Spermatology 2026

Hosted by University of Birmingham

Conference Brochure and Abstracts

29 July - 2 August 2026

Great Hall and Bramall Music Building

Contents

Welcome to the International Spermatology Symposium 2026

It is with great pleasure we welcome you to the XVth International Spermatology Symposium hosted at the University of Birmingham!

We are delighted to bring together leading researchers, early career researchers, and industry partners from around the world to share the latest advances in sperm function and reproductive health.

This symposium is a unique opportunity designed as a retreat, which brings together multiple disciplines with a vested interest in sperm to spend dedicated time together and foster interdisciplinary discussions, collaborations and dissemination of knowledge through an exciting schedule of talks, activities and shared dinners.

The University of Birmingham has a notable history in reproduction. Dr Dame Hilda Lloyd was the first woman to be elected to the GMC (1946) and President of the RCOG (1949). Hilda was significant for her concern for the 'urban poor', STIs, illegal abortions, and the *flying squads* she pioneered to help save the lives of mothers and babies. Baron Solly Zuckerman published the paper in 1951describing the arguments for and against postnatal oogenesis, and Professor Jack Cohen wrote iconic textbooks such as *Living Embryos* (1967), *Reproduction* (1977), and in 1967 published his initial theories on sperm redundancy.

During your time with us we hope you have the opportunity outside of the symposium to discover Birmingham, a city of 1.2 million people renowned for its rich industrial heritage, cultural diversity, and warm hospitality.

We greatly appreciate you joining us here in Birmingham for what is set out to be an inspiring and intellectually stimulating symposium.

We hope your time spent with us is rewarding and memorable.

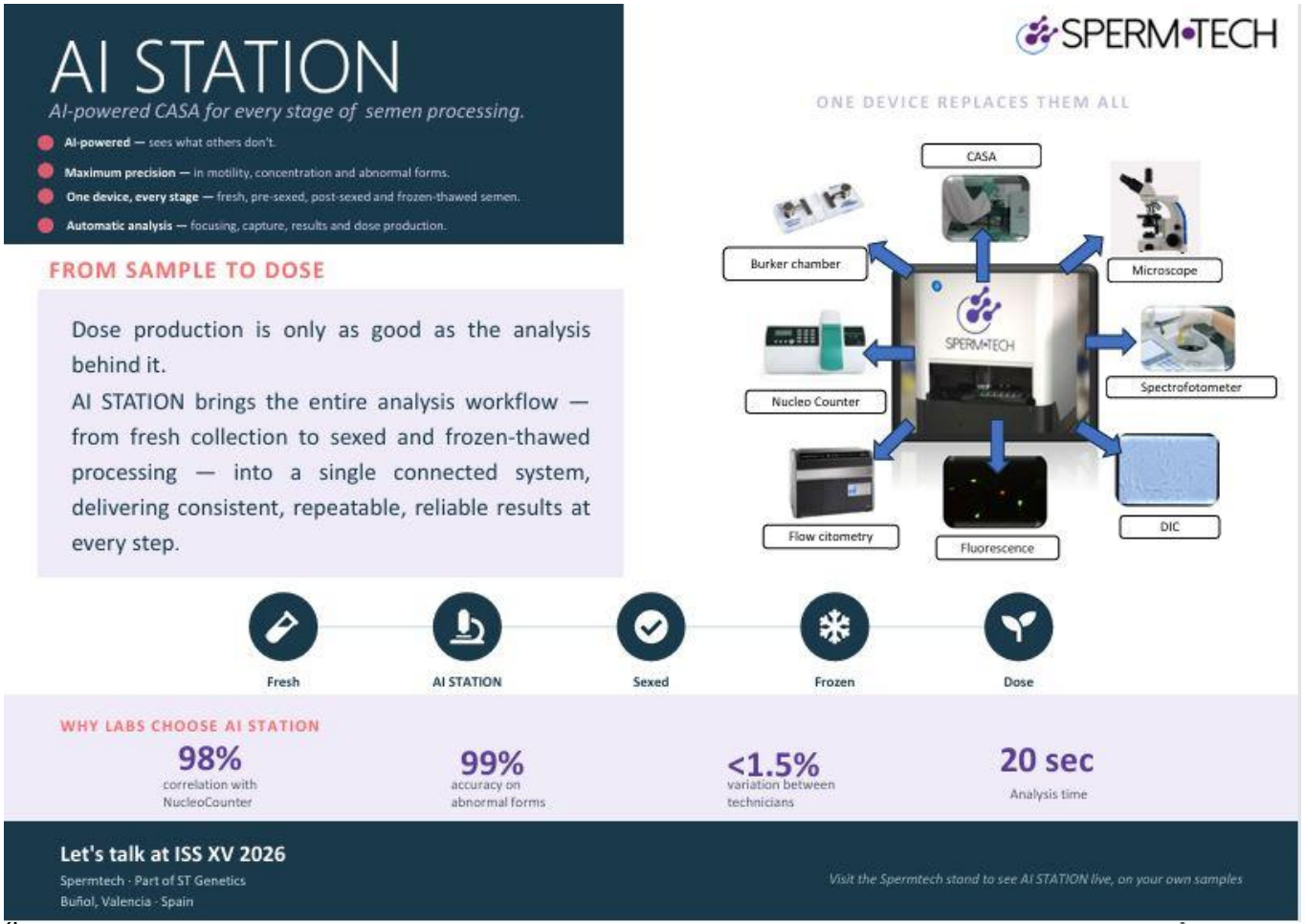
Sincerely,

Prof Jackson Kirkman-Brown

Gold Sponsor

SPERM•TECH

Sperm analysis is our business!



Silver Sponsors

CRYOLAB Ltd

Nexpring Health™

Miltenyi Biotec

truion
science for
the next generation

testhim®
Andrology Clinical Services

Powered by:
LogixX
Fertility

The three musketeers of cell sorting

Large or small, we sort them all

- MACSQuant® Tyto® Lux Cell Sorter
- MACSQuantify™ Tyto Software 3.2
- Large-cell cartridge (LC)

Come by our stand at Spermatology 2026 to find out how we can support your research!

▶ **miltenyibiotec.com/tytolux**

Miltenyi Biotec Ltd. | Almac House, Church Lane | Bisley, Surrey GU24 9DR | UK | Phone +44 1483 799 800 | Fax +44 1483 799 811 | macsuk@miltenyi.com | **www.miltenyibiotec.com**

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

Miltenyi Biotec

testhim®
Andrology Clinical Services

Male fertility diagnostics, delivered with care, speed and accuracy

Accurate testing **Fast results** **Expert guidance** **Ongoing support**

We deliver results that are secure, clear, and clinically actionable, backed by expert guidance and patient support every step of the way.

In partnership with LogixX Fertility

FITZROVIA HOSPITAL LONDON
Part of QASMC Healthcare

Bronze Sponsors

exseed

HAWKSLEY

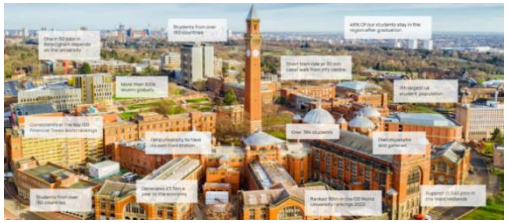
sapyen

Society for Reproduction and Fertility

Key Information

Venue Address

Alan Walters Building
University of Birmingham
Edgbaston
Birmingham,
B15 2TT



Travel Information

The University has its own train station, 'University (Birmingham)' – which is a short walk from the conference venue. Train timetables and service updates are available from the **National Rail website**.

For those driving to the conference, parking is available at the North-East Multi-Storey Car Park (B15 2SA), on floors 1-5 (the ground floor is strictly for hotel guests only). The car park is a 2-minute walk to the Alan Walters Building.

For further transport information to the venue, please see our **Travel page** on the website.

Conference Registration

The Registration Desk will be on the Ground floor of the Alan Walters Building and will be open for the duration of the conference for registration and queries. Please report to the registration desk upon arrival to receive a name badge.

Wi-Fi

To use the venue Wi-Fi, please connect to the network 'UoBGuestWiFi'. Your browser should automatically open, if not go to 'wifi.bham.ac.uk'. Follow on screen instructions, either create an account or log on with existing details. If you have any problems connecting, please ask for support at the Registration Desk.

Social Events

If you have registered for any of the social events listed below, your ticket will be included inside your conference name badge when you collect it at registration.

If you have not registered for a social event, we are unfortunately unable to offer additional tickets at this time. However, please visit the Registration Desk to be added to the waiting list. If a ticket becomes available, we will contact you by email.

Date	Time	Social Event	Location
Wednesday 29th July	6:45pm – 9pm	Drinks Reception	Alan Walters, Atrium
Thursday 30th July	6:30pm – 9pm	Indian Street Food	Vale, Shackleton Building, Infusion Terrace
Friday 31st July	7pm – 9pm	BBQ	Vale, Shackleton Building, Infusion Terrace
Saturday 1st August	7pm – 10pm	Conference Dinner	Edgbaston Park Hotel, Fry Suite

All venues are marked on the campus map on **page 19** of this conference brochure.

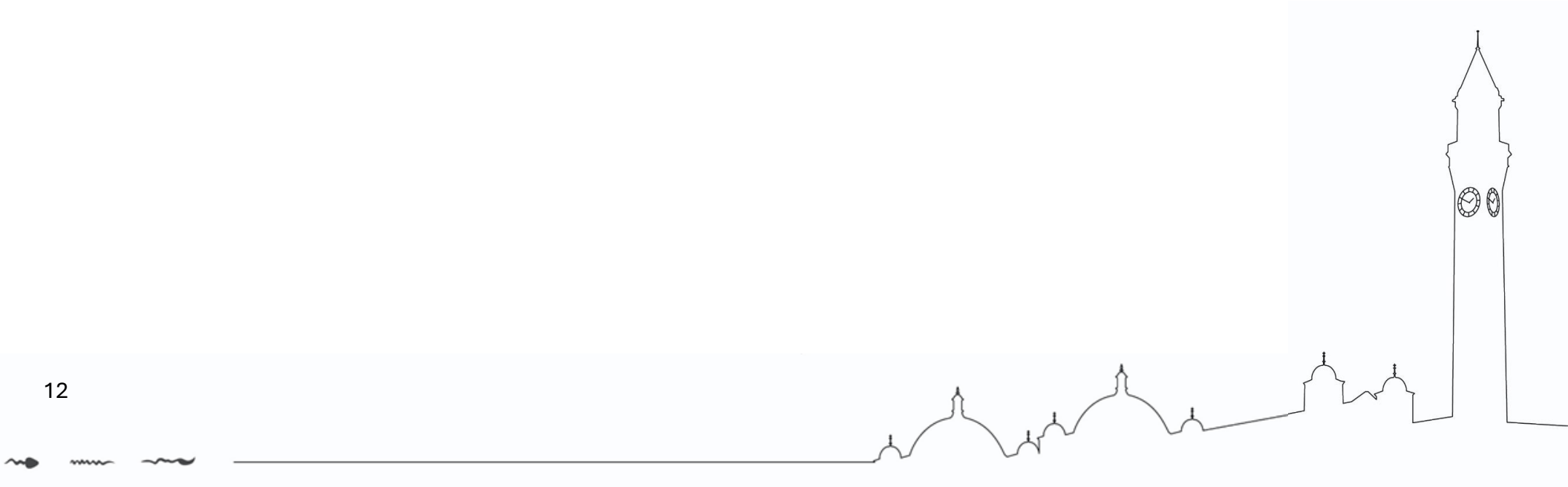
For more information on social events, please visit the **conference website.**

If you have any questions, please speak to a member of the conference team at the Registration Desk—we'll be happy to help.

Conference Programme

Wednesday 29th July

Opening Session		
Time	**Session**	**Location**
16:00 – 17:00	Registration	Atrium
17:00 – 17:30	**Introductory Address and Welcome to UoB** Jackson Kirkman-Brown, University of Birmingham, UK	G03
17:30 – 18:30	**Keynote Speaker: Thaddeus Mann Memorial Lecture** Eduardo Roldán, CSIC, Spain	G03
18:30 – 18:45	**Spermatology Then and Now** Lars Björndahl	G03
18:45 – 21:00	**Welcome Reception with Drinks and Buffet**	Atrium



Thursday 30th July - Spermatogenesis & Genetics

Time	Session	Location
8:00 – 9:00	**Registration and Refreshments**	Atrium
9:00 – 9:45	**Keynote Speaker: Selfish Sperm** Anne Goriely, University of Oxford, UK	G03
9:45 – 10:15	**O1. Machine learning–driven morphometric analysis reveals when species-specific sperm form emerges during spermiogenesis** Maria Teves, Virginia Commonwealth University, USA	G03
10:15 – 10:45	**O2. Integrated metabolomic and RNA-seq analyses suggest a biosynthetic pathway for a sperm attractant in ascidians** Manabu Yoshida, Kitazato University, Japan	G03
10:45 – 11:15	**Refreshment Break**	Atrium
11:15 – 12:00	**Keynote Speaker: When genetics fail sperm, identifying genes critical for sperm function** Leonie Herrmann, Institute of Reproductive Genetics, Germany	G03
12:00 – 12:30	**O3. Calcium-ion efflux maintains fertilizing potential of newt sperm during storage in the sperm reservoir** Akihiko Watanabe, Yamagata University, Japan	G03
12:30 – 14:15	**Lunch**	Atrium
14:15 – 15:00	**Keynote Speaker: Cryo-electron microscopy as a discovery tool in sperm biology** Tzviya Zeev Ben Mordehai, Utrecht University, Netherlands	G03
15:00 – 15:30	**O4. The spermatozoa centriole transmits a novel head kinking movement powered by tail beating** Tomer Avidor-Reiss, University of Toledo, USA	G03
15:30 – 16:00	**Refreshment Break**	Atrium
16:00 – 16:45	**Keynote Speaker: Functional study of testis-specific genes in vivo and in vitro using agenome editing approach** Masahito Ikawa, The University of Osaka, Japan	G03

16:45 – 17:15	**O5. A multi-scale view of horse sperm function: From in vivo dynamics to fertility proteomic signatures** Elie Barakat, INRAE, France	G03
18:30 – 21:00	**Evening Meal - Indian Street Food**	The Vale, Shackleton, Infusion Terrace

Friday 31st July - Ejaculation, Delivery and the Fertilisation Environment

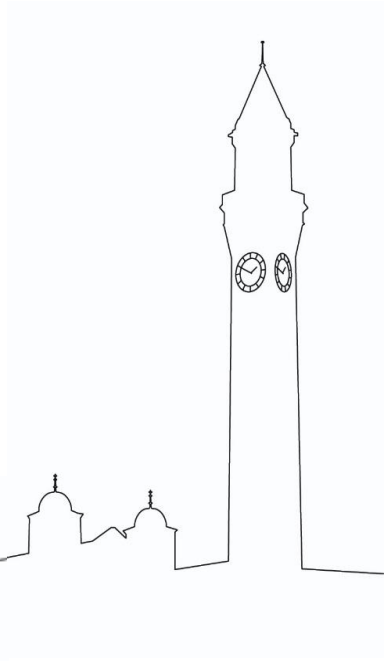
Time	Session	Location
8:30 – 9:00	**Registration and Refreshments**	Atrium
9:00 – 9:45	**Keynote Speaker: Are your Sperm Sneaky? A Squid Story** Noritaka Hirohashi, Shimane University, Japan	G03
9:45 – 10:15	**O6. Spermatozoa as in vitro toxicity model to elucidate the effect of environmental contaminants** Liana Maree, University of The Western Cape, South Africa	G03
10:15 – 10:45	**O7. A multiparametric high-throughput imaging assay to resolve distinct acrosome reaction states in human sperm** Analia Guadalupe Novero, University of Dundee, UK	G03
10:45 – 11:15	**Refreshment Break**	Atrium
11:15 – 12:00	**Keynote Speaker: Myths and Mysteries of human sperm functions** Lars Björndahl, Karolinska Institutet, Sweden	G03
12:00 – 12:30	**O8. Gallocatechin improves mitochondrial activity and motility of stored boar semen** Barbora Klusackova	G03
12:30 – 14:15	**Lunch**	Atrium
14:15 – 15:00	**Keynote Speaker: Channels Maketh the Sperm (function)** Polina Lishko, Washington University School of Medicine, USA	G03
15:00 – 15:30	**O9. Progesterone signalling in human sperm involves rapid changes in membrane potential that are controlled by an interplay of CatSper and Slo3 channels** Christoph Brenker, University of Münster, Germany	G03
15:30 – 16:00	**Refreshment Break**	Atrium
16:00 – 16:45	**Keynote Speaker: Gaining deeper insight from sperm analysis - quality, viscosity, and how the tail wags the head** Meurig Gallagher, University of Birmingham, UK	G03

16:45 – 17:15	**O10. A novel regulator of sperm motility links mitochondrial dysfunction and ferroptosis** Yingying Yin, Karolinska Institute, Sweden	G03
19:00 – 21:00	**Evening Meal - BBQ**	The Vale, Shackleton, Infusion Terrace

Saturday 1st August - Sperm Function: To the oocyte and beyond

Time	Session	Location
8:30 – 9:00	**Registration and Refreshments**	Atrium
9:00 – 9:45	**Keynote Speaker: A Billion Cells – Many Hundred Ways - Diversity Across Species** Gerhard van der Horst, University of the Westen Cape, South Africa	G03
9:45 – 10:15	**O11. Hanging in the balance: Understanding the importance of sperm ROS for pre-implantation embryo development** Joshua Deluao, Adelaide University, Australia	G03
10:15 – 10:45	**O12. Exploring geographical differences in semen quality of young men using spatial dependence analysis** Rita Rahban, University of Geneva, Switzerland	G03
10:45 – 11:15	**Refreshment Break**	Atrium
11:15 – 11:45	**O13. Ultra-rare sperm detection using an AI-enabled microwell system** Hadi Shafiee	G03
11:45 – 12:15	**O14. The effect of combined cryoprotectant strategies on the functional quality of post-thaw chicken sperm** Azindokht Babapour, Wrocław University of Environmental and Life Sciences, Poland	G03
12:15 – 14:15	**Lunch**	Atrium
14:15 – 15:00	**Keynote Speaker: Ocean Derived Chemicals in Mussels** Jonathan P. Evans, University of Western Australia, Australia	G03
15:00 – 15:30	**O15. Towards semen collection at younger age: non-invasive monitoring of puberty onset in young beef bulls at performance testing stations** Joanna Bremer, University of Inland Norway, Norway	G03
15:30 – 16:00	**Refreshment Break**	Atrium
16:00 – 16:45	**Keynote Speaker: Sheep Sperm - Optimised Breeding and Key Mammalian Model** Simon de Graaf, University of Sydney, Australia	G03

18:30 – 22:00	**Conference Dinner**	Edgbaston Park Hotel, Fry Suite

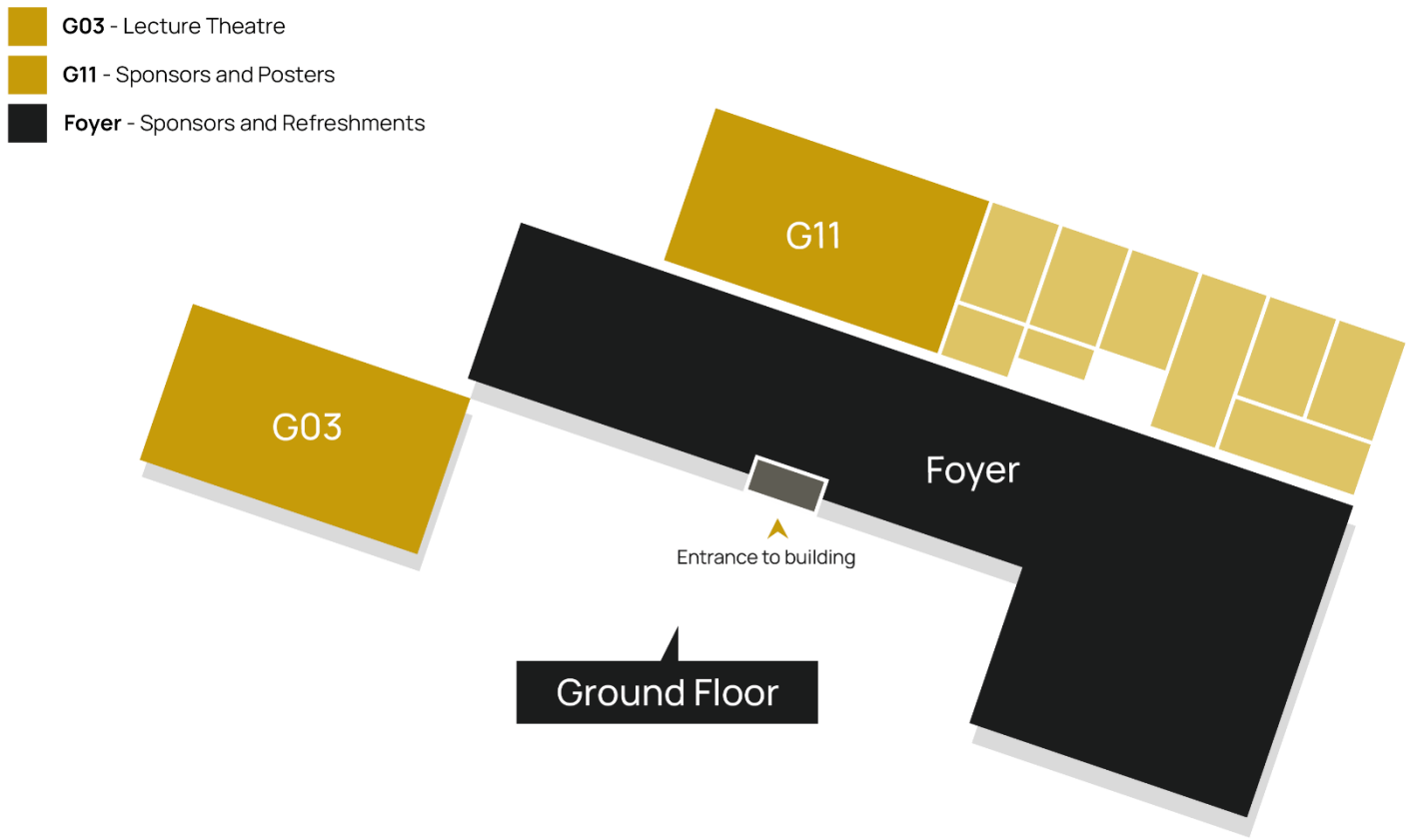


Sunday 2nd August - Sperm: Tiny Cell - Big Picture

Time	Session	Location
9:15 – 9:45	**Registration and Refreshments**	Atrium
9:45 – 10:30	**Keynote Speaker: Disentangling Paternal Effects, Sperm Quality and Aging** Irem Sepil, University of Oxford, UK	G03
10:30 – 11:00	**Refreshment Break**	Atrium
11:00 – 11:45	**Keynote Speaker: Contraception – New Perspectives** Chris Barratt, University of Dundee, UK	G03
11:45 – 12:00	**Spermatology in the Future**	G03
12:00 – 12:15	**Closing Ceremony** Jackson Kirkman-Brown & Meurig Gallagher, University of Birmingham, UK	G03
12:15 – 13:15	Packed Lunch to takeaway	Atrium

Venue Floor Plan

Alan Walters Building Ground Floor



Campus Map

The conference venue is marked as **R29** in the red zone on the campus map, which is available to view/download here.

