



**The Nettlefold Building
Winterbourne House & Botanic Gardens
The University of Birmingham**



FUTURE GENERATIONS

Centre for Human
Reproductive Science

Part of the integrated fertility services at

Birmingham Women's 
NHS Foundation Trust



**UNIVERSITY OF
BIRMINGHAM**

Acknowledgements

The BAS and local organisers would like to thank the following sponsors of BAS2011 who have made the meeting possible, please take time to encourage their future support and find out more about them.



Welcome to the Annual Conference of the British Andrology Society

It is a pleasure to welcome you to the **2011 Annual Conference of The British Andrology Society** at Winterbourne House, The University of Birmingham.

Our Society is a multi-disciplinary society and draws its membership from a broad range of backgrounds encompassing not just clinical and laboratory Andrology, but other associated fields such as Clinical Urology, Gynaecology, and Veterinary Medicine. We also have members from other scientific disciplines, including Reproductive Biology, Endocrinology, Cytology, Microbiology, Toxicology and Embryology.

Such a wide ranging membership has allowed us to take advantage of different opinions and interests within the society. Hence our annual meetings are attracting a broad range of specialities with a common interest in Andrology. The topics to be discussed in the 2011 annual meeting are promising to cater for all these different interests.

At this point I should like to thank Dr Jackson Kirkman-Brown and his team for hosting us and the splendid job that they have done to put this year's scientific program together. The preparation of an international scientific meeting is not an easy job. I am grateful to Jackson for inviting us to Birmingham and all the hard work that he has done to make this meeting happen.

I look forward to two days of high quality science, the chance to meet as many members as possible and hearing your views and opinions. Not only about the science of Andrology but also on how you want the society to further improve its activities and programs.

Alireza Fazeli

Chairman British Andrology Society

Welcome to Birmingham

Welcome to Brum!

We are the UK's Second City with a population of around 3.7 Million, but have a longer history than our modern image.

Birmingham's history goes back to 10,500 years ago, when a Stone Age settlement was formed in the Digbeth area, now better known for the Selfridges building. The Romans also left their mark on the city's landscape, with a major fort actually on the site of the University where we currently meet and many roads that cross the area.

The Birmingham Museum & Art Gallery has on display some of the 3,500 pieces of gold and silver from the 7th century Anglo-Saxon kingdom of Mercia, now famous as the "Staffordshire Hoard", which were discovered in July 2009. It is well worth a visit with free entry and many other amazing items in its collection: www.bmag.org.uk

Once famous as a 'City of a Thousands Trades' and the 'Toyshop of Europe', Birmingham was a world leader in the production of toys, pens, buckles, buttons, jewellery and guns. The ideas of the Lunar Society, a group of genius industrialists, philosophers and intellectuals including Matthew Boulton, James Watt and Erasmus Darwin not only changed Birmingham's history, but the history of the world. It is at this time we also gained the fabulous canal network giving us 'more canals than Venice' (35 miles as opposed to 26 miles!).

During the mid 19th century Birmingham gained a world reputation for a number of patents. 75% of everything written in the world was written with a 'Birmingham' pen. You can find out more about this at Winterbourne – as the house was home to the pen-making Nettlefold family. Also take time to visit the fantastic Barber Institute of Fine Arts: www.barber.org.uk which is just across the road.

Birmingham also became known as a leading model for 'municipal socialism' in Britain guided by Joseph Chamberlain, the clocktower on campus is named 'Old Joe' in his memory. At the end of the 19th century George Cadbury opened his chocolate factory and founded the Bournville village for the factory workers. JRR Tolkien was born locally and many of our landmarks are supposed to be inspirations for places in Lord of The Rings.

At the beginning of the 20th century Birmingham adapted the new way of life as electrical engineering and car manufacturers became the dominant industries in the city, we are still the home to Jaguar. Over the last 30 years however, the city's focus has shifted from being predominantly a manufacturing industry to a service economy. Former industrial properties such as the Custard Factory have been transformed into some of Birmingham's most exciting art and nightlife venues.

Now, Birmingham is a city that is energised by the youngest population in Europe, with 65,000 students at our three universities and two university colleges, it is the youngest city in Europe. We are a city of immense variety, from Michelin-starred dining to the Birmingham Royal Ballet; CBSO concerts, theatre and the major shopping venue after London, Birmingham really does offer it all. We are also within an hour's drive of Welsh mountains, Shakespeare's Stratford or the Peak District.

We hope you enjoy your time here and take the time to explore more.

Jackson Kirkman-Brown - Local Organiser



UNIVERSITY OF BIRMINGHAM

College of Medical and Dental Sciences

School of Clinical and
Experimental Medicine

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Reproduction, Genes & Development
Academic Unit
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Message from the School of Clinical and Experimental Medicine

I am delighted to welcome you to the College of Medical and Dental Sciences at the University of Birmingham. We have a long and celebrated history of scientific and clinical research into women and children's health. In my own subspecialty of Fetal Medicine we are constantly reminded of the importance of embryonic and fetal development. The influence of gametes on fetal development is unquestionable and the aims of your Society in understanding basic science and clinical research in this area is key to understanding.

I am absolutely certain that presentation of ideas, discussion and forging of new partnerships are pivotal to progress in today's scientific arena.

I wish your conference well and hope that you have a constructive, fulfilling and enjoyable experience. I hope that the meeting will provide each of you with fulfilment and the forging of both professional and social friendships.

Professor Mark Kilby
School of Clinical Endocrinology & Metabolism
College of Medical & Dental Sciences
University of Birmingham

General Information

Meeting Registration: The registration desk will be situated in the reception area of the Nicolson Building, which is adjacent to the main entrance for Winterbourne House (see map at end of booklet).

The desk will be open from 8am on Friday 16th 2011.

Admission: Entry to the building and scientific sessions is limited to badge wearers. Tickets will be issued for functions and must be presented for admission. Your badge will also provide free admission to Winterbourne House and Botanical Gardens. If you have a companion they can be admitted to Winterbourne House by paying the standard admission fee.

Presentation format: All oral presentations will be via PowerPoint. Any other presentation needs can only be catered for if speakers request in advance. Mac facilities can be available by prior arrangement.

Speakers are asked to present their presentations at least 20minutes in advance of the session in which they are speaking in.

Young Researcher Competition: A judging panel selected a number of the poster abstracts from Young Researchers for oral presentation at the meeting. A prize will be awarded for the top presentation.

Business Meetings: The AGM of the British Andrology Society will be held at 08:45 on Saturday 17th September. All delegates can attend, but only members have voting rights.

Finding the Conference Site:

Accommodation booked via us for BAS2011 is located on the University Conference Park immediately adjacent to Winterbourne House and Garden. The reception desk at Lucas House is the check-in area for your accommodation. You should have been sent separate check-in details by email from Venue Birmingham.

Winterbourne House & Garden is situated close to the acclaimed University of Birmingham's Edgbaston campus, just 10 minutes from Birmingham city centre. Please note that Winterbourne House and Garden has limited parking available on-site. All delegates should park in the free parking at the Lucas House / Hornton Grange accommodation site, which is just 50m along the road, if possible.

The Address:

University of Birmingham,
58 Edgbaston Park Road,
Edgbaston,
Birmingham B15 2RT

By Road

From the M6 motorway, leave at junction 6 (signposted Birmingham Central), at the end of the motorway, go over the flyover and join the A38, Bristol Road. The University is on your right, 2.5 miles from the city centre. Turn right into Edgbaston Park Road and follow signs for Winterbourne Botanic Garden.

From the South leave the M5 at Junction 4 (signposted Birmingham SW) to join the A38. The University is on the left, 8 miles from the motorway.

Free car parking is available across from the Winterbourne House and Gardens, at Hornton Grange. Please ensure that you are parked at the specified car park.

By Air

The nearest airport is Birmingham International Airport. The best transport system to the city centre is via train, which takes approximately 10 minutes to get to the heart of Birmingham. From Birmingham New Street Station, you can either take a taxi or a train to Winterbourne.

By Bus

To get to Winterbourne from the city centre the numbers 61 and 63 provide the most regular services, with a joint frequency of every 3-4 minutes between Birmingham City Centre and Northfield, stopping on the Bristol Road - a 5 minute walk from Winterbourne. The X64 and X62 pick up passengers from Paradise Circus and the Town Hall, and run less frequently, however they stop at fewer stops making it an express service. For all bus services, alight at the fire station/gun barrels pub on the corner of Edgbaston Park Road and the Bristol Road. For more information see www.travelwm.co.uk

By Bike

Many cyclists use the University campus and Winterbourne is easy to cycle to from most locations. We now have bike racks available at the front of the house next to the Nicolson Building. If you are a larger group of cyclists, please notify us in advance.

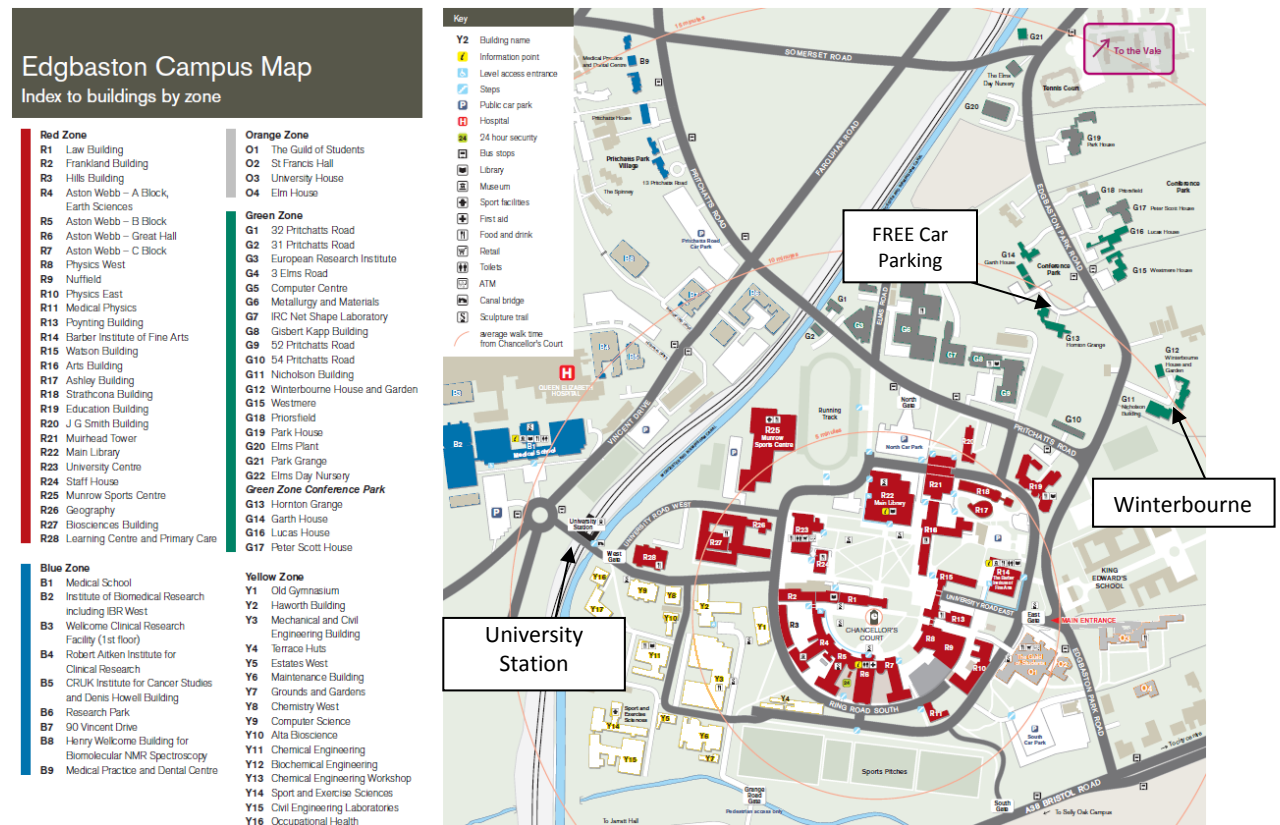
By Rail

If you are travelling by rail, the main station is Birmingham New Street Station. From there, you may have to change trains to get to **University Station**, on the Cross-City line. The trains are quite frequent from Birmingham New Street Station and run almost every 15 minutes. The University campus, 15 minutes walk from Winterbourne. However, there is no taxi rank at this station.

