Dear EAA Members,

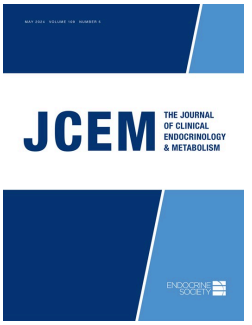
A monthly load of noteworthy andrology-related articles for you, covering reproductive system problems in all ages, and bringing some ground-breaking findings in basic research. Key words in this edition: global fertility decline, increasing paternal age, paediatric and transitional andrology (anogenital distance, cryptorchidism, transmasculine youth), hypogonadism, cryptozoospermia, guidelines in vasectomy, Peyronie disease, androgen-deprivation therapy, endocrine disrupters, evolution of genetic regulation of spermatogenesis, 'gut-germline axis', epigenetic reprogramming, DNA methylation patterns in spermatogenesis, modulation of immune system by androgens, PBX1 and Leydig cell function, testicular inflammation, uro-oncogenomic platform, microfluidic sperm sorting, 3D testis scaffold, and more.

Clinical andrology and epidemiology

The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2021 produced a demographic assessment of key fertility indicators at global and national levels from 1950 to 2021 and forecast fertility trends to 2100. Fertility is declining, with rates below replacement level in more than half of all countries in 2021, but with considerable heterogeneity. Future fertility rates will continue to decline worldwide. These changes will have far-reaching consequences due to ageing populations in higher-income countries, combined with an increasing share of livebirths among the poorest regions.

GBD 2021 Fertility and Forecasting Collaborators. Global fertility in 204 countries and territories, 1950-2021, with forecasts to 2100: a comprehensive demographic analysis for the Global Burden of Disease Study 2021. ***Lancet***. 2024 May 18; 403(10440):2057-2099.
https://doi.org/10.1016/s0140-6736(24)00550-6

The anogenital distance (AGD) is considered a postnatal readout of early fetal androgen action. This unique study found that AGD is sexual dimorphic already in the 3rd trimester and correlated with AGD in infancy. AGD is associated with body size but not circulating androgen levels at minipuberty.



Fischer MB, Mola G, Priskorn L, Scheel L, Hegaard HK, Sundberg K, Frederiksen H, Andersson AM, Juul A, Hagen CP. Longitudinal evaluation of fetal and infant AGD in healthy children: association with penile size, testosterone and DHT. ***J Clin Endocrinol Metab***. 2024 May 18:dgae342.
https://doi.org/10.1210/clinem/dgae342

The study assessed testicular volume at puberty in boys who underwent orchidopexy at 9 or at 36 months, compared to boys with spontaneous postnatal descent.
Orchidopexy at 9 m resulted in better testicular growth compared to 3 y. but did not reach the corresponding volumes of their scrotal counterparts. This indicates that earlier surgery is beneficial to testicular growth.

Kollin C, Nordenskjöld A, Ritzén M. Testicular volume at puberty in boys with congenital cryptorchidism randomised to treatment at different ages. ***Acta Paediatr***. 2024 May 11.
https://doi.org/10.1111/apa.17270

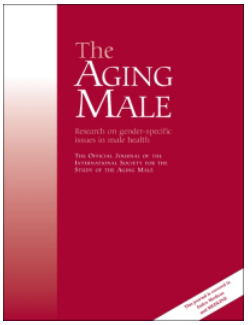
The EAA Centre in Lodz investigated changes in testicular volume, atrophy index and testicular growth in patients with different types of undescended testes (UDT) from infancy to puberty.
The study revealed continuous growth of UDTs until puberty independently of their position, including intra-abdominal.

Nowak M, Niedzielski J, Slowikowska-Hilczer J, Walczak-Jedrzejowska R, Marchlewska K. Undescended Testes Growth Potential in Relation to Testis Position from Diagnosis until Puberty. ***J Clin Med***. 2024 Apr 29;13(9):2620.
https://doi.org/10.3390/jcm13092620

Transmasculine youth (assigned female at birth) can be treated with testosterone (T), sometimes preceded by gonadotropin-releasing hormone agonist (GnRHa) for puberty blockade. This study found that the GnRHa pre-treatment in early puberty before T increases final adult height, which can be important for many transmen.

