International Network for Young Researchers in Male Fertility

Winter edition - 2015

INYRMF Newsletter

Greetings

Dear INYRMF member,
You are now reading the 2nd edition of our trimestral newsletter!
For this special winter issue, we brought many gifts for you.
First of all, we are pleased to announce that our website has had a face-lift, and now include a more dynamic and interactive interface with chat, posts and group discussions. Please come and play with it on www.youngresearch.eu to see the very nice work made by Thomas Darde, our new INYRMF board member!
A second important issue is the announcement of our 9th INYRMF meeting in Rennes, adjacent to the European Testis Workshop. You will find more information below and on the website!
Finally, we offer you a rich content to spend very nice end of year celebrations: a family affair about histones, a controversy over paracetamol, the parallel stories of two researchers on sperm that marked their time and plenty of useful information about colloquia, careers and funding.
We wish you a pleasant reading!

The INYRMF board

In this issue:

Main responsible for this issue:
Pierre Calvel
Yoni Baert

INYRMF Bulletin

We are pleased to announce the 9th INYRMF meeting that will be held in Rennes, France, on the 9th and 10th of June 2016. It will precede the 19th European Testis Workshop that will take place near Rennes, in St Malo, from the 11th to the 15th of June 2016.
As this meeting will celebrate the 10th anniversary of our network, we have prepared a very special program for you!
The meeting will take place in the prestigious EHESP School of Public Health on the scientific campus of Rennes. In this particular environment we will have the pleasure to welcome famous scientists in our field for a series of presentations and open discussions around the subject of male reproduction and fertility, but also on more general topics such as ethics in research. We will also propose a specific session to allow six selected participants to present their work during a short talk.
To get any further information on the meeting, program and registration procedure, please visit our website.
We look forward to seeing you in Rennes for the 9th INYRMF congress!

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Young researcher profile

Histone methylation : a matter of generations

When one speaks about the role of epigenetic regulations in sperm cells a lot of things are to be said but probably not so much about histones. Indeed, it is well known that somatic histones are progressively replaced by protamines during the course of spermiogenesis so that only a few remain bound as nucleosomes to sperm DNA (less than 1 % in mouse). The role of this residual part is still not clear, but previous studies have shown that methylated nucleosomes retained in sperm were mostly found on CpG-rich promoters of developmentally regulated genes, suggesting a role for nucleosomes in epigenetic inheritance across generations.

In a recent publication in *Science*, two young PhD. students, Keith Siklenka from McGill University (Canada) and Serap Erkek from Basel University (Switzerland), and their colleagues confirmed this hypothesis and demonstrated that genetically-driven modification of histones can result in transgenerational phenotypic alterations. By overexpressing the histone demethylase KDM1A in the male germline, they were able to produce viable but epigenetically altered spermatozoa, in which the level of methylated nucleosomes is reduced. These subtle alterations of the epigenome, which are called “epimutations”, led to the down-regulation of several developmentally-regulated genes in non-transgenic mice of the 2nd and 3rd generations. The observation of transgenerational effects from epigenomic variations had already been detailed at the level of DNA methylation, but this is the first time that histones have been identified as a vector of such.

When asked how mild modifications of histones can lead to such severe effects, Siklenka explains that *“these alterations disrupt gene expression during a critical window of germ cell development, creating an altered pool of sperm borne RNA that may be delivered to the embryo.”* Moreover, mature sperm also carries *“alterations in chromatin state at many genes involved in embryonic development”. “The altered gene expression in the early embryo could be a combination of these two factors”.*

This study asks the question whether such transgenerational alterations of the histone code could be environmentally-driven and have an impact on human health. *“Although the study was in mouse, the biology of spermatogenesis and histone retention is rather well conserved”* said Siklenka. *“It is quite likely that natural exposures which alter sperm histones could impact the developing embryo”.* Moreover, *“upon fertilization, material brought by sperm and oocyte sets the stage for the development of an entire organism”* added Erkek. *“In this sense, any defect associated with the early development could make one individual more susceptible to diseases”.*

Besides scientific aspects, this collaborative study has been a very motivating experience for these two young researchers. *“The complementary skills offered by the two labs and the continuous exchanges of the findings made this collaboration very fruitful”.* Moreover, *“working on this model was very exciting because it revealed us a lot about mechanisms underlying embryonic development”*, said Erkek and Siklenka, even if they confess *“they both went into the field of reproduction by accident”.* Definitely, a happy accident!