Alternatively from Birmingham New Street, a taxi to Winterbourne takes around 10 minutes but the cost may be significantly more than the taking a train to University and walking to Winterbourne.

By Foot

If you are walking, it is likely that your origin would be from the University Station. See the map. G11 – Nicolson Building at Winterbourne - is registration area. G16 – Lucas House – is accommodation.



Important Contact Numbers:

1. Winterbourne House and Gardens : 0121 414 3003
2. Private Taxi Hire: Falcon Cabs : 0121 603 6666

Friday, 16th September 2011

08:00-09:10 Registration + Breakfast Snacks

09:10 Welcome The Birmingham Team

Session 1: "What Sperm Bring to the Party" – Current Advances

Chair: Professor Sheena Lewis - BAS Treasurer

09:15 DNA Integrity and Other Genetic Insights into Sperm Quality Prof. Dagan Wells
University of Oxford

10:00 Sperm Nuclear Organisation Prof. Darren Griffin
University of Kent

10:45 Coffee, Posters & Trade Exhibits

11:00 The Role of Phospholipase C Zeta During Fertilization and its Link with Human Infertility Dr John Parrington
University of Oxford

11:45 From Flies to Men, Sperm Histones Carry the Same Embryological Signature to the Egg Dr David Miller
University of Leeds

12:30-13:30 Lunch, Posters & Trade Exhibits

Session 2: Preserving Sperm/Fertility

Chair: Dr Jackson Kirkman-Brown

13:30 Sperm Banking - Cancer & Social Banking Prof. Sheena Lewis
Queen's University Belfast

14:15 Sperm in Conservation; *If Only All Species had the Same Sperm!* Prof. Bill Holt
Institute of Zoology

14:45-15:15 Coffee, Posters & Trade Exhibits

15:15 Slow freezing and Vitrification: Sperm Storage Challenges Prof. Anna Petrunkina
University of Cambridge

16:00 Andrological Considerations in the Management of Severe Scrotal Injury Mr Richard Viney
University of Birmingham

16:45 - 17:45 Young Researcher Communications for Young Researcher Prize

Chair: Dr David J Smith

Presenters: Alexandra Amaral, Sahib Shahani, Renata Tavares, Julia Simpson

20:00 Conference Dinner

Presentation of the Brian Setchell Medal

Young Researcher Prize Presentation

Saturday, 17th September 2011

08:30-09:15 Coffee, Posters & Breakfast Snacks

08:45 BAS Annual General Meeting

09:15 **Brian Setchell Medal Lecture** Dr W Chris L Ford
Sperm, Sperm, Sperm, Egg and Sperm: Reflections on a Life *2011 Setchell Laureate*
in Andrology

Session 3: Environmental effects – “Being male - on the macro and microscale”

Chair: Dr Alireza Fazeli – BAS Chair

10:00 Toxicological Effects on the Male - Environmental Factors Affecting the Androgen/Oestrogen Balance Dr Rosemary Waring
University of Birmingham

10:45 Coffee, Posters & Trade Exhibits

11:15 Motility in Viscous Fluids: *Going Against the Flow!* Dr Eamonn Gaffney
University of Oxford

12:00 Physiological and Artificial Modulation of Sperm Motility Dr Jackson Kirkman-Brown
University of Birmingham

12:30 Lunch, Posters & Trade Exhibits

Session 4: Future Perspectives in Andrology – “Where are sperm going?”

Chair: Dr S J Publicover

13:30 Sperm Chemotaxis - the Known and Unknown Prof. U. Benjamin Kaupp
Caesar Research Centre, Germany

14:15 Sperm Competition: What Next? Prof. David Hosken
University of Exeter

14:45 Proteomics for basic biology and sperm sexing Dr Ian Brewis
University of Cardiff

15:30 Final remarks and Meeting Close

Brian Setchell Medal for Contributions to the BAS

The Brian Setchell Medal was introduced in 2007 to commemorate 30 years of the British Andrology Society being a learned society. Brian Setchell was a founding member of the BAS with a long illustrious career in the field of andrology.

This year's medal will be presented to Chris Ford at the annual conference dinner and they will provide the Society with a presentation on the following day.

Autobiography Dr Chris WCL Ford

I was born in 1947 in Sussex. I had a happy childhood and attended the local grammar school. In 1965 I went to Birmingham University to read Biochemistry and graduated with a 2:1 in 1968. I returned to Birmingham to do a PhD again in Biochemistry which I completed in 1971. I then did a 2yr post-doc in the Biochemistry Department at Edinburgh University before moving to the Johnson Research Foundation at the University of Pennsylvania, helped by a Fulbright fellowship.

I entered the world of Andrology in 1975 when seeking to return to the UK, I answered an advert from Professor Geoffrey Waites for a post-doctoral metabolic biochemist. This started a fruitful collaboration in Reading that lasted for 11 years. We investigated the mode of action of the male contraceptive alpha-cholorohydrin. We were the first to establish the stereospecificity of its action and went on to discover the related contraceptive action of 6-chloro-6-deoxysugars. Sadly both alpha-cholorohydrin and the 6-chloro-6-deoxysugars turned out to be neurotoxic and were abandoned as potential contraceptives for humans. However, they revealed the potential of the sperm isoform of glyceraldehyde 3-phosphate dehydrogenase (GAPDHs) as a contraceptive target which still attracts some research interest. It was an exciting time in Reading other people in the lab included John Walton, James Leask, Trevor Cooper, Cheng Hei Yeung and Nigel Jenkins and I benefitted from the skilled assistance of Anne Harrison.

By 1986 Geoff Waites had moved to WHO in Geneva and it was time to leave Reading. I was fortunate to be offered a Senior Fellowship in Bristol by another renowned figure, Michael Hull. This meant a change of emphasis from preventing fertile men from conceiving to helping infertile men to conceive. This presented many interesting challenges including taking a hand in managing a developing donor insemination and IVF service. One of Mike's big ideas was that the diagnosis of male fertility should depend on sperm function and not sperm numbers and we put much effort into evaluating sperm function tests. Another problem was that sperm from most potential semen donors did not survive cryopreservation and with Eileen McLaughlin we investigated this in depth. This led into an interest in calcium regulation of sperm function since elevated intracellular calcium is a characteristic of frozen-thawed sperm. John Aitken introduced me to the effects of reactive oxygen species on sperm which began another productive avenue of research which continues elsewhere to this day. I continued my interest in metabolism and the role of glucose in supporting human sperm fertility, one student being Andy Williams the present managing editor of Human Reproduction. The group in Bristol never recovered from Mike Hull's untimely death and I took early retirement in 2006.

I was able to conclude my career by returning to Birmingham to work for 2 years in Professor Chris Barratt's group where we were able to establish a role for protein S-nitrosylation in sperm.

Now, I enjoy my family, go trout fishing, cultivate my allotment and do a little freelance editing!

**Invited Speaker
Abstracts & Profiles**

Dr W Chris Ford

Centre of Reproductive Medicine and Andrology
of the University, Münster, Germany

**Sperm, Sperm, Sperm, Egg and Sperm:
Reflections on Life in Andrology**

My career in Andrology was, perhaps, an honourable failure. I will discuss if the objectives I and my contemporaries tried, but failed to reach, remain valid for the next generation of Andrologists. First, the male contraceptive. Epididymal sperm seemed the ideal target, onset of infertility would be rapid and reversibility likely since these sperm are soon replaced. Alpha-chlorohydrin (AC) demonstrated that this approach could work but was neurotoxic in higher doses. Its principal target was sperm glyceraldehyde 3-phosphate dehydrogenase (GAPDHs). This remains a valid contraceptive target but differences between observations in AC inhibited sperm and GAPDHs KO mouse sperm pose questions about energy delivery to the mammalian sperm tail. Second, sperm function testing. Important to provide an accurate prognosis for fertility but a much harder task than we realised, many tests eg CASA, hamster oocyte penetration test were tried but none were adopted widely in clinical practice. Yet avoiding unnecessary allocation of couples to assisted conception (ART) could save millions of pounds. Third, what causes sperm dysfunction, critical knowledge for devising treatment and prevention of male sub-fertility avoiding the need for expensive ART. Damage from reactive oxygen species (ROS) was a good lead but investigations were plagued by artifacts and many questions remain unanswered. Longitudinal studies of sperm quality are needed to see how sub-fertility develops. Finally how does the sperm synchronise getting ready to fertilise with its arrival at the zona pellucida, is nitric oxide secreted by the cumulus mass important and how does it influence sperm function?

Speaker Information

For more information on Chris read his Short Autobiography in the Setchell Medal section

Friday 16th September
09:15-10:00

Prof Dagan Wells

Senior Fellow in Reproductive Genetics, University of Oxford
Director, Reprogenetics UK
dagan.wells@obs-gyn.ox.ac.uk

DNA Integrity and Other Genetic Insights into Sperm Quality

It is widely accepted that traditional semen assessments are limited in the amount of prognostic information they provide. Their predictive value is, for the most part, restricted to sperm samples with the worst parameters (low concentrations and poor motilities). Since as much as 50% of all infertility is considered to be of male origin, improved methods for the evaluation of sperm samples would be extremely valuable. Genetics represents one of the most important aspects of sperm potential and consequently tests that provide an insight into the genetic competence of spermatozoa are particularly attractive. The methods of assessment currently available focus on cytogenetic (i.e. chromosome) analysis and evaluation of genomic integrity (i.e. DNA fragmentation).