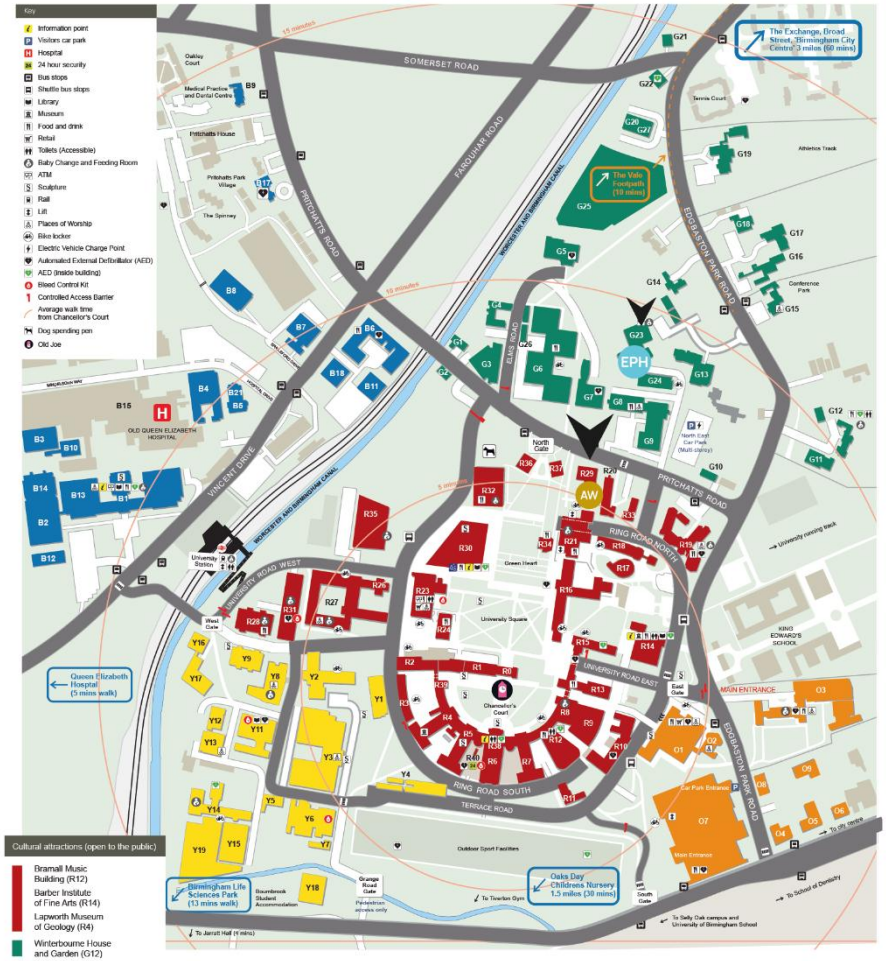
Red Zone
R29 The Alan Walters Building
R0 The Harding Building
R1 Law Building
R2 Frankland Building
R3 Hills Building
R4 Aston Webb – Lapworth Museum
R5 Aston Webb – B Block
R6 Aston Webb – Great Hall
R7 Aston Webb – Student Hub
R8 Physics West
R9 Nuffield
R10 Physics East
R11 Medical Physics
R12 Bramall Music Building
R13 Poynting Building
R14 Barber Institute of Fine Arts
R15 Watson Building
R16 Arts Building
R17 Ashley Building
R18 Strathcona Building
R19 Education Building
R20 J G Smith Building
R21 Muirhead Tower
R23 University Centre
R24 Staff House
R26 Geography & Environmental Sciences
R27 Biosciences Building
R28 Murray Learning Centre
R29 The Alan Walters Building
R30 Main Library
R31 Collaborative Teaching Laboratory
R32 Teaching and Learning Building
R33 Fry Building
R34 Cuore
R35 Molecular Sciences Building
R36 Pritchatts Lodge 1
R37 Pritchatts Lodge 2
R38 Aston Webb Dome & Semi-Circle
R39 Aston Webb Semi-Circle (West)
R40 Security

Orange Zone
O1 The Guild of Students
O2 St Francis Hall
O3 University House
O4 Ash House
O5 Beech House
O6 Cedar House
O7 Sport & Fitness
O8 Fir House
O9 Elm House

Green Zone
G1 32 Pritchatts Road
G2 31 Pritchatts Road
G3 European Research Institute
G4 3 Elms Road
G5 Computer Centre
G6 Metallurgy and Materials
G7 IRC Net Shape Laboratory
G8 Gisbert Kapp Building
G9 52 Pritchatts Road
G10 54 Pritchatts Road – The Institute of Advanced Studies
G11 Maples Nursery
G12 Winterbourne House and Garden
G13 Hornton Grange
G14 Garth House
G15 Westmere
G16 Lucas House
G17 Peter Scott House
G18 Priorsfield
G19 Park House
G20 Wolfson Advanced Glasshouses
G21 Park Grange
G22 Elms Day Nursery
G23 Edgbaston Park Hotel and Conference Centre
G24 Centre for Human Brain Health
G25 Environmental Research Facility
G26 Plasma Furnace
G27 Elms Glasshouses

Blue Zone
B1 Medical School
B2 Institute of Biomedical Research
B3 Institute of Translational Medicine
B4 Robert Aitken Institute for Clinical Research
B5 Denis Howell Building
B6 Institute of Research and Development
B7 90 Vincent Drive
B8 Henry Wellcome Building for Biomolecular NMR Spectroscopy
B9 University Medical Practice
B10 Advanced Therapies Facility
B11 Biomedical Innovation Hub
B12 Health Services Research Centre
B13 Wolfson Centre for Medical Education
B14 Medical School Extension
B17 13 Pritchatts Road
B18 Information & Computer Technology (ICT)
B21 Cancer Immunology Building

Yellow Zone
Y1 The Old Gym
Y2 Haworth Building
Y3 Y3
Y4 Terrace Huts
Y5 Estates West
Y6 Maintenance Building
Y7 Landscape Services Building
Y8 Engineering Building
Y9 Computer Science
Y11 Chemical Engineering
Y12 Biochemical Engineering
Y13 Chemical Engineering Workshop
Y14 Sport, Exercise and Rehabilitation Sciences
Y15 Civil Engineering Laboratories
Y16 Y16
Y17 Public Health
Y18 Bournbrook Sports Pavilion
Y19 NBIF



Key

AW **Alan Walters**
- Conference Venue

EPH **Edgbaston Park Hotel**
- Conference Dinner

Footpath to the Vale
- Accomodation

Keynote Speakers

Prof. Chris Barratt

Professor of Diabetes Endocrinology and Reproductive Biology, University of Dundee

Talk title: Contraception – New Perspectives

Biography: Professor Barratt is Head of the Division of Post Graduate Medicine at the Medical School, Head of the Reproductive Medicine Group at the University of Dundee as well as a clinical scientist (Hon) with NHS Tayside. He graduated with an Honours degree in Zoology and then completed a Post Graduate Certificate in Education (University of Wales, Swansea). His PhD, also in Zoology, was under the supervision of Jack Cohen at the University of Birmingham. His formative post-doctoral studies and ART experience was gained at the University of Sheffield [with Ian Cooke] where they specialized in natural cycle IVF. From 1997-2005 he was the Scientific Director of the ART Centre at the Birmingham Women's Hospital. Professor Barratt has been awarded a number of honours for outstanding contributions to the discipline e.g. Young Andrologist of the Year, the Professor Sir Robert Edwards keynote lecture at ESHRE. He was awarded a DSc from University of Birmingham and has a h index of 54 (Scopus 2021). Professor Barratt is a Fellow of the Royal Society of Edinburgh. He is a regularly invited speaker at national and international scientific conferences/workshops. He was a member of the WHO Male Fertility Semen Analysis Taskforce (for both the 4th and 5th editions) and director of the WHO (2012-2017) Male Fertility Expert Working Group which devised a new system for the diagnosis and treatment of the infertile male. He was a member of the Human Fertilisation and Embryology Authority for 6 years until 2009.Professor Barratt has been appointed to editorial board of WHO for development of new Semen Analysis manual (6th edition, publication date late 2021). He recently published the global WHO guidelines for semen reference ranges.He has been on the Editorial Board of Human Reproduction, Human Fertility, Biology of Reproduction, Human Reproduction Update, Journal of Andrology and was Editor-in-Chief of Molecular Human Reproduction from 2013-2018. Currently he is a member of the Editorial board of Physiological Reviews. Professor Barratt is coordinating the Global Initiative on Male Reproductive Health. His life's ambition is to see - live - Wales comprehensively beat the All Blacks.

Dr. Lars Björndahl

Associate Professor of Andrology, Karolinska Institutet

Talk title: Myths and Mysteries of human sperm functions

Biography: M.D.Karolinska Institutet 1982, PhD 1986 "On Sperm Nuclear Zinc and Chromatin Decondensation". Specialist in Laboratory Medicine 1992. At the Andrology Laboratory, Karolinska University Hospital 1987-2000; 2001-2005 ACU, Birmingham Women's Hospital and University of Birmingham, Honorary Senior Lecturer. 2010-2026 Senior Consultant Laboratory Physician at Karolinska. From 2013 Andrology Lab Director, head of Tissue Establishment. 2024 Associate Professor of Andrology at Karolinska Institutet. Member of the WHO editorial committee for the Semen Analysis Manual, 5th edition (2005-10) and Chief Editor of the 6th edition (2018-2021); consultant for the WHO Infertility Global Guidelines and Research Group (2012-2019). Project Leader for the Basic Semen Examination Standard ISO 23162 (2017-2021). Involved in studies on AI in basic ejaculate examination, human sperm physiology as well as development of clinically useful assessments of male reproductive functions.

Prof. Simon de Graaf

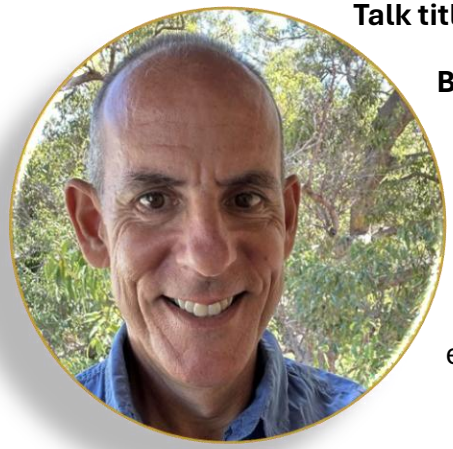
Professor of Animal Reproduction, The University Of Sydney

Talk title: Sheep Sperm - Optimised Breeding and Key Mammalian Model

Biography: Simon is recognised worldwide as an expert in livestock reproduction, artificial breeding and semen assessment. He has published over 100 journal articles and several book chapters on animal reproduction and trained over 3000 veterinary medicine, animal science and agriculture students in animal reproduction and surgical theriogenology. He is currently Professor of Animal Reproduction at The University of Sydney, Secretary General of the International Congress on Animal Reproduction, Director of The Reproduction Company Pty Ltd and an elected fellow of The Royal Society of New South Wales and the Society of Reproductive Biology.

Dr. Jonathan P. Evans

Professor of Evolutionary Biology, The University of Western Australia



Talk title: Ocean Derived Chemicals in Mussels

Biography: Jon is an evolutionary biologist whose recent research focuses on gamete-level sexual selection and how female reproductive fluids (FRF) influence fertilisation success and offspring fitness. His work uses broadcast-spawning marine invertebrates to understand how egg-derived chemical cues that attract sperm differentially bias fertilisation toward genetically compatible males. His latest research aims to understand the mechanisms that facilitate these differential interactions between sperm and FRF, which will lead to collaborative research on the possibilities of differential gene expression and haploid selection in ejaculates.

Prof. Anne Goriely

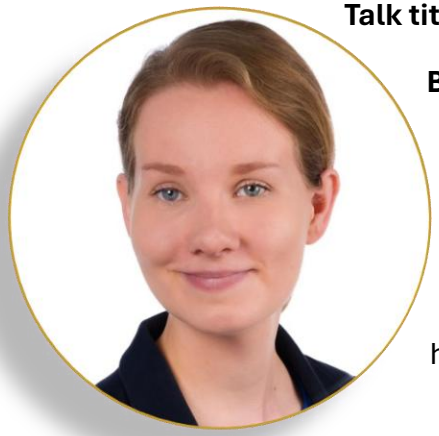
Professor of Human Genetics, University of Oxford

Talk title: Selfish Sperm

Biography: After an undergraduate degree in Engineering (Agronomy) at the Université Libre de Bruxelles (Belgium), Anne Goriely obtained a PhD studying the development of the nervous system of the Drosophila embryo. She spent 4 years in New York at Cornell Medical School and Rockefeller University, before moving to the UK to work on the nervous system development of the chick embryo. She then joined the MRC WIMM to study the origin of rare human developmental disorders associated with paternal age effects. Using a human genetics approach, her group's main interests lie in elucidating the mechanisms by which we acquire new germline mutations and the implications of such processes in health, disease and for genome evolution. Her work focuses mainly on the biology of the testis - particularly how aging affects sperm production and genome integrity.

Dr. Leonie Herrmann

Postdoctoral Scientist, Institute of Reproductive Genetics



Talk title: When genetics fail sperm, identifying genes critical for sperm function

Biography: Leonie Herrmann is a biologist specializing in male reproductive health. She began her postdoctoral career in the field of human sperm physiology at the Centre of Reproductive Medicine and Andrology in Münster, Germany. She is currently a postdoctoral scientist in the group of Professor Frank Tüttelmann at the Institute of Reproductive Genetics at the University of Münster, where her research focuses on genes and molecular pathways essential for spermatogenesis, sperm morphology, and sperm motility. Her work aims to advance the understanding of the genetic basis of human male infertility.

Prof. Noritaka Hirohashi

PI, Shimane University

Talk title: Are your Sperm Sneaky? A Squid Story

Biography: Noritaka Hirohashi is a PI at Shimane Univ. in Japan studying animal reproduction and sexual selection. He has been studying sperm acrosome reaction in sea urchins and mice. Currently, his focus is on squid biology, particularly exploring the evolution of sperm traits that are closely linked to male mating behaviors.

Dr. Masahito Ikawa

Professor, RIMD, The University of Osaka

Talk title: Functional study of testis-specific genes in vivo and in vitro using agenome editing approach

Biography: Masahito Ikawa is a Distinguished Professor at the Research Institute for Microbial Diseases, Osaka University, Japan. He received his Ph.D. in 1997 and has since made pioneering contributions to reproductive biology and genome engineering. His laboratory pioneered the application of CRISPR/Cas9 genome editing to reproductive biology, transforming functional genetic analysis in mice. His group has generated more than 400 testis-specific knockout mouse lines, identified over 100 genes essential for male fertility, and uncovered key molecular mechanisms governing spermatogenesis, sperm function, and fertilization. These discoveries have provided fundamental insights into the molecular basis of male infertility and mammalian fertilization. Dr. Ikawa has published more than 400 peer-reviewed papers and has received numerous honors, including the JSPS Prize (2012), the SSR Research Award (2017), the Ando-Tajima Award from the Japanese Association for Laboratory Animal Science (2021), and the Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology (2023). He serves on the editorial boards of several international journals and continues to promote innovative research and international collaboration in reproductive biology.

Dr. Polina Lishko

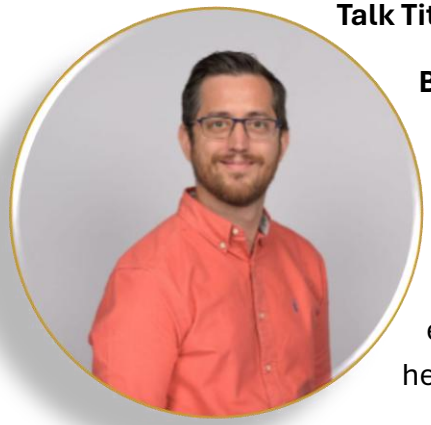
Professor of Cell Biology and Physiology, Washington University School of Medicine

Talk title: Channels Maketh the Sperm (function)

Biography: Polina V. Lishko, Ph.D., is a BJC Investigator and Professor of Cell Biology and Physiology at WashU School of Medicine. She earned her Ph.D. in Biophysics from the National Academy of Sciences of Ukraine in 2000, where she studied the molecular mechanisms of neurodegeneration and memory. Dr. Lishko completed her postdoctoral training at Harvard Medical School and Harvard University. There, she investigated the molecular mechanisms of vision in the lab of Dr. Arshavsky and later worked with Dr. Gaudet on the structure and function of mammalian TRPV channels—key sensors of pain and temperature. Her work led to the first structural description of the TRPV1 ankyrin repeat domain, shedding light on the channel's regulatory mechanisms.From 2006 to 2011, Dr. Lishko was a researcher at the University of California, San Francisco, where she studied ion channels and transporters such as CatSper, Hv1, and UCP1, elucidating their roles in reproduction and thermogenesis. Her discoveries during this period revealed critical regulatory pathways for mammalian fertility.In 2012, she joined the Department of Molecular and Cell Biology at the University of California, Berkeley, as an Assistant Professor. There, she led pioneering research on sperm motility, fertility, contraception, and ovarian aging. After earning tenure, she expanded her work to encompass age-related disorders, including neurodegeneration and vision loss. Dr. Lishko joined WashU Medicine in 2023, where she continues to investigate ion channels at the intersection of reproduction, aging, and neurodegenerative diseases.

Dr. Meurig Gallagher

Assistant Professor in the Centre for Systems Modelling and Quantitative Biomedicine



Talk Title: Gaining deeper insight from sperm analysis - quality, viscosity, and how the tail wags the head

Biography: Meurig is an Associate Professor of Interdisciplinary Healthcare Science, in the School of Medical Sciences, University of Birmingham. As part of this role, Meurig is Deputy Director of ChRS, and is the Human Fertilisation and Embryology Authority (HFEA) Research Person Responsible for the University of Birmingham (Centre 0209).

Meurig is a truly interdisciplinary researcher who integrates mathematical modelling and software with experimental methods to create new diagnostics and treatments for male infertility, and to tackle wider healthcare applications.

The main focus of Meurig's research revolves around the creation and development of the package FAST (Flagellar Analysis and Sperm Tracking), a tool that enables rapid, affordable analysis of the moving flagellum of the sperm cell. FAST is deployed with international clinical collaborators to understand key questions surrounding the role sperm play in the outcomes of fertility treatment, as well as to understand more general aspects of sperm function across species.

Prof. Eduardo Roldan

Research Professor, Spanish Research Council

Talk title: Thaddeus Mann Memorial Lecture

Biography: Eduardo Roldan is a Research Professor at the Spanish Research Council (CSIC), head of the Reproductive Biology and Evolution Group, and the director of the Germplasm and Somatic Tissue Bank of wildlife and endangered species at the National Museum of Natural Sciences (CSIC) in Madrid. Professor Roldan holds degrees in veterinary medicine (DVM), natural sciences (PhD) and philosophy (BA). Was postdoctoral fellow at the University of Hawaii, Honolulu (USA) and the AFRC Institute of Animal Physiology and Genetics Research, Cambridge (UK). He subsequently served as Senior Research Scientist at The Babraham Institute, Cambridge (UK), and as Professor and Head of the Division of Reproduction and Development at The Royal Veterinary College in London (UK). In Spain, he held senior research positions at the Department of Animal Reproduction (INIA), Madrid, and at the Institute of Biochemistry (CSIC-University Complutense), Madrid. He later joined CSIC, where he progressed through its senior scientific ranks—from Research Scientist to Senior Research Scientist and ultimately to Research Professor. He has received numerous prestigious recognitions, including awards from the People's Republic of China, the Wolfson Research Merit Award from The Royal Society of London, the University of Buenos Aires 200th Anniversary Medal and a doctor honoris causa degree from the University of Buenos Aires. He is also a Fellow of the Royal Academy of Veterinary Sciences of Spain. Professor Roldan has contributed extensively to reproductive biology, having served on the Editorial Board of Reproduction, and currently as Associate Editor for Molecular Reproduction and Development, Frontiers in Cell and Developmental Biology, Theriogenology Wild, and Scientific Reports. He leads an internationally recognized research program focused on the molecular and cellular biology of sperm formation and function, evolution of reproductive traits and genes in mammals, and the development of assisted reproductive techniques for wildlife and endangered species.

