caspase-3 and PCNA expression. Slides were analyzed semi-quantitatively, at the area of strongest reaction ("hot spot"), by two pathologist (R.T. and M.U.), on a scale from 0 to 3, depending on the percentage of reactive cells. The data was analyzed in GraphPad Prism using the Mann–Whitney test.
Results: The results have shown a statistically significant difference in the rate of apoptosis between the human teratocarcinomas and the experimental mouse model, with the mouse model showing a higher rate (more than 25% of positive cells) in 64% of tumors compared to 30% of human tumors with highest reaction. PCNA quantification has shown comparable levels of PCNA expression in the embryonal carcinoma regions of the experimental mouse model and human teratocarcinomas, while in the teratoma regions the mouse model has a higher proliferative activity defined with PCNA staining.
Some of the difference could be attributed to the fact that the study was a pilot with a relatively small sample pool, the intragroup difference in biological development and "age" between the human teratocarcinomas as well as the intergroup difference in biological development between the human and mouse model teratocarcinomas. Western Blot analysis of Caspase-3 and PCNA activity should be done to verify the results and a larger cohort should be studied.

P068

Withdrawn.

P069

Testicular microlithiasis – a link to testicular dysgenesis syndrome and testicular cancer

V. ROSTA[1,2], T. TAKÁCS[1,3] AND Z. KOPA[1]
[1]Andrology Center, Semmelweis University, Department of Urology, Budapest, Hungary; [2]Medical student, University of Szeged, Hungary; [3]Urology Department, St Bernard's Hospital, Gibraltar, UK

Background: The prevalence of testicular cancer has doubled in many populations in the last decades. Numerous evidence supports the link between testicular germ cell tumours (TGCT), cryptorchidism, hypospadias and male infertility, which fact arose the hypothesis of the common aetiology and pathogenesis in fetal life and constituted the concept of Testicular Dysgenesis Syndrome (TDS). Different features of TDS could range between a reduced sperm concentration to more serious forms of the disorder, including complex testicular failure and testicular cancer. Testicular microlithiasis has consistently been associated with infertility, carcinoma in situ and testicular cancer; however, the relation is still controversial.
Objective: The aim of our study was to investigate the possible link between microlithiasis and TDS components.
Methods: Patients in infertile couples with ultrasonographically detected testicular microlithiasis (TML) were enrolled in an ongoing prospective, single-centre, case-

control study, investigating the incidence of TDS components. Preliminary results are provided.
Results: We included data from 212 medical records from microlithiasis patients examined for an andrological indication from 2012 to 2017 in our Andrology Center. During diagnostic workup in 33 patients (15.56%) testicular cancer was revealed.
In the TGCT patient group, the mean age was 39.36 years. 27 patients (82%) had impaired sperm analysis result, azoospermia was found in 8 (24.24%) patients. 6 patients (18.18%) had a history of cryptorchidism, hypospadias was not observed in this patient group.
Conclusion: Men with TDS are at increased risk of infertility and testicular cancer compared to the general population. Our results show a possible link between TML and other TDS components and support the recommendations of the EAU guidelines, that in case of the existence of more components of TDS, close follow-up is needed, and testicular biopsy (onco-TESE) should be offered for azoospermic patients.

P070

Dorsal phalloplasty: revealing the concealed penis along with penile prosthesis implantation

O. SHAEER[1] AND K. SHAEER[2]
[1]Department of Andrology, Kasr EL Aini Faculty of Medicine, Cairo University, Egypt; [2]Kamal Shaeer Hospital, Cairo, Egypt

Background: Seventy-two percent of patients complain of shortening following penile prosthesis implantation (PPI). Dorsal phalloplasty (DP) is a minimally invasive same-session adjuvant to PPI, where a tacking suture approximates the under surface of the peno-pubic junction (PPJ) to the pubis, enhancing length.
Objective: This work describes long term follow up results of DP.
Methods: This is a controlled prospective study of 66 patients who underwent simultaneous PPI and DP (PPIDP group), and 60 underwent PPI without DP (Control group). For the PPID group, the tacking suture was placed using size-5 non-absorbable Polyester suture material, passed through the pubic periosteum then into the subcutaneous tissue and dermis of the PPJ, and left untied, followed by implantation, then tying the tacking suture. DP was evaluated as per measured gain in erect length in the PPIDP group indicated by the difference between pre-tacking and post-tacking length, maintenance of that length gain until final follow up, as well as by the difference between both groups in impression on post-implantation length compared to length before ED had set in ("longer", "same" or "shorter"), and satisfaction with length on a five-point Likert scale).
Results: PPIDP group demonstrated a 23% increase in length post-tacking, compared to pre-tacking ($p < 0.0001$), maintained up to final follow up (36 months $\pm$ 4.7). 80% of patients in the control group reported a shorter penis compared to before ED had set-in, in contrast to 6.1% in the PPIDP group. The PPIDP group reported 28.4% higher satisfaction with length, compared to the control group ($p < 0.0001$).
Conclusion: DP along with PPI improved visible length, minimized the impression of shortening and enhanced satisfaction with length.

P071

Patient and parental satisfaction and long term psychosexual outcome after hypospadias surgery

L. J. W. TACK[1], A.-F. SPINOIT[2], P. HOEBEKE[2], E. V. LAECKE[2], A. MAHMOUD[3], S. RIEDL[4,5], M. HIESS[6], U. TONNHOFER[7], A. SPRINGER[7], M. COOLS[1] AND E. V. HOECKE[8]
[1]Department of Pediatric Endocrinology and Diabetology, Ghent University Hospital, Ghent, Belgium; [2]Department of Pediatric Urology, Ghent University Hospital, Ghent, Belgium; [3]Department of Endocrinology, Ghent University Hospital, Ghent, Belgium; [4]Department of Pediatric Pulmonology, Allergology and Endocrinology, Medical University of Vienna, Vienna, Austria; [5]Department of Pediatrics, St Anna Children's Hospital, Medical University of Vienna, Vienna, Austria; [6]Department of Urology, Medical University of Vienna, Vienna, Austria; [7]Department of Pediatric Surgery, Medical University of Vienna, Vienna, Austria; [8]Department of Pediatric Psychology, Ghent University Hospital, Ghent, Belgium

Background: Genital surgery is increasingly controversial. However, parental and patient satisfaction with early hypospadias surgery and long term psychosexual outcome of hypospadias repair are understudied.
Objective: To assess long term psychosexual outcome and attitudes towards past surgery in young adult men (15–21 years) born with hypospadias.
Methods: Cross-sectional assessment of a large cohort of men who were born with hypospadias and controls. Participants filled in five questionnaires: Decision Regret Scale (DRS), Penile Perception Score (PPS), Sexual Quality of Life – Male (SQoL-M), International Index of Erectile Function (IIEF-5) and a custom-made questionnaire. DRS and a custom-made questionnaire were also completed by parents.
Results: Ninety-seven men participated in the study (75 hypospadias and 22 controls). Forty-seven percent of parents worried about their sons' fertility, sexual experience and/or pubertal development. The majority (75%) of parents indicated to have been initially shocked by the hypospadias of their son, 15% of them had great difficulties to deal with the diagnosis. Most parents found that the parents should decide together with the doctor if hypospadias repair is needed (76.4%), whereas none indicated that the child should decide, likely due to the young age at first surgery (mean 2.2 years). Most men agreed (74.7%), however, many would have preferred to take part in the decision once old enough to understand the implications of surgery. Although the majority of parents/participants had no regret, a higher number of reinterventions correlated with higher regret scores. Forty-four percent of men who had hypospadias felt that their penis looked different and was smaller than their friends' penises. Men who had hypospadias did not feel more inhibited than controls to engage with someone they find attractive (hypospadias: 13.5%; controls: 13.6%). Nonetheless, fewer men born with hypospadias had had sexual

intercourse at the time of the survey (hypospadias: 54.7%; controls: 86.4%). Twenty-five percent of men with hypospadias was worried about their fertility as compared to 14% of controls. PPS revealed more dissatisfaction regarding penile length in hypospadias cases than controls (25.3% vs. 9.1%), which was associated with smaller stretched penile length in hypospadias cases only (dissatisfied: 11 cm; satisfied: 14 cm). The location of the meatus urethrae, shape of the skin of the penis and axis in erection were causes of dissatisfaction in 17.3%, 16% and 13.3% of hypospadias cases, respectively. Seven out of 75 (9.3%) men who had hypospadias were dissatisfied about their overall genital appearance, as compared to one of 22 (4.5%) controls. One man who had hypospadias had mild erectile dysfunction (IIEF-5: 19/25) and another had sexual dysfunction (SQoL-M: 48).

Conclusion: Parents are concerned about their sons' psychosexual development and some had difficulties to deal with the diagnosis. According to patients and parents, the decision about hypospadias repair should be taken by the parents and surgeon. Dissatisfaction about the genital appearance and worries about fertility are causes of concern in young men treated for hypospadias. Sexual or erectile dysfunction is infrequent in this age group.

P072

Detection of mitochondrial Reactive Oxygen Species in live spermatozoa of infertile subjects and in cancer patients

F. CARPENTIERO[1], G. MANGONE[2], C. AZZARI[2], E. BALDI[1], L. GIOVANNELLI[3] AND M. MURATORI[1]

[1]Sexual Medicine and Andrology Unit, Department of Experimental, Clinical and Biomedical Sciences, University of Florence, Florence, Italy; [2]Division of Immunology, Section of Paediatrics, Department of Health Sciences, Anna Meyer Children's University Hospital, Florence, Italy; [3]NEUROFARBA Department, Pharmacology and Toxicology Section, University of Florence, Florence, Italy

Background: Oxidative stress occurs when the levels of Reactive Oxygen Species (ROS) overwhelm antioxidant defences and has been strongly associated with male reproductive dysfunction. Hence, determination of ROS in semen appears as an important test in male infertility work up.

Methods: In this study, we evaluated the percentage of Live spermatozoa with Oxidative Stress (LOS) in native semen samples, by using a flow cytometric technique coupling the detection of sperm mitochondrial ROS (with MitoSOX probe) with staining of dead cells (with LIVE/DEAD fixable dead cells stain kit). With this technique we evaluated LOS in 80 infertile and in 16 cancer patients (testicular and hematological cancer), afferent to our laboratory for, respectively executing routine semen analysis and cryopreserving semen before undergoing cytotoxic therapy. In infertile patients the median value (IQR) of LOS was 24.80% (16.29;33.20).

Results: After grouping the patients according to the presence/absence of clinical signs of oxidative stress (inflammation/infection, smoking habit, leukocytospermia, semen viscosity, semen bacteria), we found that LOS was 28.56% (25.01;40.79) in subjects with clinical signs of semen oxidative stress ($n = 42$) and 17.18% (12.18;21.71) in subjects without ($n = 38$, $p = 0.0001$). To verify whether LOS was able to identify patients with semen oxidative stress, we built proper ROC curves. We found that LOS predicted the presence of clinical signs of oxidative stress with a good accuracy (AUC: 0.799, CI: 0.692–0.906) and that using 22.74%, as threshold value, the TPP (true positive proportion) was 86% whereas the false positive proportion (FPP) was 21%. As known, oxidative stress is one of the main mechanism originating sperm DNA fragmentation (sDF). To verify whether live spermatozoa with mitochondrial ROS also exhibited higher levels of sDF, first we sorted live spermatozoa with and without mitochondrial ROS using a FACSAria cell sorter. Subsequently, we processed the two sorted fractions with Comet assay. As expected, we found a higher amount of sDF in the spermatozoa with mitochondrial ROS than in those without (Median Percentage Tail Intensity: $37.80 \pm 9.20\%$ vs. $27.90 \pm 4.80\%$, respectively). In the 16 cancer patients we found that LOS was 41.53% (28.71;62.10) [testicular cancer, $n = 9$: 44.14% (34.26;80.32); haematological cancer, $n = 7$: 31.22% (18.00;47.26)]. Such value was much higher than in infertile men ($p = 0.0001$), even considering only those infertile subjects presenting clinical signs of semen oxidation ($p = 0.017$). No difference was found between the two types of cancer ($p = 0.174$).

Conclusion: This study shows a new flow cytometric technique for evaluating oxidative stress in live spermatozoa. Contrary to previous similar methods, such technique does not use selected spermatozoa but native semen samples, which are much more representative of the in vivo condition. In addition, the technique resulted able to identify subjects with clinical signs of semen oxidative stress with good accuracy. With this technique we found that semen from subjects with cancer showed very high levels of oxidative stress that could explain the higher detrimental effects of semen cryopreservation observed in these patients.

P073

The role of appendix testis androgen receptor status in the hormonal treatment of undescended testis

G. DRABIK[1], T. JÓZSA[2], O. RUZSNAVSZKY[3], K. KISTAMÁS[4], B. DIENES[4], I. KOVACS[4], M. BENYÓ[1] AND T. FLASKÓ[1]

[1]Department of Urology, Faculty of Medicine, University of Debrecen, Hungary; [2]Division of Urology, Department of Pediatric Surgery, Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden; [3]Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; [4]Division of Pathology, Kenézy Gyula Hospital, Debrecen, Hungary

Background: Human chorionic gonadotropin hormonal treatment (hCG) in cryptorchidism is still debated because of its poor success rate and adverse effects. The most recent guidelines not support the use of hormonal therapy as a primary treatment option.

We compared the androgen receptor status of the appendix testis in congenital undescended and retractile

testicles to explore the causes of different hCG efficacy in several types undescended testicles.
Methods: We removed 24 appendix testes under orchiopexy of 21 boys to detect androgen receptor expression with immunofluorescence staining. In Group 1 (n = 3) appendix testes from 3 patients with unilateral and in Group 2 (n = 6) appendix testes from 3 patients with bilateral congenital undescended testis were removed. Bilateral form were treated with human chorionic gonadotropin without effect. In Group 3 (n = 12) appendix testes were examined of 12 boys with acquired undescended testis. Group 4 (n = 3) includes appendix testes from 3 young azoospermic adults previously treated with hCG because of undescended testis in their childhood and recently underwent testicular biopsy. Further seven appendix testes were removed for mRNA detection of androgen receptors with RT-PCR analysis. (3 from patients with congenital undescended testis and 4 from patients with retractile testis).
Results: In Group 3 and 4 androgen receptor expression was evaluated by immunofluorescence and immunohistochemistry staining. There was no visible androgen receptor expression in Group 1 and 2 however RT-PCR analysis revealed mRNA expression of androgen receptors both congenital undescended and retractile testicles.
Conclusion: The presence of androgen receptor of appendix testis in patients with retractile testicle and the absence of androgen receptor in patients with congenital undescended testis can be a possible cause of the effectiveness or ineffectiveness of hormonal treatment in patients with undescended testicles.

P074

Comparative analysis of semen parameters and ejaculate sediment in patients with Cystic Fibrosis and CBAVD

M. I. SHTAUT[1], E. MARNAT[2], L. SCHILEIKO[1], G. SHMARINA[1], T. SOROKINA[1], L. F. KURILO[1], S. REPINA[1,2], S. KRASOVSKY[3], M. ANDREEVA[1] AND V. CHERNYKH[1,2]
[1]Research Centre for Medical Genetics, Russian Federation, Moscow, Russia; [2]Pirogov Russian National Research Medical University, Moscow, Russia; [3]Pulmonology Research Institute of Moscow, Moscow, Russia

Background: Cystic Fibrosis (CF) and CBAVD syndrome are genetic disorders caused by CFTR gene mutations. CBAVD patients and vast majority with CF are infertile due to obstructive azoospermia resulted from bilateral obstruction of vas deferens.
Objective: The aim of the study was comparative analysis of semen parameters, ejaculate sediment and fructose content in azoospermic men with CF and CBAVD patients.
Methods: Examined group consisted of 76 azoo-/cryptospermic men, including 53 CF and 23 CBAVD patients (24.8 ± 4.6; 17–39; and 32.6 ± 8.8; 22–57 years old, respectively). Semen analysis was performed according to the WHO laboratory manual for the examination and processing of human semen (2010). Quantitative karyological analysis (QKA) of immature germ cells (IGCs) from the ejaculate sediment was done to detect germ cells at different developmental stages. Fructose in seminal plasma was measured by photometric test using commercial kit (Ferti-Pro NV, Belgium). The Mann–Whitney U test was used for the statistics analysis.
Results: No significant difference was found between CF and CBAVD groups for the following ejaculate parameters: volume, pH, viscosity, sperm and leukocytes concentration ($p \geq 0.5$). Semen volume varied from a very low to normal (CF – 0.7 ± 0.8 mL, CBAVD – 0.6 ± 0.4 mL); oligospermia was found in 93% and 91% patients of these groups, respectively. Increased acidity was characteristic for semen fluids in both CF (pH 6.1 ± 0.3) and CBAVD (pH 6.3 ± 0.5) patients. Increased viscosity was approximately equal in the groups (CF – 18.3%, CBAVD – 19.0%). Cryptozoospermia was found in 10% and 26% samples (CF and CBAVD, respectively). Furthermore, QKA IGCs, performed in 46 men with CF and in 13 CBAVD patients, allowed to detect spermatozoa (n = 0–160, 32.10 ± 40.55; n = 0–156, 37.54 ± 50.87) and/or immature germ cells (n = 0–483, 65.2 ± 81.1; n = 0–201; 49.9 ± 61.4) in the ejaculate sediment in all analyzed samples of these groups, respectively. Moderate leucospermia was revealed in 1.7% CF and 4.3% CBAVD patients, respectively. Fructose, analyzed in 27 semen samples, was decreased in 26 patients with no significant difference between groups (CF – 0.29 ± 0.69; 0–3.54 mg/ejaculate, CBAVD – 0.59 ± 0.25; 0.12–0.47 mg/ejaculate). One (4%) of CF patients presented fructose content in normal range ($N \geq 2.4$ mg/ejaculate).
Conclusion: CBAVD and azoospermic CF patients present the same degree of the obstruction of seminal ducts and the impairment of seminal vesicles with characteristic semen parameters (azoo-/cryptozoospermia, oligospermia, low pH and fructose content). Spermatozoa and IGCs, detected in the ejaculate sediment in azoospermic patients with CF/CBAVD, indicate an incomplete bilateral obstruction of seminal ducts, but not congenital absence of the vas deferens.

P075

The methylation and expression analysis of MLH1, MSH2 genes in male infertility

O. SENA AYDOS[1], T. ÖZKAN[1], Y. HEKMATSHOAR[1], S. ILGAZ[1], A. SUNGUROĞLU[1] AND K. AYDOS[2]
[1]Department of Medical Biology, Medical School, Ankara University; [2]Department of Urology, Medical School, Ankara University

Background: There is a strong association between sperm genetic damage and male infertility. One of the reasons of DNA damage is inactivation of DNA repair pathway. Like genetic changes, epigenetic changes play important roles in maintenance of genetic stability and regulation of genomic structure. For our knowledge, the methylation patterns of DNA mismatch repair genes were studied especially in cancer and some other diseases, but there was very few studies on male infertility in the literature.
Objective: In this study we aimed to investigate the methylation patterns of hMLH1 and hMSH2 genes in male infertility and also expression analysis of them to clarify the roles in fertility status and sperm DNA fragmentation.
Methods: The study was performed on 40 infertile and 8 healthy fertile men. Isolated sperm DNAs were modified by bisulfide treatment-DNA modification method and

were analyzed by Methylation Specific PCR (MSP) and sperm DNA fragmentation was analyzed by 'Comet Assay'. The expression levels of MLH1 and MSH2 were studied by real-time quantitative polymerase chain reaction (RT-QPCR).
Results: Our results showed that MLH1 and MSH2 expression levels were decreased in comparison with control group ($p \leq 0.001$). However our results showed, no methylation difference of hMLH1 and hMSH2 genes but significantly fragmentation of DNA ($p < 0.001$) in infertile men.
Conclusion: According to our data it can be suggested that low expression of MLH1 and MSH2 genes can leads to sperm DNA fragmentation and male infertility, but different mechanisms other than methylation play role in low expression levels.
This project was supported by grand 09H3330005 from Ankara University Scientific Research Projects (BAP).

P076

A differential cytokine expression profile before and after rFSH treatment in Sertoli cell cultures of men with non-obstructive azoospermia

O. S. AYDOS[1], S. HEIDARGHOLIZADEH[1], Y. YUKSELTEN[1], S. OZKAVUKCU[2], A. SUNGUROĞLU[1] AND K. AYDOS[3]
[1]Department of Medical Biology, School of Medicine, Ankara University, Ankara, Turkey; [2]Department of Obstetrics and Gynecology, Assisted Reproduction Center, School of Medicine, Ankara University, Ankara, Turkey; [3]Department of Urology, School of Medicine, Ankara University Ankara, Turkey

Objective: To evaluate the differences for the expression of cytokine profiles in Sertoli cells of non obstructive azoospermia (NOA) and obstructive azoospermia (OA) patients and the effects of FSH treatment on cytokine gene expressions.
Methods: A total of 15 azoospermic men diagnosed as OA ($n = 5$) (control group) and NOA ($n = 10$) were included in the study. NOA patients were split into two further subgroups: normal (nFSH) and high (hFSH) serum FSH levels. Primary Sertoli cell cultures were prepared from the testicular tissue samples collected during the micro-TESE procedure. Expression of cytokine gene panel (88 genes), FSH receptor (FSHR) and androgen binding protein (ABP) were evaluated by real-time PCR array analysis. FSHR protein level was measured by the Western blot.
Results: In primary cultures of Sertoli cells 7 genes were found to be increased and 13 were decreased in NOA group, when compared to OA ($p < 0.05$). When rFSH was introduced into the culture media, expression of 12 genes in the NOA group restored a comparable level to those of the control OA group. Sertoli cells in all groups responded rFSH administration with increased expression of ABP.
Conclusion: Our study was the first to measure the expression levels of these cytokine genes in Sertoli cells in relation to their influence on male infertility. Our results suggest that FSH treatment may have positive effects on Sertoli cells of non-obstructive azoospermic patients via changing the expression levels of certain cytokine genes and restoring their levels in normal Sertoli cell population. Some cytokine levels can be considered as a potential candidate for detecting NOA patients. ABP is a good marker for cell viability and functionality in primary Sertoli cell culture.
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P077

Pathology of the reproductive system in male patients with Cystic Fibrosis

S. REPINA[1,2], S. KRASOVSKY[3], G. SHMARINA[2], R. ROZHIVANOV[4], T. SOROKINA[2], L. SHILEIKO[2], M. I. SHTAUT[2], L. F. KURILO[2], T. ADYAN[1,2], A. STEPANOVA[2], A. V. POLYAKOV[2] AND V. CHERNYKH[1,2]
[1]Pirogov Russian National Research Medical University, Moscow, Russian Federation; [2]Research Centre for Medical Genetics, Moscow, Russian Federation; [3]Pulmonology Research Institute, Moscow, Russian Federation; [4]National Research Centre for Endocrinology, Moscow, Russian Federation

Background: Cystic Fibrosis (CF) is a common monogenic disease caused by CFTR gene mutations.
Methods: We examined 81 Russian men (15–69, mean 25.6 ± 7.9 years old) with CF [pancreatic sufficient (PS), $n = 42$, and pancreatic insufficient (PI), $n = 39$]. Standard and biochemical semen analysis were performed according to WHO recommendations (2010). CFTR gene was analyzed by AFLP-PCR, MLPA and DNA sequencing.
Results: Physical examination not revealed abnormalities of external genitals. 48% of patients had a delay of puberty (15 ± 1.5 years). 26% of patients had urological pathology in anamnesis. Mean level of testosterone was 4.6 ± 1.9 ng/mL ($N = 3$–12 ng/mL), 24% patients presented low level of testosterone. Total testicular volume was 26.2 ± 8.2 cm3 (TS – 12.5 ± 4.2 cm^3; TD – 13.7 ± 4.4 cm^3). Mono-/bilateral testicular hypoplasia were observed in 42% patients. Pathologic ultrasonography findings were detected in 70% patients. Diffuse alterations and cysts of epididymis, tumors of the testis were found in 53.5% and 9.3% patients, respectively. Diffuse changes/calcifications of the prostate and hypoplasia of the seminal vesicles were found in 80% and 100% patients, respectively. Azoo-/cryptozoospermia was found in 87.5% samples, moderate oligo-/asthenozoospermia and normozoospermia – in 11.1% and 1.4% samples, respectively. Azoo-/cryptozoospermia more frequently was observed in PI then in PS patients groups (100% vs. 76.5%, $p = 0.0008$, Fisher test). In 82.7% samples the acidity of the ejaculate was less than normal. Oligospermia was found in 87.5% samples and was significantly more often noted in the group of men PI CF (93% vs. 61%) ($\chi^2 = 11.58$; $p = 0.0007$). The signs of the partial arrest of spermatogenesis at prophase I of meiosis were detected in 37% patients. Patients <25 years more frequently presented normal volume of ejaculate then older ones (22.9% vs. 12.9%) and moderate oligo-/asthenozoospermia (12.9% vs. 9.7%) Biochemistry shown low fructose, citrate and α-glucosidase in 96%, 45.5% and 37.5% samples, respectively. CFTR mutations were detected in 100% alleles. F508del was commonest (48%

alleles), other common CFTR mutations were 3849 + 10kbC>T (13.0%), CFTRdele2.3(21 kb) (6.2%), E92K (4.9%),2789 + 5G>A (2.5%), others mutations (25.4%). No seminal ducts obstruction was found in 15 of 21 (71%) 3849 + 10 kb C>T mutation's carriers. A significant difference ($\chi^2 = 46.38$; $p < 0.00001$) in the mutation frequency of 3849 + 10 kb C>T was detected between men with an obstruction (9.5%) and men without obstruction (93.8%) of the vas deferens.

Most of men with CF have signs of obstruction vas deferens, aplasia/hypoplasia of the seminal vesicles (oligospermia, azoo/cryptococcemia, pH < 7.0, a low of fructose in ejaculate), impaired excretory function of the prostate gland, diffuse alterations and cysts of epididymis. Often there is a delay of puberty, hypoplasia of the testicles, disorders of spermatogenesis.

Conclusion: The presence in the ejaculate of male germ cells in all men with CF, including azoospermia, indicates not the absence of vas deferens, but a bilateral violation of their patency. PS CF, young age (<25 years) and 3849 + 10 kb C>T mutation are factors of preserved fertility in CF patients.

P079

Variants in NANOS1 are associated with male infertility

C. FRIEDRICH[1], N. KÖCKERLING[1], A. RÖPKE[1], L. HANKAMP[1], C. KRALLMANN[2], S. KLIESCH[2] AND F. TÜTTELMANN[1]

[1]Institute of Human Genetics, University of Münster, Münster, Germany; [2]Centre of Reproductive Medicine and Andrology, Department of Clinical and Surgical Andrology, University Hospital Münster, Münster, Germany

Background: Approximately 7% of the male population worldwide is affected by male infertility. Diagnosing male infertility mostly relies on semen and hormone analysis resulting in descriptive classification into oligo- and azoospermia. Genetic causes of male infertility include chromosomal aberrations, AZF deletions, CBAVD and CHH. Single-gene defects could be another reason for male infertility (1). One of the putative candidate genes is NANOS1, a human homologue of the Drosophila morphogen. In Drosophila Nanos is a key translational regulator of specific mRNAs during morphogenesis and germ cell development. In humans, three NANOS homologues (NANOS1-3) have been described. Although the NANOS2 and 3 knock out-mice are infertile, there is currently no evidence for infertility-causing mutations in these two genes in humans. In contrast, the disruption of NANOS1 has no consequences for germ cell development in mice, but NANOS1 (OMIM 608226) gene variants (in-frame deletions and missense variants) have been described to be associated with male infertility in humans (2).

Methods: A large patient cohort of well characterised infertile men ($n = 286$) with different phenotypes (non-obstructive azoospermia due to SCOS $n = 145$, meiotic arrest $n = 24$, mixed atrophy $n = 22$, no biopsy $n = 62$, and severe oligozoospermia $n = 33$) was analysed by whole exome sequencing (WES). Variants were prioritized due to their consequence on protein function, minor allele frequency (MAF <2% in dbSNP, ESP, gnomAd, ExAc) and in silico pathogenicity prediction (CADD >10, PolyPhen-2, SIFT, MutationTaster).

Results: Starting with 24 variants, in the first step, noncoding variants were excluded ($n = 16$). Then, only rare and novel variants (MAF <2%) were considered and six variants remained (2 missense variants, 4 in-frame deletions) which were present in patients with non-obstructive azoospermia ($n = 3$), SCOS ($n = 1$), severe oligozoospermia ($n = 1$) and mixed testicular atrophy ($n = 1$). One of the missense variants showed a consistent pathogenic in silico prediction while the other had a discordant result. Two of the identified in-frame deletions have formerly been described to be pathogenic in patients with SCOS.

Conclusion: Regarding our results, this study substantiates the association between rare variants in NANOS1 and male infertility. However, the variable phenotypes found so far warrant further functional analyses.

This work was carried out within the frame of the DFG Clinical Research Unit, Male Germ Cells: from Genes to Function' (CRU 326).

References: (1) Tüttelmann et al. Medizinische Genetik. 2018;30:12–20.

(2) Kusz-Zamelczyk et al. Journal of Medical Genetics 2013;50:187–193.

