

British Andrology Society

Annual Meeting
19-20 November 2009
Queen's University, Belfast

*Recent Developments and Future
perspectives in Andrology*



General Information

Meeting Registration: The registration desk will be situated in the Canada Room, First floor, Lanyon Building, Queen's University (see map at end of booklet). The desk will be open from 11:30-12:00 on Thursday 19th November 2009 until 9.15am Friday 20th November 2009.

Admission: Entry to scientific sessions is limited to badge wearers. Tickets will be issued for meals and functions and must be presented for admission.

Presentation format: Facilities will be available for PowerPoint presentations. Any other presentation needs can only be catered for if speakers request in advance. 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 prize will be awarded for the top presentation.

Business Meetings: The AGM of the British Andrology Society will be held at 08:15-09:15 on Friday 20th November 2009.

Map of Queen's University Belfast:



Welcome to the annual meeting of the British Andrology Society

The British Andrology Society (BAS) was formed as a special interest group in 1977 for scientists and clinicians working in the fields of human and mammalian reproduction with an interest in the male.

The Society is multi-disciplinary 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, together with a wide range of scientific disciplines, including Reproductive Biology, Endocrinology, Cytology, Microbiology and Embryology.

Research plays a prominent role within the Society, with areas of interest including spermatogenesis, semen analyses, sperm function, fertilisation, contraception and cryopreservation.

The British Andrology Society is closely associated with other reproductive medicine societies such as the British Fertility Society (BFS), Association of Clinical Embryologists (ACE), and the Society for Reproduction and Fertility (SRF). The Chairman of the Society is an ex-officio member of the British Fertility Society.

The British Andrology Society is also involved with the UK NEQAS, a specialist advisory group which is a group involved with the UK NEQAS quality assurance scheme in Andrology. The British Andrology Society has produced guidelines for the laboratories undertaking post-vasectomy semen analyses. The British Andrology Society has also provided guidelines for the screening of sperm donors.

Other organizations/charities that we are closely associated with and have representatives on their Committees include the National Gamete Donation Trust (NGDT), the National Quality Assurance Panel in Andrology (Royal College of Pathologists).

Dr Alireza Fazeli (Chairman)

Welcome to Northern Ireland

Welcome to Queen's University Belfast. Queen's combines the best of tradition with a progressive outlook. It is an institution with a world-class academic reputation as reflected in the award of a Queen's Anniversary Prize in 2006, the fourth time the University has been honored in this way. As a member of the Russell Group of leading UK universities, Queen's is building on its tradition of excellence to secure a future for its students - the leaders of tomorrow. It is our pleasure to host this 2009 annual conference of the British Andrology Society. We hope it is a useful time to discuss new ideas and develop new collaborations

Northern Ireland is one of Europe's most scenic destinations, just waiting to be discovered. It is a land of immense variety, with wave swept coastal drives, hazy mountains, vast open moorland and glassy lakes. Belfast, gateway city is situated within an hour's drive of rugged and unspoiled landscape, dramatic coastline and two of the top golf courses in the world.

Message from Queen's VC

I am delighted to welcome you to Queen's University Belfast. Last year we celebrated Queen's Centenary as a University. These celebrations served as a reminder of our distinguished tradition as a centre of academic excellence and of our responsibilities to society as we step with confidence into the next century.

Knowledge knows no boundaries, and the forging of partnerships and the sharing of knowledge in today's shrinking world is essential if we are to address effectively the challenges facing our wider society. Events such as the annual meeting of the British Andrology Society have a crucial role to play in this respect.

And so I wish you well for a constructive, fulfilling and enjoyable conference. I hope that this meeting will provide each of you with valuable professional and personal memories of your time at Queen's and in Northern Ireland.



Professor Peter Gregson

President and Vice-Chancellor

Thursday 19 November 2009

11:30-12:00: Registration
12:00-13:00: Lunch

13:00 Welcome - Professor Sheena Lewis

Session 1: Europe's Falling Birth Rates - stemming the tide by ART

Chair: David Miller

13:00-13:45 "*Testicular Dysgenesis and low European Fertility Rates*"
Niels Skakkebaek, Copenhagen

13:45-14:30 "*Strategies to Increase Andrology Funding opportunities in Europe*"
Lars Bjorndahl, ESHRE

14:30-15:00 Coffee, Posters & Trade Exhibits

Session 2: The Role of Sperm DNA in Improving ART Success

Chair: Sheena Lewis

15:00-15:45 "*Developing better prognostic tests -Sperm DNA*"
Ulrik Kvist, Karolinska Institute, Stockholm

15:45-16:30 "*The Role of Antioxidant Therapy in Treatment of the Male*"
Ashok Agarwal, Cleveland

16:30-17:15 "*ICSI Results with Surgery for Retrieved Sperm Versus ICSI Results with Ejaculated Sperm*"
Sherman Silber, St Louis, Missouri, USA

17:15-18:15 Young Researcher Communications

Chair: Rhiannon Lloyd

17:15-17:30 "*Establishment of a defined in vitro model for investigation of maternal interactions with gametes and embryo*"
Ahmed Aldarmhi, PhD Student, University of Sheffield, UK

17:30-17:45 "*Effect of heat shock 70KDA protein 8 on boar sperm viability*"
Najmeh Moein Vaziri, PhD Student, University of Sheffield, UK

17:45-18:00 "*Abnormalities in human sperm protamine levels (P1/P2 ratio) are associated with increased DNA fragmentation measured by the alkaline Comet assay*"
Luke Simon, PhD Student, Queens University Belfast, United Kingdom

18:00-18:15 "*Treating fresh boar sperm with cyclodextrin pre-loaded with cholesterol improves the osmotic tolerance limits*"
Cristina Tomás, PhD Student, CITA – IVIA, Segorbe (Castellón), Spain

18:15-19:00 Young Researcher Question Time

Chair: Paul Watson

The purpose of this session is to give Young Researchers of the BAS an informal environment to quiz a panel of andrologists (David Miller - Academia, Sue Avery (tbc) - Clinic and Jacqui Piner - Industry) for advice on career possibilities in andrology and ideas on how to get there. To open discussions, each panel member will be asked to answer the following question: "What is the future of Young Researchers in Andrology: Surely it is not all Doom and Gloom?". Young Researchers will then be given the opportunity to ask the panel questions of their own.

20:00 Dinner at Queens University Great Hall



**After dinner speaker Professor James Dornan,
Past Vice President Royal College of Obstetricians and Gynaecologists**

Young Researcher Prize Presentation
(£300 for best presentation)

Presentation of the Brian Setchell Medal for contributions to the BAS

Entertainment – Irish dancing and harpist by O' Malley Experience



Friday 20 November 2009

08:15-09:15 BAS Annual General Meeting

09:15-10:00 Brian Setchell Medal Lecture

Session 3: Novel Strategies in Andrology

Chair: Alireza Fazeli

10:00-10:45 “*Advances in Sperm Proteomics*”
Rafael Oliva, Barcelona, Spain

10:45-11:30 “*Male-line transgenerational responses: A new aspect of human inheritance*”
Marcus Pembrey, University College London, United Kingdom

11:30-12:00 Coffee, Posters & Trade Exhibits

Chair: Iwan Lewis-Jones

12:00-12:45 “*Artificial sperm-neither next year nor ever*”
Harry Moore, University of Sheffield, United Kingdom

12:45 Concluding remarks

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.

Professor Brian Setchell has a long-standing interest in the physiology, biochemistry and endocrinology of the testes and epididymis with particular reference to the blood-testes barrier, testicular fluids and blood flow, the descent of the testis, cryptorchidism and the effects of motility, fertilising ability, and the development of embryos in normal females made pregnant by males whose testes had been heated.

The first award of the British Andrology Society's Brian Setchell Medal was awarded to Professor Paul Watson at the Royal Veterinary College for his outstanding contribution to the research in the field of andrology.

The 2008 Brian Setchell medal is awarded to Roy Jones. The medal will be presented to Roy at the annual conference dinner and Roy will provide the Society with a presentation on the following day. The following is a short autobiography by Roy Jones, telling us about himself, his career development and his major achievements.