Reference : **Disruption of histone methylation in developing sperm impairs offspring health transgenerationally**. Siklenka K, Erkek S, et al.; *Science. 2015 Nov 6;350(6261)*

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Press Highlights

Recent developments in human reproductive risk assessment of endocrine disruptors: The effect of paracetamol on fetal testis.

The concept of endocrine disruptors (ED) was proposed after various reproductive function alterations in wildlife were linked to the intensive use of pesticides in agriculture and industrial chemicals. In humans, similarly, an increase in incidence of male reproductive disorders, such as cryptorchidism, hypospadias, low sperm count and testicular germ cell cancer, was observed. These disorders have been hypothesized to be the expression of one common underlying disorder, the testicular dysgenesis syndrome that arise during fetal life. Thus, the (human) fetal testis is a very relevant target to study the ED effects on male reproductive function using in-vitro or in-vivo systems.

In a recent publication from van den Driesche and colleagues (1) human fetal testes were exposed to clinically relevant doses and regimens of paracetamol (also known as acetaminophen) using a xenograft model. Paracetamol is the most commonly used analgesic worldwide and taken by ±50% of pregnant women. Nonetheless, maternal use of paracetamol and subsequent cryptorchidism in male offspring have been linked in several independent epidemiological studies. In this study, the authors showed that exposure to a human-equivalent therapeutic regimen of paracetamol for 1 week significantly reduced plasma testosterone and seminal vesicle weight in castrated host mice harbouring human fetal testis xenografts, whereas a 1 day treatment did not cause such alterations. These were intriguing observations given that plasma paracetamol concentrations at one hour after the final dose in exposed mice did not even reach those measured in humans after a therapeutic oral dose. Additional in utero exposure studies in rats were performed and revealed that the paracetamol-induced reduction in testosterone likely results from reduced expression of key steroidogenic enzymes (*Cyp11a1, Cyp17a1*). The authors concluded that a clinical relevant regime of paracetamol may suppress fetal testosterone production. Their results, obtained by the xenograft approach, contrast with a study that investigated the effects of paracetamol on human fetal testes in an organotypic in vitro culture system and found no effects on testosterone production after 24 to 72 hours of culture. They explained this discrepancy can be due to the difference in age of fetal testes used or caused by the limitations of the in-vitro conditions and the lack of concordance with the in-utero situation, although a recent comment was posted arguing the latter (2). In contrast, the authors believe the xenograft model nicely reflects physiological development and hence is a better alternative to study the effect of chemical exposure on human fetal testicular development. However, the xenograft model itself has several limitations: it is more time-, money- and animal-intensive and displays a higher variability. Furthermore, the metabolism of the chemicals in the rodent host can differ from that in humans.

Regardless the discussion on research models both camps agreed on advising a pragmatic approach that may involve the avoidance of protracted use of paracetamol during pregnancy where possible.

Reference: 1) van den Driesche S, et al. (2015). **Prolonged exposure to acetaminophen reduces testosterone production by the human fetal testis in a xenograft model,** *Sci Transl Med* 7, 288ra80.
2) Bernard J (2015). **Paracetamol-induced endocrine disruption in human fetal testes**, *Nat Rev Endocrinol* 11, 453-4.

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Tête-à-tête with experts: John Aitken

John Aitken's research career began with a PhD in reproductive biology from the University of Cambridge under the supervision of RV Short. Following post-doctoral positions at the Institute of Animal Genetics, University of Edinburgh and the University of Bordeaux, he joined in 1977 the Medical Research Council's Reproductive Biology Unit, University of Edinburgh, to establish a research group in gamete biology with clinical outreach into male infertility. In 1992, John was awarded an Honorary Professorship within the Faculty of Medicine of Edinburgh University and in 1998 he received an ScD degree from the University of Cambridge in recognition of his research contributions to gamete biology. In the same year he moved to the University of Newcastle, NSW, as Chair of Biological Sciences and, later, Director of the ARC Centre of Excellence in Biotechnology and Development. He is currently Pro Vice Chancellor of the Faculty of Health and Medicine, Laureate Professor of Biological Sciences, and Co-Director of the Priority Research Centre in Reproductive Science.

http://www.newcastle.edu.au/profile/john-aitken

When and why did you decide to work in Male Fertility?