P080

FSHB polymorphism as putative predictor of sperm retrieval in testicular sperm extraction

J.-F. CREMERS[1], A. S. BUSCH[2,3,4], M. SCHUBERT[1], M. ZITZMANN[1], J. GROMOLL[2], F. TÜTTELMANN[5] AND S. KLIESCH[1]

[1]Centre of Reproductive Medicine and Andrology, Department of Clinical and Surgical Andrology, University of Münster, Germany; [2]Centre of Reproductive Medicine and Andrology, Institute of Reproductive and Regenerative Biology, University of Münster, Germany; [3]International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), Rigshospitalet, Denmark; [4]Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Denmark; [5]Institute of Human Genetics, University of Münster, Germany

Background: Male infertility is often characterized impaired sperm production. The most severe form clinically presents as azoospermia or cryptozoospermia. Knowing of FSH being a key player in normal spermatogenesis, genetic variations i.e. single nucleotide polymorphisms (SNPs) of FSHB and FSHR affecting FSH action have been shown to be strongly associated with male reproduction. We hypothesized that genetic variations of FSH action might contribute to the etiology of impaired spermatogenesis and might predict testicular sperm extraction outcome.

Methods: We enrolled retrospectively 1366 azoo-/cryptozoospermic patients who underwent testicular biopsy or TESE procedure at the CeRA in Münster between 1997 and 2016. After karyotyping we assessed the association of 3 SNPs (FSHB c.-211G>T, FSHR c.-29G>A, FSHR c.2039A>G) on surgical sperm retrieval and hormone levels.

Results: We observed a significant association of FSHB c.-211G>T with TESE outcome in patients without major causative factors of azoospermia (n = 747, p = 0.004). The Odds ratio was 0.73 (95%CI: 0.53–0.995) and 0.25 (95%CI: 0.09–0.68) for the FSHB c.-211G>T heterozygotes and minor allele homozygotes, respectively, compared to the FSHB c.-211G>T wild-type homozygotes. FSHB c.-211G>T and TESE outcome were significantly associated (p = 0.004). Furthermore a significant association of FSHB c.- 211G>T and FSHR -29G>A with serum FSH levels in patients without major causative factors of azoospermia/severe cryptozoospermia (p = 0.001 and p = 0.03) could be seen.

Conclusion: In conclusion, our study indicates a strong contribution of genetic variation of FSHB and FSHR to the etiology of impaired spermatogenesis may serve as additional predictive factors prior to TESE. FSHB c.- 211G>T genotyping should be taken into consideration prior to TESE.

P081

Mouse knockout model of Tcte1 gene in human spermatogenesis

M. OLSZEWSKA[1], A. MALCHER[1], A. N. YATSENKO[2], N. POLLOCK[2], T. STOKOWY[3], M. KAMIENICZNA[1] AND M. KURPISZ[1]

[1]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland; [2]Department of OB/GYN and Reproductive Sciences, School of Medicine, University of Pittsburgh, PA, USA; [3]Department of Clinical Science, University of Bergen, Norway

Background: T-Complex-associated-Testis-Expressed 1 (TCTE1) gene is evolutionary conserved in most eukaryotic organisms, that contain motility cilia or flagella in their life cycle. In human, defects in TCTE1 gene (locus 6p21.1) are responsible for various ciliopathies. The protein is required for proper flagella functioning and is located in axoneme within nexin-dynein regulatory complex. Expression of TCTE1 is being observed in testis (highest), choroid plexus and Fallopian tube. To date, only one report described a role of Tcte1 gene in infertility. It was shown that sperm cells of Tcte1-null mice exhibited decreased ATP level required for proper dynein functioning. This defect leads to asthenozoospermia in Tcte1-null mice. Expression of mouse Tcte1 gene is being testis-enriched, starts at the stage of spermatids, with the protein localized to the flagellum.

Methods: Using CRISPR/Cas9 technique, mouse knockout line Tcte1 was created on the basis of C57Bl/6J strain. Adult animals were mated for 5 months to check their reproductive potential, with schemes: Tcte1-null male × wild type (wt) female, Tcte1-heterozygous male × Tcte1-heterozygous female, and Tcte1-null female × wt male. Only Tcte1-null males revealed no progeny at all. Other combinations showed similar results to control mating, when considering number of litters, pups, and sex ratio. Also, no differences in body parameters were found between homo-, hetero-, and wild type animals (body mass, weight and size of testes, and epididymis). Seminal analysis revealed no differences in sperm concentration, however, movement of spermatozoa in Tcte1-null males was decreased (circular pathway). Genotyping of pups was performed using tail-blood samples. At the age of 12–14 weeks, animals were sectioned, and histopathology of testes, epididymis, prostate and brain was performed. DNA, RNA and proteins were extracted (AllPrep, Qiagen; MagCore, RBC Biosciences). Genes expression was performed by RNAseq method (Illumina, NovaSeq6000), followed by RT-PCR validation. Immunocytochemistry or immunofluorescence with proper antibodies were performed to evaluate meiotic events in testis, and apoptosis occurrence in sectioned organs. Also, mutation screening was performed using whole exome sequencing (WES) and Sanger sequencing for total 200 azoospermia males.

Results: Results revealed 6 heterogeneous rare variants in 7 patients, so far. Results obtained confirm the role of Tcte1 gene mutation in male infertility, both in human as well as mouse model.

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P082

The relationship between selected indicators of obesity and fat accumulation and testosterone deficiency in aging men

I. ROTTER[1], A. RYŁ[1], K. GRZESIAK[2], A. SZYLIŃSKA[1], O. SIPAK-SZMIGIEL[3] AND M. LASZCZYŃSKA[2]

[1]Department of Medical Rehabilitation and Clinical Physiotherapy, Pomeranian Medical University in Szczecin, Poland; [2]Department of Histology and Developmental Biology, Pomeranian Medical University in Szczecin, Poland; [3]Department of Obstetrics and Gynecology, Pomeranian Medical University in Szczecin, Poland

Background: Numerous studies indicate the existence of mutual relationships between low levels of testosterone and obesity. The content of adipose tissue is related to the concentration of androgens in men and affects the balance between androgens and estrogens.

Objective: The aim of the study was to show which of the indicators used in the assessment of overweight and obesity and the accumulation of adipose tissue is the best indicator of testosterone deficiency in non-diabetic aging men. We assumed that the fat accumulation indicators that take into account biochemical parameters of lipid metabolism would be better markers of actual fat deposition in the body than those based only on anthropometric measurements.

Methods: Four hundred and fifty-five non diabetic men aged 50–75 participated in the study. The participants underwent anthropometric measurement and determination of total testosterone (TT), estradiol (E2), dehydroepiandrosterone sulphate (DHEA S), sex hormone binding protein (SHBG), fasting glucose (FPG), high-density lipids cholesterol (HDL-Ch), and triacylglycerols (TAG) in serum. The following indicators were calculated: body mass index (BMI), waist-to-hip ratio (WHR), lipid accumulation product (LAP), and visceral adiposity index (VAI).

Results: It was shown that patients with TT deficiency and without TT deficiency differed in each of the obesity indicators analyzed in the study. It was shown that each of the analyzed indicators correlated statistically significantly with TT concentration in the patients' blood serum. It was also shown that WC was the only indicator showing a statistically significant negative correlation with another hormone: DHEA-S. A logistic regression analysis was performed, explaining the most powerful predictors for testosterone deficiency. It was shown that each of the indicators showed static significance in this analysis. The index that had the highest prediction value was VAI, while the lowest was shown by WC. ROC curves were analyzed by setting cut-off points for each of the analyzed indicators. It was shown that the threshold value at which the risk of T deficiency increased was 28.71 kg/m^2 for BMI, 1.58 for VAI, 104 cm for WC, and 37.01 for LAP.
Conclusion: We believe that fat collection indicators in which biochemical parameters assessing lipid metabolism are also taken into account are better markers of actual body fat deposition than those based only on anthropometric measurements. Of particular note is the VAI index, which seems to be the most suitable biomarker of testosterone disorders in non-diabetic aging men.rn

P083

The possible role of strain-elastography in the investigation of male infertility

Z. FEJES[1], É. BARTHA[1], L. KARCZAGI[1], N. PÁSZTOR[2], I. KIRÁLY[3], B. KIS[4], K. KALMÁR[4] AND A. PALKÓ[4]
[1]Affidea Diagnostic Ltd., Szeged, Hungary; [2]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Szeged, Hungary; [3]Department of Urology, Faculty of Medicine, University of Szeged, Hungary; [4]Department of Radiology, Faculty of Medicine, University of Szeged, Hungary

Background: Infertility affects millions of men of reproductive age. Over the last decades, the incidence has been increasing worldwide, including Hungary. Testicular ultrasound examination is very important in the investigation of male infertility and complementing it by strain-elastography can help to clarify its etiology.
Methods: 23 males of infertile couples were included in the research (age 28–44, average 37.24 years). The control group consisted of healthy volunteers with normal sperm parameters (4 persons). Testicular ultrasound examination including strain-elastography was performed to determine the degree of tissue elasticity. According to our hypothesis, the development of testicular rigidity may differ in the various forms of infertility, and may correlate with pathological changes of sperm analysis. Strain ratio was calculated, reference value was taken as the elasticity value of scrotal subcutaneous fat tissue. These data were compared with the result of the sperm analysis of the patient. A GE Loqic E9 ultrasound, high-frequency (7.5–15 MHz) array linear transducer was used.
Results: We found correlations between the strain ratio values and sperm parameters. There was a significant difference between the strain ratio values of patients with normal and abnormal sperm parameters and the volumes of the testicles ($p < 0.05$, $p < 0.01$, respectively). There was no significant difference in age. The strain ratio showed a significant correlation with the non-progressive motility (rho = 0.65, $p < 0.01$) in group with abnormal sperm parameters. A strong inverse correlation between the strain ratio and the sperm concentration as well as the abnormal morphology can be measured (rho = −0.6, rho = −0.8 rho = $p < 0.05$, respectively).
However, no significant difference was observed in the case of progressive motility, but moderate inverse correlation was observed in the group with abnormal sperm parameters.
Conclusion: Our study shows that strain–elastography may provide additional objective information to support the algorithm used to investigation of male infertility. Strain-elastography contributes significantly to the accuracy of ultrasonography in the evaluation of testicles.

P084

The role of penile rehabilitation for Peyronie's, does it really work?

A. BABU, A. ALMITWALLI, B. VISSAMSETTI AND M. HILMY
York Teaching Hospital, York, UK

Background: Peyronie's disease (PD) causes penile deformity and erectile dysfunction (ED) in sexually active men with incidence of 3–9%. Penile rehabilitation is recommended (EAU 2016) to limit the progression of the disease.
Methods: A single centre analysis of management of patients with PD over a 2-year period. Data was collected retrospectively via case note review.
Results: A total of 68 patients, who had at least one follow up appointment, were seen from July 2015 to March 2018, with a mean age of 55 (23–74) years. All patients were offered to fill in a Peyronie's Disease Questionnaire (PDQ). Penile deformity ranged between 20 and 60 degrees. Vacuum treatment (SOMAcorrect – ©iMEDicare) was offered in 51 patients as primary therapy. Surgery was offered as primary treatment in 10 patients and 7 patients were discharged with no treatment. In those that received vacuum treatment there was subjective improvement seen in 49% ($N = 25$). In this group, there was a significant increase in the ability to perform penetrative intercourse, 48% ($N = 12$). Those that failed SOMAcorrect therapy were offered surgery ($N = 26$) in the form of Nesbitt's procedure. The failure group showed only a 38% improvement in the ability to perform penetrative intercourse, pre-surgery. 10 patients were offered Nesbitt's as a primary treatment method with 60% improvement in ability to perform penetrative intercourse. Pretreatment mean curvature in those that improved with SOMAcorrect was noted to be 38°. In contrast, those that failed SOMAcorrect or underwent primary surgery had a pretreatment angle of 44–45°.
Conclusion: SOMAcorrect is a valuable tool in select patients to treat PD. It has the potential to prevent significant surgical intervention in a large proportion of patients with minimal adverse effects. Preliminary results show comparable efficacy to surgery with a minimally invasive approach. Subjective outcomes are promising and it

should be considered as primary treatment method in appropriate patients.

P085

A comprehensive baseline study of erectile function using Doppler ultrasound, IIEF and EHS in patients affected by prostate cancer before laparoscopic radical prostatectomy

V. P. CORTÉS, J. M. RODRÍGUEZ, N. H. HADI, A. D. GARCÍA, À. P. VILASECA, J. L. G. SALA, D. G. ROJO, E. V. PALACIO, L. D. V. ROMAN, M. ANDRÉS ROSADO URTEAGA, P. P. SOLER, C. A. GAIRIN, C. C. ÁLVAREZ AND J. P. LÓPEZ
Hospital Universitari Parc Taulí, Sabadell, Spain

Background: Prevalence of erectile dysfunction among men between 40 and 70 years old is high and the effect on this function after radical prostatectomy is worsen. Therefore, it is important to perform a baseline assessment of these patients prior to oncological surgery.
Methods: Erectile function (EF) has been evaluated in 112 patients with prostate cancer before being submitted to radical prostatectomy. This function was evaluated by the erectile function domain of the IIEF, the scale EHS and a penile Doppler ultrasound performed in urological cabinet. In addition, the quality of life was assessed using the questionnaires of the EORTC QLQ-C30 and PR25.-C30.
Results: 50.9% of the studied population has a normal EF based on the IIEF questionnaire and 75.9% refer grade 3–4 erection in the EHS. In contrast, only 28.6% presented a Doppler ultrasound within the parameters of normality and 51.8% showed arterial insufficiency. We highlight a significant association ($p < 0.0001$) between categorized IIEF scores (normal, mild/moderate/severe dysfunction) and the value of EHS. The association between the presence of a Doppler ultrasound (normal vs. pathological) and the EHS (3–4 vs. 1–2) present a statistically significant association ($p = 0.005$). Only 35.3% of patients with EHS = 3–4 had a normal ultrasound. We found a significant association ($p = 0.043$) between penile Doppler ultrasound and EF assessed according to IIEF (≥26 vs. <26). Only 38.6% of patients with IIEF ≥ 26 had a normal ultrasound. Regarding the quality of life through the EORTC QLQ-C30 questionnaire, we found an average difference 11.31 points between the item QoL and QLQ summary score ($p < 0.0001$). Among the functions, we find a worse function emotional than the rest of functions ($p < 0.0001$) and also more insomnia symptoms regarding to the rest of symptoms ($p < 0.0001$).
Conclusion: A global assessment of erectile function is crucial in order to assess the expectations of recovery of this function after surgery and not reduce this function only to self-administered tests. The penis Doppler ultrasound can play an important role.

P086

Penile low intensity shock wave treatment for PDE5I refractory erectile dysfunction: a randomized sham-controlled trial

J. VINAY, D. MORENO, O. RAJMIL, E. RUIZ-CASTAÑE AND J. R. SANCHEZ-CURBELO
Fundació Puigvert, Barcelona, Spain

Background: Erectile dysfunction (ED) is a prevalent condition in males, affecting around 30% of men over 40 years. Lately, the use of penile low intensity shockwave treatment (LIST) has been studied on vascular ED patients, because of its potential role in modifying several pathways of ED pathophysiology. The theoretical effect of LIST is based on its ability to increase the blood supply of treated areas.
Objective: To investigate the effect of electromagnetic LIST on the erectile function of patients suffering PDE5I refractory vascular ED.
Methods: Randomized, simple-blind, sham-controlled study. 76 patients completed the study. 40 men were treated with lineal electromagnetic penile LIST (1 session/week for 4 weeks, 5000 shocks/session, using 0.09 mJ/mm^2 of energy density) and 36 were treated with a sham probe. Baseline and post-treatment (1, 3 and 6 months) evaluations were done using validated erectile function questionnaires (IIEF-EF, EHS, SEP2, SEP3 and GAQ1).
Results: Active and sham groups were similar regarding age and comorbidity load. Both groups had moderate ED, with IIEF-EF medians of 12 (IQR 8–17) in the treated group and 13 (IQR 8–17) in the sham group ($p = 0.352$). 3 months after treatment, median changes in IIEF-EF scores for active and sham groups were 3.5 (IQR 0–10) and −0.5 (IQR 8–17), respectively ($p < 0.05$). 6 months after treatment, 21 patients (52.5%) in the active group and 10 patients (27.8%) in the sham group presented EHS>2 ($p < 0.05$). At the same evaluation, 16 (40.0%) and 5 patients (13.9%) had positive answers to the GAQ-1 question ($p < 0.05$), in the treated and sham groups, respectively.
Conclusion: In vitro and in vivo studies have hypothesized 4 pathways in which shockwaves could improve cavernous tissue blood supply: neo-angiogenesis, recruitment of progenitor cells, modulation of vasodilation and nerve regeneration. Only one previous group carried out a randomized clinical trial with patients presenting refractory ED, obtaining similar results to this current study, with shorter follow up and smaller sample size. In this study, linear electromagnetic LIST improved different erectile function parameters at 3 and 6 months of follow-up, when used on refractory ED patients vs. sham treated patients. No adverse events were observed during treatment or follow-up. Currently, there is no good quality evidence, with low risk of biases, that could really asses the role of LIST as a treatment option for vascular ED. It is mandatory to carry on a multicentric clinical trial, using different energy sources, several shockwave protocols and considering different causes of ED, with a follow up of more than 1 year.

P087

Pelvic floor muscle training improves sexual function and sexual distress in women with stress urinary incontinence

A. ZACHARIOU[1], F. DIMITRIADIS[2], C. MAMOULAKIS[3], M. FILIPONI[4], P. TSOUNAPI[5], A. KARAGIANNIS[1], I. GIANNAKIS[1], P. LANTIN[6], M. MARTINEZ[1], A. TAKENAKA[5] AND N. SOFIKITIS[1]
[1]Department of Urology, Medical School, University of Ioannina, Ioannina, Greece; [2]1st Department of Urology, Medical School, Aristotle University, Thessaloniki, Greece; [3]Department of Urology, Medical School, University of Crete, Heraklion, Greece; [4]Physical Medicine and Rehabilitation Centre, Volos, Greece; [5]Department of Urology, Medical School, Tottori University, Yonago, Japan; [6]Urology Centre of the Philippines, Manila, Philippines

Background: Taking into account that the least invasive, repeatable method with the fewer side effects should always be the first choice for the treatment of stress urinary incontinence (SUI), pelvic floor muscle training (PFMT) has been recommended as the first step of conservative management of SUI.
Objective: The aim of this trial was to evaluate the effect of PFMT on sexual function and sexual distress in women with SUI. Secondary objective was to determine if any improvements in sexual function was related to increases in pelvic floor muscle strength, endurance and vaginal resting pressure.
Methods: In this randomized controlled trial, 67 women were randomly included into an intervention Group A (6 months PFMT) and 64 women were randomly included into a control Group B (observation group; negative control group). All women completed a 3-day-bladder diary both at the beginning and at the end of the 6-month trial. Pelvic muscle strength, the number of incontinence episodes and the number of pads used were evaluated, as well. All women were assessed with the Female Sexual Function Index (FSFI) and Female Sexual Distress Scale-Revised (FSDS-R) in the beginning and at the end of the study. Statistical analysis was performed using a Chi-square test (Yates correction).
Results: Differences in baseline variables of sexual function, incontinence episodes, and pelvic floor muscle strength were not significant between groups A and B ($p > 0.05$). At the end of the 6-month period there was a statistically significant improvement in all domains of FSFI in Group A compared with Group B. Eleven women in Group A and two women in Group B reported that their sexual difficulties were resolved at the end of the trial-period. The greater the increase in pelvic floor muscle strength was, the higher the quantitative markers of the sexual function were.
Conclusion: PFMT improves significantly sexual function and sexual distress in women suffering from SUI.

P088

Erectile dysfunction is the main determinant of depression in men with chronic spinal cord injury

A. BARBONETTI, S. D'ANDREA, A. MARTORELLA, E. MINALDI, S. FRANCAVILLA AND F. FRANCAVILLA
Medical Andrology Unit, Department of Life, Health and Environment Sciences, University of L'Aquila, L'Aquila, Italy

Background: Depression is the most common psychological issue following a spinal cord injury (SCI) and is associated with significant disability, mortality and healthcare costs. The loss of global functional independence (FI) and social/work status, along with bladder, bowel and sexual dysfunctions, are expected to contribute to psychological consequences of SCI. Additional putative determinant of depression, such as hypogonadism and low levels of vitamin D (25(OH)D), could also be involved, due to their high prevalence in men with SCI and the demonstrated relationship with depression in the general population.
Objective: As SCI mainly occurs in sexually active young men, rating the improvement of sexual function as a high priority in improving their quality of life, we hypothesized that erectile dysfunction (ED) could represent a major determinant of depression in this population.
Methods: Fifty-seven consecutive male patients (47.0 ± 17.4 years) admitted to a rehabilitation program because of traumatic chronic SCI underwent clinical and biochemical evaluations, including assessment of depressive status by Beck Depression Inventory-II (BDI-II), erectile function by International Index of Erectile Function-5 (IIEF-5), global and bowel-bladder FI by the spinal cord independence measure (SCIM), measurements of total testosterone (TT) and 25(OH)D levels. Free T (FT) levels were calculated by the Vermeulen formula.
Results: Depression (BDI-II score $\geq$14) was reported by 17 subjects (29.8% of the study population). They exhibited significant lower levels of TT, calculated FT and 25(OH)D, a higher prevalence of ED, a more severe bowel-bladder dysfunction and were engaged in a significantly poorer leisure time physical activity (LTPA) when compared to non-depressed men. Specific SCI-related variables, such as level and completeness of the injury and the impairment degree in the global FI, were not significantly different between the two groups. At the multiple logistic regression analysis, including all significant predictors of depression selected by the univariable analysis, depression exhibited a significant independent association only with ED (OR = 19.00; $p = 0.004$) and, to a lesser extent, with a more severe impairment of bowel-bladder function. No significant association was found with testosterone and 25(OH) D levels. Depression was observed in 14 out 32 subjects (43.7%) with ED and only in 3 out 25 subjects (12.0%) without ED ($p = 0.018$).
Conclusion: Healthcare providers should be aware of the importance of managing ED in spinal cord-injured men, as it represents a major independent determinant of their depressive status, which, in turn, may hinder physical rehabilitation programs and exacerbate SCI-related physical health issues. As spinal cord-injured men suffer from a purely organic neurogenic ED, they could also represent an interesting clinical model for shedding light on a causative link between ED and depression.

P089

Psychosexual correlates of unwanted sexual experiences (USE) in women consulting for female sexual dysfunction (FSD) according to their timing across the life span

E. MASEROLI[1], I. SCAVELLO[1], B. CAMPONE[2], V. D. STASI[1], S. CIPRIANI[1], F. FELCIAI[1], V. CAMARTINI[1], A. MAGINI[1], G. CASTELLINI[2], V. RICCA[2], M. MAGGI[1] AND L. VIGNOZZI[1]

[1]Sexual Medicine and Andrology Unit, Department of Experimental, Clinical and Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy; [2]Psychiatric Unit, Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, Florence, Italy

Background: Approximately 20% of women experience Unwanted Sexual Experiences (USE) worldwide. USE survivors are prone to experiencing psychiatric symptoms and sexual dysfunction (SD).

Objective: This study aimed to evaluate the prevalence and correlates of reported USE at different ages in a sexual medicine setting.

Material and Methods: We retrospectively studied 200 heterosexual women attending our clinic for SD. All patients completed the Middlesex Hospital Questionnaire (MHQ), Female Sexual Function Index (FSFI), Female Sexual Distress Scale (FSDS-R) and Body Uneasiness Test (BUT).

Results: Forty-seven women (23.5%) reported USE, which occurred in childhood, adolescence and adulthood in 9% (n = 18), 7.5% (n = 15) and 7% (n = 14) of cases, respectively. USE women showed significantly higher MHQ total score (p = 0.024), free-floating anxiety score (MHQ-A: $p < 0.0001$) and phobic anxiety symptoms score (p = 0.047) when compared with non-USE women. USE women also showed significantly lower FSFI Orgasm domain (p = 0.001), Arousal (p = 0.044) and Satisfaction scores (p = 0.025), and a higher FSDS-R total score (p = 0.003). After adjusting for confounders, only the difference in FSFI Orgasm (F = 6.02, p = 0.015) and FSDS-R total scores (F = 6.41, p = 0.012) retained statistical significance. When considering BUT, USE patients showed significantly higher Global Severity Index (p = 0.021), Weight Phobia (p = 0.025), Body Image Concerns (p = 0.025), Avoidance (p = 0.042), Depersonalization (p = 0.032), Positive Symptom Total (p = 0.045) and Positive Symptom Distress Index (PSDI) scores (p = 0.022) than non-USE patients. All the differences retained statistical significance in a fully-adjusted model. Then, we compared women who never experienced USE with women reporting USE in childhood, adolescence and adulthood. Women reporting USE in adulthood showed significantly higher MHQ-A score and lower FSFI Orgasm score when compared to patients not reporting USE (F = 7.76, p = 0.006 and F = 8.52, p = 0.004 respectively). On the other hand, patients reporting USE in adolescence showed significantly lower FSFI Arousal (F = 5.58, p = 0.019), Orgasm (F = 10.32, p = 0.002), Satisfaction (F = 5.35, p = 0.022) and Total scores (F = 8.16, p = 0.005) and a higher FSDS-R Total score (F = 11.31, p = 0.001) when compared to patients not reporting USE. Moreover, patients reporting USE in adolescence showed significantly lower FSFI Arousal (F = 4.89, p = 0.035), Orgasm (F = 8.24, p = 0.008), Satisfaction (F = 5.79, p = 0.023) and Total scores (F = 9.19, p = 0.005) when compared to patients reporting USE during childhood. Finally, patients reporting USE during childhood showed significantly higher BUT-GSI (F = 6.16, p = 0.015), BUT-DEP (F = 11.97, p = 0.001) and BUT-PSDI scores (F = 6.66, p = 0.012) when compared with patients not reporting USE. All these differences were adjusted for confounders.

Conclusion: Investigating a possible history of USE and its timing is important for a complete assessment of FSD and may reveal specific comorbidities to target in clinical management. Women reporting USE show higher anxiety symptoms, sexual distress and body image concerns and a worse orgasm functioning when compared to non-USE women. USE has a greater negative impact on orgasm functioning and sexual satisfaction when perpetrated in adolescence than in childhood; conversely, women reporting USE in adulthood and childhood show, respectively, higher anxiety symptoms and worse body perception when compared to non-USE women.

P090

Outcome of medical and psychosexual interventions for Vaginismus: a systematic review and meta-analysis

E. MASEROLI[1], I. SCAVELLO[1], G. RASTRELLI[1], E. LIMONCIN[2], S. CIPRIANI[1], G. CORONA[3], M. FAMBRINI[4], A. MAGINI[1], E. A. JANNINI[2], M. MAGGI[1] AND L. VIGNOZZI[1]

[1]Sexual Medicine and Andrology Unit, Department of Experimental, Clinical and Biomedical Sciences "Mario Serio", University of Florence, Italy; [2]Endocrinology and Medical Sexology, Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy; [3]Endocrinology Unit, Medical Department, Azienda Usl Bologna Maggiore-Bellaria Hospital, Bologna, Italy; [4]Gynecology and Obstetrics Unit, Department of Experimental, Clinical and Biomedical Sciences "Mario Serio", University of Florence, Italy

Background: Although Vaginismus (V) is a condition with a great impact on psycho-sexological well-being, the evidence on the efficacy of therapeutic interventions is lacking.

Objective: The aim of this meta-analytic study is to review all information regarding V treatment, including data from observational studies and from RCTs comparing active treatment vs. controls.

Methods: Systematic search was conducted of MEDLINE, EMBASE, and ClinicalTrials.gov. Two independent meta-analyses of observational studies and RCTs were performed. For RCTs, only those having no treatment (waiting list control or placebo) as the comparator were considered eligible. The primary outcome was the success rate (number of successes/total sample) in the completion of sexual intercourse.

Results: Thirty-nine observational studies (1601 women) and 3 RCTs (264 women) were included in the final analyses, respectively. The combination of the results obtained from the observational studies showed that treating V is associated with the completion of sexual penetrative

intercourse in 78% of cases, independently of the therapy used [success rate 0.78 (0.73–0.82)]. When only moderate or strong quality studies were considered, success rate was 0.81 (0.71–0.88). In the meta-analysis of RCTs, the use of psychological interventions showed a trend towards a significantly better result vs. the comparator [MH-OR 10.27 (0.79;133.5); $p = 0.075$]. Neither the diagnostic methods for V used as inclusion criteria, the origin of V (primary, secondary or both), its duration, the mean age of the enrolled women or the involvement of the partner in the intervention exerted a significant effect on the therapeutic outcome.

Clinical Implications: Psychological therapy, pharmacological therapy, pelvic floor physiotherapy and removal of hymenal remnants are all successful treatment for V. Due to the limited evidence available, great caution is required in the clinical interpretation of results.

Strengths and Limitations: We only selected studies specifically enrolling patients with V, and analyses were performed on an intention to treat approach. The main limitations are the small number of trials in the meta-analysis of RCTs and the lack of a comparison group in the meta-analysis of observational studies, which cannot rule out that V could be meaningfully affected by a placebo effect.

Conclusion: The meta-analysis of observational studies indicated that women with V benefit from a range of treatments in almost 80% of cases; no approach has proven superior to the others in allowing the achievement of penetrative intercourse. The meta-analysis of RCTs documented a trend towards higher efficacy of active treatment vs. controls.