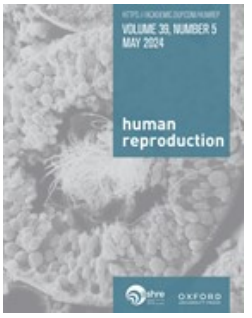
Persky RW, Apple D, Dowshen N, Pine E, Whitehead J, Barrera E, Roberts SA, Carswell J, Stone D, Diez S, Bost J, Dwivedi P, Gomez-Lobo V. Pubertal Suppression in Early Puberty Followed by Testosterone Mildly Increases Final Height in Transmasculine Youth. ***J Endocr Soc (JES)*** 2024 May 2;8(6):bvae089.
https://doi.org/10.1210/jendso/bvae089

Based on large cohort studies, incl. EMAS, this collaborative effort established INSL3 reference range for adult men. INSL3 is constitutively secreted by functional Leydig cells with little intra-individual variation, but slowly declines with age from 35 years. Reduced INSL3 is significantly associated with increasing age-related morbidity.



Anand-Ivell R, Heng K, Antonio L, Bartfai G, Casanueva FF, Maggi M, O'Neill TW, Punab M, Rastrelli G, Slowikowska-Hilczer J, Tournoy J, Vanderschueren D, Wu FC, Huhtaniemi IT, Ivell R. Insulin-like peptide 3 (INSL3) as an indicator of leydig cell insufficiency (LCI) in Middle-aged and older men with hypogonadism: reference range and threshold. ***Aging Male.*** 2024; 27(1):2346322.
https://doi.org/10.1080/13685538.2024.2346322

Two distinct subgroups of cryptozoospermic patients (sperm conc. < 0.1 million/ml) were revealed based on testicular volume, tissue composition, and FSH levels. The two subgroups could represent a general response mechanism to the reduced output of sperm, which may be associated with a progressive phenotype.



Schülke LC, Wistuba J, Nordhoff V, Behre HM, Cremers JF, Kliesch S, Di Persio S, Neuhaus N. Identification of two hidden clinical subgroups among men with idiopathic cryptozoospermia. ***Hum Reprod***. 2024 May 2;39(5):892-901.
https://doi.org/10.1093/humrep/deae013

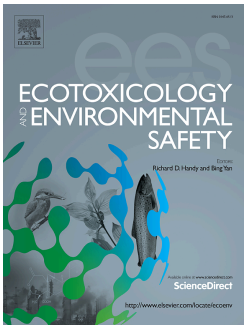
Two studies of special interest for urologists published in Andrology. The first study evaluated expectations of patients with Peyronie disease before treatment, which can be unrealistic. The authors underlined the importance of assessing patients' expectations before starting treatment. The second paper is an expert analysis of surgical vasectomy guidelines and alternative methods.

Schäfer L, Cremers JF, Witschel B, Schüttfort V, Nieder TO, König F, Vetterlein MW, Gild P, Dahlem R, Fisch M, Kliesch S, Soave A. What do patients with Peyronie's disease expect from therapy? A prospective multi-center study. ***Andrology***. 2024 May;12(4):821-829.
https://doi.org/10.1111/andr.13538
Pelzman D, Honig S, Sandlow J. Comparative review of vasectomy guidelines and novel vasal occlusion techniques. ***Andrology***. 2024 May 22.
https://doi.org/10.1111/andr.13665

Androgen deprivation therapy (ADT) and radiotherapy are used to manage prostate cancer after surgery. The length of ADT treatment was studied in the RADICALS-HD trial, and the results are described in two articles in *The Lancet*. The longer course ADT resulted in a 6% benefit in metastasis-free survival but that was counterbalanced by side-effects, including a high risk of life-long hypogonadism.