I did not start out in life wanting to become an andrologist. On leaving school at the age of 16 my first choice of a career was in real estate – the family business. After a few painful months I realized that this was not the career for me and decided to go back to school […]. My first choice of subjects to study at advanced level were Art, English and Geography but my headmaster convinced me that the only subject that I really excelled in was Chemistry and I should combine this with Botany and Zoology to make up the trio of subjects conventionally taken at this advanced level […]. I threw myself into these subjects with gusto and, three A-levels later, was destined for the University of London to take a BSc in Zoology, for no better reason that this was the swinging 60s and London was in full swing.

During the course of this degree, I found the embryology component of our curriculum was the most interesting. While the descriptive components of early embryonic development were well established, we still had very little idea of the underlying mechanisms that determined cell position and fate during embryogenesis. Thus at the conclusion of my BSc, I decided to undertake a Masters degree in Embryology and Mammalian Reproduction at the University College of North Wales, instigated by one of the pioneers of reproductive science, FW Rogers Brambell […]. Towards the end of this degree I wrote a speculative letter to another giant in reproductive science, Dr Roger Short who, at the time, was a Reader in the Department of Veterinary Clinical Studies at the University of Cambridge […]. Roger applied for a PhD scholarship from the Medical Research Council (MRC) to study embryonic diapause in the roe deer (*Capreolus capreolus*) and, to my great delight, the application was successful. Suddenly, I found myself catapulted from complete intellectual obscurity to 1970's Cambridge, which, at the time, was an international hot bed of reproductive research. […] I have very vivid memories of this time, when reproductive science was in its prime and the characters who drove it were larger than life. Roger Short in particular was, and still is, the most inspirational mentor that anyone could have wished for. After Cambridge, I secured a position at the University of Edinburgh to conduct research on blastocyst implantation with another colossus of reproductive science, Anne McLaren […]. Following a further post-doctoral fellowship at the University of Bordeaux and a very instructive year with the World Health Organization's Human Reproduction Unit, […] I had been recruited to the MRC Reproductive Biology Unit to work on the biochemistry of embryo implantation.

However, after spending several months in gynecology wards hoping that someone would drop the odd milligram endometrial tissue into my stainless steel bowl, I finally came to the realization that if I was to make any headway at all in reproductive medicine, I needed to find a cell type that I could access directly, without depending on the largesse of my clinical colleagues. As a direct response to this need for clinical material I took my first faltering steps into andrology with the encouragement of Roger Short and one of the leading figures in modern laboratory andrology, David Mortimer.

At this time, I was joined by Edwina Rudak from Pat Jacobs' laboratory in Hawaii and she introduced me to the zona-free hamster oocyte penetration test, introduced by another scientific giant, Ryuzo Yanagimachi. Yana's endless energy, powers of observation and spirit of inquiry had identified this heterologous in vitro fertilization assay and I focused on developing its clinical application; not so much as a diagnostic test (it was far too complex a procedure to be used in routine clinical practice) but as a bioassay that would allow us to investigate the molecular mechanisms that regulate sperm function. I reasoned that if we understood, at a biochemical level, why the spermatozoa generated by infertile males have lost their competence for fertilization, then we could use this information to gain insights into the etiology of this condition and possibly even develop some rational therapeutic strategies. In this venture I was aided by a team of highly talented research assistants and inspired by some brilliant clinicians and scientific colleagues. I remember being particularly stimulated by a single lecture I attended at an unforgettable meeting in Taormina organized by my close friend and colleague, Giancarlo D'Agata. This paper was given by Vide Hansson from the University of Oslo and addressed the biochemical mechanisms underpinning spermatogenesis. Following this talk, I was absolutely certain that the way forward was to define defective sperm function in biochemical terms; the rest of my life has been spent trying to achieve this solitary aim.

Can you name the greatest success(es) in your career?

