

Dear EAA Members,

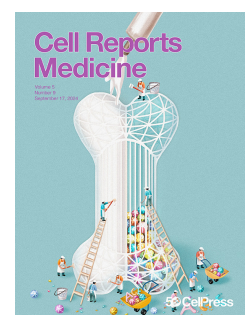
It is our pleasure to share with you the latest EAA literature digest, with interesting articles worth reading. Keywords for this issue: RANKL and spermatogenesis, T in transwomen, TART, Klinefelter syndrome, TESE and histology, microplastics in semen, genetic program for spermatogenesis, Y-chromosome in DSD subjects, *PLCZ1* variants, genomic landscape of TGCT, mechanisms of sperm and egg fusion, PIWI-interacting RNA, lncRNAs in azoospermia, IGF2BP1 and spermatogenesis, IGF2 mRNA in spermatozoa, VEGF-dependent testis vascularisation, amitrole exposure and testis effects, plant sperm biology, a quick method for histopathological analysis of mutant mice testicles, a simple method for selecting high-quality sperm in marmosets, and a new microscopy technique to image proteins and chromosomes in intact cells.

Clinical andrology and epidemiology

Treatment with the RANKL inhibitor denosumab, normally used to treat osteoporosis, increased sperm concentration in a subgroup of infertile men with serum AMH ≥ 38 pmol/L at baseline. Denosumab increased also testicular weight and inhibin B in a humanized RANKL mouse, and stimulated germ cell proliferation in *ex vivo* testis cultures.

Andreassen CH, Holt R, Juel Mortensen L, Knudsen NK, Nielsen JE, Poulsen NN, Yahyavi SK, Boisen IM, Cui Z, Ongaro L, Hjerresen JP, Toft BG, Hasselager T, Jørgensen NR, Bernard DJ, Juul A, O'Brien C, Jørgensen A, Blomberg Jensen M. Denosumab stimulates spermatogenesis in infertile men with preserved Sertoli cell capacity. *Cell Rep Med*. 2024 Oct 3:101783.

<https://doi.org/10.1016/j.xcrm.2024.101783>



Transgender women who underwent gonadectomy have lower serum testosterone (T) than cisgender women. This study assessed the feasibility of dosing T to reach female physiological range in trans-women, and found that T supplementation is feasible but with personalized titration. The treatment seems safe in the short term but will require controlled trials in larger cohorts.

Gieles NC, Kroon MAGM, Both S, Heijboer AC, Kreukels BPC, den Heijer M. Addition of testosterone to endocrine care for transgender women: a dose-finding and feasibility trial. *Eur J Endocrinol*. 2024 Aug 30;191(3):279-287. <https://doi.org/10.1093/ejendo/lvae103>

The long-term evolution of gonadal function was analysed in men with classic 21-hydroxylase deficiency (21OHD), with or without testicular adrenal rest tumours (TART). Testosterone levels were frequently below the normal range but they increased over time, together with LH, while inhibin B declined, especially in men with TART. The study highlights the need to consider gonadal suppression from glucocorticoid treatment.

Auer MK, Büyükerzümü D, Lottspeich C, Bidlingmaier M, Rieger E, Nowotny H, Tschaidse L, Auchus RJ, Reisch N. Prevalence of adrenal rest tumors and course of gonadal dysfunction in a clinical sample of men with congenital adrenal hyperplasia: a longitudinal analysis over 10 years. *Eur J Endocrinol*. 2024 Aug 30;191(3):370-380. <https://doi.org/10.1093/ejendo/lvae112>

This study examined karyotypes in spermatogonia and Sertoli cells in non-mosaic 47,XXY Klinefelter patients pre-and post-puberty. The data suggest that germ cells lose the extra X chromosome during embryonic, fetal, or neonatal life, while Sertoli cells lose it around puberty.

Gül S, Vloeberghs V, Gies I, Goossens E. Testicular mosaicism in non-mosaic postpubertal Klinefelter patients with focal spermatogenesis and in non-mosaic prepubertal Klinefelter boys. *Hum Reprod*. 2024 Oct 1;39(10):2210-2220. <https://doi.org/10.1093/humrep/deae192>

This retrospective analysis of 526 TESE patients compared the fresh specimen and the histology sample for the presence of sperm. Concordance was found in 70,7% and discordance in 29,3% of patients. To improve diagnostic accuracy, the authors suggest taking a larger biopsy for histopathology and performing cytological evaluation of the TESE cell suspension.

