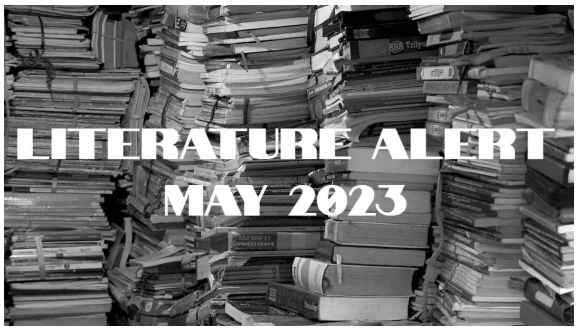


EUROPEAN ACADEMY OF ANDROLOGY

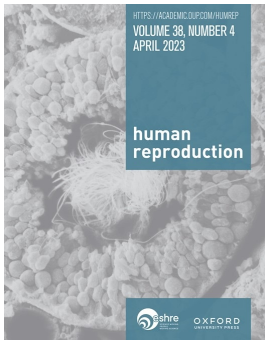
Dear EAA Members,

Some material for your continuous andrological education. Keywords in this edition: intrauterine insemination, testis volume and obesity, INSL3, testosterone therapy, incl. transgenders, testicular cancer, incl. new EAU guidelines, Klinefelter syndrome, human pangenome, novel AR transcription mechanism, testosterone in mammoths, regulation of DND1 (in mice), prenatal hypoxia and teratoma, spermatogonial differentiation, WWC2, germ cell chromatin remodelling (DOTL1, MEIOSIN), a new tool to study murine spermiogenesis, long post-mortem sperm survival.

Clinical andrology and epidemiology

Testis volume and metabolic parameters were evaluated in 268 children and adolescents followed-up for obesity. Significantly smaller testis volume was found in overweight/obese boys, especially those with hyperinsulinemia or insulin resistance.

Cannarella R, Caruso M, Condorelli RA, Timpanaro TA, Caruso MA, La Vignera S, Calogero AE. Testicular volume in 268 children and adolescents followed-up for childhood obesity-a retrospective cross-sectional study. ***Eur J Endocrinol***. 2023 Apr 5;188(4):331-342.
https://doi.org/10.1093/ejendo/lvad033



It is not necessary to perform intrauterine insemination immediately after semen processing so there is more time available to choose the optimum work-flow. In this study, a long interval (180 min) resulted in a shorter time to pregnancy and a slight improvement in cumulative ongoing pregnancies.

Statema-Lohmeijer CH, Schats R, Lissenberg-Witte BI, Kostelijk EH, Lambalk CB, Vergouw CG. A short versus a long time interval between semen collection and intrauterine insemination: a randomized controlled clinical trial. ***Hum Reprod.*** 2023 May 2;38(5):811-819.
https://doi.org/10.1093/humrep/dead044

Journal of Clinical Medicine

The authors generated an algorithm helping to predict failure of intrauterine insemination, using the parameters significantly associated with the lack of pregnancy: male age, sperm count, swim-up alkaline Comet, female obesity and long duration of infertility.

Garcia-Grau E, Oliveira M, Amengual MJ, Rodriguez-Sanchez E, Veraguas-Imbernon A, Costa L, Benet J, Ribas-Maynou J. An Algorithm to Predict the Lack of Pregnancy after Intrauterine Insemination in Infertile Patients. ***J Clin Med***. 2023 Apr 30;12(9):3225.
https://doi.org/10.3390/jcm12093225

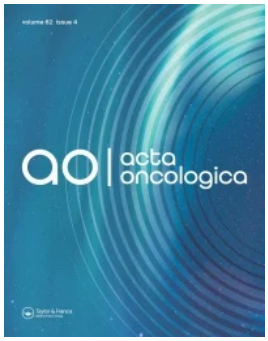
This study investigated the concomitant changes in serum concentrations of INSL3, testosterone (T) and LH during testicular suppression, and found that INSL3 complements well T as a Leydig cell marker during therapeutic suppression and in surveillance of illicit use of androgens.

Albrethsen J, Østergren PB, Norup PB, Sønksen J, Fode M, Kistorp C, Nordsborg NB, Solheim SA, Mørkeberg J, Main KM, Juul A. Serum Insulin-like Factor 3, Testosterone and Luteinizing Hormone in Experimental and Therapeutic Testicular Suppression. ***J Clin Endocrinol Metab.*** 2023 May

26:dgad291. Epub ahead of print.
https://doi.org/10.1210/clinem/dgad291

Using carotid-femoral pulse wave velocity, this study evaluated arterial stiffness in transgender men receiving long-term testosterone therapy and found greater aging-related aortic stiffening, which can increase the risk of cardiovascular events.

