EAA Literature Alert
Edition May 2022

Welcome to the May edition of the andrology literature review. Keywords for this edition: kisspeptin, sperm selection and retrieval, testosterone, androgen receptor, Inhibin A inactivation, Klinefelter syndrome, DDX3Y/X, testicular biopsy, ageing, prostate cancer, sperm capacitation, and more. As previously, recent publications of the EAA Training Centres were prioritised.

Clinical andrology and epidemiology

The first study of the effects of kisspeptin on human male bone metabolism. The clinical part was a placebo-controlled study in 26 men receiving kisspeptin. The experimental in vitro part examined human male osteoblasts and osteoclasts treated with kisspeptin. The data showed that kisspeptin promotes osteogenic differentiation of osteoblast progenitors and inhibits bone resorption in vitro and is a good candidate for treatment of osteoporosis.

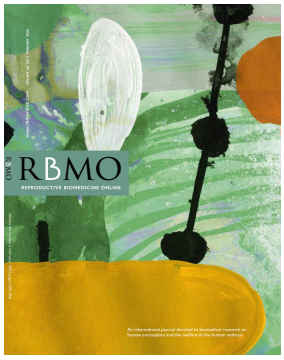
Comninos AN, Hansen MS, Courtney A, Choudhury S, Yang L, Mills EG, Phylactou M, Busbridge M, Khir M, Thaventhiran T, Bech P, Tan T, Abbara A, Frost M, Dhillo WS. Acute Effects of Kisspeptin Administration on Bone Metabolism in Healthy Men. ***J Clin Endocrinol Metab (JCEM)***. 2022 May 17;107(6):1529-1540. PMID: 35244717. https://doi.org/10.1210/clinem/dgac117

This meta-analysis performed by a group of experts, including EAA Academicians, demonstrated that hormone therapy was not associated with improved sperm retrieval (SSR) rates in patients with hypergonadotropic hypogonadism, except marginal improvement in eugonadal men with non-obstructive azoospermia (NOA). Therefore, hormone therapy should not be routinely used prior to SSR.

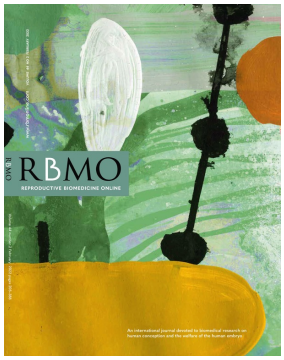
Tharakan T, Corona G, Foran D, Salonia A, Sofikitis N, Giwercman A, Krausz C, Yap T, Jayasena CN, Minhas S. Does hormonal therapy improve sperm retrieval rates in men with non-obstructive azoospermia: a systematic review and meta-analysis. ***Hum Reprod Update***. 2022 May 8:dmac016. Epub ahead of print. PMID: 35526153. https://doi.org/10.1093/humupd/dmac016

This study investigated the utility of magnetic-activated cell sorting (MACS) for sperm selection for intracytoplasmic sperm injection (ICSI). Although MACS sperm selection slightly increased the cumulative live birth rates per embryo transferred compared with the reference group, this change was not clinically meaningful. MACS should not be recommended indiscriminately to all infertile patients undergoing ICSI with donated oocytes as a sperm processing add-on.

Gil Juliá M, Hervás I, Navarro-Gomezlechon A, Quintana F,

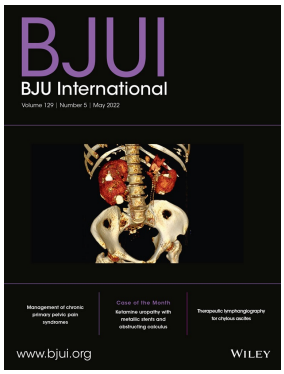


Amorós D, Pacheco A, González-Ravina C, Rivera-Egea R, Garrido N. Cumulative live birth rates in donor oocyte ICSI cycles are not improved by magnetic-activated cell sorting sperm selection. ***Reprod Biomed Online (RBMO)*** 2022; 44(4): P677-684. PMID: 35184950. https://doi.org/10.1016/j.rbmo.2021.09.024



Recent publications from the EAA Centre in Brussels dealt with fertility preservation in patients with Klinefelter syndrome. We highlight here the study of early-diagnosed patients who were followed after testis tissue preservation. The data confirmed that harvesting testicular tissue did not have a long-term impact on their gonadal development.