Dr. Irem Sepil

Royal Society Research Fellow, University of Oxford

Talk title: Disentangling Paternal Effects, Sperm Quality and Aging

Biography: Irem is an evolutionary biologist interested in reproductive ageing, nongenetic parental effects, sexual selection, and life-history theory. Her postdoctoral research examined an overlooked aspect of male reproductive ageing: the decline in ejaculate performance with age. Now a Royal Society Dorothy Hodgkin Fellow at the University of Oxford, she investigates how paternal age and diet affect offspring fitness. Using fruit flies, her group aims to reveal the ejaculate-mediated mechanisms linking paternal condition to offspring outcomes.

Prof. Gerhard van der Horst

Emeritus Professor, University of the Western Cape

Talk title: A Billion Cells – Many Hundred Ways - Diversity Across Species

Biography: Professor Gerhard van der Horst is an internationally recognized reproductive biologist and physiologist. A founding member of the Reproductive Biology Society of South Africa (1984), he has played a leading role in advancing reproductive science both nationally and globally. In the 1990s, he co-developed and marketed the sperm motility analysis program SMQ, which became widely used in the study of sperm function. He has served two terms as President of the Physiology Society of Southern Africa and chaired the International Union of Physiological Sciences (IUPS) in South Africa, representing the country at the 1997 IUPS meeting in St Petersburg. He later convened and chaired the 9th International Symposium on Spermatology, held in Cape Town in 2002.Professor van der Horst has published more than 150 peer-reviewed papers, contributed to four book chapters, and presented over 250 conference papers worldwide. He is the only person to have twice received the Senior Research Award from the University of the Western Cape and has been honored with the Ernst Oppenheimer Memorial Travel Award. Over the past fifteen years, he has presented research and workshops in 45 countries and supervised more than 50 MSc and PhD students.He serves as Senior Consultant to Microptic SL in Barcelona, contributing to the development of Computer-Aided Sperm Analysis, and as Research Associate at the National Zoological Gardens (SANBI) in Pretoria. He holds honorary professorships in Physiology and Animal Physiology at the University of Stellenbosch and serves as Associate Editor for Reproduction in Domestic Animals and the International Polish Andrology Journal.Renowned for his expertise in comparative spermatology, Professor van der Horst has studied sperm from over 100 species, from invertebrates such as sea urchins to vertebrates including fish, mammals, and humans, making significant contributions to our understanding of reproductive biology.

Dr. Zeev-Ben-Mordehai

Associate Professor, Utrecht University

Talk title: Cryo-electron microscopy as a discovery tool in sperm biology

Biography: Tzviya Zeev-Ben-Mordehai is an associate professor at Bijvoet Centre for Biomolecular Research of Utrecht University in the Netherlands.An expert in cellular structural biology of mammalian gametes. Zeev-Ben-Mordehai received her PhD from the Weizmann Institute of Science in 2008. In 2015 she established her group in the Division of Structural Biology of Oxford University. Since 2017 her group is part of Bijvoet Centre for Biomolecular Research of Utrecht University. Her group combines biochemistry, biophysics, and advanced cryo-electron microscopy to unravel the molecular basis of fertilization. Among the group achievements are the first in-cell structures of mammalian sperm flagella (EMBO J 2021 PMID 33694216), structure of conserved protein arrays that tether mitochondria to the underlying cytoskeleton (PNAS 2021 PMID 34737233). By developing a cryo-electron microscopy single-particle-analysis approach that allows resolving atomic resolution structures of multi-protein complexes without isolating them from the cell, the group resolved the native axonemal doublet microtubules structure from sperm (Cell 2023 PMID 37327785, Nature 2025 PMID 39743588).

Oral Presentations

O1: Machine learning–driven morphometric analysis reveals when species-specific sperm form emerges during spermiogenesis

*** This abstract will also be displayed as a poster, on posterboard 33 ***

Clara Agudo-Rios[1], Anthony Valverde[2], Eduardo R S Roldan[1], **Maria Teves**[3]

[1]Museo Nacional de Ciencias Naturales, CSIC, [2]Costa Rica Institute of Technology, San Carlos Campus, [3]Virginia Commonwealth University

Spermiogenesis is a complex and dynamic differentiation process essential for sperm development. During this process, round spermatids undergo modifications that allow them to differentiate into elongated spermatids, ultimately leading to mature species-specific spermatozoa. Despite its importance, knowledge of the mechanical, molecular, and cellular bases underlying this process remains limited. Traditionally, spermatid maturation steps have been identified using morphological criteria in histological sections of seminiferous tubules examined by light microscopy, primarily based on nuclear and acrosomal changes. More recently, immunofluorescence and electron microscopy have enabled improved characterization. There is now an urgent need for objective methods to categorize spermatid steps in vitro in order to (a) identify underlying species-specific changes, (b) advance induced differentiation from pluripotent stem cells, and (c) detect the onset of sperm abnormalities. Quantitative morphometric analyses offer a powerful alternative. Here, we developed an unsupervised machine-learning pipeline to objectively classify spermatid maturation steps based on morphological variables of spermatid structures. We further extend this approach to identify the key steps at which distinct species-specific patterns emerge. Three mouse species (Mus musculus, Mus spretus, and Mus spicilegus), which differ in mature sperm shape, size, and function, were used as models.

Germ cells (n=5 males of each species) were labeled with DAPI (nucleus) and anti-acetylated tubulin (manchette). Each cell was imaged using fluorescence microscopy (nucleus, manchette) and phase contrast (entire cell). Shape and size were analyzed for each structure using NMA software v1.20.0. Morphometrics of entire cell, nucleus and manchette of each spermatid steps were combined with Principal Component Analysis (PCA), k-means clustering and Linear Discriminant Analysis (LDA) to objectively characterize progression within and between species.

Changes followed a continuous and conserved trajectory in all species. Yet, interspecific morphological divergence was not uniformly distributed along the process. Informative interspecific differences emerged during steps 11-12 in the shape and dimensions of the three structures analyzed (entire cell, nucleus and manchette). In shape, diversity was identified in overall angle profiles and specific segments 0 and 1. In morphometry, differences were uncovered in nuclear minimum diameter, entire cell maximum Feret and bounding height, and in the manchette's area and minimum diameter. Inclusion of manchette descriptors enhanced the discriminative capacity of models that included only entire cell and nuclear parameters, improving separation of steps and reducing overlap of the three species. k-means clustering following PCA achieved a mean accuracy of 91.7% for the discrimination of steps between species.

Our findings identified spermiogenesis steps 11-12 as a key developmental window in which species-specific sperm morphology is established. By linking quantitative morphology with developmental timing, this framework provides a robust basis for integrative studies that combine morphometric, molecular and evolutionary approaches to investigate how selective pressures influence sperm form and function through their effects on spermiogenesis.

O2: Integrated metabolomic and RNA-seq analyses suggest a biosynthetic pathway for a sperm attractant in ascidians

*** This abstract will also be displayed as a poster, on posterboard 35 ***

Manabu Yoshida[1], Mitsuru Jimbo[3], Kaoru Yoshida[2]

[1]Kitazato University College of Liberal Arts and Sciences, [2]Toin University of Yokohama, [3]Kitasato University School of Marine Biosciences

Study Question: To identify intermediate metabolites and candidate genes involved in SAAF biosynthesis using integrated metabolomic and transcriptomic analyses.

What is already known: Sperm chemotaxis toward the egg contributes to successful fertilization. In ascidians, which belong to the phylum Chordata, sulfated steroids released from the egg function as sperm activating and attracting factors (SAAFs). Although the structure of SAAF in Ciona robusta (also known as C. intestinalis type I) has been elucidated, its biosynthetic pathway remains unknown. The structure of SAAF in Ascidia sydneiensis is highly similar to that in Ciona. Elucidation of this pathway is expected to provide insights into both the mechanism of sperm chemotaxis and the evolution of fertilization.

Study Design and Methodology: We combined metabolomic and transcriptomic approaches to investigate SAAF biosynthesis. Metabolomic analyses were conducted using outsourced profiling, followed by targeted MS/MS to identify precursor and intermediate metabolites. In parallel, publicly available RNA-seq data from C. robusta oocytes at different developmental stages were reanalyzed. RNA-seq reads were re-quantified using Salmon, and genes expressed during oocyte maturation were extracted and classified according to stage-dependent expression patterns. Functional annotation was then used to identify candidate genes involved in sterol metabolism and sulfation.

Results: Metabolomic analysis detected precursor molecules containing zero or one sulfate group, indicating that sulfation occurs during the later stages of oocyte maturation. In addition, multiple hydroxylated sterol intermediates were identified, suggesting active modification of cholesterol-derived precursors during maturation. Transcriptomic analysis revealed stage-dependent expression of genes associated with sterol metabolism, including cytochrome P450s (CYPs), hydroxysteroid dehydrogenases (HSDs), and

sulfotransferases. Genes involved in steroid backbone modification were predominantly expressed at intermediate stages, whereas genes related to sulfation and terminal modification showed higher expression at later stages.

Limitations: This study is based on transcriptomic reanalysis and metabolite identification without direct functional validation. The assignment of candidate genes relies on annotation-based inference, and the proposed pathway remains to be experimentally validated.

Conclusion: These findings support a biosynthetic model in which steroid-like precursors are sequentially modified and then sulfated to form SAAF. Integrating metabolomic and transcriptomic data provides a coherent framework linking chemical intermediates to candidate biosynthetic genes, offering new insights into the molecular basis of sperm chemotaxis in ascidians.

O3: Calcium-ion efflux maintains fertilizing potential of newt sperm during storage in the sperm reservoir

Reiji Teraoka[1], Nozomi Sato[1], Haruna Kanno[1], Eriko Takayama-Watanabe, **Akihiko Watanabe**[1]

[1]Yamagata University

Sperm storage is a critical event in various reproductive strategies of animal species. It sometimes prolongs for months to years depending on species, whereas long time storage has a risk to damage fertilizing potential of the sperm. It is unclear how the sperm's potential for fertilization is maintained during the long storage period. Japanese-red bellied newt, Cynops pyrrhogaster undergo internal fertilization using sperm stored in the female reservoir. Spermatogenesis continuously progresses in warm seasons, and in winter, meiotic division of spermatogenic cells is ceased, while post meiotic cells progress spermiogenesis to become fertile sperm. After spermiation, sperm are stored in the vas deferens for several months, and then, transferred to female. Fertile sperm of the newt maintain sub-micromolar levels of intracellular Ca2+ in the midpiece, which makes them responsive to the sperm motility initiating substance (SMIS) at fertilization. As Ca2+ influx possibly occurs through Ca2+ permeable channels of the newt sperm, Ca2+ efflux suggestively concerns the maintenance of appropriate levels of intracellular Ca2+ in the midpiece. Here, we investigated the significance of Ca2+ efflux to keep sperm fertility of the newt during storage in the vas deferens. Intracellular Ca2+ was detected by a Ca2+ indicator, Fluo8H, only in the midpiece, in both the midpiece and the principal piece, or only in the principal piece depending on individual vas deferens sperm. Calcium-ion in the principal piece disappeared in response to SMIS in many sperm, and the disappearance was suppressed by inhibitors of plasma membrane Ca2+-ATPases (PMCAs), caloxin 2A1, and inhibitors of Na+-Ca2+ exchangers (NCXs), sn6 and SEA0400. Inhibitors of calmodulin, ophiobolin A, and calpain, PD150606, suggested that the Ca2+ efflux through PMCA was activated initially by calmodulin and supplementarily by calpain. When sperm were incubated in the Steinberg's salt solution, relatively small Ca2+ influx occurs to gradually increase intracellular Ca2+ in the principal piece. The inhibitors of PMCA and NCX activities promoted the increases of intracellular Ca2+.The inhibitors also suggested that PMCA and NCX prevented acrosome reaction from occurring spontaneously. In the sperm stored in the vas deferens, decreasing activities of calmodulin and calpain is suggested to increase the Ca2+ in the principal piece and make sperm likely to undergo acrosome reaction spontaneously. These results suggest that Ca2+ efflux through PMCA and NCX comprehensively maintains fertilizing potential of the newt sperm during months of storage in the sperm reservoir.

O4: The spermatozoa centriole transmits a novel head kinking movement powered by tail beating

Tomer Avidor-Reiss[1]

[1]University of Toledo

Spermatozoa evolved an atypical centrosome with novel structures that are uniquely dynamic. What the function of these unique features is unknown. We propose that the atypical centrosome serves as a transmission system that delivers power from tail beating to generate a novel head-kinking movement.

The spermatozoa centrosome includes a splayed (atypical) distal centriole with two unique rod-like structures that scaffold the right and left sides of the distal centriole. During bovine sperm swimming, the distal centriole rods slide. This sliding correlates with sperm tail beating and head kinking. This head-kinking is observed during the swimming of human, bovine, and rabbit spermatozoa. Furthermore, this hypothesis is supported by studies of the essential function of sperm centriole protein POC1B.

POC1B has multiple functions in sperm formation, and its loss results in abnormal sperm morphology and motility, making it difficult to study. We introduced a subtle mutation to overcome this, deleting 78 amino acids from POC1B's C-terminus. We used rabbits as a genetic model because, unlike mice, rabbit sperm centrioles functionally resemble those in human sperm. Homozygous POC1B mutant spermatozoa are fertile, free-swimming, and have normal morphology; however, their centrioles lack POC1B, indicating the essential role of the POC1B C-terminus in centriolar localization in spermatozoa. Preliminary data suggest that mutant sperm have short, static, fused centriole rods, indicating a critical role for POC1B in rod formation and sliding. In rabbit spermatozoa, tails beat to the right, and heads kink to the left, and these directions correlate. The directionality and correlation in the mutant are the same as in the wild type. However, the mutants have reduced ranges of tail beating and head kinking, indicating that rod fusion limits the freedom of axoneme movement. Also, the mutant spermatozoa have reduced cycle times for tail beating and head kinking. Finally, the fertility of POC1B mutant males was reduced in sperm competition experiments, suggesting that centriole rod sliding provides a reproductive advantage. Overall, this study supports the hypothesis that the sperm centrosome is a transmission system between the tail and head, coordinating tail beating and head kinking.

We propose that spermatozoa head kinking can serve as a novel biomarker for diagnosing unexplained male infertility and as a tool for selecting sperm with higher fertilization potential.

O5: A Multi-scale View of Horse Sperm Function: From In Vivo Dynamics to Fertility Proteomic Signatures

Elie Barakat[1], Theo Prudhomme[1], Erika Caldas[1], Fabrice Reigner[2], Philippe Barrière[2], Thierry Blard[2], Juliette Cognié[1], Marie-Veronique Demattei[1], Isabelle Grasseau[1], Richard Eloy[1], Guillaume Tsikis[1], Valerie Labas[3], Daniel Tomas[3], Audrey Prezelin[3], Ana-Paula Teixeira-Gomes[3], Simon De Graaf[4], Xavier Druart[1]

[1]INRAE, CNRS, Université de Tours, PRC, Nouzilly, France, [2]Centre INRAE Val De Loire, [3]INRAE, Université de Tours, [4]The University of Sydney

Can integrated sperm imaging and proteomics identify conserved fertility mechanisms and enable translational targeting of soluble adenylyl cyclase for non-hormonal contraception?

Sperm function depends on tightly regulated molecular pathways controlling motility, capacitation, and fertilization competence. While in vitro assays and proteomics have identified candidate fertility markers, their physiological relevance in vivo remains incompletely understood. Cross-species conservation of sperm proteins suggests fundamental reproductive mechanisms, yet their functional translation to fertility outcomes is still limited. Soluble adenylyl cyclase (sAC) is a key regulator of cAMP signaling during capacitation and represents a promising non-hormonal contraceptive target, but its in vivo relevance and translational potential require further validation.

This work integrates multi-scale approaches across species. In vivo sperm imaging was performed to characterize sperm behavior and migration dynamics within the mare reproductive tract. Parallel proteomic analyses were conducted on sperm subcompartments (head and flagellum) to identify region-specific protein signatures associated with fertility. Comparative proteomics across 12 mammalian species was used to identify conserved and species-specific molecular determinants. Functional validation focused on pharmacological inhibition of sAC using specific inhibitors, assessing effects on motility, capacitation markers, and fertilization competence. The horse was used as a translational model due to its physiological and reproductive similarities, enabling in vitro and in vivo assessment of contraceptive potential.
In vivo imaging revealed a minor proportion of sperm cells that was able to reach the tip of the uterine horn, highlighting functional heterogeneity not captured by standard in vitro assays. Proteomic analyses identified compartment-specific protein signatures, with flagellar proteins enriched in motility-related pathways and head proteins associated with fertilization processes. Several proteins correlated with fertility outcomes, suggesting potential biomarkers. Cross-species analysis revealed a conserved core sperm proteome alongside species-specific adaptations. Functional studies demonstrated that sAC inhibition significantly impaired sperm motility,

capacitation-associated signaling. In the equine model, these effects were consistent and reproducible, supporting the relevance of targeting sAC across species.In vivo imaging remains technically constrained and limited in throughput. Proteomic correlations with fertility require validation in larger cohorts. Pharmacological studies rely on inhibitor specificity, and long-term in vivo contraceptive efficacy and reversibility were not fully assessed.

This work provides an integrated framework linking sperm behavior, molecular composition, and fertility across species. It identifies conserved proteomic signatures and validates sAC as a central regulator of sperm function. The translational findings in the horse support the development of non-hormonal male contraceptive strategies targeting sAC. More broadly, combining in vivo imaging with proteomics offers a powerful approach to uncover physiologically relevant fertility mechanisms and accelerate reproductive innovation.

O6: Spermatozoa as in vitro toxicity model to elucidate the effect of environmental contaminants

Liana Maree[1], Shannen Keyser[1], Daniel Marcu[2], Morgan Davidse[1], Monique Bennett[1], Leslie Petrik[1]

[1]University Of The Western Cape, [2]University of East Anglia

With a global increase in environmental pollution and exposure risk, it has become paramount to assess the health of ecosystems and its potential detrimental effect on living organisms, including reproductive health. Spermatozoa are highly specialized cells that have the capacity to be an in vitro toxicology model due to its inherent compartmentalized structure, various measurable characteristics and dose-response to toxicants. Furthermore, spermatozoa depend on and are influenced by the external environment, e.g. by reacting to signals in the female reproductive tract, but also renders it vulnerable to environmentally-induced damage. Contaminants of emerging concern (CECs) have received increased attention during the past three decades due to its potential detrimental effect on human and ecosystem health. Due to the difficulty in removing CECs from the environment and the fact that it bioaccumulates in higher trophic levels, screening of CECs for its potential toxicity has become a precedence.
We investigated the use of human and marine invertebrate spermatozoa as a potential model for toxicity screening after exposure to selected CECs (pharmaceuticals and pesticides) to determine which sperm characteristics are most sensitive to be used as a screening tool and which exposure level has the most severe impact.

Using a highly motile sperm subpopulation, selected via double density gradient centrifugation from human semen, standardized protocols were applied to assess various sperm functional parameters after 1-4 hours of exposure to either individual or a collective mixture of chemicals. The outcome of the human experiments was applied to marine invertebrates, by assessing the effect of individual and mixtures of CECs on spermatozoa extracted from Pacific oyster and Cape sea urchin as well as the fertilizing ability of its gametes.

It is evident from our results that commonly used semen and sperm parameters such as percentage total motility is insufficient to capture the often subtle effects induced by chemical exposure. More advanced and sensitive measures, such as sperm kinematics, induced hyperactivation, viability, mitochondrial membrane potential (MMP) and presence of reactive oxygen species (ROS) indicated a significant detrimental effect on sperm function. Most chemicals exerted a dose-response effect on sperm parameters, with the higher concentrations resulting in the most negative effects ($P<0.05$); however, for at least one of the chemicals, the most detrimental effects were found at the lowest concentration tested, suggesting a non-monotonic dose-response. In marine invertebrates, environmentally-

relevant concentrations of individual contaminants had no effect on sperm functional parameters, while the collective mixture with the highest concentration of contaminants had a detrimental effect on various sperm characteristics. Exposure of gametes to individual or combined contaminants had no effect on fertilization success; however, short-term exposure could mask potential effects at later developmental stages.

Due to spermatozoa being sensitive to oxidative stress, mitochondrial damage and energy metabolism, the effects of CECs found in other cell types are also discernable in these gametes, making them candidates for use as a robust model for assessing toxicity. While previous literature has clearly indicated the negative impact of CECs on reproductive health, especially male fertility (sperm production and function), we propose using the other side of the double-edged sword, in that spermatozoa could be used as a bioindicator in eco- and repro-toxicology studies that due to its sensitivity to environmental conditions.

O7: A Multiparametric High-Throughput Imaging Assay to Resolve Distinct Acrosome Reaction States in Human Sperm

*** This abstract will also be displayed as a poster, on posterboard 36 ***

Analia Guadalupe Novero[1], Hannah Travers[1], Radoslaw Lukoszek[2], Anthony Richardson[3], Zoe C Johnston[1], Christopher Barratt[1], Sarah Martins Da Silva[1]

[1]Ninewells Hospital and Medical School, University of Dundee, [2]National Phenotypic Screening Centre, School of Life Sciences, University of Dundee., [3]Drug Discovery Unit, University of Dundee

Study Question: Can a novel imaging-based assay distinguish between distinct acrosome reaction states in human sperm and improve the assessment of sperm function?