The transmission of a haploid chromosome complement is the single most important purpose of the spermatozoon. Embryos that inherit an incorrect number of chromosomes (aneuploidy) will fail to implant, result in a miscarriage or, more rarely, produce a child with an aneuploid syndrome, such as Down's. Recombination is an essential mechanism for the maintenance of accurate chromosome segregation during meiosis. However, significantly lower rates of meiotic recombination have been observed in infertile men. This may be the underlying cause of the increase in sperm chromosome abnormality rates observed in various groups of infertile/subfertile men. The patients most at risk of elevated sperm aneuploidy include those with Klinefelter syndrome, structural rearrangements (e.g. translocations), severe morphologic defects and nonobstructive azoospermia. Aneuploidy testing should be seriously considered in these patient populations. Such tests are also likely to provide useful information for patients with a history of unexplained recurrent pregnancy loss or repeated IVF failures, some of which turn out to be linked to aneuploidy. Diagnosis of an increased risk of sperm chromosome aneuploidy may reduce risk to the offspring (preimplantation genetic diagnosis may be advisable) and in some cases reduce the financial and emotional burden of recurrent IVF failure. Such analyses may also have value for assessing the chromosomal status of sperm produced following chemotherapy treatment.

The link between DNA damage and reduced reproductive potential has been clearly established in animals and also has strong experimental and clinical support in the human. Murine spermatazoa with induced DNA damage are associated with poor embryo development and reduced implantation. Perhaps even more alarming are the reported post-natal impacts, affecting growth, longevity and susceptibility to tumours. In humans much of this data is still lacking, but it seems clear that high levels of DNA damage are associated with impaired fertility, an elevated incidence of miscarriage and an increased risk of disease in offspring. Multiple studies have indicated that the assessment of DNA integrity in sperm may be a useful marker of fertility, independent of (although in some cases associated with) standard semen parameters. The etiology of sperm DNA damage is likely to be multi-factorial, originating from processes such as abortive apoptosis, failure to resolve DNA breaks created during chromatin remodelling and due to oxidative stress. Well-designed, large scale studies are now urgently required to quantify the impact of sperm DNA damage and verify the value of screening methods.

There are several limitations of the current technologies used for sperm DNA fragmentation analysis. They are either labour-intensive, require expensive instrumentation, or are poorly adapted for use in the IVF clinic. However, the most important shortcoming, which affects all existing methods of DNA damage evaluation, is that the spermatozoa are destroyed by the testing process. Thus far, no method for the selection of viable spermatozoa, free of DNA damage, has been devised.

This would obviously be a highly attractive possibility, as even the worst samples tend to have a few spermatozoa with intact DNA. A method of identification that preserved viability would allow such spermatozoa to be used for ICSI, even when only forming a small fraction of the sperm population. We have developed a novel peptide stain with DNA-damage binding properties that may fulfil this purpose. Investigations of the stain are ongoing, but initial data is encouraging.

In summary, genetic evaluation of sperm samples yields useful information, additional to, and independent of, insights gleaned using routine semen assessments. Further studies will be necessary in order to maximise the value (clinical and counselling) of these technologies, but these forms of genetic analysis appear to have great potential. Additionally, emerging methods that preserve sperm viability, allowing selection of individual sperm for fertilisation, and techniques that assess other aspects of sperm genetics, such as genomic imprinting and other forms of epigenetic alteration, hold much promise for the future.

Speaker Information

Dagan Wells studied at University College London (UCL), obtaining bachelors and doctoral degrees in Genetics. He has been actively involved in preimplantation genetic diagnosis (PGD) and the study of human gametes and embryos for two decades, conducting his first PGD cases in 1992. After a time spent supervising molecular diagnostics at the UCL Centre for PGD in London, Dagan moved to the United States and joined Reprogenetics, one of the largest providers of PGD services in the USA. In 2003 he initiated Reprogenetics' highly successful single gene PGD program, testing embryos for numerous serious inherited conditions. Dagan later joined the faculty of Yale University Medical School, where he spent four years as an Assistant Professor, before returning to the UK in October 2007. His research group is now located in the Nuffield Department of Obstetrics and Gynaecology at the University of Oxford.

Dagan's current research program is focused on increasing understanding of the molecular processes underlying gametogenesis and preimplantation development. His laboratory has a strong translational emphasis and is actively involved in the development of new PGD methods that are more comprehensive and more reliable than those in current use. His research also aims to create novel techniques for improving the success rates of in vitro fertilization (IVF) treatment. Dagan's research program has attracted funding from multiple public and private organizations, including the National Institutes of Health (NIH) and the Medical Research Council (UK).

Dagan's work has been recognized with several awards, including the American Society for Reproductive Medicine (ASRM) General Program Prize, European Society for Human Reproduction and Embryology (ESHRE) Basic Science Prize and the Society for Assisted Reproductive Technologies (SART) Prize. He has also been an author of more than 120 peer-review publications and book chapters. Dagan currently serves on the Editorial Boards of several international medical journals, including Molecular Human Reproduction, Reproductive Biomedicine Online and Prenatal Diagnosis. Additionally, he is Director of Reprogenetics-UK, an independent UK-based company offering state-of-the-art PGD services. Dagan is a Fellow of the Royal College of Pathologists (UK) and holds a New York State Dept of Health PGD laboratory director license.

Friday 16th September
10:00-10:45

Prof Darren Griffin

School of Biosciences,
University of Kent,
Kent, CT2 7NZ, UK

Sperm Nuclear Organisation

The spermatozoon is the smallest cell in the human body and, in order to package its chromatin into such a small space, a radical nuclear reorganisation is essential. In biochemical terms the histone proteins are gradually replaced by protamines however wholesale alteration in the spatial and temporal topology of the chromosome territories is also apparent. Notable among these changes is a migration of the sex chromosomes to the centre of the nucleus and the formation of a "chromocentre" (where the centromere sequences reside) next to the sex chromosome body. Numerous studies have implicated the role of gross genomic rearrangements in male infertility e.g. constitutional aneuploidy, translocations, inversions, Y chromosome deletions, elevated sperm disomy and DNA damage. Positional alterations of chromosome territories have been associated with disease phenotypes (e.g. laminopathies, cancer) in somatic cells. It seems reasonable to hypothesise therefore that alterations in nuclear organisation may also be associated with male factor infertility. We have assayed the nuclear address of (peri-) centromeric loci for 18 chromosomes (including X and Y), of 30 sub-telomeric loci, of the TTAGGG telomeric repeat itself and a selection of other loci common used for aneuploidy screening. We have examined normal donor males, men with severe male factor infertility and men with Hodgkin's lymphoma and testicular cancer undergoing chemotherapy treatment. We have used six colour probe sets and an analysis programme algorithm developed in-house. In nearly all experiments we find that all loci in the control groups adopt defined positions with the centromeres and sex chromosomes central and with the telomeric and subtelomeric sequences some distance from the nuclear periphery on average. We have observed subtle rearrangements associated with infertility, cancer and cancer treatment. However considering the gross impairment of spermatogenesis in the test groups (evidenced by compromised semen parameters and increased chromosome abnormalities), the observed differences in nuclear organisation compared to the controls are modest. A defined pattern of nuclear reorganisation in sperm heads therefore appears to be a remarkably robust process, even if spermatogenesis is severely compromised.

Speaker Information

Darren Griffin graduated from the University of Manchester in Genetics and Cell Biology (1988). He did his PhD in Human Genetics under Joy Delhanty, graduating in 1992, then post-doctoral research in Cleveland Ohio and The University of Cambridge under Terry Hassold and Malcolm Ferguson-Smith respectively. In 1998 he won his first academic appointment at Brunel University, rising to Senior Lecturer in 2001 and Professor in 2004. In 2004 he joined the University of Kent as a Reader in Genetics, being promoted to Professor of Genetics in 2007. In 2000 he was admitted as a Fellow of the Institute (now Society) of Biology and in 2002 he was awarded a post-graduate certificate of Higher Education. In 2008 he was awarded both a fellowship of the Royal College of Pathologists and Doctor of Science from the University of Manchester. He was awarded the Institutional Teaching Prize for his work on eLearning, and in supervising PhD students and has recently also been shortlisted for Research Project of the Year by the Times Higher Education supplement. He is Vice president of the International Chromosome and Genome Society and a member of ESHRE. He has over 100 publications on the chromosomes of humans, pigs, fish and birds; he makes regular TV and public lecture appearances and lives in Canterbury with his wife and two sons.

Friday 16th September
11:00-11:45

Dr John Parrington

J. Parrington¹, K. Coward², J. Kashir², C. Jones², M. Konstantinidis², D. Wells², P. Grasa¹, C. Young¹, B. Heindryckx³, P. De Sutter³, R. A. Fissore⁴

¹Department of Pharmacology, University of Oxford, Oxford, United Kingdom; ²Nuffield Department of Obstetrics & Gynaecology, University of Oxford, Oxford, United Kingdom; ³Department of Reproductive Medicine, Ghent University Hospital, Ghent, Belgium; ⁴Department of Veterinary and Animal Sciences, University of Massachusetts, University of Massachusetts, Amherst, United States

The Role of Phospholipase C Zeta During Fertilization and its Link with Human Infertility

Mammalian oocytes appear to be activated by a sperm-specific phospholipase C, PLCzeta (PLCzeta) (Parrington et al., 2007). In our recent studies we have identified the pattern of localization of PLCzeta in human and rodent sperm and found this to be in line with PLCzeta being the endogenous oocyte activation factor (Grasa et al., 2008; Young et al., 2009). We have also shown that in infertile men with defects in oocyte activation, PLCzeta is abnormally localized in the sperm. Molecular analysis of genomic DNA from infertile patients identified a point mutation in the PLCzeta gene in an infertile male exhibiting normal sperm morphology, but defective oocyte activation capability, leading to a significant change in a conserved Histidine at position 398 in the catalytic region of the protein to a Proline (H398P), that may inhibit normal function (Heytens et al., 2009). Most recently, we have identified another novel mutation in the PLCzeta gene in the same patient. We show that both the novel mutation and H398P are heterozygous mutations, both occurring on separate alleles, with H398P inherited from the patient's father. We also show for the first time the inheritance of a male factor infertility mutation passed on by the patient's mother to her son. Here we also report the identification of PLC ζ orthologues in pufferfish species *Takifugu rubripes* (Fugu) and *Tetraodon nigroviridis* (Tetraodon) (Coward et al., 2011). Unexpectedly in these species PLC ζ is expressed not in the testis, but in ovary and brain. Injection of pufferfish PLC ζ cRNA into mouse eggs failed to trigger Ca²⁺ oscillations, unlike medaka PLC ζ cRNA. Our findings provide the first evidence that PLC ζ may be expressed in the egg, rather than the sperm, in some vertebrate species, and that its mechanism of action and physiological role at fertilization may differ in different vertebrate species.