Braye A, Böhler S, Vloeberghs V, De Boe V, De Schepper J, Gies I, Goossens E. Testicular biopsy for fertility preservation in early-diagnosed Klinefelter patients: patient characteristics and long-term follow-up. ***Reprod Biomed Online***. 2022 May;44(5):889-895. PMID: 35282994. https://doi.org/10.1016/j.rbmo.2022.01.014



This study from the EAA Centre in Copenhagen showed that optimised contralateral biopsy procedure improved the detection rate of GCNIS in patients with testicular germ cell cancer and minimized their risk of developing metachronous cancer. Performing contralateral biopsies is beneficial for the patients' care and should be offered as a part of the management.

Rajpert-De Meyts E, Jørgensen N, Petersen JH, Almstrup K, Aksglaede L, Lauritsen J, Rørth M, Daugaard G, Skakkebaek NE. Optimised detection of germ cell neoplasia in situ in contralateral biopsy reduces the risk of second testis cancer. ***BJU Int.*** 2022 May 16. Epub ahead of print. PMID: 35575005. https://doi.org/10.1111/bju.15774

A new publication from EMAS Consortium. This prospective study did not find an association between endogenous sex hormone levels or CAG repeat length polymorphism in the AR gene and cognitive ageing in men. These data suggest that sex steroid levels do not affect visuospatial function, visual memory, or processing speed in middle-aged and older men.

Overman MJ, Pendleton N, O'Neill TW, Bartfai G, Casanueva FF, Forti G, Rastrelli G, Giwercman A, Han TS, Huhtaniemi IT, Slowikowska-Hilczer J, Lean ME, Punab M, Lee DM, Antonio L, Gielen E, Rutter MK, Vanderschueren D, Wu FC, Tournoy J. Reproductive hormone levels, androgen receptor CAG repeat length and their longitudinal relationships with decline in cognitive subdomains in men: The European Male Ageing Study. ***Physiol Behav.*** 2022 Apr 26;252:113825. PMID: 35487276. https://doi.org/10.1016/j.physbeh.2022.113825

This interesting study addressed the question whether men's testosterone levels correlate with the number of sexual partners. Based on data from the large NHANES cohort, the author found that this correlation is contingent upon age: It is positive for younger men but negative for older men.

Bozick R. Number of sexual partners and serum testosterone levels in a population-based sample of unpartnered heterosexual men. ***Andrology***. 2022 May 10. Epub ahead of print. PMID: 35537138.

https://doi.org/10.1111/andr.13195

COVID-19

Low testosterone (T) levels in males have been linked with increase in proinflammatory cytokines and adverse COVID-19 outcomes. This study showed that T therapy did not help older men and was not associated with decreased odds of hospitalization or intensive care unit admission/mechanical ventilation.

Baillargeon J, Kuo YF, Westra J, Lopez DS, Urban RJ, Williams SB, Raji MA. Association of testosterone therapy with disease progression in older males with COVID-19. ***Andrology***. 2022 Apr 29. Epub ahead of print. PMID: 35486968.
https://doi.org/10.1111/andr.13193

Androgenetics

Homozygous *INHA* inactivation caused by a 2 bp deletion (c.208_209delAG, R70Gfs*3), which led to a truncated INHA protein was identified in two Turkish infertile azoospermic brothers with hypospadias, low serum testosterone, high gonadotropins and AMH, and undetectable inhibin concentrations. The heterozygous parents had normal sex development and reproduction.

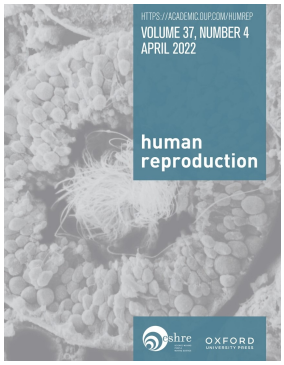
Arslan Ates E, Eltan M, Sahin B, Gurpinar Tosun B, Seven Menevse T, Geckinli BB, Greenfield A, Turan S, Bereket A, Guran T. Homozygosity for a novel INHA mutation in two male siblings with hypospadias, primary hypogonadism, and high-normal testicular volume. ***Eur J Endocrinol.*** 2022 Mar 23;186(5):K25-K31. PMID: 35235537.
https://doi.org/10.1530/eje-21-1230

Commentary by I. Huhtaniemi: The first report on homozygous INHA inactivation in humans. ***Eur J Endocrinol.*** 2022 May 19;187(1):C1-C2.
https://doi.org/10.1530/eje-22-0330

This study from the EAA Centre in Tartu and GEMINI Consortium evaluated clinical value of secondary findings (SFs) identified in exome sequencing of patients with non-obstructive azoospermia (NOA). They found identifiable SFs in 3.6% NOA patients, and possible association with future health problems, e.g. familial cancer syndromes or cardiovascular disease.