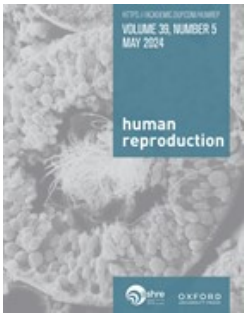
Parker CC, Clarke NW *et al* et RADICALS investigators. Adding 6 months of androgen deprivation therapy to postoperative radiotherapy for prostate cancer: a comparison of short-course versus no androgen deprivation therapy in the RADICALS-HD randomised controlled trial. ***Lancet*** 2024; (May 16),
https://doi.org/10.1016/S0140-6736(24)00548-8
Parker CC, Kynaston H, *et al* et RADICALS investigators. Duration of androgen deprivation therapy with postoperative radiotherapy for prostate cancer: a comparison of long-course versus short-course androgen deprivation therapy in the RADICALS-HD randomised trial. ***Lancet*** 2024; (May 16),
https://doi.org/10.1016/S0140-6736(24)00549-X
Commentary by Pollack & Dal Pra: Androgen deprivation therapy combined with postoperative radiotherapy for prostate cancer management.
https://doi.org/10.1016/S0140-6736(24)00802-X

Glyphosate is among the most commonly used herbicides. It has been detected in human seminal plasma in concentrations four times higher than in blood. In men with measurable glyphosate, oxidative stress index and biomarkers were significantly higher.



Vasseur C, Serra L, El Balkhi S, Lefort G, Ramé C, Froment P, Dupont J. Glyphosate presence in human sperm: First report and positive correlation with oxidative stress in an infertile French population. ***Ecotoxicol Environ Saf***. 2024 Jun 15;278:116410.
https://doi.org/10.1016/j.ecoenv.2024.116410

Over the last decades, mean paternal ages at childbirth have risen drastically, alarming researchers. This article unveiled that in many countries this is not unprecedented, and the paternal age trend is U-shaped.



Willführ KP, Klüsener S. The current 'dramatically' high paternal ages at childbirth are not unprecedented. Hum Reprod. 2024 Apr 3:deae067.
https://doi.org/10.1093/humrep/deae067

Andro-(epi)genetics

Genome-wide DNA methylation changes during human spermatogenesis and alterations in infertile men were examined in this excellent study. The imprinted gene methylation is stable, but a global decline occurs in primary spermatocytes followed by selective re-methylation to establish a spermatids/sperm-specific methylome, which correlates with expression of several genes. In disturbed spermatogenesis, germ cells exhibited considerable DNA methylation alterations.

Siebert-Kuss LM, Dietrich V, Di Persio S, Bhaskaran J, Stehling M, Cremers JF, Sandmann S, Varghese J, Kliesch S, Schlatt S, Vaquerizas JM, Neuhaus N, Laurentino S. Genome-wide DNA methylation changes in human spermatogenesis. ***Am J Hum Genet.*** 2024 May 11:S0002-9297(24)00132-0.
https://doi.org/10.1016/j.ajhg.2024.04.017

This important study described the conserved evolutionary origin of a core genetic program regulating spermatogenesis in flies, mice, and humans. The analysis uncovered also 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, Shekhar Misra C, 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, 13: RP95774 (reviewed preprint).
https://doi.org/10.7554/eLife.95774.1

Translational and basic andrology

A ground-breaking study. Altering gut bacteria in male mice induced metabolic changes in the testes, reduced sperm count and affected sperm small RNAs. Some of the offspring had lower body weight and increased mortality. This effect was limited to the first generation and reversible. The authors suggest the existence of 'gut-germline axis' in mice, which is sensitive to environmental exposures and can influence placental development by yet unknown mechanism.

Argaw-Denboba A, Schmidt TSB, Di Giacomo M, Ranjan B, Devendran S, Mastrorilli E, Lloyd CT, Pugliese D, Paribeni V, Dabin J, Pisaniello A, Espinola S, Crevenna A, Ghosh S, Humphreys N, Boruc O, Sarkies P, Zimmermann M, Bork P, Hackett JA. Paternal microbiome perturbations impact offspring fitness. ***Nature***. 2024 May;629(8012):652-659.
https://doi.org/10.1038/s41586-024-07336-w

An advance towards gametogenesis in vitro. The Saitou lab established a strategy for inducing epigenetic reprogramming and differentiation of pluripotent stem cell (PSC)-derived human PGC-like cells (hPGCLCs) into mitotic pro-spermatogonia or oogonia. They found that BMP signalling is a key driver of these processes, and that TET1 demethylase-deficient hPGCLCs differentiate into extraembryonic cells.