What is already known: The acrosome reaction is a critical step in fertilization, yet current assays classify sperm in a binary manner (reacted vs. non-reacted). Hence, limiting the ability to study intermediate or dysfunctional states associated with infertility. Most existing approaches rely on CD46 (a marker of the inner acrosomal membrane)–based flow cytometry, or PSA (Pisum sativum agglutinin, a lectin that labels the acrosomal matrix)–based imaging to assess acrosomal status, but these markers have never been combined, restricting the ability to resolve distinct reaction states.Furthermore, the lack of standardized assays and defined functional thresholds limits the ability to distinguish between fertile and infertile sperm and hampers the development of non-hormonal contraceptives.

Methodology: We developed a multiparametric imaging-based assay combining CD46 immunolabeling, an antibody that detects exposure of the inner acrosomal membrane, and PSA staining, a lectin that labels the acrosomal matrix. Human sperm from normozoospermic donors were capacitated for 3 h and stimulated to undergo acrosome reaction using both non-physiological (A23187 a calcium ionophore) and physiological stimuli (P4: progesterone; PGE1: prostaglandin E1; and NH_4Cl: ammonium chloride). D46 and an amine-reactive viability dye were applied prior to fixation, whereas PSA and nuclear staining were applied post-fixation. Samples were analysed using high-content imaging (Operetta system), enabling high-throughput quantification across hundreds of cells per condition. This allows standardized and quantitative assessment of acrosome reaction patterns. The assay was validated against flow cytometry, demonstrating consistency between imaging-based and established quantitative approaches.

Results: This approach enables the classification of sperm into three distinct states: non-reacted (PSA-positive, CD46-negative), fully reacted (PSA-negative or restricted to the equatorial segment and CD46-positive), and partially reacted (PSA- and CD46-positive). Notably, physiological stimulation resulted in a higher proportion of partially reacted sperm compared to ionophore-induced conditions, which predominantly produced an all-or-none response.These findings reveal previously unrecognized heterogeneity in acrosome reaction dynamics that is not captured by conventional assays, such as flow cytometry. This provides a framework for defining functional sperm phenotypes beyond conventional classifications. Importantly, this framework enables the investigation of whether partially reacted states are associated with physiological induction or specific infertility phenotypes characterized by an increased proportion of non-reacted sperm. In addition, it provides a quantitative platform to assess the efficacy of compounds targeting sperm function for non-hormonal contraception.

Furthermore, the use of high-content imaging enables the analysis of thousands of sperm per condition, compared to the limited number typically assessed by conventional microscopy, thereby increasing statistical robustness and enabling large-scale, high-throughput applications.

The assay was validated against CD46-based flow cytometry, demonstrating consistency between our novel imaging-based assay and established quantitative approaches.

Limitations: This study relies on in vitro assays, and further studies are required to establish direct correlations between acrosome reaction patterns and fertilization outcomes in clinical settings. In addition, while the assay provides a platform to evaluate compounds targeting sperm function, its predictive value for in vivo contraceptive efficacy remains to be determined.

Conclusions: We present a novel, high-throughput, multiparametric assay that resolves distinct acrosome reaction states in human sperm. This approach provides a more nuanced assessment of sperm function and holds potential for improving the diagnosis of male infertility, as well as for evaluating the efficacy of non-hormonal contraceptive strategies. Importantly, it may contribute to the definition of functional thresholds required to distinguish fertile from infertile sperm and to guide non-hormonal contraceptive development.

O8: Gallocatechin improves mitochondrial activity and motility of stored boar semen

Barbora Klusackova[1,2], Alexis Jones[2], Erica Mantle[2], Natalie Zelenkova[1,2], Zuzana Pilsova[1,2], Aneta Pilsova[1,2], Mohammad Liman[3], Michal Zigo[2], Miriam Sutovsky[2], Pavla Postlerova[1,4], Dietmar Holm[3], Peter Sutovsky[2,5]

[1]Department of Veterinary Sciences, University of Life Sciences Prague, [2]Division of Animal Sciences, University of Missouri, [3]Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, [4]Laboratory of Reproductive Biology, Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV, [5]Department of Obstetrics, University of Missouri

Study Question: Does gallocatechin supplementation improve sperm motility, mitochondrial membrane potential and membrane integrity in the extended boar semen during room-temperature storage?

What is already known: Catechin derivatives are polyphenolic flavonoids with strong antioxidant activity and reported anti-inflammatory and neuroprotective properties. Gallocatechin is naturally present in plant sources including green tea (Camellia sinensis) and black wattle (Acacia mearnsii) bark. Previous studies have suggested that catechin-derived compounds may improve semen preservation, including but not limited to cryopreservation by limiting oxidative stress and supporting sperm homeostasis. However, the effect of gallocatechin supplementation on extended boar semen during liquid storage is yet to be explored.

Study Design and Methodology: Ejaculated boar semen was extended in Preserv®xtreme and supplemented with gallocatechin at final concentrations of 5 µM, 10 µM or 15 µM. Samples of final concentration 1×10^8 spermatozoa/mL and total volume of 10 mL were stored at room temperature up to 10 days and evaluated daily. Sperm motility and kinetic parameters were assessed daily by using computer-assisted sperm analysis (CASA). Mitochondrial membrane potential and membrane integrity were evaluated by imaging flow cytometry using JC-1 and Calcein Violet staining, respectively. The effects of gallocatechin alone were compared with untreated controls and with combined supplementation of gallocatechin with $ZnCl_2$ and NaHS, both of which had beneficial effect on stored boar spermatozoa in published studies.

Results: Supplementation with 5 µM gallocatechin significantly improved total and progressive sperm motility compared with controls during storage. This concentration also enhanced key kinetic parameters, including curvilinear velocity, average path velocity, straight-line velocity, beat cross frequency and straightness. Furthermore, imaging flow cytometry showed improved mitochondrial membrane potential in the 5 µM gallocatechin group, suggesting better preservation of mitochondrial function. In contrast, higher concentrations of gallocatechin showed reduced or no beneficial effects, indicating a dose-dependent response, possibly a reductive stress induction

with higher concentrations of gallocatechin. Combined supplementation with gallocatechin, $ZnCl_2$ and NaHS did not produce additional improvement.

Limitations: This was an in vitro study using liquid-stored boar semen. Fertility outcomes were not assessed, and the outcomes may have been influenced by extender composition and storage conditions.

Conclusions: Low dose gallocatechin supplementation improved the quality of stored boar semen, particularly sperm motility, kinetic parameters and mitochondrial membrane potential. The results suggest that optimized gallocatechin supplementation may benefit sperm functional stability/homeostasis during liquid storage. These findings highlight its potential application in livestock assisted reproduction, although further studies evaluating fertility outcomes and mechanisms of action are required.

Funding: This research was funded by was supported by grants number 2020-67015-31017 and 2021-67015-33404 from the USDA National Institute of Food and Agriculture, and seed funding from the University of Missouri College of Agriculture, Food and Natural Resources (CAFNR). B.K., N.Z., Z.P., A.P., and P.P. were supported by the Ministry of Education, Youth and Sports of the Czech Republic under the INTER-EXCELLENCE II program, subprogram INTER-ACTION (LUAUS25072) and the Internal Grant Agency of the Czech University of Life Sciences in Prague (SV25-2-21230). M.L and D.H. were supported by the Red Meat Research and Development Trust of South Africa project committee (RMRD SA, 26/03/2020) and by the “Translational Medicine Research Theme” of the Faculty of Veterinary Sciences, University of Pretoria.

O9: Progesterone signalling in human sperm involves rapid changes in membrane potential that are controlled by an interplay of CatSper and Slo3 channels

Michelina Kierzek[1], Isabel Wagner[1], Timo Strünker[1], **Christoph Brenker**[1]

[1]Centre of Reproductive Medicine and Andrology, University of Münster

Activation of the sperm-specific Ca2+ channel CatSper by progesterone evokes rapid changes in intracellular Ca2+ in human sperm that result in changes in the pattern of the flagellar beat and are absolutely required for fertilization. However, the mechanisms shaping the progesterone-induced Ca2+ signals have remained elusive.

We used quantitative kinetic fluorimetry with fast voltage-sensitive fluorescent indicators to investigate how progesterone affects the membrane potential (Vm) of human sperm. Additionally, we employed the FASTM technique to simultaneously record changes in both Vm and intracellular Ca2+. We combined this approach with patch-clamp recordings from human sperm and super resolution microscopy to unravel the molecular underpinnings of the interplay of CatSper and Slo3 both on a functional and structural level.

Progesterone evokes a rapid pulse-like depolarization and repolarization. We show that the depolarization is caused by Ca2+ influx through CatSper, which pulls Vm away from a resting membrane potential (Vrest) of -65 mV set by the sperm-specific K+ channel Slo3. We further show that Vm- and Ca2+-dependent mechanisms limit the CatSper-mediated Ca2+ influx, thereby promoting repolarization and enabling K+ efflux through Slo3 channels to restore Vrest. Moreover, patch-clamp recordings of sperm from donors and CatSper-deficient patients indicate that the channels are functionally strongly coupled, suggesting a colocalization of both channels within Ca2+ signalling domains. This finding was corroborated by colocalization studies using super resolution microscopy.

Our findings provide experimental evidence that progesterone signalling in human sperm is regulated by negative feedback on CatSper and involves a dynamic interplay between CatSper and Slo3 within Ca2+ signalling domains to control Vm. We anticipate that our novel kinetic, quantitative Vm recording and Vm/Ca2+-multiplexing techniques will reveal additional molecular mechanisms underlying CatSper-mediated Ca2+ signalling in human sperm, both in health and disease.

O10: A novel regulator of sperm motility links mitochondrial dysfunction and ferroptosis

*** This abstract will also be displayed as a poster, on posterboard 31 ***

Yingying Yin[1], Zhenhua Zhang[1], Hongbin Liu[2]

[1]Karolinska Institute, [2]Shandong University

Male infertility contributes to nearly half of infertility cases worldwide, and impaired sperm motility is a major cause. However, the molecular mechanisms underlying asthenozoospermia remain incompletely understood. Here we identify a leucine-rich repeat containing sperm protein, termed LRCSP1, as a previously uncharacterized regulator of sperm motility. LRCSP1 is highly enriched in mouse testis and dynamically expressed during spermatogenesis. Genetic deletion of Lrcsp1 resulted in complete male infertility without affecting spermatogenesis. Mutant sperm exhibited severe motility defects accompanied by annulus displacement and mitochondrial abnormalities in the sperm midpiece. Ultrastructural analyses revealed disrupted mitochondrial organization. Functional assays demonstrated decreased mitochondrial membrane potential, increased reactive oxygen species and reduced ATP production. Multi-omics analyses identified significant alterations in mitochondrial pathways and lipid metabolism. Importantly, ferroptosis-related pathways were strongly activated, suggesting that ferroptotic damage contributes to mitochondrial dysfunction in sperm. Together, these findings reveal a previously unrecognized mechanism linking ferroptosis to sperm motility defects. These findings reveal a previously unrecognized mechanism linking ferroptosis to sperm motility defects. All animal experiments were conducted in accordance with the ethical guidelines approved by Shandong University and Karolinska Institute. The authors declare no competing interests.

O11: Hanging in the balance: Understanding the importance of sperm ROS for pre-implantation embryo development

*** This abstract will also be displayed as a poster, on posterboard 34 ***

Joshua Deluao[1,2], Hannah Lyons[1,2], Macarena Gonzalez[1,2], Mark Nottle[1,2], Nicole McPherson[1,2,3]

[1]Adelaide University, [2]Robinson Research Institute, [3]Genea

Introduction: Reactive oxygen species (ROS) mediated damage is a major contributor to male infertility, implicated in up to 80% of cases of male factor infertility. Excessive ROS are well known to induce sperm DNA damage, lipid peroxidation and reduced motility. Paradoxically, physiological ROS levels are essential for normal sperm function, including capacitation and fertilisation. How deviations on either side of this physiological window affect fertilisation and early embryogenesis remains poorly defined. Emerging evidence suggests that sperm-derived non-coding RNAs may mediate paternal environmental effects on embryo development. In other organisms, members of the miR 30 family are critical regulators of spermatogenesis and early developmental processes, raising the possibility that sperm oxidative status may influence embryogenesis via RNA-mediated mechanisms. This study aimed to define the optimal ROS range required for normal sperm function, fertilisation and early embryo development, and to determine whether perturbations in sperm ROS levels alter embryo outcomes and miR 30a expression following fertilisation.

Methods: Spermatozoa from 8-10-week-old CBAF1 mice (N=13) were incubated for 1h in 5nM, 50nM carbonyl-cyanide-m-chlorophenyl-hydrazone (CCCP) to increase ROS; and 5µM, 100µM manganese-(III)-tetrakis-(4-benzoic-acid)-porphyrin-chloride (MnTBAP) to decrease ROS. Sperm motility was assessed using computer-assisted sperm analysis, superoxide (MitoSoxRed), intracellular ROS (CellRoxGreen), lipid peroxidation (BODIPY) and mitochondrial membrane potential (MMP – JC-1) were assessed by flow cytometry, while sperm miR30a abundance assessed by qPCR. Embryo development was assessed at 24h and 96h post-in vitro fertilisation using time lapse microscopy, and blastocyst cell lineage measured by Nanog/Oct4/DAPI immunofluorescence. Superoxide levels were also assessed in PN3-PN5 zygotes to assess if sperm oxidative status affected zygotic oxidative status.

Results: Sperm superoxide levels were modulated as intended, increasing with CCCP and decreasing with MnTBAP in a linear manner (R^2 = 0.96). Excessive reduction of ROS with 100 µM MnTBAP significantly impaired sperm function, reducing intracellular ROS (p = 0.01), mitochondrial membrane potential (p = 0.04) and motility (p = 0.02), while sperm lipid peroxidation remained unchanged. Importantly,

elevating sperm ROS with 5 nM CCCP resulted in a significant increase in sperm miR 30a expression ($p = 0.0067$). MnTBAP reduced 2 cell rates ($p = 0.03$), blastocyst formation ($p = 0.03$), embryo survival ($p = 0.0008$), and delayed blastocyst expansion ($p = 0.01$), with associated disruptions to embryo morphokinetics ($p = 0.0003$). CCCP reduced 2 cell rates at both concentrations tested (50 nM $p = 0.0008$; 5 nM $p = 0.061$). Zygotic superoxide levels were significantly decreased following fertilisation with MnTBAP treated sperm (5 µM $p = 0.003$; 100 µM $p = 0.004$). Total epiblast cell number was reduced following exposure to both MnTBAP (5 µM $p = 0.028$) and CCCP (50 nM $p = 0.003$).

Conclusion: These findings demonstrate that sperm ROS operate within a critical physiological window, where both excessive and insufficient levels compromise sperm function and early embryo development. The increased abundance of miR30a following elevated ROS exposure suggests a potential sncRNA mediated mechanism linking paternal oxidative status to embryonic gene regulation, warranting further investigation. Together, this data challenges the current dogmathat oxidative stress reduction is universally beneficial. Instead, the focus should be on optimising, rather than simply minimising, the sperm oxidative environment. A more nuanced understanding of the interface between ROS pathology and physiology in early embryogenesis has important implications not only for paternal preconception care, but also for refining laboratory conditions in assisted reproduction.

O12: Exploring geographical differences in semen quality of young men using spatial dependence analysis

Rita Rahban[1,2], Hugo-Alejandro Santa-Ramírez[3], Alfred Senn[1,2], Eric Stettler[1,4], Idris Guessous[3,5], Stéphane Joost[3,5,6], Serge Nef[1,2]

[1]University Of Geneva, Department of Genetic Medicine and Development, [2]Swiss Centre for Applied Human Toxicology, [3]Geneva University Hospitals, Unit of Population Epidemiology, [4]Swiss Armed Forces Joint Staff, [5]Group of Geographic Information Research and Analysis in Population Health, [6]École Polytechnique Fédérale de Lausanne (EPFL), Group of Geospatial Molecular Epidemiology (GEOME)

Study question: Are semen quality parameters randomly distributed across space, or do they exhibit spatial patterns that may reflect underlying environmental influences?

Summary answer: Semen quality parameters are not randomly distributed in Switzerland but exhibit distinct spatial clustering, with identifiable hotspots and coldspots.

What is known already: Regional differences in semen quality across Europe suggest environmental influences, but most studies are limited to urban areas with small samples and low geographic precision, leaving gaps in understanding precise geographical patterns at a national level.

Study design and methodology: This is a cross-sectional study including 2'677 men aged 18-22 from the general Swiss population who were recruited between 2005 and 2018 during military conscription. Each participant provided a semen sample and completed a questionnaire on health and lifestyle, including demographic details on their residence. Georeferenced data were used to compute the level of spatial dependence using the local Getis-Ord Gi* statistic with a 25 Km spatial lag to identify clusters of high versus low semen parameter values across Switzerland. The parameters assessed included semen volume, sperm concentration, total sperm count (TSC), and sperm morphology. Participants belonging to the same Getis-Ord* class for each semen parameter formed a persistent cluster. To assess differences in environmental exposure between high and low persistent clusters, land use data within a 500 m radius of each participant's place of residence were extracted from national registries for the periods of 1992-1997 and 2004-2009.

Results: Semen quality parameters are not randomly distributed across Switzerland, with distinct local clusters of high values (hotspots) and low values (coldspots) identified. A persistent low-value cluster was observed in central western Switzerland. Analysis of

national land use data revealed significant differences in agriculture land types between clusters: fodder and field crops accounted for 52% of the land use in the coldspot, compared to 28.3% in the hotspot and 30.1% in the non-significant cluster. Additionally, the mean proportion of built areas (housing and infrastructure) near participants' residences was significantly lower in the coldpot (32.6%) compared to the hotspots (53.3%).

Limitations, reasons for caution: Although the study population is nationwide and broadly representative, the sample size used for individual-level spatial analyses limits statistical power.

Conclusions: To our knowledge, this is the first study to apply spatial dependence methods to investigate semen quality parameters using individual-level data at a national scale. This approach can be extended to larger datasets to further explore the relationship between environmental factors and reproductive health. The insights gained may inform public health policy by supporting more targeted environmental monitoring and preventive strategies.

O13: Ultra-rare sperm detection using an AI-enabled microwell system

Arafeh Bigdeli[1], Manoj K Kanakasabapathy[1], Azim Parandakh[1], Prudhvi Thirumalaraju[1], Jindong Feng[1], Krushna K Madhavan Giri[1], Jay Petel[2], Berhan Bogale[2], Nursel Keklik Karabulut[1], Hemanth Kandula[1], Niveditha Kovilakath[1], Jagannathan Adanakottai Viswanathan[1], Lokeshwari Polur Sundarrajan[1], Komal Krishna Annarapu[1], Alexandra Berger Eberhardt[3], Charles Bormann[4], Martin N Kathrins[3], **Hadi Shafiee**

[1]Department of Medicine, Harvard Medical School, [2]Division of Reproductive Endocrinology and Infertility, Mass General Brigham, [3]Department of Urology, Mass General Brigham, [4]Department of Obstetrics and Gynecology, Mass General Brigham

Background: Non-obstructive azoospermia (NOA) represents a severe form of male infertility in which surgical sperm retrieval is the only viable route to biological parenthood. However, the clinical success of these interventions is fundamentally limited by the stochastic and labor-intensive nature of the manual microscopic search for ultra-rare testicular sperm, leaving nearly half of NOA patients without a biological reproductive option despite surgery. This bottleneck highlights the urgent need for a scalable, high-throughput approach capable of reliably detecting and non-destructively recovering sperm from specimens that would otherwise be discarded as unusable.

Objective: To determine whether high-throughput microwell interrogation is required for ultra-rare sperm detection beyond AI-based approaches.

Methodology: We developed an integrated hardware–software platform combining a microengineered microwell array with AI-assisted image analysis. The microwell architecture spatially confines testicular cell suspensions into thousands of indexed compartments (~4,000 wells per device), reducing focal depth from ~600 µm in conventional droplets to ~3–5 µm and enabling rapid, large-area imaging. A deep learning model trained and validated on >1 million microscopy images was used for sperm detection, with inference speeds exceeding ~60 frames per second. Detected sperm were indexed by microwell coordinates, enabling targeted navigation and retrieval using standard intracytoplasmic sperm injection (ICSI) micromanipulators. Performance was evaluated using low-concentration sperm-spiked microTESE samples (1,000 sperm/mL) and 8 clinically discarded tissue specimens from NOA patients previously classified as sperm-negative after extensive manual search. Detection performance was compared across droplet-based search, microwell-only (hardware) analysis, and AI-assisted analysis.

Results: In spiked samples, microwell confinement alone enabled a step-change in search throughput, reducing time to first sperm detection from ~36 minutes to ~30 seconds and increasing detection rates by more than two orders of magnitude compared to

conventional droplet-based microscopy. This demonstrates that resolving the physical search space is sufficient to markedly improve detection efficiency when sperm are present at low but detectable concentrations.
However, under clinically relevant ultra-low concentration conditions, this increase in throughput exposed a new limitation. In a controlled comparison using 8 clinically negative specimens, microwell-based manual review (hardware-only) remained insufficient, failing to detect sperm in 7 out of 8 samples within a defined search window. In contrast, AI-assisted analysis applied to the same microwell datasets enabled consistent detection, identifying an average of 21 sperm per specimen and an average of 15 seconds to detect the first sperm.Separately, when compared against standard-of-care (SOC) manual search where all 8 samples had previously been classified as sperm-negative, the AI-microwell device enabled identification of sperm in 7 out of 8 specimens. All sperm identified in the clinically negative patient specimens were independently reviewed and confirmed by experienced embryologists, supporting the reliability of AI-assisted detection.