Roy Jones PhD, The Babraham Institute, Cambridge

After graduating from Queen's University Belfast in 1969, I set sail (literally) for Liverpool to do a PhD under Tim Glover in his newly established Unit of Reproductive Biology in the School of Veterinary Science. Tim had a 'sink or swim' philosophy with students and we were left very much to our own devices. It was a hard experience but one which taught me the virtues of self-reliance. In 1972 I moved to the Animal Research Station, University of Cambridge, on 2-year postdoctoral fellowship with Thaddeus Mann that eventually turned into 5 years. I arrived at the same time as Joe Tash from Chicago who was doing his PhD. Joe was given a project on cAMP and I was asked to investigate the effects of oxygen free radicals (now known as ROS) on sperm survival. Both topics subsequently grew into major areas of importance. It was at ARS that I met some of the 'giants' in sperm biology. Besides Thaddeus, there was Hector Dott, Cyril Adams and Chris Polge on the staff with frequent visitors like M-C Chang, Mike Bedford, Brian Setchell, Roger Short, Bob Edwards and Ryuzo Yanagimachi. Chang was especially inspirational and I was always impressed by the way he took time to talk to students and young scientists about their research.

In 1976 I moved to Boston USA on a Ford Foundation Fellowship with Don Fawcett and David Hamilton. Harvard was a very competitive place with people under considerable pressure to obtain grants to support their salaries. I hadn't experienced this intensity of research before (12 hour days in the laboratory were common), but I found a niche and spent a year learning electron microscopy. In Boston I shared a laboratory briefly with Trevor Cooper before he moved back to the UK and on to Munster. At the end of my year Fawcett commented 'you've taken some nice pictures'. Praise from Fawcett was praise indeed and I was happy with that. From Boston I took a Lalor Foundation fellowship to NIH, Bethesda, where Richard Sherins had established a male infertility clinic and I had my first experience of working on human material. At this time human sperm research was at a very rudimentary level and I found it frustrating. I was accustomed to large quantities of boar, ram and bull sperm on demand and I did not find working in a clinical setting attractive.

Nonetheless, we demonstrated human sperm susceptibility to ROS and its relationship to low fertility. In 1978 I returned to the Babraham Institute, Cambridge, where I remained for the next 30 years with periodic sojourns to the University of Naples. The core funding provided at Babraham fostered a culture of curiosity-driven basic science that was more to my liking and we were able to range over a variety of topics on sperm maturation, capacitation, sperm sexing and signalling events at fertilization. Our focus was always on the sperm plasma membrane and lately we have been applying high resolution microscopy procedures and biophysical techniques to understanding its structure and organisation, especially at the moment of fertilization.

The people I respected in science were always bench scientists, not desk drivers or paper shufflers. I decided early on that I would stay close to experimental work and I was fortunate to be able to do this as I had loyal and dedicated support from research assistants, students and postdocs. You may not get rich in science but one of the pleasing compensations is that you make friends all over the world. I am honoured to receive this award and I thank the BAS for its support and generosity over the years.

Invited Speaker Profiles

Niels Skakkebaek MD, DMSc

Professor and Head of the Development of Growth and Reproduction at Copenhagen University Hospital (Rigshospitalet). Professor Skakkebaek is a pioneer in the field of carcinoma in situ and pathogenesis of testicular cancer and is also internationally renowned within the fields of Andrology and paediatric endocrinology.

Lars Björndahl, ESHRE

Lars Björndahl, MD and PhD from Karolinska Institutet in Stockholm, Sweden. Specialist in Clinical Chemistry. After basic studies at the Department of Physiology contributed to the development of a clinical andrology laboratory at the Karolinska University Hospital. Former Scientist (hon Senior Lecturer) 2001-2005 and also Director of Andrology 2004-2005 at the Assisted Conception Unit, Birmingham Women's Hospital, and the University of Birmingham. Past Co-ordinator of the Special Interest Group in Andrology of ESHRE; President of the Nordic Association for Andrology.

Ulrik Kvist, Karolinska Inst, Stockholm

Ulrik Kvist, M.D. Ph.D., The Center of Andrology and Sexual Medicine, Karolinska Institutet and Karolinska University Hospital, Huddinge, Stockholm, Sweden. Head of the Andrology Laboratory. Lecturer in Physiology and Andrology. Specialist in Clinical Genetics and Clinical Chemistry. Trained in Urology and Gynecology. Sperm chromatin and physiological importance of the sequence of ejaculation are main interests in Andrology.

Ashok Agarwal, PhD, HCLD

Director of the Clinical Andrology Laboratory and Reproductive Tissue Bank, and the Director of Research at the Center for Reproductive Medicine. He holds these positions at The Cleveland Clinic Foundation, where he is a Professor at the Lerner College of Medicine of Case Western Reserve University and, since 1993, Senior Staff in the Glickman Urological & Kidney Institute, OB-GYN and Women's Health Institute, Anatomic Pathology, and Immunology.

Sherman Silber, MD

Sherman Silber is a renowned pioneer in microsurgery and infertility. He has contributed major scientific breakthroughs to our understanding of quantitative sperm production, epididymal physiology, and the successful treatment of the severest forms of male and female sterility. He performed the world's first microsurgical vasectomy reversal in humans, as well as the first testicle transplant and the world's first successful ovary transplant in humans. He developed, along with his Brussels colleagues, the TESE-ICSI technique for retrieving a few sperm from hopelessly sterile men who appear to be creating no sperm and thereby achieve normal pregnancy rates. His research also includes the study of reproduction and fertility in zoo animals and endangered species in the wild. He directs one of the most successful high tech programs in the world for fertilization in couples with severe infertility problems.

Rafael Oliva, Barcelona

Presently Professor at the Faculty of Medicine of the University of Barcelona, and Consultor Geneticist at the Hospital Clínic, Barcelona, Spain. Medical studies in 1984 and PhD in 1986 by the University of Barcelona. Post-Doctoral Fellow 1986-1989 at the Department of Medical Biochemistry, University of

Calgary, Canada. Staff Scientist 1989-1990 at the Human Genome Center, Lawrence Berkeley Laboratories, Berkeley, CA, USA. Current main Projects: Molecular basis of male infertility. Sperm Genomics and Proteomics. Protamines, nuclear proteins and epigenetics.

Marcus Pembrey, University College London

Marcus Pembrey is a clinical geneticist, Emeritus Professor of Paediatric Genetics at the Institute of Child Health, University College London, and a visiting Professor at the University of Bristol UK, where he was Director of Genetics within the Avon Longitudinal Study of Parents and Children – ALSPAC (www.alspac.bris.ac.uk) until 2006.

Harry Moore, University of Sheffield

Professor Harry Moore has worked for many years on mammalian fertility and early embryogenesis. He currently co-directs (with Prof Peter Andrews), the Centre for Stem Cell Biology at the University of Sheffield which undertakes multidisciplinary research on fundamental and applied aspects of human embryonic stem cell (hESC) biology. His lab has so far derived 8 hESC lines for distribution by the UK Stem Cell Bank and is currently deriving clinical grade cell lines for several potential therapeutic applications. Since 1992 he has held a personal chair in Reproductive Biology in the Section of Reproductive and Developmental Medicine and the Department of Molecular Biology and Biotechnology at the University of Sheffield.

Professor Jim Dornan - After Dinner Speaker

Professor Jim Dornan is a graduate of Queen's University of Belfast. Having been a fetal medicine trainee at Queen's University in Kingston, Canada, he returned to Northern Ireland where he has until recently been Director of Fetal Medicine at the Royal Maternity Hospital. From being the Northern Ireland Fellows' Representative on the Royal College of Obstetrician and Gynaecologists Council, he is Immediate-Past Vice President of the RCOG. He has also been President of the Belfast Medical Students Union Association, something of which he is particularly proud. His research interests include assessment of fetal wellbeing, prenatal screening and diagnosis.

Invited Speaker Abstracts

Roy Jones

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Sperm-egg binding molecules at fertilization

One of the most vexatious problems in the field of fertilization research is elucidating the early molecular events during sperm-egg binding and fusion. While there is much information about the cell biology of fertilization in a wide range of vertebrate and invertebrate species, the identity of the molecules involved in gamete recognition and their mechanism of action, especially in mammals, remain contentious. This information is crucial for assessing the fertilizing capacity of a particular sperm sample in a clinical laboratory and, in the long-term, for designing new anti-fertility agents for human and animal use.

