Von Behring-Röntgen-Symposium 2022

The Epididymis

Organizers: A. Meinhardt, R. Middendorff, T. Berger

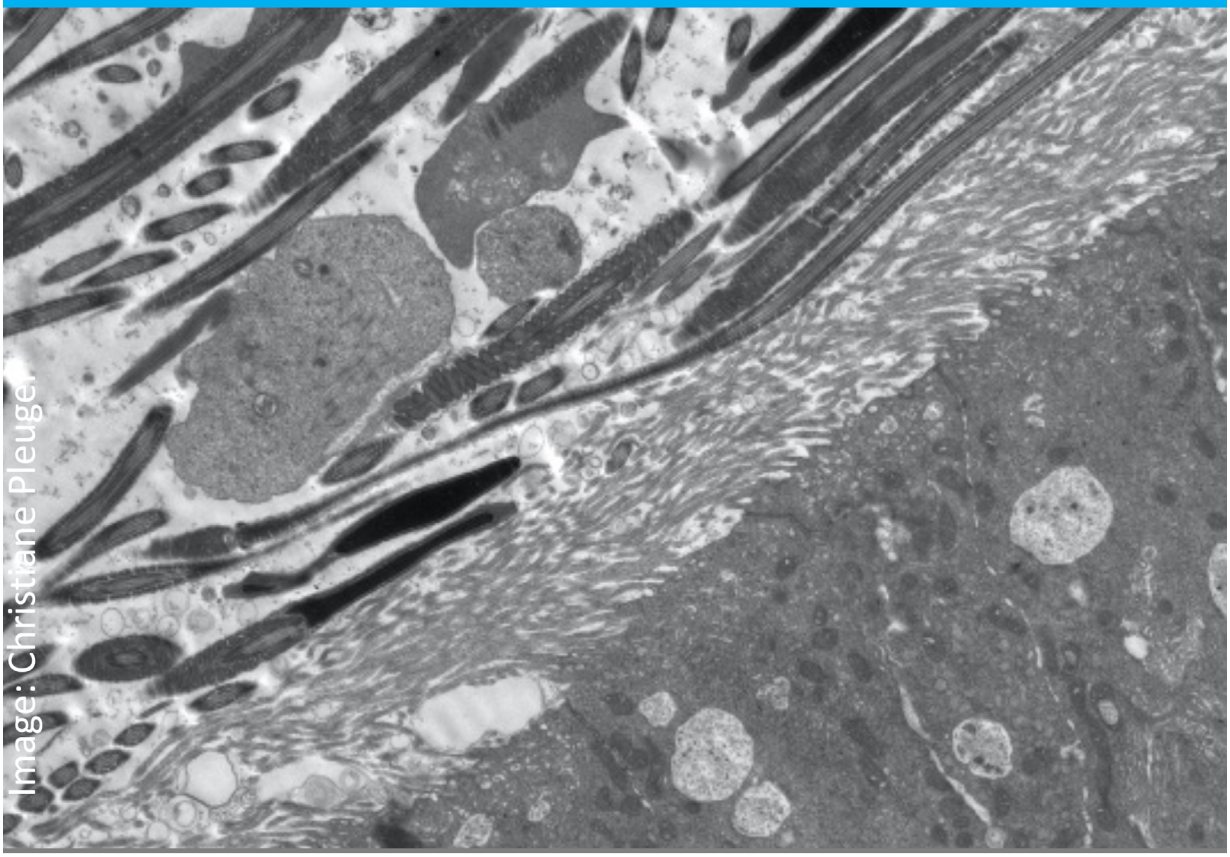

Image: Christiane Pleuger

4 - 7 September 2022 | Giessen | Germany

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www.epididymis2022.de

Dear Colleagues!

It is a great pleasure for us to welcome you to the von Behring-Röntgen Symposium 'The Epididymis' in Giessen. This forum will allow an intensive exchange of recent advances in the exciting field of epididymis research with a faculty of leading experts. Participants from all continents will present their latest research during the workshop ranging from clinical to basic science aspects in a highly interactive atmosphere. There is much opportunity for discussions and fun in an open atmosphere aided by social events and options to contribute by short oral presentations and posters displayed in two poster sessions.

Beside engaging in an exciting scientific program, we cordially invite you to informally continue your discussions during the breaks (food and drinks provided), social events such as our welcome reception, the congress dinner, a boat tour on the river Lahn or a guided tour through the historic city of Marburg.

The meeting venue is in the historic teaching building of the Medical Faculty of Justus-Liebig University. Being a lively university city, Giessen is located centrally in Germany, only 70 min train ride away from Frankfurt airport. The workshop venue is in walking distance to the original 19th century laboratories of Justus von Liebig, founder of the world's first major school of chemistry and patron of our university, in the "Liebig Museum", as well as the "Mathematikum", the world's first mathematical science centre.

Foremost, the generous support of the von Behring-Röntgen Foundation should be highlighted. The foundation supports research in the medical schools of Giessen and Marburg university and offers support for international symposia. The workshop, however, would not have been possible without the efforts of the Scientific Committee and Local Organising Committee as well as staff members and students of our institutes to whom we are especially grateful.

Welcome to Epididymis 2022!

On behalf of the LOC,

Andreas Meinhardt
Ralf Middendorff
Thomas Berger

Workshop Organisation

Andreas Meinhardt
Ralf Middendorff
Thomas Berger

Local Organising Committee

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Thomas Berger
Sudhanshu Bhushan
Monika Fijak
Aileen Harrer
Jörg Klug
Pia Jürgens
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Ralf Middendorff
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Andreas Meinhardt, Giessen, Germany
Ralf Middendorff, Giessen, Germany
Brett Nixon, Newcastle, Australia
Bernard Robaire, Montreal, Canada
Winnie Shum, Shanghai, China

Venue

Medizinisches Lehrzentrum Seltersberg
Klinikstraße 29, 35392 Giessen, Germany

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The following companies are acknowledged for scientific information material and give-aways:

Miltenyi Biotec
NEW ENGLAND BioLabs Inc.
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Promega

Oral presentations of invited speakers

The time slot is either 30 minutes (20 minutes talk and 10 minutes discussion) or 20 minutes (15 minutes talk and 5 minutes discussion). For a smooth run through the program we would appreciate presenters sticking to the schedule.

Short oral presentations selected from submitted abstracts

The time slot for short orals is 15 minutes, 10 minutes for the talk and 5 minutes for the discussion. For a smooth run through the program, we would appreciate presenters sticking to the schedule.

Files for oral presentation

All speakers are asked to approach the media desk on Sunday and hand in their presentation file on a USB stick, if possible. Make sure that your presentation is running under Microsoft Office Powerpoint 2016 that will be installed on the presentation laptop. If you cannot hand in your presentation on Sunday, please use the coffee break prior to your session at the latest. The beamer is preset at a 16:10 format (16 wide, 10 high).

Poster presentations

The poster boards are set up in theatre 1 downstairs in the basement. Poster boards are 150 cm high and 120 cm wide. Each board is marked; sufficient pins for installing the poster are available. In consideration of a smooth hand-over to the prior and following event, we would appreciate respecting the designated time:

Time for installing posters: Sunday, September 4th, 17:00 – 17:30

Time for removing posters: Wednesday, September 7th, 10:30 – 11:00

Awards

The best poster presentation and the best short oral presentation will be awarded, respectively. Presence at the award ceremony is a prerequisite for being considered.

Baggage storage

Your baggage can be stored at the venue in a baggage room. Please be aware that we cannot take liability for your belongings stored there.

Arrival

The venue building “Medizinisches Lehrzentrum” (MLZ) is located in 10 minutes walking distance from Giessen main railway station (“Gießen Bahnhof”). Train schedules (e.g. from Frankfurt airport) can be viewed on https://www.bahn.com/en (booking online or on platform). Please note that as of now (August 2022), wearing a mask is mandatory on public transport in Germany. For the few who will arrive by car the car park on the clinic campus is a good choice (see map on page 6).

Car parking fee	< 30 minutes	free
	per hour or part thereof	€ 1.50
	daily rate	€ 10

Corona measures

Wearing a mask is recommended.

Bus transfer to Social Activities and Workshop Dinner

Bus stop: Klinikstraße, opposite MLZ building, across the street
Buses leave on time.

Privacy statement

Photos are taken during the meeting and can be displayed for reporting purposes on websites associated with the meeting, social media, and distribution among the participants. If you object, please inform the LOC.

Venue MLZ (Medizinisches Lehrzentrum, Klinikstrasse 29

Giessen Main Station "Bahnhof"

Footbridge Shortcut for Pedestrians

Only public access for cars

No public access for cars

Public car park

Bus stop: Departure for workshop dinner

Sunday, September 4th, 2022

14:00-16:00 **Registration**

16:00-16:15 **Welcome & Opening of the Workshop**

Andreas Meinhardt, Workshop Organisation
Lars Wittek, President of the *Behring-Röntgen Foundation*
Wolfgang Weidner, Dean of the Faculty of Medicine, Justus Liebig University

16:15-17:00 **Opening Keynote Lecture**

Chair: Bernard Robaire

Lumicrine factors in the regulation of spermatogenesis
Masahito Ikawa, Osaka, Japan

17:00-17:30 **Coffee Break & Installation of Posters**

17:30-19:00 **Presentation of Even-numbered Posters**

19:00 **Welcome Reception with Dinner (on site)**

Monday, September 5th, 2022

9:00-10:30 **Session I: Human Epididymis and Epididymitis**

Chairs: Terry Turner & Jörg Klug

9:00-9:30 Acute epididymitis - a clinician's perspective
Adrian Pilatz, Giessen, Germany

9:30-10:00 Male genital tuberculosis
Ekaterina Kulchavenya, Novosibirsk, Russia

10:00-10:30 Cell identity in the human proximal epididymis
Ann Harris, Cleveland, USA

10:30-11:00 **Coffee Break**

11:00-12.30 **Short Oral Presentations**

Chair: Fabrice Saez

11:00-11:15 **SO1** - Urinary phthalate metabolites and small non-coding RNAs from seminal plasma extracellular vesicles among men undergoing infertility treatment.
Richard Pilsner, Detroit, USA