Limitations: This study was performed on a limited subset of discarded, cryopreserved specimens. While the platform enables coordinate-based identification and retrieval, prospective studies are required to evaluate the functional suitability of detected sperm for ICSI and clinical outcomes.

Conclusions: Microwell-based confinement resolves the primary physical bottleneck in sperm search by enabling high-throughput interrogation of microTESE specimens. However, this increase in throughput introduces a secondary analytical bottleneck, where AI-assisted detection becomes essential for identifying ultra-rare sperm. Together, these findings demonstrate that reliable detection in NOA requires both high-throughput hardware and AI-based analysis, establishing a complementary framework for rare cell detection in reproductive medicine.

O14: The effect of combined cryoprotectant strategies on the functional quality of post-thaw chicken sperm

*** This abstract will also be displayed as a poster, on posterboard 32 ***

Azindokht Babapour[1], Skarlet Napierkowska[1], Pilar Vallejo-Soto[2], Prof. Manuel Hidalgo[2], Agnieszka Partyka[1], Prof. Wojciech Niżański[1]

[1]Department of Reproduction and Clinic of Farm Animals, Wroclaw, [2]Veterinary Reproduction Group, University of Cordoba

Study Question: This study aimed to evaluate whether non-permeable cryoprotectants combined with a reduced concentration of permeable cryoprotectant can improve post-thaw chicken sperm quality.

What is already known: Semen cryopreservation is essential for genetic resource conservation, but it is associated with cellular damage, oxidative stress, and reduced post-thaw sperm quality. The selection of appropriate cryoprotective agents (CPAs) is a key factor in improving cryopreservation outcomes. Permeable CPAs, such as DMF, can protect sperm by reducing intracellular ice formation but may also induce toxicity depending on concentration and exposure time. In contrast, non-permeable CPAs, including sugars and polymers, act as osmoprotectants and may help reduce cryodamages. However, the combined effects of permeable and non-permeable CPAs, as well as their optimal concentrations, remain unclear.

Study Design and Methodology: Semen was collected from ten Green-legged Partridge roosters by dorso-abdominal massage twice per week. After pooling, samples were diluted and cryopreserved using a double-straw method at a 1:1 ratio in BHSV as the basic extender supplemented with 6% DMF (control group). Experimental treatments included Ficoll (0.5 and 1 mM), raffinose (50 and 100 mM), and dextran (40,000 MW; 25% and 30%), each supplemented with 3% DMF. Cryopreservation was performed by exposing samples to liquid nitrogen vapor for 10 minutes on the rack, followed by plunging into liquid nitrogen. Thawing was carried out in a 37 °C water bath for 30 seconds. Post-thaw sperm quality was evaluated using CASA for motility and velocity parameters. Flow cytometry was used to assess apoptosis, mitochondrial membrane potential, acrosome integrity, DNA fragmentation, lipid peroxidation (LPO), intracellular calcium levels, and plasma membrane integrity using specific fluorescent probes.

Results/ Findings: No significant differences were observed in total, progressive, or slow motility across groups. However, velocity parameters (VAP, VCL, and VSL) were significantly increased in raffinose 50mM and dextran 25% groups compared to control ($p<0.05$).

Also, raffinose 100mM significantly increased VCL compared to the control group ($p<0.05$). Most flow cytometry parameters, including apoptosis, mitochondrial activity, acrosome integrity, and DNA fragmentation, remained unchanged across treatment groups. Lipid peroxidation in live spermatozoa was significantly reduced in several groups, including Ficoll at both 0.5 and 1mM concentrations, dextran 25% ($p<0.05$), and raffinose 100mM ($p<0.01$). Additionally, some treatments like Ficoll 0.5mM and Dextran 25% improved intracellular calcium regulation, reflected by reduced proportions of live spermatozoa with high Ca levels. However, while Ficoll 0.5mM, and raffinose-treated groups, particularly raffinose 50mM, showed reduced sperm viability compared to the control, other treatments such as Ficoll 1mM and dextran groups improved viability compared to raffinose 50mM.

Limitations: This study was limited to in vitro evaluation of sperm functional parameters, and further studies are needed to clarify the underlying mechanisms, particularly the relationship between improved motility and reduced viability observed in some treatments.

Conclusions: Overall, basic motility parameters were maintained at levels comparable to the control across all groups, while several treatments improved sperm velocity and reduced oxidative stress and calcium-related damage, indicating enhanced functional quality after thawing. However, these beneficial effects were not consistently reflected in sperm viability. Among the tested treatments, dextran 25% exhibited an overall improvement across multiple functional parameters, including sperm velocity, oxidative stress, and calcium regulation. Additionally, Ficoll 1mM demonstrated a balanced protective effect, maintaining sperm viability while reducing oxidative stress. Whereas raffinose 50mM primarily enhanced velocity parameters but was associated with reduced sperm viability.

O15: Towards semen collection at younger age: non-invasive monitoring of puberty onset in young beef bulls at performance testing stations

Joanna Bremer[1], Birgitte Narud[1], Michał Maj[2], Michael Alexander Riegler[3], Terkel Hansen[4], Møyfrid Therese Myhre Harbak[4], Geir Klinkenberg[4], Kristian Heggelund[5], Solvei Cottis Hoff[5], Morten Søberg[5], Olav Lie[5], Elisabeth Kommisrud[1]

[1]CRESCO, Centre for Embryology and Healthy Development, University of Inland Norway, [2]Data Science Solutions, [3]Simula Research Laboratory, [4]Department of Biotechnology and Nanomedicine, SINTEF Industry, [5]TYR

Breeding companies need tools to monitor puberty onset that are fast, safe, and operable by any technician. Methods currently available for assessing reproductive development in young bulls are neither safe nor practical enough for routine use. Manual scrotal circumference (SC) measurement is typically performed once at around 12 months during breeding soundness evaluation, however many companies have discontinued it altogether due to animal handling risks. Blood-based hormone analysis, while informative, is too labour-intensive to perform routinely. Consequently, the peripubertal window, during which testicular development and the onset of steroid hormone production determine future reproductive potential, is rarely monitored systematically. Earlier identification of bulls ready for semen collection would shorten the generation interval and increase genetic gain, but achieving this requires a novel monitoring approach.

Performance testing stations provide a unique setting for this: bulls of multiple breeds are present for an extended period, allowing repeated sampling across the peripubertal window. We monitored 80 beef bulls of five breeds (Aberdeen Angus, Charolais, Hereford, Limousin, Simmental) monthly over 8 consecutive months, covering ages 6 to 15 months, in collaboration with TYR, Norway's national beef breeding organisation. Scrotal circumference was measured manually using scrotal tape, while LiDAR depth imaging data were simultaneously captured via iPhone 16 Pro (iOS 26). The depth images form the training dataset for a deep learning model currently under development to detect and measure SC by LiDAR only. The goal is to integrate this model into an easy-to-use mobile application suitable for routine field use, without the need for specialised equipment. In parallel, tail hair samples were collected for LC-MS/MS quantification of six hormones: five spanning the steroidogenesis pathway (DHEA-S, DHEA, androstenedione, testosterone, and estradiol) and cortisol as a marker of stress response. Both whole-hair and segmental analyses are planned to resolve the temporal hormone dynamics that single-timepoint blood sampling cannot capture. Hair segments reflect hormone incorporation during growth, enabling retrospective measurements of their levels from a single sample.Manual SC growth curves have been completed for all 80 bulls, and iPhone LiDAR depth images have been successfully captured across all monitoring visits. An LC-MS/MS method for

simultaneous quantification of all six hormones in tail hair has been developed and validated on pilot samples by SINTEF, confirming reliable extraction and detection sensitivity across the full steroid panel. Initial hormone profiling will focus on a selected subset of Limousin and Angus samples, chosen to capture both breed-level contrast in peripubertal hormonal trajectories and the effect of hair colour, as Limousin (brown) and Angus (black) represent the two contrasting coat colours among the five breeds studied. Hair colour has previously been shown to influence testosterone levels measured in hair, making this comparison particularly informative for method validation. Individual tail hair growth rate was measured in two bulls by shaving, yielding a rate of 2–3 cm/month, consistent with values reported in the literature. Full calibration across all animals was not feasible, limiting the temporal precision of segmental hormone assignment at the individual level. This combined approach could provide breeding companies and performance testing stations with practical, non-invasive tools for routine longitudinal monitoring of reproductive development. The result would be earlier and more accurate identification of bulls ready for semen collection, improving both breeding efficiency and animal welfare.

Poster Presentations

P1: In vitro evaluation of the interaction between sea urchin soluble adenylyl cyclase and the sperm-specific Na+/H+ exchanger

Fernando Aranda Lozano[1], PhD Takuya Nishigaki[1]

[1]Instituto de Biotecnología

This study aims to determine whether sea urchin soluble adenylyl cyclase interacts with the sperm-specific Na+/H+ exchanger and is hyperpolarization-regulated through it.

In many metazoans, sperm motility is activated through the cAMP cascade after release into an external environment with an ionic composition that differs from that of the gonads. In mammals, cAMP is mainly synthesized by soluble adenylyl cyclase (sAC), which physically interacts with the sperm-specific Na+/H+ exchanger (sNHE) to regulate motility. In marine species such as sea urchins and ascidians membrane potential controls cAMP production through an unknown mechanism. Because sNHE contains a voltage-sensing domain activated by hyperpolarization, it may provide the missing link between membrane potential and sAC-mediated cAMP synthesis.

Recombinant sea urchin sAC and sNHE fused to fluorescent proteins are heterologously expressed in HEK293 cells to evaluate their interaction using visible immunoprecipitation (VIP) assays. In parallel, untagged proteins are co-expressed with a fluorescent cAMP biosensor to determine whether membrane hyperpolarization regulates sAC activity through sNHE. Hyperpolarizing stimuli are applied while monitoring changes in biosensor fluorescence as a readout of intracellular cAMP accumulation. Comparative analyses will be performed in cells expressing sAC alone or together with sNHE to assess the exchanger's contribution to voltage-dependent regulation.

This ongoing study has some limitations. The proteins are examined outside their native sperm environment, and additional sperm-specific factors required for their function may be absent in the heterologous system. Positive findings will require validation in spermatozoa to confirm physiological relevance.

This work will determine whether the physical coupling between sNHE and sAC described in mammalian sperm is conserved in sea urchins. It will also provide insight into how membrane hyperpolarization triggers cAMP production and initiates sperm motility after spawning. Understanding this signaling pathway may reveal conserved principles of sperm activation across species and broaden current knowledge of reproductive physiology.

P5: Single-cell RNA sequencing identifies a high-resolution transcriptional landscape and molecular signatures of human sperm in male infertility

Stefan S Du Plessis[1], Yamna Khurshid[1], Shabana Anjum[2], Mohammed Uddin[3], Fawzia AlObeidly[2], Temidayo Omolaoye[2]

[1]Research and Graduate Studies, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai Health, [2]College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai Health, [3]Center for Applied and Translational Genomics, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai Health

Single-cell RNA sequencing identifies a high-resolution transcriptional landscape and molecular signatures of human sperm in male infertility

Study question: Can single-cell RNA sequencing (scRNA seq) generate a high-resolution transcriptional map of human sperm and identify molecular signatures associated with male infertility?

What is already known: Male infertility contributes to nearly half of all infertility cases, yet the molecular mechanisms underlying sperm dysfunction remain poorly understood. Conventional semen analysis provides limited functional and molecular information, while bulk RNA sequencing masks transcriptional differences between individual spermatozoa. To our knowledge, no previous study has comprehensively characterized the transcriptome of individual human sperm using scRNA seq. This approach offers an unprecedented opportunity to resolve sperm heterogeneity and identify molecular pathways associated with normal and impaired fertility.

Study design and methodology: This study generated a single-cell transcriptional map of human sperm from normozoospermic, asthenospermic, and oligozoospermic samples (n = 6). Cell viability was assessed using propidium iodide and acridine orange staining and quantified using an automated cell counter. Samples with ≥85% viability were diluted to 700–1200 cells/µL and processed using the 10x Genomics Chromium Next GEM Single Cell 3' v3.1 platform. Following Gel Bead-in-Emulsion (GEM) generation and reverse transcription, amplified cDNA libraries were prepared and sequenced at a target depth of 50,000–100,000 reads per cell. Transcript alignment and unique molecular identifier (UMI) quantification were performed using the Cell Ranger pipeline. Downstream analyses included dimensionality reduction using UMAP, clustering, differential gene expression analysis, and pathway enrichment to identify molecular signatures associated with sperm quality.

Results: scRNA seq identified three transcriptionally distinct cell clusters by UMAP, revealing substantial transcriptional heterogeneity within human sperm samples. Differential gene expression analysis identified cluster-specific marker genes, supporting distinct transcriptional states. Comparative pathway enrichment analysis demonstrated enrichment of mitochondrial respiratory chain complex I, oxidative phosphorylation, and NADH dehydrogenase pathways in asthenozoospermic samples, while oligoasthenozoospermic samples showed enrichment of immune- and inflammation-associated pathways, including calprotectin complex formation and Toll-like receptor signaling. These findings demonstrate distinct molecular alterations associated with different forms of male infertility.

Limitations: The study included a limited number of samples (n = 8), which may reduce generalizability. Functional validation of identified genes and pathways was not performed, and larger cohorts are required to confirm these transcriptomic signatures.

Conclusions: This study establishes a high-resolution single-cell transcriptomic landscape of human sperm and reveals transcriptional heterogeneity associated with male infertility. These findings provide new insights into human sperm biology and lay the foundation for future investigations into the molecular mechanisms of impaired fertility.

P7: mRNA-Based Therapy: A New Approach to Restore Sperm Fertility in a Mouse Model of Male Infertility

Paul Fourquin[1], Maellys Thurloy-Havaux[1], Elise Giang[2], Court Magali[1], Florence Appaix[1], Jean-Luc Duteyrat[3], Loana Torres[1], Geneviève Chevalier[1], Emeline Lambert[1], Celia Tebbakh[1], MCU-PH Zine-Eddine Kherraf[1], PU-PH Pierre Ray[1], Bernard Verrier[2], Claire Monge[2], Dr Christophe Arnoult[1], **Jessica Escoffier**[1]

[1]Université Grenoble Alpes, Equipe "Génétique, Epigénétique et Thérapies de l'Infertilité", IAB, INSERM 1209, CNRS UMR 5309, [2]Université Claude Bernard Lyon 1 - Laboratoire de Biologie Tissulaire et d'Ingénierie Thérapeutique, UMR 5305, Université Lyon 1, CNRS, IBCP, [3]ENS de Lyon, CNRS, Université Lyon 1, PLATIM,UMR 5242

Infertility is an increasing public health concern, affecting approximately one in six couples worldwide. Current treatments remain largely palliative, with success achieved in only one out of two couples, and do not address the underlying molecular and genetic causes of infertility. This limitation is particularly critical in male infertility, where non-obstructive azoospermia (NOA) and severe oligozoospermia (SO) represent the most severe disorders of spermatogenesis and are frequently associated with genetic defects. NOA, characterized by the complete absence of spermatozoa in the ejaculate due to impaired spermatogenesis, is among the most challenging forms of male infertility, with very limited therapeutic options available.

Gene therapy offers the possibility of correcting such defects by introducing a functional copy of a mutated gene into germ cells to restore production of the missing protein. Although this approach has shown remarkable efficacy in several inherited diseases, its application to infertility is ethically and legally restricted because genomic modifications introduced into germ cells may be transmitted to future generations. To avoid heritable genetic alterations and any associated eugenic concerns, germline gene modification is prohibited in humans in most countries.

The recent development of messenger RNA (mRNA)-based therapeutics, highlighted by the success of COVID-19 vaccines, has opened new perspectives for the treatment of genetic diseases. Unlike DNA, mRNA remains in the cytoplasm and is translated transiently without entering the nucleus or integrating into the genome. This enables temporary restoration of protein expression while preserving the integrity of the patient's genetic heritage. Such a strategy is particularly attractive for spermatogenesis, a dynamic and highly regulated process in which transient correction during specific developmental stages may be sufficient to restore fertility.

In this context, we hypothesize that targeted delivery of therapeutic mRNA to the adult testis using lipid nanoparticles (LNPs) enables transient and functional restoration of spermatogenesis in severe genetic forms of male infertility while remaining compatible with

future clinical application. LNPs have become the reference technology for in vivo nucleic acid delivery because of their efficiency, safety, and versatility. Produced by microfluidic formulation, LNPs encapsulate mRNA within ionizable lipids that protect it from nuclease degradation and clearance by the mononuclear phagocyte system. Additional phospholipids and stabilizing components promote hydrosolubility, low immunogenicity, efficient cellular uptake, and endosomal escape. Since the approval of Onpattro in 2018 and the widespread use of mRNA-based COVID-19 vaccines, LNP technology has become a clinically validated platform, with more than 40 formulations currently in clinical development.

To validate this approach, adult mouse testes are transfected with LNPs encapsulating mRNA encoding the reporter protein EGFP. Expression efficiency and kinetics are assessed using in vivo and ex vivo two-dimensional and three-dimensional imaging, together with histological analyses and evaluation of sperm production. Experiments are performed in sexually mature mice rather than immature animals, as used in most previous studies, in order to better reproduce the physiological and clinical conditions encountered in adult patients.

Therapeutic efficacy and safety are then evaluated in two genetically relevant mouse models of human infertility: Kash5 knockout mouse, which recapitulates NOA, and Armc2 knockout mouse, a model of OATS syndrome. Restoration of spermatogenesis, sperm motility, and fertility serves as functional readouts of treatment efficacy.

This project lies at the interface of nanomedicine and reproductive biology and establishes the basis for a first-in-class, non-integrative molecular therapy for severe genetic male infertility. By combining therapeutic mRNA with clinically validated LNP technology, this strategy offers a promising and ethically acceptable alternative to conventional gene therapy for patients with currently unmet medical needs.

P8: Antioxidant Therapy in Couples of Advanced Reproductive Age: Protocol for a Multicentre Prospective Observational Cohort Study

Jane McDonnell[1,2,3], Pru Sweeten[1,2,4], Jo Master[2,4], Molly Toogood[2,4], Tessa Morgan[1,2,3], Helen Badouin[2,5], Rachel Rodgers[1], Harleen Kaur[2,6], Shannon Kim[1,2], Nicole Kellow[7], Devora Lieberman[4], Michael Costello[1,2], Rebecca Deans[1,2], Geoff De lulis[8], John Aitken[8,9], Meurig Gallagher[10], Jackson Kirkman-Brown[10], Roger Hart[11,12,13], **Fabrizzio Horta**[1,2,4,14]

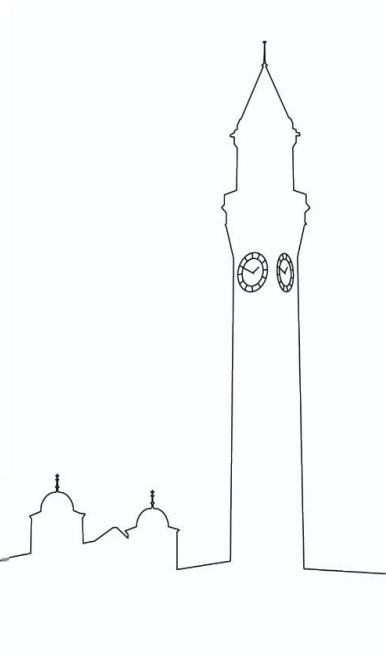
[1]Fertility & Research Centre, The Royal Hospital for Women, [2]Discipline of Women's Health, School of Clinical Medicine, The University of New South Wales, [3]School of Medicine, University of Notre Dame, [4]City Fertility, Research Support Unit, [5]Unité de Formation et de Recherches, Université de Nantes, [6]Andrology, NSW Health Pathology, [7]Department of Nutrition, Dietetics and Food, Monash University, [8]Centre for Reproductive Science, School of Environmental and Life Sciences, College of Engineering, Science and Environment, University of Newcastle, [9]Infertility and Reproduction Research Program, Hunter Medical Research Institute, , [10]Centre for Human Reproductive Science, School of Medical Sciences, College of Medicine and Health, University of Birmingham and Birmingham Women's Fertility Centre, Birmingham Women's and Children's NHS Foundation Trust, [11]Fertility Specialists of Western Australia, [12]Division of Obstetrics and Gynaecology, School of Medicine, University of Western Australia, [13]City Fertility, [14]Department of Obstetrics and Gynaecology, Monash University

Background: Idiopathic male infertility describes abnormalities of a semen analysis where no underlying aetiology is identified. An important contributor of sperm's competence is the integrity of sperm DNA. Oxidative stress can cause sperm DNA damage and factors such as male aging are linked to increased sperm DNA damage. Importantly, fertilised oocytes can repair DNA damage from spermatozoa, however, this DNA repair response is affected by oocyte ageing. It is therefore suggested that the impact of sperm DNA damage in spermatozoa is more marked in women of advanced reproductive age. Hence, it is relevant to investigate antioxidant therapy in men partnered with women of advanced reproductive age.

Aim: To evaluate the effectiveness and safety of nutraceutical product FTC0098 (containing pollen extracts, zinc and vitamin E) on semen quality, sperm health makers (i.e. sperm DNA damage, oxidative DNA damage) and reproductive outcomes in infertile couples with idiopathic male infertility and where the woman is >35 years old.

Methods: This is the protocol for a multi-centre prospective observational study with pre- and post-test design, which plans to enrol 55 male patients aged 20-60 who meet inclusion criteria, pending ethics approval. Male patients will receive the nutraceutical product FTC0098 for 16 weeks and sperm function will be assessed three times over a 3.5-month period. Data on sperm quality and reproductive health outcomes will be obtained from medical records, and statistical analyses will be conducted on both an intention-to-treat and per-protocol basis using multivariate methods such as MANOVA. Post-hoc exploratory analyses will also be conducted, including generalised mixed models to account for cofounding factors. Data will be analysed using SAS and/or SPSS software.