Over the years a lot of attention has focused on receptors on the zona pellucida (ZP) surrounding the egg, possibly because it is a relatively simple structure consisting of 3-4 major glycoproteins depending on the species. In the mouse model, extensive biochemical and genetic analyses indicate that the different glycoproteins have different functions. ZP3 glycoprotein is primarily concerned with recognition of acrosome intact sperm whereas ZP2 serves to bind and retain acrosome reacted sperm. Both these functions appear to be associated with the carbohydrate moieties of the ZP glycoproteins although there is still much debate about the nature of the sugars involved and how they are modified during the block to polyspermy. In contrast, the sperm surface membrane is much more complex and dynamic, consisting of diverse lipids and glycoproteins. Many candidate zona binding molecules have been proposed but few, if any, have stood up to serious scrutiny. Unlike the egg, sperm are subjected to an ever changing environment during their passage from the testis to the oviduct and in the process encounter a range of different cell-types. All of these cells must be ignored if the fertilizing spermatozoon is to find and bind specifically to the ZP. Since they cannot respond to external signals by synthesising new protein, sperm do the next best thing, they modify and reposition existing molecules. This leads to a hierarchy of responses during maturation and capacitation that ensures important molecules for zona binding, penetration and fusion are only activated at the correct time and in the correct place.

In this lecture I shall describe some of our own and other studies on identification of zona binding molecules on sperm and how application of genetic, biophysical and high resolution microscopy techniques are providing new insights into understanding this aspect of mammalian fertilization.

Niels E. Skakkebaek

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Testicular Dysgenesis and Low European Fertility Rates

Recent research has revealed that testicular germ cell cancer, and some cases of undescended testis, poor semen quality and hypospadias may be biologically linked as a testicular dysgenesis syndrome (TDS). However, it is important to remember that several other biological mechanisms may lead to the same disorders. The evidence of TDS comes from a number of clinical, epidemiological and experimental animal studies, which are in line with the hypothesis that these disorders are of fetal origin. Core evidence comes from studies of the precursor to testicular germ cell cancer - the carcinoma in situ cell - which is assumed to be a transformed gonocyte, arrested during the fetal differentiation process. The factors involved remain to be determined, but impaired Sertoli- and Leydig cell functions may be involved. The most severe cases of TDS may be due to rare genetic or chromosomal disorders, e.g. SRY mutations or 45,X/46,XY karyotype. However, the bulk of TDS cases may be due to environmental factors. Recently, a rat model for a TDS-like syndrome was described in offspring exposed in utero to certain phthalates. However, humans are exposed to much smaller doses of phthalates and human exposures are never limited to one or two endocrine disrupters. Our food, water, air and cosmetics contain mixtures of a high number of agents, which individually may be present in tiny amounts, but nevertheless may constitute a risk, particularly during the most sensitive periods, which we believe are the fetal period and childhood. We speculate that some of the endocrine disrupters may be responsible for the increase in TDS related symptoms, at least in Caucasian populations. The quantitative role of TDS for male reproductive health in general remains to be determined.

Obviously, there are several other etiologies to male infertility, including genetic factors. Furthermore, men with isolated hypospadias without other genital abnormalities seem to have semen quality similar to men in the general population. However, most cases of testicular maldescent and perhaps all cases of testicular germ cell cancer may be due to testicular dysgenesis.

A hypothesis will be presented showing that an increasing number of men with poor semen quality may contribute to the current low fertility rates in Europe.

13:45-14:30

Lars Bjorndahl

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Karolinska University Hospital, Huddinge
Stockholm, Sweden

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Strategies to Increase Andrology Funding opportunities in Europe

Research in Andrology and Reproductive Medicine has not expanded to the same extent as the clinical practice of ART. Although the knowledge about male reproductive functions slowly increases, other fields of medical science attract more basic funding.

The practical success of IVF and ICSI has meant that treatment of childlessness has improved rapidly in recent decades. However, with regard to the male side investigation and treatment has in general been reduced to sperm retrieval and ICSI. Underlying disorders are usually not considered.

With regard to laboratory investigations, still too many laboratories performing basic semen analysis perform substandard work, and even more clinicians lack pertinent information to interpret laboratory results properly. The very common term “male factor infertility” can therefore be called in question.

It is not very likely that there are only a few disorders explaining the main part of “male factor infertility”. Multicenter studies could be necessary to gather sufficient numbers of patients in subgroups related to different causes for reproductive disorders. However, to make such studies possible, there is an urge for standardization and quality control in laboratory work as well as clinical investigations.

Basic to strategies to increase andrology funding opportunities is therefore how we unite, standardize and quality control and train as well clinical as laboratory skills. Mixed with the ambitions to increase the scientific abilities of Europe are the wish of the EU to increase collaboration between groups in different countries – networking between groups is therefore favoured. Different examples of EU projects will be given in the presentation.

Ulrik Kvist

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Stockholm, Sweden

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Developing better prognostic tests – Sperm DNA

The assessment of DNA damage in the male germ line and the study of its consequences have received considerable attention. Hitherto, affected sperm DNA has been reported associated with impaired fertilization and reduced pregnancy rate after natural and assisted reproduction, disrupted preimplantation development, increased rates of abortions and increased rates of disease in children and young adults – e.g. cancer and complex neurological conditions. However, key questions, including clinical significance, remain to be answered

It appears that the best predictive value of sperm DNA tests was obtained for unselected spermatozoa from samples used for IUI: only 3% of the couples became pregnant if the DNA Fragmentation Index (DFI) of spermatozoa in raw semen was above 30%, and 24% of the couples became pregnant when the DFI was below 30%. However, the picture is more complicated since selected spermatozoa used for insemination showed “normal” DFI – only some 4% of these spermatozoa had increased DFI, even among spermatozoa that were selected from “bad” samples with high DFI in raw semen. The conflict here is that a poor DFI of spermatozoa in raw semen is related to poor result with IUI, while a good DFI after preparation is not related to the IUI outcome. Thus spermatozoa from ejaculates characterized by high DFI carry a defect that cannot be revealed with the DFI measure.

The original protocols were developed and validated for somatic cells and not for spermatozoa. None of the protocols take in consideration, neither that the availability of the sperm DNA is completely different due to the fundamentally different degree and type of chromatin stabilization (disulfide bridge dependent, zinc dependent), nor that these changes are influenced by the ejaculatory sequence and the time of exposure to seminal plasma of varying composition in vitro. In vitro, superstabilization of the sperm chromatin is likely to change the accessibility of DNA for methods measuring DNA defects. Altogether, biological as well as methodological differences between somatic cells and spermatozoa opens for significant causes for especially false negative results.

It appears that a “measured” defect DNA is a true bad, sign whereas a “normal DNA” could be either a good sign or a “falsely negative sign” as evidenced from studies on the TUNEL, COMET and Flow cytometry of Acridine Oranged incorporation after acid provocation.

Progress in this area will therefore need fundamental improvements in our understanding of the chromatin structure, the causes and consequences of DNA damage in the male germ line and the development of robust diagnostic tests that can be incorporated into the clinical assessment of male infertility patients.

15:45-16:30

Ashok Agarwal

Center for Reproductive Medicine, Cleveland
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The Role of Antioxidant Therapy in Treatment of the Male

Male infertility continues to be a clinical challenge of increasing significance. While male factors such as decreased semen quality are responsible for about 25% of all infertility issues, the etiology of suboptimal semen quality is poorly understood. Many physiological, environmental, and genetic factors have been implicated, including oxidative stress.

Oxidative stress is induced by reactive oxygen species (ROS), or free radicals, and although ROS are required for critical aspects of sperm function, excessive levels of ROS can negatively impact sperm quality. The origin of ROS generation, and the etiologies of increased ROS in men with suboptimal sperm quality have only recently been elucidated, offering multiple targets for potential therapy. The speaker will present a critical review of the available literature describing the role of antioxidants in the treatment of male factor infertility.

Sherman Silber

St Louis, Missouri, USA
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ICSI Results with Surgery for Retrieved Sperm Versus ICSI Results with Ejaculated Sperm

This lecture will begin with 1) diagnostic testis biopsy and the basics of spermatogenesis in oligospermic and normospermic men, 2) describe the use of sperm retrieval techniques and intracytoplasmic sperm injection (ICSI) for azoospermia, and 3) discuss the differences in embryo quality, chromosomal abnormalities, and pregnancy rates with ICSI using testis sperm versus epididymal and ejaculated sperm for men with varying degrees of spermatogenic deficit.