11:15-11:30 **SO2 -** Androgen-regulated epididymis barrier function in the human proximal epididymis
Shih-Hsing Leir, Cleveland, USA

11:30-11:45 **SO3 -** Restoration of segmented mouse and rat epididymal transcriptional profiling data in a relaunch of the mammalian reproductive genetics database
Daniel Johnston, Bethesda, USA

11:45-12:00 **SO4 -** Investigation of GM1-enriched murine epididymosomes with segmental expression and lumicrine regulation
Danielle Sosnicki, Ithaca, USA

12:00-12:15 **SO5 -** Functional and structural particularities regarding the transit of the epididymal duct between neighboring segments in rodents and men
Thorben Hau, Giessen, Germany

12:15-13:30 Lunch (on site)

13:30 Bus Departure for Both Social Activities from Congress Site

Alt. 1 Giessen: boat tour on the river Lahn and guided tour of the city
Alt. 2 Marburg: guided tour „Emil von Behring - Savior of Children“
(Return bus from Marburg: 17:00 at Elisabeth Church)

18:00-19:30 Presentation of Odd-numbered Posters with Beer, Wine & Nibbles

Tuesday, September 6th, 2022

9:00-10:20 Session II: Immunobiology and Immunopathology of the Epididymis

Chairs: Mark Hedger & Aileen Harrer

9:00-9:20 The role of the activins in epididymal function and immunopathology
Rukmali Wijayarathna, Melbourne, Australia

9:20-9:40 The immune context of the mammalian epididymis
Rachel Guiton, Clermont-Ferrand, France

9:40-10:00 Key players in immune surveillance of the epididymis
Maria Agustina Battistone, Boston, USA

10:00-10:20 Region-specific epididymal responses to inflammation: insights from experimental models of epididymitis
Erick Silva, Botucatu, Brazil

10:20-11:00 Coffee Break

11:00-12:30 Short Oral Presentations

Chair: Christina Avellar

11:00-11:15 **SO6 -** The regional distribution of resident immune cells shapes distinct immunological environments along the murine epididymis
Christiane Pleuger, Giessen, Germany

11:15-11:30 **SO7 -** Uropathogenic Escherichia coli infection leads to the formation of tertiary lymphoid organs in the epididymis
Hiba Hasan, Giessen, Germany

11:30-11:45	**SO8 -** Investigating retinoic acid synthesis and signaling in the epididymal epithelium *Cathryn Hogarth, Wodonga, Australia*
11:45-12:00	**SO9 -** DNA integrity alteration in mouse spermatozoa after short exposure to dibutyl phthalate or bisphenol AF and remediation by an antioxidant oral supplementation. *Elisa Hug, Clermont-Ferrand, France*
12:00-12:15	**SO10 -** Runx transcription factors are necessary for maintenance of proper differentiation of epididymal epithelial cells *Petra Sipilä, Turku, Finland*
12:15-12:30	**SO11 -** Do wireless communications networks impact the male reproductive system? *Geoffry De Iuliis, Newcastle, Australia*
12:30-14:00	**Lunch**
14:00-15:30	**Session III: Epididymis and Sperm Maturation**
	Chairs: Brett Nixon & Thomas Berger
14:00-14:30	Shifting the paradigm of sperm maturation; global (phospho)proteomic profiling of epididymal sperm *David Skerrett-Byrne, Newcastle, Australia*
14:30-15:00	*Paternal exposures, epididymal maturation, and epigenetic programming of sperm* *Michael Golding, College Station, USA*
15:00-15:30	Regionalized mapping of biological functions in the epididymis using large-scale *in situ* approaches *Charles Pineau, Rennes, France*
15:30-16:10	**Coffee Break**
16:10-17:40	**Short Oral Presentations**
	Chair: Colin Conine
16:10-16:25	**SO12 -** Epididymal primary cilia induce intracellular calcium signaling and gene expression in response to fluid shear stress *Sepideh Fakhari, Québec City, Canada*
16:25-16:40	**SO13 -** Using cryo-electron tomography to study the cytoplasmic droplet *Tzviya Zeev-Ben-Mordehai, Utrecht, Netherlands*
16:40-16:55	**SO14 -** Epididymal acquired sperm miRNAs and male fertility *Natalie Trigg, Philadelphia, USA*
16:55-17:10	**SO15 -** Comprehensive protein profiling of seminal CD63+ small extracellular vesicles unveils their biological relevance and tissue-specific origin *Meritxell Jodar Bifet, Barcelona, Spain*

17:10-17:25	**SO16 -** Oxytocin may recover impaired epididymal contractile function in patients with spinal cord injury who wish to father children *Andrea Mietens, Giessen, Germany*
17:25-17:40	**SO17 -** Mapping the interaction between mouse epididymal protease inhibitor (EPPIN) and the seminal vesicle secreted protein 2 (SVS2) *Noemia Mariani, São Paulo, Brasil*
18:00	**Bus Departure from Congress Site**
18:30	**Congress Dinner at the "*Lokschuppen*" Marburg**

Wednesday, September 7th, 2022

9:00-10:30	**Session IV: Physiology and Cell Biology of the Epididymis** *Chair: Joël Drevet & Ralf Middendorff*
9:00-9:30	Illuminating cell dynamics in murine epididymis by live cell imaging *Tsuyoshi Hirashima, Singapore*
9:30-10:00	Host defense functions of the epididymal amyloid matrix *Gail Cornwall, Lubbock, USA*
10:00-10:30	Motility of efferent duct cilia aids passage of sperm cells through the male reproductive system *Heymut Omran, Münster, Germany*
10:30-11:00	**Coffee Break**
	Chair: Barry Hinton
11:00-11:30	Epididymal injury and morphological sperm anomalies in a high fat diet (HFD) model *Rosanna Chianese, Naples, Italy*
11:30-12:15	Breakout sessions to discuss future of research on the epididymis
12:15-12:30	Reports back from breakout sessions
12:30-12:45	**Award Presentations, Farewell**
12:45	**Adjourn**

Lumicrine factors in the regulation of spermatogenesis

Masahito Ikawa

Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 5650871, Japan

CRISPR/Cas9 system has opened a new era for reverse genetics. In 2013, we developed the efficient gene knockout (KO) system in mice by injecting the plasmids expressing humanized Cas9 and single guide RNA into zygotes. Now it is replaced by electroporation of oocytes with CAS9/crRNA/tracrRNA ribonucleoprotein complex for simple genome editing (knockout, point mutation, tag insertion, etc). Altogether, we have knocked out 321 testis enriched genes and analyzed their phenotypes in vivo. Whereas 219 of the KO mouse lines were fertile and did not show any severe phenotypes, 9 KO mouse lines showed lethality. The remaining 93 KO mouse lines showed infertility or severe subfertility. By analyzing the molecular mechanism of infertility phenotypes, we have recently elucidated the lumicrine system. Testicular NELL2 goes through the efferent duct's lumen and stimulates the ROS1 signaling pathway in the epididymal epithelial cells. Thus differentiated epididymal epithelial cells secrete proteases such as OVCH2 that mediates sperm ADAM3 trimming and sperm maturation. Finally, I would like to introduce recent findings on another molecule required for lumicrine pathway.

Acute epididymitis - a clinician's perspective

Adrian Pilatz

Department of Urology, Pediatric Urology and Andrology, Justus-Liebig-University Giessen, Giessen, Germany

Acute epididymitis is the most common infection of the male genitals. It can occur at any age and is predominantly of bacterial origin. Clinically, pain and swelling are in the focus in the acute situation. Rapid initiation of empirical antibiotic therapy is necessary to minimise complications. In addition to spreading to the testis in the sense of epididymo-orchitis, secondary testicular infarction with the need for semi-castration and, as a long-term consequence, sub/infertility in young men are particularly feared. The clinical lecture is intended to provide an overview, especially for basic scientists with an interest in translational research, of which questions are relevant for patients. In addition, it should give an insight into which basic requirements must be present on the clinical side in order to establish a prospective patient cohort in the long term and to set it up successfully for translational projects.

Male genital tuberculosis

Ekaterina Kulchavenya

Novosibirsk Research TB Institute, Novosibirsk, Russian Federation

Introduction
Urogenital tuberculosis (UGTB) still has an actuality. The registered drop in the incidence rate is mostly due to the defects of official statistic.
Material and methods
In this study 456 UGTB patients, who were diagnosed between 2003 to 2015 in Siberia, were enrolled.
Results
Most common form was TB of the urinary system - 314 patients (68.8%), genital TB was diagnosed in 22.4%. Generalized UGTB, when both kidney and genitals were hurt, was registered in 41 patients (9%). A significant decrease a share of nephroTB was found: 2008 - 82.2%; 2015 - 48.6% ($\chi 2 = 12.71$; $P = 0.0004$). On the contrary, if in 2003 the proportion of patients with genital TB was 18% (18 cases), in 2015 this form was diagnosed in 30 (29.1%) ($\chi 2=3.46$; $P=0.06$).
The proportion of prostate TB varied from 0 in 2003 and 7.1% in 2008 up to 54.2% in 2013. In 2003, TB of the epididymis was diagnosed in 100% among male genital TB. In 2013 and 2015 - TB of the epididymis had every forth patient the genital TB. In total, for all years, tuberculosis of the scrotal organs was diagnosed in 26 patients (41.9% of all male genital tuberculosis cases). The combination of TB of the scrotum and prostate TB also varied from year to year significantly - from 17.3% to 35.7%. Generalized UGTB was diagnosed with a minimum proportion of 1.4% in 2008, then there was an upward trend with a maximum rate in 2015 - 22.3% ($\chi 2 = 29.38$; $P < 0.0001$).
Conclusion
Patients with UGTB for a long time are misdiagnosed.