Cunha FS, Bachega TASS, Costa EMF, Brito VN, Alvares LA, Costa-Hong VA, Verardino RGS, Sircili MHP, de Mendonça BB, Bortolotto LA, Domenice S. Arterial Stiffness in Transgender Men Receiving Long-term Testosterone Therapy. ***J Endocr Soc.*** (JES) 2023 Mar 17;7(5):bvad040.
https://doi.org/10.1210/jendso/bvad040



This trial evaluated metabolic profile and bone mineral density (BMD) in testicular cancer survivors treated with testosterone or placebo for mild Leydig cell insufficiency. The therapy did not change BMD, there was only clinically irrelevant increase in type 1 procollagen (P1NP).

Jørgensen PL, Kreiberg M, Jørgensen N, Juul A, Oturai PS, Dehlendorff C, Lauritsen J, Wagner Y, Rosenvilde J, Daugaard G, Medici CR, Jørgensen NR, Bandak M. Effect of 12-months testosterone replacement therapy on bone mineral density and markers of bone turnover in testicular cancer survivors – results from a randomized double-blind trial. ***Acta Oncol 2023***, in press.
https://doi.org/10.1080/0284186x.2023.2207218

The 2023 update of the EAU guidelines on testicular cancer (TC) includes: a new section covering oncology treatment and quality of life thereafter; new text regarding venous thromboembolism prophylaxis; an update of the WHO 2022 pathological classification; and the revalidation of the 1997 IGCCCG prognostic risk factors.

Patrikidou A, Cazzaniga W, Berney D, Boormans J, de Angst I, Di Nardo D, Fankhauser C, Fischer S, Gravina C, Gremmels H, Heidenreich A, Janisch F, Leão R, Nicolai N, Oing C, Oldenburg J, Shepherd R, Tandstad T, Nicol D. European Association of Urology Guidelines on Testicular Cancer: 2023 Update. ***Eur Urol.*** 2023 May 12:S0302-2838(23)02732-X. Epub ahead of print.
https://doi.org/10.1016/j.eururo.2023.04.010

This somewhat controversial study found a lower prostate cancer (PCa) risk for men smoking cigarettes, attributed to lower uptake of PSA testing by smokers. By contrast, smokers, particularly obese men, had higher PCa mortality.

Jochems SHJ, Fritz J, Häggström C, Järvholm B, Stattin P, Stocks T. Smoking and Risk of Prostate Cancer and Prostate Cancer Death: A Pooled Study. ***Eur Urol.*** 2023 May;83(5):422-431. doi: 10.1016/j.eururo.2022.03.033. Epub 2022 May 4. PMID: 35523620.
https://doi.org/10.1016/j.eururo.2022.03.033

A unique study describing the hypothalamus-pituitary-thyroid (HPT) axis and thyroid ultrasonographic appearance in patients with Klinefelter syndrome (KS) throughout the life span. In all ages, KS patients had increased morpho-functional abnormalities of the thyroid gland, combined with a central feedback dysregulation sustained by the effect of hypogonadism on pituitary D2 deiodinase.

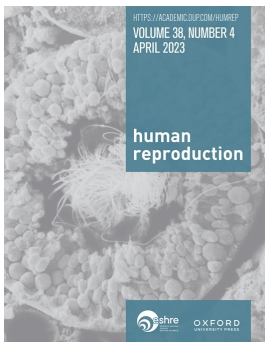
Carlomagno F, Minnetti M, Angelini F, Pofi R, Sbardella E, Spaziani M, Aureli A, Anzuini A, Paparella R, Tarani L, Porcelli T, De Stefano MA, Pozza C, Gianfrilli D, Isidori AM. Altered thyroid feedback loop in Klinefelter syndrome: a cohort study from infancy through the transition into adulthood. ***J Clin Endocrinol Metab***. 2023 May 22:dgad281. Epub ahead of print.
https://doi.org/10.1210/clinem/dgad281

A collection of articles has been put together in ***Endocrine Connections*** following the 3rd Workshop on Klinefelter Syndrome, XXX and XYY (Sept 2022, Leiden).

The previous EAA alert announced two first articles. Here is the second batch of studies of relevance for andrologists.

Biochemical identification of prepubertal boys with Klinefelter syndrome by combined reproductive hormone profiling using machine learning
Madsen A, Juul A, Aksglaede L
Unique plasma metabolite signature for adolescents with Klinefelter syndrome reveals altered fatty acid metabolism
Davis SM, Urban R et al.
Osteoporosis and bone metabolism in patients with Klinefelter syndrome
Grande G, Graziani A, Di Mambro A, Selice R, Ferlin A

Androgenetics



Bi-allelic loss-of-function variants in *INSL3* and *RXFP2* result in bilateral cryptorchidism and male infertility, whereas heterozygous variant carriers are phenotypically unaffected but can at most be regarded as a risk factor for cryptorchidism. The findings have diagnostic value for patients with familial/bilateral cryptorchidism.