Approximately 1 out of every 200 men in any population (even excluding those who have had a vasectomy) is azoospermic. Approximately 20% of couples in the United States are infertile, and 25% of all infertile couples have a low sperm count. About 2% of infertile couples have azoospermia. Thus, azoospermia represents approximately 8% of the cases of male infertility.

We classify azoospermia as obstructive and nonobstructive. Obstructive azoospermia can be secondary to a vasectomy, congenital absence of the vas deferens (CBAVD), accidental surgical interruption of the vas or epididymis during a hernia or hydrocele operation, or primary epididymal blockage from previous infections. In all of these cases there is normal spermatogenesis in the testes. Most of these, with the exception of congenital absence of the vas (CBAVD), are amenable to microsurgical repair. In fact, because of ICSI, virtually any man with obstructive azoospermia can now father his own child, with the only limitation being the fertility of the wife.

A simplified clinical quantitative evaluation of the testicle biopsy is based on the normal histology and kinetics of spermatogenesis in humans. The quantitative testicle biopsy is predictive of mean sperm count in the ejaculate. Using an exponential curve, the number of mature spermatids per tubule can be used to predict the anticipated sperm count. In the absence of obstruction, the correlation is remarkably close. When there are less than three mature spermatids per tubule, the patient is virtually always azoospermic.

A simple “window” scrotal exploration under local anesthesia (just like for diagnostic testis biopsy) is performed for sperm retrieval. For obstructive azoospermia, we prefer to use epididymal sperm, although testicular sperm is necessary when there is vasa efferentia or rete testis blockage. The advantage of epididymal sperm as a first choice is that it freezes easily and represents a simple, clean, easy, and indefinite supply of sperm for the laboratory without need for future invasive procedures. As we shall see now, it also results in better ICSI pregnancy rates than testis sperm.

Often, there is only one specific area of the proximal epididymis where motile sperm can be retrieved, and this can be found more easily through microsurgery than via a blind-needle stick (which, in truth, is more painful than this microsurgical MESA procedure). If more distal, less motile or non-motile senescent sperm are used, then MESA would paradoxically give worse results.

An important warning is that for nonobstructive azoospermia, epididymal sperm can never be retrieved because the walls are collapsed. Nonetheless, for nonobstructive azoospermia, an open testicular biopsy performed under the microscope can still be accomplished in the same manner under the same type of local anesthetic with the patient wide-awake and minimal postoperative discomfort.

Shortly after introducing sperm retrieval for obstructive azoospermia in 1986, we made the observation in non-obstructive azoospermia that even in men with the most severe spermatogenic defects (causing complete azoospermia), there were frequently a very minute number of sperm sparsely present in an extensive testicular biopsy, and these occasional testicular sperm could be used for ICSI. In 1993, we coined this procedure “testicular sperm extraction” or TESE. This approach was based on our quantitative studies of spermatogenesis dating back to the late 1970s. Examination of the testicular histology of azoospermic, oligospermic, and normospermic men

shows that the number of sperm in the ejaculate is directly correlated to the number of mature spermatids found quantitatively in the testis. The average mature spermatid count per tubule in a large amount of tubules is predictive of the sperm count in the ejaculate. Intriguingly, the majority of patients with complete azoospermia have a few mature spermatids in their testis histology. There is some minute presence of spermatogenesis in 60% of azoospermic men. However, the amount of spermatogenesis present in these men is below the threshold (three mature spermatids per tubule) necessary for these few sperm to spill over into the ejaculate. It was enough sperm for ICSI, however.

Unnecessary confusion exists in TESE with testicular sperm, mature spermatids, and round spermatids. Sperm tails are seldom seen on histology, and only the thicker sperm head shows up in thin sections, and usually only an oval-shaped head is observed. Mature spermatids at TESE are no different in appearance than sperm. The solution in cases with no sperm seen on TESE is not to look for "round spermatids." We never see round spermatids in the absence of mature spermatids, which at TESE are what appear to be sperm. The solution is to search for the few sperm that are sparsely and diffusely present, not for round cells.

The formidable testicular deterioration that has been observed with overly aggressive TESE procedures is caused by either direct interference with microvascular supply of the seminiferous tubules or, even more commonly, increased intratesticular pressure because of minor amounts of bleeding within the enclosed tunica albuginea. The tunica albuginea is a very nonflexible enclosure. A small degree of intratesticular bleeding causes a noticeable increase in intratesticular pressure, which can be readily observed by those doing conventional, multiple-testicle biopsy samplings for TESE. Furthermore, the closure of open biopsies with the usual nonmicrosurgical suture, particularly in a running manner with conventional TESE, further compromises the intratesticular volume and thereby adds to the increased pressure. TESE must be performed in an aggressive but non-destructive manner, and I will describe the technique.

Our early studies in 1994 and 1995 demonstrated that the major determinant of success with ICSI was not the quality or origin of the sperm, but instead the age and fertility of the wife. However, a detailed review of delivery rates with ICSI in couples with varying degrees of severity of spermatogenic defect, as well as fluorescence *in situ* hybridization (FISH) studies of both sperm and embryos derived from this sperm, indicates that sperm may also have an impact on ICSI results. Our data confirm no significant increase in sperm aneuploidy with oligospermia, but about twice the rate of sperm aneuploidy in nonobstructive azoospermia.

The high rate of mosaic embryos observed as a result of TESE-ICSI may be more related to defects in the sperm centriole than to a higher incidence of numerical chromosome abnormalities. Our TESE-ICSI-derived embryos had no greater incidence of aneuploidy than ICSI with ejaculated sperm from men with higher sperm production rates. However, a dramatically increased rate of mosaic errors was found in these embryos because of abnormal mitosis, which could be related to defects in the sperm centriole. Severe spermatogenic defects, as in nonobstructive azoospermia, may result in a higher percentage of mosaic and chaotic mosaic embryos, causing less efficient implantation and live birth rates, and testis-derived sperm may give somewhat lower success than proximal epididymal ejaculated sperm. However, the negative impact of sperm factors is only modest when compared to the negative impact of the female's age (causing aneuploidy). Nonetheless, in men with obstructive azoospermia with normal spermatogenesis, proximal epididymal sperm gave better results than distal sperm (more senescent), and also better results than testicular sperm (more immature). Thus, for obstructive azoospermia, MESA is preferable to TESE if it is performed microscopically and proximally.

10:00-10:45

Rafael Oliva

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Advances in Sperm Proteomics

Different proteomics approaches are presently being applied to andrology. The basic steps in most proteomic analysis at present are the protein or peptide extraction from the sample, the separation of the proteins or peptides and the application of mass spectrometry analysis and database comparisons to identify the different proteins or peptides. Essentially two mass spectrometry approaches have been applied to the study of the sperm cell: initial two-dimensional (2D) separation of proteins followed by MALDI-TOF (matrix assisted laser desorption ionization - time of flight) and LC-MS/MS (liquid chromatography - mass spectrometry / mass spectrometry). In a MALDI-TOF analysis the proteins are typically excised from the gel, digested with trypsin and the ratio of mass to charge of the resulting peptides determined, which upon comparison to database data results in the identification of the protein. The other technique, LC-MS/MS, combines the solute separation power of HPLC (high performance liquid chromatography), with the detection power of a mass spectrometer. LC-MS/MS experiments usually generate peptide primary structure (sequence) information from each peptide in the initial mix.

As a result of the recent application of proteomics to andrology, catalogs of hundreds to thousands of proteins and proteomic 2D maps are now available from human and animal model sperm cells, seminal fluid secretions, epididymis, testis, and prostate, among others. Of importance, for the human sperm cell over 1000 proteins are presently known either as derived from 2D maps (Pixton et al., *Hum Reprod* 2004; 19: 1438-1447; de Mateo et al., *Proteomics*. 2007 7:4264-77; Oliva et al., *Proteomics* 9: 1004–1017) or from the LC-MS/MS catalogues (Aitken and Baker, *Int. J. Androl.* 2008, 31: 295-302; Baker, et al., *Proteomics Clin. Appl.* 2007, 1:524–532; de Mateo S, Oliva, Estanyol JM, unpublished results). The compositional information provides a reference for subsequent basic research and potential clinical applications. Some unexpected results have been the identification of DNA binding proteins with a potential epigenetic function. In addition, one of the applications of proteomics is the identification of proteins present with a differential abundance in the sperm cell of infertile patients. The identification of these proteins provides an important tool towards the identification of the pathogenic mechanisms involved in male infertility and also has a potential towards the development of new prevention and treatment strategies.