Speaker Information

Dr Parrington was educated at Downing College, Cambridge and received a BA in Natural Sciences (Zoology). He obtained his PhD from the Imperial Cancer Research Fund and London University, having worked on the molecular mechanisms underlying the interferon signalling pathway in Ian Kerr's laboratory. Dr Parrington moved to the MRC National Institute for Medical Research where he began studying the molecular events underlying fertilization, a theme which he continued at University College London as an MRC Career Development Fellow and MRC Senior Non-Clinical Fellow. Dr Parrington currently holds a joint Tutorial Fellowship and College Lectureship at Worcester and Exeter Colleges, respectively, and a University Lectureship in Pharmacology, at Oxford University.

Dr Parrington's principal research interest is in using biochemical and molecular strategies within a multidisciplinary approach to study key questions in cell signalling, particularly how calcium signalling governs key physiological events. He has been centrally involved in demonstrating that the physiological agent of egg activation in mammals appears to be a novel, sperm-derived phospholipase C with distinctive properties - PLCzeta. He is currently involved in identifying and characterising the NAADP receptor and other components of the cADPR and NAADP signalling pathways at the molecular level. Most recently he played a central role in identifying the two pore channels as endolysosomal calcium channels and integral components of the NAADP receptor.

Dr Parrington was until recently General Secretary of the Society for Reproduction and Fertility. He also has a diploma with distinction in Science Communication from Birkbeck College, London. He has written articles about science and educational issues for The Guardian and New Scientist as well as preparing reports about scientific issues for the public for the Wellcome Trust, the Royal Society and the British Council. He presented the Charles Darwin Award Lecture at the BA Festival of Science at the University of Salford.

Friday 16th September
11:45-12:30

Dr David Miller

Abdul Elnfati¹, David Iles² and David Miller¹.
Leeds Institute of Genetics, Health and Therapeutics, University of Leeds.
Institute for Integrative and Comparative Biology, Faculty of Biological Sciences,
University of Leeds

From Flies to Men, Sperm Histones Carry the Same Embryological Signature to the Egg

Unlike somatic cell nuclei, the sperm nucleus is unusual in being transcriptionally inert although its chromatin appears to be 'poised' for gene expression. This state of suspended animation arises because during spermiogenesis, the major nucleosome based DNA packaging seen in all somatic cells and up to the round cell stage in spermatogenesis is progressively replaced firstly by testis-specific histones, then by transition proteins and finally by protamines. The almost 20 fold greater DNA packaging density afforded by protamines allows the spermatozoon to fit the paternal genome into the smallest possible volume. Once in the egg, sperm chromatin decondenses and the protamines are rapidly displaced by maternal histones. Until recently, it was thought that the substitution of histones by protamines in mammalian sperm was complete until reports appeared showing that some histones are retained and in non-random positions reflecting their developmental (embryological and spermatogenic) functions. There are also many modified histones in the sperm nucleus, including acetylated and methylated residues on H3 and H4 and histone variants, including H2AX, H3.3 and CENPA. The modified histones frequently appear in bivalent motifs resembling the chromatin arrangement in embryonic stem cells and indicating a close relationship between paternal epigenetic marks on sperm and developmental potential in the embryonic inner cell mass. Many of these observations are also recapitulated in the sperm of the fruit-fly, *Drosophila melanogaster* which undergo a similar switch from histone to protamine based packaging but which retain some histones nonetheless. Like human (and probably mouse sperm chromatin), fly sperm histones tend to associate with exonic regions and display particularly strong enrichment in the exons of developmentally important genes. They also package genes involved in spermatogenesis. Hence in both mammals and flies, sperm histones package elements of two distinct but developmentally important genes, namely those involved in spermiogenesis and those destined for expression in the developing embryo. The likelihood that sperm convey an epigenetic message to the egg 'writ large' in the way the paternal genome is packaged is becoming more compelling. This signature may in part be related to the methylation state of sperm DNA but by no means exclusively so.

Arpanahi A et al, 2009 Endonuclease-sensitive regions of human spermatozoal chromatin are highly enriched in promoter and CTCF binding sequences. *Genome Res* 2009 19 1338-1349.

Saida et al, 2011. Key gene regulatory sequences with distinctive ontological signatures associate with differentially endonuclease accessible mouse sperm chromatin. *Reproduction*, DOI REP-10-0536 [pii] 10.1530/REP-10-0536.

Speaker Information

I graduated with honours in Cell Biology from Glasgow way back in 1978; then I did a PhD in Dental Biochemistry at Liverpool, examining the non-collagenous proteins of human dentine (awful project). From there, I went to Kent where I studied tubulins in slime moulds and trypanosomes before returning to Liverpool to work on heat shock proteins and desiccation tolerance in brine shrimps. Finally, I moved to Leeds where I began work on stress proteins in human sperm and also on new non-invasive methods of prenatal diagnosis. That was in 1989 and I've been here ever since. I am now Reader in Molecular Andrology where my work is focused primarily on nucleic acid structure and composition of human, mouse and now fly sperm. My University now wants to turn me and all other Readers into Associate Professors (which many of us are resisting of course).

Friday 16th September
13:30-14:15

Prof Sheena Lewis

Centre for Public Health,
Queen's University Belfast

Sperm Banking - Cancer & Social Banking

As the number of men of reproductive years presenting with cancers increases, the need for long term gamete storage also rises. The incidence of cancer (particularly testicular cancer) has increased across Europe over the past decade and so too has life expectancy of affected males. The survival rate after 5 years is now 96% and within the group of testicular cancer survivors, 77% desire to have children so this quality of life issue becomes increasingly consequential. Infertility can be caused by cancer and exacerbated by chemotherapy causing further damage to spermatogenesis and sperm quality. To date, our knowledge of the effects of cancer or chemotherapy on male reproduction is lacking and our ability to cryopreserve their sperm successfully is limited. As sperm DNA integrity is increasingly recognised as a promising measure of male fertility potential, there is an urgent need to clarify these issues. We need to characterise the effects of cancer and chemotherapy on these sensitive sperm DNA biomarkers and to establish baseline criteria for cryopreservation in order to provide an effective, economical service for patients and health trusts alike. In this session, we will examine the studies determining the effects of sperm cryobanking on semen, sperm DNA integrity and male fertility of cancer survivors.

In our 21st century Society of poor lifestyle choices and delayed parenthood, the need to protect future male fertility has never been greater. The male genome is vulnerable with assaults from environment, occupation, lifestyle and disease and exacerbated by negligible protection from the sperm's minimal cytoplasmic resources and limited repair. The pros and cons of social sperm banking will also be raised for discussion.

Speaker Information

Over the past two decades, Sheena has led the Reproductive Medicine research group at Queens University, Belfast. Her research has focused on male infertility and in particular sperm DNA fragmentation testing where her goal has been to identify causes of and treatments for male infertility by developing novel biomarkers. Sheena is Chair of the Andrology special interest group of ESHRE, the treasurer of the British Andrology Society, a member of the executive committee of the British Fertility Society and past Vice Chair of the Irish Fertility Society. Sheena is a regular invited speaker at ART conferences and has published over 80 full papers and numerous reviews and chapters.

Friday 16th September
14:15-14:45

Prof Bill Holt

Institute of Zoology,
Zoological Society of London,
Regent's Park, London NW1 4RY

Sperm in conservation; if only all species had the same sperm!

Wildlife extinctions are now thought to be occurring more rapidly than at any time in the history of the earth, mainly because of human activities. These include agriculture, urbanisation, deforestation and a myriad other processes. While it is impractical to think that reproductive technologies could be used on a grand scale to help save large numbers of species from extinction, the judiciously targeted use of some simple technologies should be useful in helping to support the viability of small populations facing genetic problems such as inbreeding. In fact, it has long been proposed that banks of frozen spermatozoa could be used as adjuncts to captive breeding programmes, thus allowing genetically valuable males to continue contributing to the gene pool long after their death. Unfortunately, this scenario has yet to be realised at a practical level. Much of the problem lies with the difficulty of establishing successful cryopreservation techniques for different species. While it is true that the technique of semen freezing in domestic bulls is highly effective, it is not possible to transfer the same protocols to cattle-like species with the same degree of success. Even transferring the protocols to domestic sheep and pigs is a major problem, and the most widely used cryoprotectant, glycerol, can be regarded as a contraceptive for some birds species. Working with completely different taxonomic groups requires a complete rethink of our approaches to cryopreservation technologies. Marsupials are an interesting case in point as many species are threatened and there is considerable interest in establishing genetic resource banks for species such as the Tasmanian devil, koala and several wallaby and kangaroo species. However, nearly all marsupials lack the cysteine groups within sperm protamines that confer stability to the nucleus. Possibly as a consequence of this, it has so far proved impossible to cryopreserved marsupial spermatozoa with any significant success. In addition there is the unexplained observation that marsupial spermatozoa seem to require more than double the cryoprotectant concentrations typically used with eutherian species; for example, more than 15% glycerol is required for wallaby and kangaroo sperm cryopreservation in order for any motility to be retained after freezing and thawing. It seems that the realistic development of working genetic resource banks for wildlife conservation still, unfortunately, requires dedicated and species-focused research approaches. If all species had the same sperm life would be so much easier!

Speaker Information

Bill Holt is Head of Reproductive Biology at the Institute of Zoology, Zoological Society of London. The focus of the institute is to underpin the conservation of threatened species by the development of fundamental scientific knowledge about their biology, and also to develop techniques that can be useful in practice. The research group has therefore focused on methods for the assessment and preservation of gametes, especially spermatozoa, in a variety of species. Fundamental research into sperm quality has produced novel insights into the phenotypic differentiation of sperm subpopulations in terms of their motility, DNA structure and selection in the female reproductive tract. For the last decade, the research group has also been attempting to understand the biology of natural sperm storage mechanisms in the female reproductive tract on the basis that such understanding might translate into better practical tools for the long-term preservation of spermatozoa. To date we have shown that the mammalian oviduct has a significant capacity to protect and store spermatozoa, and that this is correlated with the stimulation of novel gene transcription and oviductal protein synthesis as soon as the sperm arrive at the storage sites.

Friday 16th September
15:15-16:00

Prof Anna Petrunkina

Cambridge Institute for Medical Research,
University of Cambridge

Slow freezing and Vitrification: Sperm Storage Challenges

Cryo-storage of spermatozoa has become an indispensable part of reproductive biotechnology and medicine. However, when applied to many species, current cryo methods lead to very significant declines in sperm population viability and function after thawing. So far, advances in cryopreservation procedures have been made largely via empirical rather than analytical approaches. One problem emanating from these empirical approaches is that the extenders and procedures used have varied markedly, even within species, and controlled studies comparing different techniques have rarely been carried out. Another problem is that when analytical biophysical approaches have been applied, severe discrepancies between theory and practice have often been revealed. Clearly a better understanding of the cellular and molecular processes that accompany sperm cryopreservation is required, as well as investigation of the mechanisms by which cryopreservation alters sperm function and influences selection of sperm with higher fertilizing potential. Such studies should focus especially on the processes involved in sperm volume regulation, sperm-oviduct interaction, capacitation and cellular signalling. An interesting recent advance has been the development of permeant cryoprotectant-free vitrification as a practical procedure in human assisted reproduction.