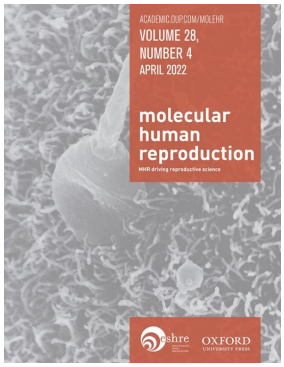
Kasak L, Lillepea K, Nagirnaja L, Aston KI, Schlegel PN, Gonçalves J, Carvalho F, Moreno-Mendoza D, Almstrup K, Eisenberg ML, Jarvi KA, O'Bryan MK, Lopes AM, Conrad DF; GEMINI Consortium, Punab M, Laan M. Actionable secondary findings following exome sequencing of 836 non-obstructive azoospermia cases and their value in patient management. ***Hum Reprod.*** 2022 May 10:deac100. Epub ahead of print. PMID: 35535697.
http://dx.doi.org/10.1093/humrep/deac100

The study identified six biallelic variants in *HFM1* in infertile men with non-obstructive azoospermia (NOA) and meiotic arrest. The data suggest that HFM1 may regulate meiotic crossover formation in a dose-dependent manner. In the mouse



models, the study showed that HFM1 variants could impair spermatogenesis and oogenesis with a varying degree of severity.

Xie X, Murtaza G, Li Y, et al et Shi Q. Biallelic *HFM1* variants cause non-obstructive azoospermia with meiotic arrest in humans by impairing crossover formation to varying degrees. ***Hum Reprod.*** 2022 May 8:deac092. Epub ahead of print. PMID: 35526155. https://doi.org/10.1093/humrep/deac092



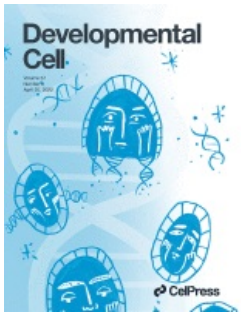
This study reported a rare homozygous *SYCP1* frameshift mutation in two men with severe oligozoospermia. This mutation decreases the ability of the truncated SYCP1 protein to bind DNA. The data suggest that a small number of DNA double-strand breaks are not efficiently repaired in germ cells resulting in the oligozoospermia phenotype.

Nabi S, Askari M, Rezaei-Gazik M, Salehi N, Almadani N, Tahamtani Y, Totonchi M. A rare frameshift mutation in SYCP1 is associated with human male infertility. ***Mol Hum Reprod.*** 2022; 28(4):gaac009. PMID: 35377450. https://doi.org/10.1093/molehr/gaac009

This cross-sectional study found that serum undercarboxylated-osteocalcin (ucOCN) and rs2247911 polymorphism of *GPRC6A* (putative OCN receptor) were associated with higher serum testosterone (T) in a cohort of infertile men, suggesting the involvement of ucOCN-GPRC6A axis in the regulation of T production by the testis.

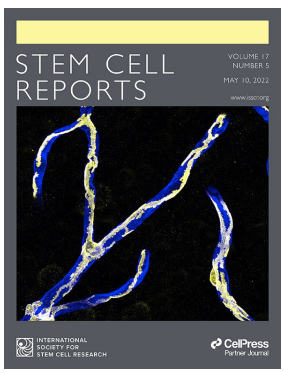
Jawich K, Rocca MS, Al Fahoum S, Alhalabi M, Di Nisio A, Foresta C, Ferlin A, De Toni L. RS 2247911 polymorphism of GPRC6A gene and serum undercarboxylated-osteocalcin are associated with testis function. ***J Endocrinol Invest.*** 2022 Apr 28. doi: 10.1007/s40618-022-01803-9. Epub ahead of print. PMID: 35482214. https://link.springer.com/article/10.1007/s40618-022-01803-9

Translational and basic andrology



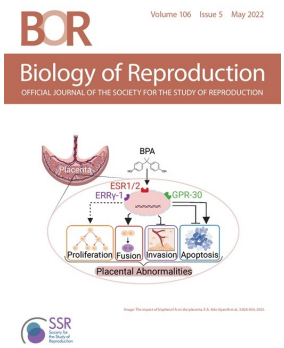
This international study investigated testicular changes conferred by aging by means of single-cell RNA-seq profiling. Age-related dysregulation was modest in spermatogonia but severe in somatic cells, including inflammation and aberrant metabolic and steroid signalling in multiple cell types. The extent of dysregulation correlated with body mass index in older but not in younger men.