P091

Masturbation has a positive impact on long-term functional outcome after nerve-sparing radical prostatectomy

S. DUMLER[1], V. GOETHE[1], A. BANNOWSKY[2], M. KRON[3], H. SCHULWITZ[1], J. GSCHWEND[1] AND K. HERKOMMER[1]
[1]Klinikum rechts der Isar, Technical University of Munich, Germany; [2]Imland Klinik Rendsburg, Germany; [3]Institute of Epidemiology and Medical Biometrics, University of Ulm, Germany

Background: Penile rehabilitation after nerve-sparing radical prostatectomy (nsRP) influences urinary continence (UC) and erectile function (EF). To date little is known about the association between masturbation and functional outcome after RP.

Objective: Aim of this study was to evaluate the association between masturbation and the recovery of UC and EF in patients after nsRP.

Methods: Patients after nsRP (11/2013–3/2016) with preoperative International Erectile Function Score-Erectile Function (IIEF-EF) of ≥22 and/or Erection Hardness Score (EHS) of ≥3 without neo- or adjuvant therapy were included in this analysis. Patients were asked in their follow-up questionnaires 6, 12 and 24 months after their RP if they masturbated (yes = m, no = nm), if they used any pads and if the pads were dry or wet. Their EF was assessed via EHS and/or IIEF-EF and to evaluate the EF more accurately patients were also asked if they had morning erections (yes: occasionally, almost always, always; no: almost never, never). Primary outcome was UC defined as use of 0 or 1 dry safety pad per day and good EF defined as EHS ≥ 2 or IIEF-EF ≥ 17 after RP. Furthermore importance of sexuality was investigated by asking the patients how important sexuality has been for them before and after RP.

Results: 292 patients who were preoperatively potent with median age of 64.1 years (range 45.2–77.6 years) at RP were included. Their median frequency of sexual intercourse in the last 4 weeks before RP was 4.0. 98.9% of the patients stated that sexuality has been important for them at time of RP. After nsRP m- patients stated clearly more often that sexuality has been important for them than nm-patients at any time in their follow-up (6 months – m: 93.5%; nm: 83.6%, 12 months – m: 96.8%; nm: 86.7%, 24 months – m: 93.0%; nm: 83.6%). In m-patients at 6 months follow-up UC was observed clinically significantly more often with 80.5% compared to 63.8% in nm patients. A difference was also found at 12 and 24 months follow-up but was decreasing over time (m: 82.4%; nm: 72.2%, m: 84.9%; nm: 78.7% respectively). Differences in EF measured by EH-Score increased over time (6-months m: 45.9%; nm: 33.3%, 12 months – m: 63.0%; nm: 41.8%, 24 months – m: 68.0%; nm: 44.1%). Slightly more masturbating patients had an IIEF-EF Score ≥17 within their long-term follow-up (12 months – m: 27.5%; nm: 21.1%, 24 months – m: 37.6%; nm: 35.1%). Moreover m-patients had clinically significantly more often morning erections in their 12 months (m: 44.0%; nm: 28.8%) and 24 months (m: 48.5%; nm: 35.6%) long-term follow-ups.

Conclusion: Masturbating patients had a clinically significantly better functional outcome after nsRP. Further studies are needed to get better evidence.

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P092

Sex hormone-binding globulin is associated with androgen deficiency features independently of total testosterone

G. RASTRELLI[1], G. CORONA[2], S. CIPRIANI[1], E. MANNUCCI[3] AND M. MAGGI[1]
[1]Sexual Medicine and Andrology Unit, Department of Experimental Clinical and Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy; [2]Endocrinology Unit, Medical Department, Azienda Usl Bologna Maggiore-Bellaria Hospital, Bologna, Italy; [3]Diabetology, University of Florence and Careggi Teaching Hospital, Florence, Italy

Background: It is recognized that total testosterone (TT) does not sufficiently describe androgen status when sex hormone-binding globulin (SHBG) is altered. However, in humans, evidence supporting the existence of a hypogonadism due to low T bioactivity is scanty.

Objective: The aim of the study was to assess whether changes in SHBG levels, independently of TT, are associated with subjective and objective androgen-dependent parameters.

Methods: Cross-sectional observation. Two thousand six hundred and twenty-two men (aged 51.1 ± 13.5 years) attending a Sexual Medicine and Andrology Outpatient Clinic for sexual dysfunctions. All patients underwent a standardized diagnostic protocol before starting any treatment. Clinical and biochemical parameters have been collected. Higher ANDROTEST score has been used as a comprehensive marker of more severe hypogonadal symptoms. Prostate-specific antigen (PSA) and haematocrit have been used as objective surrogate markers of T bioactivity.
Results: After adjusting for TT and lifestyle, SHBG showed a significant positive association with ANDROTEST score [B = 0.79 (0.61; 0.96), $p < 0.0001$]. Conversely, higher SHBG, independently of TT, was negatively related to PSA [B = −0.86 (−0.83; −0.89); $p < 0.0001$] and haematocrit [B = −0.64 (−0.88; −0.40); $p < 0.0001$], after adjustment for the aforementioned confounders along with age and body mass index. Furthermore, a relationship between SHBG and lipids or blood pressure was found, with lower SHBG levels associated with a worse metabolic profile, independently of TT.
Conclusion: Higher SHBG, independently of TT, is associated with either subjective or objective androgen deficiency features. This indicates that besides a hypogonadism due to an impaired T production, a hypogonadism due to a lower biological activity of T does exist.

P093

Clinical characteristics of men complaining premature ejaculation together with erectile dysfunction: a cross-sectional study

G. RASTRELLI[1], S. CIPRIANI[1], G. CORONA[2] AND M. MAGGI[1]
[1]*Sexual Medicine and Andrology Unit Department of Experimental Clinical and Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy;*
[2]*Endocrinology Unit, Medical Department, Azienda Usl Bologna Maggiore-Bellaria Hospital, Bologna, Italy*

Background: Erectile dysfunction (ED) and premature ejaculation (PE) are the most common sexual complaints worldwide. PE is present in up to 30% of men with ED.
Objective: To assess whether men complaining both ED and PE have peculiar clinical features as compared with men reporting ED or PE only.
Methods: A consecutive series of 4024 men (mean age 51.2 ± 13.2 years) seeking medical care for sexual dysfunction at the Sexual Medicine and Andrology Unit of the University of Florence was studied. The population was categorized according to their symptoms into ED-only (n = 2767; 68.8%), PE-only (475; 11.8%) and ED-PE (782; 19.4%). Several clinical parameters were collected, including information obtained by the structured interviews SIEDY and ANDROTEST, validated for the evaluation of the pathogenic components of sexual dysfunction and the symptoms of hypogonadism, respectively. Penile colour Doppler ultrasound (PDCU) parameters were also collected.
Results: As compared with PE alone, ED-PE was characterized by a higher prevalence of sexual complaints, including impaired morning erections [OR = 5.8 (4.1; 8.3)], decreased sexual desire [OR = 2.6 (1.8; 3.7)], decreased ejaculate volume [OR = 2.7 (1.8; 4.0)] and sexual abstinence [OR = 2.1 (1.2; 3.7)]. Conversely, ED-PE and ED-only men had similar prevalence of sexual symptoms. In ED-PE men, the characteristics of ED (severity, onset, worsening) were similar to ED-only men, whereas the characteristics of PE (severity, frequency, onset) were milder than in PE-only men. As compared with ED-only men, ED-PE patients were characterized by a lower frequency of symptomatic hypogonadism (total testosterone <8 nmol/L and hypogonadal symptoms according to ANDROTEST). ED-PE men had a milder organic component for sexual dysfunction, as detected by a lower SIEDY scale 1 score, than ED-only men. Conversely, SIEDY scale 1 was higher in ED-PE men than PE-only ones. Accordingly, ED-PE men had a significantly higher prevalence of hypertension, diabetes and cardiovascular (CV) diseases [OR = 1.8 (1.1; 3.0), 2.7 (1.3; 5.6) and 2.7 (1.1; 6.5), respectively], as compared with PE-only subjects. In addition, ED-PE men showed a worse dynamic peak systolic velocity at PDCU [B = −12.0 (−17.7; −6.2)] and a greater 10-year estimated CV risk [B = 3.8 (2.5; 5.1)] as compared with PE-only patients. Conversely, the prevalence of comorbidities and PDCU parameters were similar in ED-PE and ED-only men, although ED-only men showed a higher estimated CV risk. Concerning psychological features, ED-PE had more severe symptoms of somatized anxiety and milder symptoms of phobic anxiety. No differences were found between ED-PE and ED-only men.
Conclusion: Men complaining ED and PE together have clinical characteristics similar to ED-only patients, whereas they differ from PE-only men. In particular, ED-PE patients report more sexual symptoms and have more severe cardiometabolic comorbidities than PE-only men. Our results confirm that, from a clinical point of view, ED-PE men might be considered (and managed) as those with only ED, considering PE as a secondary complaint more than a separate condition.

P094

GLP-1 sustains human sperm motility via mechanism that involves the stimulation of Akt phosphorilation

M. SANTORO[1], R. CASTIGLIONE[2], G. PELUSO[3], G. MORRONE[3], S. AQUILA[1] AND R. D'AGATA[2]
[1]*Centro Sanitario, Department of Pharmacy, University of Calabria, Rende (CS), Italy;* [2]*Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy;* [3]*Unit of Physiopathology of Reproduction, Annunziata Hospital, Cosenza, Italy*

Background: Glucagon-like peptide-1 (GLP-1) is an incretin hormone that increases insulin sensitivity, by the activation of the GLP-1R in β-cells. However it is now known that the GLP-1R is expressed in a variety of tissues other than pancreas. In these tissues, GLP-1 affects organ specific function. This has aroused interest in the non-metabolic actions of this peptide. There are also evidences of male gonad as target of GLP-1. In this study we characterized the effect and impact of GLP-1 on sperm performance and the molecular mechanism that mediates this effect.

Methods: We purified sperm of normospermic men using Percoll gradient. Sperm parameters were evaluated in absence and in the presence of increasing concentration of exendin-4, a GLP-1 analog, (from 300 pM to 50 nM). Motility and vitality were evaluated at the beginning and at the end of each incubation time. Concomitantly, cell lysates were also prepared for WB analysis of phosphorylated and total Akt.
Results: Exendin-4 (300 pM) incubated for 2 h stimulated significantly progressive motility in all samples examined, although the degree of motility improvement in the whole study varied from sample to sample. Analog exposure was able to sustain the motility and progressive motility and after 2 h the motility was higher than the pre-incubation levels. No significant effect on vitality was observed. This dose of exendin resulted in an increased phosphorylation of Akt by 110% of controls. On the contrary an higher concentration of agonist, 50 nM, induced a significant and marked decrease on sperm motility and vitality, and dephosphorylated significantly the p-Akt. Effect of PI3K/Akt inhibitor on sperm. We incubated purified spermatozoa for 4 h in presence of increasing doses of wortmannin (5–20 μM) an inhibitor of the inducer Akt phosphorilation, PI3K. Wortmannin coincubated with exendin-4 resulted in a decrease of exendin-4 induced effect on spermatozoa motility of around 20%. The inhibitor exposure negated the exendin-4 induced Akt activation.
Conclusion: So we demonstrated: (i) that GLP-1 added to spermatozoa in vitro counteracted the time dependent loss of motility; (ii) This effect occurs via mechanism that involves the stimulation of Akt phosphorylation as demonstrated by wortmannin exposure which abrogated the exendin-4 Akt activation; (iii) GLP-1 is a new spermatozoa prosurvival inducer.

P095

Hypogonadal men with type 2 diabetes benefit from testosterone treatment in terms of weight reduction and improved glycemic control: 10-year real-life data from a registry study

F. SAAD[1,2], A. HAIDER[3], K. HAIDER[3], G. DOROS[4] AND A. TRAISH[5]
[1]Medical Affairs Andrology, Bayer AG, Berlin, Germany; [2]Gulf Medical University, Ajman, UAE; [3]Private Urology Practice, Bremerhaven, Germany; [4]Boston University School of Public Health, Boston, MA, USA; [5]Boston University School of Medicine, Boston, MA, USA

Background: Prevalence of hypogonadism in men with type 2 diabetes mellitus (T2DM) can be as high as 50%.
Methods: Registry of hypogonadal men established in 2004 in a single urologist's office. Men had testosterone ≤12.1 nmol/L and symptoms. In 776 patients, 286 (36.9%) had type 2 diabetes diagnosed and treated elsewhere (diabetes center).
133 received TU 1000 mg/12 weeks (T-group) following an initial 6-week interval. 153 had opted against TTh and served as controls (CTRL). Measurements were performed 1–4 times a year for up to 11 years.
Mean changes over time between groups were compared by mixed effects model for repeated measures with random effect for intercept and fixed effects for time, group and their interaction and adjusted for age, weight, waist circumference, fasting glucose, blood pressure and lipids to account for baseline differences between groups.
Results: Mean age: 61.8 ± 5.4 years (T-group), 64.2 ± 4.6 years (CTRL). Mean (medican) follow-up was 6.9 (7) years in the T-group and 7.3 (8) years in CTRL. HbA1c progressively decreased from 8.8 ± 0.9 to 5.9 ± 0.3% after 10 years in the T-group with statistical significance vs. previous year for the first 8 years. In CTRL, HbA1c increased from 7.7 ± 0.6 to 9.5 ± 0.9%, estimated adjusted difference between groups: −4.6% ($p < 0.0001$ for all). Fasting glucose decreased from 7.6 ± 1.1 to 5.3 ± 0.1 mmol/L in the T-group with statistical significance vs. previous year for the first 3 years. In CTRL, fasting glucose increased from 6.2 ± 0.6 to 6.9 ± 1.0 mmol/L, estimated adjusted difference: −2.2 mmol/L ($p < 0.0001$ for all). Fasting insulin decreased from 29.6 ± 4.2 to 15 ± 4.8 μU/mL with statistical significance vs. previous year for the first 7 years. In CTRL, fasting insulin increased from 26.5 ± 2.6 to 37 μU/mL, estimated adjusted difference: −23.1 μU/mL ($p < 0.0001$ for all). HOMA-IR decreased from 10.2 ± 2.0 to 3.6 ± 1.2 after 10 years with statistical significance vs. previous year for the first 5 years. In CTRL, HOMA-IR increased from 7.4 ± 1.4 to 11.4, estimated adjusted difference: −9.4 mmol/L ($p < 0.0001$ for all). Weight decreased from 113.1 ± 13.7 to 89.6 ± 9.0 kg after 10 years in the T-group ($p < 0.0001$) with statistical significance vs. previous year for the first 9 years. In CTRL, mean weight remained stable, estimated adjusted difference: −22.2 kg ($p < 0.0001$).
Waist circumference decreased from 111.1 ± 7.4 to 99.2 ± 5.5 cm after 10 years in the T-group ($p < 0.0001$) with statistical significance vs. previous year for the first 9 years. In CTRL, waist circumference fluctuated but dropped during the last 4 years from 115.1 ± 12.8 at baseline to 111.8 ± 13.0 cm at 10 years (NS vs. baseline), estimated adjusted difference: −13.5 cm ($p < 0.0001$). In the T-group, 106 (80%) achieved HbA1c <6.5%, and 116 (87%) achieved HbA1c <7% at last measurement. In CTRL, no patient achieved either HbA1c <6.5% or HbA1c <7.0%. All but 1 man had an increase in HbA1c. In the T-group, 4 patients (3%) died. In CTRL, 24 patients (15.7%) died. 50 non-fatal MACE occurred (26 myocardial infarctions, 24 strokes).
Conclusion: Long-term treatment with TU in hypogonadal men with T2DM resulted in improved glycemic control and weight loss. Correcting hypogonadism in men with T2DM sustainably supports standard diabetes treatment.

P096

Andrology assessment and fertility study in patients with type 1 diabetes mellitus (T1D)

M. TENUTA, A. PETROZZI, D. GIANFRILLI, C. POZZA, F. PALLOTTI, M. G. TARSITANO, S. MORANO, R. BUZZETTI, A. F. RADICIONI, D. PAOLI, F. LOMBARDO, A. M. ISIDORI AND A. LENZI
Department of Experimental Medicine, Sapienza University, Rome, Italy

Background: Type 1 diabetes mellitus (T1D) is a chronic disease responsible for systemic complications at various levels. Andrological involvement in the disease has often been overlooked by clinical trials and it is of secondary importance in patient evaluation. Recently, according to the increasing incidence of pathology in children and adolescents, the scientific community is changing opinion and scientific evidences suggests that T1D effects may alter andrological physiology in many aspects, even if the exact pathogenetic mechanisms of such involvement are not yet fully known.
Objective: The aim of this work was to evaluate the reproductive health of T1D patients with a case-control study on 36 T1D patients vs. 31 non-diabetic patients (ND).
Methods: All patients performed: seminal fluid examination, hormone assays of the gonadal axis, seminal biochemistry, sperm DNA fragmentation (SDF) and testicular US; sexual aspect was also investigated through IIEF-15 questionnaire.
Results: The two groups were comparable by age (T1D 33.1 $\pm$ 6.8 years vs. ND 31.8 $\pm$ 3.9 years), BMI and testicular volumes. Comparison of the seminal parameters between T1D and ND group, respectively, revealed: non linear motility 11.6 $\pm$ 9.0% vs. 6.8 $\pm$ 3.8% ($p = 0.006$) positively correlated with the HbA1c values ($p = 0.006$); straight motility 37.7 $\pm$ 14.2% vs. 44.7 $\pm$ 8.6% ($p = 0.018$); atypical forms: 91.4 $\pm$ 4.4% vs. 88 $\pm$ 2.9% ($p = 0.002$); DNA fragmentation index (DFI): 14.9 $\pm$ 7.4% vs. 10.0 $\pm$ 2.0% ($p = 0.002$); no significant differences were found in total sperm concentration and in seminal biochemical markers between the two groups. Regarding hormone levels, only SHBG was increased in the T1D group vs. controls ($p < 0.001$). IIEF15 results did not reveal differences between the two groups. These results show that in diabetic patients there is a qualitative alteration of sperm motility, probably caused by both down-regulation of glucose membrane transporters and lack of energy substrates of glycolytic pathway, which are essential for flagellar movement. Furthermore, high DFI, probably due to an increase in cellular oxidative stress, could represent an important data in the evaluation of reproductive outcome.
Conclusion: Our data show that T1D patients had qualitative alterations of seminal fluid, which may be responsible for increased risk of infertility. For this reason, andrological assessment in the diabetic patient is certainly not something to be overlooked. The data available to date are not sufficient to standardize this aspect in the study of T1D complications, however its understanding could lead, in selected cases, the clinician to ask for targeted diagnostic investigations for a better patient follow-up.

P097

Changes in classical sperm parameters in a 6 years period in Hungary

C. CSÍKI[1,2], A. KAPOSI[1,3], N. DARABOS[1,4] AND Z. KOPA[1]
[1]Department of Urology, Andrology Centre, Semmelweis University, Budapest, Hungary; [2]Department of Urology, Szent Borbála Hospital, Tatabánya, Hungary; [3]Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary; [4]Department of Urology, Petz Aladár County Teaching Hospital, Győr, Hungary

Background: The prevalence of infertile relationships is increasing in developed western countries. In approximately 50% of infertile couples, a male factor is also present. In these geographical regions sperm concentration, sperm count and male fertility potential are decreasing. Publications usually analyze longer than 10 years' time periods. Evaluating this worsening tendency, the question raised if the decrease in sperm parameters could also be detected in shorter time intervals?
Methods: A retrospective analysis was performed on a 6 years period between 2011 and 2017 in our Andrology Centre. Semen samples of patients seeking medical treatment for infertile relationships were examined according to the WHO V criteria. Semen volume, sperm concentration, progressive motility, normal sperm morphology, leukocyte concentration were assessed, additionally, the prevalence of azoospermia and over 40 M/mL sperm concentration were calculated. Statistical analysis was performed using linear regression analysis and correlation coefficient *t*-test. For endpoints examination *t*-test (previously *f*-test of equality of variances) and chi-squared dichotomous variables analysis were used.
Results: 3.818 semen samples were analyzed during the research period. The mean age of the patients showed a slightly declining tendency over the years, mean patient age in the 6 years period was 38.67 years (19–74 years). Mean semen volume was found with a declining trend, decreased from 3.6 to 3.2 mL in the observed period. Total mean values of sperm concentration, progressive motility and normal sperm morphology were 52.28 M/mL, 20.61%, and 4.61%, respectively. Mean sperm concentration between 2011 and 2017 was found 55.4, 49.5, 52.3, 54.2, 50.4 and 51.9 M/mL, respectively. Mean progressive motility changed from 24.3% in 2011 to 18.7% in 2016, showing a statistically significant decrease ($p = 0.027$) in every consecutive year. Mean normal sperm morphology varied between 4.2% and 5.3%, ($p = 0.041$). Leukocytospermia showed a significant change ($p = 0.031$) in this 6 years' time interval; decreasing from 0.5 M/mL in 2011 to 0.3 M/mL in 2016. Prevalence of azoospermia was in 2011 and 2016 7.42% and 10.63%, respectively, showing a clear increasing trend but found no statistically significant ($p = 0.058$). The number of patients with sperm concentration over 40 M/mL showed a statistically significant decrease from 46.86% to 40.67% ($p = 0.035$).
Conclusion: During decades worsening of semen quality and deterioration in sperm parameters have already been confirmed. Findings of publications and studies revealing longer time intervals could be supported by our retrospective analysis also in shorter periods. According to our findings statistically significant negative changes could be observed in semen volume and progressive motility also in a 6 years' time interval. Sperm concentration was not significantly changed in this shorter time, interestingly normal sperm morphology and leukocytospermia were observed with a significant improvement during the last 6 years. However, increasing prevalence of azoospermia was demonstrated, but statistical significance could not be obtained. On the other hand, the rate of the patients with sperm concentration over 40 M/mL was found significantly decreasing. Our data support previous findings in the overall increase in/subfertility rate in our geographic region calling the attention to preventive measurements.

P098

Testicular microlithiasis – correlation with testicular dysgenesis syndrome and fertility parameters in infertile couples

T. TAKÁCS[1,2], V. ROSTA[1,3] AND Z. KOPA[1]
[1]Department of Urology, Andrology Center, Semmelweis University, Budapest, Hungary; [2]Urology Department, Gibraltar, St Bernard's Hospital, British Overseas Territory, UK; [3]Medical student, University of Szeged, Hungary

Background: Testicular microlithiasis (TML) has consequently been linked to testicular germ cell tumours and male infertility, however the relation is still disputed. Publications mostly focus on oncological issues. Recently observations assumed a link between cryptorchidism, hypospadias, Leydig and Sertoli cell dysfunction manifested in later sub/infertility, testicular cancer and hypogonadism. The hypothesis of testicular dysgenesis syndrome (TDS) has been raised supposing a common foetal pathogenesis. Although the clinical relevance of TML is still controversial the obvious association with testicular cancer might suppose a significant correlation with other TDS components.
Objective: The aim of our study is to determine the association between TML and classical sperm parameters, and the presence of TDS components in males in infertile couples.
Methods: Preliminary results of a prospective, cross-sectional controlled study are present to compare fertility data of TML positive (TML+) patients to age matched TML negative (TML) controls in our Andrology Centre. Results are correlated with the data of voluntary sperm donors (DON) to represent unscreened fertile population. Microscopic (WHO 5th Manual) and computer assisted sperm analysis (CASA Microptic System), scrotal colour Duplex ultrasound examinations were performed. The presence of TDS components was also assessed. Statistical analysis was performed using independent two-sample *t*-test, Welch test and chi-square test.
Results: 217 patients from infertile couples were detected with TML (TML+ group, mean age: 34.7 years) between 2012 and 2017. Classic sperm parameters and the presence of TDS components were compared to 30 TML negative (TML− group, mean age: 34.9 years) patients from infertile couples and 25 voluntary sperm donors (DON group, mean age: 27.1 years).
Lowest mean sperm concentration (14.31 M/mL), progressive motility (13.19%) and normal sperm morphology (2.74%) was found in the TML+ group. Sperm concentration and progressive motility were significantly higher in the TML− group (28.45 M/mL and 24.6%, respectively, $p = 0.029$ and $p = 0.027$), normal morphology was found also higher (4.4%) lacking statistical significance. Moreover significantly higher parameters were obtained in the DON group [77.32 M/mL ($p = 0.01$), 66.33% ($p = 0.02$) and 10.85% ($p = 0.009$), respectively].
The rate of oligozoospermia/azoospermia was 76.8% and 30.9% in the TML+ group compared to 40% and 15% in the TML− patients ($p = 0.09$ and $p = 0.011$, respectively). No oligozoospermia or azoospermia was diagnosed in the DON group. The average number of TDS components was 2.44 in the TML+ patients group, meanwhile it was 1.15 in the TML− group vs. 0.08 in the DON group ($p = 0.001$ and $p < 0.001$, respectively).
Conclusion: Sperm concentration and progressive motility were lower in the TML+ group compared to TML− and DON group, confirming a statistically significant negative correlation, meanwhile the rate of oligozoospermia and azoospermia were significantly higher in TML+ patients. TDS components occurred in a significantly higher number in the TML+ group. Our results support the theory that TML can be a component of TDS and TML+ patients are at higher risk of infertility. Our data call the attention to the clinical impact of TML and indicates andrological assessment of the TML positive patients.

P099

Expression of GPER in biopsies of human testicular tissue and laser microdissected seminiferous tubules of normal and disturbed human spermatogenesis

R. WALCZAK-JĘDRZEJOWSKA[1], E. OSZUKOWSKA[2], M. BRYŚ[3], E. FORMA[3], K. MARCHLEWSKA[1], R. MAKSYM[4], P. WŁODARSKI[4], J. ROGOWSKI[1], J. J. SŁOWIKOWSKA-HILCZER[1] AND K. KULA[1]
[1]Department of Andrology and Reproductive Endocrinology, Medical University of Lodz, Poland; [2]II Clinic of Urology, Medical University of Lodz, Poland; [3]Department of Cytobiochemistry, University of Lodz, Poland; [4]Department of Histology and Embryology, Center for Biostructure Research, Medical University of Warsaw, Poland

Background: Most estrogenic actions described so far are mediated through two estrogen nuclear receptors (ESR1, ESR2). More recently new membrane-bound G protein-coupled estrogen receptor 1 (GPER) has been demonstrated and its expression in multiple tissues has been described. However, only few studies concern its expression in human testis.
Objective: The aim of this study was to determine the expression of GPER in testicular tissue and laser microdissected seminiferous tubules obtained from testes with normal spermatogenesis (NOR) and Sertoli cell only syndrome (SCO) and to analyse its possible relationship with hormonal status of the subjects.
Methods: Archival testicular biopsies, Bouin fixed, paraffin embedded, obtained from obstructive and non-obstructive azoospermic men (NOR $n = 21$, SCO $n = 31$) were studied. Immunohistochemistry against GPER was performed to visualize the site of its expression. Moreover, the GPER gene expression was studied in the biopsy samples and in laser microdissected seminiferous tubules (NOR $n = 9$; SCO $n = 10$) using real-time quantitative PCR. Serum levels of FSH, LH, testosterone (T) and oestradiol (E) were measured and the T/LH and E/T ratios were calculated. Additionally, gene expression of anti-Mullerian hormone (AMH) was analysed in laser microdissected tubules as marker of Sertoli cell maturation status. Relative copy number values (number of copies of GPER and AMH mRNA per 1000 copies of RPS29 mRNA – house-keeping gene) were calculated.