Cell identity in the human proximal epididymis

Ann Harris

Department of Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH, USA

Species-specific differences in the anatomy and physiology of the male genital ducts are well documented. In human, efferent ducts, which connect the head of the epididymis (caput) with the testis, have a different function and transcriptome from the caput, and despite the lack of septa separating the head, body (corpus) and tail (cauda) of the human epididymis, each region is functionally specialized. We previously generated bulk transcriptomic data for the head, body and tail regions and epithelial cells derived from them. More recently we focused on the proximal epididymis since it has a critical role in sperm maturation, and established a single cell atlas by scRNA-sequencing and immunofluorescence. We reveal transcriptional signatures for at least 8 cell clusters, which identify the individual roles of principal, apical, narrow, basal, clear, halo, and stromal cells in the epididymis. A marked cell type–specific distribution of function is seen along the duct with local specialization of individual cell types integrating processes of sperm maturation. Of particular relevance to epididymis physiology are the cell populations that express the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CFTR transcripts are abundant in a sub-population of efferent duct cells but most notably in clear cells within the caput epithelium. We examined the comparative identity of these CFTR-high cells in different epithelial cells types that are key to CF pathology.

The role of the activins in epididymal function and immunopathology

Rukmali Wijayarathna

Hudson Institute of Medical Research, Clayton, Victoria, Australia

The activins (A and B) and their binding protein, follistatin, play crucial roles in development, inflammation and immunoregulation throughout the body. In the epididymis, activin expression is highest in the initial segment and proximal caput and progressively declines towards the cauda and vas deferens, while the converse is true for follistatin. The activin-follistatin balance is critical for regulating coiling of the duct during epididymal development. In-situ hybridization has localised activin A expression principally to immune cells in the epididymal interstitium, while activin B is produced by epithelial cells in the initial segment. Activin A is implicated in the regulation of mononuclear phagocyte function and immune responses in the caput, and stimulates the expression of the key immunoregulatory protein, indoleamine 2,3-dioxygenase in this region. Conversely, the increase of activin A that occurs in the cauda during bacterial and autoimmune epididymitis drives inflammation and fibrosis, causing damage to the ductal epithelium and obstruction. Activin A is also implicated in the recruitment of lymphocytes to the cauda epididymis during inflammation. Consequently, while the activin-follistatin axis is crucial for maintaining normal epididymal structure and function, disruption of this balance during inflammation has deleterious effects on male fertility.

The immune context of the mammalian epididymis

Rachel Guiton

GReD Laboratory, CNRS UMR 6293 - INSERM U1103, Clermont Université, Clermont-Ferrand, France

Long neglected, the immune context of the epididymis is now recognized as a crucial topic in the field of reproductive health. Contrary to the testis in which immune tolerance is the rule, the epididymis has to deal with both immune tolerance to spermatozoa and immune response to ascending pathogens by still unknown mechanisms. Our previous results identified the immune cells that populate the mouse epididymis at steady state and we have now described the dense lymphatic network that drains the organ and which is also used for the immune cells traffic. The identification of the epididymal draining lymph node in which the immune response must be triggered will be the next step to have the complete table of the epididymal immune organization. Another aspect of our research aims at optimizing sperm antigen-based immunocontraceptive strategies, with the objective to control the overgrowth of a wild pest rodent (*Arvicola terrestris schermann*). A group of sperm antigens were identified as new putative efficient targets. Our increasing knowledge of the mouse immunity and the discovery of these targets were combined in a new project with the objective to obtain a proof of concept for the future transposition of the immunocontraceptive strategy to human. Using the mouse model, our preliminary results look promising and will allow for molecular studies, which are clearly limited in the wild rodent model.

Key players in immune surveillance of the epididymis

Maria Agustina Battistone

Massachusetts General Hospital - Harvard Medical School, Boston, MA, USA

Mononuclear phagocytes (MPs) play an active role in the immunological homeostasis of the epididymis. We previously showed that epididymal CX3CR1+ MPs can internalize and process antigens. Here, we examined whether the lack of functional CX3CR1 in homozygous mice (CX3CR1EGFP+/+, KO) alters the ability of MPs to initiate immune responses against harmful antigens during epididymitis. Confocal microscopy showed that intraepithelial KO MPs displayed a significant reduction in the number of luminal-reaching projections. Immunophenotype characterization showed no differences in the percentage of macrophages or dendritic cells between KO and control MPs. Flow cytometry showed no overall impairment in antigen capture 1h after ovalbumin injection, however, confocal microscopy revealed reduced internalization in intraepithelial KO cells. We also observed a higher number of MPs with the monocytic signature and CD103+ dendritic cell accumulation in the KO epididymis after LPS injection. Together, we show morphological alterations in MP luminal projections in CX3CR1-deficient mice, which displayed an impaired antigen sampling, and an immunophenotypic shift in response to injury. Additionally, CX3CR1 deletion induces defective cell-cell communication between MPs and CD103+ cells and indicates that MPs are the gatekeepers of the immunological blood-epididymis barrier. By identifying immune mechanisms in the epididymis, our study may lead to new diagnostics and therapies for male infertility.

Region-specific epididymal responses to inflammation: insights from experimental models of epididymitis

Erick Silva

Department of Biophysics and Pharmacology, Institute of Biosciences, São Paulo State University, Botucatu-SP, Brazil

The epididymis is a mosaic organ defined by region-specific microenvironments presenting distinct morphology, cell composition, and gene expression crucial to promoting sperm maturation. The epididymal mosaic also encompasses the regionalized distribution of subsets of immune cells and the expression of innate immune elements, such as Toll-like receptors (TLRs), antimicrobial genes (e.g., members of the WFDC family), and inflammatory mediators. The distinct immune environment of the epididymal regions seems essential to its proper function and governing responses to stressors. Given the epididymis' high exposure to bacterial infection, which causes a male-fertility threatening condition known as epididymitis, understanding the spatial events eliciting inflammatory responses throughout the organ is crucial to developing adjuvant strategies to prevent permanent tissue damage and poor sperm outcomes. Our studies explore pathogen-associated molecular patterns from clinically relevant bacteria, such as lipopolysaccharide (LPS) and lipoteichoic acid (LTA), as pharmacological inducers of experimental epididymitis in mice via activation of TLR4 and TLR2/TLR6 to dissect the inflammatory responses of proximal and distal epididymis, including (i) temporal changes in the expression of inflammatory mediators and Wfdc genes, (ii) contributions of TLR-associated signaling pathway in such responses, and (iii) impact on reproductive outcomes.

Shifting the paradigm of sperm maturation; global (phospho)proteomic profiling of epididymal sperm

David Skerrett-Byrne

College of Engineering, Science and Environment, The University of Newcastle, NSW, Australia

A distinctive feature of the functional maturation of mammalian spermatozoa in the epididymis is that it occurs in the absence of de novo gene transcription and protein translation, thus reliant on a substantive remodelling of the intrinsic sperm proteomic architecture, the scale of which is yet to be fully understood. Here, we define these proteomic changes using label-free quantitative mass spectrometry; reporting an unprecedented 6045 proteins and 9686 site-specific phosphorylation events. Contrary to the belief that epididymal maturation is driven by the uptake of additional proteins, we demonstrate that sperm shed over 56% of their proteins during this process, producing a refined fertilisation-competent cell. This reduced proteomic complexity of sperm aligned with activation of functions including sperm motility and capacitation. In accounting for how these changes influence sperm function, we demonstrate that RHOA, a small GTPase, is acquired by maturing spermatozoa (2x increase), with a concomitant downregulation or complete loss of RHOA repressing proteins, including ARHGAP18 and GDI-1 in mature sperm. Pharmacological inhibition of RHOA activity, resulted in a compromised ability of mature spermatozoa to undergo an acrosome reaction (~40% reduction). Such data represent an important paradigm shift in our understanding of male fertility regulation and suggests that capacitation is a subtle molecular switch fine-tuning sperm function in preparation for fertilization.

Paternal exposures, epididymal maturation, and epigenetic programming of sperm

Michael Golding

Department of Veterinary Physiology & Pharmacology, Texas A&M University, College Station, Texas, USA

Until recently, we did not anticipate that paternal lifestyle could impact offspring developmental outcomes. However, epigenetic mechanisms of paternal inheritance are now an emerging area of interest in our efforts to understand various developmental defects, including those associated with fetal alcohol spectrum disorders. Using a mouse model, studies by our group reveal that chronic preconception paternal alcohol exposures program nonlinear, dose-dependent changes in offspring fetoplacental growth, with long-lasting consequences on adult health and metabolism. However, how the memory of preconception alcohol exposures transmits through sperm and impacts early embryonic life remains unknown.

Transition through the luminal environment of the epididymis promotes sperm maturation and the ability to capacitate in the female tract. However, recent studies indicate that the epididymal epithelium also directs critical aspects of epigenetic programming in sperm, enabling the transmission of nongenomic information from father to offspring. In this talk, I will focus on epigenetic mechanisms of inheritance occurring during the epididymal maturation of sperm, their alteration as a consequence of chronic alcohol exposure, and how these changes influence placental development and function in the next generation. I will also discuss the resilience of the paternal epigenetic program and how paternal stressors may program hermetic growth responses in the next generation.

Regionalized mapping of biological functions in the epididymis using large-scale in situ approaches

Charles Pineau

Univ Rennes, Inserm, EHESP, Irset (Institut de Recherche en Santé, Environnement et Travail) - UMR_S 1085, Rennes F-35042 Cedex, France

The strikingly complex structural organization of the male reproductive system and gamete production in vivo creates particular difficulties for studies of its organization, function and regulation. The use of classical molecular and cell biology approaches to unravel this complexity has proved problematic and has led over the past decade to a greater reliance on high-quality Omics analyses as a prelude to the in vitro and in vivo testing of hypotheses.
In mammals, spermatozoa released from the testis are morphologically complete but neither motile nor fertile. They acquire their fertilizing capacity during transit through the epididymis. There a complex maturation process occurs that involves a substantial molecular remodeling at the surface of the gamete and is based on interactions of the spermatozoa with the epididymal microenvironment.
Most studies carried out on epididymal maturation have been performed on sperm samples or tissue extracts collected from the caput, corpus, and cauda regions. This generally leads to the loss of information on the precise in situ localization of the studied molecules. A large number of metabolic events, particularly lipid metabolism, are suspected to be involved in the epididymal maturation process and need to be localized for a better understanding of their biological role.
In this presentation, we will discuss the use of MALDI imaging mass spectrometry (IMS), spatial segmentation and metabolite annotation tools to explore the distribution of metabolites throughout the length of the rat epididymis. We will also discuss how 3D MALDI IMS can access and determine the volumetric distribution of metabolites detected in the organ.
Ongoing work to integrate our high quality in situ lipid distribution dataset with large-scale quantitative proteomes obtained for the three main epididymal regions will be presented. This work aims at identifying the key enzymes involved in lipid metabolism within the organ. This work opens new perspectives for elucidating the role of lipid metabolism in sperm maturation during its transit through the epididymis.