10:45-11:30

Marcus Pembrey^{1,2}

Lars Olav Bygren^{3,6}, Gunnar Kaati⁴, Sören Edvinsson⁵, Kate Northstone²,
The ALSPAC Study Team², Michael Sjöström⁶, Jean Golding².

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Male-line transgenerational Responses: a new aspect of human inheritance

Transgenerational effects of maternal nutrition or other environmental 'exposures' are well recognised but not down the male line, where epigenetic inheritance or some other novel inheritance mechanism would have to be postulated. We have reported earlier historical associations of longevity and diabetic deaths with paternal ancestors' food supply in mid childhood (Bygren LO et al Acta Biotheoret. 2001;49:53, Kaati G et al EJHG 2002;10:682). Using the Avon Longitudinal Study of Parents and Children (ALSPAC, www.alspac.bristol.ac.uk) we identified 166 fathers who reported starting smoking before age 11 years and showed, after correcting for confounders, that their sons (but not daughters) had a greater body mass index at 9 years than offspring of fathers with later onset of smoking. Sex-specific effects were also shown in two of three historical Överkalix cohorts from northern Sweden; paternal grandfather's food supply was only linked to the mortality rate of grandsons, whilst paternal grandmother's food supply was only associated with the granddaughters' mortality rate (Pembrey et al EJHG 2006;14:159. Senn 2006 & Bygren et al EJHG 2006;14:1149). This 'criss-cross' sex-specific transmission of information across the generations through the same set of fathers creates a situation that is 'internally controlled' for social patterning confounders at least down to the fathers. Further analysis adjusting for the early-life social circumstances of the probands (grandchildren) themselves shows that the male line transgenerational effects persist (Kaati et al EJHG 2007;15:784). These transgenerational effects were observed with exposure only at specific periods during the paternal ancestor's development - mid childhood (both grandparents) and fetal/infant life (grandmothers), but not during either grandparent's puberty.

It is early days in the study of male-line transgenerational responses in humans, but some coherence is emerging with respect to observed outcomes. They have features of the Metabolic Syndrome which some regard as a 'mal-adaptation' to modern lifestyles. In addition to the cardiovascular and diabetic risks observed in the Överkalix cohort and the ALSPAC data linking mid childhood paternal smoking with raised body mass index in sons, in a Taiwan study paternal betel nut (*Areca catechu*) chewing has been linked to early onset of the metabolic syndrome in the offspring (Chen et al Am J Clin Nutr 2006; 83: 688). It is possible that 'uncertainty stress' triggers a default 'survival' mode of gene expression in descendants.

Currently the mediating molecular mechanisms are unknown. We are not claiming that it is epigenetic inheritance (i.e. where the DNA sequence itself is unchanged), only that this mechanism is a good candidate. We hypothesise that these transmissions are mediated by the sex chromosomes, X and Y and that the non-recombining region of the Y can preferentially transmit environmentally-induced epigenetic states or reversible DNA changes to the next generation(s).

12:00-12:45

Harry Moore

Centre for Stem Cell Biology and Reproductive Developmental
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Artificial sperm-neither next year nor ever

Human embryonic stem cells (hESCs) are pluripotent and there is great hope that stem cell technology can be utilised to treat degenerative disease as the cells that differentiate from hESCs show often remarkable similarity to specific cells in various organs. However, when it comes to the potential development of gametes from hESCs, there is considerable ethical debate. Indeed germ cells derived from hESCs have been dubbed 'artificial gametes' although cell phenotypes for other tissues have not been given such an 'artificial' connotation. An *in vitro* model to investigate the earliest development of human germ cells and gametes would be a valuable tool to study the origins of testicular cancer, effects of toxicants on reproduction and might ultimately lead to clinical therapies to overcome infertility. The discovery of induced pluripotent stem (iPS) cells means that in the future cells could initially match the genetic composition of the patient.

Gametes in mammals are derived from a founder population that is segregated early in embryogenesis and specified to become primordial germ cells (PGCs). These cells migrate to the genital ridge of the developing foetus and undergo gametogenesis to eventually form spermatozoa or oocytes. Several studies have demonstrated that ESCs in the mouse can differentiate to PGCs and subsequently early gametes and blastocysts. Recently, immature sperm cells derived from ESCs in culture have generated live mice. Human ESCs most likely display a similar developmental capacity. But it remains unclear as to the extent of gamete development from hESCs *in vitro*. In our lab we can demonstrate that 3-D culture of hESCs can form a niche for male germ cell development, and although post-meiotic differentiation is very rare it occasionally occurs. We are now exploring new co-culture models to improve gametogenesis from hESCs but the developmental capacity of *in vitro* derived sperm remains unclear.

**Young Researcher
Communication Abstracts**

List of Young Researcher Competition Oral Presentations (4 finalists)

Ahmed Aldarmahi

University of Sheffield, United Kingdom

Establishment of a defined *in vitro* model for investigation of maternal interactions with gametes and embryos

Najmeh Moein Vaziri

University of Sheffield, United Kingdom

Effect of heat shock 70kDa protein 8 on boar sperm viability

Luke Simon

Queens University Belfast, United Kingdom

Abnormalities in human sperm protamine levels (P1/P2 ratio) are associated with increased DNA fragmentation measured by the alkaline Comet assay

Cristina Tomás

Centro de Investigacion y Tecnologia Animal, Instituto Valenciano de Investigaciones Agrarias, Spain

Treating fresh boar spermatozoa with cyclodextrin pre-loaded with cholesterol improves their osmotic tolerance limits

Aldarmahi, A¹, Elliott, S¹ and Fazeli, A¹

¹*Academic Unit of Reproductive and Developmental Medicine,
The University of Sheffield, United Kingdom*

**ESTABLISHMENT OF A DEFINED *IN VITRO* MODEL FOR
INVESTIGATION OF MATERNAL INTERACTIONS WITH GAMETES
AND EMBRYOS**

The mechanisms underlying gamete and embryo interaction with the maternal environment are not well understood. Several studies have indicated that local responses are generated by female reproductive tract towards spermatozoa, oocytes and embryos. However, no defined model exists to allow detailed and systemic investigation of maternal communications with gametes and embryos currently. Here we aimed to establish an *in vitro* model, based on boar sperm interaction with a porcine telomerase-immortalised oviductal epithelial cell line (TERT-OEC), to further understand the nature of this process and evaluate different factors that may affect it. Boar spermatozoa diluted in Beltsville thawing solution were washed using a Percoll gradient and diluted to 10⁶ spermatozoa/ml in Tyrode's albumin lactate and pyruvate solution. Washed spermatozoa were co-cultured with the porcine TERT-OEC line at 37°C, 5 % CO₂, 95% humidity for 24 hours. Then, RNA was extracted, purified and cDNA was synthesised and used for quantitative real-time PCR. Genes such as heat shock 70 kDa protein 8 (HSPA8), Adrenomedullin (ADM) and prostaglandin E Synthase (PGES) that have been reported to be altered in oviduct in response to spermatozoa were considered as end points of the assay. All the samples were treated in triplicate and the values obtained were normalised to the beta-actin housekeeping gene using the delta-delta Ct method. The results showed an alteration in gene expression in response to spermatozoa (ADM and PGES, $P < 0.005$). Different genes showed varied responses to increasing sperm concentrations and dead spermatozoa were not as effective as live spermatozoa in the induction of alterations within the oviductal transcriptome. These data indicate the feasibility of the establishment of a defined *in vitro* model for investigation of maternal interactions with gametes and embryos. Further experiments are in progress to characterise other factors such as sperm source and cell passage alterations that can influence transcriptome changes in the maternal tract in response to gametes and embryos.

Moein Vaziri, N¹, Elliott, S¹, Pockley, G¹ and Fazeli, A¹

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EFFECT OF HEAT SHOCK 70KDA PROTEIN 8 ON BOAR SPERM VIABILITY

Introduction: Many female species store spermatozoa in the reproductive tract prior to fertilisation. Using quantitative proteomic approaches, we have reported alterations in the abundance of the constitutively-expressed member of the 70 kDa stress protein family (Hsc70, HspA8) in response to spermatozoa in oviductal epithelial cells. This study examines the potential physiological influence of HspA8 by determining its effects on boar sperm viability.