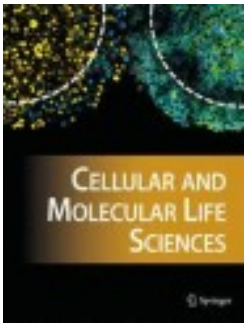
Murase Y, Yokogawa R, Yabuta Y, Nagano M, Katou Y, Mizuyama M, Kitamura A, Puangsricharoen P, Yamashiro C, Hu B, Mizuta K, Ogata K, Ishihama Y, Saitou M. In vitro reconstitution of epigenetic reprogramming in the human germ line. ***Nature***. 2024 May 20.
https://doi.org/10.1038/s41586-024-07526-6

Commentary in Nature: Lab-grown sperm and eggs: 'epigenetic' reset in human cells paves the way.
https://doi.org/10.1038/d41586-024-01404-x

This remarkable work constructed a single-cell transcriptomic atlas from 17 tissues in mouse and explored the effects of sex and androgens on the molecular programs and cellular populations. Among the main findings: immune gene expression and immune cell populations are modulated by androgens. The authors developed a pipeline to dissect the androgen–AR axis and provided a web tool (https://casadbtools.com/andr_effect).

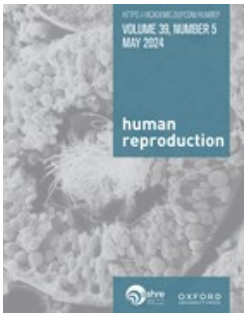
Li F, Xing X, Jin Q, et al et Bai F, Gao D. Sex differences orchestrated by androgens at single-cell resolution. ***Nature***. 2024 May;629(8010):193-200.
https://doi.org/10.1038/s41586-024-07291-6

PBX1, a transcription factor, previously linked to a human developmental disorder comprising genitourinary abnormalities, was in this study identified as a regulator of Leydig cell differentiation and testosterone (T) production in mice. Conditional deletion of Pbx1 in Leydig cells caused testicular dysgenesis and sterility. Loss of Pbx1 affected the fate decisions of progenitor Leydig cells, altered the transcription of genes associated with steroidogenesis, and decreased T levels.



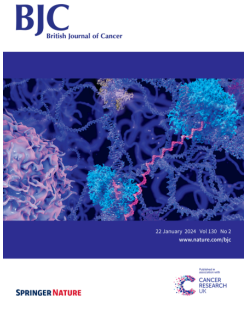
Wang FC, Zhang XN, Wu SX, He Z, Zhang LY, Yang QE. Loss of PBX1 function in Leydig cells causes testicular dysgenesis and male sterility. ***Cell Mol Life Sci.*** 2024 May 9;81(1):212.
https://doi.org/10.1007/s00018-024-05249-5

Chemokine signalling pathway CCR2/CCL2 is increased in experimental autoimmune orchitis, and it is modulated by activin A, mainly in macrophages. Consequently, inhibition of CCR2 or activin A could serve as a potential therapeutic strategy for reducing testicular inflammation.



Hasan H, Peng W, Wijayarathna R, Wahle E, Fietz D, Bhushan S, Pleuger C, Planinić A, Günther S, Loveland KL, Pilatz A, Ježek D, Schuppe HC, Meinhardt A, Hedger MP, Fijak M. Monocytes expressing activin A and CCR2 exacerbate chronic testicular inflammation by promoting immune cell infiltration. ***Hum Reprod.*** 2024 May 22:deae107.
https://doi.org/10.1093/humrep/deae107

Immune cell infiltration is common in testicular germ cell tumours (TGCT). This study analysed the T cell subtypes in TGCT. The main finding of rare Treg and Tfh cells suggests their involvement in TGCT pathobiology.