The greatest successes of my career have been where the above strategy has borne fruit. Undoubtedly, my greatest single success has been to reveal the role played by oxidative stress in the etiology of defective sperm function. Unknown to me at the time, Thaddeus Mann, had highlighted the vulnerability of spermatozoa to oxidative stress in a series of classic papers he published with Roy Jones in the 1970s, largely on large domestic animals. Quite independently, I discovered the same vulnerability in a clinical setting and set about defining the source of the reactive oxygen species and their impact on specific aspects of sperm function. Ultimately, this approach has led to the development of a set of diagnostic tools to measure oxidative stress in the male germ line and the formulation of antioxidants, which appear to be efficacious. As a consequence there are thousands, if not hundreds of thousands, of subfertile males now taking antioxidant therapy in the hope that it will improve their chances of conception. One day I hope we shall secure definitive evidence to support such intervention […]. Giving all infertile males antioxidant therapy is a dangerous and wasteful activity.

Another area where we have successfully defined a biological defect and then resolved its biochemical basis, concerns cases of infertility where motile spermatozoa fail to exhibit an affinity for the zona pellucida. In a collaborative project with my colleague, Brett Nixon and PhD students, Kelly Asquith, Kate Redgrove and Elizabeth Bromfield, we have demonstrated that such functional defects are commonly associated with a loss of the molecular chaperone HSPA2 from the sperm proteome […]. A third area of success involved my collaboration with Mark Baker, a talented biochemist who joined my group around a decade ago to establish an advanced proteomics facility, which has revolutionized the study of sperm cell biology […] and opened up completely new avenues of research that would have otherwise remained unappreciated and unexplored.

Can you name a moment of failure and explain which lesson you learned from it?

Happily, there have not been too many outright failures. One that immediately comes to mind is known in my laboratory as the Yun-factor, after a very talented PhD student (Yun-Hwa Lee) who isolated a soluble factor from the caput epididymis that can reversibly silence mitochondrial membrane potential in spermatozoa. I have expended the lives of several technicians and students on the molecular characterization of this factor but have still not resolved its chemical composition. If there are any postdocs or potential PhD students out there, who are ready for a challenge, then this is the project for you! It would be a very powerful discovery if we could nail it.

Which advice(s) would you give to young researchers in Male Fertility?

I advise all of the young recruits to our laboratory that they are about to embark on a career with an extremely high failure rate. With grant success hovering around 10% in most developed countries, a large number of talented individuals are bound to lose out. The survivors will exhibit a variety of attributes and those that seem the most important to me are listed below.

Be creative. Clearly the most successful scientists, in andrology or any other branch of science, have to be intensely creative if they are to be successful. One of the hallmarks of creativity is the capacity to generate novel insights and this, in turn, is achieved by the juxtapositioning of disparate pieces of information in order to create a novel synthesis that open up new avenues of exploration (see Arthur Koestler's *The Act of Creation* for anyone interested in the psychological origin of ideas).

Read voraciously. With the internet bringing the world's scientific literature to your desktop there can be no excuse for not being well informed not just about your immediate field of interest but in the fundamentals that underpin our understanding of cell biology and biochemistry.

Be relevant. The landscape of biomedical research is changing rapidly. No longer can we pursue topics just because they seem intellectually interesting. In the new world we have to address a pressing issue of relevance to society and, for andrologists, this will be in the areas of animal production, reproductive oncology, infertility and contraception […].

Be resilient. Scientific research is a difficult career to pursue not least because the road ahead contains as many disappointments as it does successes. The key is to be sufficiently bolstered by the successes to accept the disappointments with equanimity and grace. There will be many times when you will receive a broadside from the lumbering galleons of mediocrity and great ideas will be unreasonably criticized and […] equally, there will be times when the criticisms are justified and you have to admit you were wrong.

Exercise humility. A really positive feature of successful laboratories is that the individual members feel secure enough to be able to present ideas and criticism without fear of retribution. Surrounding yourself with colleagues who are able to objectively comment on your ideas without malice or too much enthusiasm, is one of the keys to a successful career in science.