Results: Immunohistochemical localisation of GPER was found in Sertoli, Leydig cells in NOR and SCO and all types of germ cells, except mature spermatozoa in NOR.
In SCO group expression of AMH and GPER mRNA in laser microdissected tubules was higher vs. NOR although the latter increase was not significant (AMH: 17.0 ± 11.7; Median = 15.8 for SCO vs. 1.3 ± 1.9; Median = 0 for NOR; $p = 0.001$; GPER: 30.2 ± 17.5; Median = 33.6 for SCO vs. 15.8 ± 16.1; Median = 7.8 for NOR). FSH and LH serum levels were significantly higher in NOR vs. SCO group while levels of estradiol and T/LH ratio were significantly lower. In NOR a moderate positive correlation between E and GPER mRNA expression in testicular tissue ($R = 0.5$; $p = 0.03$) and between FSH and GPER mRNA expression in laser microdissected tubules ($R = 0.67$; $p = 0.04$) were observed. Similarly in SCO a high positive correlation between FSH and GPER transcripts ($R = 0.78$; $p = 0.005$) were present in laser microdissected tubules. And additional positive moderate correlation was seen in this group between mRNA expression for GPER and AMH ($R = 0.6$; $p = 0.03$).
Conclusion: (i) In SCO seminiferous tubules a higher mRNA expression of GPER and AMH was observed in comparison with NOR. (ii) In NOR and SCO a positive correlation between GPER mRNA expression in seminiferous tubules and serum FSH levels was observed. (iii) In SCO seminiferous tubules the positive correlation between mRNA expression of GPER and AMH was seen. (iv) It may suggest an involvement of GPER action in functional and maturational status of Sertoli cells in impaired spermatogenesis.
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P100

Sperm retrieval in Klinefelter syndrome patients – first Hungarian data

A. BATA[1], T. WENINGER[1] AND Z. KOPA[2]
[1]Department of Urology, Jahn Ferenc Teaching Hospital, Budapest, Hungary; [2]Department of Urology, Andrology Center, Semmelweis University Budapest, Hungary

Background: Although Klinefelter syndrome (KS) is the most common numerical sex chromosome abnormality with a prevalence of about 1–2 in 1000 males, only limited series are reported in the literature. The syndrome is characterized by small but firm testes, gynecomastia, elevated FSH and LH levels, and reduced testosterone. In about 90% of the cases, azoospermia is found. Until 1996 there was no chance for KS patients to father a child but since this date, surgical or microsurgical sperm retrieval (testicular sperm extraction – TESE) and intracytoplasmic sperm injection (ICSI) allow and has been more widely used to achieve a pregnancy.
Objective: The aim of our study is to evaluate the microsurgical sperm retrieval of Klinefelter's syndrome patients under treatment in our Andrology Center.
Methods: Clinical data of 39 KS patients treated between 2009 and April 2018 in the Semmelweis University's Andrology Center (Budapest) were processed. Following a WHO V. semen analysis age, body composition, the most important endocrine parameters (FSH, LH, and Testosterone) and scrotal ultrasound (US) findings were analyzed. Surgical sperm retrieval was performed by microdissection technique. Samples were checked on site and then cryopreserved.
Results: Clinical data of 39 KS patients showed an average age of 32.2 (±7.5) years. In 37 patients (95%) non-obstructive azoospermia was found, while in 2 cases (5%) spermatozoa (severe oligo-astheno-teratozoospermia) could be detected. Average testis volume was 2.7 (±1.7) mL. Endocrinoligical profile showed hypergonadotropic hypogonadism; mean FSH and LH IU/L level was 31.7 (±7.7) IU/L and 17.6 (±6.9) IU/L, respectively, while mean total testosterone was revealed as 6.67 (±2.8) nmol/L. 11 (28%) patients were seen with microlithiasis on scrotal ultrasound examination. Only 15/37 (40%) azoospermic patients requested the offered microdissection TESE. Mean age of the patients underwent microTESE was 31.4 years, mean right and left testis volume were similar, 3.7 mL. Mean FSH and LH levels were elevated, 31.2 and 15.4 IU/L, respectively, mean total testosterone showed 6.6 nmol/L. Microlithiasis could be detected in 6 cases (40%). Sperm retrieval rate was found 20% (3/15 patients).
Conclusion: According to international data, sperm retrieval rate is approximately 50% in KS patients. Previous publications showed a negative correlation with age, however, according to the latest meta-analysis, SRR was found independent of age, testis volume and serum hormone levels. Our results do not reflect the 50% sperm retrieval rate published in international studies and multicenter trials. Much lower (9%) SRR was reported by Rohayem et al. (2015) in adolescent population when LH exceeded 17.5 IU/L and total testosterone was found below 7.5 nmol/L. In our adult patient series LH and total testosterone were found 15.4 IU/L and 6.6 nmol/L, respectively, raising the suspicion that highly elevated LH and rather low TT levels could explain lower SRR. Multicenter, international trials would be necessary to evaluate SRR data in European countries.

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Fatherhood after testicular cancer – a retrospective study

A. PETROZZI, F. PALLOTTI, G. SENOFONTE, T. CARLINI, A. LENZI, D. PAOLI AND F. LOMBARDO
Laboratory of Seminology – "Loredana Gandini" Sperm Bank Department of Experimental Medicine – Medical Pathophysiology Section, "Sapienza" University of Rome, Italy

Background: While testicular tumors are rare in the general population, they are the most common cancer in men of reproductive age (second to fifth decade of life), with a rising trend over the last 30 years. The use of chemo- and radiotherapy and refinement of surgical techniques may affect fertility and sexual health of cancer survivors, especially impairing spermatogenesis. The best option to preserve the potential reproductive capacity is semen cryopreservation before any gonadotoxic treatment. Banked semen can be used to achieve fatherhood through artificial reproductive techniques (ART). Although ART are widely used, cumulative use rate of frozen semen is low.

Objective: The aim of this study is to evaluate the effectiveness of our Sperm Banking program, measured as the cumulative use rate of cryopreserved samples and success rates of IVF and ICSI treatments using cryopreserved semen in terms of live births. As a secondary aim, we will evaluate natural fecundity according to hormonal and sperm parameters.
Methods: We have conducted a retrospective study on a cohort of 663 patients attending our Seminology Laboratory Sperm Bank at the Sapienza University of Rome's Department of Experimental Medicine, between 2003 and 2009 for sperm cryopreservation following a diagnosis of testicular cancer. The average length of follow up was 10 years. Our data refer to: semen parameters, hormonal levels, use rate of cryopreserved samples and number of born children. Statistical analysis was carried out with Statistical Package for the Social Sciences (SPSS) 24.0, and a p value <0.05 has been considered significant.
Results: Most testicular cancer patients showed a spermatogenesis recovery but unfortunately, up to 10% of them experienced azoospermia after antineoplastic treatment. Total use rate of cryopreserved semen was 14.4%, 20% of which achieved fatherhood with ART. 64.2% of our testicular cancer patients discarded their cryopreserved semen in a mean time of 4.0 ± 2.5 years. About 22% of them achieved fatherhood naturally.
Conclusion: According to literature data our study indicates a relative low use rate of sperm cryopreservation. Moreover it demonstrates that a remarkable percentage of testicular cancer patients can achieve fertility. ART is a viable option for patients who underwent more aggressive treatments. However, cryopreservation of seminal fluid represents a fundamental health tool, and an important psychological help especially for those patients with a permanent impairment of spermatogenesis.

P103

Comparison of the sexual-steroid concentration after transdermal application of two various testosterone gels in two different dosages in hypogonadal men

A. SAHI, M. ZITZMANN AND S. KLIESCH
Department of Clinical and Surgical Andrology, Centre of Reproductive Medicine and Andrology, University Hospital Münster

Background: For testosterone (t)-substitution in Germany various t-gels are available, e.g. Testogel® and Testotop®. The T-concentration of those gels is different: Testogel® has an concentration of 1% testosterone, Testotop-gel has 2.5%. Question: do serum t-levels differ between the two various compounds?
Methods: Four hundred and eighty-three hypogonadal patients [166 prim. hypog./127 sek. hypog./190 functional hypog. (not caused by testicular/hypothalamic-pituitary disorders)], treated with t-gel (Testogel®50 mg (n = 345), 100 mg (n = 46); Testotop®62.5 mg (n = 46), 125 mg (n = 45). Serum levels of t/free t, dihydro-t and estradiol baseline, after 3 and 12 months were checked. Safety parameters: Hematocrit, blood pressure, prostate size/PSA.
Results: Treatment with testosterone gel generally ($p < 0.001$) and dosage dependant ($p = 0.012$) led to an increase of t-levels [7.7 ± 2.7 nmol/L to 18.3 ± 12.2 nmol/L (3 months), 18.5 ± 13.2 nmol/L (12 months)]. Higher age of patients was associated with lower t-levels under therapy ($p = 0.021$). Testogel 50 mg/d and Testotop 62.5 mg/d led to equivalent t-levels (17.4 ± 10.6 nmol/L vs. 16.5 ± 5.0 nmol/L, $p = 0.52$), Testogel 100 mg/d led to lower t-levels than Testostop 125 mg/d (20.9 ± 12.2 nmol/L vs. 26.3 ± 14.1 nmol/L, $p = 0.051$); corresponding results for free t, dihydro-t. Estradiol-levels increased with BMI and t-levels (both $p < 0.001$). Hematocrit increased with t- and estradiol-levels as well as BMI ($p = 0.001$, $p < 0.001$, $p = 0.002$). No significant changes in blood pressure and prostate levels.
Conclusion: Testosterone replacement therapy with both gels led to dosage dependant t-levels. Aromatization is i.a. BMI-affected. Old men appear to have a poorer absorption.

P104

Protective role of testicular hormone INSL3 from atrophy and weakness in skeletal muscle

A. FERLIN[1], L. DE TONI[2], A. AGOULNIK[3], G. LUNARDON[2], A. ARMANI[4], S. BORTOLANZA[4], B. BLAAUW[4], M. SANDRI[4,5] AND C. FORESTA[2]
[1]Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy; [2]Department of Medicine, University of Padova, Padova, Italy; [3]Department of Human and Molecular Genetics, Herbert Wertheim College of Medicine, Florida International University, Miami, Florida, USA; [4]Department of Biomedical Sciences, University of Padova, Padova, Italy; [5]Venetian Institute of Molecular Medicine, Padova, Italy

Background: Androgens are primarily involved in muscle growth, whilst disease-driven muscle wasting is frequently associated with hypogonadism. The peptide hormone Insulin-like peptide 3 (INSL3), produced by Leydig cells of the testis, displays anabolic activity on bone, a target tissue of androgens, and its plasma concentrations are diminished in male hypogonadism. Here we tested the role of INSL3 on muscle mass regulation, in physiological and pathological conditions.
Methods: The effect of INSL3 was investigated in vitro on differentiated myotubes from C2C12 cells. The role of INSL3/RXFP2-signaling was assessed in vivo through the analysis of muscle loss after denervation in Rxfp2−/− mice.
Results: INSL3 promotes protein synthesis through the Akt/mTOR/S6 pathway in C2C12 cell line. Next, studies on Rxfp2−/− mice showed that INSL3 is required to prevent excessive muscle loss after denervation. Mechanistically, denervated Rxfp2−/− mice lacked the compensatory activation of the Akt/mTOR/S6 pathway and showed an abnormal activation of the ubiquitin-proteasome system. In addition, the lack of INSL3 activity resulted in reduced contractile force.
Conclusion: INSL3/RXFP2 axis exerts a role in protein turnover, likely contributing to muscle wasting in male patients affected by hypogonadism.

P105

Microsurgical Testicular Sperm Extraction in 750 cases of non obstructive azoospermia: sperm retrieval and success in single centre – an Indian study

P. DHARMARAJ, C. M. ASHRAF, C. KULKARNI AND M. SHYLAJA

CRAFT Hospital and Research Centre, Kodungallur, Kerala, India

Objective: We describe our micro-TESE experience in a large group of men with Non-Obstructive Azoospermia (NOA) and poor prognosis for Sperm Retrieval (SR), and critically analyse the method s results and limitations.

Methods: An ART facility was setup in a tertiary care centre to perform SR using microsurgery. 750 men with NOA underwent micro-TESE while their female partners received ovarian stimulation for Oocyte Pickup (OCP). Micro-TESE was performed on the day prior to OCP, and surgically-retrieved testicular sperm were used for sperm injections. We assessed sperm retrieval rates, operative aspects, and ICSI outcomes.

Results: The success of micro-TESE at obtaining testicular sperm for Intracytoplasmic Sperm Injection (ICSI) was 54.4% with no major complications. Sperm were obtained in 73.6% of cases in which clearly dilated seminiferous tubules were seen, with minimal tissue excision which facilitated laboratory processing. Patients with successful and failed retrievals did not differ with respect to baseline characteristics, and presence of varicocele. Retrieval rates differed pertaining to testicular histology category. Also, retrieval rates were higher (53.1% vs. 35.6%) in patients who received medication to boost testosterone production prior to micro-TESE compared with those who did not. Sperm injections resulted in normal fertilization and embryo cleavage of 61% and 75%, respectively. A cumulative clinical pregnancy rate per ICSI cycle of 29.78%, with an implantation rate of 19%was achieved.

Conclusion: Micro-TESE is a valid method of SR in NOA. It yields sustainable results in poor prognosis azoospermic patients, with minimal damage to the testes. Our experience with micro-TESE applied to the most difficult cases of azoospermia is very reassuring, and we advocate that micro-TESE should be the method of choice in such cases.

P106

The polymorphic exon 1 androgen receptor CAG repeat and X-chromosome inactivation status in transsexuals

S. SH. KHAYAT[1], L. F. KURILO[1], E. A. BLIZNETZ[1], A. V. POLYAKOV[1], M. I. SHTAUT[1], N. YU. KUZINA[1], H. D. MAGOMEDOVA[1], N. V. SHILOVA[1], N. D. KIBRIK[2] AND V. B. CHERNYKH[1,3]

[1]Research Centre for Medical Genetics, Russian Federation, Moscow, Russia; [2]Serbsky Federal Medical Research Centre for Psychiatry and Narcology, Moscow, Russia; [3]Pirogov Russian National Research Medical University, Moscow, Russia

Background: Transsexualism (TS) is considered as a form of sex development disorder. According to 10th revision of The International Classification of Diseases (ICD), TS is referred to as a gender identity disorder, which is characterized by a persistent desire to live and be accepted as a member of the opposite sex (F64.0).

Methods: We performed a clinical and genetic examination of 36 unrelated patients with transsexualism 12–50 years old (female-to-male TS, $n = 20$; male-to female TS, n-16). Chromosome analysis was performed on cultured peripheral blood lymphocytes according to conventional cytogenetic methods using standard GTG-banding technique. Molecular analysis of the CAG repeat polymorphism of exon 1 of androgen receptor, AR/HUMARA gene (Xq13) was performed in 6 patients (M-F transsexuals, $n = 2$, and F-M transsexuals, $n = 4$). The CAG repeat length was determined by fluorescent polymerase chain reaction. The X-chromosome inactivation (XCI) was evaluated by the methyl-sensitive restriction analysis of the AR CAG alleles.

Results: No chromosomal abnormalities were detected in examined cohort of patients, as well as mismatches between their karyotype and the phenotype. The following AR (CAG)*n* genotypes/alleles were identified: 20/21, 20/23, 12/24, 18/26, 22, 24. Skewed and random X-chromosome inactivation: 79%/21%, 85%/15%, 67/33 and 62/38, respectively.

Conclusion: Further studies of the AR gene and the X-chromosome inactivation will make it possible to identify the association of the TS with AR CAG repeat polymorphism in the androgen receptor gene, characterization of XCI status.

P107

A comparative study of 2 sperm selection techniques for ICSI by TESE in obstructive azoospermia

E. C. MONDRAGÓN, A. M. R. SÁNCHEZ, L. A. D. MONTERROSAS, M. Y. O. GUADARRAMA AND A. K. AMBE

Centro Especializado para la Atención de la Mujer SC

Background: Testicular sperm extraction (TESE) is a minimal invasive procedure with low morbidity commonly used in men with azoospermia for assisted reproduction techniques (ART). Azoospermia may be explained by obstructive and non-obstructive factors. In both cases, processing this material is laborious because the presence of other cells (erythrocytes, immature cells, etc.) difficulting sperm selection by reducing motility and recovery and furthermore, the presence of those cells may cause damage by formation of reactive oxygen species. To process TESE obtained tissue mechanic and enzymatic methods are utilized, however neither of those eliminate residual cellular debris. After processing, sperm selection is the cornerstone to achieve better results in ICSI. The most common utilized technique for sperm selection after TESE is 24 h tissue culture before ICSI but even so cellular debris is still present. An alternate sperm selection technique is by density gradient which has been routinely utilized in ejaculated sperm showing a lower degree of sperm DNA fragmentation and more mature cells recovered. However, its use in TESE and ICSI has not been widely studied.

Objective: To compare safety and efficacy of density gradients technique vs. culture for sperm selection in TESE.
Methods: Prospective comparative cohort study that include 173 ICSI cycles with TESE because of obstructive azoospermia. In all cases, the tissue was process by mechanical technique. They were divided in 2 groups 1: Sperm selection by density gradients (Isolate, Irvine scientific) and 2: Sperm selection by 24 h tissue culture. After selection ICSI was performed. Fertility, cleavage, and pregnancy rates as well as birth weight where analyzed between groups.
Results: There were no significant differences in demographic characteristics between groups. Fertilization rate had a statistic difference between groups (group 1 vs. Group 2:41.74% +1 23.7 vs. 32.06 + - 22.3 $p = 0.03$). There were no differences in cleavage, pregnancy and birth weight rates between groups. However, all studied variables showed a tendency to improve in group 1.
Conclusion: Density gradient is a safe, useful and easy sperm selection technique for ICSI with TESE and may improve reproductive results.

P108

Outcome of Intracytoplasmic Sperm Injection (ICSI) using cryopreserved testicular sperm from azoospermic infertile men with varicocele

M. AL-HUSSINI[1], R. SALEH[1,2], R. E. EL-DAWLA[1], M. Y. ABDELRAHMAN[3] AND M. FAWZY[2]
[1]Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Sohag University, Sohag, Egypt; [2]Ajyal Hospital, Sohag, Egypt; [3]Department of Obstetrics and Gynecology, Faculty of Medicine, Sohag University, Sohag, Egypt

Objective: To study the outcome of ICSI using cryopreserved testicular sperm from infertile men with varicocele-associated non-obstructive azoospermia (NOA).
Methods: Prospective controlled clinical study. The study included 40 infertile men with NOA who underwent ICSI with cryopreserved testicular sperm. Twenty two patients had clinically palpable varicocele (group 1) and 18 had no detectable abnormality in their genital examination (group 2). Diagnosis of varicocele was confirmed by scrotal color Doppler ultrasound.
Diagnosis of azoospermia was confirmed by repeated absence of sperms in centrifuged semen pellets. Men with abnormal hormonal profiles, genetic abnormalities or genitourinary anomalies suggestive of obstructive pathology were excluded. Couples with known female factor infertility were excluded. ICSI was done using mature MII oocytes with good morphology. The pronuclear formation was assessed at 17 ± 1 h following injection. Embryo quality was assessed on day 3 (67 ± 2 h) and day 5. Positive clinical pregnancy was considered upon finding of gestational sac(s) and fetal pulsation by trans-vaginal US, 15 days after BHCG assessment. Results were presented as mean $\pm$ standard deviation (SD) for continuous variables and frequency and percentage (%) for categorical variables. p-value <0.05 was significant.
Results: Men's age was 37.8 ± 5.4 years in group 1 and 34.5 ± 7.5 years in group 2 ($p = 0.07$). Female partner's age was 27.9 ± 5 years in group 1 as compared to 26.3 ± 3.7 years in group 2 ($p = 0.35$). Out of the 22 patients with varicocele, 12 (54.5%) had grade 3 and 10 (45.5%) had grade 2. Mean $\pm$ SD of number of retrieved oocytes (MII) were 9.1 ± 6.2 in group 1 and 11.8 in group 2 ($p = 0.13$). Fertilized oocytes were 4.2 ± 3.9 in group 2 and 6.7 ± 4.5 in group 2 ($p = 0.08$). Good quality embryos at day 3 were 2.6 ± 1.8 in group 1 and 3.1 ± 1.7 in group 2 ($p = 0.29$). Good quality embryo at day 5 were 2.2 ± 1.7 in group 1 and 2.8 ± 2.2 in group 2 ($p = 0.30$). Clinical pregnancy was recorded in 7/22 cases (31.8%) in group 1 as compared to 12/18 (66.7%) in group 2 [OR: 0.23; 95% Confidence Interval (0.06–0.88); $p = 0.03$].
Conclusion: Pregnancy outcome following ICSI was significantly reduced on using cryopreserved testicular sperms from infertile men with varicocele-associated NOA. Future research is warranted to study whether this selected category of patients would benefit from varicocele repair before recommending ICSI.

P109

Virtually stained sperm quantitative imaging and selection for ICSI

A. SHALEV[1], I. BARNEA[1,2] AND N. T. SHAKED[1,2]
[1]Tel-Aviv University; [2]Q-ART Medical Ltd.

QART Medical develops a novel workstation and a disposable cartridge for virtually stained sperm quantitative imaging and selection, initially intended for ICSI procedures.
The stain-free imaging modality utilizes digital holographic microscopy, allowing visualize live unstained sperm cells and their organelles and quantitatively characterize their 2D or 3D morphology, as well as their mass and volume.
We have shown that this method generates equivalent results as obtainable with standard microscopy images of stained sperm cells. We have also shown that the 3D characterization of individual sperm cells and their organelles yields results that are highly correlative with the 5-level grading of the Acridine Orange (AO) assay for assessing DNA fragmentation.

P110

Effect of prolonged treatment with PDE5-inhibitors on Endothelial Dysfunction in vascular diseases and in vascular risk conditions: a systematic review analysis and meta-analysis of randomized double-blind placebo-controlled trials

S. D'ANDREA, A. BARBONETTI, C. CASTELLINI, A. MARTORELLA, E. MINALDI, S. NECOZIONE, F. FRANCAVILLA AND S. FRANCAVILLA
Department of Life, Health and Environmental Sciences, Andrology Unit, University of L'Aquila, L'Aquila, Italy

Objective: The aim was to challenge the contention that a chronic use of phosphodiesterase-5 (PDE5) selective inhibitors (i) might reduce endothelial cell (Ec) dysfunction

(Dy) in patients with vascular diseases or vascular risk conditions.

Methods: We performed a systematic review and meta-analysis of randomized double-blind placebo-controlled trials (RCTs) dealing with a prolonged use of PDE5i in all areas of medicine. The risk of bias and quality of selected trials was assessed using the Cochrane algorithm and Jadad quality score. The fixed or random effect model and standardised mean differences, as well as heterogeneity, were estimated. The publication bias was graphically explored through funnel plot: a symmetric inverted funnel shape arises from a "well-behaved" data set, in which publication bias is unlikely. Meta-regression models and subgroup analysis were conducted to investigate between study heterogeneity. A systematic search was performed in MEDLINE, EMBASE, CINAHL, SCIENCE DIRECT and the Cochrane Library to identify RCTs. Eligibility criteria for selecting studies. RCTs reporting measures of EcDy and/or of Ec activation (Ac) were analyzed.

Results: Overall 450 subjects were allocated to PDE5i, and 442 to placebo in 12 RCTs. Flow-mediated dilation (FMD) of the brachial artery showed an overall improvement after PDE5i ($p < 0.0001$). The elevated and not correctable heterogeneity ($I^2 = 92\%$) and the asymmetry of the Funnel plot suggesting a publication bias favoring the publication of positive studies only, questioned the results. PDE5i had no effect on EcDy assessed in resistance vessels by peripheral (digital) arterial tonometry. Blood level of endothelin-1 (ET-1) was reduced after treatment ($p = 0.03$) but the effect disappeared after correcting for publication bias and for heterogeneity. The effect of treatment on biomarkers of EcAc was inconsistent.

Conclusion: Continuous treatment with PDE5i failed to show a significant effect on endothelial function. The large heterogeneity and the low quality of most trials denied the use of PDE5i to improve endothelial cell function in patients with vascular diseases or vascular risk conditions. Systematic review registration. PROSPERO registration: CRD42017055399.

P111

Long-term consequences of cryptorchidism – status of sperm DNA and conventional sperm parameters

M. FRĄCZEK[1], M. KAMIENICZNA[1], M. BUDZINSKA[1], L. WOJNAR[2], L. GRZESKOWIAK[3], K. GILL[4], M. PIASECKA[4] AND M. KURPISZ[1]

[1]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland; [2]Clinic of Urology and Oncological Urology, Poznan University of Medical Sciences, Poznan, Poland; [3]Certus Private Hospital, Poznan, Poland; [4]Department of Histology and Developmental Biology, Pomeranian Medical University, Szczecin, Poland

Background: The production of spermatozoa and synthesis of steroids are two important function of testes, which can be disrupted by harmful effects of increased temperature during uncorrected testis(es) descent. There is a growing tendency of cryptorchidism incidence which is the most common urogenital disorder among male newborns. The association of cryptorchidism with testicular cancer and infertility in adulthood is well documented. Many adult infertile patients with cryptorchid history have oligozoospermia or asthenozoospermia. The higher incidence of azoospermia has also been revealed especially in bilateral cryptorchidism and delayed surgical intervention (orchidopexy). Despite conventional sperm parameters, a set of non-conventional tests should be investigated to increase our knowledge about possible mechanisms involved in infertility caused by increased scrotal temperature. Among non-conventional sperm parameters, the evaluation of sperm DNA status seems to be the most controversial.

Objective: The aim of this study was to assess the sperm DNA integrity in infertile men with cryptorchid history.

Methods: The studied group consisted of: 20 infertile men with cryptorchidism in childhood, 40 infertile men not exposed to continuous hyperthermia and 20 individuals with proven fertility as controls. All semen samples were subjected to routine semen analysis according to WHO 2010 criteria. The sperm chromatin structure assay (SCSA) in all semen specimens has been performed.

Results: There was a deterioration in all standard sperm parameters in both infertile groups, as compared with the control group. This effect was the highest in men with history of cryptorchidism. Interestingly, the percentage of sperm with DNA fragmentation emitting strong red fluorescence as well as the percentage of sperm with high DNA staining emitting strong green fluorescence in the group of cryptorchidism were significantly higher than the same measured in the other groups studied.

Conclusion: Cryptorchidism has a serious negative consequences for fertility status in adult men. Heat-induced spermatogenic damage has a deleterious effect on quality of spermatozoa which has been reflected by both conventional and non-conventional sperm tests. The sperm DNA integrity disorders can be a direct reason of fertility disturbances in men with undescended testis(es) in childhood.

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P112

Testicular function and comorbidity among patients with negative prostate biopsy

A. KOMIYA, S. SAKAMOTO, K. KAWAMURA, T. IMAMOTO AND T. ICHIKAWA

Department of Urology, Chiba University Graduate School of Medicine, Chiba-She, Japan

Objective: We have previously reported that decreased testicular function was associated with lower urinary tract symptoms. The objective of the present study is to identify incidence of hypogonadism among patients with negative prostate biopsy and relationship between testicular function and comorbidity.

Methods: From 1744 patients who underwent prostate biopsy between January 2001 and January 2017, we enrolled 546 patients with negative prostate biopsy for suspicious prostate cancer and at least one measurement of serum total testosterone in this study. We investigated the incidence of hypogonadism. The relationship between the testicular function and comorbidity was analyzed by Student's *t*-test.

Results: Mean (± SD) and median age of the patients are 69.9 ± 7.3 and 67 years old. The serum prostate specific antigen (PSA) levels were 10.27 ± 3.96 and 7.34. Free to total PSA ratios were 19.65 ± 8.68 and 24.70%. Serum levels of total testosterone (TT) were 4.68 ± 4.92 and 4.68 ng/mL. Serum luteinizing hormone (LH) levels were 44.1 ± 61.0 and 6.12 mIU/mL. Serum follicle-stimulating hormone levels were 10.67 ± 8.93 and 7.86. Total prostate volume was 48.2 ± 24.5 and 42.7 mL. The transition zone volume was 27.3 ± 19.1 and 22.7 mL. Incidence of hypogonadism (TT < 3.0 ng/mL) was 76/546 (13.9%). LH was higher than normal limit in 96/302 (31.8%). TT and LH showed correlation to age (coefficient −0.1416 and 0.3568, $p = 0.0061$ and <0.001, respectively). Medical histories were found in 375 patients from our database. Among these, 16 cases had malignancy other than prostate cancer, 11 had asthma, 28 had diabetes mellitus (DM), 15 had hyperuricemia, 36 had hyperlipidemia (HL), 103 had hypertension (HT), 12 had arrhythmia, 8 had ischemic heart disease (IHD) and 190 did not had any apparent medical history. In the patients with DM, HL, HT or any sort of comorbidity, testicular function (TT) was lower than in those with no apparent medical history as controls (3.94 ± 1.27 vs. 4.68 ± 1.81, $p = 0.0078$; 4.15 ± 1.45 vs. 4.67 ± 1.81, $p = 0.0519$; 4.25 ± 1.59 vs. 4.76 ± 1.83, $p = 0.0090$; 4.44 ± 1.70 vs. 4.80 ± 1.86, $p = 0.0527$, respectively). In the patients with HT or any co-morbidity, serum LH levels were higher than controls (5.55 ± 4.66 vs. 3.36 ± 2.57, $p = 0.0285$; 6.75 ± 7.35 vs. 4.95 ± 2.71, $p = 0.0285$, respectively). In cases with malignancy, serum LH was lower than controls (4.85 ± 2.42 vs. 6.07 ± 5.98, $p = 0.0201$). Furthermore, the patients with HT was older than controls (68.6 ± 6.6 vs. 64.4 ± 7.83, $p < 0.001$).

Conclusion: In the patients with negative prostate biopsy, a certain part of cases had hypogonadism. Presence of DM, HL, HT or any sort of comorbidities was associated with decreased testicular function, which may be due to its age-related decline. In the clinical practice, testicular function should be monitored in addition to PSA levels. Special attention should be paid to concurrent medical comorbidity to maintain patients' health.

P113

Sexual dysfunctions in homosexual males: a systematic review and meta-analysis

A. BARBONETTI, S. D'ANDREA, A. MARTORELLA, S. FRANCAVILLA AND F. FRANCAVILLA

Medical Andrology Unit, Department of Life, Health and Environment Sciences, University of L'Aquila, L'Aquila, Italy

Background: A few studies have explored the prevalence of sexual dysfunctions in homosexual males. Exclusion of homosexuals from the largest population studies and the lack of well validated tools to explore sexuality in non-heterosexual individuals could contribute to explain this knowledge gap.

Objective: We carried out a systematic review with meta-analysis of the available case-control studies in order to verify possible differences in the occurrence of major sexual dysfunctions between homosexual and heterosexual males.