Nie X, Munyoki SK, Sukhwani M, Schmid N, Missel A, Emery BR, DonorConnect, Stukenborg JB, Mayerhofer A, Orwig KE, Aston KI, Hotaling JM, Cairns BR, Guo J. Single-cell analysis of human testis aging and correlation with elevated body mass index. ***Dev Cell.*** 2022 Apr 18:S1534-5807(22)00244-1. Epub ahead of print. PMID: 35504286. https://doi.org/10.1016/j.devcel.2022.04.004



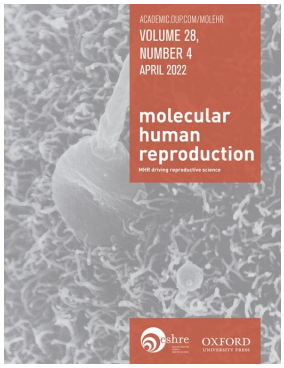
Using fluorescence-activated cell sorting (FACS) followed by single-cell RNA sequencing, this study showed that fibroblast growth factor receptor 3 (FGFR3) is expressed by human PGCs during the first and second trimester. FGFR3 is repressed as PGCs differentiate into primordial oocytes but stays active in male germ cells.

Chitiashvili T, Hsu FM, Dror I, Plath K, Clark A. FGFR3 is expressed by human primordial germ cells and is repressed after meiotic initiation to form primordial oocytes. ***Stem Cell Reports.*** 2022 May 5:S2213-6711(22)00204-1. doi: 10.1016/j.stemcr.2022.04.015. Epub ahead of print. PMID: 35594860. https://doi.org/10.1016/j.stemcr.2022.04.015



This study in a mouse model showed negative effects of maternal malnutrition during gestation and lactation on the fertility of the male offspring. Interestingly, spermatogenesis and sperm parameters were quantitatively normal, but spermatozoa exhibited an altered capacitation and decreased ability to fertilise oocytes.

Lord T, Law NC, Oatley MJ, Miao D, Du G, Oatley JM. A novel high throughput screen to identify candidate molecular networks that regulate spermatogenic stem cell functions. ***Biol Reprod.*** 2022 Mar 4:ioac048. Epub ahead of print. PMID: 35244684.
https://doi.org/10.1093/biolre/ioac048



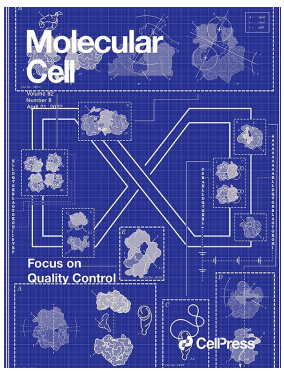
Sperm capacitation requires an increase in both intracellular calcium ($[Ca^{2+}]_i$) and pH (pH_i), but it is unknown if pH increases naturally during human sperm capacitation. This study found that acrosomal pH progressively increases during capacitation. Alkalinization of the acrosome may be a key step in the path toward the acrosome reaction.

Carrasquel Martínez G, Aldana A, Carneiro J, Treviño CL, Darszon A. Acrosomal alkalinization occurs during human sperm capacitation. ***Mol Hum Reprod***. 2022 Mar 8;28(3):gaac005. doi: 10.1093/molehr/gaac005. PMID: 35201340.
https://doi.org/10.1093/molehr/gaac005

SCIENTIFIC REPORTS

DDX3Y encodes an RNA helicase important for translational control of the cell cycle in human premeiotic male germ cells. This study showed species differences in primate testis-specific DDX3Y transcript variants, and suggested a positive selection for transcripts that increase stability and translation efficiency.

Vogt PH, Rauschendorf MA, Zimmer J, Drummer C, Behr R. AZFa Y gene, DDX3Y, evolved novel testis transcript variants in primates with proximal 3´UTR polyadenylation for germ cell specific translation. ***Sci Rep*** 2022 May 27;12(1):8954. PMID: 35624115.
https://doi.org/10.1038/s41598-022-12474-0



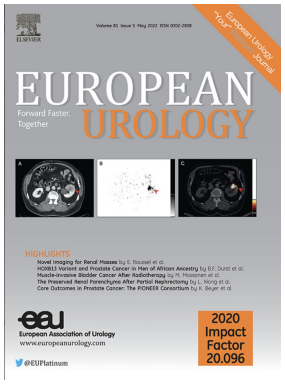
If you want to learn more about the function of the sex chromosome-encoded protein homologs, DDX3X and DDX3Y, read this paper, which showed differences in their ATPase activity and regulation of mRNA translation.