Illuminating cell dynamics in murine epididymis by live cell imaging

Tsuyoshi Hirashima

Mechanobiology Institute, Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, SIngapore

Dynamical and mechanical aspects of cells in epididymis remain less well studied despite their importance for the understanding of development and physiology. Live imaging provides a powerful tool to investigate the cellular dynamics underlying morphogenetic and homeostatic processes of tissues, thus contributing to the quantitative understanding of epididymis studies. In this talk, I will present two topics, in which the fluorescence live imaging led to the finding of characteristic cellular behaviors in murine epididymis. The first topic is the morphogenesis of murine embryonic epididymis (2014, Cell Rep; 2019, Development). Under an organ culture condition, we found that the epididymal tubule cells counteract compressive forces caused by cell division to produce polarized contractile forces, eventually leading to an oriented cell arrangement. This finding suggests an unprecedented system in which the polarized mechano-responsive movement of cells organizes the maintenance of the tube morphology. The second topic is the epididymal and sperm dynamics in adult mice (unpublished). I have established intravital mouse imaging systems using two-photon microscopy, and found that the sperm collectives generate turbulent flow in the cauda of epididymis via the interaction with the epithelial cells. This finding leads to a better understanding of the link between collective sperm dynamics and physiological processes, and opens the door to bridging many different disciplines.

Host defense functions of the epididymal amyloid matrix

Gail Cornwall

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The epididymis plays a critical role in protecting sperm from invading pathogens that can ascend the male tract, causing inflammation and infertility. The epididymis relies heavily on antimicrobial proteins (AMPs) to defend against these pathogens, but it remains unclear mechanistically how these AMPs function. We previously established a nonpathological, functional amyloid is in the epididymal lumen and contains the amyloid forms of four CRES subgroup members, a reproductive subgroup within the family 2 cystatins of cysteine protease inhibitors. Our studies show that various maturational states of CRES amyloids and the epididymal amyloids exhibit potent antimicrobial activity against bacterial strains that cause epididymal infections in men suggesting the amyloids play important roles in host defense. We show CRES amyloids and the epididymal amyloids use several mechanisms including bacterial trapping, disruption of bacterial membranes, and promotion of unique bacterial ghost-like structures to defend against pathogens. We also demonstrate that the CRES monomer and immature assemblies of the epididymal amyloid matrix transitioned into advanced structures in the presence of bacteria, suggesting their amyloid-forming/shape-shifting properties allows for a rapid reaction to a pathogen and provides an inherent plasticity in their host defense response. Together, our studies reveal new mechanistic insight into how the male reproductive tract defends against pathogens.

Motility of efferent duct cilia aids passage of sperm cells through the male reproductive system

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Motile cilia line the efferent ducts of the mammalian male reproductive tract. Several recent mouse studies have demonstrated that a reduced generation of multiple motile cilia in efferent ducts is associated with obstructive oligozoospermia and fertility issues. However, the sole impact of efferent duct cilia dysmotility on male infertility has not been studied so far either in mice or human. Using video microscopy, histological- and ultrastructural analyses, we examined male reproductive tracts of mice deficient for the axonemal motor protein DNAH5: this defect exclusively disrupts the outer dynein arm (ODA) composition of motile cilia but not the ODA composition and motility of sperm flagella. These mice have immotile efferent duct cilia that lack ODAs, which are essential for ciliary beat generation. Furthermore, they show accumulation of sperm in the efferent duct. Notably, the ultrastructure and motility of sperm from these males are unaffected. Likewise, human individuals with loss-of-function DNAH5 mutations present with reduced sperm count in the ejaculate (oligozoospermia) and dilatations of the epididymal head but normal sperm motility, similar to DNAH5 deficient mice. The findings of this translational study demonstrate, in both mice and men, that efferent duct ciliary motility is important for male reproductive fitness and uncovers a novel pathomechanism distinct from primary defects of sperm motility (asthenozoospermia).

Epididymal injury and morphological sperm anomalies in a high fat diet (HFD) model

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Seminiferous tubule morphology, blood-testicular barrier integrity, normal sperm concentration and motility parameters are all aspects of male reproductive health impaired by High Fat Diet (HFD).
The epididymal epithelium shows cell-to-cell junctions, able to form the blood-epididymal barrier (BEB), a finely organized structure that actively participates in the establishing the optimal microenvironment needed for epididymal sperm maturation.
Until today, HFD implications in the epididymal field and, as a consequence, in sperm functionality have not yet been investigated.
Hence, we analyze HFD dependent effects on epididymal epithelium and BEB integrity, using a HFD obesity male mouse model. Our results show that HFD induced a severe deregulation of Adherens (AJs) and GAP epididymal junctions. In addition, sperm head morphological anomalies, low epididymal motility rate acquisition and, finally, abnormal acrosomal distribution of IZUMO1, the main glycoprotein involved in sperm-oocyte interaction, were also observed during sperm epididymal transit.
Interestingly, the oral administration of Lactobacillus rhamnosus IMC501, Lactobacillus brevis and Bifidobacterium lactis HN019 mix of probiotics in HFD mice significantly reverted epididymal epithelium anomalies as well as sperm morphological and motility parameters.
Our results collectively demonstrate that interkingdom cellular communication could be an important mechanism to prevent obesity, improving male reproductive health.

SO1 Urinary phthalate metabolites and small non-coding RNAs from seminal plasma extracellular vesicles among men undergoing infertility treatment

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Background
Emerging data in rodents suggest that cargo of extracellular vesicles (EVs) in the male reproductive tract are susceptible to environmental factors. However, such investigations are limited in humans. Thus, we evaluated the association between urinary phthalate metabolites and small non-coding RNA (sncRNAs) of seminal plasma EVs (spEV) among men partners of couples receiving clinical infertility care.
Methods
We conducted sncRNA sequencing of EVs in 96 seminal plasma samples collected as part of the Sperm Environmental Epigenetics and Development Study (SEEDS). Associations with normalized micro RNA (miRNA), transfer RNA fragments (tRFs), piwi-interacting RNA (piRNA) counts were assessed using EdgeR (FDR<0.05).
Result
The metabolites of DEHP, DOP, DBP, DiBP, DiNP and DiNCH were associated with normalized read counts from 23 unique ncRNA transcripts (7 miRNAs (pre & mature); 6 tRFs; and 10 piRNAs), most (78%) of which displayed increased expression patterns. miRNA and tRFs gene targets were enrichment in vesicle-mediated transport and developmental-related ontology terms, such as tyrosine kinase, head development, and cell morphogenesis. piRNAs were enriched in pseudogenes of genes important in EV cargo transfer and embryonic development.
Conclusion
This is the first study to associate phthalate and their replacement exposures to altered spEV sncRNA profiles. Future studies are needed to determine their impact on reproductive outcomes.

SO2 Androgen-regulated epididymis barrier function in the human proximal epididymis

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Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene are a critical cause of male infertility, and >95% of males with cystic fibrosis (CF) are infertile due to congenital bilateral absence of the vas deferens (CBAVD) and absence of the distal epididymis. This results in obstructive azoospermia and leads to infertility. Previously, we showed that CFTR contributes to the maintenance of transepithelial electrical resistance (TER) in human epididymis epithelial (HEE) cells, and testosterone or synthetic androgen methyltrienolone treatment increased the TER of HEE cells isolated from the caput region. In this study, we further compared efferent duct (ED) and caput HEE cells in terms of their response to physiological concentrations of testosterone and dihydrotestosterone (DHT), and investigated the underlying mechanism of the androgen-regulated epithelial barrier and the genes involved in the function. Our results showed that the ED and caput HEE cells had similar levels of TER (range, 200-300 ohms-cm2) at baseline, and DHT and testosterone treatment increased the TER by 35-40% in both cells. RNA-sequencing and qRT-PCR validation facilitated identification of androgen-regulated genes that may have important roles in normal epididymis function. The epididymis barrier is regulated by androgen in the proximal epididymis. Identifying androgen-regulated genes may enable elucidation of how CFTR-dependent processes in the epididymis impair fertility in CF.

SO3 Restoration of segmented mouse and rat epididymal transcriptional profiling data in a relaunch of the mammalian reproductive genetics database

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The Mammalian Reproductive Genetics Database was established at the University of Washington in 1997. It was designed to disseminate tissue and cell gene expression data of reproductive tissues, including the segmented mouse (10 segments) and rat epididymis (19 segments) published by Turner, Johnston and Jelinsky between 2005 and 2007. It was a powerful research resource in the reproductive biology community. At the time of shutdown in 2012, the database was visited by researchers approximately 11K times per year. A new web portal has been developed, designed “The Mammalian Reproductive Genetics Database, version 2”. The segmented epididymal data from the original database were recovered and incorporated in the new version of the database. A summary of epididymal segmentation in the mouse and rat, the resulting transcriptional profiling datasets, examples of utility, and the data location on the new database will be presented to the field.