Results: Results on sperm quality will be reported as mean with corresponding 95% confidence intervals and p-values. Reproductive outcomes such as clinical pregnancy and live birth will be also assessed.



P9: Exploring the relationship between oxidative stress, sperm parameters, and molecular biomarkers in men presenting for fertility preservation

Fabrizzio Horta[1,2], Annrose Joseph[1,2], Harleen Kaur[1,3], Rachael Rogers[1], Pru Sweeten[1,2], Jo Master[1,2], Ria Reaz[1,3], Rebecca Deans[1,2]

[1]Fertility & Research Centre, Royal Hospital for Women, [2]Discipline of Women's health, School of Clinical Science, University of New South Wales, Sydney, Australia, [3]NSW Health Pathology

Background: Male infertility is increasingly recognised as a condition not solely defined by abnormal semen parameters, but also by underlying molecular and biochemical defects within spermatozoa. Oxidative stress is reported in 30–80% of male infertility cases, with excessive reactive oxygen species (ROS) negatively affecting sperm function through impaired motility, DNA damage, protein oxidation, and lipid peroxidation. These changes may directly compromise reproductive potential yet remain undetected by conventional semen analysis, which does not assess oxidative damage or chromatin integrity. A more comprehensive understanding of sperm health therefore requires investigation of oxidative stress alongside molecular biomarkers and functional measures of sperm activity, including flagellar movement and energetic performance.

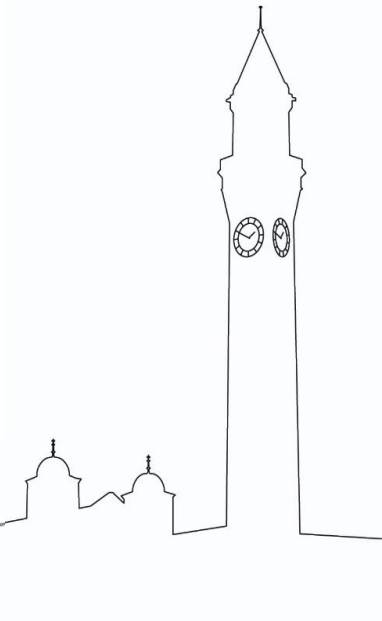
Aim: This study aimed to investigate the relationship between oxidative stress, molecular sperm biomarkers, and post-thaw semen quality in cryopreserved samples obtained from fertility preservation patients.

Methods: Cryopreserved semen samples from fertility preservation patients were analysed (n = 40). Conventional semen parameters were assessed according to WHO 2021 guidelines. Oxidative stress was evaluated using static oxidation-reduction potential (sORP) with the MiOXSYS system. Sperm DNA damage was measured using both the HaloSperm assay and sperm chromatin structure assay (SCSA) on the Sysmex Cube 6 platform. In addition, a novel Halo-8OHdG assay was used to detect oxidative DNA lesions in relation to sperm DNA fragmentation. To further explore the relationship between molecular biomarkers and sperm functional status, sperm flagellar movement was assessed using Flagellar Analysis and Sperm Tracking (FAST).

Results: Cryopreservation was associated with a significant decline in sperm quality, including reduced conventional semen parameters and increased sperm DNA fragmentation ($p < 0.005$). Higher oxidative stress was also significantly associated with poorer post-thaw sperm quality ($p < 0.005$), supporting its potential role as a key contributor to cryodamage. In contrast, patient-related variables including age, abstinence period, and duration of sample storage were not significantly associated with post-thaw semen

quality or molecular sperm health measures. The different assays used to assess sperm DNA damage showed no significant differences in their overall findings, suggesting consistency across methodologies in detecting sperm chromatin injury.

Conclusions: Assessment of oxidative stress and sperm DNA damage provides clinically relevant information beyond standard semen analysis in fertility preservation settings. These findings support the inclusion of oxidative stress and molecular biomarker testing as part of a more comprehensive evaluation of sperm quality in cryopreserved samples. Such approaches may improve understanding of sperm dysfunction and help refine diagnostic and clinical decision-making in male fertility preservation programs.



P10: Effects of Temperature Variation on Human Sperm Motility Characteristics

Hisham Alahwany, **Jonathan John**, Jackson Kirkman-Brown, Meurig Gallagher

[1]University of Birmingham

Background: Temperature is thought to play a role in directing sperm movement, particularly during navigation within the reproductive tract. In laboratory and clinical settings, sperm cells are often exposed to varying temperature conditions throughout processing and analysis. However, the precise effects of these changes on the flagellum—the structure responsible for propulsion—are not yet fully understood. Investigating how sperm respond to temperature shifts, and whether their function can recover, is important for both scientific understanding and clinical reliability.

Methodology: Semen samples were collected from donors with informed consent obtained. Samples were prepared using a swim-up technique at 37°C for 30 minutes. They were then subjected to a controlled temperature cycle, cooling from 37°C to 25°C before being reheated back to 37°C using a calibrated heating stage. Sperm motion was recorded using phase contrast microscopy and analysed with FAST software.

The following motility characteristics were assessed:
Flagellar beat frequency (fBF)
Arc wavelength (fAWL)
Arcwavespeed (fAWS)
Power (p30)
Head straight-line velocity (VSL)
Curvilinear velocity (VCL)

Results: Lower temperatures are expected to be associated with a reduction in several motility measures, including beat frequency, wave speed, velocity, and power, suggesting a negative relationship with temperature. Arc wavelength is anticipated to show minimal variation across conditions, indicating that temperature is likely to influence the rate of movement rather than the structural pattern of the flagellar wave. Following reheating to 37°C, all motility measures are expected to return to levels comparable to their initial state.

Conclusion: Temperature changes are expected to have a noticeable but reversible effect on sperm motility. It is anticipated that sperm will regain normal function after short-term cooling, suggesting that brief temperature fluctuations during handling are unlikely to significantly impact diagnostic outcomes.

P11: Context-dependent sperm motility: environmental shaping of performance across reproductive modes in fishes

Vitaliy Kholodnyy, Anatolii Sotnikov, Borys Dzyuba, Serhii Boryshpolets

[1]University of South Bohemia in České Budějovice Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses

Sperm motility is a central determinant of fertilization success, yet it is often treated as an intrinsic property of the cell rather than as a dynamic behavior emerging from interactions with the surrounding environment. This distinction is particularly important in externally fertilizing species, where sperm are activated abruptly and function within a narrow temporal window under rapidly changing physicochemical conditions.

In such systems, spermatozoa encounter a complex reproductive microenvironment shaped by ovarian fluid, egg-associated signals, and local physical properties such as viscosity. These factors may influence not only classical motility parameters (e.g., velocity), but also trajectories, spatial distribution, and ultimately the probability of sperm–egg encounter. However, the extent to which these effects are conserved or differ across species and reproductive modes remains insufficiently resolved.

Here, we investigate how the reproductive environment modulates sperm behavior across externally fertilizing freshwater fish species with contrasting spawning strategies (rainbow trout, common carp, and sterlet). Using computer-assisted sperm analysis (CASA), microcapillary chemotaxis tests, and controlled in vitro fertilization, we quantified sperm trajectories, velocity, spatial patterns, and fertilization success under different conditions. Our results show that exposure to reproductive fluids consistently alters sperm trajectories and promotes local accumulation near ovarian fluid sources, and also affects fertilization outcomes. The magnitude and dynamics of these responses vary among species, but they consistently yield distinct spatial patterns with a similar functional outcome: increased likelihood of sperm–egg encounter.

In addition to biochemical cues, the medium's physical properties also play a significant role. Increased viscosity alters both swimming speed and trajectory structure, further shaping sperm distribution in space. Together, these findings indicate that sperm behavior emerges from the combined effects of chemical and physical components of the reproductive environment.

To place these observations in a broader biological context, we compare these patterns with our data from internally fertilizing systems, including sharks and humans. Despite fundamental differences in reproductive mode, these systems reveal analogous functional outcomes, where environmental conditions modulate sperm behavior to enhance the probability of reaching the egg.

Taken together, our results support a view of sperm motility as a context-dependent and plastic trait, rather than a fixed characteristic of the cell. Although our study focuses on selected species and experimental conditions, it points to consistent, context-dependent patterns of sperm behavior. Recognizing this interaction improves our understanding of reproductive strategies and has potential implications for both basic reproductive biology and applied fields such as aquaculture and assisted reproduction.

The work was carried out with the support of VVI CENAKVA Research Infrastructure (LM2018099, MEYS CR, 2023–2026) and the Czech Science Foundation (Project 26-20614S).

P13: Profiling of compounds targeting key signalling proteins in sperm using a viscous media swim-up (VSU)

Franziska Mattes[1],Meurig Thomas Gallagher[2], Jackson C. Kirkman-Brown[2], Jule Brauckmann[1], Vesna Bojovic[1], Johanna Hessel[1], Christoph Brenker[1], Timo Strünker[1], Jessica Patricia Kurzella[1]

[1]Centre of Reproductive Medicine and Andrology, Molecular Reproductive Physiology, University of Münster, [2]Centre for Human Reproductive Science, School of Medical Sciences, College of Medicine and Health, University of Birmingham

New strategies for non-hormonal contraception are based on compounds that disable sperm motility. However, standardized assays to assess the action of these compounds are missing. To fertilize the egg, sperm have to be able to swim across a landscape of varying viscoelastic properties. For example, to transition from the vagina into the uterus, sperm must pass through the viscous mucus secreted by the cervical canal, which seems to filter out sperm with impaired motility. Previously, a direct swim-up method through a viscous barrier – viscous media swim-up (VSU) – was develop for sperm selection in vitro. We advanced and standardized the VSU technique. Moreover, we used the VSU to investigate whether and how molecules acting on proteins controlling the motility of human sperm affect their migration in a viscous environment.

For this purpose, the VSU was performed on ejaculates of normozoospermic donors. The ejaculate was overlaid with a 2 cm viscous barrier (1% methylcellulose, 4000 cP) that was overlaid with a low-viscosity buffer. Sperm were allowed to swim out of the ejaculate across the viscous layer into the buffer for one hour at 37°C, and the VSU-Index was determined, indicating the fraction of sperm able to overcome the viscous barrier. For pharmacological testing, the viscous layer was supplemented with different inhibitors, e.g., TDI-11861 to inhibit the soluble adenylate cyclase (sAC). We show that inhibition of the sAC by TDI-11861 decreases the VSU-Index in a dose-dependent fashion. We also examined the action of compounds acting on ion channels and membrane transporters on the VSU-Index.

Our results provide new insights into the pharmacological modulation of sperm motility in viscous environment. Moreover, we show that the VSU can be used as a powerful technique in basic research as a drug-profiling tool for new non-hormonal contraceptives.

P14: Viscous penetration assay – the ability of sperm to travel through viscous media and the flagella waveform properties of the sperm that travel furthest

Rachel Mckernan[1], **Jackson Kirkman-Brown**[1], **Meurig Gallagher**[1], **Johnathan John**[1]

[1]University of Birmingham

Background: Currently, we do not have a clear understanding of the difference between fertile and infertile sperm. This is an essential question to answer in terms of infertility treatment, as well as in the context of sperm-targeted contraception. In order to reach the oocyte and achieve fertilisation, sperm cells must be able to travel through the viscous fluid of the female reproductive tract. It is important that we understand the specific properties of the cells that enable them to travel through the viscous environment.

Aim: Quantify how the flagellar waveform changes as sperm travel through viscous fluid.

Methods: We designed an experimental protocol to assess the differences between sperm that can travel varying distances through viscous media. Donors consented to participate in ChRS research. Samples were collected from two distinct groups: fertile and sub-fertile men. A 1% methylcellulose solution was added to a commercial IBIDI chamber and capped with a Luer lock to create an air-locked system. Once the media had settled, liquefied semen was added to the chamber. The chamber was then capped and left for 25 minutes at 37 degrees Celsius, 6% CO2. Following incubation, FAST videos were captured of the sperm swimming along the chamber surfaces at distances of 3cm, 2cm and 1cm. FAST data will be analysed to determine the differences in flagella waveforms between sperm that can reach different distances.

Limitations: There are some limitations to this experimental set up, including ensuring consistent imaging locations and FAST capturing the higher numbers of sperm at shorter distances; however, these challenges can be combated. A 3-D printed mould can allow the precise location to be marked on the slide for image location and future versions of FAST software will be able to detect higher numbers of sperm per field.

Conclusions: Performing the viscous penetration assay in IBIDI chambers may provide valuable insights into flagella waveform properties of cells that are able to travel further through viscous fluid. This could support future advancements in assisted reproductive technology by helping to identify the most suitable sperm to select for fertilisation. Additionally, there is potential for this approach to improve diagnostic testing for sub-fertile men through tracking flagella waveforms.

P15: Temperature-Mediated Changes in Bacterial Growth and Semen Quality During Boar Semen Storage

Sara Miguel Jiménez[1], Úrsula Álvarez-Martín[1], Raquel Ausejo-Marcos[1]

[1]Magapor

This study assessed boar semen storage at 5 and 10 °C as a strategy to remove antibiotics from extenders, monitoring bacterial growth and sperm quality preservation.

Antimicrobial resistance represents a major global concern, prompting efforts to reduce antibiotic use in animal production. In the European Union, the use of antibiotics in semen extenders is no longer mandatory, encouraging the search for effective alternatives. Low-temperature semen storage has been proposed as a strategy to limit bacterial growth without antibiotics. However, it is essential to determine the impact of reduced temperatures on sperm quality, particularly in boar semen, which is known to be highly sensitive to cold shock and chilling injury.

Semen was collected from 4 boars (one ejaculate per boar; n=4) and extended with antibiotic-free Spermax extender (Magapor S.L.) to 2×10^6 sperm/mL in 80 mL AI doses. Semen doses were inoculated with 3×10^3 CFU/mL of 6 different bacterial strains (Serratia marcescens, Providencia rettgeri, Proteus vulgaris, Pseudomonas aeruginosa, Burkholderia cepacia and Cedecea davisae), separately, plus a non-spiked control. Then, samples were stored directly at 5, 10 and 16 °C for up to 6 days. Sperm quality (total motility, viability, and acrosome integrity) and bacterial count, after culture in tryptic soy agar (TSA) for 24h at 37 °C, were evaluated on days 1, 3, and 6.

Bacterial growth showed a clear dependence on both temperature and incubation time. Across all strains, cell counts increased from day 1 to day 6, with higher values observed at 16 °C. Low temperature (5 °C) markedly slowed bacterial proliferation, although none of the strains were completely inhibited. At 10 °C, growth was moderate and progressive, whereas incubation at 16 °C promoted rapid increases, frequently reaching maximum levels by day 3 or day 6. S. marcescens and C. davisae displayed the highest overall growth and the greatest responsiveness to temperature, while P. aeruginosa and P. vulgaris showed more limited increases, particularly at lower temperatures. Control samples exhibited minimal growth, with slightly higher counts at 16 °C. Overall, increasing temperature amplified time-dependent differences in bacterial growth. Motility increased with temperature under low contamination levels but declined over time and decreased sharply under high bacterial loads. Sperm membrane and acrosome integrity remained above 70 % in all samples.

This study was limited to a small number of bacterial strains and controlled laboratory conditions, which may not fully reflect field or clinical environments. Bacterial loads and incubation times were fixed, and interactions among mixed microbiota were not evaluated. Bacterial growth was strongly influenced by temperature and incubation time, with higher temperatures markedly accelerating proliferation across all strains. Lower storage temperatures (5 °C and 10 °C) effectively limited bacterial growth. Species-specific responses highlighted differences in temperature sensitivity and growth potential. Despite increased bacterial loads, sperm membrane and acrosome integrity remained preserved, whereas motility declined over time under high contamination levels. Importantly, storage at 10 °C appears to be a viable alternative for semen preservation, as it reduces bacterial proliferation while maintaining acceptable sperm quality. This approach could contribute to controlling bacterial growth without relying on antibiotics.

P16: Off-target activation of CatSper limits the utility of the Slo3 inhibitor VU0546110 and its derivatives in human sperm

Teresa Mittermair[1], Jens Münchow[2], Louisa Temme[3], Prof. Timo Strünker[1], Christoph Brenker[1]

[1]Centre of Reproductive Medicine and Andrology, University of Münster, [2]Institute of Pharmaceutical and Medicinal Chemistry, Pharmacampus, University of Münster, [3]Institute of Pharmacy, University of Hamburg

Slo3 is the principal potassium channel in mouse and human sperm. The voltage-gated, sperm-specific channel regulates the membrane potential in sperm and thereby other ion channels and transporters. It is absolutely required for sperm function and fertilization in mice and humans. Thereby, it is a good target for male contraceptives. However, its molecular physiology remains ill-defined due to a lack of selective pharmacological tools. Recently, the compound VU0546110 was introduced as the first selective Slo3 inhibitor. While currently used to study Slo3, its potential off-target effects in human sperm have not been fully characterized. We aimed to characterize the function of VU0546110 on Slo3 and CatSper, the principal calcium channel in human sperm. We also characterized 30 commercially available VU0546110-derivatives to find a more selective Slo3 inhibitor.
We analyzed the effects of VU0546110 and 30 commercially available derivatives using electrophysiological and fluorometric approaches. We performed patch-clamp recordings to determine the inhibitory effect of the compounds on Slo3. CatSper activity was assessed by kinetic fluorimetry using sperm from fertile donors and from infertile men carrying a homozygous deletion of the CATSPER2 gene to verify CatSper-dependent responses. To establish a rapid screening strategy for improved Slo3 inhibitors, Ca^{2+} responses were compared between sperm extracellular solutions containing low- and high-potassium, enabling us to identify compounds affecting Slo3-activity.

Here, we demonstrate that VU0546110 exhibits profound off-target effects. It evokes CatSper-mediated Ca^{2+}-signals in human sperm and simultaneously inhibits Slo3 and activates CatSper. This dual action limits the compound's utility, as it is difficult to decipher if its action on human sperm is due to inhibition of Slo3, activation of CatSper, or a combination of both. To address this, we tested 30 derivatives of VU0546110 to identify improved tool compounds. We identified two derivatives which show a reduced potency and efficacy for CatSper activation, while preserving the inhibitory action on Slo3. These compounds may be used to gain insights into the physiology of Slo3 in human sperm. We also introduce a new novel screening approach for Slo3-selective inhibitors that allows us to identify candidate compounds for further characterization.

P17: Formation of a complex between TMEM217 and the sodium-proton exchanger SLC9C1 is important for mouse sperm motility and male fertility

Rie Iida-Norita[1], **Haruhiko Miyata**[1], Akinori Ninomiya[1], Chihiro Emori[1], Maki Kamoshita[1], Chen Pan[1], Haoting Wang[1], Masahito Ikawa[1]

[1]Research Institute for Microbial Diseases, The University of Osaka

Sperm motility is crucial for male fertility and is tightly regulated by signaling processes within the flagellum. Slc9c1 encodes a sperm-specific Na^+/H^+ exchanger (sNHE/SLC9C1) that is localized to the flagellum and is essential for both sperm motility and male fertility. Unlike other Na^+/H^+ exchangers, SLC9C1 uniquely contains a voltage-sensing domain (VSD), whose physiological roles in mammals remain largely unclear. In this study, we identified Tmem217, a gene encoding a transmembrane protein localized to the sperm flagellum, by analyzing genes that coevolve with Slc9c1. Knockout (KO) of Tmem217 in mice caused defects in sperm motility and resulted in male infertility, closely resembling the phenotype observed in Slc9c1 KO mice. Co-immunoprecipitation and structural prediction analyses suggested that TMEM217 interacts with SLC9C1 through its VSD. Further investigation revealed that, in mature spermatozoa lacking TMEM217, both SLC9C1 and its associated protein, soluble adenylyl cyclase (sAC), were absent, leading to disruption of the cAMP signaling pathway. Notably, treatment with cAMP analogs restored both motility and fertilizing ability of Tmem217 KO spermatozoa in vitro, confirming the critical role of TMEM217 in regulating cAMP production. These results demonstrate that the interaction between TMEM217 and SLC9C1 via the VSD is essential for maintaining the proper structure and function of the SLC9C1–sAC–cAMP signaling axis in mature spermatozoa.

P18: Effect of bicarbonate included in the semen extender on boar sperm characteristics and DNA damage after storage at 17˚C

Hossain Md Iqbal[1], Kenji Shimizu[2], Yasushi Kuwahara[2], **Tetsuma Murase**[1]

[1]Gifu University, [2]Fuji Nojo Service

Study questions: The study aimed to examine the effects of bicarbonate included in the extender for storage of boar spermatozoa at 17˚C on sperm motility, viability, morphology and DNA damage after storage.

What is already Known: Liquid stored porcine semen is used for artificial insemination, and all extenders contain bicarbonate to the best of our knowledge. During summer season, boar spermatozoa are more advanced in the capacitated status and more spermatozoa are DNA-damaged. Calcium, bicarbonate and albumin are known to be responsible for capacitation. It was reported that with bicarbonate, premature capacitation progresses during storage of boar spermatozoa, and thus excluding bicarbonate was effective in reducing premature capacitation. Damage of DNA in the spermatozoa have been recognized recently as important parameter in addition to the standard sperm characteristics for fertility.

Study Design and Methodology: Sperm-rich fraction of semen from four boars was extended with Beltsville Thawing Solution containing bicarbonate (BTS) and BTS containing HEPES in place for bicarbonate (BTS-H) at 12 folds and stored at 17˚C for up to 28 days. Total and progressive sperm motility and viability were assessed on Day 3 and 10 of storage (Day 0 = semen collection), and DNA damage on Day 1, 7, 14, 21 and 28. In a separate experiment using 4 boars, sperm morphology was examined on Day 5 and 15. Sperm motility was evaluated using a computer-assisted semen analysis system. Sperm viability was assessed by SYBR14/PI staining. Glutaraldehyde-fixed spermatozoa were examined under the phase contrast microscope for morphology. Acridine orange staining was performed by a method combined from Tajeda et al. (1984) and Evenson (2022) for DNA damage.