Speaker Information

Professor Anna Petrunkina studied physics at St Petersburg's University, graduating in 1992 with a Master degree. Anna took a second postgraduate specialization in Biomedical Engineering at the Medical School of Hanover, followed by a PhD on biophysical methods in sperm biology at the Veterinary School of Hanover. After completing her PhD she continued to work as senior research scientist and lecturer at the Veterinary School of Hanover.

Anna's research interests have focused primarily on understanding the mechanisms that regulate sperm function. The main aims of her research have been to identify factors and pathways which facilitate sperm adaptation and response to their environment, in particular related to cell volume regulation, capacitation and sperm-oviduct interaction.

In 2005 Anna completed her Habilitation (DSc) in Reproductive Biology. In 2007, she took up her present position at the Cambridge Institute for Medical Research as Head of Flow Cytometry, to direct the provision and development of scientific technology services with respect to flow cytometric applications.

Apart from research interests in andrology, Anna retains an active research interest in the promoting of mathematical and computational analysis in reproductive biology. She is active as a reviewer for leading international journals and has published over 50 papers, reviews and book chapters. In 2009 Anna was appointed as Associate Editor of *Reproduction, Fertility, Development*. In 2011 she was awarded a personal Professorship by the Veterinary School of Hanover.

Friday 16th September
16:15-16:45

Mr Richard Viney

School of Cancer Sciences,
University of Birmingham

Andrological Considerations in the Management of Severe Scrotal Injury

The use of improvised explosive devices (IED), the use of body armour and improvements in immediate medical aid to victims of IEDs. Has led to a pattern of injuries that we have not seen before. Individuals are surviving massive injuries to their appendages with relative sparing of the torso. From a urological perspective, we are seeing extensive blast injuries to the external genitalia. We have reviewed the patterns of injury sustained by the most injured personnel evacuated to the Centre of Defence Medicine at UHB with particular focus on the urological injuries. We describe the changes in surgical management of these injuries, in particular those to the scrotum with focus on securing future fertility.

Speaker Information

Born and educated in the West Midlands I won a scholarship to study medicine at Birmingham University. After qualifying in 1995 and completing my house jobs, I taught anatomy for a year at Birmingham Medical School. I then undertook my basic surgical training at University Hospitals Birmingham (UHB) Foundation Trust whilst completing my MRCS with both the Royal Colleges of Edinburgh and England. I then entered the higher surgical training programme in the Midlands with sub-specialist training in urological cancer surgery. Just prior to completing my training I took a three year sabbatical. In this time I completed an MSc in Health Care Policy and Management with my dissertation on applying statistical process control tools in surgery and worked towards my PhD on an MRC grant supported project in gene and immunotherapy in prostate cancer with CRUK at the School of Cancer Sciences at Birmingham University. On completing my higher surgical training I was appointed as consultant urological surgeon at UHB and as a senior lecturer in urology at Birmingham University.

Saturday 17th September
10:00-10:45

Dr Rosemary Waring
Birmingham Toxicology Consortium,
University of Birmingham

Toxicological Effects on the Male - Environmental Factors Affecting the Androgen/Oestrogen Balance

In men the main determinant of sexual function is the level of androgens, the steroids which determine male characteristics. However, all men also have small amounts of oestrogens, the female-determining steroids. If the ratio of androgens to oestrogens is altered, then this can have physiological effects. Oestrogens are synthesised from androgens via the enzyme aromatase – the reaction is a ‘critical pathway’ which is irreversible and is the only available synthetic route. Compounds which activate aromatase eg resorcinol, may increase oestrogen levels and therefore have feminising effects while aromatase inhibitors, such as some plasticisers, may also alter the androgen/oestrogen ratio. Oestrogens are inactivated and removed from the system by the process of sulphonation which requires active sulphotransferase enzymes and produces oestrogen sulphonates, the circulating inactive forms of the hormones. Chemicals such as the naturally occurring flavonoids, found in soy, fruit and vegetables, and environmental contaminants such as alkyl phenols all inhibit oestrogen sulphonation and hence give enhanced levels of the active feminising oestrogens. The androgen/oestrogen ratio can therefore be affected by both dietary components and by a range of environmental pollutants.

Speaker Information

Prof. Rosemary Waring is a Reader in Human Toxicology at the University of Birmingham and has a long-term interest in the effects of steroids on the brain. Funded by the Food Standards Agency and the EU, she and her group have focused on environmental compounds which affect steroid metabolism (endocrine disrupters, EDs). She was co-ordinator of the 5th Framework project ‘ENDOMET’ (part of the ‘CREDO’ cluster) on the potential for plasticisers to act as EDs and was a member of the recent EFSA panel on the danger to human health of the endocrine disrupter bisphenol-A, used in formation of plastic bottles. The group have recently designed in vitro biomarker profiles which can be used to identify possible endocrine disrupting compounds and are applying toxicogenomics to determine the effects of environmental pollutants on steroid metabolism.

Saturday 17th September
10:00-10:45

Dr Eamonn Gaffney

The Centre for Mathematical Biology,
University of Oxford

Motility in Viscous Fluids: *Going Against the Flow!*

Mammalian spermatozoa motility is a subject of growing importance, due to rising human infertility and the possibility of improving animal breeding. Fluid and continuum dynamics offers many opportunities to provide novel insights concerning the mechanics of these specialised cells, especially utilising a synergistic combination of imaging and theory in exploring sperm motility. Examples of the information that can be extracted from imaging will be highlighted together with its mechanical interpretation. In addition, we will explore the influence of flagellar compliance in highly viscous media and its impact on cell swimming behaviours.

Speaker Information

Eamonn Gaffney pursued Theoretical High Energy Physics for a Ph.D. at The University of Cambridge, subsequently followed by a change in academic direction with a Wellcome Trust Post-Doctoral Fellowship at The Centre for Mathematical Biology within The University of Oxford's Mathematical Institute. This was followed by a faculty position at The School of Mathematics, University of Birmingham, before returning to Oxford and The Centre for Mathematical Biology in 2006. During this time Eamonn has developed research interests in modelling and simulating cellular and physiological systems, with a keen focus on sperm mechanics in recent years.

Saturday 17th September
11:15-12:00

Dr Jackson Kirkman-Brown

Centre for Human Reproductive Science (ChRS), School of Clinical & Experimental Medicine, University of Birmingham; Birmingham Women's Fertility Centre, Birmingham Women's NHS Foundation Trust

Physiological and Artificial Modulation of Sperm Motility

Internal fertilization is reliant upon successful sperm navigation of the female tract. Timely location of the oocyte in what is a complex highly invaginated series of moist opposed surfaces is a challenge at which only tens of sperm ever succeed.

Many groups have examined the role for a chemotactic 'attractive egg' effect upon sperm, but most have neglected to consider the role that the viscosity of the mucous layers, which coat the entire tract and through which sperm must swim, plays in both sperm selection and ongoing modulation of their behaviour. From allowing sperm to enter through the cervix during the ovulation phase, to denying them entrance through action of the female contraceptive pill, viscous effects are fundamental in controlling the migrating sperm population throughout the tract. The physiological effects of viscosity are also crucial to consider when designing and extrapolating data from in vitro experiments to the in vivo situation.

To add to this complexity, it is not only secretions of the female tract, but also directly the epithelial cells that can further modulate the behaviour of the cells and their calcium signalling and vice versa. We are only just beginning to understand the signals occurring and how they may affect fertility.

Speaker Information

Jackson studied for a BSc in Biological Sciences (Animal) in Birmingham, doing a project on biocontrol of insects. He stayed in Birmingham for his PhD after seeing a studentship collaboration between the Assisted Conception Unit and Biosciences, working between Steve Publicover, Masoud Afnan and latterly Chris Barratt. In his PhD Jackson developed many on the single-cell $[Ca^{2+}]$ imaging and analysis techniques the group are still well known for today, in examination of progesterone and oestrogen induced signals in human sperm.

Following his PhD Jackson moved to UMASS Medical School, Worcester, MA, to work with Professor Harvey Florman. Here he explored the new world (for him) of working with mice and molecular biology! The project included work on TRPC channels and the TRPC2 k/o mouse. Following on from this research Jackson returned to Birmingham to complete some further postdoctoral work from his PhD and in 2004 moved to a new NHS-based post.

Jackson is now Science Lead for Birmingham Women's Fertility Centre and Director for The Centre for Human Reproductive Science, which unites research in the area across the University and The NHS. His research group is very multidisciplinary with engineers, mathematicians and chemists working alongside cell biologists to unravel key physiological events in gamete maturation through to the early embryo.

Saturday 17th September
13:30-14:15

Prof. U. Benjamin Kaupp
Cancer Research Centre, Germany

Sperm Chemotaxis - the Known and Unknown

Eggs attract sperm by releasing chemical factors. Sperm of the sea urchin *Arbacia punctulata* respond to the binding of a single molecule of chemoattractant with a brief Ca^{2+} spike. We have studied the molecular basis for this exquisite sensitivity. The chemoattractant receptor, a guanylyl cyclase (GC) which synthesizes cGMP, is densely packed covering almost the entire surface of the flagellum (ca. 1,000,000 copies/flagellum). The density is similar to that of rhodopsin in the disc membrane. Binding of chemoattractant to the trimeric receptor is controlled by negative cooperativity, which allows sperm to operate over a 100,000-fold range of concentrations without becoming saturated. Chemoattractant and receptor form a relatively long-lived complex that synthesizes only a few molecules of cyclic GMP/s, i.e. receptor signaling is of low gain. However, cyclic GMP activates in a non-cooperative fashion a unique pseudo-tetrameric cyclic nucleotide-gated K^+ channel that displays nanomolar cGMP sensitivity. A single chemoattractant molecule causes the cell to hyperpolarize by about 1-2 mV. The small hyperpolarization activates a sufficient number of Ca_v channels. The nature of the Ca^{2+} entry channel is not known leaving alone its mechanism of activation. At rest, the receptor GC is multiply phosphorylated. Signaling is terminated by inactivation of the receptor within 0.3 s via rapid dephosphorylation of the GC. The changes in $[\text{Ca}^{2+}]$ control the flagellar beat and thereby the swimming path. At rest, sperm swim on regular circles. In a chemical gradient, the periodic stimulation while swimming in circles is translated into a periodic modulation of the path curvature. Alternating periods of high and low curvature give rise to a motility pattern of drifting circles that move towards the source of the chemoattractant.