Methods: Recombinant bovine HspA8 was generated in our laboratory using an E.coli over-expression system and its effects on boar sperm viability was determined. For this, boar sperm samples (n=9) diluted in Beltsville thawing solution were washed using a Percoll gradient and diluted to 10⁶ spermatozoa/ml in Tyrode's albumin lactate and pyruvate solution. Diluted semen samples were incubated with 0, 0.1, 0.5 and 1 µg/ml of HspA8 for 0, 24 and 48 hr at 39°C and 100% humidity. Sperm viability was assessed by fluorescent microscopy on the basis of membrane integrity using Calcein-Am and ethidium homodimer.

Results: HspA8 enhanced sperm viability after 24 hr, although this effect was concentration dependent (34±1%, 37±1, 43±1 and 39±2% using 0, 0.1, 0.5 and 1 µg/ml of HspA8 respectively*). These findings were verified using an alternative assay (SYBR-14 and Propidium iodide) in which sperm viability was assessed after 15 min coincubation with 0 and 0.5 µg/ml of HspA8 (65.26±1.43 and 72.16±1.53% using 0 and 0.5 µg/ml of HspA8, respectively). The effect of HspA8 on sperm viability was also determined by pre-incubating sperm under capacitating condition for 0, 3, 6 and 9 hr, at which time 0.5 µg/ml of HspA8 was added and viability immediately assessed (11.5±3.5, 7±2.31, 3±2 and 0.6±2.4%, respectively).

Conclusions: These findings indicate that our recombinant bovine HspA8 can enhance boar sperm viability as after just 15 minutes of coincubation. Furthermore, it appears that the influence of HspA8 on sperm viability diminishes after pre-incubation of sperm under capacitating conditions.

*Data are means±SEM

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ABNORMALITIES IN HUMAN SPERM PROTAMINE LEVELS (P1/P2 RATIO) ARE ASSOCIATED WITH INCREASED DNA FRAGMENTATION MEASURED BY THE ALKALINE COMET ASSAY

Prior to spermatogenesis, around 85% of the histones in the sperm nucleus are replaced with protamines. This histone-protamine replacement process results in sperm chromatin compaction and is also necessary for transcription silencing during the latter stages of spermatogenesis. In the human, protamines are comprised of two types represented as protamine-1 (P1) and protamine-2 (P2). Variations in sperm protamine expression are associated with male infertility. Similarly, sperm DNA integrity is also important for male fertility. This study has been designed to evaluate variations in P1 and P2 content and to see if there is an association with DNA fragmentation. A total of 69 infertility patients including 35 men attending an Andrology laboratory for diagnosis, 17 men undergoing IVF treatment and 17 men undergoing ICSI treatment were recruited into the study. Sperm DNA fragmentation was measured by the alkaline Comet assay and 10 million spermatozoa were used for the extraction of protamines. The protamines were separated in acid-urea polyacrylamide gels and the P1/P2 ratio was calculated by comparing the intensities of the P1 and P2 bands. The subjects were stratified into six groups based on their P1/P2 ratio. Based on previous reports, subjects with P1/P2 ratio (between 0.8 to 1.0) were considered to be normal. DNA fragmentation was significantly elevated in patients with both very low $57.3 \pm 5.8\%$ and very high P1/P2 ratio ($59.6 \pm 17.6\%$) versus those with normal P1/P2 ratio ($30.1 \pm 5.4\%$, $P = <0.05$). DNA fragmentation showed a significant inverse correlation with very low P1/P2 ratio ($r = 0.42$, $P = <0.001$), very high P1/P2 ratio ($r = 0.23$, $P = <0.001$), and total protamine content (P1+P2/DNA) ($r = 0.20$, $P = <0.001$). These data demonstrate that poor and rich protamination is associated with increased DNA fragmentation.

We gratefully acknowledge Hamilton Thorne Biosciences (USA) and the Ministerio de Ciencia e Innovación (Spain, BMC2006-03479) for funding the project.

Tomás, C¹, de Mercado, E², Blanch, E¹, Gómez-Izquierdo, E² and Mocé, E¹

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**TREATING FRESH BOAR SPERMATOZOA WITH CYCLODEXTRIN
PRE-LOADED WITH CHOLESTEROL IMPROVES THEIR OSMOTIC
TOLERANCE LIMITS**

During freezing and thawing cells are exposed to both hyperosmotic and hyposmotic conditions that are potentially harmful to the sperm plasma membrane. The aim of this investigation was to study the osmotic tolerance limits of fresh boar spermatozoa pre-treated with methyl- β -cyclodextrin loaded with cholesterol (MCLC) by evaluating the sperm plasma membrane integrity after incubation in several anisomotic solutions. Fresh sperm-rich fractions from 5 fertile boars stored at 17 °C for 16 h were used. After seminal plasma elimination (800g/10 min), sperm concentration was adjusted to 1000×10^6 spermatozoa/mL and the sample was split into two subsamples: control or MCLC-treated (1 mg /120 x 10^6 spermatozoa, 15 min/17°C). Aliquots from each subsample were exposed (5 min/17°C) to 7 different anisomotic BTS-BSA (0.6%) solutions (50, 150, 300, 425, 600, 800, 2400 mOsm), returned to isosmotic conditions and then incubated for 10 min at 37°C before evaluating sperm membrane integrity by flow cytometry using dual staining (Sybr-14/PI). Data were expressed as live spermatozoa (%) and analyzed with a mixed model ANOVA. There were significant differences in live spermatozoa observed among control and MCLC-treated samples for both hyposmotic solutions (50 mOsm: 17.92% vs. 67.34% \pm 4.93, 150 mOsm: 48.70% vs. 79.76% \pm 4.93, for control and MCLC-treated respectively; P<0.0001) and hyperosmotic solutions (600 mOsm: 41.96% vs. 56.32% \pm 4.93, 800 mOsm: 36.78% vs. 50.02% \pm 4.93, for control and MCLC-treated respectively; P<0.05). In conclusion, our data suggest that treatment of fresh boar spermatozoa with cyclodextrin pre-loaded with cholesterol widens their osmotic tolerance limits and this could improve the resistance of boar spermatozoa to the freeze-thaw process.

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List of Poster Presentations

Blanch, E, Tomás, C, Mocé, E

Evaluation of alternative cryoprotectants for boar sperm freezing

de Mercado, E, Gómez, E, Sanz, E, Hernández, M, Gómez, J, González-Bulnes, A and Sánchez R

Relation of the seminal plasma proteins of Iberian pig with sperm freezability

Emerson, G, Hughes, C, Mocanu, E

Cryopreserved Semen for Oncology Patients: Its use and reproductive outcome in an Irish cryopreservation unit

Miller, D, Arpanahi, A, Brinkworth, M, Elnefati, A, Iles, D, Krawetz, S, Paradowsak, A, Saida, M, Steger, K, Tedder, P

Differential packaging of mammalian sperm chromatin reveals a global epigenetic signature: could the same apply to insect sperm?