Results/Findings: Analyses by ANOVA revealed that the main effects of the storage time or the extender type on total and progressive motility, viability and morphology (percent spermatozoa with intact acrosomes, with proximal and distal cytoplasmic droplets and tail abnormality) as well as their interaction were not significant. Significant main effects of both the storage time and the extender type on the percent spermatozoa with damaged DNA were found with no significant interaction. The percent spermatozoa with damaged DNA were consistently lower with BTS-H compared to BTS. The results show that the inclusion of bicarbonate may act as an influential factor that increases DNA damage during storage. These results suggest that bicarbonate contained in the extender increases DNA damage

during storage up to 28 days but that sperm motility, viability and morphology are not remarkably affected during storage up to at least 10 days.

Limitations: Mechanisms leading to the increase in DNA damage of boar spermatozoa by bicarbonate included in the extender during storage remained unclear. Further experiments need to be carried out to clarify the mechanisms.

Conclusions: Bicarbonate may promote DNA damage of boar spermatozoa during storage at 17˚C without compromising sperm motility, viability or morphology. Because DNA damage is one of the important factors that determine fertility of spermatozoa, reduction or exclusion of bicarbonate from the extender might be a tool to use for boar semen preservation, particularly during summer when DNA damage is higher. It would be possible that bicarbonate might have promoted capacitation status of spermatozoa during storage leading to the excess generation of oxidative stress damaging DNA. However, the mechanisms underlying bicarbonate-promoted DNA damage remained to be clarified.

P19: Testis expressed 50 is essential for maintaining sperm acrosome integrity during epididymal transit

Chisato Nagai[1]

[1]National Cerebral And Cardiovascular Center

Testis expressed 50 (Tex50) is a testis-enriched gene with an unknown role in male fertility, and we investigated the basis of infertility in Tex50 knockout (KO) male mice, which exhibit normal spermatogenesis and apparently intact acrosome formation in the testis. In mammals, spermatogenesis is completed in the testis, whereas spermatozoa acquire fertilizing ability and undergo further maturation during epididymal transit. Defects in acrosome biogenesis commonly cause globozoospermia, which is typically associated with abnormal spermatogenesis and malformed testicular spermatozoa. Multiple globpzoospermia-related genes have been identified, and their disruption leads to acrosomal defects arising within the testis. However, the molecular mechanisms that maintain sperm structural integrity after spermatogenesis, particularly during epididymal transit, remain poorly understood. Tex50 KO mice were generated using CRISPR-Cas9-mediated gene disruption and assessed male fertility, spermatogenesis, sperm morphology, motility, and acrosome integrity. Testicular and epididymal spermatozoa were analyzed by light microscopy, scanning and transmission electron microscopy, and fluorescence imaging using acrosome- and mitochondria- reporter transgenic mouse lines. Expression and localization of globozoospermia-related and sperm membrane proteins were examined by immunoblotting and immunofluorescence. To determine TEX50 localization during spermiogenesis, Tex50-mCherry knock-in mice were generated and analyzed across defined stages of sperm development and after the acrosome reaction. Tex50 KO male mice were completely infertile despite normal testis weight, spermatogenesis, and morphologically intact testicular spermatozoa. In constant, cauda epididymal spermatozoa from KO mice exhibited globozoospermia-like phenotypes, including loss of acrosomes, abnormal head and tail structures, and complete loss of motility. Acrosome formation and the localization of globozoospermia-related proteins were normal in the KO testis but were markedly disrupted in epididymal spermatozoa. TEX50-mCherry localized around the acrosome during early spermiogenesis and subsequently relocated to the post-acrosomal region of the sperm head, persisting after the acrosome reaction. These findings indicate that TEX50 is dispensable for acrosome formation in the testis but is essential for maintaining sperm acrosome integrity during epididymal transit. We were unable to identify TEX50-interacting proteins or directly assess molecular mechanisms underlying sperm acrosome destabilization in the epididymis. Further biochemical and functional analyses will be required to define TEX50-dependent pathways. Our study identifies TEX50 as a critical sperm membrane protein required for preserving sperm acrosome integrity and morphology during epididymal transit. Unlike previously characterized globozoospermia-related mouse models, TEX50 deficiency causes post-testicular sperm malformations despite normal spermatogenesis. These findings reveal a quality-control mechanism

acting during epididymal maturation and provide new insights into male infertility caused by defects due to spermatozoa migration from the testis to the epididymis. TEX50 may represent a diagnostic marker and potential therapeutic target in reproductive medicine.

P20: Objective measurement of bovine sperm hyperactivated motility using a viscous medium containing sodium hyaluronate

Yousuke Naniwa[1], Masashi Kinukawa[1], Kyoko Uchiyama[1]

[1]Livestock Improvement Association of Japan, Inc.

Study Question: Can a sodium hyaluronate-based viscous medium enable computer-assisted sperm analysis to objectively quantify hyperactivated motility of bovine sperm as an indicator of fertilizing potential?

What is already known: Current quality standards for frozen bovine semen rely on the post-thaw motile sperm rate, which shows a limited correlation with conception rates following artificial insemination. Hyperactivated motility, acquired during capacitation, is essential for penetrating the cumulus cell layer and zona pellucida during fertilization and is therefore considered a functional indicator of fertilizing potential. However, hyperactivated sperm exhibit rapid, erratic trajectories, such as figure-of-eight and star-spin patterns, that conventional CASA systems cannot reliably track. Visual identification of hyperactivation from high-speed video recordings has been attempted, but this approach is labour-intensive, subjective, and impractical for routine bull fertility assessment.

Study Design and Methodology: Frozen semen straws from 20 one-year-old Holstein bulls (two ejaculates per bull) were thawed and washed by centrifugation to remove cryoprotectants. Among three previously reported hyperactivation-inducing media, the most reproducible formulation was selected and modified to enhance hyperactivation induction within a shorter incubation period. Washed sperm were incubated in this medium at 38 °C under air, and motility was assessed at 0, 0.5, 1, 1.5, 2, 2.5, and 3 hours of incubation. Immediately before each measurement, aliquots were diluted two-fold in medium containing either 0% sodium hyaluronate (non-viscous control) or 0.5% sodium hyaluronate (viscous treatment), pre-warmed to 38 °C, and loaded into 20-μm- deep chamber slides for CASA recording. In the non-viscous control, hyperactivated sperm were identified visually by a trained observer from recorded video sequences based on characteristic figure-of-eight and star-spin movement patterns; immotile, motile, and hyperactivated sperm were counted separately.

Results/Findings: In the non-viscous control, hyperactivated sperm were first detected at 1 hour of incubation, with the hyperactivation rate peaking at 1.5 hours and gradually declining thereafter through 3 hours. In the viscous medium, violin plots of curvilinear velocity (VCL) and lateral head amplitude (ALH) distributions revealed the progressive emergence of two distinct subpopulations from 1 hour onward, with separation becoming most pronounced at 1.5 hours. Scatter plot analysis of individual sperm at 1.5 hours (n = 18,571

from 20 bulls) identified threshold values of approximately VCL > 100 µm/s and ALH > 5 µm that effectively separated the fast, high-amplitude subpopulation from the remaining cells. The proportion of sperm exceeding both thresholds in the viscous medium was significantly correlated with the visually determined hyperactivation rate in the non-viscous control ($r = 0.872$, $P < 0.01$), confirming that CASA-derived parameters in viscous medium can reliably capture hyperactivation status.
Limitations: Only two ejaculates per bull from 20 animals were used, which may limit statistical power. The threshold values for VCL and ALH were determined by visual inspection of scatter plot density rather than statistical modelling, and may require validation across different CASA systems, protocols, and breeds.

Conclusions: A sodium hyaluronate-based viscous medium successfully enabled objective, CASA-based quantification of hyperactivated motility of bovine sperm. By reducing overall sperm velocity while preserving the motility differences between hyperactivated and non-hyperactivated populations, this approach overcomes the fundamental limitation of conventional CASA in tracking erratic hyperactivated trajectories. Compared with visual assessment, this method offers substantial advantages in objectivity, reproducibility, and labour efficiency, making it suitable for routine screening applications. Future studies should examine the relationship between CASA-derived hyperactivation rates and field fertility data to establish its predictive value for bull selection.

P21: Injection into immature oocytes rescues spermatocyte arrest at mid-diplotene or later in azoospermic mutant mice

Narumi Ogonuki[1]

[1]RIKEN BioResource Research Center

Study Question: At which stage can arrested spermatocytes be rescued by injection into meiotic oocytes?

What is already known: In mice, offspring can be produced from primary spermatocytes using microinjection techniques. However, chromosome segregation abnormalities that occur after injection significantly reduce the birth rate (Kimura et al., 1998; Ogura et al., 1998). We improved these chromosomal segregation errors by artificially reducing the size of the oocyte cytoplasm, thereby increasing the live birth rate to 20%, and successfully producing live offspring from azoospermic mice with meiosis arrest (Ogonuki et al., 2022). These findings suggest that at least some types of spermatocyte arrest mutations could be rescued by injection into immature oocytes.

Study Design and Methodology: Adult azoospermic mice from nine strains carrying spermatocyte arrest mutations were used as spermatocyte donors. The most advanced stage of spermatocytes from each mutant strain was assessed by chromosome spread analysis. The spermatocytes at the most advanced stage of each strain were injected into MI oocytes. About half of the recipient ooplasm was removed from the recipient oocytes to ensure more stable chromosome segregation during meiosis. The spermatocyte-injected oocytes were allowed to mature in vitro to the MII stage, and their ooplasm was replaced with the ooplasm from fresh MII oocytes. After activation with SrCl2, the reconstructed oocytes that reached the 2-cell stage were transferred to pseudopregnant females. On embryonic day 19.5, recipient females were euthanized, and their uteri were examined for live fetuses.

Results/ Findings: Based on the chromosome spread analysis of spermatocytes, we classified the spermatocyte arrest mutants into three classes: Class 1, mutants with spermatocyte arrest at the mid-diplotene or later stages; Class 2, those at the early diplotene stage; and Class 3, those at the pachytene stage. All four Class 1 mutants could resume normal meiosis following injection into MI oocytes, as evidenced by the birth of normal offspring. Similarly, one of the two Class 2 mutants could be rescued, but the other could not. By contrast, none of the three Class 3 mutants supported full-term development because of complete implantation failure, indicating that the reconstructed embryos carried severe chromosomal aberrations.

Limitations: Although the number of mutant strains used in this study was too small to draw definitive conclusions, the results are nonetheless consistent: the more advanced the spermatocyte stage, the higher the probability of rescue.

Conclusions: In humans, a considerable proportion of spermatogenic arrest occurs at the primary spermatocyte stage. Spermatocyte injection might represent a potential therapeutic strategy for treating human male-factor infertility due to azoospermia in the future.

P22: Marked proteomic differences in spermatozoa subjected to selection procedures in couples with unexplained infertility

Marina Leiva[1], Meritxell Jodar[1,2], Maria Corredor[1], Mar Domingo[1], Marta Guimerà[3], Aina Borràs[3,4], Dolors Manau[3,4], Juan Manuel Corral[5], Judit Castillo[1], **Rafael Oliva**[1]

[1]Molecular Biology of Reproduction and Development Research Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Fundació de Recerca Clínic Barcelona (FRCB), Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona (UB), [2]Biochemistry and Molecular Genetics Service, Hospital Clínic Barcelona, [3]Gynecology, Obstetrics and Neonatology Clinical Institute (ICGON), Hospital Clínic Barcelona, [4]Gynecology, Human Reproduction and Women´s Health Research Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), [5]Urology Service, Andrology Unit, Nephrology and Urology Clinical Institute (ICNU), Hospital Clínic Barcelona

Study Question: Do sperm selection procedures in infertile patients result in proteomic differences in the selected sperm and could those be useful to assess sperm quality?

What is already known: Sperm selection procedures are routinely applied during Assisted Reproduction Technologies (ART) with the goal to improve sperm parameters. While discontinuous density gradient centrifugation (DGC) is the most commonly used method, passive selection procedures are also being used in the clinic, although differential proteomic and molecular studies comparing both methods were needed.

Study Design and Methodology: The sperm quality and molecular maturity of sperm selected by 40–80% DGC were compared to those obtained with the application of ZyMot (TM) Multi passive device in 29 normozoospermic infertile patients, as evaluated through differential proteomics, protamine determinations, seminal parameters and sperm DNA fragmentation.

Results: We found that passive selection significantly improved motility, vitality, and morphology ($p < 0.0001$) as compared to both DGC and neat semen and reduced the proportion of sperm with DNA fragmentation ($p < 0.001$). Proteomic analysis identified 1,882 sperm proteins showing differential abundance in the passively-selected fraction, as compared to neat semen (p-value< 0.05, FC ≥ 1.5). Further analysis of the differential proteins indicated that passively-selected sperm were enriched in proteins linked to essential functions, such as acrosome reaction and the maintenance of the redox balance, which is crucial for a proper chromatin integrity. In addition, a significant reduction of protamine 2 precursor was also observed, revealing a more mature state of the chromatin. In contrast, DGC-selected sperm exhibited a proteomic profile equivalent to neat semen, with no significant differences detected.

Conclusions: The results obtained support the use of passive sperm selection to enhance sperm quality by favouring molecular traits leading to improved fertilization.

Funding: Supported by a grant from the Instituto de Salud Carlos III (ISCIII; PI20/00936) to RO. JC is Serra Húnter fellow. The authors belong to COST Action Andronet CA20119, supported by COST (European Cooperation in Science and Technology, www.cost.eu).

P25: Establishing a robust screening platform to identify putative CatSper inhibitors using Ca^{2+} influx assay in human sperm samples

Anna Maria Saari MacDonald[1], Sean Brown[2], Christopher Barratt[1]

[1]Reproductive Medicine Research Group, Faculty of Health, University of Dundee, [2]University of Abertay
Biological complexity of CatSper as a drug target

CatSper is a sperm-specific cation channel with a highly complex multisubunit architecture, often referred to as the "CatSper complex" or "CatSperasome." To date, high-resolution structural determination and successful reconstitution of the native channel have remained elusive. This highlights the importance of studying CatSper in its native context, as animal models (e.g., mouse) do not fully recapitulate human CatSper physiology due to significant functional differences. Consequently, biologically relevant functional assays in human sperm, coupled with rational compound selection, are essential.

The strict sperm specificity of CatSper and its absence from somatic tissues make it an attractive target for non-hormonal male contraception; however, this specificity is accompanied by considerable biological and pharmacological complexity.

Assay validation and pharmacological controls: To establish assay robustness, a chemically diverse panel of compounds—comprising clinically approved drugs, ion channel modulators, and signalling pathway inhibitors—was screened. This strategy enabled evaluation of a wide range of pharmacological effects on progesterone (P4)-induced Ca^{2+} responses without presupposing mechanism of action. Direct measurement of Ca^{2+} influx provides a reliable functional readout of CatSper activity; however, selectivity against other ion channels (e.g., SLO3, hERG) requires orthogonal validation.

Ca^{2+} influx assay workflow: Semen samples from normozoospermic donors were collected and processed following liquefaction. Motile spermatozoa were isolated using density gradient centrifugation (40%/80%, ~1600 rpm, 20 min), followed by washing and centrifugation (10 min). The resulting pellet was resuspended in human tubal fluid (HTF0) supplemented with 0.3% human serum albumin (HSA).

Cells were adjusted to 1×10^7 cells/mL and incubated with Fluo-4 AM at 37 °C for 30 min. After washing to remove excess dye, cells were dispensed into 384-well plates. Fluorescence was measured using a FLUOstar OMEGA plate reader, and signals were analysed to quantify intracellular Ca^{2+} dynamics.

Results: Progesterone elicited a maximal Ca^{2+} response (~100%), similar to the inactive CatSper analogue (C2), confirming assay specificity. Positive controls, including $ZnCl_2$ and TS175a (derivative of TS150a), significantly reduced Ca^{2+} influx (~30–45%), validating assay sensitivity.

Cardiovascular agents (Ca^{2+} channel blockers/ACE inhibitors) generally showed limited inhibition, with responses clustering around ~90–115%. Nifedipine induced partial suppression (~75–80%), whereas flunarizine slightly increased responses (>110%), indicating inconsistent effects on CatSper.

NSAIDs (COX-inhibitors) exhibited minimal impact (~90–100%), suggesting little involvement in CatSper modulation.
Na^+/K^+ channel blockers/Slo3 inhibitors produced variable effects: quinidine and bupivacaine were near control levels (~95–110%), whereas clofilium tosylate and 3,4-dichlorobenzamil reduced Ca^{2+} influx (~50–90%), indicating compound-specific activity.
Phosphodiesterase inhibitors showed heterogeneous responses. IBMX, etazolate, and pentoxifylline reduced Ca^{2+} signals (~50–70%), while others remained closer to baseline. Additional modulators displayed moderate to weak inhibition overall, reinforcing the rarity of potent CatSper blockers.

Conclusions: A validated Ca^{2+} influx assay provides a robust functional readout of CatSper activity in human sperm and enables identification of candidate inhibitors. The low frequency of strong inhibitors highlights the intrinsic biological complexity of this target. These findings underscore the importance of high-throughput screening in native human sperm, supported by orthogonal validation approaches—including electrophysiology, functional motility assays, and genetic models (e.g., CatSper-KO sperm)—to accelerate identification of highly selective CatSper inhibitors.

P27: Beyond the head: tail-aware sperm segmentation using fine-tuned AI computer vision models

Sina Shafiezadeh[1], Alex Lyttle[2], Louise Brown[2], James Tyrell[2], Jackson Kirkman-Brown[1], Meurig Gallagher[1]

[1]Department of Metabolism and Systems Science, University of Birmingham, [2]Advanced Research Computing Team, University of Birmingham

Background: Accurate and informative assessment of sperm motility is essential for diagnosing male infertility and optimising Assisted Reproductive Technologies (ART) such as in vitro fertilisation (IVF). While Computer-Aided Sperm Analysis (CASA) systems have been available for decades, they focus on tracking sperm heads, neglecting the tail, which carries the critical information about motility patterns and functional integrity needed to draw insight into these sensitive cells.

Recent advances in computer vision AI models have demonstrated remarkable performance on instance segmentation of everyday objects. However, their performance often degrades in specialised medical domains, including sperm segmentation, due to their rapid pace, thin segments, and lack of image sharpness on a small scale.

Methodology: In this study, we fine-tuned the YOLO-26 (You Only Look Once) model on a dataset of 625 microscopic sperm videos (one frame per video) to segment individual sperm cells in complex video data, enabling a more comprehensive analysis that includes both head and tail structures.

Results: Our model achieves a mean average precision (mAP@50) of approximately 90%, indicating strong performance in detecting and segmenting the majority of sperm at the pixel level under moderate overlap criteria. However, performance declines to around 50% at stricter thresholds (mAP@90), highlighting the inherent difficulty of tiny object segmentation. In particular, sperm tails occupy only a small number of pixels, so even slight spatial deviations can lead to substantial penalties in the evaluation metrics.

Conclusion: These findings suggest that while modern segmentation models are promising for sperm analysis, the inherent difficulty of small object segmentation remains a key limitation. Future work should focus on specialised architectures and training strategies tailored to complex microscopic data, to bridge this gap, and provide a more holistic tool for infertility diagnostics.

P28: Understanding the prevalence of HPV infections in men referred for diagnostic andrology

Michael Spurway[1], Jo Parish[1], Jackson Kirkman-Brown[1]

[1]University of Birmingham

Currently, there is limited evidence describing the prevalence of human papillomavirus (HPV) infection in men undergoing investigation for infertility. This study aims to characterise the prevalence of undiagnosed HPV infection in men from the Birmingham population who have been referred for diagnostic andrology following difficulty achieving pregnancy with a partner.

Approximately one in six couples experience difficulty conceiving without assisted reproduction, and in up to 30% of cases the cause remains unexplained. This diagnostic uncertainty contributes additional psychological burden for affected couples. In both men and women, fertility may be impaired by a range of intrinsic and extrinsic factors, including undiagnosed or subclinical genitourinary infections. HPV is a sexually transmitted infection, and it is estimated that the majority of sexually active individuals will acquire at least one genital HPV type during their lifetime. While research in women's health has appropriately focused on oncogenic HPV types associated with cervical cancer, emerging evidence suggests that both low- and high-risk HPV strains may adversely affect sperm function and reduce the likelihood of conception.

This study investigates the presence of HPV infection, specific HPV genotypes, and associated pro-inflammatory markers in semen samples. Participants are recruited from men attending Birmingham Women's Hospital for diagnostic andrology and complete an anonymised questionnaire detailing personal and partner history of genitourinary infections, as well as HPV vaccination status. The findings aim to determine whether HPV represents a significant risk factor for unexplained male-factor infertility in this population, with potential implications for future diagnostic strategies and non-invasive therapeutic interventions.

P29: Functional testing of a novel non-hormonal contraceptive

Teodora Stoyanova[1], Eve Gumerova[1], Hannah Travers[1], Anthony Richardson[2], Suzanne Norval[2], Shruti Kane[3], Radoslav Lukoszek[3], Sarah Martins da Silva[1], Christopher Barratt[1], Zoë Johnston[1]

[1]Reproductive Medicine Research Group, Faculty of Health, University of Dundee, [2]Drug Discovery Unit, Faculty of Life Sciences, University of Dundee, [3]National Phenotypic Screening Centre, Faculty of Life Sciences, University of Dundee

Contraception options are limited, and most are associated with various side effects. Currently the only approved methods aimed at sperm cells are condoms and vasectomy, which have varying degrees of success. Thus, there is an urgent need for the development of novel contraception with a high level of protection and minimal side effects. There are gaps in understanding of fundamental sperm physiology, as well as a lack of biologically relevant assays which hinders the development of novel therapeutics that target male gametes.