Castellano S, Tondo F, Bulbul O, Aprea S, Monti E, Carnesi E, Levi Setti PE, Albani E. Rate of testicular histology failure in predicting successful testicular sperm extraction. *Front Endocrinol* (Lausanne). 2024 Oct 10;15:1466675. <https://doi.org/10.3389/fendo.2024.1466675>

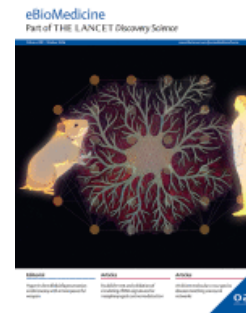
The authors developed a deep learning (DL) model to associate testicular grayscale ultrasound (TG-US) images with histology. The study analysed TG-US images from 353 azoospermic men in connection with histology after TESE. The accuracy for predicting spermatozoa presence was 83.5%, and for identifying maturation arrest vs SCOS was 92.1%.

Hu JY, Lin ZZ, et al et Gao Y, Wang Z. Prediction of testicular histology in azoospermia patients through deep learning-enabled two-dimensional grayscale ultrasound. *Asian J Androl*. 2024 Oct 4. <https://doi.org/10.4103/aja202480>



Microplastics are widespread environmental pollutants. This study confirmed the presence of multiple microplastics in human semen and urine in all men studied. The microplastics were negatively associated with sperm quality, with the strongest effect exerted by exposure to polytetrafluoroethylene (used for non-stick pans).

Zhang C, *et al* et Duan S, Cao J, Huang H. Association of mixed exposure to microplastics with sperm dysfunction: a multi-site study in China. ***EBioMedicine***. 2024 Oct;108:105369. <https://doi.org/10.1016/j.ebiom.2024.105369>



Androgenetics

This elegant multicentre study reported the deep evolutionary conservation of a core genetic program regulating spermatogenesis in flies, mice, and humans, based on network analysis of the spermatocyte transcriptomes in several species. The analysis uncovered 161 previously unknown spermatogenesis genes and three new potential genetic causes of human infertility.



Brattig-Correia R, Almeida JM, Wyrwoll MJ, Julca I, Sobral D, Misra CS, Di Persio S, Guilgur LG, Schuppe HC, Silva N, Prudêncio P, Nóvoa A, Leocádio AS, Bom J, Laurentino S, Mallo M, Kliesch S, Mutwil M, Rocha LM, Tüttelmann F, Becker JD, Navarro-Costa P. The conserved genetic program of male germ cells uncovers ancient regulators of human spermatogenesis. ***eLife***. 2024 Oct 10;13:RP95774. <https://doi.org/10.7554/elife.95774>
Commentary in eLife: <https://elifesciences.org/articles/95774#digest>

A comprehensive analysis of DNA/RNA sequences and DNA methylation in patients with 46,XX DSD, Klinefelter syndrome (47,XXY), male controls (46,XY) and female controls (46,XX) was performed in association with clinical measurements. The study found variable Y chromosome segment lengths, refining our understanding of the genetic composition in 46,XX DSD.



Berglund A, Johannsen EB, Skakkebaek A, Chang S, Rohayem J, Laurentino S, Hørlyck A, Drue SO, Bak EN, Fedder J, Tüttelmann F, Gromoll J, Just J, Gravholt CH. Integration of long-read sequencing, DNA methylation and gene expression reveals heterogeneity in Y chromosome segment lengths in phenotypic males with 46,XX testicular disorder/difference of sex development. ***Biol Sex Differ***. 2024 Oct 8;15(1):77. <https://doi.org/10.1186/s13293-024-00654-8>

Using samples and data from the UK NHS and the English 100,000 Genomes Project, this study described genomic elements underlying TGCT pathogenesis, and correlations between genomic alterations and histological TGCT subtypes. Among new findings: identification of a subgroup of adult TGCTs undergoing late whole genome duplication, and evidence for HLA loss as a prevalent mechanism of immune disruption in seminomas.



Ní Leathlobhair M, et al. et Murray MJ, Wedge DC, Verrill C; Testicular Cancer Genomics England Clinical Interpretation Partnership Consortium; Genomics England Research Consortium. Genomic landscape of adult testicular germ cell tumours in the 100,000 Genomes Project. ***Nat Commun***. 2024 Oct 26;15(1):9247. <https://doi.org/10.1038/s41467-024-53193-6>

Variants of PLCZ1 with compound heterozygous mutations, including 3 previously reported and 5 novel mutations, were identified in 5 patients experiencing total fertilisation failure (TFF) after ICSI. Functional and conformational alterations were investigated, some showing inability to trigger typical Ca²⁺ oscillations.

Li Q, Guo J, Huang G, Wu N, Chen S, Dai J, Zhang X, Zhang G, Zhi W, Yan J, Zheng R, Yan F, Yan Z, Wu L, Wu S, Ji Z, Zeng J, Lin G, Li B, Xu W. Novel PLCZ1 compound heterozygous mutations indicate gene dosage effect involved in total fertilisation failure after ICSI. *Reproduction*. 2024 Sep 16;168(4):e230466.