Dicke AK, Albrethsen J, Hoare BL, Wyrwoll MJ, Busch AS, Fietz D, Pilatz A, Bühlmann C, Juul A, Kliesch S, Gromoll J, Bathgate RAD, Tüttelmann F, Stallmeyer B. Bi-allelic variants in INSL3 and RXFP2 cause bilateral cryptorchidism and male infertility. Hum Reprod. 2023 May 19:dead105. Epub ahead of print.
https://doi.org/10.1093/humrep/dead105

A landmark triple publication from the Human Pangenome Consortium. (1) Liao et al. demonstrated that using their pangenome datasets resulted in 34% fewer errors in calling small variants (especially in repetitive DNA regions), (2) Guarracino et al. used their data to analyse acrocentric p-arms, including Robertsonian rearrangements, and (3) Vollger et al. improved mapping of segmental duplications, including those on the sex chromosomes.

For a useful summary of these 3 articles, see the accompanying "News & Views" editorial: Massarat et al. Human pangenome supports analysis of complex genomic regions. ***Nature*** 11 May 2023:
https://www.nature.com/articles/d41586-023-01490-3

Translational and basic andrology

Two interesting papers in one of the latest ***Nature*** issues:

This study uncovered a novel mechanism explaining how mutations in *DAAM2* cause the androgen insensitivity syndrome. In response to dihydrotestosterone, DAAM2 co-localised with the androgen receptor in the nucleus to form actin-dependent transcriptional droplets.

Knerr J, Werner R, Schwan C, Wang H, Gebhardt P, Grötsch H, Caliebe A, Spielmann M, Holterhus PM, Grosse R, Hornig NC. Formin-mediated nuclear actin at androgen receptors promotes transcription. ***Nature***. 2023 May;617(7961):616-622.

The cover story confirmed a close relation between African elephants and

extinct mammoths. The male mammoths had similar androgen-dependent episodes of aggression (musth), which enhance mating success.

Cherney MD, Fisher DC, Auchus RJ, *et al.* Testosterone histories from tusks reveal woolly mammoth musth episodes. ***Nature***. 2023 May;617(7961):533-539. Erratum: Nature, PMID: 37138076.

DND1 is essential to maintain germ cell identity. This mouse KO model study distinguished two male germ cell populations during late gestation's cell cycle arrest: one with low DND1 expression (predominant) and the other subpopulation with higher levels of DND1 targeting epigenetic and translational regulators.

Ruthig VA, Hatkevich T, Hardy J, Friedersdorf MB, Mayère C, Nef S, Keene JD, Capel B. The RNA binding protein DND1 is elevated in a subpopulation of pro-spermatogonia and targets chromatin modifiers and translational machinery during late gestation. ***PLoS Genet.*** 2023 Mar 1;19(3):e1010656.
https://doi.org/10.1371/journal.pgen.1010656

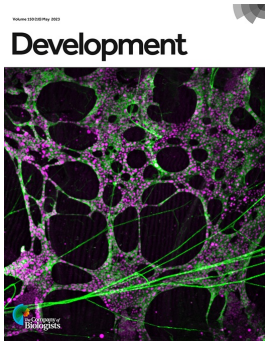
In 129/SvJ mice heterozygous for Ter, ~70% of the unilateral teratomas arise in the left testis, likely because of different vasculature. In 129/SvJ *Dnd1*$^{Ter/+}$ mice exposed in utero to low oxygen conditions the incidence of bilateral teratomas vastly increased, suggestive that hypoxia causes a delay in male germ cell differentiation that promotes teratoma initiation.

Bustamante-Marin XM. Capel B. Oxygen availability influences the incidence of testicular teratoma in *Dnd1Ter/+* mice. ***Front Genet.*** 2023 Apr 26;14:1179256.
https://doi.org/10.3389/fgene.2023.1179256

Testis maturation during the fetal to neonatal transition in mice was investigated by performing scATACseq on multiple time points.

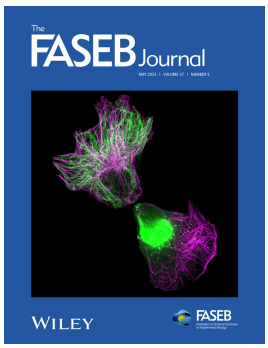
The authors identified several novel subpopulations, of both germ cell and somatic origin, and defined candidate target cell types and genes of several genome-wide association study (GWAS) signals, including those associated with testosterone levels.