A parent compound that reduces sperm motility was selected from a commercially available library as the start of a drug expansion series. To explore the contraceptive potential of the synthesised lead compound, its effect on total and progressive motility in both aqueous (AM) and viscous (VM) medium was determined using CASA Ceros II. Additionally, the effect on hyperactive motility in AM was examined. Compound effect on motility in raw semen was also evaluated. Hyaluronic acid binding after swim up through drugged VM and zona pellucida binding following incubation with the lead compound were used to interrogate residual sperm function. The effect on tyrosine phosphorylation, which has been linked to capacitation, was examined. To better understand potential issues with human dosing, a wash out assay was developed to evaluate whether effects on motility are maintained after compound removal. Finally, the effect on rat sperm motility was assessed to validate animal species to be used in future toxicity studies.

The lead compound tested in this study was found to significantly reduce total, progressive and hyperactive motility in AM and VM, functions essential for the movements of the sperm cell through the female reproductive tract. The effect was also evident after short incubations with raw semen, which simulated exposure of the sperm cells to the compound in the vagina. Treatment significantly reduced levels of tyrosine phosphorylation, which could be linked to lower capacitation levels. The compound significantly reduced hyaluronic acid and zona pellucida binding, mimicking the processes leading to egg fertilization. After incubation with the compound and subsequent wash out some of the sperm motility was recovered. Although control levels of motility were not reached, results highlight the need for careful human dosing to ensure maximum drug effect with user error. The lead compound reduced motility in rat sperm, suggesting it is a suitable species for toxicity testing.

The compound used in this study was derived from a phenotypic screen and as such the mode of action is yet unknown. This hinders the selection of appropriate experimental controls. Furthermore, extensive toxicology studies would be required to enable the use of the compound as a non-hormonal contraceptive in humans.

In this study we explored a novel compound derived from a phenotypic screening campaign for its use as a non-hormonal contraceptive that acts in the female reproductive tract against the sperm cell. In vitro assays showed the compound significantly downregulated various functions considered essential for successful fertilization such as motility, capacitation and zona pellucida binding. Furthermore, we explored some of the next steps of drug development, looking at compound wash out simulating under-optimum dosing in the human as well as validating a suitable animal species for toxicity testing. Together these results highlight the strong potential of a novel compound to be used as a non-hormonal contraceptive which could expand family planning options for people worldwide.

P30: Is hyaluronic acid–based sperm selection superior to conventional IVF (cIVF), a natural sperm selection technique?

Melissa Durgahshree Tharmalingam[1], **Melinda Ling Hou Chan**[1], **Yee Sheng Chong**[1]

[1]Kk Women's and Children's Hospital

Background: Physiological intracytoplasmic sperm injection (PICSI) facilitates sperm selection based on hyaluronic acid (HA) binding, reflecting sperm maturity and DNA integrity. While PICSI has been associated with reduced miscarriage rates, its clinical utility remains uncertain in the context of non-severe male factor infertility, where conventional IVF (cIVF) enables physiological sperm selection. At KKIVF, hyaluronic binding assays are not routinely performed during diagnostic semen analysis due to intra-individual variability. Patients with semen parameters suitable for cIVF, poor ICSI fertilization and usable blastocyst rates were offered PICSI and cIVF. This study compares embryological and clinical outcomes between PICSI and cIVF in patients with semen parameters suitable for cIVF.

Methodology: This retrospective sibling oocyte study included 117 IVF cycles (2023–2025), excluding those using frozen gametes. Patients underwent their 1st–4th cycles. Retrieved oocytes or cumulus oocyte complexes (COCs) were allocated to PICSI or cIVF on the day of oocyte retrieval. For PICSI, sperm selection was performed using the PICSI® dish, whilst for cIVF approximately 200,000 sperm were introduced per well of a 4 well dish containing 1–4 COCs, denudation was performed atleast 2.5 hours post-insemination. Patients were stratified by female age: 30–34 (n=29), 35–37 (n=44), 38–39 (n=31), and 40–42 (n=13). Outcomes included fertilization, blastulation, usable embryos, and clinical outcomes following single embryo transfer. Kruskal-Wallis and Wilcoxon signed rank test were performed at 0.05 statistical significance.

Results: Mean male age differed significantly in the 30–34 group compared to older cohorts (35.5 ± 3.8 vs 39.5 ± 5.8, 40.6 ± 5.4, 41.2 ± 4.0; p=0.0095), while oocyte yield was comparable across groups. Oocyte allocation, maturity, and normal and abnormal fertilization rates were similar between PICSI and cIVF in all age strata.In women aged 30–34, cIVF was associated with significantly higher usable cleavage-stage embryo rates (39.7 ± 6.6% vs 35.3 ± 8.8%, p=0.021), blastulation rates (43.3 ± 5.8% vs 40.4 ± 6.7%, p=0.037), and usable blastocyst rates (39.7 ± 6.6% vs 34.5 ± 7.1%, p=0.01) compared to PICSI. No significant differences were observed in the 35–42 age groups. Clinical pregnancy (16.7% [1/6] vs 0% [0/1]) and live birth rates (16.7% [1/6] vs 0% [0/1]) favored cIVF in the 30–34 cohort. In combined older cohorts, cIVF demonstrated lower clinical pregnancy (28.6% [4/14] vs 37.5% [3/8]) and live birth rates (50% [2/4] vs

100% [3/3]), with a higher miscarriage rate (50% [2/4] vs 0% [0/3]) compared to PICSI; statistical testing was not performed due to small sample size.

Conclusion: PICSI does not confer embryological advantage over cIVF in patients aged 35–42, supporting the use of cIVF where feasible. In younger patients (30–34), cIVF demonstrated superior embryological outcomes, potentially reflecting lower male age (35.5 ± 3.8 years) and better semen quality. cIVF reduces oocyte manipulation and its consequences. While clinical outcomes in older patients trend toward benefit with PICSI, these findings are limited by sample size. Larger, prospective studies incorporating semen parameters are warranted. In appropriately selected patients, cIVF represents a less invasive and cost-effective strategy without compromising outcomes.

P31: The comparative assessment of physiological and chemical inducers of the acrosome reaction in human sperm

Hannah Travers[1], Analia Novero[1], Radoslaw Lukoszek[2], Anthony Richardson[3], Zoe Johnston[1], Christopher Barratt[1], Sarah Martins Da Silva[1]

[1]Reproductive Medicine Research Group, School of Medicine, University Of Dundee, [2]National Phenotypic Screening Centre, School of Life Sciences, University of Dundee, [3]Drug Discovery Unit, Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, Dundee, United Kingdom

Study Question: What are the comparative effects of physiological and chemical acrosome inducers on sperm motility, viability and acrosome reaction levels?

What is already known: The acrosome reaction (AR) is crucial to sperm gaining functional competence for fertilisation. Exocytosis of the acrosomal vesicle exposes zona binding proteins, facilitating binding with the oocyte. The percentage of sperm capable of undergoing the AR is measured in-vitro using various artificial inducers, many of which do not accurately replicate in-vivo conditions.Commonly used inducers such as calcium ionophores do not fully replicate physiological conditions. Progesterone (P4) alone is used to induce the acrosome reaction despite being insufficient to robustly induce physiological acrosome reaction. The lack of a robust and reproducible AR assay, and defined thresholds, limit its use in fertility diagnostics and contraceptive development.

Methodology: Semen samples from healthy volunteer donors were prepared by density gradient centrifugation. Following preparation, cells were capacitated for 3 hours before triggering the acrosome reaction.

We compared sperm motility, viability and acrosome reaction levels from a range commonly used inducers of the acrosome reaction as below:
Calcimycin A23187(10μM and 30μM)
Ionomycin (10μM, 20μM and 50μM)
P4 (4μM and 10μM)
PGE1 (5μM)
Triple Mix (P4 10μM, PGE1 5μM and NH4Cl 30mM)

Motility assessments were performed using CASA (CEROS II). The cells were labelled with a live/dead marker to assess viability and CD46 antibody for measuring the acrosome reaction. Presence of the live/dead markers and CD46 antibody was measured using Novocyte Flow Cytometry and results analysed in FlowJo.

Results: Results demonstrated substantial variability in responses between AR inducers. Notably, despite ionomycin at 50uM producing an AR rate exceeding 40%, this also resulted in an almost entirely immotile cell population and elevated levels of cell death (26%). Thus, introducing the potential for data error through overestimating the level of AR due to high levels of cells death. This is likely due to its non-physiological mechanism of action, inducing acrosome reaction through excessive calcium influx and membrane disruption. Reduced concentrations of ionophore were less detrimental to cell viability but showed a significantly reduced level of acrosome reaction of around (~10% at 10uM). A23187, in contrast showed higher motility values and lower rates of cell death, whilst still achieving an acrosome reaction rate of around 30%. This suggests a better balance of induction efficiency and cell preservation. Regarding physiological inducers, we found that P4 used in isolation showed minimal rates of acrosome reaction in comparison to our triple mix solution, indicating that P4 alone is not a sufficient physiological inducer of the AR in human sperm.

Limitations: The main limitation of this work is that flow cytometry assessment of the AR only detects reacted or unreacted cells meaning the dynamic nature of the AR and any intermediate states are not captured. Another limitation is that the clinical relevance of the results is yet to be determined as the relationship between induced AR rates and fertilisation potential has not been established.

Conclusions: This study provides evidence that ionomycin, whilst effective at inducing AR, compromises sperm viability, limiting its suitability as an inducer. A23187 appears to offer a better response, supporting AR whilst better preserving viability. However, both are non-physiological inducers, and their biological relevance is unclear. The proposed use of our novel substance, triple mix, provides a more physiological relevant approach to inducing the AR. Improved understanding of the AR response in the fertile and infertile populations will allow thresholds for sperm competence to be defined, with applications in fertility diagnostics and the development of non-hormonal contraception.

P32: Do two different non-sterile sperm metallic counting chambers provide accurate estimates of sperm concentration compared to known target values provided by UKNEQAS?

Bryan Woodward[1]

[1]X&Y Fertility

What is known already: The WHO manual for the laboratory examination of semen recommends use of the new improved Neubauer counting chamber for assessing sperm concentration. However, on the day of treatment or sperm freezing, many IVF clinics use non-sterile metallic counting chambers to provide an estimate of sperm concentration, accepting the higher measurement uncertainty compared to the gold standard of the Neubauer. One counting chamber (the Makler®) was launched in the 1980s and became well established in IVF labs across the world. In 2025, a new counting chamber (the SpermScope®) became available. Whilst similar in design, validation of the new chamber is needed to check if performance is acceptable.

Study design and methodology: Physical samples of sperm in 10% formalin (n=40) were provided by the UK National External Quality Assessment Service (UKNEQAS) for Reproductive Science. The target concentration in all samples had been pre-established via UKNEQAS. Sample concentration was assessed in replicate for the two counting chambers: the Makler® (Sefi Medical Instruments, Israel) and the SpermScope® (Cryolab, UK). For each counting chamber, 10ul fluid was transferred onto the 100 square grid etched on the base portion, covering an area of $0.01mm^2$. A circular coverslip was then overlaid, providing an estimated depth of around 10um between the grid and the lower surface of the cover slip. Concentration was estimated by counting all 100 squares and dividing this number by 10. The study took place in an HFEA-licensed clinic by one andrologist, using a calibrated pipettor and sterile tips. Statistical comparisons used Student's T-tests (significance set a $P<0.05$) and correlations.

Results: When compared to the established UKNEQAS target values, there were significant differences between both concentrations reported from the Makler® ($P<0.05$) and the SpermScope® ($P<0.05$). Regarding correlations against the UKNEQAS target values, the SpermScope® performed better than the Makler® ($R^2 = 0.77$ vs $R^2 = 0.68$). When the concentrations provided by the Makler® and the SpermScope® were compared to one another, there were no significant differences ($P>0.05$). For the 40 samples, 72.5% (29/40) showed a difference in concentration of 5 million/ml or less between the two counting chambers. Using the same phase contrast optics, the SpermScope® field of view was slightly darker, and this increased contrast may have helped in identifying sperm where low concentrations were present.

Limitations: This study incorporated a relatively low sample number (n=40), although this was sufficient for statistical comparison. Whilst neither counting chamber is CE-marked, the SpermScope® is UKCA (UK Conformity Assessed) marked. Neither device is recommended by the WHO for diagnostic semen analysis, although both may find use in IVF labs.

Conclusion: Neither the Makler® counting chamber or the SpermScope® counting chamber provide accurate estimates of sperm concentration compared to known target values provided by UKNEQAS. However, since both counting chambers provide similar results, the SpermScope® counting chamber could be used instead of the Makler® counting chamber, and in the study had a slightly higher correlation to target values.

P33: Analysis of sperm DNA integrity between the above- and below-average fertility bulls

Danhui Yang[1], Samantha Small[2], Jonason Francisco[2], Neil Standley[1], Kylie Price[2], **ZhenZhong Xu**[1]

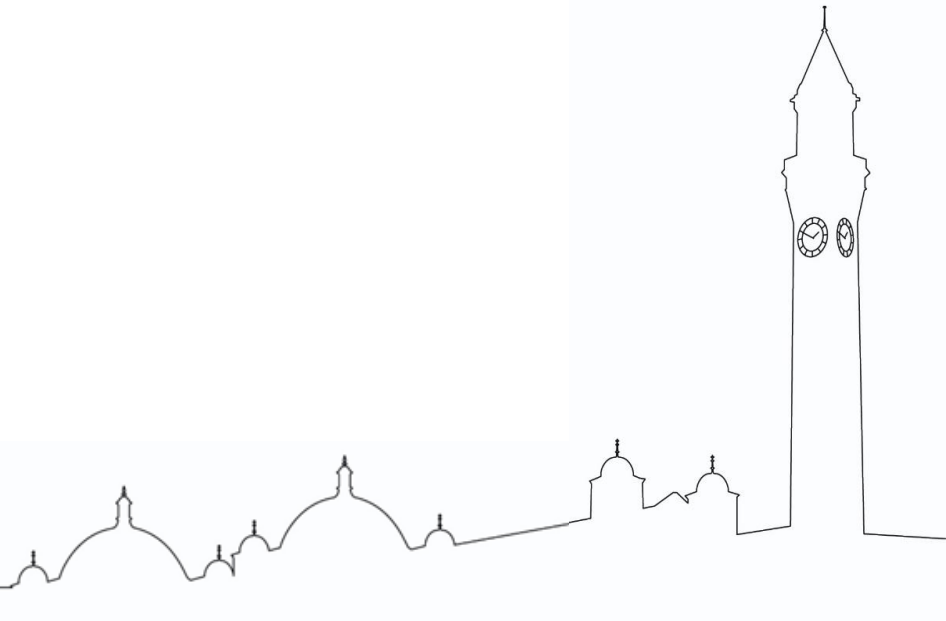
[1]Livestock Improvement Corporation, [2]Malaghan Institute of Medical Research

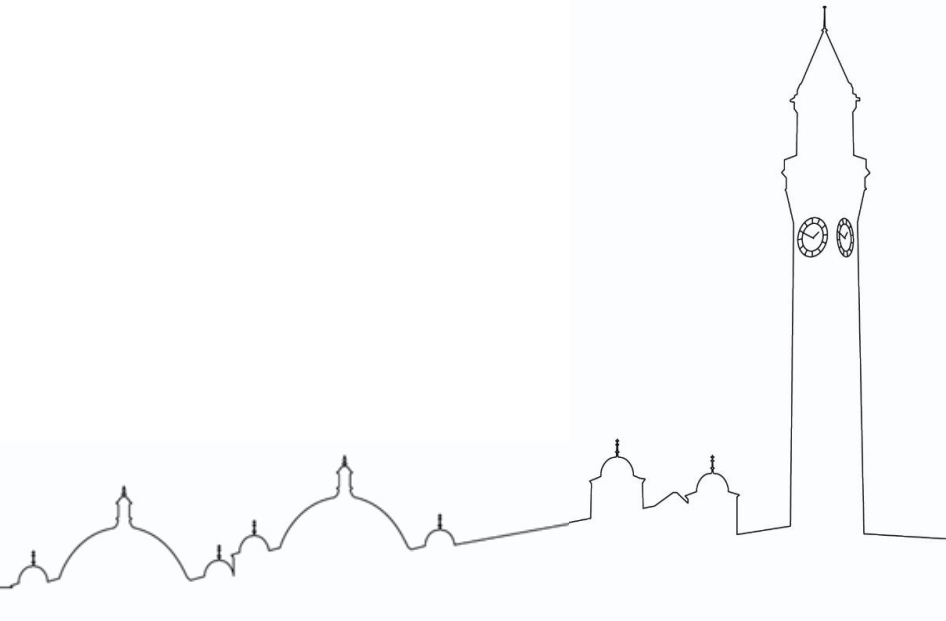
The objectives of this study were to validate the Sperm Chromatin Structure Assay (SCSA) for assessing bull sperm DNA fragmentation and to evaluate the association between DNA fragmentation index (DFI) and bull fertility measured as the field non-return rate (NRR). Changes in DFI with bull age were also investigated. A total of three experiments were conducted. The first experiment determined whether fresh or frozen semen was more suitable for DFI testing and showed that either sample type was suitable. Frozen semen straws were chosen for the second and third experiments because they allowed the analysis of samples from bulls that had been culled. In experiment two, semen from 13 bulls that had a history of poor NRR and 17 bulls with normal fertility were analysed. Results showed that 4 of the 13 low-fertility bulls (~30%) had DFI > 5%, whereas none of the 17 normal-fertility bulls had DFI > 5%. In experiment three, another semen batch from 12 of the low-fertility bulls used in experiment two was analysed to determine changes in DFI with bull age. This second batch was selected based on a collection date as far apart as possible from the first batch. In addition, semen straws from another 16 normal-fertility bulls were analysed to increase the power to detect a low false positive rate, defined as the probability that a normal-fertility bull would have a high DFI. Only two bulls showed both high DFI and poor NRR for both batches (~20%). Two additional bulls that had poor NRR at a young age have since changed classification from poor to average fertility. Overall, results from this retrospective study indicate that while high DFI is most likely associated with poor NRR, most bulls with poor NRR have normal %DFI. Among the low-fertility bulls included in the analysis, changes in DFI class with bull age were small, with approximately 80% remaining in same DFI class for both batches ($r = 0.794$, $P = 0.002$). Among the 33 bulls with normal fertility, three had high DFI (9% false positive rate). In summary, approximately 20% of the low-fertility bulls can be identified using the DFI test with a 5% cut-off.

Author Index

C

D



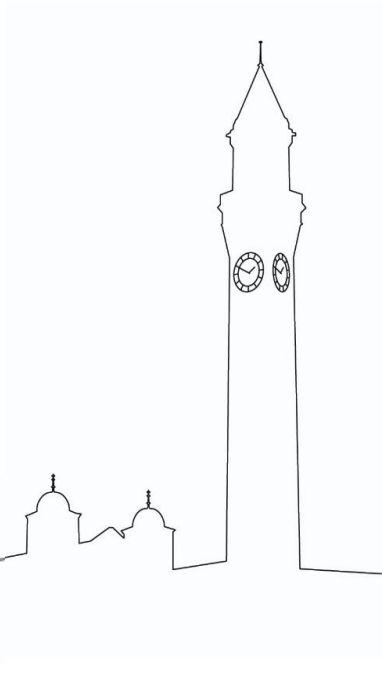


I

J

K

L



M

N

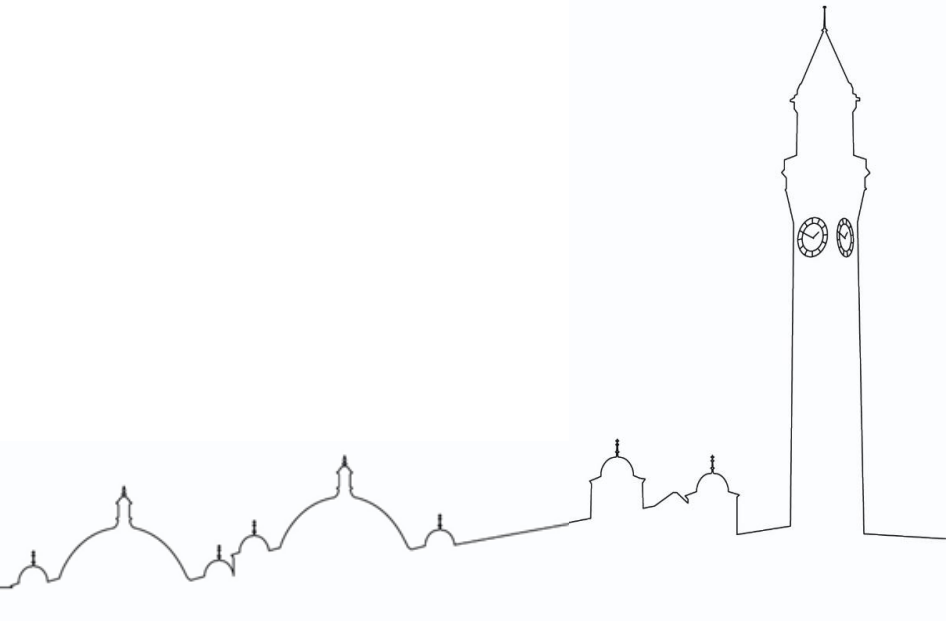
R

S

T

U

V



W

X

Y

Z