SO4 Investigation of GM1-enriched murine epididymosomes with segmental expression and lumicrine regulation

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The murine epididymis has distinct segments which provide opportunity to identify specific epididymal functions and their underlying mechanisms. Here, we investigated segmental expression of the ganglioside GM1, which is known to play key roles in sperm capacitation and acrosome exocytosis. Direct fluorescence labeling of GM1 was performed on epididymis tissue cryosections from wildtype (WT) mice and imaged via confocal microscopy, revealing high expression in segment 2 of the caput epididymis in principal cells, clear cells, and luminal extracellular vesicles (EVs) with relatively reduced expression in the rest of the organ. To determine if GM1 originated from the epididymis or from the testis/sperm, we examined azoospermic mouse models and performed efferent duct ligation (EDL) procedures on WT mice. GM1 localization in the epididymides of the azoospermic models and WT mice post-EDL was altered with loss of expression specific to Segment 2, but GM1-enriched EVs were still present indicating epididymal origin. Transcriptome profiling revealed segmental de-differentiation with loss of lumicrine signaling. By quantifying fluorescence intensity of labeled GM1 on sperm heads isolated from epididymal segments 1-3, we found GM1 increased upon transit through segment 2. We hypothesize that these EVs are epididymosomes that transfer GM1 to sperm and are involved in sperm maturation.

SO5 Functional and structural particularities regarding the transit of the epididymal duct between neighboring segments in rodents and men

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The epididymis ensures sufficient maturation, transport and storage of sperm released by the testis. In addition to the classical regions (caput, corpus, cauda) of the epididymis, segments could also be defined by connective tissue septa (CTS). In this context it is of interest that in an animal model of luminal ascending infection, bacteria were found to be restricted exactly to one segment three days after treatment and were not present in the upstream segment (Stammler et al., 2015). Information about the three-dimensional (3D) organization of the epididymal duct in the context of segmentation is barely available. To investigate the course of the epididymal duct in rodents and men while passing the CTS from one to the adjacent segment, the epididymis and the duct itself were re-constructed using new imaging tools. The created 3D reconstruction demonstrated a clear separation of neighboring segments and differences in duct folding. Transit from one segment to the other was shown to take place in a broader area of dense connective tissue. When using the 3D dataset, a specific folding of the epididymal duct could be revealed in the intersegmental part consisting of a central U- or Ω-shape almost completing a 180-degree turn (x-y plane). The duct then leaves, both in the caput and cauda direction, the x-y plane in a wider loop. Our findings of the duct folding might be relevant for a well-orchestrated sperm maturation and avoiding infertility caused by ascending infections.

SO6 The regional distribution of resident immune cells shapes distinct immunological environments along the murine epididymis

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The epididymis faces contrasting immunological challenges (immune tolerance towards spermatozoa vs. immune reactivity against pathogens) and thus, healthy organ function depends on a tightly controlled immune balance. Inflammation-associated tissue damage is seen in the cauda, but not in the caput, and resulting duct obstruction has severe impact on fertility. Using a mouse model of acute bacterial epididymitis, we have analyzed the disease progression at cellular and transcriptional levels. To understand the underlying reasons for region-specific differences in immune responses, we untangled the heterogeneity of resident immune cell populations under steady-state conditions by scRNA-seq analysis of extravascular CD45+ cells. In total, 12 different immune cell subsets were identified, ranging from mononuclear phagocytes (macrophages, dendritic cells and monocytes) to lymphocytes (NK, B and T cells). Macrophages constitute the majority of resident immune cells and could be further stratified into several distinct subsets. Intriguingly, the opposing ends of the epididymis showed striking differences in their immunological landscape. These findings indicate that resident immune cells are strategically positioned along the epididymal duct to provide different immunological milieus required to maintain tissue integrity required for sperm maturation, and to adequately tackle invading pathogens ascending from the urogenital tract.

SO7 Uropathogenic Escherichia coli infection leads to the formation of tertiary lymphoid organs in the epididymis

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Uropathogenic Escherichia coli (UPEC) is a pathogen commonly isolated from male urogenital tract infections, including epididymitis. In human and mouse models, the cauda epididymis is predominantly affected. We here report for the first time the detection of tertiary lymphoid organs (TLO) in the mouse cauda epididymis after infection with UPEC. Following ligation of the vas deferens in C57BL/6J mice, either PBS (sham control) or UPEC (strain CFT073) were bilaterally injected into the vas deferens. Mice were sacrificed 1, 3, 5, 10, 14, 18, 21 and 28 days (n=3-5 animals per time point) after infection. Our results show elevated mRNA expression of the TNF/lymphotoxin (LT) ligand-receptor family members, which play critical roles in high endothelial venule formation. Determination of the mRNA expression of the TLO associated chemokine ligands Cxcl13, Ccl19 and Ccl21 in the cauda epididymis revealed elevated expression (~80-, 30-, 70-fold, respectively) 14 days post-infection (p.i.). Immunofluorescence examination showed distinct organized zones of B and T cells, high endothelial venules, proliferating B cells as well as isotype-switched plasma cells from day 14 p.i. The presence of isotype switched B cells was confirmed by flow cytometric analysis. These changes were not detected in the caput or corpus of UPEC infected epididymis. These data establish that several TLO-associated features and mediators are present in the mouse cauda epididymis following UPEC infection.

SO8 Investigating retinoic acid synthesis and signaling in the epididymal epithelium

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Epididymitis is an acute inflammatory reaction to an infection. Normal epididymal function is not always regained following epididymitis and male infertility is a common long-term consequence of the more severe or untreated cases. This project aimed to further our understanding of the mechanisms triggering the onset of epididymitis. We investigated whether retinoic acid (RA) played an important role in protecting epididymal epithelial function and whether the epididymal epithelium could synthesize RA. Analysis of our genetically modified mouse line, the RAR-DN mice, revealed that in the absence of RA activity in principal cells, epididymitis occurs, indicating that RA is an important factor in preventing this disease. We observed an influx of inflammatory mediators, including macrophages, into the RAR-DN epididymis and the epithelial cells underwent squamous metaplasia. Our immunohistochemistry studies revealed that RA can be synthesized by the epididymal epithelium in a cell- and region-specific manner, with ALDH1A1 likely important for generating RA in principal cells while ALDH1A2 appears to produce RA in the epididymal immune cells. This project has revealed RA as a key regulator of epididymal function and suggests that this molecule is important for controlling epididymal immune cell fate. Future studies will focus on the downstream targets of RA at the onset of epididymitis and whether synthetic retinoids could be a viable treatment strategy for this disease.

SO9 DNA integrity alteration in mouse spermatozoa after short exposure to Dibutyl Phthalate or Bisphenol AF and remediation by an antioxidant oral supplementation

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Many studies showed negative effects of environmental pollutants on spermatogenesis. However, potential alterations of spermatozoa in the post-testicular compartment have not been extensively evaluated. It is of particular importance as the epididymal tubule is opened to the systemic compartment due to a dense vascular network and a weak blood-epididymal barrier. Any toxic molecule entering the blood compartment may therefore be harmful to maturing spermatozoa.
This study was conducted to investigate the damaging effects on epididymal spermatozoa of a short exposure with low doses of two pollutants, with a focus on sperm DNA integrity. The protective effect of an oral antioxidant supplementation was also tested. Male mice (C57B6) were exposed to either Dibutyl Phthalate (DBP) or Bisphenol AF (BPAF), added to their drinking water (50mg/kg BW/day), for 14 days with or without oral supplementation.
None of the pollutants significantly affected sperm viability, motility nor acrosome integrity. However, both molecules had a strong effect on sperm DNA integrity by significantly increasing the percentage of spermatozoa with oxidised DNA as well as the percentage of spermatozoa with decondensed DNA. A significant increase in the number of spermatozoa with abnormal morphological features and DNA fragmentation was observed only after BPAF exposure. Interestingly, our study showed that the majority of these harmful effects could be corrected with oral antioxidant supplements.

SO10 Runx transcription factors are necessary for maintenance of proper differentiation of epididymal epithelial cells

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We have previously generated a Dicer1 cKO mouse model, where Dicer1 was deleted from the proximal epididymis. The lack of DICER1 caused dedifferentiation of the epididymal epithelium around the age of 5 weeks. As DICER1 is an enzyme in the miRNA processing pathway, it affects wide variety of signaling pathways. In order to specify factors required for the maintenance of differentiated epididymal epithelium in the proximal epididymis in this model, we performed RNA-seq analysis from Dicer1 cKO epididymides and data showed that the loss of runt related transcription factor 1 and 2 (Runx1 and -2) expression coincides with epithelial dedifferentiation.
For further studying the role of RUNX transcription factors in the epididymal epithelium, we generated deletions of both of the RUNXs in the mouse epididymal cell line (ΔRunx cells). Deletion of Runx1 and -2 caused the morphology of ΔRunx cells to change towards more mesenchymal appearance. ΔRunx cells also failed to form organoids in 3D cultures. Accordingly, pathway analysis from RNA-seq data from the ΔRunx cells revealed a significant enrichment of GO terms related to epithelium morphogenesis and cell adhesion. Moreover, the lack of RUNXs affect negatively cell adhesion and increase invasion capability of the cells. Our results demonstrate that RUNX transcription factors are important for the maintenance of proper epididymal epithelial cell identity.

SO11 Do wireless communications networks impact the male reproductive system?

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Mobile communication devices and other appliances such as Wi-Fi routers/emitters, are ubiquitously incorporated into our living environment, emitting radiofrequency, and more recently, '5G' millimetre wave, electromagnetic energies (EME). Their omnipresent nature together with rapid advancements and subsequent roll out of new technologies is driving a public demand for improved understanding of potential health impacts associated with current and emerging technologies. A fundamental hindrance toward reaching a conclusion on safety is that despite some well documented biological impacts, there is no clear mechanistic understanding of how such non-ionising energy elicits these effects. The concern is deepened in the context of male reproduction given the proximity of the reproductive system to stored mobile devices and the recent global decline in semen parameters. Poor reproductive outcomes are linked to increased genetic defects and losses in sperm function, both caused by cellular incursions, facilitated by oxidative stress. We have found that sperm nucleic acids and protein thiol substrates undergo oxidation during EME exposures. Similarly, the mature sperm of EME exposed mice also harbor alterations in epigenetic, small non-coding RNA profiles. These losses in sperm quality occur without obvious alterations in the testis of exposed animals, thus highlighting the potential vulnerability of the epididymis to EME and propagating a potential impact on male reproductive health.