<https://doi.org/10.1530/rep-23-0466>



Basic and translational andrology

The molecular mechanisms of sperm and egg fusion remain poorly understood. Using an AlphaFold-Multimer screen, the authors predicted a conserved trimeric sperm complex composed of Izumo1, Spaca6, and Tmem81, and demonstrated that Tmem81 is essential for male fertility in zebrafish and mice. The complex formation creates the binding site for the egg fertilization factor Bouncer in zebrafish or JUNO in mammals.

Deneke VE, Blaha A, Lu Y, Suwita JP, Draper JM, Phan CS, Panser K, Schleiffer A, Jacob L, Humer T, Stejskal K, Krssakova G, Roitinger E, Handler D, Kamoshita M, Vance TDR, Wang X, Surm JM, Moran Y, Lee JE, Ikawa M, Pauli A. A conserved fertilization complex bridges sperm and egg in vertebrates. *Cell*. 2024 Oct 10:S0092-8674(24)01093-6.

<https://doi.org/10.1016/j.cell.2024.09.035>

Commentary in Nature: Ledford H. AlphaFold reveals how sperm and egg hook up in intimate detail. Three sperm proteins work together as matchmakers to enable fertilization in vertebrates.

<https://doi.org/10.1038/d41586-024-03319-z>



The PIWI-interacting RNA (piRNA) pathway, incl. the PIWI protein MIWI2 (PIWIL4) blocks transposons via DNA methylation during germline development in male mice. This study elucidated the mechanism of the piRNA-directed LINE1 DNA methylation, which requires a developmentally timed two-factor authentication process.

Dias Mirandela M, Zoch A, Leismann J, Webb S, Berrens RV, Valsakumar D, Kabayama Y, Auchynnikava T, Schito M, Chowdhury T, MacLeod D, Xiang X, Zou J, Rappsilber J, Allshire RC, Voigt P, Cook AG, Barau J, O'Carroll D. Two-factor authentication underpins the precision of the piRNA pathway. *Nature*. 2024 Oct;634(8035):979-985.

<https://doi.org/10.1038/s41586-024-07963-3>



RNA-seq analysis was used to screen for differentially expressed lncRNAs between azoospermic (NOA) and control males. The study identified MEG3 as promoting cell autophagy and apoptosis in vitro, with the proposed mechanism of competing with microRNA (miR)-21. Additional findings demonstrated that MEG3-miR-21-SPRY1-NF- κ B pathway probably acted as a feedback loop leading to azoospermia.

Fang X, Lu X, Ma Y, et al et, Guo H, Xiong H, Song W. Possible involvement of a MEG3-miR-21-SPRY1-NF- κ B feedback loop in spermatogenic cells proliferation, autophagy, and apoptosis. *iScience*. 2024 Sep 10;27(10):110904.

<https://doi.org/10.1016/j.isci.2024.110904>



This study explored the function of an RNA binding protein IGF2BP1 in spermatogenesis in a germ-cell specific knockout mouse model. Deficiency of Igf2bp1 resulted in decreased numbers of undifferentiated spermatogonia and increased apoptosis. Further research showed that IGF2BP1 regulates Lin28a mRNA, which is essential for expansion of undifferentiated spermatogonia.



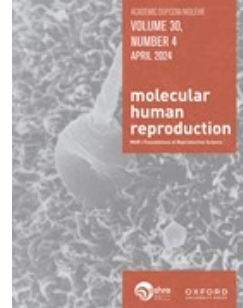
Luo J, Yang C, et al et Zhou Z, Li Z, Yao C. RNA-binding protein IGF2BP1 is required for spermatogenesis in an age-dependent manner. *Commun Biol*. 2024 Oct 21;7(1):1362.
<https://doi.org/10.1038/s42003-024-07055-y>

For basic scientists who want to learn more how IGF2BP1 stabilises RNA - this study revealed that phosphorylation modulates low-affinity interaction networks in disordered linkers to regulate RNP condensate formation and RNA metabolism.



Hornegger H, Anisimova AS, Muratovic A, Bourgeois B, Spinetti E, Niedermoser I, Covino R, Madl T, Karagöz GE. IGF2BP1 phosphorylation in the disordered linkers regulates ribonucleoprotein condensate formation and RNA metabolism. *Nat Commun*. 2024 Oct 20;15(1):9054.
<https://doi.org/10.1038/s41467-024-53400-4>

This study suggests that IGF2 mRNA in spermatozoa provides critical support for early embryo development. The authors suggest that pre-ART IGF2 mRNA levels in sperm may be used as a marker to predict the chances of obtaining good blastocysts for infertile couples undergoing ART.