Simon, L, Brunborg, G, Lutton, D, McManus, J, Lewis, S.E.M

Clinical significance of sperm DNA damage and DNA adducts measured by alkaline Comet assay in assisted reproductive outcome

Tomás, C, Blanch, E, Mocé, E, Fazeli, A

Treatment of boar sperm with cholesterol-loaded cyclodextrins prior to cryopreservation improves the sperm binding ability to oviductal epithelial cells in vitro

EVALUATION OF ALTERNATIVE CRYOPROTECTANTS FOR BOAR SPERM FREEZING

Glycerol protects cells during freezing but cells suffer from osmotic stress during its addition and removal. Other cryoprotectants have been shown to be more permeable to plasma membrane than glycerol and their use in freezing protocols could result in higher cell survival. The aim of the present work was to evaluate alternative cryoprotectants to glycerol (GLY) for boar sperm cryopreservation, (i.e. ethylene glycol (EG), propylene glycol (PG) and dimethyl sulphoxide (DMSO)). Boar ejaculates (N=4) were frozen with lactose-egg yolk extender supplemented with 0.5 % Orvus ES Paste and different concentrations of the cryoprotectants evaluated: GLY 0.41M, EG 0.25M, EG 0.50M, PG 0.50M, PG 0.75M, DMSO 0.50M and DMSO 0.75M. Semen was packaged in 0.5 mL straws, frozen in a programmable freezer (Carvajal et al. 2004 J.Androl. 25:389-396) and thawed at 37°C for 30 sec. Sperm viability was evaluated by flow cytometry using a dual fluorescence stain (SYBR-14 and propidium iodide). Total (TM) and progressive (PM) sperm motility were assessed using a CASA system. Sperm viability for GLY ($60 \pm 2.83\%$), EG 0.25M ($59 \pm 2.83\%$) and EG 0.50M ($60 \pm 2.83\%$) was higher than for the other treatments ($35 \pm 2.83\%$ to $49 \pm 2.83\%$)($P<0.05$). Total and progressive motility for GLY (TM= $51 \pm 10.28\%$, PM= $43 \pm 9.82\%$) and EG 0.25M (TM= $48 \pm 10.28\%$, PM= $39 \pm 9.82\%$) were superior to the rest of the cryoprotectants (TM= $13 \pm 10.28\%$ to $34 \pm 10.28\%$, PM= $10 \pm 9.82\%$ to $28 \pm 9.82\%$) ($P<0.05$). In conclusion, ethylene glycol provided results similar to glycerol and it could be used as an alternative cryoprotectant for boar sperm freezing.

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Poster

de Mercado, E¹, Gómez, E¹, Sanz, E¹, Hernández, M¹,
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**RELATION OF THE SEMINAL PLASMA PROTEINS OF IBERIAN PIG
WITH SPERM FREEZABILITY**

Differences in seminal plasma (SP) protein profiles have been associated with low and high sperm freezability in several species and, specifically in boars, with different in vivo fertility rates. The objective of this study was to investigate the relationship between the protein profile of Iberian pig SP and sperm freezability. Two aliquots from 36 fertile Iberian boar ejaculates were centrifuged to recover the SP (5000g/30min, supernatant stored at -80°C until analysis) or the sperm pellet (2400g/3min) which was resuspended in freezing extender fructose-egg yolk-glycerol (1×10^9 cells/mL) in 0.5 mL plastic straws and frozen using computer-controlled freezing equipment. The freezability of the samples was evaluated 30 min after thawing by calculating the mean sperm motility (CASA) and by fluorescence microscope analysis of plasma membrane integrity (SYBR14/PI). Two groups of sperm were determined according to freezability (H: high freezability, n=20; L: low freezability, n=16) by means a cluster analysis and data were analyzed using the GLM procedure of SAS. The SP protein profile was analyzed by capillary electrophoresis using lab-on-a-chip technology (Agilent®, USA). A protein of 15 kDa, which its molecular weight indicates that could belong to the spermadhesin family proteins, was more abundant ($P < 0.05$) in SP samples collected from Iberian pigs with higher sperm freezability. On the other hand, one protein of 25 kDa was more abundant ($P < 0.05$) in SP samples from pigs with lower sperm freezability. In conclusion, the study suggests differences in the seminal plasma protein profiles from Iberian pigs with low and high semen freezability.

Supported by PEA 2008/232(113) ITACYL, Junta de Castilla y León

Emerson, G¹, Hughes, C¹ and Mocanu, E¹

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CRYOPRESERVED SEMEN FOR ONCOLOGY PATIENTS: ITS USE AND REPRODUCTIVE OUTCOME IN AN IRISH CRYOPRESERVATION UNIT

Introduction: As survival rates among patients with malignancies have significantly increased over the last decades (1), a strong demand for sperm banking for male oncology patients is evident. Due to the reality that future reproductive outcome after chemotherapy is hard to predict, numerous studies have shown the benefits of cryopreservation for males prior to undergoing these multidrug regimens (2,3,4).

The National Gamete Cryopreservation at the Human Assisted Reproduction Ireland (HARI) is the main referral centre for the majority of sperm banking procedures in Ireland. The aim of this study is to assess the use rate and assisted reproductive outcome of the cryopreserved semen of cancer patients in our facility over a 7 year period (1998-2005).

Method: A retrospective analysis of our semen cryopreservation database and chart review.

Results: We identified a total of 540 oncology males seeking sperm cryopreservation prior to cancer therapy (ages between 16-50 years). A total of 1026 semen samples were frozen for possible future use. On follow up, 47 (9%) males have been reported to us as non survivors of their cancers and 21 (4%) requested disposal with 8 males transferring their samples to other storage facilities. Ninety four (17%) males returned for semen analysis following cancer therapy and a further 39 patients (7.2%) have returned for treatment at our ART unit, undertaking a total of 66 treatments of IVF/Intracytoplasmic sperm injection (ICSI) and frozen zygote transfers (FZT) cycles. Fourteen IVF's, 46 ICSI's, 6 FZT's were undertaken resulting in 61 transfers with a clinical pregnancy rate of 50%, 37%, 33%, respectively.

Conclusion: These results suggest the effective promotion of sperm cryopreservation is essential. It contributes to the lessening of the personal risk for infertility and while uptake appears modest, success rates after therapy are excellent. Of note, pregnancy outcomes appear reduced in the ICSI group compared to the IVF group confirming the need to pursue new tools in evaluating the impact of certain cancers, adjuvant therapies and cryopreservation techniques on already suboptimal sperm.

References:

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Poster

Miller, D, Arpanahi, A, Brinkworth, M, Elnefati, A, Iles, D, Krawetz, S, Paradowsak, A, Saida, M, Steger, K and Tedder, P

Universities of Leeds, United Kingdom; Bradford, United Kingdom; Giessen, Germany; Wayne State, USA

DIFFERENTIAL PACKAGING OF MAMMALIAN SPERM CHROMATIN REVEALS A GLOBAL EPIGENETIC SIGNATURE: COULD THE SAME APPLY TO INSECT SPERM?

Introduction: Spermatozoal chromatin is more highly condensed than somatic chromatin by being repackaged into protamines during spermiogenesis. However, some spermatozoal chromatin remains in a nucleosomal configuration, the function of which is unknown. We have taken a fresh look at human and mouse sperm to understand why this differential packaging phenomenon occurs.

Methods: Using combinations of salt extraction followed by restriction endonuclease digestion (SRD) or limited micrococcal nuclease digestion (MND) of detergent permeabilised human and mouse sperm nuclei, DNA solubilised by these treatments were compared with their corresponding insoluble residues by comparative genome hybridization (CGH) microarray analysis. A parallel, anti-histone, ChIP based promoter analysis was also undertaken on human sperm chromatin and a preliminary investigation on the chromatin composition of sperm from the fruit-fly, *Drosophila melanogaster* is reported.

Results: We found consistent soluble (S) and insoluble (I) partitioning profiles from the ejaculates of several men and from mouse epididymal spermatozoa suggesting strong enrichment of gene sequences in the soluble fractions from both human and murine sperm. Closer inspection of the data revealed that the enrichment was stronger for promoter sequences and CTCF binding sites. A clear, non-spermatogenic developmental ontology is also apparent in the soluble promoter sequences. The ChIP based promoter analysis on human sperm chromatin revealed many other highly significant gene ontologies including and in addition to development. We have also detected histones in the mature sperm of *Drosophila*, suggesting that differential packaging of gene sequences may also be applicable to insect sperm.