SO12 Epididymal primary cilia induce intracellular calcium signaling and gene expression in response to fluid shear stress

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The primary cilium (PC) is a cellular antenna that regulates cell polarity, differentiation, and proliferation in response to shear stress and downstream calcium signaling. While we detected PC at the surface of non-differentiated and principal cells of the epididymis at prepubertal stages, their role as shear stress mechanosensors remains unexplored. By combining in silico fluidic modeling with in vitro biofluidic strategies, we assessed the molecular response of ciliated epididymal principal cells to luminal shear stress. Immortalized distal caput epididymis principal cells (DC2) were cultured on fluidic microplates and subjected to static conditions or to a 1 dyn/cm2 fluid shear stress. While shear stress triggered a rapid increase of intracellular Ca2+ in DC2 cells, this response was abrogated following the pharmacological impairment of primary ciliogenesis with CiliobrevinD. According to RNA-sequencing profiling, the expression of 63 genes was significantly increased in responseto shearar stress (Fold change >2, FDR< 0.05), including Early growth response (EGR) 2/3. Pathway analysis identified MAPK, TNF, and TGF-beta as the signaling pathways the most responsive to shear stress in epididymal cells. Our findings identify PC as a fluid-mechanosensor that may contribute to epididymis development in response to testicular-derived fluid and/or the first wave of spermatozoa.

SO13 Using cryo-electron tomography to study the cytoplasmic droplet

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Morphologically, the most obvious change during sperm epididymal maturation is the migration of the cytoplasmic droplet from the sperm neck towards the end of the midpiece. Depending on the species, mature sperm may lose this organelle during epididymal maturation. Since the cytoplasmic droplet translocation coincides with gaining motility, it was speculated that the cytoplasmic droplet plays a role in motility acquisition. Details of the cytoplasmic droplet translocation mechanism and how this relates to gaining motility are scarce. Our group has pioneered the use of cryo-electron tomography to study mature mammalian sperm at the molecular level. We develop and apply multiple workflows that enable imaging of intact mammalian sperm for the first time without fixation, dehydration and heavy metal staining. Our data show that mammalian sperm flagella are modified across scales – from large accessory structures that increase the effective size and rigidity of the entire assembly, to extensive microtubule inner proteins that reinforce the microtubules themselves. We are now turning our attention to epididymal sperm and developing approaches to study how the cytoplasmic droplet interact with the motor apparatus.

SO14 Epididymal acquired sperm miRNAs and male fertility

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Sperm small RNAs delivered to the zygote during fertilization have emerged as an important non-genetic contributor to offspring phenotype via the regulation of embryonic gene expression. Interestingly, small RNAs are delivered to sperm during post testicular maturation in the epididymis. Notably, during this transit a subset of microRNAs (miRNAs) are gained by sperm, that have been linked to sperm competency in mammals. Here, we utilized Cre:lox conditional genetics to selectively ablate Dgcr8 in male germ cells (GC-Dgcr8) and the epididymal epithelium (Epi-Dgcr8), respectively, to investigate the function of sperm miRNAs in male fertility. In line with the loss of Dgcr8, a key miRNA biogenesis enzyme, mature sperm from these mice display altered miRNA profiles compared to controls. Interestingly, sperm from GC-Dgcr8 males regain many miRNAs that are lost in testicular sperm during epididymal transit. Conversely, sperm from Epi-Dgcr8 males displayed reduced abundance of epididymal acquired miRNAs. A loss of miRNA either in the testis or epididymis also leads to varying levels of fertility. GC-Dgcr8 males are subfertile while Epi-Dgcr8 males are unable to sire offspring. While investigation of this infertility in Epi-Dgcr8 males revealed reduced sperm motility, sperm were able to support embryo development to blastocyst stage via intracytoplasmic sperm injection. Future analysis will focus on how sperm miRNA loss influences early embryo gene expression.

SO15 Comprehensive protein profiling of seminal CD63+ small extracellular vesicles unveils their biological relevance and tissue-specific origin

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Seminal plasma seems to play important roles in male fertility in addition to simply being a medium to carry spermatozoa. The transcriptional and translational silent spermatozoa produced in the testis are able to incorporate proteins required for sperm function from the small extracellular vesicles (sEV) released by the epididymis and by the different accessory sex glands into seminal plasma. Ultracentrifugation (UC) of seminal plasma in a 25-30% sucrose cushion followed by CD63-magnetic beads selection was applied to purify an enriched population of sEVs with reduced non-sEVs contamination. A total of 832 proteins were detected using liquid chromatography separation and tandem mass spectrometry identification of peptides in seminal CD63+ sEVs from normozoospermic men. Gene ontology enrichment analysis showed that proteins identified are involved in processes such as sperm motility and fertilization suggesting that, among the heterogeneous population of seminal EVs, the specific population of CD63+ sEVs is essential for reproductive success. The identification of some of these proteins in seminal CD63+ sEVs from post-vasectomy men confirms their extra-testicular origin. In addition, we detected subsets of proteins with exclusive epididymal origin, as well as specific of the prostate and seminal vesicles. This reveals the participation of all these tissues on the CD63+ sEV release and opens a window to the study of the role of gland-specific sEV in male fertility.

SO16 Oxytocin may recover impaired epididymal contractile function in patients with spinal cord injury who wish to father children

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During the emission phase of ejaculation, sperm is driven from its storage site, the cauda epididymidis, through the vas deferens by strong contractions. These contractions mainly depend on sympathetic action mediated by the neurotransmitter noradrenaline. Life imaging of defined segments of the rat and human epididymis showed an effect of oxytocin with a uniquely strong and rapid response. We developed a novel imaging analysis method to assess the complex nature of the contractile response, to quantify multidirectional contractions and to visualize them as heat maps. The distal epididymis showed a concentration-dependent contraction to oxytocin that was inhibited by oxytocin antagonists atosiban and cligosiban, but not by an arginine vasopressin 1A antagonist. The alpha-1 adrenoreceptor antagonist tamsulosin inhibited the response to noradrenaline in both rat and human tissue, whereas the effect of oxytocin was unimpaired. Our data point to an important role of oxytocin in the ejaculatory process and support oxytocin signaling as an alternative potent pathway for emission-related contractions independent of noradrenaline signaling. Oxytocin-based medications could be a promising non-adrenergic treatment option for ejaculatory disorders due to impaired sympathetic and noradrenergic signaling like e.g. in case of spinal cord injury. Stimulating the oxytocin pathway in the epididymis may rescue fertility in paraplegic patients by recovering emission-related contractions.

SO17 Mapping the interaction between mouse epididymal protease inhibitor (EPPIN) and the seminal vesicle secreted protein 2 (SVS2)

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Seminal plasma proteins (SPP) are crucial for the regulation of sperm function after ejaculation. In humans, the major SPP is semenogelin 1 (SEMG1), which modulates sperm function upon binding to the epididymal protease inhibitor (EPPIN) on the sperm surface, an event considered a target for male contraception. EPPIN is expressed in the testis and epididymis, and its presence in spermatozoa is associated with sperm maturation. We previously showed that mouse EPPIN interacts with seminal vesicle secreted protein 2 (SVS2), ortholog to human SEMG1, on mature spermatozoa. Here, we investigated the interaction between mouse EPPIN and SVS2 using in vivo and in vitro approaches. We observed that EPPIN remains bound to the mouse spermatozoa in the female reproductive tract (vagina, uterus near cervix, and uterus near oviduct) upon ejaculation and colocalizes with SVS2 in the sperm head and flagellum. Protein-protein interaction assays demonstrated that the SVS2 sequence R98-G375, spanning its C-terminal long repeats, contains the EPPIN binding sequence. Our findings shed new light on EPPIN biology as a conserved docking site for SPP with a crucial role in sperm function after ejaculation. Further studies are needed to pint-point the residues in SVS2 critical to modulating sperm function and clarify whether these regions overlap with the binding sequence required for EPPIN binding.

P18 Deep imaging of blood and lymphatic networks in the mouse epididymis: a complex system that could partly explain the peculiar immune biology of the mammalian epididymis

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Long considered an accessory tubule of the male reproductive system, the epididymis is proving to be a key determinant of male fertility. In addition to its secretory role in ensuring functional maturation and survival of spermatozoa, the epididymis has a complex immune function. Indeed, it must manage both peripheral tolerance to sperm antigens foreign to the immune system and the protection of spermatozoa as well as the organ itself against pathogens ascending the epididymal tubule. Although our knowledge of the immunobiology of this organ is beginning to accumulate at the molecular and cellular levels, the organization of the blood and especially the lymphatic networks of this tissue, important players in the immune response, remains largely unknown. In the present report we have taken advantage of a VEGFR3:YFP transgenic mouse model. Using high-resolution 3D-imaging following organ clearing via the 3DISCO technique coupled with multiplex immunodetections of lymphatic (LYVE-1, PDPN, PROX1) and/or blood (PLVAP/Meca32) markers, we provide for the first time a simultaneous deep 3D view of the lymphatic and blood epididymal vasculature in the mature adult mouse as well as during postnatal development.

P19 Effects of the bitter substance denatonium benzoate on the smooth muscle cell contractions in the most distal part of the epididymal duct

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Major functions of the epididymis are maturation, transport and storage of spermatozoa. The most distal part of the epididymal duct (EpD) primarily stores the spermatozoa until ejaculation and is involved in their emission by strong and complex noradrenaline (NA)-mediated smooth muscle cell (SMC) contractions.
It was suggested in various tissues that bacterial metabolites can modulate SMC contractions. In the present study using mouse tissue, we have now tested whether the bitter substance denatonium benzoate (DNT) has an impact on SMC contractions in the most distal part of the EpD (segment 10) devoid of spontaneous contractions. For visualization and analysis of potential effects we used classical live imaging and in addition a newly developed method that allowed us to quantify multidirectional contractions and to display them using heat maps.
No contractions were induced by DNT per se, but pretreatment with DNT prevented or markedly reduced the complex contractions initiated by NA. DNT even diminished ongoing NA-induced contractions and nearly completely abolished the effect of a second NA treatment. Further experiments suggest that DNT directly affects the smooth muscle layer.
Thus, in case of bacterial epididymitis the relaxing effect of bitter substances on contractions especially in the most distal part of the EpD could promote the ascent of infections.