Methods: Case-control studies reporting Odds Ratio (OR) for erectile dysfunction (ED) and/or premature ejaculation (PE) in homosexual and in heterosexual males were analyzed. The quality of the studies was evaluated by the New Castle Ottawa scale. Data were combined using random effect models. The Cochrane Chi-square (Cochrane Q) statistic and the I-square test were used to analyze heterogeneity. The publication bias was graphically explored through funnel plot, and Duval and Tweedie's "trim-and-fill" test was used to correct possible publication bias. We obtained the PROSPERO registration number CRD42017068955.

Results: The selection process yielded only four studies with eligibly criteria for analysis, that gave information on 1807 homosexual and 3955 heterosexual males. The pooled OR estimated showed that homosexuals exhibit a significantly higher prevalence of ED (OR = 1.47, 95%CI: 1.00, 2.15; $p = 0.05$) and a significantly lower prevalence of PE (OR = 0.72, 95%CI: 0.52, 1.00; $p = 0.05$). However a significant heterogeneity among included studies was observed both in the analysis of ED (I-square = 67%, $p = 0.03$) and in PE (I-square = 66%, $p = 0.03$). Funnel plot suggested a possible publication bias in the analysis of ED but not PE. The "trim-and-fill test" identified a possible "missing study" on the right side of funnel plot of ED analysis; when this additional putative study was included in the analysis, adjusted pooled OR was 1.6 (95%CI: 1.1, 2.3; $p = 0.01$), with persistent significant heterogeneity (I square = 65%, $p = 0.04$).

Conclusion: Homosexual males seem to exhibit a higher prevalence of ED and a lower prevalence of PE when compared to heterosexuals. The use of different diagnostic tools and recruitment strategies might explain the large heterogeneity among the available studies.

P114

Serum homocysteine levels in men with and without erectile dysfunction: a systematic review and meta-analysis

A. SANSONE[1], A. CIGNARELLI[2], M. SANSONE[1], F. ROMANELLI[1], G. CORONA[3], D. GIANFRILLI[1], A. M. ISIDORI[1], F. GIORGINO[2] AND A. LENZI[1]

[1]Department of Experimental Medicine, Section of Medical Pathophysiology, Food Science and Endocrinology, Sapienza – University of Rome, Rome, Italy; [2]Department of Emergency and Organ Transplantation, Section of Internal Medicine, Endocrinology, Andrology, and Metabolic Diseases, University of Bari Aldo Moro, Bari, Italy; [3]Endocrinology Unit, Medical Department, Azienda Usl Bologna Maggiore-Bellaria Hospital, Bologna, Italy

Objective: Elevated levels of serum homocysteine (Hcy) have been associated with cardiovascular diseases and endothelial dysfunction, conditions closely associated with erectile dysfunction (ED). This meta-analysis was aimed to assess serum Hcy levels in subjects with ED compared to controls in order to clarify the role of Hcy in the pathogenesis of ED.

Methods: Medline, EMBASE, and the Cochrane Library were searched for publications investigating the possible association between ED and Hcy. Results were restricted by language but no time restriction was applied. Standardized mean difference (SMD) was obtained by random effect models.
Results: A total of 9 studies were included in the analysis with a total of 1320 subjects (489 subjects with ED; 831 subjects without ED). Pooled estimate was in favor of increased Hcy in subjects with ED with a SMD of 1.00, 95% CI 0.65–1.35, $p < 0.0001$. Subgroup analysis based on prevalence of diabetes showed significantly higher SMD in subjects without diabetes [1.34 (95% CI 1.08–1.60)] compared to subjects with diabetes [0.68 (95% CI 0.39–0.97), $p < 0.0025$ vs. subgroup w/o diabetes].
Conclusion: Results from our meta-analysis suggest that increased levels of serum Hcy are more often observed in subjects with ED; however, increase in Hcy is less evident in diabetic compared to non-diabetic subjects.
Prospero registration number: CRD42018087558.

P115

Venous-occlusive erectile dysfunction therapy – new indications for inhibitors of angiotensin convertase enzyme

R. OVCHINNIKOV, A. POPOVA AND S. GAMIDOV
Department of Andrology and Urology, National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I. Kulakov, Moscow, Russia

Background: Venous-occlusive erectile dysfunction (VOD) is one of the most difficult for treatment. Efficacy of present pharmacotherapy is less than 60–75%. Aim of the study is to improve treatment results of patients with erectile dysfunction (ED) and to develop new effective method for VOD treatment.
Methods: Two hundred and thirty-seven patients with isolated VOD (mean age 28.1 ± 7.3 years) were evaluated with International Index Erectile Function (IIEF), penile color Doppler duplex ultrasound (CDDU), laboratory tests. Patients with arterial hypertension were excluded. All patients were treated with 10–40 mg inhibitor of angiotensin convertase enzyme (fosinopril) during 3 months. Measurement of blood pressure and pulse rate were performed daily, IIEF questioning and penile CDDU – monthly. The statistical significance of the change in variables was calculated using the Wilcoxon test. Critical level $p = 0.05$ was established for all criteria.
Results: Domain "Erectile Function" IIEF increased from 17.4 ± 6.2 to 24.3 ± 5.7 after 3 months of fosinopril therapy ($p < 0.05$). Pulse rate and systolic blood pressure were constantly, but diastolic blood pressure decreased from 78.2 ± 6.7 to 68.3 ± 7.2 mm Hg ($p < 0.05$). No serious or severe adverse events (hypotension) occurred. During penile CDDU end diastolic velocity (EDV) in cavernous arteries significantly decreased from 16.3 ± 5.1 to 4.8 ± 3.8 cm/s. Resistance index increased from 0.63 ± 0.09 to 0.76 ± 0.2 after 3 month of treatment with fosinopril ($p < 0.05$).
Conclusion: Daily use of individually selected doses of inhibitor of angiotensin convertase enzyme (fosinopril) resulted in significant increasing erectile function domain score of IIEF after 3 months of treatment. This increasing is achieved due to improving of venous penile circulation proved by CDDU (decrease of EDV and resistance index). Taking into consideration, that isolated VOD in absence of arterial hypertension is noticed mainly in young patients, suggested new effective and cheap method of conservative treatment of ED may become a treatment of choice in many patients with VOD.

P116

Meta-analysis of results of testosterone therapy on sexual function based on International Index of Erectile Function Scores

S. CIPRIANI[1], G. CORONA[2], G. RASTRELLI[1], A. MORGENTALER[3], A. SFORZA[2], E. MANNUCCI[4] AND M. MAGGI[1]
[1]*Andrology and Sexual Medicine Unit, Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy;* [2]*Endocrinology Unit, Medical Department, Azienda Usl Bologna Maggiore-Bellaria Hospital, Bologna, Italy;* [3]*Men's Health Boston, Harvard Medical School, Boston, MA, USA;* [4]*Andrology and Sexual Medicine Unit, Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy; Diabetes Agency, Careggi Hospital, Florence, Italy*

Background: The interpretation of available clinical evidence related to the effect of testosterone (T) treatment (TTh) on sexual function has been inconsistent, in part due to the use of different and self-reported measures to assess outcomes. The International Index of Erectile Function (IIEF) is the most frequently used validated tool to assess male sexual function.
Objective: To perform a meta-analysis of available data evaluating the effect of TTh on male sexual function using IIEF as the primary outcome.
Methods: An extensive Medline, Embase, and Cochrane search was performed including all placebo-controlled randomized clinical trials enrolling men comparing the effect of TTh on sexual function. Out of 137 retrieved articles, 14 were included in the study enrolling 2298 participants, with a mean follow-up of 40.1 week and mean age of 60.2 ± 6.5 year. Using IIEF-erectile function domain (IIEF-EFD) as the outcome, we found that TTh significantly improved erectile function compared with placebo [mean difference = 2.31 (1.41;3.22) IIEF-EFD score, $p < 0.0001$]. Patients with more severe hypogonadism (total T < 8 nmol/L) reported greater changes in final IIEF-EFD score when compared with those with a milder T deficiency [total T < 12 nmol/L; 1.47 (0.90;2.03) and 2.95 (1.86;4.03) for total T < 12 and <8 nmol/L, respectively, Q = 5.61, $p = 0.02$]. The magnitude of the effect was lower in the presence of metabolic derangements, such as diabetes and obesity. Other aspects of sexual function, as evaluated by IIEF subdomains, were also improved with TTh including libido, intercourse satisfaction, orgasm, and overall sexual satisfaction.

Conclusion: TTh significantly improves erectile function and other sexual parameters as measured by IIEF in hypogonadal men. These results argue that sexual dysfunction should be considered a hallmark manifestation of T deficiency, since those symptoms can be significantly improved with normalization of serum T. In addition, these results suggest that TTh alone may be considered a reasonable treatment for hypogonadal men with milder degrees of erectile dysfunction, whereas the addition of other treatments, such as phosphodiesterase type 5 inhibitors, may be more appropriate for men with more severe erectile dysfunction.

P117

Is the insulin resistance a regulator on the levels of insulin and testosterone in seminal plasma in men with metabolic syndrome

K. ANANIEVA-TODOROVA[1], V. RILCHEVA[2] AND V. ILIEVA[2]

[1]Medical University, Pleven, UMHAT "D-r G. Stranski", University Clinic of Endocrinology and Metabolism; [2]Clinical Institute for Reproductive Medicine, Pleven

Background: The morbidly weight gain is associated with multiple hormonal interactions with potential negative effect on male fertility. It is not clearly understand if visceral adiposity or Insulin Resistance (IR), or both together, is the key mediator for decreasing testosterone (T) synthesis and activity in men with metabolic syndrome (MS).

Objective: To establish the influence of IR on the semen levels of Insulin (Ins) and T and such as on the semen concentration, motility and morphology in men with MS.

Methods: A pilot, prospective study was done among obese men with IR without knowing fertile problems on age between 20 and 40 years. According to their BMI the participants were divided into three groups: first group (G1), of non obese subjects with BMI $\geq 19.0 \leq 24.9$ kg/m^2, (n1 = 30); second group (G2) of overweight, pre-obese subjects with BMI $\geq 25.0 \leq 29.9$ kg/m^2, (n2 = 31) and third group (G3) of overweight subjects with BMI $\geq 25.0 \leq 29.9$ kg/m^2, (n3 = 29). Semen samples were collected and assayed for Ins, T and standard semen analysis as well.

Results: Obesity was associated with significantly increased serum insulin levels and IR, but with no significant difference in serum testosterone levels. Significantly severe inverse correlations between serum levels of Ins and T, as well as between BMI and serum T were established. There were found positively correlation between serum and seminal concentration of Ins. A moderately strong negative correlation between concentrations of Ins and T in seminal plasma was confirmed. The significant differences in the average sperm concentration and teratozoospermia index were observed by comparison between obese and non obese men.

Conclusion: This pilot study showed that obesity and IR in complex, adversely affects sperm parameters such as decreased sperm motility and increased teratozoospermal index. The pandemic spread of obesity necessitates further studies to clarify the additional links and mechanisms that are manifested at an early stage and are related to violations of reproductive function in obese men.

P118

Feasibility of spermatogenesis temperature optimization with innovative electronic device

R. MAKSYM[1,2], J. SZYMAŃSKI[1,2], Ł. KONARSKI[3] AND M. RABIJEWSKI[1]

[1]Department of Reproductive Health, Center of Postgraduate Medical Education, Poland; [2]St Sophia Hospital, Warsaw, Poland; [3]CoolTec sp z o o, Wrocław, Poland

Background: The fraction of couples struggling with infertility has rapidly increased in developed countries and emerge as a widespread social disease. Male factor infertility is a burden for up to 8% of men worldwide. The most common manifestation of male infertility is low sperm quality i.e. reduced spermatozoa concentration, mobility or changes in their structure. Impaired sperm production is influenced by long-lasting overheating of the testicles caused by varicocele, wearing a tight-fitting underwear, frequent use of the sauna, spending many hours driving a car or operating computer. The outer position of the testicles is necessary for their temperature to be approx. 2°C lower than the temperature of the interior of the body, since spermatogenesis requires a temperature of about 34.5–35.5°C. It has been shown that elevated temperature causes apoptosis of spermatogonia and sperm maturation impairment. Many attempts was done to decrease testicular temperature for fertility improvement so far. Publication mention diverse solutions: cold or ice packs, cold showers, devices that moisture scrotal surface or wash scrotum with cool air. Promising results of this attempts did not cause real breakthrough in infertility treatment due to obstacles that was met. Devices were uncomfortable or stationary and never before allow precise set-up of cooling range.

Methods: We present preliminary results of feasibility and effectiveness trial of testicle temperature optimization with CoolMen, an innovative, electronic device that constantly measure testicle temperature and uses Peltier modules to transfer thermal energy from scrotal surface.

Results: Unlike other male fertility enhancement methods, the CoolMen device continually optimizes the testicle's temperature and thus could enable significant and long lasting improvement in semen parameters, contributing to increased fertility of the pair. It is a non-pharmacological, safe and easy-to-use unit. The specific target group for this device is sedentary men (office workers, IT specialists, drivers), but also patients with varicocele. The device is developed with support of a grant of the Polish National Center for Research and Development's (NCBiR) "Fast Track" program no POIR.01.01.01-00-0966/16. Trial of the device will start in 2018 and all results will be collected to end of 2019.

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Seminal suPAR levels as marker of abacterial male accessory gland inflammation in hypogonadism

D. MILARDI[1,2], G. GRANDE[1,2], C. AUTILIO[3], F. MANCINI[2], L. D. MARINIS[4], R. MARANA[2], C. ZUPPI[3], A. URBANI[3], S. BARONI[3] AND A. PONTECORVI[1,2]
[1]*Teaching and Research Hospital "Agostino Gemelli" Foundation, R – Division of Endocrinology Rome, Italy;* [2]*International Scientific Institute "Paul VI" Rome, Italy;* [3]*Catholic University – Institute of Chemistry and Clinical Biochemistry Rome, Italy;* [4]*Teaching and Research Hospital "Agostino Gemelli" Foundation – Division of Endocrinology Rome, Italy*

Background: Recent evidences suggest that hypogonadism is an important risk factor for lower urinary tract symptoms and benign prostatic hyperplasia. Several papers have discussed the role of chronic inflammation in the development of BPH, which may be modulated by the hypogonadal state. Soluble urokinase-type plasminogen activator receptor (suPAR), known protein marker of systemic inflammation, can be assayed in the seminal plasma and represents a reliable and sensitive marker of inflammation for the male accessory gland inflammation (MAGI).
Objective: The aim of this study has been to investigate if seminal suPAR is elevated in MAGI with hypogonadism and if suPAR represent an useful marker of abacterial inflammation in hypogonadism.
Methods: We included in the study twenty male patients aged between 25 and 55 year-old with secondary postsurgical hypogonadism. The same patients were also evaluated after a 3-month of testosterone replacement therapy (TRT), to evaluate the effect of androgen replacement therapy on suPAR. Ten fertile men have been enrolled as a control group in the protocol. SuPAR concentrations were assayed on seminal plasma using an enzyme-linked immunosorbent assay (ELISA) kit.
Results: Hypogonadic patients presented significantly increased levels of seminal suPAR respect to controls (86.1 ± 36.8 vs. 55.2 ± 20.0 ng/mL, $p < 0.05$). TRT in hypogonadic patients has been associated with a significant reduction of suPAR levels as reported in the control group (50.9 ± 22.91 vs. 86.1 ± 36.8 ng/mL $p < 0.05$).
Conclusion: These results confirm the role of suPAR as a protein marker of MAGI and support the hypothesis that hypogonadism induces a state of inflammation in male accessory glands which is involved in male infertility. Moreover demonstrated that testosterone treatment probably exerts a positive effect on MAGI and infertility as documented by reduction of suPAR levels in hypogonadic treated patients.

P120

The rates of sperm obtaining and pregnancy, by microTESE surgery, among our azoospermic patients whose FSH levels are higher than 6 mIU/mL

U. OZTEKIN[1] AND M. CANIKLIOĞLU[2]
[1]*Bozok University, Yozgat, Turkey;* [2]*Sinop Atatürk City Hospital, Sinop, Turkey*

Background: We aimed to present the rates of sperm obtaining and pregnancy, by microTESE surgery, among azoospermic patients whose FSH levels are higher than 6 mIU/mL.
Methods: A hundred patients with non-obstructive azoospermia and whose FSH levels were ≥6 mIU/mL and which hadn't underwent any hormonotherapy before surgery, were examined retrospectively. All surgical procedures were performed by the same urologist. MicroTESE procedure was performed under a microscope image. The antimesenteric side of Tunica Albuginea was incised. The samples were taken from the more brilliant and bigger seminiferous tubules in upper, middle and lower poles of testicles. The spermatozoa were distinguished by enzymatic method or manually. All specimens also were examined histopathologically. The cases were classified as Sertoli cell only syndrome (SCOS), maturation arrest (MA), hypospermatogenesis and tubular hyalinisation, by the same pathologist. The cases which sperm obtaining were achieved, underwent intra-cytoplasmic sperm injection (ICSI) procedure.
Results: The mean value for FSH was 20.86 mIU/mL. The cases showed histopathological distribution as SCOS 56%, MA14%, hypospermatogenesis 7% and tubular hyalinisation 6%. We weren't able to acquire 16 patients' histopathologic results. The rate of sperm obtaining was 32%. After transfer procedure 17 cases (17% in the whole group, 53.1% in transfer group) got pregnancy.
Conclusion: MicroTESE in azoospermic patients with FSH levels ≥6 mIU/mL is a reliable surgical procedure. Our clinic's result are compatible with the literature.

P121

TESE in patients with Klinefelter's syndrome

D. JEZEK[1,2], D. HAUPTMAN[2], Z. ZIMAK[2], Z. KASTELAN[2] AND P. STANIC[3]
[1]*Department of Histology and Embryology, University of Zagreb, School of Medicine, Zagreb, Croatia;* [2]*University Hospital Zagreb, School of Medicine, Zagreb, Croatia;* [3]*Outpatient Clinic "Repromed", Zagreb, Croatia*

Background: Klinefelter's syndrome is present in 11% of patients with azoospermia and in 0.7% of infertile men with oligozoospermia. The backbone of the syndrome lies in the excess X chromosome. The clinical picture of Klinefelter's syndrome is characterized by eunuchoid phenotype, poor development of secondary sexual characteristics, gynecomastia and mental retardation. The patient's testicles are often drastically reduced in size, with a very strong consistency. However, in 50% of cases, there are also focal points with preserved spermatogenesis.

Objective: The aim of this paper is to present our experience in cryopreservation of testicular tissue in infertile patients with Klinefelter syndrome and azoospermia.
Methods: Since the founding of the Testicular Tissue Bank at University Hospital "Zagreb" in 2013 until today, over 180 patients have been operated for azoospermia. Of these, 8 patients were diagnosed with Klinefelter's syndrome. Age of patients ranged from 18 to 36 years. All patients were subjected to the open testicular biopsy with a multiple tissue cryopreservation for the possible TESE/ICSI procedure.
Results: In general, patients had a significantly reduced testicular volume (range: 1.3–2.2 cm^3), elevated FSH (14.79–51.90 mIU/L) and LH (10.9–22.6 mIU/L) and slightly reduced or mostly normal testosterone levels (5.6–29.6 nmol/L). In 5 patients, histological analysis demonstrated a picture of "mixed atrophy" of seminiferous tubules with some focal active spermatogenesis (including mature spermatids and spermatozoa). In 3 patients the parenchyma was extensively altered bearing seminiferous tubules lined only with Sertoli cells or fibrous tissue ("tubular shadows"). It should be pointed out that the mature spermatids and spermatozoa were also found in one patient of advanced age (28 years). The couple was able to conceive (TESE-ICSI). The prenatal diagnostics indicated a normal male foetus.
Conclusion: Our data suggest that the "classical" open biopsy of the testis could be a good alternative to mTESE, even in the cases of Klinefelter's syndrome.

P122

Prevalence of type 2 diabetes (T2DM) and prediabetes in hypogonadal men with or without testosterone treatment for up to 12 years, and effects on anthropometric parameters and glycemic control

F. SAAD[1,2], G. DOROS[3] AND A. YASSIN[2,4,5]
[1]Medical Affairs Andrology, Bayer AG, Berlin, Germany; [2]Gulf Medical University, Ajman, UAE; [3]Boston University School of Public Health, Boston, MA, USA; [4]Institute for Urology and Andrology, Norderstedt, Germany; [5]Dresden International University, Dresden, Germany

Background: Hypogonadal men have a high prevalence of T2DM and prediabetes.
Methods: In a controlled registry study of 505 hypogonadal men in a urology office, 146 (29%) had T2DM and 253 (50.2%) prediabetes. Mean age: 61.4 ± 9.7 years, mean BMI: 30.7 ± 4.1 kg/m^2. 321 men received testosterone therapy (TTh, T-group) with TU 1000 mg/12 weeks for up to 12 years. 184 men had opted against TTh and served as controls (CTRL). 8-year data are reported for the total group, regardless of presence of T2DM and/or prediabetes.
Results: Weight (kg) decreased from 99.4 ± 13.4 to 91.8 ± 11.4 in the T-group ($p < 0.0001$) and increased from 91.4 ± 10.5 to 96.5 ± 12.4 in CTRL ($p < 0.0001$ for both). Waist circumference (cm) decreased from 107.2 ± 9.6 to 100.3 ± 9.0 in the T-group and increased from 99.8 ± 9.1 to 104.7 ± 8.3 in CTRL ($p < 0.0001$ for both). BMI (kg/m^2) decreased from 31.5 ± 4.3 to 29.0 ± 3.8 in the T-group and increased from 29.2 ± 3.2 to 30.7 ± 4.0 in CTRL ($p < 0.0001$ for both).
Fasting glucose (mmol/L) decreased from 6.1 ± 2.0 to 5.2 ± 1.3 in the T-group ($p < 0.0001$) and remained stable from 5.4 ± 2.1 to 5.4 ± 2.2 in CTRL (NS). HbA1c decreased from 6.5 ± 1.2 to 6.1 ± 1.1% in the T-group and increased from 6.0 ± 0.7 to 6.2 ± 0.8% in CTRL ($p < 0.0001$ for both). The triglyceride:HDL ratio, a surrogate parameter for insulin resistance, decreased from 6.8 ± 3.4 to 4.6 ± 2.5 in the T-group ($p < 0.0001$) and increased from 4.0 ± 2.4 to 5.9 ± 2.35 in CTRL ($p < 0.0001$ for both). 74 of 94 patients (78.7%) with T2DM in the T-group had their last measured HbA1c <6.5%, but only 9 of 52 (17.3%) men in CTRL, regardless of concomitant treatment modalities. Of the prediabetes patients in CTRL, 38.5% had a progression to T2DM during the observation time. Since all injections of TU in the T-group were administered in the office and documented, there was a 100% treatment adherence.
Conclusion: In an unselected cohort of men presenting to a urology office and diagnosed with hypogonadism, almost 80% had impaired glucose metabolism manifest as type 2 diabetes or prediabetes. Impaired glucose metabolism may be an important feature of hypogonadism. Long-term TTh with testosterone undecanoate injections in hypogonadal men with a high prevalence of T2DM or prediabetes resulted in weight loss and improvements in glycemic control whereas untreated controls experienced worsening of all parameters.

P123

Hypogonadal men with diabetes mellitus type 1 (T1DM) benefit from long-term testosterone therapy (TTh) – real-world experience from a registry study

F. SAAD[1,2], A. HAIDER[3], K. HAIDER[3], G. DOROS[4] AND A. TRAISH[5]
[1]Medical Affairs Andrology Bayer AG, Berlin, Germany; [2]Gulf Medical University, Ajman, UAE; [3]Private Urology Practice, Bremerhaven, Germany; [4]Boston University School of Public Health, Boston, MA, USA; [5]Boston University School of Medicine, Boston, MA, USA

Background: Few studies have assessed prevalence of hypogonadism in T1DM. Holt et al. reported a prevalence of 9.5% (JCEM 2014; 99: E1655–E1660), Grossmann et al. found 7% (JCEM 2008; 93: 1834–1840). From a registry assessing effectiveness and safety of TTh, we analyzed a subgroup of 21 patients with T1DM followed for up to 7 years.
Methods: Men presenting to a urological office with various complaints were screened for the presence of hypogonadism and, if found to have total testosterone ≤12.1 nmol/L, offered TTh. Those who had received at least 1 year of treatment with testosterone undecanoate 1000 mg injections (TU) were entered into a prospective, observational, cumulative registry study. 21 men had T1DM according to patient history. TU was administered in 3-month intervals following an initial 6-week interval for up to 7 years. At each or each other visit, anthropometric and metabolic parameters were measured.

Results: Mean age was 55.7 ± 4.3 years, mean follow-up 63 ± 23 months. Fasting glucose declined from 5.9 ± 0.9 to 5.3 ± 0.2 mmol/L, change from baseline −0.6 ± 0.2 mmol/L ($p < 0.001$). HbA1c decreased progressively from 7.9 ± 0.8 to 5.7 ± 0.5% ($p < 0.0001$), change from baseline −2.2 ± 0.1% with statistical significance compared to the previous year for all 7 years. Triglyceride:HDL ratio, a surrogate parameter of insulin resistance, declined from 6.0 ± 2.3 to 3.0 ± 0.8 ($p < 0.0001$) with statistical significance compared to the previous year for the first 3 years. In all patients, the last measured HbA1c was lower than baseline, in 20/21 patients, the last measured HbA1c was <6.5%. Weight decreased from 98.4 ± 15.1 to 80.6 ± 6.9 kg. Change from baseline at 7 years was −12.5 ± 1.0 kg, percent change from baseline −12.4 ± 1.2%. Waist circumference decreased from 102.3 ± 7.6 to 89.8 ± 4.3 cm. Change from baseline was −8.5 ± 0.5 cm. BMI decreased from 31.9 ± 4.6 to 26.5 ± 2.1 kg/m^2, change from baseline −4.1 ± 0.3 kg/m^2 ($p < 0.0001$ for all measures). Since all injections were administered in the office, there was a 100% adherence to testosterone therapy. There were no major adverse cardiovascular events during the full observation time.
Conclusion: Hypogonadal men with T1DM showed meaningful and sustained improvements in glycemic control as well as weight loss when receiving long-term treatment with testosterone.

P124

Involvement of Retinoic Acid Receptor α and of all-trans Retinoic Acid in the physiopathology of varicocele-associated male infertility: their action on human sperm metabolism

M. SANTORO[1], V. RAGO[1], R. MALIVINDI[1], D. D. ROSE[1], G. D'AGATA[2], F. I. MONTALTO[1] AND S. AQUILA[1]
[1]Department of Pharmacy and Sciences of Health and Nutrition, University of Calabria, Italy; [2]Department of Clinical and Experimental Medicine, University of Catania, Italy

Background: Testicular varicocele is the most common cause of male infertility with an occurrence frequency of >30% in men with primary infertility and up to 81% in men with secondary infertility. Nevertheless, the mechanism/s by which varicocele affects fertility remain undetermined. Varicocele influences testicular function in a variety of ways, in spermatogenesis, in semen and sperm quality. Recently, we showed that this pathology induces damage in male gamete at molecular level, opening a new chapter in the already multifactorial pathophysiology of varicocele, complicating this issue.
Considerable evidence showed that the retinoic acid receptor α (RARα) and its all-trans retinoic acid (ATRA) ligand, the active form of vitamin A, play key roles in sperm maturation. Although, the Vitamin A is required for fertility and normal spermatogenesis, the mechanisms that drive its action are not defined yet. Western blot and immunogold electron microscopy showed that RARα expression was restricted to the neck region and the middle piece. RARα content was reduced in varicocele sperm. ATRA influenced positively sperm performance, however responsiveness to ATRA was reduced in varicocele sperm. Further, ATRA promoted cholesterol efflux and survival in sperm. The anatomical location of the receptor in the middle piece, where are present the mitochondria, led us to hypothesize a role also in sperm metabolism. To further define the ATRA significance in male gamete and in the varicocele physiopathology, we tested for the first time its action on human sperm metabolism. Sperm energy management is an intriguing issue since it appears that sperm regulate its own metabolism independently by the systemic regulation. During capacitation, energy demand increases showing an overall energy expenditure. It may be hypothesized that RARα co-working with other factors stimulate some enzymatic activities providing additional metabolic fuel to sustain capacitation. Nature has endowed spermatozoa with striking cellular peculiarities given its essential role in the propagation of life, but with a single, irreversible chance to fertilize an egg; its metabolism needs to be fine-tuned and independent of the systemic regulation.
Results: Our previous studies demonstrated that substances which induced capacitation reduced triglycerides content while concomitantly some enzymatic activities related to the energy expenditure increased. The evaluation of the triglycerides content and lipase activity suggest that ATRA influences the lipid metabolism. The modulation of glucose-6-phosphate dehydrogenase activity, concomitantly with a reduction of the glucose content, highlight a role of ATRA on glucose metabolism. Interestingly, we discovered that ATRA treatment was able to reprogram sperm metabolism towards that of the capacitation status providing compelling evidence of the efficacy of vitamin A as therapeutic tool in improving sperm quality. However, the same effects were not observed in varicocele sperm, indicating that they have difficulty to switch into the capacitation.
Conclusions: These novel findings further confirm the importance of vitamin A in male fertility and add new insights into the retinoids complex biological framework. Collectively, ATRA administration in procedures for artificial insemination or dietary vitamin A supplementation might represent a promising therapeutic approach for the management of male infertility.