Conclusions: We conclude that mammalian sperm contain a less condensed chromatin that is accessible to endonucleases and enriched in gene regulatory regions including promoter sequences and CTCF binding sites. This DNA is likely to form a subset of histone-bound DNA sequences in sperm nuclei that may have some significance to the developing embryo of mammals and flies

Poster

Simon, L¹, Brunborg, G², Lutton, D³, McManus, J³ and Lewis, S.E.M¹

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CLINICAL SIGNIFICANCE OF SPERM DNA DAMAGE AND DNA ADDUCTS MEASURED BY ALKALINE COMET ASSAY IN ASSISTED REPRODUCTIVE OUTCOME

Sperm DNA damage impacts negatively on assisted reproductive technology (ART) outcomes and shows promise as a more robust biomarker than conventional semen parameters. In this study we determined the usefulness of sperm DNA fragmentation as a prognostic test for ART success. DNA fragmentation in native semen and the 90% fraction of prepared spermatozoa was measured from a total of 239 couples [149 IVF and 90 ICSI] by the alkaline Comet assay and correlated with fertilization rates, embryo quality, clinical pregnancy and early pregnancy loss. In addition to existing sperm DNA strand breaks, DNA adducts present in the spermatozoa were converted into strand breaks using the formamidopyrimidine DNA glycosylase enzyme and also measured by the Comet assay. After IVF treatment, there was a significant decrease in fertilization rates and embryo quality when DNA fragmentation was above 60% in native semen or above 40% DNA fragmentation in prepared spermatozoa. Couples who failed to achieve a pregnancy also had higher DNA fragmentation than those couples who achieved pregnancies. When DNA adducts were added to existing strand breaks the total DNA fragmentation in spermatozoa was markedly higher and there was also a significant difference between those who were or were not successful in achieving a pregnancy. Above a threshold value of 48% DNA fragmentation in prepared sperm there was a significant decrease in pregnancy rate. After ICSI treatment, DNA fragmentation was not significantly associated with fertilization rates, embryo quality or clinical pregnancy in either native semen or prepared spermatozoa until DNA adducts were converted into strand breaks and then the total DNA fragmentation measured showed a significant difference between those who were or were not achieved a pregnancy. In conclusion, we observed a negative relationship between DNA fragmentation and ART outcome parameters in both IVF and ICSI.

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Poster

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TREATMENT OF BOAR SPERM WITH CHOLESTEROL-LOADED CYCLODEXTRINS PRIOR TO CRYOPRESERVATION IMPROVES THE SPERM BINDING ABILITY TO OVIDUCTAL EPITHELIAL CELLS *IN VITRO*

Cyclodextrins are capable either of removing or incorporating cholesterol into the sperm plasma membrane. The aim of this investigation was to evaluate the porcine oviductal epithelial cell (OPEC) binding capacity of frozen-thawed boar sperm pre-treated with methyl- β -cyclodextrin loaded with cholesterol (MCLC) using an *in vitro* OPEC-sperm binding assay. Pooled sperm-rich fractions from four boars were either non-treated (control) or treated with 1 mg MCLC/ 120×10^6 sperm (15 min/17°C). Thereafter samples were frozen in lactose-egg yolk extender with 3% glycerol (1000×10^6 sperm/mL) in 0.5 mL straws using computer-controlled freezing equipment. After thawing, the sperm plasma membrane integrity was evaluated with dual staining (Sybr-14/PI) under fluorescence microscopy. Thawed sperm were washed with a Percoll gradient and the concentration adjusted to 100×10^6 cells/mL in TALP_s (TALP supplemented with serum) before co-incubation (1:1; v: v) with trypsinized OPEC (2×10^6 cells/mL) in TALP_s (30 min/37°C/on rotation). Spermatozoa bound to 250 fixed OPEC were counted by light microscopy. Data were expressed as % live sperm after thawing and number of bound sperm/OPEC and analyzed in a mixed multifactorial model ANOVA. Live sperm were similar between treatments (59.38 ± 4.09 and 62 ± 4.09 for control and MCLC-treated sperm; $P=0.67$) but higher number of spermatozoa bound per OPEC were observed for MCLC-treated compared with control sperm (17.85 ± 1.31 and 15.16 ± 1.31 , respectively; $P<0.0001$). In conclusion, our data suggest that exposure of fresh boar sperm to cyclodextrin pre-loaded with cholesterol prior to freeze-thaw process improves the binding capacity of frozen-thawed boar sperm to OPEC *in vitro*.

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Notes

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Activities

Belfast

If you wish to explore Belfast and learn about the history and landmarks – there are a variety of tours available.

To get the best views take one of the open top tours, these will take you through the city centre, out to Titanic Quarter where you can see the infamous cranes of Harland & Wolff, then to the architecturally impressive Parliament Buildings Stormont, through the leafy suburbs of East Belfast where the iconic writer C S Lewis spent his childhood, not forgetting the Political Wall Murals in West Belfast depicting the story of Belfast's colourful past.

For numbers less than eight why not get a more personal insight and take a traditional Black Taxi Tour, concentrating on the West of the City, the driver as your guide will take to the notorious Shankill and Falls areas of Belfast.

The Antrim Coast & Giants Causeway

The Causeway Coastal Route is rated as one of the Top Five Road Trips worldwide. The road starts in Carrickfergus and takes you to Larne, the gateway to the nine Glens of Antrim, a romantic realm of rivers, woodland and cascading waterfalls. The road takes you around the coast to the unique Giants Causeway. Formed over 60 million years ago, when molten lava cooled suddenly on contact with water, it is an awe inspiring landscape of mostly hexagonal basalt columns. Also, within easy reach of Bushmills, the oldest 'legal' distillery in the world!

The Walled City of Derry

Londonderry, also known as Derry is an ancient city and situated less than two hours from Belfast. The rich cultural and architectural heritage is reflected in the city's three names: Derry, from old Irish *Doire*, a reference to the oak grove where Saint Columba founded a monastery around 546 AD, Londonderry, the name granted during the seventeenth century Plantation of Ulster, and The Walled City, a modern nomenclature reflecting its status as one of the best preserved walled cities in Europe. Today you can stroll along the walls and explore some of the many intriguing sights. Saint Columba's Anglican Cathedral, the beautiful Guildhall and the Craft Village. Across the city is Saint Eugene's Roman Catholic Cathedral, dating from 1873. Derry also has the Harbour, Tower and Workhouse Museums and is a host of many atmospheric pubs throughout the old town.

The Fermanagh Lakes

Situated in the west, The Fermanagh Lakeland's are an area of natural beauty, the twin lakes of Lough Erne cover one-third of Fermanagh. The lower Lough is a splendid curve of open water, five miles wide, narrowing to the isthmus where Enniskillen stands. The upper Lough is a myriad of islets inter-cut by the sinuous curves of the River Erne. Fermanagh affords great opportunities for boating, everything from cruising to canoeing, plenty of fishing in the lakes and rivers. Also, the ferry from Trory goes across to Devenish Island, one of the most important monastic sites in Northern Ireland. Fermanagh is also host to the famous Marble Arch Caves and Castle Coole, perhaps the most stately of all the National Trust properties in Northern Ireland which was designed by James Wyatt for the Earl of Belmore and completed in 1798.

The Mourne Mountains & Down

Towering above Newcastle in the south east corner of Northern Ireland are the beautiful mountains of Mourne. Distinctive with 12 summits rising to above 2,000 ft the Mournes offer spectacular views of the surrounding countryside. The Mourne Mountains provide the every outdoor activity - hill walking, cycling, climbing, golf, horse riding, and fishing - the perfect holiday location. Nearby Downpatrick is the home to the impressive and historic Down Cathedral and the grave of St Patrick, Patron Saint of Ireland.

Affectionately known as 'The Linen Homelands', County Down is still the centre for Irish Linen. The Ferguson Linen Centre in Banbridge and the award winning Irish Linen Centre and

Museum at Lisburn follow the history of the flax flower fabric from the 17th century to the present day. Down has many museums and records of times past across the County, including the perfectly preserved Down County Museum at Downpatrick's Old Jail and the superb Ulster Folk and Transport Museum near Holywood.

Dublin

Situated just two hours south by high speed train, Dublin is world renowned. Dublin is a medieval city where the charming and cosmopolitan converge in delightful diversity. Fine museums and art galleries chronicle Dublin's long and historical past, while the pubs and cafes buzz with traditional and contemporary entertainment. Just a 20 minute journey takes you from the bustling city to the charming coastal towns and villages which dot the lovely coastline of Dublin County, where you can enjoy craft shopping, water sports, seafood dining and picturesque walks against the spectacular backdrop of Dublin Bay. With its Viking remains and atmospheric cobbled alleys and Georgian squares, it is notorious for literary landmarks – from the James Joyce Tower and the Writers museum to the famous Gate and Abbey Theatre to Trinity College. Not to forget the many traditional Irish pubs and of course 'the craic'.

Lagan Boat Cruise

The tours we offer aim to provide a totally different perspective of Belfast from the river, harbour area and the lough to tourists, local residents, and school and community groups. Commentary focuses on the constantly changing mixture of heritage and modern developments on the river, history of the area, conservation issues and the impact of new development on the river environment. The tours also provide a welcome and relaxing respite from the hustle and bustle of city life. The M.V. 'Joyce Too' is fully weatherproof and heated.

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