Speaker Information

Prof. Kaupp studied Chemistry and received his PhD in 1979 at the Max-Volmer-Institute of the Technical University Berlin. From 1988 to 2007, Kaupp was Director of the Institute of Neuroscience and Biophysics (INB) of the Research Centre Jülich. Since 2008, Kaupp is Scientific Director of the Center of Advanced European Studies and Research (caesar) that is associated with the Max Planck Society. He is also a Scientific Member of the Max Planck Society. He is Professor for Biophysical Chemistry at the University of Cologne and Professor for Molecular Neurobiology at the University of Bonn. Kaupp's research interests lie in the fields of sensory physiology, molecular neurobiology, sperm chemotaxis, and ion channels. He discovered the molecules of cyclic nucleotide-gated (CNG) and hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels. His scientific contributions became common knowledge in textbooks for biochemistry, neurobiology, and physiology.

Saturday 17th September
14:15-14:45

Prof. David Hosken

Biosciences, University of Exeter

Sperm Competition: What Next?

Parker's realisation that male-male competition could continue after copulation and that this could profoundly influence male behaviour, morphology and physiology was a major advance in evolutionary biology. However, in spite of considerable research, a number of basic issues remain unresolved, although recent experimental advances are allowing researchers to delve further into some of these areas. I will discuss some outstanding questions and suggest ways to address them.

Speaker Information

David Hosken did his PhD at the University of Western Australia before moving to Zurich as a Postdoc. This was followed by an Alexander von Humboldt Fellowship in Konstanz, a Postdoc at the University of Chicago, and an Assistantship back in Zurich, before moving to the University of Exeter as a Reader in 2004. He was promoted to Professor of Evolutionary Biology in 2010 and is the current Director of the Centre for Ecology and Conservation. Hosken primarily works on sexual selection, including sperm competition. He is also interested in evolutionary conflicts, inbreeding and bat ecology.

Saturday 17th September
14:45-15:30

Dr Ian Brewis

School of Medicine, Cardiff University

Proteomics for basic biology and sperm sexing

Proteomics technologies for the global analysis of proteins have matured significantly in recent years. This talk will introduce the range of proteomics workflows commonly used for protein identification and quantification. Perhaps the key challenge for fundamental research is to move from lists of identified proteins to informed understanding of biological function. Using the mammalian sperm cell at fertilization as an exemplar I argue that proper subcellular fractionation and solubilisation strategies offers critical advantages to achieving this goal. In relation to understanding initial gamete recognition events at fertilization (capacitation, zona binding and acrosomal exocytosis) it is imperative to study the sperm surface proteome by using purified plasma membrane fractions. Whilst this task is challenging there are now strategies at our disposal to achieve comprehensive coverage of the proteins at the sperm surface and the data produced by ourselves in close collaboration with Bart Gadella (Utrecht University, The Netherlands) will be presented. Proteomics also offers considerable opportunities for more applied research in fields such as biomarker discovery. We were formerly closely involved in an extended commercial project with Ovasort Ltd (2005-2009) interested in sperm sexing. The approach aimed to discover sex-linked surface proteins that might be differentially expressed in X- and Y-chromosome bearing sperm cells. Identification of such biomarkers will enable the development of relatively cheap kits for sperm sorting based on differential surface protein characteristics. If such kits result in the enrichment of X- or Y-bearing cells they will be hugely important to livestock industries.

Speaker Information

Ian Brewis is a Senior Lecturer and has been at Cardiff University since 2003. He has three distinct roles: Academic Lead for Cardiff University CBS Proteomics Facility, Course Director for an MSc Bioinformatics Programme and he also leads his own research group interested in mammalian sperm cells. He was recruited to Cardiff to achieve funding and establish a new Proteomics Facility from scratch and now leads an ISO-accredited Facility. A major commercial interaction with Ovasort Ltd was interested in developing new approaches for sperm-based sex selection in livestock industries (2005-2009). Ian was formerly Scientific Director but is no longer involved with Ovasort. Ian's personal research focuses on the proteomic and functional characterisation of molecular mechanisms in mammalian sperm cells at fertilization and this work is conducted as part of a long-standing collaboration with Dr Bart Gadella (Utrecht University, The Netherlands).

**Young Researcher
Selected
Short Presentations**

Alexandra Amaral

Alexandra Amaral^{1,2}, Judit Castillo¹, Josep Maria Estanyol³, José Luís Balleca⁴, João Ramalho-Santos² and Rafael Oliva¹

1Human Genetics Group, IDIBAPS, Faculty of Medicine, University of Barcelona, Spain; 2Biology of Reproduction and Human Fertility Research Group, CNC, University of Coimbra, Portugal; 3Proteomics unit, Faculty of Medicine, University of Barcelona, Spain; 4Clinic Institute of Gynecology, Obstetrics and Neonatology, Clinic Hospital, Barcelona, Spain.

Human sperm tail proteome suggests novel metabolic clues

Our knowledge on human sperm proteomics has largely increased in the last decade, contributing to a better understanding of sperm function. Still, there is certainly a lot to be learned, and the use of up-to-date techniques is warranted. The aim of this work was to characterize the subcellular proteome of human sperm tail, and hopefully identify less concentrated proteins (not found in whole cell proteome studies).

Sperm were isolated from normozoospermic semen samples (n=4), and depleted of any contaminating leukocyte. Tails fractions were obtained by sonication followed by sucrose-gradient ultracentrifugation, and their purity was confirmed by various techniques. Proteins were run on SDS-PAGE and trypsinized after cutting the gel into small pieces. Peptides were further separated and identified by LC-MS/MS.

We have identified 1065 proteins, more than half of which not previously described in human sperm. As expected, the majority of proteins were localized to the mitochondrion, the cytoplasm and the cytoskeleton. The categorization of proteins according to their function revealed two main groups: proteins related with metabolism and energy production (25%); proteins related with cell movement (14%). Although we are still analyzing our data, we have already made some interesting observations. For instance, it seems that human sperm express various enzymes involved in lipidic metabolism, suggesting that the use of fatty acids as fuel may be more preponderant than previously thought. Interestingly, and for the first time, we have identified proteins implicated in peroxisomal biogenesis/proliferation. The complete analysis of our data is expected to suggest novel sperm metabolic attributes.

Supported by a grant from the “Ministerio de Ciencia e Innovación” (BFU2009-07718) to RO and a postdoctoral fellowship from the “Fundação para a Ciência e a Tecnologia” (SFRH/BPD/63120/2009) to AA.

Sahib K. Shahani

¹S.K. Shahani, ²S.G. Revell., ¹C.G. Argo., ¹R.D. Murray.

¹University of Liverpool School of Veterinary Science, Leahurst Campus, Chester High Road, Neston.
CH64 7TE.

²Genus Breeding Limited, Freezing Unit, Llanrhydd, Ruthin, Denbighshire LL15 2UP.

Assessment of sperm motility and its relationship to bull fertility

The aim of this study was to verify the metabolic pathways by which the sperm generates energy to support their motility and to investigate the relationship among the sperm progressive motility (PM), ZO_2 (μ l oxygen consumed by 10^8 live sperm/hour), mitochondrial membrane potential and bull non-return rates (NRRs). The sperm motility characteristics in 4 commercial AI bulls were measured using Computer Assisted Semen Analyzer (CASA) in fresh (FR) and frozen-thawed (FT) spermatozoa diluted in the medium containing glycolytic inhibitors 2-deoxy-D-glucose (DOG) and iodoacetamide (IAM) and respiratory inhibitor valinomycin (VAL).

CASA recorded motility parameters were not significantly different in FR and FT spermatozoa treated with DOG and VAL compared to control (CON) but decreased significantly in IAM. In FR semen: VAP, VSL and VCL were significantly lowered in treated than the un-treated sperm but this trend was only found in IAM treated FT spermatozoa. Sperm ALH increased and STR and LIN decreased in DOG treated samples while these values were reversed in VAL samples, confirms the participation of mitochondria in sperm hyperactivation and capacitation. Insignificant positive correlation existed between the PM and ZO_2 ($r=0.8$, $r=0.15$) and org:grn fluorescence (org:grn) ratio ($r=0.6$, $r=0.5$) of FR and FT semen respectively. When sperm PM was compared with their 49 day NRRs, sperm PM was correlated positively ($r=0.3$) with NRR. In addition, org:grn ratio ($r=0.5$) correlated with NRR better than ZO_2 ($r=0.2$).

Conclusions: Bull sperm can maintain similar level of motility when controlled generating their energy either from glycolytic or respiratory pathways. Sperm hyperactive motility was associated with mitochondrial function. Positive relationship of sperm progressive motility, ZO_2 and org:grn ratio with bull NRRs reveals that these sperm characteristics may be useful to predict bull fertility.

Renata Tavares

Tavares R. 1, Sousa A.P.1,2, Velez de la Calle J.F.3, Figueiredo H.4, Almeida V.5, Almeida-Santos T.2, Ramalho-Santos J.1,2,6

1 Center for Neuroscience and Cell Biology, University of Coimbra, Largo Marquês de Pombal, 3004-517 Coimbra, Portugal

2 Human Reproduction Service, University Hospitals of Coimbra, 3000 Coimbra, Portugal

3 In Vitro Fertilization Unity, Pasteur Saint-Esprit Clinic, 29200 Brest, France

4 Laboratório FIV, Centro Hospitalar V.N. Gaia, 4400-129 V.N. Gaia, Portugal

5 Department of Zoology and Anthropology, Faculty of Sciences, University of Oporto, and Centro de Estudos de Infertilidade e Esterilidade (CEIE), 4050-345 Oporto, Portugal

6 Department of Life Sciences, University of Coimbra, Largo Marquês de Pombal, 3004-517 Coimbra, Portugal

Simultaneous analysis of human sperm morphology and chromatin status – a new and simple approach

Sperm chromatin status/nuclear DNA integrity has been pointed out as an important marker of male fertility. However, and despite the well-implemented assays used for its evaluation, the long-standing protocols and expensive reagents and equipment make it impossible to employ as a routine semen parameter in standard Andrology laboratories. Recently, we developed a simple and hasty method to analyze sperm chromatin status in field conditions using the Diff-Quik assay, an assay commonly used to assess sperm morphology worldwide.

Using different Diff-Quik-like stains we evaluated both sperm morphology and the presence of abnormal dark nuclear staining in human sperm from 4 ART centers. Additionally, TUNEL assay was also carried out in the same samples. Significant correlations were observed for sperm morphology and dark nuclear staining between stains, as well as among TUNEL-positive sperm and dark sperm nuclei. Associations between the percentage of abnormal dark sperm nuclei and seminal parameters, embryo development rate, embryo quality and clinical pregnancy were also established. A value of 32% abnormal staining is thus proposed as a predictive cut-off for embryo development and pregnancy.