P125

Evaluation of Endocrine Profile and Semen Quality in a cohort of newly diagnosed male with Multiple Sclerosis (MS) who started the first MS therapy: a prospective study

G. BURGIO[1], E. D'AMICO[2], R. A. CONDORELLI[1], A. ZANGHI[2], F. PATTI[2] AND A. E. CALOGERO[1]
[1]Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy; [2]Department of Neurology, University of Catania, Catania, Italy

Background: Multiple sclerosis (MS) is one of the most common causes of chronic neurological disability in young adults and middle-aged men. It affects women two to three times more frequently than men, but the clinical course in the latter, in which the disease begins later in life

than women, is more rapid and severe. Male infertility has been linked to an increased risk of several diseases such as tumors, cardiovascular diseases, and overall increased mortality, but the risk of autoimmune diseases such as MS has not been deeply explored. Moreover, it is described that men with MS have lower serum testosterone levels and higher disability. Overall, poor attention has been paid to the reproductive and sexual health of men with MS. In the last 5 years, new drugs with different mechanisms of action are now available (called disease-modifying treatments, DMTs), in addition to the older injectable agents (ß-interferons and glatiramer acetate). These include teriflunomide, dimethyl-fumarate, natalizumab, fingolimod, alemtuzumab, and ocrelizumab, and several new compounds are being developed. All the approved medications have mainly anti-inflammatory effects and increasing evidence indicates that all of them are more effective in the early phases of disease development. Poor data is available on the new released treatments for MS and their potential effects on reproductive and sexual health of men with MS.
Methods: We designed a prospective-12 month-interventional study at the MS center of the University of Catania. We will assess the fertility status of men who were recently diagnosed with relapsing remitting MS (RRMS), before starting their prescribed disease modifying treatments of MS (DMTs). The enrolled men with RRMS will be followed up for 12 months from the time of enrollment to assess any changes in their fertility profile. The endocrine profile, hypothalamic–pituitary–testis axis and semen quality will be evaluated. MS disease activity will be assessed by clinical and radiological measures. Moreover, a brief and complete cognitive assessment will be performed. In the end, vesicular emptying will be assessed with transrectal ultrasound before and after ejaculation, in order to investigate neurological defects still not known in SM patients.
Results: Preliminary results have shown that mean levels of total testosterone (TT) were 3.08 ± 2.3 ng/mL (v.n. 2.3–10.1). Vesicular ejection fraction (VEF) was reduced bilaterally with a mean rate of 16.1% (normal values >21%). Correlation analysis showed negative correlation between TT levels and Expanded Disability Status Scale (EDSS) at onset (Pearson = −0.788; $p < 0.001$) and number of spinal cord lesions and VEF (for both Pearson = −0.854; $p < 0.001$). Further data are being collected to elaborate on these results.

P126

Prevalence of hypogonadism in males affected by Heart Failure with reduced or preserved ejection fraction and relationships with oxidative stress

C. BRUNO[1], E. VERGANI[1], A. SILVESTRINI[2], E. MEUCCI[2], M. A. NICOLAZZI[3], A. M. FAVUZZI[3] AND A. MANCINI[1]
[1]Division of Endocrinology, Department of Internal Medicine, Catholic University of the Sacred Heart, Rome, Italy; [2]Institute of Biochemistry and Clinical Biochemistry, Catholic University of the Sacred Heart, Rome, Italy; [3]Division of Internal Medicine and Cardiovascular Diseases, Department of Internal Medicine, Catholic University of the Sacred Heart, Rome, Italy

Background: Chronic heart failure (CHF) is defined as a clinical syndrome based on an unbalance between cardiac output and metabolic requirements of organism. The main classification of CHF relies on left ventricular ejection fraction (LVEF), evaluated by echocardiography; two main kinds of CHF were described: heart failure with reduced ejection fraction (HFrEF), with LVEF < 40%, and heart failure with preserved ejection fraction (HFpEF), with LVEF > 50%. Both the conditions present multi-hormonal deficiencies with high prevalence. On the other hand, anabolic hormones are known to regulate oxidative stress in different manners, exerting pro-oxidative effects or regulating antioxidant systems.
Objective: Therefore, we aimed to explore: (i) the prevalence of testosterone (T) deficiency in patients with HFrEF or HFpEF, (ii) the correlations between serum T with metabolic parameters and index of oxidative stress, trying to better understand the possible molecular consequences of hormonal derangement in these conditions.
Methods: A total of 50 male patients were enrolled. Among them, twenty patients with HFrEF, aged 42–88 years (mean 69.45) and thirty patients with HFpEF, aged 59–90 years (mean 77.7), were recruited. The diagnosis of CHF was established according to the current guidelines of the European Society of Cardiology. Patients with end stage renal disease, liver cirrhosis, neoplastic or autoimmune diseases were excluded. Clinical, anthropometric and echocardiographic evaluation were obtained, including the main risk factors for cardiovascular disease and NYHA classification. A morning blood sample was collected and immediately centrifuged, with aliquots stored at −80°C until assayed. We evaluated metabolic (glycaemia, insulinemia, total-HDL-LDL-cholesterol) and hormonal parameters (fT3, fT4, TSH, IGF-1, Testosterone, DHEAS); hormones were assayed with CMIA method. Total Antioxidant Capacity (TAC), as a parameter of Oxidative Stress, was measured with a spectrophotometric method, using the system H_2O_2-metmyoglobin and the chromogen ABTS. TAC was expressed as LAG (sec), as the latency time in the appearance of radical species. T deficiency was defined as serum T level <3 ng/mL, according to the criteria of T.O.S.C.A. Registry (Bossone et al., Intern. Emerg. Med., 2018).
Results: Deficit of Testosterone was observed in 30% of HFrEF patients and in 37% of HFpEF patients.
TAC, expressed by LAG, showed a significant direct correlation with serum T in HFpEF patients ($r^2 = 0.24$, $p = 0.02$). In HFrEF, no correlations between TAC and serum T levels were found.
Conclusion: While testosterone deficiency showed a similar prevalence in the two kinds of CHF, only in patients with HFpEF T correlated with antioxidant capacity, despite age and comorbidities differences in the two groups, suggesting different physiopathological mechanisms. These results underline the role of T as a modulator of antioxidant systems in CHF.

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Effects of common environmental pollutants on human sperm functions: an in vitro study

S. MARCHIANI[1], L. TAMBURRINO[2] AND E. BALDI[2]
[1]*Azienda Opsedaliero Universitaria Careggi, Florence, Italy;* [2]*Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy*

Background: Decline of fertility is a growing worldwide concern and about the 15% young couples experience difficulty with a male factor contributing for about half of cases. A high proportion of male infertility is unexplained or idiopathic. Recent literature suggests the potential role of the environmental contaminants which has a toxic effect accumulating inside the body and causing multi-organs alterations, especially in female and male reproductive systems.

Objective: To investigate the in vitro effects of two environmental toxicants, cadmium chloride (CdCl2, a heavy metal contained in different foods and in smoking tobacco) and Dibutyl phthalate (DBP, an organic compound commonly used as plasticizer), on sperm quality and functions.

Methods: The in vitro effects of $CdCl_2$ at the concentration of 10 μM and DBP at the concentration of 200 μM have been tested on Swim up selected spermatozoa incubated for 24 h at 37°C 5%CO2. Sperm progressive and total motility (measured by optical microscopy), kinetic parameters and hyperactivated motility (measured by the computerized-assisted analysis system CASA), viability (by eosin-nigrosin staining), spontaneous or progesterone-induced acrosome reaction (evaluated in viable spermatozoa by FITC-labeled A Arachis hypogea (peanut) Lectin staining) and basal and progesterone-induced intracellular calcium levels (by spectrophotometric method using the Fura 2 probe) have been evaluated. Data have been expressed as variation of treated samples respect to controls.

Results: $CdCl_2$ exposure determined a statistical significant decrease of progressive ($\Delta = -31.1\%$, $n = 34$, $p < 0.05$) and total motility ($\Delta = -14.4\%$, $n = 34$, $p < 0.05$) as well as hyperactivated motility ($\Delta = -28.6\%$, $n = 18$, $p < 0.05$). Sperm viability was slightly reduced ($\Delta = -6.4\%$, $n = 11$, $p < 0.05$) following $CdCl_2$ incubation, but the reduction was not comparable to the motility decrease observed in the same samples. Treatment with $CdCl_2$ determined a significant increase of spontaneous acrosome reaction ($\Delta = +78.9\%$, $n = 9$, $p < 0.05$) and a reduction of acrosome reaction following progesterone challenge (ARPC, $\Delta = -86.2\%$, $n = 9$, $p < 0.05$). Basal intracellular calcium levels were significantly increased ($\Delta = +16.4\%$, $n = 5$, $p < 0.05$) following $CdCl_2$ incubation, whereas no alterations in progesterone-stimulated calcium levels were observed. DBP exposure significantly reduced the percentage of sperm progressive and total motility ($\Delta = -22.3\%$, $n = 14$ and $\Delta = -17.5\%$, $n = 14$, respectively, $p < 0.05$) and, in a less severe way, also viability ($\Delta = -11.2\%$, $n = 11$, respectively, $p < 0.05$). Sperm hyperactivated motility resulted to be decreased following DBP incubation ($\Delta = -37.2\%$, $n = 14$, respectively, $p < 0.05$).
Finally, DBP exposure determined a significant increase of spontaneous acrosome reaction ($\Delta = +78.0\%$, $n = 10$, $p < 0.05$) and a reduction of acrosome reaction following progesterone challenge (ARPC, $\Delta = -70.6\%$, $n = 10$, $p < 0.05$).

Conclusion: Our results demonstrate that the in vitro exposure to $CdCl_2$ or DBP of human spermatozoa can compromise sperm quality and functions which are needed for the fertilization process, suggesting that these environmental toxicants, which can be found both in semen and in the female reproductive tract, are potentially dangerous for male fertility status.

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Evaluation of micronutritional status in patients with idiopathic male infertility

A. TSUKANOV, D. TURCHANINOV, T. JUNACKAJA, D. SATYBALDIN AND N. RUDCHENKO
Omsk state medical university, Omsk, Russia

Background: Infertility in marriage is an important medical and social problem affecting demographic foundations of society. In the structure of infertile marriages male factor according to WHO is 50%. Among the variety of reasons that lead to the reduction of sperm quality, attention is drawn to the high level of idiopathic infertility, which varies according to different authors from 30% to 75%.
Intent research. Evaluation the micronutritional status of patients with idiopathic male infertility in the form of Omsk.

Methods: Sixty-six men participated in the study, 66 of them with idiopathic form of male infertility, 30 men in the control group without the disease of the reproductive system.
We have analyzed the food status of patients and measured the level of micronutrients in the blood. The analysis is based on the qualitative composition of food products.

Results: In the group with infertility showed a reduction of vitamin A 0.23 μg/mL, in the control group – 0.56 μg/mL ($p < 0.005$); vitamin D group infertility 16.8 ng/mL, in controls – 32.8 ng/mL ($p < 0.001$); folic acid group with infertility 2.3 ng/mL, in controls – 16.4 ng/mL ($p < 0.0001$). In the group with infertility found selenium reduction: 18.9 μg/L, in the control group – 88.5 μg/L ($p < 0.01$). Average zinc content in a group of infertile 9.7 mmol/L, in control – 14.7 mmol/L ($p < 0.05$). These vitamins and minerals have a leading role in spermatogenesis. Reducing the level of vitamin E and C are not detected.

Conclusion: Micronutritional factor can be regarded as the main without another men' diseases with pathospermia. In addition, the presence of nutritional factor can be background aggravating condition with the presence of another causes of pathospermia.

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Are men ready to use thermal male contraception? Acceptability in two French populations: New fathers and new providers

M. AMOUROUX[1], R. MIEUSSET[2,3], R. DESBRIERE[4], P. OPINEL[5], G. KARSENTY[6], M. PACI[7,8], S. FERNANDES[9], B. COURBIERE[8,10] AND J. PERRIN[8,10]

[1]*Département Universitaire de Médecine Générale Faculté de Médecine de Marseille Aix-Marseille Université;* [2]*Université Toulouse III-Paul Sabatier, Human Fertility Research Group, Toulouse;* [3]*Andrologie-Médecine de la Reproduction, Hôpital Paule de Viguier, CHU de Toulouse, Toulouse;* [4]*Service de Gynécologie-Obstétrique, Pôle Parents Enfants, Hôpital Saint-Joseph, 26 Bd de Louvain, Marseille;* [5]*Centre Hospitalier Pays d'Aix, Aix en Provence;* [6]*Service d'Urologie et Transplantation Rénale, Aix Marseille Université, CHU La Conception, Marseille;* [7]*Aix Marseille Université, INSERM, GMGF UMR_S 910, Marseille;* [8]*AP-HM La Conception, Centre Clinico-Biologique d'Assistance Médicale à la Procréation CECOS, Marseille;* [9]*Laboratoire de Santé Publique, 20-27 bd Jean Moulin, Marseille;* [10]*Aix Marseille Université, CNRS, IRD, Avignon Université, IMBE UMR 7263, Marseille, France*

Background: Since the 1970s, international research has been actively pursued mainly on hormonal male contraception and at a less extent on thermal male contraception (TMC). Although the efficacy of TMC has been proven in a still limited population, its acceptability has not been studied in either potential users or in potential prescribers.

Methods: A cross-sectional descriptive multicenter study of 2 populations was designed to represent potential male users of TMC (new fathers, NF) and potential prescribers of TMC (new providers, NP) and conducted between November 2016 and February 2017.

The participants completed a 3-part survey to determine the i) socio-demographic profile of participants; ii) personal experience with contraception; and iii) knowledge, interest and preference for male contraception, particularly TMC. For NP only, the survey included a fourth part to evaluate the professional experience with male contraception.

Results: Participation rate: 51% for NF (305) and 34% for NP (300, with 97 men (male new providers, MNP) and 203 women (female new providers, FNP)). For NF and NP, TMC was known by 3% and 15%, respectively (including 26% of population of MNP and 10% of FNP, $p < 0.01$). After reading information on TMC, NF were significantly less ready to try TMC (29%) than NP (40%) ($p < 0.01$). The 3 main advantages of TMC for the NF were: "natural" (52%), "without side effects" (38%) and "non-hormonal" (36%). The main disadvantages were "lengthy wear time" (56%), "daily wearing of the undergarments" (43%), and "concern about possible discomfort" (39%).

Conclusion: Young male and female providers have a limited knowledge of male contraception, are keen for more information, and would generally prescribe TMC to their patients. The successful development of male contraception use would require distribution of better information to potential users and providers.

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Andrological microsurgery at the University of Debrecen

M. BENYÓ[1], J. DOCS[1], M. MOLNAR[1,2], G. DRABIK[1] AND T. FLASKO[1]

[1]*Department of Urology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary;* [2]*Department of Neurology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*

Background: Although microsurgery seems to be the most effective treatment modality for properly selected patients with azoospermia, varicocele or benign testicular lesions; its' widespread in Central Europe is slower. A possible explanation can be the higher cost of surgical equipment and longer learning curve of these procedures. Still there is no extra reimbursement for covering these extra costs for andrological microsurgery in most of the aforementioned countries as in case of laparoscopic interventions. This is the explanation why György Papp performed the first microsurgical vasoepididymostomy in Hungary in 1982 but the modern andrological microsurgery was launched only 27 years later by Zsolt Kopa in 2009. Till nowadays Zsolt Kopa is the most experienced in andrological microsurgery in our country. Matyas Benyo was the next person in Hungary who has performed modern andrological micro-TESE in 2013.

Objective: The aim of the retrospective analysis is to demonstrate the necessary initial steps and equipment to launch microsurgical procedures and to present the microsurgical procedures performed in the second most experienced microsurgical center in Hungary.

Methods: The basic microsurgical skills were learned at the Department of Operative Techniques and Surgical Research, Faculty of Medicine, University of Debrecen, Debrecen, Hungary (head Norbert Nemeth). During that 3 weeks long individually tutored and guided education basic suturing and knotting was learned on plastic gloves using 5/0–8/0 threads. Preparation skill was improved on chicken's thigh. Anastomosis of an artery is created on the same trainer first, and then all the steps above were further practiced on living rat. Final goal of the course was a successful kidney autotransplantation and vaso-vasostomy on the same animal. Further practical skills were obtained on a 6 months long practical education at the Centre of Andrology, Department of Urology, Semmelweis University (head Zsolt Kopa).

Results: Between October 2013 and April 2018 141 microsurgical procedures were performed at University of Debrecen Department of Urology by two surgeons (M.B.: $n = 118$, Gy.D.: $n = 23$). During that period 53 microsurgical varicocelectomies, 74 microsurgical testicular sperm extraction (micro TESE) procedures, 11 microsurgical epididymal sperm aspirations (MESA) and 3 microsurgical organ sparing testicular surgeries were performed. There were no severe intra- or postoperative complications linked to the procedure. One epididymitis and 7 varicocele reoccurrences were registered after micro-varicocelectomy, three epididymitises, one subcutaneous and one intratesticular haematoma formation occurred after microTESE surgeries. All the organ sparing testicular surgery cases had benign lesions on final histology. The long term outcome of the first 40 microTESE and first 8 MESA procedures have been assessed (October 2013–April 2016). We've started with conventional TESE in most of the cases of nonobstructive azoospermia, and after a prompt analysis with negative result we've proceeded to microsurgery resulting in lower micro TESE and better conventional TESE sperm retrieval rate: TESE success rate 79% (28/22); microTESE success rate: 40% (40/16). MESA was successful in 100% (8/8).

Conclusion: Microsurgery is safe and effective method which should be introduced in the everyday andrological practice of each experienced center, but it requires a longer learning curve to reproduce the outcome of the skilled microsurgeons.

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The levels of selected hormone and protein in serum and in prostate tissue homogenates in men with benign prostatic hyperplasia and metabolic disorders

M. LASZCZYŃSKA[1], K. GRZESIAK[1], A. RYŁ[2], I. BARANOWSKA-BOSIACKA[3], I. ROTTER[2], O. SIPAK[4], W. RATAJCZAK[1], M. PIASECKA[1] AND M. SŁOJEWSKI[5]
[1]Department of Histology and Developmental Biology, Pomeranian Medical University, Szczecin, Poland; [2]Department of Medical Rehabilitation and Clinical Physiotherapy, Pomeranian Medical University, Szczecin, Poland; [3]Department of Biochemistry and Medical Chemistry, Pomeranian Medical University, Szczecin, Poland; [4]Department of Obstetrics and Gynecology, Pomeranian Medical University, Szczecin, Poland; [5]Department of Urology and Urological Oncology, Pomeranian Medical University, Szczecin, Poland

Background: Benign prostatic hyperplasia (BPH) and metabolic disorders are important contributors to the health-related quality of life (HRQoL) of aging men. Many studies have dealt with the association between BPH and metabolic syndrome (MetS). This connection may result from the fact that changes in hormone levels and metabolic disorders predispose to BPH. The purpose of the study presented here was to assess the relationship between changes in the levels of selected hormones in serum and prostate tissue homogenate with regard to metabolic disorders in patients with diagnosed, surgically treated BPH.
Methods: The study involved a group of 154 men with a diagnosis of BPH, aged 52–75 years, who had undergone transurethral resection of the prostate (TURP). The patients were divided into two groups: with metabolic syndrome (MetS), and without MetS, diagnosed on the basis of the International Diabetes Federation (IDF) 2005 criteria. The men included in the study were patients at the Clinic of Urology and Urological Oncology, Pomeranian Medical University in Szczecin, Poland. The serum levels of the hormones -total testosterone (TT), free testosterone (FT), insulin (I), dehydroepiandrosterone sulfate (DHEAS), estradiol (E2), luteinizing hormone (LH), and sex hormone binding globulin (SHBG) and insulin-like growth factor-1 (IGF-1) – were determined using the ELISA method. Prostate tissue sections obtained from the patients during transurethral resection of the prostate were frozen in liquid nitrogen. We determine the levels of the same hormones.
Results: There was a statistically significant difference between the groups in terms of serum SHBG levels, but not in the prostate tissue SHBG levels. A similar relationship was observed with regard to IGF-1, the serum levels of which were significantly higher in patients with MetS. MetS had an effect on the ratio of hormone levels in serum to their levels in the prostate tissue. Correlations between the levels of biochemical parameters and the levels of hormones in serum and the prostate tissue of BPH patients with and without MetS demonstrate that serum SHBG levels correlated weakly with waist size and TG levels.
Conclusion: The occurrence of MetS in BPH patients was associated with changes in the levels of hormones and proteins. These changes, however, were not always equivalent to changes in the levels of these parameters in prostate tissue. It should also be mentioned that MetS in BPH patients had an influence on a quantitative balance between the levels of SHBG in serum and prostate tissue.

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M-TESE – our experience 2012–2018

J. K. WOLSKI[1,2], G. KAPUŚCIŃSKI[1], K. KOZIOŁ[1], R. WYBAN[1], K. GAWRON[1], A. STRZALKOWSKA[1], M. IGNASZAK[1], K. PAPIS[1], M. IZDEBSKA-KSIĄŻEK[1], M. RADZIEWICZ-PIGIEL[1], E. STACHOWIAK[1], K. KLIMCZEWSKA[1], K. KUBIAK[1], K. LEŚNIEWSKA[1], S. TRUBACZ[1], H. MARSZAŁ[1], M. LIGAJ[3], J. OSTROWSKA[4], J. FARYNA[4], D. STARCZEWSKI[1], A. AUGUSTYNIAK[1], J. URBAŃCZYK[1], M. CHRUŚCIEL[1], A. SUCHECKA[1], B. BIARDA[1] AND P. LEWANDOWSKI[1]
[1]Fertility Clinic Novum, Warsaw, Poland; [2]Department of Urology-Oncology, Maria Sklodowska-Curie Memorial Cancer Center, Warsaw, Poland; [3]Department of Pathology, Maria Sklodowska-Curie Memorial Cancer Center, Warsaw, Poland; [4]Center of Pathology, Warsaw, Poland

Background: Microsurgical Testicular Sperm Extraction (M-TESE) first reported by Schlegel (1999), allowed incorporated into the assisted reproduction In Vitro Fertilization-Intracytoplasmic Sperm Injection (IVF-ICSI) the next group of men with nonobstructive azoospermia, with previous negative biopsies.
Methods: This is our experience during 2012–2018. M-TESE biopsies were performed among the high selected, nonhomogeneous group of 217 men with nonobstructive azoospermia; age 17–60 (mean 33 years). For 35 men M-TESE was the first biopsy (28 – Klinefelter syndrome 47 XXY; 2 – DSD 46,XY; 1 – post-TURED; 2 – YdelAZF C, 2-testicular hypoplasia), for 177 – second, for 5-third.
Results: When low testosterone level was noticed, patients before biopsy had hormonal stimulation of spermatogenesis (6–12 weeks): androgen + antiestrogen (Adamopoulos, 1993) with the addition of vasodilator and trace elements with vitamins, in some cases injections of LH/FSH were done. The levels of LH, FSH and testosterone in the blood serum were controlled during therapy. All M-TESE procedures were performed under general anesthesia, as the day-case-surgery. The operating microscopes Seiler Evolution XR6 (94), Carl Zeiss S7 (6), Leica M860 2 × 2 (117) were used (magnification 20–25×). From both gonads the testicular tissue were collected from 3 levels (Weidner, 2012). The current evaluation with following conservation at liquid nitrogen (−196°C) for the future IVF were done immediately after surgery and the routine histological study (conservation in Bouin's fluid, Johnsen score) in each case was done. Sperm were found in 57/187 patients (30.5%), in subgroups: 47,XXY 4/28 (14%); no sperm were

noticed in DSD 46,XY (2). 70 IVF-ICSI procedures have been done, 20 pregnancy and 4 miscarriage were noticed. Until then, 16 children were born, 1 pregnancy is observed. M-TESE, testicular biopsy using the operating microscope, increases the chance to find sperm in men with nonobstructive azoospermia, finally allows incorporated them into the reproduction protocol – IVF-ICSI.

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Sperm chromatin maturity in infertile and healthy normozoospermic men

M. PIASECKA[1], K. GILL[1], M. KUPŚ[1,2,3], A. ROSIAK[1,3], J. JAKUBIK[1], Ł. PATORSKI[1,4], R. KURZAWA[3,5], M. FRĄCZEK[6] AND M. LASZCZYŃSKA[1]
[1]Department of Histology and Developmental Biology, Pomeranian Medical University in Szczecin, Poland; [2]Department of Urology an Oncological Urology, Regional Specialist Hospital in Szczecin, Poland; [3]VitroLive Fertility Clinic in Szczecin, Poland; [4]Department of Gynecology, Endocrinology and Gynecological Oncology, SPSK1, Pomeranian Medical University in Szczecin, Poland; [5]Department of Procreative Health, Pomeranian Medical University in Szczecin, Poland; [6]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland

Background: The etiopathogenesis of male infertility is multifactorial and in many cases has a molecular background. Therefore, the routine seminological diagnostic tests are an insufficient tool in the assessment of male fertility. There is a need to introduce supplementary and more sophisticated assays to evaluate sperm molecular biomarkers and discover their relations to reproductive outcomes. Sperm chromatin fragmentation (SDF) is being recognized as a predictive factor for the spermatozoa's ability to fertilize an oocyte, embryo development, implantation and achieving a pregnancy. Moreover, SDF appears important for health of the offspring. Currently, developmental failure in the spermatogenic remodeling process, uropathological, genetic, environmental and lifestyle factors are involved in etiology of SDF.
Methods: Our study was designed to determine SDF in infertile subjects with abnormal semen characteristics (n = 182) as well as in healthy men with normal standard semen parameters (n = 114). Furthermore, we decided to establish the correlation between standard semen parameters and SDF. Traditional semen characteristics of ejaculated spermatozoa were evaluated according to recommendations of Worth Health Organization (WHO, 2010) while the extent of SDF was assessed using sperm chromatin dispersion (SCD) test (Halo test).
Results: Statistically less subjects (17.03%) with normal levels of SDF (≤15% sperm cells – correlation to high fertility potential) and statistically more subjects (35.17%) with high level of SDF (>30% sperm cells – correlation to low fertility potential) were found in infertile men compared to normospermic men (57.90%, 5.26% respectively). The study groups did not differ in intermediate level of SDF (16–30% sperm cells – correlation to moderate fertility potential) (Chi^2 test). The higher proportion of spermatozoa with DNA fragmentation ($p < 0.000001$) was noted in infertile men (median 23.00%, range 7.00–76.00) than in normozoospermic men (median 14.00% range 4.00–42.00) (Mann–Whitney U test). Sperm chromatin damage negatively correlated with sperm concentration (rs = −0.289), total number of spermatozoa (rs = −0.243), sperm morphology (rs = −0.533), sperm progressive motility (rs = −0.554), and eosin-negative- and Hos-test-reactive sperm cells (rs = −0.492, rs = −0.535 respectively) but positively correlated with teratozoospermia index (rs = 0.433).
Conclusion: Our study discovered a significant difference in the prevalence and median value of SDF between infertile and normozoospermic men as well as the existence of a relationship between SDF and conventional sperm characteristics. The results obtained suggested that abnormal standard sperm parameters coexisted with molecular chromatin abnormalities. The SDF measured by the sperm chromatin dispersion should be considered as a complementary and useful tool in clinical prediction of male infertility. The used test have shown potential to discriminate infertile men from healthy normozoospermic men. There is no doubt that verification of sperm chromatin integrity is essential in the correct therapeutic management which should lead to the restoration of the natural fertility of the patient or enrollment him into assisted reproduction technology procedures, or too exclude him from this procedures due to the high level of DNA damage.