Islam R, Heyer J, Figura M, Wang X, Nie X, Nathaniel B, Indumathy S, Hartmann K, Pleuger C, Fijak M, Kliesch S, Dittmar F, Pilatz A, Wagenlehner F, Hedger M, Loveland B, Hotaling JH, Guo J, Loveland KL, Schuppe HC, Fietz D. T cells in testicular germ cell tumors: new evidence of fundamental contributions by rare subsets. ***Br J Cancer***. 2024 Apr 22.
https://doi.org/10.1038/s41416-024-02669-9

This study re-analysed the single-cell RNA-sequencing libraries of 34 post-pubescent human testes to generate an integrated atlas of germ cell differentiation. In contrast to rodents, a fetal-like state 0B of spermatogonial stem cells (SSC) seems to be maintained as a reserve population.

Bush SJ, Nikola R, Han S, Suzuki S, Yoshida S, Simons BD, Goriely A. Adult Human, but Not Rodent, Spermatogonial Stem Cells Retain States with a Foetal-like Signature. ***Cells***. 2024 Apr 24;13(9):742.
https://doi.org/10.3390/cells13090742

This study detected 12 types of microplastics in all investigated canine and human testes, highlighting the pervasive presence of microplastics in the male reproductive system, with potential consequences on male fertility.

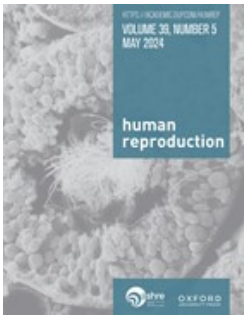
Hu CJ, Garcia MA, Nihart A, Liu R, Yin L, Adolphi N, Gallego DF, Kang H, Campen MJ, Yu X. Microplastic presence in dog and human testis and its potential association with sperm count and weights of testis and epididymis. ***Toxicol Sci.*** 2024 May 15:kfae060.
https://doi.org/10.1093/toxsci/kfae060

Methodology

The authors developed the Uro-oncogenomics Viewer (UncoVer; https://uncover.genouest.org), a web resource for sharing, and exploring SSO data sets in uro-oncology. UncoVer has a user-friendly interface that is compliant with modern web standards, and is accessible from any device.

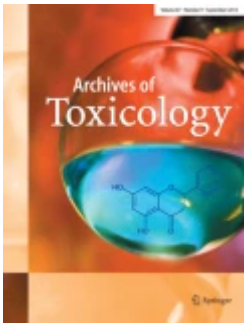
Lecuyer GCV, Lardenois A, Chalmel F; Uro-oncogenomics Viewer Group. UncoVer: A Web-based Resource for Single-cell and Spatially Resolved Omics Data in Uro-oncology. ***Eur Urol Oncol***. 2024 Apr 29:S2588-9311(24)00100-7.
https://doi.org/10.1016/j.euo.2024.04.008

Semen specimens from 50 normozoospermic and 50 non-normozoospermic men were sorted using microfluidic sperm sorting (MSS), density-gradient centrifugation and swim-up methods. The MSS selected sperm with improved parameters, lower DNA fragmentation, and higher fertilizing potential.



Sheibak N, Amjadi F, Shamloo A, Zarei F, Zandieh Z. Microfluidic sperm sorting selects a subpopulation of high-quality sperm with a higher potential for fertilization. ***Hum Reprod.*** 2024 May 2;39(5):902-911.
https://doi.org/10.1093/humrep/deae045

This study developed a neonatal mouse 3D testicular co-culture scaffold model that allows assessment of various aspects of male developmental reproductive toxicity. Di-n-butyl phthalate (DBP), an environmental contaminant, was used as a model compound to illustrate the utility of the model. DBP induced a concentration-dependent reduction in the number of Leydig cells and testosterone levels.



Almamoun R, Pierozan P, Karlsson O. Mechanistic screening of reproductive toxicity in a novel 3D testicular co-culture model shows significant impairments following exposure to low-dibutyl phthalate concentrations. ***Arch Toxicol.*** 2024 May 20.
https://doi.org/10.1007/s00204-024-03767-6