Suen HC, Rao S, Luk ACS, Zhang R, Yang L, Qi H, So HC, Hobbs RM, Lee TL, Liao J. The single-cell chromatin accessibility landscape in mouse perinatal testis development. ***eLife***. 2023 Apr 25;12:e75624.
https://doi.org/10.7554/eLife.75624.sa0



A new analysis of spermatogonial (SPG) differentiation done by experts from three groups! The authors found that undifferentiated SPG (PIWIL4^{+}) are quiescent, and only GFRA1^{+} SPG are active in the cell cycle. They also described a novel subset of early differentiating SPG, suggesting that the first generation of differentiating spermatogonia arises early during the epithelial cycle.

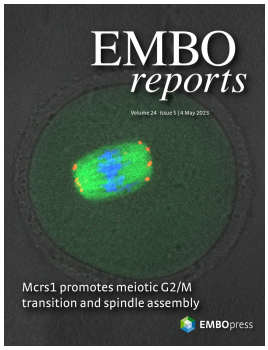
Capponi C, Palazzoli M, Di Persio S, Fera S, Spadetta G, Franco G, Wistuba J, Schlatt S, Neuhaus N, de Rooij D, Vicini E. Interplay of spermatogonial subpopulations during initial stages of spermatogenesis in adult primates. ***Development***. 2023 May 15;150(10):dev201430. Epub 2023 May 24.
https://doi.org/10.1242/dev.201430



This study comprehensively investigated the WWC2 expression and function in mammalian spermatogenesis and male fertility. The authors found the cell-specific expression of WWC2 in spermatocytes, and identified rare *WWC2* variants in the background of disturbed spermatogenesis.

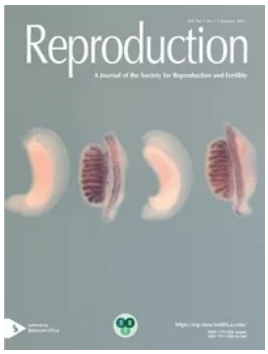
Höffken V, Di Persio S, Laurentino S, Wyrwoll MJ, Terwort N, Hermann A, Röpke A, Oud MS, Wistuba I, Kliesch S, Pavenstädt HJ, Tüttelmann F, Neuhaus N, Kremerskothen I. WWC2 expression in the testis: Implications for spermatogenesis and male fertility. ***FASEB J.*** 2023 May;37(5):e22912.
https://doi.org/10.1096/fj.202200960R

Using a KO mouse model, this study showed that DOT1L, (the H3K79-methyltransferase) controls chromatin remodelling in spermatids and subsequent reorganization and compaction of the spermatozoon genome. Spermatozoa in *Dot1l*-KO



mice have decreased head compaction and motility, leading to impaired fertility.

Blanco M, El Khattabi L, Gobé C, Crespo M, Coulée M, de la Iglesia A, Ialy-Radio C, Lapoujade C, Givelet M, Delessard M, Seller-Corona I, Yamaguchi K, Vernet N, Van Leeuwen F, Lermine A, Okada Y, Daveau R, Oliva R, Fouchet P, Zivvat A, Pflieger D, Cocquet J. DOT1L regulates chromatin reorganization and gene expression during sperm differentiation. ***EMBO Rep***. 2023 Apr 26:e56316. Epub ahead of print.
https://doi.org/10.15252/embr.202256316



This study suggests that STRA8 and MEIOSIN are both meiosis initiation factors in mammals, but their transcription is epigenetically regulated differently from each other.

Ishihara T, Fenelon JC, Griffith OW, Ishiguro KI, Renfree MB. Conserved H3K27me3-associated chromatin remodelling allows STRA8 but not MEIOSIN expression in mammalian germ cells. ***Reproduction***. 2023 Mar 24;165(5):507-520.
https://doi.org/10.1530/rep-22-0286

Methodology

GCAT TACG GCAT *genes*

A great new research tool created! The authors generated a round spermatid-specific *Acrv1-iCre* transgenic mouse line, which can conditionally knockout a gene with a >95% efficiency.

The line is a useful tool to study the function of genes during spermiogenesis, and to produce embryos with a paternally deleted allele without causing early spermatogenesis defects.

Gobé C, Ialy-Radio C, Pierre R, Cocquet J. Generation and Characterization of a Transgenic Mouse That Specifically Expresses the Cre Recombinase in Spermatids. ***Genes***. 2023; 14(5):983.
https://www.mdpi.com/2073-4425/14/5/983

Case report of the month

A testis was obtained 13 hours post-mortem from a suddenly deceased man. Multiple biopsies provided viable and motile sperm up to 106 hours *post-mortem*, extending the currently recommended time frame.

Thomas J, Bidhan J, Ledesma BR, Bitran J, Ramasamy R. A case report on the prolonged viability of postmortem human testicular sperm. ***F&S Reports***, 17 March 2023.
https://doi.org/10.1016/j.xfre.2023.03.005

European Academy of Andrology
Office: Szent István Krt. 7., 1055, Budapest, Hungary