P20 Impact of lipopolysaccharide- and lipoteichoic acid-induced inflammation on the transcript levels of Toll-like receptor pathway-related genes in the mouse epididymis

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Toll-like receptors (TLRs) are key molecules initiating immune responses to microbial infections. The epididymis expresses different TLRs, such as TLR4 and TLR2/TLR6, which recognize lipopolysaccharide (LPS) and lipoteichoic acid (LTA) from Gram-negative and Gram-positive bacteria, respectively, and their associated signaling molecules. We showed that LPS- and LTA-induced epididymitis differentially modulate cytokine responses in mouse initial segment (IS) and cauda epididymidis (CD). Here, we investigated whether the expression of TLR pathway-related genes is modified in the IS and CD as part of tissue responses to LPS or LTA. Male C57BL/6 mice were euthanized, and their IS and CD were incubated in DMEM media containing sterile-saline (control) or ultrapure LPS (0.05 ug/ml) or LTA (1 ug/ml) for 3 h. Tissues were processed for RT-qPCR studies to evaluate the mRNA levels of Tlr1, Tlr2, Tlr4, Tlr6, MyD88, Trif, Cd14, Cd36, Traf3, and Traf6, and Hprt (endogenous control). LPS increased the transcripts levels of Tlr2, Myd88, Trif, Cd14, and Traf3 in both the IS and CD, whereas Traf6 was upregulated in the CD only. Conversely, LTA (1 ug/ml) upregulated the transcript levels of Traf3 in the IS only. The differential outcomes of LPS- and LTA-induced epididymitis on the expression of key genes associated with the TLR signaling pathway indicate the existence of region-specific mechanisms triggered by activation of TLR4 and TLR2/TLR6.

P21 Analysis of the pathomechanisms of regionalized immune response in bacterial epididymitis

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Epididymitis is a urogenital disease often caused by infection with uropathogenic E. coli (UPEC). Despite the fact that the epididymis consists of one single convoluted duct, the infection results in vastly distinct immune responses within proximal (initial segment, caput) and distal regions (corpus and cauda). This study aims at analyzing the pathomechanisms behind the different magnitude of immune responses observed by employing a mouse model in which acute bacterial epididymitis was elicited by intravasal UPEC-injection.
Disease progression (day 1-14) was characterized using histological staining, RT-qPCR of Tnfα, Il10 and Il17 and immuno staining of neutrophils (Ly6G+), monocytes (Ly6C+) and macrophages (F4/80+). Our data show a massive influx of neutrophils and monocyte-derived macrophages into the interstitial and epithelial compartment within distal regions that positively correlates with histopathological alterations, i.e. epithelial damage. Simultaneously, expression of inflammatory cytokines was upregulated more than 100-fold within the cauda and persisted on high levels up to 14 days compared to sham mice. In contrast, the epithelial integrity remained intact within proximal regions and an arising inflammation appeared to be immediately resolved.
Our data indicate that the epididymal regions differentially regulate the initial production of inflammatory mediators and together with infiltrating immune cells are key for accelerated or resolved inflammatory processes.

P22 Biomechanical properties of the developing Wolffian duct: Role of the Extracellular Matrix and Protein Tyrosine Kinase 7

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Biomechanical properties of biological tubes play key roles during their development. We measured Young's Modulus (stiffness) along different points of the capsule surrounding the mouse Wolffian duct (WD) from embryonic day (E) E14.5 to E18.5. Results show that Young's modulus did not differ along the capsule but increased from 500Pascals (Pa)-600Pa at E14.5 to 1000Pa-1500Pa at E18.5. Changes in stiffness are the result of changes in the underlying ECM, which in turn, exert a biomechanical force that contributes to WD coiling. We showed previously that Protein Tyrosine Kinase 7 (Ptk7) regulated ECM integrity. Therefore, we tested the hypothesis that Ptk7 regulates the stiffness of the capsule. Using instrumental indentation (SquisherJoy software), we examined the spatial distribution of compressive forces along different points of the WD capsule as described above of conditional knockout Ptk7 mice (Ptk7cKO) E15.5 and E18.5. We observed an increase of Young's Modulus from Ptk7cKO E18.5 capsule (1361+/-200Pa) compared to Ptk7cKO E15.5 (685+/-79 Pa), which was also significantly higher when compared to control E18.5 capsule (849+/-106Pa). Our data would suggest that the increase in stiffness we observe in the capsule of Ptk7cKO WDs is due to the loss of ECM integrity as previously shown. Therefore, maintenance of capsule stiffness, which is dictated by the underlying ECM, plays an important role during Wolffian duct morphogenesis.

P23 Formation of sperm transporting pathway between the testis and epididymis among vertebrates

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The structure of the male genital tract is diverse among vertebrates, but its development remains unclear, especially in the rete region between the testis and epididymis. We here investigated the testis–mesonephros complex of mouse, rabbit, chicken, and frog (Xenopus tropicalis) by immunohistochemistry for markers such as Ad4BP/Sf-1 (gonadal somatic and rete cells in mammals) and Pax2 (mesonephric tubules) and three-dimensional analysis. In all investigated animals, testis cords were bundled at the mesonephros side. Rete cells positive for Ad4BP/Sf-1 (mouse, rabbit) or Pax2 (chicken, frog) were clustered at the border region between the testis and mesonephros. The cluster possessed two types of cords; one connected to the testis cords and the other to the mesonephric tubules. The latter rete cords were contiguous to the tip of the mesonephric tubules or Bowman's capsules in the mouse, rabbit, and chicken but to nephrostomes where the mesonephric tubules opened on the surface of the mesonephros in the frog. In conclusion, this study showed that mammals, avian species, and frogs commonly develop the bundle between the testis cords (testis canal) and the cluster of rete cells (lateral kidney canal), indicating that these animals share basic morphogenesis in the male genital tract. The connection site between the rete cells and mesonephric tubules is suggested to have changed from the nephrostome to the Bowman's capsule during vertebrate evolution from anamniote to amniote.

P24 Relevance of Epididymal Duct Contractions before Puberty

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Background
Smooth muscle cell (SMC) contractions in the adult epididymal duct (EpD) are crucial for the transport of immotile spermatozoa and the expulsion of stored sperm cells during ejaculation. This is reflected by an increasing epididymal SMC thickness from the transporting proximal parts towards the sperm-storing distal part of the EpD.
Former observations, however, suggested spontaneous contractions even before puberty.
Aim
In this regard, we investigated the existence, character, and functional relevance of such prepubertal EpD contractions.
Methods/Results
Live imaging revealed spontaneous contractions in the EpD of postnatal rats. In combination with histology, it was confirmed that these contractions transport exfoliated epithelial cells within the lumen of the EpD in rat and man. This luminal transport was affected by relaxing or contracting pathways.
In contrast to the adult, the prepubertal rat EpD was characterized by a homogenous SMC thickness over the complete EpD length. Preliminary results revealed comparable characteristics of the SMC layer in man.
Conclusion
In conclusion, the contractions of the postnatal EpD might be of special importance for the regulation of organized waste disposal within the lumen and could prevent obstruction-induced infertility during early developmental stages.

P25 In silico protein characterization of the epididymis for determining potential immunocontraception targets

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The epididymis is a tissue of interest for the development of novel male contraceptives since it is where spermatozoa mature in an immune-privileged site, and gain the ability to capacitate and undergo the acrosome reaction, two functions crucial to the fertilization of an oocyte. Immunocontraception is an approach that involves the development of contraceptive vaccines that leverages the immune system in order to prevent fertilization by inhibiting the function of target proteins involved in gamete production & maturation. Although there are some unknowns at the time, such as immune response variations, immunocontraception remains a promising approach with merits of the absence of hormonal disturbance, and the avoidance of irreversible procedures such as vasectomy for men. To explore the opportunities and understand the complexities of the epididymis as a tissue of interest with possible immunocontraception target proteins, an in silico approach involving the use of several integrated computational tools for the functional annotation of epididymis specific proteins was conducted. The study being a comprehensive bioinformatics analysis identified 10 epididymis specific immunocontraceptive targets, demonstrating the role and impact of in silico techniques in accelerating potential male contraceptive discovery in specific tissues such as the epididymis. Further wet lab studies into these targets is required to evaluate and capitalize on their immunocontraception potentials.

P26 Developing a non-invasive predictive model for testicular tissue histology of men with azoospermia

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Men with azoospermia who wish to have children undergo testicular sperm extraction (TESE). The success rate of TESE is only around 60% and histological analysis is the only method used for predicting sperm retrieval. In contrast to this method, magnetic resonance imaging (MRI) is non-invasive and can provide whole testis imaging, parameter mapping, and concentrations of metabolites in the tissue. To test how testicular histology correlates with MRI parameters 35 samples of testicular tissue were obtained via TESE and they underwent 7T MRI, specifically diffusion tensor imaging (DTI), magnetization transfer imaging (MTI), and magnetic resonance spectroscopy (MRS). Samples were then histologically processed, analyzed, and divided into groups based on their mean Johnsen score (JS). The apparent diffusion coefficient (ADC) was higher in samples with a lower mean JS which reflects the higher level of water diffusion in tissue with lower cell density. Fractional anisotropy (FA) was lower in tissues with the lowest cell density which could reflect less restricted water diffusion. The concentrations of glutamate (Glu) and myo-Inositol (m-Ins) were significantly higher in histological groups with higher JS. ADC and FA values as well as concentrations of Glu and m-Ins could, thus, be useful in predicting histological groups of testicular tissue. Intercellular Glu and intracellular m-Ins signaling could be important for the regulation of spermatogenesis and warrant further research.