Concluding, any Diff-Quik-like stain can easily, reproducibly and routinely monitor human sperm chromatin status if properly optimized. Furthermore, since this method is regularly used to evaluate sperm morphology, it does not entail additional costs to the laboratory.

Julia Simpson

Julia Simpson¹, Roger Sturme² and Stuart Humphries¹

¹Department of Biological Sciences, University of Hull, Hull, HU6 7RX

²Centre for Biomedical Research, Hull York Medical School, University of Hull, Hull, HU6 7RX

What did the egg say to the sperm? Possibilities for chemical communication between mammalian egg and

Chemotaxis in sperm has been well documented in externally fertilising marine species, however sperm-egg chemical communication in mammalian species is more complex and remains unclear. Hyperactivated mammalian sperm have been shown to undergo chemotaxis and that this can be influenced by the presence of cumulus-oocyte complexes, suggesting a chemo-attractant effect. Progesterone secreted by the cumulus cells associated with oocytes affects hyperactivated sperm motility and has been postulated to act as a cue to aid sperm navigation towards the cumulus-oocyte complex at the site of fertilisation. Such a guidance mechanism might impact fertilisation success. The concentration of the attractant required to induce changes in sperm motility appears to be variable and species specific. Our study therefore investigated the influence of an increasing number of mature oocytes on the swimming behaviour of bull sperm, with particular focus on velocity and direction. Using a Dunn Chemotaxis Chamber sperm motility was recorded. ANOVA was performed to test for difference in sperm velocity. No significant differences in sperm velocity were observed in the presence of 0, 1, 2 or 3 oocytes. Furthermore, no observable directional change i.e. turn and run movement or drifting circles was found. These findings suggest that bull sperm chemotaxis requires more complex conditions than hyperactivated sperm being in close proximity to mature oocytes and cumulus cells at least in an in vitro system.

Poster Abstracts

Hadi Alavi, University of South Bohemia, Czech Republic
Sperm morphology, ultrastructure and motility in polyploid weatherfish

Marta Baptista, University of Coimbra, Portugal
Effect of Multifunctional Compounds in Contraception and Sexually Transmitted Diseases

Hermes Gadelha, University of Oxford
The counterbend phenomenon: a generic property of the axoneme and other crosslinked filaments

Pavan Kachhwaha, University Hospitals Coventry & Warwickshire
Sperm DNA Fragmentation: Relationship with Embryology Parameters in ART

Walaa Ramadan, University of Oxford
The effect of age and epididymal maturation upon levels and localisation patterns of the oocyte activation factor phospholipase C zeta (PLC ζ) in mouse sperm

Farnaz Shapouri, Royan Institute for Reproductive Biomedicine, Iran
Toll like receptors and paternal interaction with none self-entities

Linda Lefievre, University of Birmingham
Store operated channels mediate rapid Ca²⁺ influx in progesterone-stimulated human sperm

Stephen Publicover, University of Birmingham
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Modern treatment of penile cancer: Results from a Supraregional Centre

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Sperm morphology, ultrastructure and motility in polyploid weatherfish

Introduction: Polyploids can originate either from alterations of meiotic or mitotic processes in specimens within a species (autopolyploidy) or by hybridization among species (allopolyploidy). Moreover, naturally or artificially polyploidy fish exhibit differences in terms of testicular development and sperm morphology, motility, velocity and fertilizing ability. In the present study, sperm morphology and motility traits were studied on naturally triploid and tetraploid weatherfish, *Misgurnus fossilis* (Teleostei, Cobitidae).

Material and methods: *Fish.* Males of weatherfish were captured from the floodplain area pools on the upper reaches of the Lužnice River (tributary of the Vltava River, Elbe River basin, North Sea drainage) close to Majdalena (48° 97' N; 14° 86' E) in South Bohemia, Czech Republic.

Evaluation of ploidy level. The ploidy level of each specimen was assessed by flow cytometry based on the relative DNA content in cell nuclei.

Sperm morphology. Sperm morphology and ultrastructure were studied using scanning and transmission electron microscope and morphological parameters were measured.

Sperm motility assessment. The sperm motility was evaluated after activation in 45 mM NaCl, 5 mM KCl, 20 mM Tris, pH 8.5. Computer-assisted image analysis was used to measure percentage of sperm motility and sperm velocity.

Results and conclusions: Triploid and tetraploid weatherfish were observed in the present study. Larger size of head, longer flagellum, and higher number of mitochondria were observed in spermatozoa of triploid compared to tetraploid weatherfish, but no any other ultrastructural differences were registered between them. Spermatozoon of weatherfish lacked an acrosome and consisted of a head (containing DNA), midpiece (place for mitochondria and proximal and distal centrioles) and a flagellum with 9 + 2 microtubular structure. Sperm velocity was significantly lower in tetraploid weatherfish compared to that of the triploid, but sperm motility did not differ. The stepwise linear regression showed significant negative correlations between sperm velocity and length of sperm head ($r = -0.92$, $p < 0.01$). The present study suggested no abnormalities of sperm morphology in triploid and tetraploid weatherfish, but revealed the effect of polyploidy on sperm velocity possibly through changes of size of sperm head, number of mitochondria and probably initial ATP content.

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Effect of Multifunctional Compounds in Contraception and Sexually Transmitted Diseases

According to the World Health Organization there are 340 million new cases of Sexually Transmitted Diseases (STD's) as well as 3 million people newly infected with HIV (Human Immunodeficiency Virus) every year and the treatments represent a financial burden, particularly in developing countries. In addition, there has been an exponential increase in the world population growth, also linked to unintended pregnancies. These two problems are exacerbated by the poverty, hunger and lack of health care that exist particularly in less developed regions. The failures in the development of compounds that act against both unwanted pregnancies and STD's stimulated further efforts to identify new cheap, well-tolerated, effective and easy-to-use dual function compounds.

In the present study we report a systematic *in vitro* evaluation of the contraceptive and microbicidal potential of cationic surfactants in correlation with their cytotoxicity in epithelial cells. Collaborations with Dr. Teresa Almeida Santos (Human Reproduction Service, University Hospitals of Coimbra), Dr. Winchil Vaz and Dr. Otilia Vieira (Chemical Department and Center for Neuroscience and Cell Biology, University of Coimbra, respectively) were vital for the development of the work. The results suggest the use of *in vitro* studies to assess the cytotoxicity of certain compounds, in order to confirm their dual action potential. The validation of *in vitro* studies is very important since these techniques are cheaper, quicker, simpler and safer than those usually used to test the toxicity of a substance *in vivo*. Concluding, this information would be helpful in the design of more effective and less harmful surfactants that could be used in vaginal gels.

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The counterbend phenomenon: a generic property of the axoneme and other crosslinked filaments

Cilia and flagella are ubiquitous in biology as a means of motility and constitute one of the most incredible engineering works of nature. Their inner core, namely the axoneme, consists of a remarkable phylogenetically conserved cytoskeletal structure, typically composed by 9+2 microtubules arranged cylindrically and interconnected by radial spokes and nexin bridges. Despite this ubiquity and importance, the details of how each structural component within the flagellum is orchestrated to generate bending waves is far from fully understood, while the elastic material contribution from the intricate three-dimensional architecture plays a crucial role and is generally unknown. The resulting structural effects from the elastic cross-linking proteins are non-negligible and give rise to important large-scale shearing behaviour, such as the paradoxical counterbend phenomenon - found when a rat sperm flagellum that have been rendered passive was forced externally in different parts by a micromanipulator. We demonstrate that mechanics and modelling can be utilised to interpret observations of this intrinsic shear signature, allowing the evaluation of bulk material properties, such as bending and shears stiffness. We develop a biomathematical model capable of elucidating counterbend behaviour, providing an explicit mathematical demonstration of the counterbend effect as a generic property of any cross-linked filament bundle, as the axoneme. Further understanding on the difference between the elastic cross-links response and pure elastic resistance is also provided, as we show the counterbend form cannot be captured by the commonly used Euler-Bernoulli beam theory.

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Sperm DNA Fragmentation: Relationship with Embryology Parameters in ART

INTRODUCTION: Sperm chromatin is packed tightly within the sperm head and is therefore prone to DNA damage. Previous studies have suggested that embryology parameters, such as fertilisation, embryo quality and assisted reproduction techniques (ART) outcomes, are related to sperm DNA damage. We undertook this investigation in order to improve male patient diagnosis and outcomes within our centre.

OBJECTIVE: To investigate whether sperm DNA fragmentation, as measured by a sperm chromatin dispersion test, is related to semen parameters and IVF or ICSI fertilisation rates, embryo quality, blastocyst formation and pregnancy.

METHOD: Sperm DNA fragmentation was measured using the Halosperm® kit according to the manufacturer's instructions. In order to perform the analysis ideally a minimum of 5 million sperm per ml was required. The analysis was performed on freshly ejaculated treatment samples surplus to clinical requirement on the day of egg collection. A DNA Fragmentation Index (DFI) was calculated for each sample by calculating the proportion of sperm with a positive signal. Samples from 34 couples undergoing ART (IVF: n = 16; and ICSI: n = 18) were measured and the results were analysed in terms of semen parameters, embryo quality and ART outcomes.

RESULTS: A number of interesting trends were observed. As expected from previous studies, there was a negative correlation between DFI and both sperm concentration and progressive motile count, but this was not statistically significant. A decline in fertilisation rate was apparent with increasing DFI in IVF but not ICSI patients. Although the day 2 embryo quality and pregnancy rates were similar irrespective of DFI, the chances of blastocyst formation on day 5 appeared reduced with increasing DFI.

CONCLUSIONS: Expansion of this study to increase the number of patients may result in more significant results, based on the trends observed. However, this study does not support DFI as highly predictive of embryology parameters in ART.

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The effect of age and epididymal maturation upon levels and localisation patterns of the oocyte activation factor phospholipase C zeta (PLC ζ) in mouse sperm

The fundamental process of mammalian oocyte activation is regulated by the sperm-specific protein phospholipase C zeta (PLC ζ). When introduced into the oocyte at gamete fusion, PLC ζ initiates a series of intracellular inositol-triphosphate-generated calcium oscillations which induce release from meiotic arrest and initiate molecular and cellular processes underlying oocyte activation and gene expression in the early embryo. Since reduced amounts, abnormal localisation patterns, and aberrant forms of PLC ζ in human sperm have been linked to certain types of infertility, there is much interest in investigating how this key protein is expressed in developing sperm. Prior studies have identified multiple localisation patterns of PLC ζ in mouse and human sperm, suggesting differential functional roles. In the mouse, PLC ζ was identified in both the acrosomal and post-acrosomal regions, with the latter population thought to be predominantly responsible for egg activation. Whether the relative level and localisation pattern of PLC ζ changes in the mouse as maturing sperm pass through the epididymis remains unknown, as does the potential effect of age. In the current study, an anti-mouse PLC ζ antibody was used to immunostain epididymal sperm from mice of different ages (6, 16 and 32 weeks, n = 15), and at three stages of epididymal maturation. Three PLC ζ localisation patterns were identified: acrosomal only, post-acrosomal only, or both acrosomal and post-acrosomal. Preliminary analysis indicates that the total level of PLC ζ in the sperm head does not change significantly with either epididymal maturation, or male age. However, significant changes in specific localisation pattern appear to occur with both epididymal maturation and male age. These findings will not only develop our understanding of how PLC ζ is expressed within developing sperm, but may also provide clues as to how the aging process may influence oocyte activation ability in human males.