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Analysis of selected parameters of oxidative stress and sperm apoptosis in semen of men exposed to both external or internal genital heat stress

M. FRĄCZEK[1], M. BUDZINSKA[1], M. KAMIENICZNA[1], M. PIASECKA[2], A. HAVRYLYUK[3], K. GILL[2], L. WOJNAR[4], L. GRZESKOWIAK[5], M. KUPS[6], J. NAKONECHNYY[7], A. NAKONECHNYY[8], V. CHOPYAK[3] AND M. KURPISZ[1]
[1]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland; [2]Department of Histology and Developmental Biology, Pomeranian Medical University, Szczecin, Poland; [3]Danylo Halytsky Lviv National Medical University, Department of Clinical Immunology and Allergology, Lviv, Ukraine; [4]Clinic of Urology and Oncological Urology, Poznan University of Medical Sciences, Poznan, Poland; [5]Certus Private Hospital, Poznan, Poland; [6]Department and Clinic Urology and Urological Oncology, Pomeranian Medical University, Szczecin, Poland, VitroLive Fertility Clinic, Szczecin, Poland; [7]Department of Urology, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine; [8]Department of Pediatric Surgery, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Background: The molecular mechanism by which scrotal hyperthermia compromise the fertilizing potential of human germ cells is complex and multifactorial, and it remains unclear.
Objective: To improve the understanding of the pathophysiology of male subfertility/infertility caused or complicated by the most controversial male infertility entities such as varicocele and cryptorchidism, we simultaneously evaluated a set of conventional (standard semen analysis)

and non-conventional sperm parameters, including oxidative stress indicators (total antioxidant capacity, catalase, superoxide dismutase, malondialdehyde, mitochondrial superoxide anion) as well as apoptotic markers (phospholipid scrambling, phosphatidylserine externalization, mitochondrial transmembrane potential, DNA fragmentation).
Methods: The research was conducted in a group of 150 men at reproductive age. The participants have undergone routine fertility work-up including medical history and physical examination. The studied male cohort was classified into one of five the following study groups: infertile patients with varicocele, infertile patients with cryptorchidism in childhood, professional drivers, infertile patients not exposed to continuous hyperthermia and fertile individuals as control.
Results: The strong deterioration in routine sperm parameters was demonstrated in men exposed to both external and internal thermogenic factor; and this effect was the strongest in the group with cryptorchidism. Analysis of subcellular sperm parameters have revealed an apoptosis-induced phenotype in all the hyperthermic groups. However, the significant increase in the percentage of spermatozoa with impaired mitochondrial transmembrane potential was observed only in the group of infertile patients with history of cryptorchidism. Additionally, an enhanced oxidative stress response visible as the increased number of live sperm producing mitochondrial superoxide anion and the decreased total antioxidant capacity was demonstrated. Moreover, the percentage of TUNEL-positive sperm was negatively correlated with total antioxidant capacity in the group of varicocele.
Conclusion: Our preliminary results support previous premises that prolonged testicular/scrotal hyperthermia leads to the strong deterioration of semen quality. The induction of intrinsic, mitochondrial-dependent sperm apoptosis can contribute as an important pathogenic mechanism by which genital heat stress can alter a status of human germ cells. Oxidative stress is primarily involved in this process.
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Multifarious clinical presentations of male hypogonadotropic hypogonadism and their reproductive challenges

D. PALANISAMY, S. FAIZI AND M. ASHRAF
CRAFT Hospital and Research Centre, Kodungallur, Kerala, India

Background: The incidence of hypogonadotropic hypogonadism (HH) is rare. HH can be broadly classified as congenital and acquired. Medical hormonal therapy is being successfully used for the treatment of men with HH and infertility, however the pregnancy outcomes in these couples has been noted to have divided results.
Objective: To evaluate the reproductive outcomes in infertile men with HH.
Methods: 32 infertile males with HH and normal female partners over a period of 6 years were included in the study. Detailed evaluation in terms of etiology, investigations such as semen analysis, hormonal profile, sperm DNA fragmentation index and sperm FISH were sent for indicated cases. Reproductive outcomes including natural conception, conception following intra uterine insemination (IUI), IVF/ICSI with ejaculate sperms and surgically retrieved sperms by testicular sperm aspiration (TESA) and micro dissection testicular sperm extraction (MTESE) was noted.
Results: Out of 32 couples, 5 conceived naturally and 3 conceived following intrauterine insemination (IUI). Out of the 24 cases requiring IVF/ICSI, in 16 couples ejaculate sperms sufficed, while 8 patients needed surgical sperm retrieval (3 cases – TESA and 5 cases – MTESE followed by ICSI).
Conclusion: Patient tailored approach in terms of clinical presentation and selection of appropriate investigations along with individualisation of therapy (medical/surgical) can result in good pregnancy rates in patients with infertile males with HH.

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Role of CAG repeat length polymorphism of androgen receptor in male-to-female transsexualism: a systematic review and meta-analysis

A. BARBONETTI, S. D'ANDREA, A. MARTORELLA, S. FRANCAVILLA AND F. FRANCAVILLA
Medical Andrology Unit, Department of Life, Health and Environment Sciences, University of L'Aquila, L'Aquila, Italy

Background: Transsexualism is characterized by lifelong discomfort with the assigned sex and a strong identification with the opposite sex. The cause of transsexualism is unknown, but it has been suggested that an aberration in the early sexual differentiation of various brain structures may be involved. A genetic contribution has been hypothesized, and genes involved in the biosynthesis and activity of sex steroids might be good candidates. Some studies have associated Male to Female (MtF) transsexualism to longer CAG repeats of Androgen Receptor (AR), leading to lower androgen activity during dimorphic sexual differentiation of the brain.
Objective: As this topic still remain controversial, the aim of the present study is to clarify the relationship between MtF transsexualism and CAG repeat polymorphism of AR through a systematic review of literature with meta-analysis of available case-control studies.
Methods: Case-control studies reporting mean ± standard deviation (SD) of AR CAG repeat length and/or odds ratios (ORs) for long CAG repeat (>19 repeats) were analyzed. The Cochrane Chi-square (Cochrane *Q*) statistic and the *I*-square test were used to analyze heterogeneity and, in the absence of heterogeneity, data were combined using fixed effect models. Publication bias was graphically explored through funnel plots, and Duval and Tweedie's "trim-and-fill" analysis was used for its correction.
Results: The selection process yielded five studies with eligibly criteria for analysis, that gave information on 655 MtF transsexual subjects and 1100 heterosexual males. Studies were of good quality, as evaluated by the New Castle Ottawa scale. The overall standardized mean difference

(SMD) showed no significant differences in AR CAG repeat length between the two groups (pooled SMD 0.10; $p = 0.06$; I-square = 0%). Similarly, MtF transsexual subjects had an increased, but not significantly higher, risk to exhibit long AR CAG repeats (pooled OR 1.19; $p = 0.12$; I-square = 0%). The symmetrical shape of funnel plot suggested the absence of significant publication bias among the studies investigating the difference in CAG repeat mean length. Although asymmetry in funnel plot of ORs for long CAG repeats suggested a possible publication bias, its correction by the "trim-and-fill test" (identifying two putative "missing studies" on the left side of the distribution), did not significantly change the crude result (adjusted pooled OR: 1.14; $p = 0.22$).

Conclusion: This meta-analysis demonstrates the absence of statistically significant differences in the AR CAG repeat polymorphism between MtF transsexual subjects and controls. Further studies are warranted to better clarify the possible contribution of genetic factors in gender dysphoria.

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The hemizona binding assay on human sperm with fragmented DNA

M. MAZILINA[1], E. KOMAROVA[2], I. MEKINA[2], E. LESIK[2], A. GZGZYAN[2] AND V. BARANOV[1,2]

[1]Saint-Petersburg State University, Saint Petersburg, Russia; [2]The Research Institute of Obstetrics, Gynecology and Reproductology named after D.O.Ott, Saint Petersburg, Russia

Background: The mammalian oocyte extracellular matrix known as the zona pellucida (ZP) acts as a barrier to accomplish sperm fusion with the female gamete. Although penetration of the ZP is a limiting event to achieve fertilization, this is one of the least comprehended stages of gamete interaction. The hemizona assay (HZA) has been developed as a diagnostic test for the tight binding of human spermatozoa to the human ZP to predict fertilization potential.

Methods: In the present study the two matching hemizona halves are functionally equal surfaces allowing controlled comparison of binding from a fertile control (sperm donors) vs. a test sample, with reproducible measurements of sperm binding obtained from a single oocyte. Semen samples were prepared by density gradient centrifugation and swim-up methods. After incubation, oocytes were evaluated for numbers of tightly bound sperm. The ratio between test and control sperm was calculated. According to the data of other researchers we assessed <35% bound spermatozoa as a low binding. We evaluated the sperm DNA fragmentation measured by the deoxynucleotidyl transferase-mediated dUTP nick end labeling assay (TUNEL) in 213 spermatozoa after tight-binding with ZP.

Results: Among 213 tightly bound spermatozoa 27 were with DNA fragmentation (12.7%).

Conclusion: Thus, we can assume that the fraction of bound spermatozoa after density gradient and swim-up consisted mainly of motile and morphologically normal sperm, but some of them carried DNA breaks. These spermatozoa are able to bind with ZP and could participate in fertilization.

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Avanafil enhances the outcome of a surrogacy program

I. GIAKOUMAKIS[1], D. DAFNIS[1], F. DIMITRIADIS[2], A. ZACHARIOU[3], A. KARAGIANNIS[3], P. TSOUNAPI[4], I. GIANNAKIS[3], S. SKOUROS[3], P. LANTIN[5], M. MARTINEZ[5], A. TAKENAKA[4] AND N. SOFIKITIS[3]

[1]Mediterranean Fertility Institute, Chania, Crete, Greece; [2]1st Urology Department, Aristotle University, Thessaloniki, Greece; [3]Department of Urology, University of Ioannina, Ioannina, Greece; [4]Department of Urology, Tottori University, Yonago, Japan; [5]Department of Urology, Urology Institute of Manila, Philippines

Objective: Recent report has suggested a beneficial effect of sildenafil or vardenafil on semen quality (Curr Pharm Des. 2009;15:3506. Br J Urol Int 2010;106:1181). We evaluated the effect of avanafil administration on embryonic development and the pregnancy rate in a surrogate program. Considering that in a surrogacy parenthood program global legislation requires at least one of the intended parents to provide (in most cases the male partner) his/her own genetic material, it is imperative to discover new pharmaceutical agents to increase semen quality in order to achieve high pregnancy rates in the surrogate women.

Methods: Twenty-five couples (Group A) were selected. The female partner could not produce oocytes of appropriate quality for assisted reproductive technology (ART). Thus donor oocytes were used. Donor oocytes were collected and processed for ooplasmic injections of spermatozoa (ICSI techniques) recovered from the male partner. In the above-selected couples, all male participants were oligo-astheno-teratospermic and pregnancy was not achieved after transferring the generated blastocysts (using ART techniques) into the surrogate females. Subsequently each male participant received avanafil (25 mg × 2/day; taking into consideration the duration of the half-life of avanafil) for 90 days. Within a month, following the completion of the above 90-day-treatment by each man, all couples participated in a new ART program (new ICSI procedures).

Results: Within group A prior to avanafil treatment, 151 oocytes were injected and 14 blastocysts were developed. After avanafil treatment 161 oocytes were injected and 46 blastocysts were developed. The percentage of motile spermatozoa (%) and the percentage of developed blastocysts (after 106 h of culture, post-ICSI) to the total number of injected oocytes was significantly larger after the treatment with avanafil compared to the respective values prior to the avanafil treatment. Seven out of the 25 surrogate females achieved pregnancy after the 2nd ART procedure.

Conclusion: Our results suggest that avanafil treatment results in the generation of embryos (post-ICSI) with significantly higher potential for in vitro development up to the blastocyst stage probably due to amelioration of defects in genetic or epigenetic sperm factors.

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The impact of sperm DNA fragmentation on male fertility and ICSI outcome in cases of donated oocytes

S. ANTONOULI[1], A. PAPATHEODOROU[2], Y. PANAGIOTIDIS[2], S. PETOUSIS[2], S. A. NOTTOLA[3], G. MACCHIARELLI[1], M. G. PALMERINI[1], S. BIANCHI[1] AND Y. PRAPAS[2]

[1]Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy; [2]Iakentro Fertility Centre, IVF Laboratory, Thessaloniki, Greece; [3]Department of Anatomy, Histology, Forensic Medicine and Orthopedics, La Sapienza University of Rome, Rome, Italy

Background: In Assisted Reproductive Technologies, a modern aspect of the so called 'male factor' that causes infertility is the high sperm DNA fragmentation (SDF) [1]. The conventional semen analysis provides a limited prediction of the male fertility potential and the right diagnosis of infertility is not always ensured [2].

Objective: Due to this recent attention in forecast accuracy of male infertility, this study was designed to evaluate whether the assessment of the sperm nuclear DNA integrity has a predictive value for the clinical outcome in oocyte donation cycle and whether it is correlated with the semen parameters.

Methods: A total of 150 recipients (<50 years old), matched with their donors were included in the study. Sperm semen sample obtained from male partners of couples who underwent ICSI for an infertility treatment with donated oocytes were assessed by conventional sperm analysis. SDF was evaluated by Halosperm kit, a sperm chromatin dispersion test (SCD). The relations between DNA damage and male epidemiological factors (age, height, weight), standard semen parameters (concentration, total and forward motility, morphology), embryological and clinical parameters (fertilization rate, total blastocyst number, good quality blastocyst number, clinical pregnancy) were analyzed.

Results: DNA fragmentation index (DFI) was positively correlated with advanced male age ($r = 0.23$, $p < 0.05$) and negatively correlated with total sperm and forward motility ($r = -0.29$, $r = -0.27$, respectively; $p < 0.05$). DFI was not significantly correlated with pregnancy outcome in oocyte donation cycles ($r = -0.05$, respectively; $p > 0.05$). Choosing good quality blastocysts, it was detected a trend to develop high quality embryos when DFI was lower ($r = -0.20$, respectively; $p = 0.08$).

Conclusion: Although SDF does not significantly affect the IVF outcome in oocyte donation cycles, in cases of advanced paternal age a high DFI result may adversely affect the final outcome.

References: [1] Gat, I., et al. Sperm DNA fragmentation index does not correlate with blastocyst aneuploidy or morphological grading. PloS one 12 (2017): e0179002.

[2] Petousis, S., et al. Fluorescence in situ hybridisation sperm examination is significantly impaired in all categories of male infertility. Andrologia 50 (2018): e12847.

P140

Human live sperm morphology assessment by digital holographic microscope

M. KAMIENICZNA[1], M. FILIPIAK[2], A. MOCZULSKI[2], E. STACHOWSKA[2] AND M. KURPISZ[1]

[1]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland; [2]Department of Metrology and Measurement Systems, Institute of Mechanical Technology, Faculty of Mechanical Engineering and Management, Poznan University of Technology, Poznan, Poland

Background: Sperm morphology evaluation is a part of standard routine semen analysis. The dried, fixed and stained smears are examined using light microscopy to determine the percentage of morphologically normal spermatozoa. There are many computerized methods to investigate stained sperm images. Computer analysis is more quantitative and reproducible than manual analysis. Different methods of staining can, however cause numerous artifacts. The last hope for reducing false morphological assessment lies in live cells investigation. High-magnification systems appear to improve morphological investigations (e.g. motile sperm organelle morphology examinations-MSOME). Distinct possibilities can be provided by digital holography (DH). Using digital holographic microscopy (DHM) we can perform non-invasive and quantitative analysis of morphometric parameters of human spermatozoa. With DH the whole three-dimensional volume can be reconstructed from a single image (the hologram) without mechanical realigning of the optical system. All spermatozoa present in the volume can be investigated.

Objective: The aim of this study was to identify morphometric profiles of human live sperm cells from healthy fertile men and infertile men by DHM.

Methods: The morphology of human semen was studied using a digital holographic microscope DHM-T1000 (Lyncée Tec SA, Lausanne, Switzerland) and the software Koala V4. Ten ejaculates from fertile and ten from infertile men were evaluated. The investigation was carried out with intact spermatozoa and prepared by standard swim-up and Percoll techniques, which allows for the selection of the most viable and morphologically normal spermatozoa. The values of head length, width, height, length of mid-piece and tail were measured for each spermatozoon, as well as the presence of vacuoles. Further, the motility was assessed. For fertile patients intact sperm and separated spermatozoa by swim-up and Percoll gradient were examined. For infertile men the intact spermatozoa were studied.

Results: The sperm head parameters differed between the groups investigated (fertile and infertile). In the group of infertile men we have observed a wider range of values for height and length and a different distribution than for fertile men. Statistical comparisons of the sperm morphology revealed differences between samples depending on the method of sperm separation (swim-up, Percoll 47%, Percoll 90% sperm fractions). The study of the presence of sperm head vacuoles showed a strong correlation between the presence of vacuoles and the type of motility of the spermatozoa investigated. A lower number of vacuoles was observed in spermatozoa with a progressive type of motility.

Conclusion: We conclude that the DH method enables more accurate, quantitative and objective morphological measurements of live unlabeled sperm cells. We can precisely monitor the quality of sperm cells after swim-up or Percoll selection. By means of the phase-contrast holographic microscopy we may also objectively and quantitatively characterize sperm head vacuoles. The main advantage of DHM is the possibility to investigate all sperm cells in the certain volume of the sample tested and not only in single plane.

P141

Flaccid penile acceleration as a screening tool for arterial element of erectile dysfunction

M. W. RAGAB[1], A. S. ALAHWANY[1], H. ALAZZIZI[2] AND I. FAHMY[1]

[1]Department of clinical and surgical Andrology, Cairo University, Egypt; [2]Radiology Department, Cairo University, Egypt

Background: Real-time color Doppler/duplex ultrasound (CDDU) after intra-corporal injection (ICI) with vasoactive substance to induce erection is considered a minimally invasive method to evaluation of penile hemodynamics assess the vascular element of erectile dysfunction (ED). Evaluation of penile hemodynamics is beneficial in differentiation between psychogenic and vasculogenic ED, evaluation of post-traumatic ED, ED not responding to medical treatment and subjective documentation of vasculogenic ED. However, ICI may be complicated with priapism, penile pain and local complications at the site of injection. Arterial wave acceleration measured in the flaccid state i.e. flaccid penile acceleration (FPA), has been found previously to be correlated with ICI induced peak systolic velocity (PSV). Additionally, ICI induced PSV ≤30 cm/s was previously found to be an indicator for arteriogenic ED. We hypothesize that mean FPA can predict low ICI induced PSV and therefore minimize the need for ICI and its related complications.

Objective: This study aimed to implement a specific and sensitive FPA cut-off value to be used for screening the arterial component of ED defined by ICI induced PSV ≤30 cm/s.

Methods: This prospective study comprised 100 self-reported ED patients who presented to the Andrology clinic of Cairo University and 30 control subjects, in the period between April 2014 and December 2017. Medical history taking, clinical and laboratory evaluation was done. Mean FPA was calculated from both cavernosal arteries at the level of penoscrotal junction using a 10 MHz linear array transducer of CDDU device (model: MCMD01AA, Siemens Medical Solution, NY, USA). Followed by dynamic measurement of peak-systolic velocity (PSV), end-diastolic velocity (EDV) at 5, 10, and 20 min intervals after induction of erection by intra-corporal injection of 20 μg of PGE1. Receiver operating curve (ROC) was used to determine FPA cut-off value.

Results: A positive correlation between FPA and ICI induced PSV ($r = 0.446$, $p = 0.001$) was observed. A PSV ≤30 cm/s was found in 44/100 of ED patients. Mean FPA for those patients was 1.1 ± 0.7 m/s^2, which was found to be significantly lower than controls (mean FPA = 3.3 ± 1.4 m/s^2, $p = 0.001$). A FPA value ≤1.26 m/s^2 was found to be predictive of PSV ≤30 cm/s (sensitivity = 77.3%, specificity = 73.2%, positive predictive value = 69.4% and negative predictive value = 80.4%, AUC = 0.8).

Conclusion: We concluded that FPA shall not be considered as a reliable tool for screening of arterial component of ED.

P142

Patients attitude to Erectile Dysfunction in 2 central-eastern European Centers

J. GIBA[1,2], S. GÁSPÁR[3], T. WENINGER[1,4] AND Z. KOPA[1]

[1]Andrology Centre, Department of Urology, Semmelweis University, Budapest, Hungary; [2]Semmelweis University, Faculty of Medicine, Budapest, Hungary; [3]Department of Urology of University of Medicine and Pharmacy of Târgu Mureș/Marosvásárhely, Romania; [4]Jahn Ferenc Teaching Hospital, Department of Urology, Budapest, Hungary

Background: Erectile dysfunction (ED) affects approximately half of men over the age of 40 years. More than half of the affected males failed to discuss the problem with medical doctors. The introduction of Phosphodiesterase type-5 inhibitors (PDE5i) resulted in an increasing public awareness of ED. Theoretically increasing patient's awareness should lead to increased number of patients seeking medical care. As medical students during clinical practice we evaluated ED patient's awareness in 2 central-eastern European Centres.

Methods: In an international cross-sectional case series study patients seeking medical treatment on ED were asked about the duration of sexual dysfunction before visiting an andrologist in 2 Andrology Centres. Questionnaires focused also on the attitudes: with whom they discussed about ED, patient's awareness about treatment options, or have they ever used whether proprietary drugs or unofficial, widely promoted "potency enhancer" products. Before appropriate medical workup used drugs were evaluated including efficacy, treatment satisfaction and side effects. The survey was started in January 2018, preliminary data are reported.

Results: 74 consecutive patients affected by erectile dysfunction (ED) were interviewed. The mean age of the patients was 54.55 years (24–79 years). Mean duration of erectile dysfunction complaints before seeking andrological treatment was 3.88 years (0.2–10). 45.95% of the patients haven't discussed about his ED neither with partner or friends nor with medical doctors. 64 (86.49%) of the patients have ever heard about official PDE5i medications before with 121 substance records (83 sildenafil, 21 tadalafil, 10 vardenafil and 7 avanafil). 28 (43.75%) of them have tried at least one of these drugs before medical consultation, 49 substance records contained 29 sildenafil, 10 tadalafil, 6 avanafil and 4 vardenafil. On a 1–4 Grade scale was used to evaluate patient's satisfaction, where 1 means dissatisfaction, 2- weak, 3- moderate and 4- good satisfaction. The mean patient satisfaction grade was found similar for all substances, ranging between 2.5 and 3.0. 11 patients (39.29%) reported any side effect, in the frequency of the appearance: flushing, nasal congestion, headache,

epigastric discomfort, xerostomia and visual side effects. 53 (71.62%) of the enrolled patients was informed about non-proprietary potency increasing drugs, 8 (15.09%) of them have even tried it. The most popular non-proprietary potency increasing drugs were: Kamagra, Dragon Power, Man Pride, Liderin and Potemix. The mean patient satisfaction grade was found 2.3. Side effects were reported in 25% of the cases.

Conclusion: Interestingly, patients consulted andrologist almost after 4 years of ED duration. Half of the affected men have never discussed the problem with anyone. Almost 90% of the patients have heard about PDE5i medications and half of them have even tried at least one of them. The awareness of the non-proprietary potency increasing drugs is lower, 72% of the patients have ever heard about these products, and 15% have even tried any of them. However, the relatively low patient number (74) does not allow to draw significant conclusions, but we suppose, that even this initial stage interesting information can be collected about patients attitudes to ED in our region.

P143

Leptin concentration in seminal plasma of infertile men and relationship to semen parameters and some hormonal levels

E. NADA[1], M. E. TAIEB[2], A. E. SAIED[3], H. IBRAHIM[4] AND E. HEGAZY[4]

[1]Dermatology, Venereology and Andrology Department, Faculty of Medicine, Sohag University, Egypt; [2]Dermatology, Venereology and Andrology Department, Faculty of Medicine, Aswan University, Egypt; [3]Clinical and Chemical Pathology Department, Qena Faculty of Medicine, South Valley University, Egypt; [4]Dermatology, Venereology and Andrology Department, Qena Faculty of Medicine, South Valley University, Egypt

Background: Leptin is a 167 – amino acid polypeptide hormone, identified in 1994 by positional cloning in the mouse and human. This hormone is secreted by the white adipose tissue in proportion to body energy (fat) stored. Leptin functions as a satiety factor in the regulation of body weight. So far, many studies have pointed to a direct role of leptin in the control of male reproductive function. However, in contrast to its well proven effects in female fertility.

Methods: One hundred male partners from infertile couples were included in the study. Based on clinical examination, spermiogram was divided into five groups: 20 men with azoospermia, 20 men with oligozoospermia, 20 men with oligoasthenoteratozoospermia, 20 men with normozoospermia. Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), inhibin and testosterone and leptin were measured.

Results: After adjustment for body mass index, there was a negative correlation between serum levels of leptin and inhibin B, total testosterone ($r = -0.189$, $p = 0.009$ and $r = -0.250$, p 0.001 respectively) but there was no correlation between leptin and classical sperm characteristics.

Conclusion: Our results therefore demonstrate a link between leptin and testicular function, independently of FSH and LH, possibly involving testosterone through a regulation of Leydig cell function.

P144

Sperm DNA fragmentation index (DFI) does not increase with increasing number of treatment cycles

A. PRAHL, A. GIWERCMAN, M. BUNGUM, A. STENQVIST, S. MALIC VONCINA AND Y. LUNDBERG GIWERCMAN

Molecular Reproductive Medicine, Department of Translational Medicine, Lund University, Malmö, Sweden

Background: Sperm DNA strand breaks, measured as DNA fragmentation index (DFI) by sperm chromatin structure assay (SCSA) is a clinically useful marker of male infertility, and a couple's chance of pregnancy. However, it is still unclear if high DFI is more common in men going through many IVF treatment cycles compared to those achieving pregnancy in the first.

Objective: We wanted to investigate if men undergoing numerous IVF/ICSI cycles had higher amount of sperm DNA strand breaks than those entering first treatment cycle, indicating selection of men with high level of DNA damage in repeated treatment failures.

Methods: Prospective, clinical study, in which $n = 2968$ men were consecutively enrolled during the period 2007-2018, at Reproductive Medicine Centre, Malmö, Sweden. Sperm samples were collected and analyzed by SCSA before each cycle. Analysis of the flow cytometric data was carried out using dedicated software.

Results: In the first cycle 2460 DFI values, second cycle 1402 DFI values, third cycle 755 DFI values, fourth cycle 190 DFI values, and in cycles 5–7 87 DFI values were available for analysis. The mean DFI value for the first, second and third cycles were 16%, respectively. In the fourth cycle the mean DFI was 19%, and the 5th–7th 17%. There was no statistically significant difference in DFI between the cycles (p trend = 0.058). When IVF and ICSI treatment were treated separately, the same pattern was seen In the ICSI-group even a small statistically significant decrease of DFI from cycle 1 to cycle 3–4 was observed.

Conclusion: Men who have undergone many IVF/ICSI are not presenting with higher DFI than those who enter the assisted reproduction treatment.

P145

Inhibin-B and FSH are the best predictors of male infertility

K. JANKOWSKA AND W. ZGLICZYŃSKI

Center of Medical Postgraduated Education, Department of Endocrinology, Bielanski Hospital, Warsaw, Poland

Objective: Sperm cells are produced in the testes by the Sertoli cells. These cells produce Inhibin B and they are stimulated by FSH. Can Inhibin B be a good marker of spermatogenesis? Should we use other marker (FSH?, testosterone?)

Methods: Design: Prospective study. Patients: We examined 28 patients with infertility. Techniques: Semen analysis and hormonal analysis was performed. Semen analysis

was performed according to World Health Organization guidelines (WHO 2010). Hormone analysis include: FSH (follicle stimulating hormone), LH (luteinizing hormone), testosterone, prolactin, TSH and inhibin B. We analysed the dependencies between semen parameters and hormones, especially inhibin B.

Results: The sperm count was significantly and positively correlated with Inhibin B ($r = 0.4$, $p < 0.0001$). The Inhibin B was negatively correlated with FSH ($r = -0.6$, $p < 0.0001$).

The lower was the concentration of inhibin B, the lower was the number of sperm in the semen. There was also a relationship between seminogram and FSH – the higher was the FSH, the lower was the number of sperm. There was no relationship between the number of sperm and the concentration of LH, testosterone, TSH, prolactin.

Conclusion: It seems that we can use the value of inhibin B and FSH to assess the intensity of spermatogenesis. The decreased concentration of inhibin B correlates with the number of sperm (the lower the concentration of inhibin B the lower the efficiency of spermatogenesis) and with FSH (the higher FSH, the lower the sperm count). High levels of FSH and reduced levels of inhibin B clearly indicate impairment of spermatogenic function in addition to the testes. The concentration of testosterone is not good predictor of spermatogenesis. (Inhibin B and testosterone are produced from different types of cells in the testis).

P146

Extra-corporeal trans-septal penile prosthesis implantation for extreme cases of corporeal fibrosis (Shaeer's Implantation)

O. SHAEER[1] AND K. SHAEER[2]

[1]*Department of Andrology, Kasr El Aini Faculty of Medicine, Cairo University, Egypt;* [2]*Kamal Shaeer Hospital, Cairo, Egypt*

Objective: Penile prosthesis implantation into scarred corporal bodies is one of the most challenging procedures in prosthetic urologic surgery. Neglected ischemic priapism or infection and extrusion of a penile implant result in fibrosis of the corpora cavernosa. Despite the advent of many excellent techniques that require experienced hands, some cases remain where implantation fails. This work presents experience with extra-corporal implantation as a last resort.

Methods: After failure of alternative techniques, extra-corporal implantation is resorted to. The corpus spongiosum is identified by palpating the catheter and held up between two fingers. Diathermy knife is used to cut a longitudinal window into one corpus cavernosum, through the septum and into the contralateral corpus cavernosum. A single semirigid implant rod is inserted through the window at the base of the penis, half-way through. The two limbs of the rod are bent upwards towards the glans, to assume a U-shape. The limbs of the U are brought together at mid-shaft by a gathering suture: size-5 Polyester suture passed through the corpora cavernosa and septum, then tied around the limbs of the implant. Finally, the tips of the U are anchored under the glans using the same suture material.

Results: Out of 8 cases of implant infection and extrusion operated upon, the extra-corporal implant allowed coital relationship in seven, followed up for 10–18 months. Enclosure of the implant in the rigid fibrous window, and anchoring the tips under the glans prevented anterior and posterior migration/extrusion. In one case, infection occurred and the implant had to be removed. Re-implantation with the same method was performed 6 months later, and the implant survived adequately.

Conclusion: Extra-corporal implantation can salvage cases with corporal fibrosis when all alternative methods fail.