P27 The enzyme GSK3α, a male fertility kinase in mammals, is essential for sperm maturation in the epididymis

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In mammals, testicular spermatozoa are immotile and lack the ability to fertilize eggs: motility and fertilizing ability develop during their passage through the epididymis. After ejaculation, sperm undergo hyperactivation in the female reproductive tract - a motility transition that is required for sperm penetration of the egg. Both epididymal initiation of motility and hyperactivation are essential for male fertility. The two processes are unique to mammals. Motility initiation, hyperactivation, and metabolic activation involve changes, cAMP, calcium and pH acting through protein kinases and phosphatases. However, we still do not fully understand, how sperm acquire motility in the epididymis and how it is altered during fertilization.

It has long been thought that cAMP-mediated PKA activation was largely responsible for motility initiation and sperm hyperactivation. Work in our laboratory has now shown that the serine threonine protein phosphatases PP1γ2 and PP2B and the protein kinase GSK3α play essential roles in the biochemical pathway involved in sperm maturation. These enzymes profoundly alter or limit the action of cAMP in sperm. Loss of any one of the three proteins by targeted gene disruption in mice impairs epididymal sperm maturation resulting in male infertility. The fact that isoforms of these proteins are highly conserved and present only in mammalian sperm further supports the notion that they are involved in biological processes unique to mammals.

The enzymes GSK3α and GSK3β, encoded by two genes, play important roles in tissues and cells in a wide range of organisms. They are virtually identical in their catalytic functions, substituting for each other in most tissues. One allele alone of any of the two genes is sufficient to maintain normal cellular and tissue functions, highlighting the redundancy of the two isoforms. Remarkably GSK3α is essential only in testis and sperm. Its function cannot be replaced by GSK3β. Either global or testis-specific knockout of the gene for GSK3α results in male infertility.

The gene for GSK3α is present in many species except birds. We have shown that in the non-mammalian sperm that we have tested so far - sea urchin, horseshoe crab, Xenopus and zebra fish - contain only GSK3β despite the presence of GSk3α in their genomes. Because the gene for GSK3α is absent in birds, avian sperm contain only GSK3β. Interestingly, sperm from the monotremes, echidna and duck-billed platypus, do not contain GSK3α. Yeast two-hybrid identified a microtubule-binding protein, CenPV, as a GSK3α-specific binding protein. CenPV is highly expressed in differentiating spermatids and is present in mature sperm. Specific binding to CenPV is consistent with the observation that GSK3α is localized along the sperm flagellum. While sperm lacking GSK3α are infertile, inhibition of GSK3 in normal sperm also impairs in vitro fertilization of eggs. The phospho-proteome of sperm lacking GSK3α shows that proteins involved in energy metabolism are affected. Of note is the glycolytic enzyme hexokinase, impaired annulus, and localization of the monocarboxylate transporter MCT2 and its associated glycoprotein basigin. Our data also show that PP1γ2, PP2B, GSK3α and PKA and high calcium in immature sperm are essential components of the signaling mechanism regulating maturation of sperm in the epididymis and its ability to fertilize eggs in the female reproductive tract.

P28 The loss of polysialic acid shows an impaired contractile phenotype of smooth muscle cells in the epididymal duct linked with a dilated rete testis

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Polysialic acid is a homopolymer of α 2,8-linked sialic acid, which is known to be essential for the development of the brain, for example. The chains can be synthesized by two polysialyltransferases, ST8SiaII and ST8SiaIV. Previous studies showed neuronal damage and a progressive hydrocephalus in polysia knockouts. Mice were smaller and 80% of them died within the first four weeks after birth (Weinhold et al. 2005). During postnatal development, the contractile areas of the testis (Hachem et al. 2021) and epididymis (Simon et al. 2015) exhibit high amounts of polysialic acid.
We now investigated contractile cells in the testis and epididymis of polysialyltransferase-deficient mice (st8sia2-/-; st8sia4-/- and st8sia2+/-; st8sia4-/-) in comparison to wildtype mice (st8sia2+/+; st8sia4+/+) at postnatal days 7.5-9.5.
In both, the testis and epididymis, the smooth muscle cells (SMCs) of seminiferous tubules and the epididymal duct showed an impaired contractile phenotype in knockout mice, indicated by immunostainings using different SMC markers. In addition to reduced expression of structural proteins, cGMP-dependent protein kinase I (PKGI), essential for SMC contractility, was reduced in the testis and even undetectable in the epididymal duct. Most strikingly, however, was a massively dilated rete testis.
We postulate that disturbed contractility of the epididymal duct in polySia knockout mice results in a back pressure of luminal fluid towards the rete testis.

P29 The role of the epididymis in linking paternal exposures to alteration of the sperm sncRNA profile

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Paternal exposure to environmental stressors is known to elicit distinct changes to the sperm small noncoding RNA (sncRNA) profile; modifications that can have significant post-fertilization consequences. Despite this knowledge, there remains limited mechanistic understanding of how paternal exposures modify the sperm sncRNA landscape. To address this question, our laboratory has exploited tractable exposure models in which male mice have been subjected to acute administration of reproductive toxicants (acrylamide), stress hormones (corticosterone), or elevated ambient temperature; challenges that each elicit robust changes in the sperm sncRNA profile. In the case of acrylamide, we traced the differential accumulation of stress-responsive sncRNAs to coincide with sperm transit of the proximal (caput) segment of the epididymis, wherein acrylamide exposure altered the expression of several transcription factors implicated in the expression of acrylamide-sensitive sncRNAs. We have also identified extracellular vesicles (epididymosomes) secreted from the caput epididymal epithelium in relaying altered sncRNA profiles to maturing spermatozoa, the implications of which manifest in the form of dysregulated gene expression during early embryonic development following fertilization by acrylamide-exposed sperm. Overall, these data provide mechanistic links to account for how environmental insults can alter the sperm epigenome and compromise the transcriptomic profile of early embryos.

P30 Heterogeneous macrophage populations exist in the entire male reproductive tract

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The male reproductive tract (MRT) is comprised of the testis, epididymis, vas deferens, seminal vesicles, prostate, urethra, and penis. In almost all organs, macrophages serve as sentinel cells with essential functions in tissue homeostasis, tissue repair, and inflammation resolution. Information about the macrophage (Mϕ) population in the MRT is scarce. Thus, we characterized the Mϕ (F4/80+CD11b+) throughout the MRT in mice using flow cytometry. The proportion of the Mϕ population in relation to CD45+ total leukocytes in adults was highest in the testis (~60%) and epididymis (~55%) and lowest in the penis (~3%). During development, all organs contain a distinct subset of F4/80hiCD11blo Mϕ at the first week of age. After the 3rd week of age, a second Mϕ population arises, which is constituted in the penis and testis of F4/80hiCD11bhi and in the other organs of F4/80loCD11bhi Mϕ. Next, we examined the heterogeneity of Mϕ populations by flow cytometry and immunofluorescence based on CD206 and MHCII expression. We observed four Mϕ populations, namely CD206+MHCII−, CD206+MHCII+, CD206−MHCII+, and CD206−MHCII−, with varying proportions in the MRT. In conclusion, our results indicate that the MRT contains distinct subsets of Mϕ populations, likely to reflect fulfilling organ-specific homeostatic functions.

P31 Junctional endocytosis promotes cholesterol clearance through occludin-facilitated apical absorption of lipophilic cargo and plays an essential role in the epididymis

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Epithelial endocytosis is essential for tissue homeostasis, and junctional endocytosis plays an essential role. While the involvement of epithelial endocytosis in regulation of physiological homeostasis at non-junctional membranes is known, the role of junctional endocytosis in epithelial microenvironment homeostasis is unclear. Occludin is a junctional protein and its deletion mutation causes male infertility in mice. In the occludin-null mice, we found that male infertility was due to impaired sperm maturation and fertilisation ability. In the epididymis of these mice, the luminal microenvironment was disordered, such as increased pH of luminal fluid, accumulated cholesterol and secreted apolipoproteins, accompanying impaired transepithelial transport function. Immunofluorescent assays showed that occludin promotes endocytosis at epithelial tight-junctions and binds directly to apolipoprotein APOJ and facilitates APOJ-chaperoned cargo intracellular trafficking and lysosomal degradation. Overall, we propose that occludin promotes junctional endocytosis and cholesterol clearance from the epithelial lumen, thereby, maintaining the luminal microenvironment homeostasis, especially lipid homeostasis, in the epididymis. In this way, sperm maturation and male fertility are ensured.

P32 Regulation of Low Calcium Homeostasis in the Epididymis and Its Implication in Male Reproduction

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The epididymis plays an important role in sperm maturation and male fertility. The luminal microenvironment established by epithelial cells is critical for epididymal function. Low Ca2+-homeostasis in the epididymis is a crucial factor ensuring sperm maturation. Our research aims to characterise the factors necessary for a favorable epididymal microenvironment and the mechanisms regulating it, particularly as they relate to the low Ca2+-homeostasis. Our previous studies have shown that both the vitD-related TRPV6-TMEP16A channel-coupler and the vitK-dependent GGCX-mediated carboxylation of matrix Gla protein (MGP) regulate Ca2+-homeostasis in the epididymis in a spatially complementary manner. And we found that carboxylated-MGP plays an essential role in promoting Ca2+-dependent protein aggregation in a biphasic manner. An SNP in the human GGCX gene was found to associate with asthenozoospermia. In this study, we provide further evidence to support the notion that luminal matrix Ca2+ functions as a cofactor for carboxylated-MGP scavenging of metabolites in the microenvironment of mouse epididymis. The MGP-mediated aggregation with a secretory apolipoprotein was altered by changing the in-vitro Ca2+ concentrations biphasically. Integrative analyses suggested the involvement of apolipoprotein receptors and vitamin-signaling in the absorption of MGP-bound aggregates. These findings suggest that low Ca2+ homeostasis in the epididymis ensures sperm maturation and male reproduction.

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