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Toll like receptors and paternal interaction with none self-entities

Introduction: Infections of the male genitourinary tract may contribute to infertility by adversely affecting sperm function. The ability to discriminate non-self from self agents is a central feature of Immune System. Innate immune recognition is mediated by receptors known as pattern-recognition receptors (PRRs). These molecules detect pathologic microorganisms through recognition of invariant pathogen-associated molecular patterns (PAMPs). The most prominent members of the PRRs are the Toll-like receptors (TLRs). To date, 10 functional members of these receptors have been discovered in human. TLRs are known to detect ligands from g+ / g- bacteria and viruses. There is increasing evidence that many of the interactions between the immune and reproductive systems involve the Toll-like receptors (TLRs). There for, the objective of this investigation is the expression of TLRs 1-10 in different regions of male reproductive tract.

Materials and Methods: RT-PCR was used to show the existence of all TLRs genes and Q-PCR analysis used to investigate the relative expression of TLRs 2, 3 and 4 genes in TESE- and TESE+ patients. Immunoblot analysis was used for detect TLR2, 3 and 4 on spermatozoa.

Result: All TLRs expressed in different part of the human male reproductive tract. Existence of TLR2, 3 and 4 in spermatozoa has been shown by using western blot. Q-PCR has shown relative TLR2 expression in TESE- patient is lower than TESE+ patient. But, there was no difference between TLR3 and 4 expression in TESE+ compared to TESE- by using Q-PCR.

Conclusion: Presence of most TLRs in the human male reproductive tract provides broad spectrum detection of bacteria and viruses that may enter the tract to protect both spermatozoa and the epithelial linings of reproductive organs. Q-PCR analysis in patients undergoing TESE may indicate that TLR2 expression in testis is under effect of spermatozoa but spermatozoa have no effect on TLR 3 and 4 expressions in testis.

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Store operated channels mediate rapid Ca²⁺ influx in progesterone-stimulated human sperm

Progesterone, a major component of follicular fluid and cumulus cell secretions, induces rapid activation of Ca²⁺ influx in human sperm, causing 'immediate' [Ca²⁺]_i transient followed by a prolonged plateau of elevated [Ca²⁺]_i. It has been shown recently that progesterone activates Catsper channels in the principal piece of the flagellum, potentially mediating at least part of this non-genomic steroidal effect. We have observed previously that progesterone mobilises stored Ca²⁺ in human sperm, potentially activating Ca²⁺ influx through store-operated channels (SOCs). We have investigated expression of store operated channels proteins in human sperm and their participation in the response to progesterone.

Store-operated Ca²⁺ influx requires both a membrane Ca²⁺ permeable channel (Orai and/or TRPC) and a mechanism by which the Ca²⁺ content is monitored (STIM). Western blotting and immunolocalisation confirmed that both components are present in human sperm and that STIM and Orai proteins are co-localised at the sperm neck/mid piece. When sperm were pretreated with 5 μM 2-APB, which potently enhances the activation of SOCs caused by Ca²⁺ store mobilisation, the progesterone-induced [Ca²⁺]_i transient in the sperm neck region was significantly enlarged. 2-APB pretreatment also recruited a 'late' elevation of [Ca²⁺]_i, which initiated 10-30 s after application of progesterone and was generated in the midpiece. Similarly loperamide, another agonist of SOCs, greatly prolonged the [Ca²⁺]_i transient and enhanced the sustained phase of the response to progesterone, resulting in increased levels of hyperactivated motility.

We propose that the progesterone-induced Ca²⁺ transient in human sperm includes an early component, reflecting activation of Catsper channels in the principal piece of the flagellum and subsequent component(s) involving Ca²⁺ entry through SOCs present in the sperm neck and mid piece, where the channel proteins and their activator (STIM) are co-localised. Activation of SOCs in human sperm, either by store mobilisation or by a more direct mechanism, may provide a mechanism for rapid Ca²⁺ influx into the sperm neck/midpiece, leading to modulation of motility.

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Intracellular alkalinisation and Ca^{2+} store mobilisation activate different 'types' of hyperactivation in human sperm

$[\text{Ca}^{2+}]_i$ is a key regulator of the crucial transition of mammalian sperm motility from activated to hyperactivated. In mouse and bovine sperm hyperactivation occurs upon elevation of intracellular pH induced by NH_4Cl , which, activates CatSper channels in the flagellum. 4-aminopyridine (4-AP) is a highly potent activator of hyperactivation in human sperm (0.1-2.0 mM) leading to a robust and persistent hyperactivation of motility, as assessed by computer-assisted semen analysis (CASA). 4-AP induced a persistent elevation of $[\text{Ca}^{2+}]_i$ in human sperm that showed similar dose-dependence to hyperactivation. Since 4-AP is a weak base, we predicted that it acted similarly to NH_4Cl , through cytoplasmic alkalinisation. Measurement of pHi (with BCECF) showed that 4-AP caused a rapid increase in pHi of 0.2-0.3 units. However, 25 mM NH_4Cl had an almost identical effect on pHi and also increased $[\text{Ca}^{2+}]_i$ (though to a lesser extent) but had a negligible effect on the proportion of hyperactivated cells as measured by CASA (n=50, similar results obtained with 2 different CASA systems) whether assessed as % hyperactivation or by assessing individual components of motility (VCL, VAP, ALH). The effect of 4-AP under identical conditions was robust. In contrast, both NH_4Cl and 4-AP caused a significant increase in penetration of methylcellulose (a mucus substitute) as assessed by the Kremer test. (n=17)

Analysis of $[\text{Ca}^{2+}]_i$ changes induced by 4-AP showed a focus of Ca^{2+} elevation at the sperm neck/midpiece which was often associated with marked asymmetric flagellar bending in this region. In cells recently (5-10 min) exposed to EGTA-buffered (<0.1 μM Ca^{2+}) saline 4-AP induced a $[\text{Ca}^{2+}]_i$ transient. Hyperactivation induced by 4-aminopyridine was similarly transient (10-15 min) under these conditions though motility persisted for 20-30 min. Pre-treatment with 20 μM bisphenol (to empty Ca^{2+} stores) before exposure to 4-aminopyridine largely occluded the $[\text{Ca}^{2+}]_i$ response seen in low Ca^{2+} saline ($\approx 5 \mu\text{M}$).

We propose that there are at least two different components of $[\text{Ca}^{2+}]_i$ signaling that contribute functionally different effects to hyperactivation of human sperm. 4-AP mobilizes stored Ca^{2+} in addition to its effects on pHi and this is crucial for its robust effect on hyperactivation assessed by CASA. The associated bending at the sperm neck probably contributes to occurrence of sperm tracks that are classified as hyperactivated by CASA. This behaviour has been observed in sperm during penetrating of the cumulus and zona (Drobnis et al, Dev Biol 130; 311-23, 1988). However, the effect of pHi elevation by NH_4Cl , leading to Ca^{2+} influx through CatSper channels, though barely detectable by CASA, effectively promotes penetration of mucus.

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Subcellular localisation and proteomic analysis of serine/threonine protein dephosphorylation in capacitating

Spermatozoa undergo a series of capacitation events prior to fertilization that are mediated by signalling events involving protein phosphorylation. We have previously reported bicarbonate-dependent S/T dephosphorylation of five proteins (96, 90, 64, 55, 105 kDa) in boar sperm using different phospho-(S/T) kinase substrate antibodies. In this study subcellular fractionation of sperm heads and tails was performed. We found that the 96 and 64kDa proteins are localised to the head and that the 90 and 55kDa proteins are in the tail.

Advanced proteomic analysis (GeLC-MS) of whole sperm identified 37 proteins, including AKAP4, AKAP3 and Hsp70 as candidates for the dephosphorylated bands. Western blotting showed that the 96, 90 and 55kDa proteins were detected by AKAP4 Ab and localised to the principal piece of the sperm tails. This is in contrast to the localisation of the 96 protein in the head. Therefore AKAP4 was excluded as one of the possible 96kDa protein candidates. AKAP3 was another candidate for the 96 and 90kDa proteins. Western blotting using anti-AKAP3 Ab showed non-specific binding to the sperm proteins but IIF results showed that AKAP3 proteins were localised to the apical ridge of the head. HSPA1L protein was a possible candidate for the 64kDa dephosphorylated protein. HSPA1L Ab detected a 64kDa protein by blotting which localised to the equatorial subsegment region of the sperm head. This is consistent with the localisation detected by Akt and PKA antibodies. Sperm proteins were immunoprecipitated by PKA Ab and immunoblotted with HSPA1L Ab. A 64kDa protein was not pulled down whereas 96 and 90kDa proteins were immunoprecipitated.

In conclusion, the 96 and 64kDa proteins are localised to the head whereas the 90 and 55kDa proteins are tail proteins. Proteomic analysis has revealed a number of candidates for the dephosphorylated proteins and studies are ongoing to provide definitive identification.

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Modern treatment of penile cancer: Results from a Supraregional Centre.

Introduction: Penile cancers are uncommon and in the UK they are referred to specialist regional centres.

Methods: We performed a retrospective review of all cases referred to our centre between 2005 and 2010. We examined the role of initial biopsy, prophylactic lymph node dissection (LND) and the value of the proposed sub-classification of T2 disease.

Results: We reviewed 108 patients, median age 62.5 years (range 35-90). 54 patients (50%) were biopsied before referral and 35 (65%) of these had T2 disease or worse. We subdivided T2 cancers into T2a and T2b based upon spongiosum/cavernosal invasion. Of those who underwent LND, 88% of T2a and 75% of T2b patients had positive nodes. Median survival for T2a and T2b disease was 15 months and 6 months respectively. 76 patients (70%) were defined as high risk according to EAU guidelines¹. 38 (50%) of these underwent LND. 25 high risk patients (33%), with negative examination and radiology did not undergo prophylactic LND. To date no patients have developed disease progression. Remaining patients had metastases, were unfit or declined surgery.

Conclusion: EAU guidelines recommend no biopsy in obvious penile tumours¹. In our cohort many patients are having unnecessary biopsies for obvious (T2) disease, resulting in delay. Sub classification of T2 disease shows no difference in nodal status, but does reflect overall prognosis, which may be important during the counselling process. EAU guidelines recommend prophylactic LND for high-risk disease, but in our cohort its role appeared limited in those without evidence of nodal disease.

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