P147

Cardiac autonomic neuropathy and bone mineral density in subjects with diabetes

G. DEFEUDIS[1,2], T. FEOLA[1], E. MADDALONI[2], R. POFI[1], C. DI DATO[1], E. GIANNETTA[1], P. POZZILLI[2], A. LENZI[1] AND A. M. ISIDORI[1]

[1]*Department of Experimental Medicine, Sapienza University of Rome;* [2]*Unity of Endocrinology and Diabetes, Department of Medicine, Campus Bio-Medico University of Rome*

Background: Cardiac autonomic neuropathy (CAN) and bone fragility are critical diabetes-related complications. The pathophysiological link between bone fragility and diabetes has not been fully clarified. Bone remodeling is regulated by various neuronal inputs, including sympathetic tone, which is known to inhibit bone mass accrual, while the parasympathetic nervous system (PNS) is a positive regulator of bone mass accrual.

Objective: So, as CAN has an impact on PNS, directly linked to bone remodeling, the aim of this study was to evaluate the relationships of BMD in subjects with type 2 diabetes (T2D) and CAN.

Methods: A total of 55 subjects (age: 64.8 ± 6.9 years) with T2D (mean ± SD disease duration ± 12.8 ± 7.7 years) were evaluated with CAN tests, using Neurotester Meteda® elaborating three simple noninvasive tests on the heart rate response to deep breathing, to standing, and to the Valsalva manoeuvre, and dual-energy X ray absorptiometry (DXA).

Results: No significant correlations were found evaluating BMD in subjects with T2D and CAN.

Conclusion: Anyway, to our knowledge, the present study is the first to evaluate BMD with DXA in a population of patients with T2D and CAN. For sure, it opens the way for larger studies to further define the role of CAN on BMD.

P148

HDL Cholesterol levels are associated to total testosterone levels in subjects with andrological diseases

G. DEFEUDIS[1,2], S. BRIGANTI[1], E. MADDALONI[1], S. PIERALICE[1], S. MANFRINI[1], N. NAPOLI[1] AND P. POZZILLI[1]

[1]*Unity of Endocrinology and Diabetes, Department of Medicine, Campus Bio-Medico University of Rome;*

[2]*Department of Experimental Medicine, Sapienza University of Rome*

Background: It is well known that the testosterone replacement treatment (TRT) in hypogonadal subjects leads to a reduction of all the components of lipids. Less clear but numerous data are available on the ratio of the individual components of the lipid trim and total testosterone levels (TT), in the presence of andrological diseases, not in TRT.

Objective: The aim of this study was to evaluate the relationship between the levels of TT and the components of lipid trim (total cholesterol, triglycerides, HDL, LDL) in a population of men with different types of andrological diseases.

Methods: Forty-four men (*n*: 44) (mean age: 58.3 ± 10.5 years; BMI: 28.3 ± 4.7 kg/m^2), were the subjects of which a complete lipid profile was available and were evaluated for erectile dysfunction (ED) (72%), hypogonadism (2.27%), loss of libido (2.27%), premature ejaculation (2.27%), induratio penis plastica (2.27%), and other andrological diseases (testicular pain, anorgasmia, infertility) 18%.

Results: The HDL was found to be directly related to TT levels [β +5.6 (95% CI: 1.9–9.2), $p = 0.004$]. This association was found to be significant even after adjustment for age and use of statins. Total cholesterol, triglycerides and LDL cholesterol did not correlate with TT.

Conclusion: Preliminary data from this study showed that only HDL cholesterol was found to be significantly related to TT; this finding shows how TT plays an important role on a cardiovascular biomarker such as HDL cholesterol and that this relationship requires further studies that clarify its pathophysiological meaning.

P149

Restoring and long term maintaining reproductive health in central hypogonadal men with intermittent testosterone and gonadotropin substitution

L. TŰŰ AND K. NAS
Endocare Institute Budapest, Hungary

Case Report: The 27-year-old panhypopituitary male patient is undergoing our treatment since 2008. He was examined for lack of adolescence and decreased growth in the Children's Endocrine Center previously. Laboratory tests showed lack of adenohypophysis hormone production. Corticosteroid, levothyroxine, and testosterone replacement therapy was started.

We converted the previously introduced testosterone replacement to a combination of gonadotropins (recombinant FSH + HCG) for 6 months. During the treatment, physiological production of his own testosterone could be evaluated. Semen testing indicated normozoospermia (sperm count 55 million/mL, 220 million/specimen) with good morphology and motility. After restoring fertility, a continuous treatment regimen was induced with intermittent testosterone supplementation for 3 months, interrupted by a combination of recombinant FSH + HCG for 4 weeks every 4th month. Repeated sperm testing indicated normozoospermia (sperm count 25 million/mL, 100 million/specimen). The serum testosterone remained optimal during the gonadotropin combination after discontinuation of the hormone replacement therapy.

Conclusion: Intermittent interruption of testosterone replacement by short-term gonadotropin substitution is a cost-effective method for long-term preservation of fertility in male patients with central hypogonadism.

P150

The effect of pelvic varicose on the prostate in a chronic animal experiment

A. TSUKANOV, N. RUDCHENKO, I. SEZINA, A. KUZOVKIN AND D. AHMETOV
Omsk state medical university, Omsk, Russia

Background: The problem of the effect of varicose veins of the pelvic organs in men on the condition of the prostate gland is more negotiable.

Objective: Our study is for establish the effect of pelvic varicose on the prostate in a chronic animal experiment.

Methods: We firstly established a model of rabbit's experimental pelvic vein stasis. For modelling were selected 36 adult male rabbits weighing 2.6–3.2 kg. Three groups of animals were formed: Control group: 4 rabbits (for studying ultrasound and radiologic anatomy). Group 2 (16 animals): vs. sacralis medialis was ligated, and then progesterone was injected intramuscularly. Group 3 (16 animals) were false-operated, and then progesterone was injected too. Ultrasonic duplex scanning was held after 1, 3 and 6 months of experiment: diameter of the veins in the pelvis was measured, the fact of blood reflux in veins under the manual compression was recorded. Animals were outputted from experiment in the same time, with following venography. Prostate tissues were examined for histological examination.

Results: Persistent venous congestion was registered in the second group. Histological examination at 1 month after surgery revealed the infiltration, the weakening of the stroma of the prostate gland, the appearance of lymphocytes and segmented leukocytes, full-blooded vessels expand and overflow acini their secret. After 3 months progressed stromal edema, resulting in a narrowing of the excretory ducts acini. In their lumen – leukocytes and desquamated epithelial cells. Histiocytes, and plasma cells were found in the interstices around the acini. After 6 months, the main process is the transformation of macrophages into fibroblasts, sealing and thickening of the collagen fibers have been reported. In the excretory ducts acini – scarring. There are no signs of vein varicose and changes in prostate in the third group of animals.

Conclusion: Morphological manifestations of regional hypertension in the pelvis are infiltration, destruction of cells in the prostate and sclerogenesis.

P151

Stuttering priapism in patient treated with different psychopharmacotherapy – a case report

G. ROSTA, S. TURBA, C. LINTNER AND E. BREINL
Universitätsklinikum St. Pölten – Universitätsklinik für Urologie, St. Pölten, Austria

Background: Priapism is a pathologic condition representing the painful erection of the penis without sexual

stimulation, lasting up to 4 h. Stuttering priapism is characterized by repetitive episodes of prolonged erections. Although stuttering priapism is an uncommon condition overall, it occurs commonly in certain groups, such as patients under psycho-pharmacotherapy. Available literature has indicated drug-related causes account for 15–41% of total cases. Anti-psychotics and antidepressants are well known for this side effect, hypothesized to be associated with the blockade of alpha 1 receptors

Case report: We report a case of 8 times recurrent low-flow priapism in a 22 years old Caucasian male – without other obvious risk factors – between 2016 and 2018. Because of a psychiatric reason he was treated by trazodone, which was discontinued because of recurrent priapism in 2017. Until 2018. February the patient was maintained on quetiapine 50 mg nightly and Sertraline 100 mg daily, which pharmacotherapy was also interrupted because of the same reason. Psychiatric medication withdrawal has not resulted in symptoms relief after 8 weeks. Each occasion conservative management by blood aspiration and intracavernous injection of etilefrine was effective. As prophylactic treatment daily 5 mg of 5-alpha-reductase inhibitor (LE:3) was introduced and 5 mg tadalafil-3 times/week (LE:3) was planned, which drug has not yet been approved by the national insurance commission in Austria.

Conclusion: With this case, we would like to highlight the importance of sexual side effects of the antipsychotics and antidepressant medications. Patients with complex psychotropic regiments are at a higher risk for recurrent priapism. Prevention of future episodes are challenging; controlled studies are needed regarding the efficacy and safety of these treatments.

P152

Skills and knowledge in the evaluation of sexual disorders by different medical specialists

D. GARCIA-ROJO, N. HANNAOUI, C. ABAD, M. CAPDEVILA, V. PAREJO, J. MUÑOZ-RODRIGUEZ, A. PRERA, E. VICENTE, J. L. GONZALEZ-SALA, A. DOMINGUEZ, C. CENTENO, L. DE VERDONCES, M. ROSADO, P. PLANELLES, A. FERRAN AND J. PRATS
Urology Department, Parc Tauli Hospital Universitari, Universitat Autonoma Barcelona, Sabadell, Spain

Background: We analyze the knowledge of different medical specialists in relation to knowledge of the sexual dimension of their patients, as well as the attitudes and skills to listen and treat it.

Methods: We used of a previous validated FSP questionnaire: F = frequency with which the subject is confronted with the problem. S = given seriousness for the professional exercises. P = problems of skills to bring a response in terms of theoretical knowledge's. The questionnaire is quoted 0 (rarely or few), 1 (sometimes or fairly) or 2 (often or many). Survey done with 62 different medical specialists: 19 gynecologists, 15 general practitioners, 13 urologists, 11 general surgeons and 4 medical oncologists. Comparison of means were performed with Student *t* test and subsequent pairwise comparison using the technique of minimal significant difference.

Results: (number = mean) (A) Frequency: (a) personal awareness: higher in urologists (1.77), medical oncologists (1.5) and gynecologists (1.33) than in general surgeons (0.64) ($p = 0.001$); (b) professional confrontation with the sexual complaint: usual for urologists and general practitioners, less in medical oncologists and gynecologists and rarely in general surgeons ($p = 0.00$); (c) attitude in case of patient demand: similar in all the specialists ($p = 0.257$). Urologists have a more proactive attitude (1.54) than gynecologists (1.05) and general surgeons (0.64) ($p = 0.03$). (B) Seriousness: to be listening and reactive to sexual demand is more important for urologists than the remaining specialists. (C) Problems: theoretical knowledge: good in urologists (1.54) than the other specialists (gynecologists 1.0) ($p = 0.026$); technical skills low in general practitioners and general surgeons and good for urologists ($p = 0.005$). The majority of the physicians believe that there should be a for specific consultation of sexology is opened in their hospital (90%) ($p = 0.777$). Men think very important to listen their patients' sexual complaints ($p = 0.02$) and have greater knowledge sufficient to manage their sexual problems ($p = 0.012$).

Conclusion: Urologists believe have greater proactive and good knowledge to treat the sexual problems of their patients compared than other physicians. It should encourage and conduct continuing education specific to different medical specialists, in order to have a proactive and sufficient knowledge about the sexual quality of life of the patients.

P153

Investigation of antioxidant profiles in blood and seminal plasma of infertile men

P. MIRABI[1] AND S. H. ALAMOLHODA[2]
[1]*Infertility and Reproductive Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran;* [2]*Midwifery and Reproductive Health Research Center, Department of Midwifery and Reproductive Health, School of Nursing and Midwifery, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

Background: Oxidative stress has a major role in the etiology of infertility with a negative effect on the quality and performance of sperm. Non-enzymatic antioxidants play an important role in protecting against oxidative damage and lipid peroxidation.

Objective: The aim of this study was to investigate the presence of rare elements and antioxidant profiles in the blood and seminal plasma of infertile men.

Methods: This is a review study to assess the Rare Elements and Antioxidant Profiles in Blood and Seminal Plasma of Infertile Men. We searched Google Scholar, Cochrane, and Science Direct and Med Line for identifying relevant analytic studies. We used the key words: infertile men, antioxidant Profiles, stress oxidative.

Results: In infertile men, zinc and selenium levels of semen are lower than fertile. Zinc levels have a significant positive correlation with the number, motility and natural form of sperm. Selenium has a significant relationship with sperm motility. There is a positive relationship

between zinc with calcium and potassium in the semen. The amount of semen with free testosterone has a significant relationship. The total glutathione, glutathione oxidative and glutathione reductase decreased in the semen of the infertile men. There is a meaningful relationship between glutathione total with sperm motility, between glutathione oxidative with sperm form and sperm motility and glutathione reductase, with motility of sperm and sperm count. Total superoxide dismutase, antioxidant capacity, vitamin E and C concentrations were significantly lower in semen of infertile men. However, the level of oxidation stress such as malondialdehyde, phospholipid hydroperoxide and 8-hydroxy-2deoxy-guanosine are higher in the semen of the infertile men. In the infertile men's blood sample, total antioxidant capacity, carotenoids and vitamin E are significantly lower.

Conclusion: Evaluation of oxidative profiles of blood plasma and semen can play an important role in evaluating sperm production and performance and as an infertility biomarker for men.

P154

Impact of the ponderal loss caused by the gastroplasty surgery in the reproductive hormonal profile and seminal analysis – case series

J. H. DANTAS JR., P. J. DE MEDEIROS, C. A. BRITTO, R. T. M. DE PAIVA, D. A. SERAFIM, P. H. B. DE AVILA, N. C. C. FERREIRA AND G. D. DE AZEVEDO

Hospital Universitário Onofre Lopes, Universidade Federal do Rio Grande do Norte, Natal, Brazil

Background: The global prevalence of obesity has increased since the 1980s, reaching 13% of obese adults by 2014, according to data from the World Health Organization. Excessive adipose tissue has a direct impact on gonadal function, both men and women. Secondary hypogonadism associated with obesity may occur in male patients, therefore, weight loss may improve male hormonal profile and one of the strategies used for this is to perform bariatric surgery for selected cases. In order to evaluate the impact of the weight loss caused by this surgery on the reproductive and hormonal profiles of obese patients, we performed a case series for this purpose.

Methods: This single-center study is a retrospective case series in which 7 patients with a Body Mass Index (BMI) above 45 kg/m^2 and undergoing gastroplasty surgery between 2008 and 2011 were selected. All patients underwent preoperative tests for total testosterone, FSH, LH and seminal analysis, and then, all this patients were submitted to a surgery by the Roux-en-Y technique. Six months after the procedure, the patients repeated a second series of exams.

Results: In the preoperative tests, 6 of the 7 patients had total testosterone below 300 ng/dL, suggesting hypogonadism. After the surgical procedure, all volunteers had elevations in total testosterone levels and 5 of the 6 patients had levels above 300 ng/dL. FSH and LH hormone levels oscillated between elevation and decrease, as well as seminal analysis. Furthermore, 02 patients who were an azoospermic and another with cryptozoospermia before surgery, started to present 1.0 million sperm and 52 million spermatozoa respectively, on the second seminal analyzes obtained 6 months after the performance of the gastroplasty.

Conclusion: The marked weight loss caused by bariatric surgery using the Roux-en-Y technique led to a significant increase in testosterone and with promising effects in 02 patients with severe seminal changes. More studies will be necessary in order to better understand the effects caused by weight loss in male reproductive profile.

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Low levels of neutral alpha-glucosidase in men with infections caused by Chlamydia trachomatis

D. TSANEV[1], S. PETROV[1,2], N. AYVAZOVA[1,3], D. MARTINOV[1,3], A. DIKOVA[1], G. PAVLOVA[1] AND E. KONOVA[1,4]

[1]Medical Centre, Clinical Institute for Reproductive Medicine, Pleven, Bulgaria; [2]Medical University of Plovdiv, Plovdiv, Bulgaria; [3]Department of Biology, Medical University of Pleven, Pleven, Bulgaria; [4]Center for Reproductive Health, Medical University of Pleven, Pleven, Bulgaria

Background: Chlamydia trachomatis is one of the most common sexual transmitted diseases. It has been proved that it can cause epididymitis in men. Activity of neutral alpha-glucosidase (NAG) isolated from seminal plasma, is a useful parameter to determine damage of epididymal function.

Objective: We believe that NAG activity can be measured like analytical method, to determine whether the infection caused by Chlamydia trachomatis has reached the epididymis.

Methods: Diagnostic of Chlamydia trachomatis were made by serological test for IgG and IgA antibodies. Semen samples for NAG were collected from subjects by masturbation into a sterile container. Further analysis of NAG were made in accordance with the WHO laboratory manual.

Results: We researched a small group of 4 individuals and three of them were with oligozoospermia and one with azoospermia. One of the patients with oligozoospermia reported that he was treated Chlamydia trachomatis infection not long ago, a confirmation test was made. The other three were newly tested for Chlamydia trachomatis IgG and IgA antibodies. All of the patients were test for NAG activity. Two of the newly tested patients with oligozoospermia were negative for IgG and IgA antibodies, the one with azoospermia were positive. The control sample were negative as well. The NAG activity test of the person with newly diagnosed infection with Chlamydia trachomatis and the one with soon cured infection showed low levels of NAG activity <20 mIU/ejaculate. The other two samples were >20 mIU/ejaculate.

Conclusion: Chlamydia trachomatis may damage the epididymal function of the patients. Simultaneous test of Chlamydia trachomatis and NAG activity may give us information about the progress of the infection. Although larger group is needed for further analysis and confirmation, our initial researched showed that there is a relation

between Chlamydia trachomatis infection and the low levels of NAG.

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Immediate curative and permanent treatment for premature ejaculation (Alaa Aglan Operation)

A. AGLAN
Ain Shams University, Cairo, Egypt

Background: Premature ejaculation (PE) is a common male sexual disorder, although there is some medications used for this problem but there is no definite medical treatment and current surgical options is not so effective with high recurrence and low success rate.
Methods: The present study shows the effectiveness of cutting bulbospongiosus muscle bilaterally and frenulur delta excision for treatment of premature ejaculation and delay time of ejaculation in normal persons.
Results: This study was started since 6/4/2011 to 6/4/2016 (60) patient underwent this study and were operated with success rate 96.6% with immediate result after 1st intercourse usually 3 weeks after surgery, the intra-vaginal ejaculation latency time increased up to (200–1000%), patients with time less than 2 min usually reach 8 min some of them reach 20 min, patients time more than 5 min increased up to 15–20 min and some of them up to 30 min the result is permanent, in this study we explain also why some patients not respond to local anesthesia applied to glans and approved that clinically.

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Imipramine treatment of retrograde ejaculation – case report

S. PETROV[1,2], D. TSANEV[2], D. MARTINOV[2,3], N. AYVAZOVA[2,3] AND E. KONOVA[2]
[1]Medical University Plovdiv, Bulgaria; [2]Clinical institute for Reproductive Medicine – Pleven; [3]Medical University Pleven, Department of Biology

Background: Retrograde ejaculation in long term diabetes mellitus is a rare neurodegenerative complication. It is an abnormal passage of ejaculate into the bladder after orgasm, associated with dysfunction of the internal urethral sphincter mechanism, caused by autonomic neuropathy in long term history of diabetes mellitus.
Case report: A 39-years old male patient with a history of diabetes mellitus type 1 since 10th year of age, initiated with insulin treatment from the beginning and secondary infertility since 2010 (one achieved pregnancy in 25th years of age with a different woman). The patient presents symptoms of diabetic neuropathy: numbness and pain in legs, retrograde ejaculation and erectile dysfunction. No effect of treatment with PDE-5 inhibitors for erectile dysfunction. No history of mumps, urogenital tract infections, operative procedures. Height: 176 sm. Weight: 82 kg. BMI: 26 kg/m^2. Volume of the testicles appr. 20 mL for both. No evidence of varicocele. Symptoms of distal diabetic neuropathy: reduced thermal-touch and vibration sensitivity. Reserved pulsations of the peripheral arteries. Inadequate control of diabetes – HbA1c 8.7%. Sperm analysis (13.02.2017): Aspermia. Procedure for retrograde ejaculation was performed with approx. 10 spermatozoa in visual field of the sediment. FSH: 2.28 mIU/mL (1.27–19.26), Testosterone: 3.55 ng/mL (1.75–7.81), Inhibin B: 160.33 pg/mL ($N > 100$). Negative results for microbiological, Ureaplasma, Mycoplasma and Chlamydia trachomatis infections were found. Corrections of the doses of insulin aspart and detemir were done. Initiating therapy: Adnrositol, Seanergix for Him, Milgamma *N* caps., and Thiogamma tb. Use of PDE-5 inhibitors before sexual intercourse was prescribed. At the second visit patient still presented complaints of retrograde ejaculation. No effect of PDE-5 inhibitors on erectile dysfunction. Improvement of diabetes control was registered with HbA1c 8.1% (still unsatisfactory results). Sperm analysis (27.06.2017): Aspermia. Procedure for retrograde ejaculation was performed with over 200 spermatozoa gr. D in visual field of the sediment.
Initiating therapy with Imipramine (Tofranil) 25 mg – 1 tb./p.o. after consultation with a psychiatrist. Therapy supporting spermatogenesis was continued with Tribulus terrestris 1000 mg, and Wellman conception. One week after initiating therapy with Imipramine retrograde ejaculation was overcome (telephone visit). Imipramine treatment was performed intermittently with collaboration with the psychiatrist. At the third visit retrograde ejaculation did not persist and the sperm analysis (10.01.2018) established: hypospermia: 0.1 mL with 687 million spermatozoa; morphology: 3%; motility: 0% gr. A, 17% gr. B, 23% gr. C, 60% gr. D; vitality: 39% vital spermatozoa. A Cryopreservation of the sperm was suggested.
Conclusion: Retrograde ejaculation is caused by developed diabetic neuropathy secondary to diabetes mellitus type 1 in 5–18% of cases. Different therapeutic approaches are present, whether medical or surgical, with limited success rates. As a medical approach imipramine is used with approximately 38.5% efficacy as a monotherapy and as a combination with pseudoephedrine with efficacy up to 61.5%. In our case imipramine has a satisfactory effect achieving antegrade ejaculation 1 week after initiating the treatment. As a first line therapeutic approach satisfactory glycaemic control should be targeted with HbA1c levels of below 7%.

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Self mutilation – penis self-clawing

I. ALBERT[1], L. SZŰCS[1], G. BÓGYI[1] AND P. FEHÉR[2]
[1]Medical Centre, Hungarian Defence Forces Budapest, Hungary; [2]Károli Gáspár University of the Reformed Church Budapest, Hungary

A 45-year-old male patient was delivered by the ambulance. The patient cut off his penis with his own hands, he made self-mutilation. We didn't find the cutted penis. After the wound care, the dagger was sucked through the skin during symphysis.
After his surgical anamnesis, his testes was removed 13 year ago because of an accident, where his penis was also damaged. Since the surgery, he has been receiving hormone replacement therapy. In the beginning, daily

2 × 40 mg Andriol tablets (testosterone undecanoate) were ordered, then Nebido injection (1000 mg testosterone undecanoate) every 3 months. During the high-dose testosterone injection therapy, after the third week of administration, the patient had 8–9 spontaneous erections without sexual desire everyday for weeks. He was really nervous, aggressive and exhausted. He also felt convulsive pain in his penis, in his groin and in his lower abdomen, which often appeared in the most unlikely situations. The patient couldn't sleep at that time. The continuous pain and the mentioned complaints led to self-aggression, self-mutilation.

The side effects of testosterone therapy may be depression, mood problems, insomnia, restlessness, aggression or irritability. In this case, several urological and andrological tests have been accomplished, but the side effects of testosterone have never occurred. In our opinion, the psychological disturbances of high doses of testosterone treatment, self-agitation, have led to self-mutilation, so we suggest the immediate stop of high-dose testosterone therapy in similar cases.

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Converting partial orchiectomy to total orchiectomy due to intraoperative pathology result: a case report

S. SARI[1], V. SELMI[1], U. OZTEKIN[1], A. GÜREL[1] AND M. CANIKLIOĞLU[2]

[1]*Bozok University, Faculty of Medicine, Yozgat, Turkey;*
[2]*Sinop State Hospital, Sinop, Turkey*

Background: Testis tumors are the most frequent cancers between 15 and 35 years male. Between 15 and 19 years male, the most frequent cancer after leukemia. Seminoma is the most frequent type of testis tumors. Seminoma is seen in 50% of the patients. Seminomatous tumors are usually seen in 30–40 years, non seminomatous tumors are seen in 20–30 years.

Radical orchiectomy is recommended in the management of testis tumors. Partial orchiectomy is recommended in case of metachrone contralateral testis tumor, solitary testis, <20 mm testis tumor size or <30% of testis size. Testosterone levels must be normal in these patients. Punch biopsy is taken from the adjacent tumor tissue and radiotherapy is performed in the management of these patient.

Objective: In our study we aimed to present a patient for whom we planned partial orchiectomy and we converted to total orchiectomy due to intraoperative pathology result.

Case Report: Twenty-three years old single male patient admitted to our polyclinic for lower testis volume. In the physical examination left testis volume was smaller than right testis. A mass was detected in the left testis. In the Doppler ultrasonography 8 × 5 mm hypoechoic and 3 mm adjacent cystic lesions in the anterior parenchymal of the left testis were reported. Doppler activity was seen in the hypoechoic lesion. In the magnetic resonance imaging for correlation, left testis volume was low, 10 × 5 mm lobule contoured T2 hyperintense lesion was detected. 3 mm adjacent cystic lesion was also detected. The thoracal and abdomen computerised tomography (BT) were normal. AFP, B-Hcg and LDH levels were normal. In the spermiogram, volume was 2 mL, sperm count was 32 million/mL, progressive motility was 20%. Partial orchiectomy was planned. Preoperative cryopreservation was recommended. After preoperative tests, operation planned. After left inguinal incision, spermatic cord was found and clamped. The mass in the left testis was excised. With adjacent tissue biopsy, the material was sent to frozen examination. Frozen examination was suspicious for malignancy and we made total orchiectomy. Patient was discharged postoperative first day. The pathology was malign germ cell tumor (embryonal carcinoma). Macroscopic tumor diameter was 0.6 cm. Wide necrosis area was present. Tumoral invasion was present in tunica albuginea. 100% embryonal carcinoma was present in tumor. Germ cell in situ neoplasia accompanied embryonal carcinoma. There was no tumoral tissue in rete testis, epididymis and spermatic cord. Patient was referred to medical oncology for chemotherapy.

Conclusion: Total orchiectomy is the surgical management for testis tumor. Partial orchiectomy is recommended in case of metachrone contralateral testis tumor, solitary testis, <20 mm testis tumor size or <30% of testis size with normal testosterone levels. Tumor and adjacent tumor tissue biopsy is important. The result of biopsy can change the surgical treatment.

P160

A rare case report: testicular amputation

V. SELMI[1] AND M. CANIKLIOĞLU[2]

[1]*Bozok University Urology Department, Yozgat, Turkey;*
[2]*Sinop Ataturk State Hospital, Sinop, Turkey*

Background: Genital trauma is classified as blunt and penetrating, degloving and thermal by the aetiological mechanism. Traffic accidents, violence, gunshot and stab wounds, animal bites, self-mutilation and sports accidents are the most frequent causes. Blunt scrotal trauma can cause testicular rupture, scrotal haematoma testicular dislocation and testicular haematocoele. If surgery is performed within 72 h, 80% of ruptured testes can be saved without orchiectomy.

Case Report: An 11 years-old boy with blunt scrotal trauma was consulted in emergency service. The boy was wearing shorts with no underpants and riding a bicycle and he had tried to make an acrobatic move. He had fallen on the rear wheel. His right testicle was stuck, ruptured and amputated. His parents had first checked the boy and then noticed that the right testicle was missing. They had found the testicle in a muddy condition and cleaned it with water and soap and put in a jar filled with water. Nearly 2 h were passed when the patient was consulted. The patient was stable. He had no abdominal pain or other injuries. The vas deferens was outside of the scrotum but no active bleeding site was seen. A color scrotal Doppler ultrasonography was performed. The perfusion was weak and coming to an end just the level of external orifice of the inguinal canal. Initially, we decided to replant the testicle but it was not possible because of the transportation and preservation conditions. So we decided to make an inguinal exploration. We made an inguinal incision and tried to find the stump of the spermatic cord. Although it

was screened on Doppler ultrasonography we could not be able to find it. Then we incised proximally and opened the inguinal canal. We found the stump on the level of the internal orifice and ligated. After bleeding control we put a Penrose drain and sutured the scrotum. There was no complication and we removed the drain on the first day after the operation. The patient was discharged on the second day. Male external genitalia traumas usually appear between 6 and 12 years old boys because of falls, sports accidents and fights. Blunt scrotal trauma accounts 85% of testicular injury and about half of these injuries result with testicular rupture or serious damage which require early surgical intervention. Most of the cases reported in the literature are the result of genital self-mutilation. Early access to the hospital with obeying the transportation rules can help to microsurgical replantation of the injured testis. Lin et al. reported two cases of testis replantation. He defined the procedure as debridation of the necrotic parts of the testicular vessels, anastomosing the testicular artery first and then testicular vein microscopically. Finally, he performed microscopic two layer vasovasostomy. The important points of replanting the tissue are the transportation conditions, time to surgery and ischemia time. Although cavernous tissue of the penis is sensitive to anoxia, testes are much more sensitive. Thus, testis should be replanted first rather than penis in the patients with genital trauma.

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