Cannarella R, Rando OJ, Condorelli RA, Chamayou S, Romano S, Guglielmino A, Yin Q, Hans TG, Mancuso F, Arato I, Bellucci C, Luca G, Lundy SD, La Vignera S, Calogero AE. Sperm-carried IGF2: towards the discovery of a spark contributing to embryo growth and development. *Mol Hum Reprod*. 2024 Sep 12;30(9):gaae034.
<https://doi.org/10.1093/molehr/gaae034>

This study explored functions of MAPK signalling in embryonic testis cords in Oct4-eGFP transgenic mouse testes. They found an essential role for VEGF-dependent MEK1/2 signalling in promoting vasculature, indicating that FGF signalling through MEK1/2 regulates Sertoli cell organisation in the developing mouse testis.



Blücher RO, Lim RS, Ritchie ME, Western PS. VEGF-dependent testicular vascularisation involves MEK1/2 signalling and the essential angiogenesis factors, SOX7 and SOX17. *BMC Biol*. 2024 Oct 1;22(1):222.
<https://doi.org/10.1186/s12915-024-02003-y>

Effects of developmental exposure to herbicide amitrole were studied in rodents in parallel to animals exposed perinatally to TH-suppressing methimazole (MMI). Both chemicals caused hypothyroidism and disruption of germ and Sertoli cell development, resulting in smaller testes and reduced seminiferous tubule diameter.



Draskau MK, Strand IW, Davila RA, Ballegaard AR, Pedersen M, Ramhøj L, Rising S, Tran KM, Axelstad M, Bowles J, Rosenmai AK, Spiller CM, Svingen T. Perinatal exposure to environmental chemicals that disrupt thyroid function can perturb testis development. *Environ Pollut*. 2024 Oct 14;363(Pt 1):125117.
<https://doi.org/10.1016/j.envpol.2024.125117>

A bit far from the human, but sperm quality is important for plant embryo development and organ formation. Fertilization by a sperm cell with genetic deficiency led to abnormal root development in both embryos and seedlings, signifying a persistent paternal effect on plant development.



Cheng T, Liu Z, Li H, Huang X, Wang W, Shi C, Zhang X, Chen H, Yao Z, Zhao P, Peng X, Sun MX. Sperm-origin paternal effects on root stem cell niche differentiation. *Nature*. 2024 Oct;634(8032):220-227.
<https://doi.org/10.1038/s41586-024-07885-0>

A new quick method for histopathological analysis of mutant mice testicular sections (H&E) using automated segmentation and cellular composition analysis of six mouse reproductive mutants of DAZ and PUMILIO gene family. The analysis uncovered novel evidence supporting the synergistic interaction of Sertoli PUM1 and PUM2 in maintaining average testis size, and redundant roles of DAZ family proteins DAZL and BOULE in meiosis.

Ao N *et al*, Xu EY. Rapid detection of mouse spermatogenic defects by testicular cellular composition analysis via enhanced deep learning model. **Andrology**. 2024 Oct 7.

<https://doi.org/10.1111/andr.13773>

Common marmoset (*Callithrix jacchus*) is a valuable nonhuman primate model for experimental research. The authors identified characteristics of high-quality semen in this species, and validated using computer-assisted sperm analysis (CASA) and the swim-up method. The study defines robust, statistically supported reference values for evaluating marmoset semen samples.

Sadeghi N, Mustoe A, Ross CN, McCarrey JR, Hermann BP. Benchmarks defining high-quality sperm in the common marmoset (*Callithrix jacchus*). **Andrology**. 2024 Oct 22.

<https://doi.org/10.1111/andr.13782>

We usually do not highlight preprints but this one is extraordinary! The authors created a new technology; “expansion in situ genome sequencing (ExIGS)” that enables sequencing of genomic DNA and the localization of the nuclear protein in intact single cells. ExIGS may serve as a platform for connecting nuclear abnormalities to changes in gene regulation across disease contexts, including andrology.

bioRxiv
THE PREPRINT SERVER FOR BIOLOGY

Labade AS, Chiang ZD, Comenho C, Reginato PL, Payne AC, Earl AS, Shrestha R, Duarte FM, Habibi E, Zhang R, Church GM, Boyden ES, Chen F, Buenrostro JD. Expansion in situ genome sequencing links nuclear abnormalities to hotspots of aberrant euchromatin repression. **bioRxiv** (PREPRINT)

<https://doi.org/10.1101/2024.09.24.614614>

Commentary in Nature: Ledford H. Microscopy technique allows scientists to image how proteins and chromosomes interact in an intact cell.

<https://rb.gy/46vs3e>

European Academy of Andrology

Office: Szent István Krt. 7., 1055, Budapest, Hungary

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