Shen H, Yanas A, Owens MC, Zhang C, Fritsch C, Fare CM, Copley KE, Shorter J, Goldman YE, Liu KF. Sexually dimorphic RNA helicases DDX3X and DDX3Y differentially regulate RNA metabolism through phase separation. ***Mol Cell***. 2022 May 10:S1097-2765(22)00385-9. Epub ahead of print. PMID: 35588748.
https://doi.org/10.1016/j.molcel.2022.04.022

Androgen deprivation therapy is administered in prostate cancer patients to inhibit tumour growth. This study demonstrated that androgen

receptor (AR) blockade in T cells prevented T cell exhaustion and improved responsiveness to immunotherapy. The findings established that intrinsic AR activity in T cells represents a novel mechanism of immunotherapy resistance.

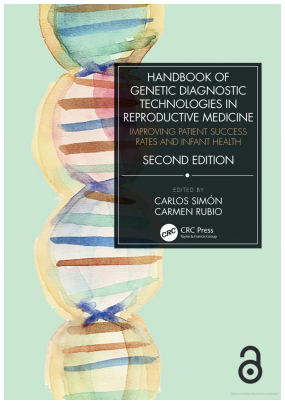
Guan X, Polesso F, Wang C, Sehrawat A, Hawkins RM, Murray SE, Thomas GV, Caruso B, Thompson RF, Wood MA, Hipfinger C, Hammond SA, Graff JN, Xia Z, Moran AE. Androgen receptor activity in T cells limits checkpoint blockade efficacy. ***Nature.*** 2022 Mar 23. Epub ahead of print. PMID: 35322234.
https://doi.org/10.1038/s41586-022-04522-6



Targeting the androgen receptor inhibits prostate cancer, but the tumour often recurs as castration-resistant prostate cancer (CRPC). This study found that CRPC-like cells and neuroendocrine cells are present early in the tumour and are not exclusively the result of selection during androgen deprivation therapy.

Cheng Q, Butler W, Zhou Y, Zhang H, Tang L, Perkinson K, Chen X, Jiang XS, McCall SJ, Inman BA, Huang J. Pre-existing Castration-resistant Prostate Cancer-like Cells in Primary Prostate Cancer Promote Resistance to Hormonal Therapy. ***Eur Urol.*** 2022 May;81(5):446-455. PMID: 35058087.
https://doi.org/10.1016/j.eururo.2021.12.039
Editorial comment: Ali A, Baena E. The Needle in the Haystack: The Presence of Castrate-resistant Prostate Cancer Cells in Hormone-naïve Prostate Cancer. ***Eur Urol.*** 2022 doi: 10.1016/j.eururo.2022.02.004.

Book of the month



A multi-chapter handbook addressed to all specialists dealing with diagnosis and management of infertility and other reproductive disorders in females and males, including children. The focus is on genetic technologies, epigenetics (incl. sperm) and genetic counseling. The book contains 32 chapters, written by 91 authors, incl. P.R., Bennett, J. Blanco, A. Capalbo, E. Cicinelli, T. Hassold, E. Hoffmann, A. Kuliev, D.A. MacIntyre, M.K. Skinner, A. Thornhill, R. Vento-Tormo, R. Zemet, and others.

Edited by: C. Simón and C. Rubio
Handbook of Genetic Diagnostic Technologies in Reproductive Medicine. Improving patient success rates and infant health.
Second Edition
Publisher: CRC Press
Publication year: 2022
ISBN: 978-0-367-45718-1 (print), 978-1-003-02494-1 (e-book)
DOI: 10.1201/9781003024941

Case report of the month

GCAT TACG GCAT genes

This report presents a rare case of mosaicism of 47,XXY with the additional female cell line 46,XX.

The patient was a male adolescent who presented with painless bilateral gynaecomastia and had a history of hypospadias and cryptorchidism.

Hovnik T, Zitnik E, Avbelj Stefanija M, Bertok S, Sedej K, Bancic Silva V, Battelino T, Groselj U. An Adolescent Boy with Klinefelter Syndrome and 47,XXY/46,XX Mosaicism: Case Report and Review of Literature. ***Genes.*** 2022; 13(5):744.
https://doi.org/10.3390/genes13050744

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