Seize every opportunity to promote your ideas. A key element of a successful career is be a powerful advocate for your laboratory and the ideas it embraces. It does not matter whether the target audience is academia, the health service, funding agencies or industrial companies, it is invariably critical that you are able to compose a compelling narrative that elicits support from all these sources. It is also essential that you actively engage with these constituencies - do not just wait for the world to come to your door.

With these thoughts in mind, I wish all of you embarking on a scientific career in andrology all the very best of fortune. Although this is a road less-travelled, our field is still in it is infancy and there is still a lot of exciting territory to discover!

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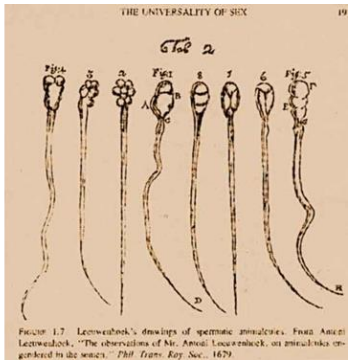
Memoirs: Antonie van Leeuwenhoek - the discoverer of spermatozoa!



Pictures from left to right: Painting of Leeuwenhoek by Jan Verkolje (Rijksmuseum, Amsterdam), Replica of the microscope used by Leeuwenhoek (Museum Boerhaave, Leiden), Leeuwenhoek's drawings of different types of sperm cells. (Originally published in: "The Observations of Mr. Antoni Leeuwenhoek, on animalcules engendered in the semen," Philosophical Transactions of the Royal Society of London, 1679). Sources: http://www.vanleeuwenhoek.com/ (left), http://patrickmurfin.blogspot.be/2015/09/the-dutchmans-gizmo-and-invisible.html (middle), https://museumboerhaave.wordpress.com/2011/07/29/replica-van-leeuwenhoek-microscope-english-version/ (right),

The name **Antonie van Leeuwenhoek** (1632-1723) is often linked to the rudimental microscopes that allowed the first observations of cells and organisms. He is therefore considered to be the father of microscopy and the precursor of microbiology.

Antonie van Leeuwenhoek was born in Delft, The Netherlands. His father was a basket maker and his mother's family were beer brewers. Antonie started his career as a merchant and only began his scientific ventures, for which he was entirely untrained, at the age of 39. At this time, he constructed the first of his simple microscopes with magnifying power 270 and a resolution of 1.4 micron. No one made better instruments until the 19th century! By means of this invention, he made numerous discoveries. He was the first to describe protozoa, bacteria, the histology of teeth, etc. He was also the first to conduct rigorous observations on sperm. In fact, a medical student, Johan Ham, told him he has seen "animalcules" in human seminal fluid that had been generated by putrefaction. Leeuwenhoek, however, supposed them to be a normal component of semen and made the first detailed description of spermatozoa of many species, including man. From his beautiful drawings one can perceive that van Leeuwenhoek could recognize the existence of sperm subpopulations with distinct morphology!

Despite his lack of scientific education, he always presented his results without confusing facts with his speculations. His style was and is still considered as a model for all researchers.

References: 1) Houtzager HL (1983). **Antonie van Leeuwenhoek**, *Europ J Obstet Gynec reprod Biol* 15, 199-203.
2) Karamanou M et al. (2010). **Anton van Leeuwenhoek (1632-1723): Father of micromorphology and discoverer of spermatozoa**, *Revista Argentina de Microbiología* 42, 311-314.

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Highlighted websites

INYRMF's new website

Check out the new layout of our website: ***www.youngresearch.eu***

Scientific colloquia: mark your calendar!

Future fertility for the male child and adolescent with cancer: best practice, research breakthroughs and current dilemmas Münster (Germany), 13th – 14th May 2016 ***www.eshre.eu***	Deadline! 12th of April 2016
SSR 2016 Annual Meeting - Systems Biology of Reproduction San Diego, California, USA, 16th-20th July 2016 ***www.ssr.org/16Meeting***	Deadline! 15th of February 2016
41st meeting of the American Society of Andrology New Orleans, Louisiana, USA, 2nd-5th April 2016 ***www.andrologysociety.org***	Deadline for Abstract! 17th of January 2016
Annual Meeting of the European Society for Human Reproduction and Embryology Helsinki (Finland), 3rd – 6th July 2016 ***www.eshre.eu***	Deadline for Abstract! 1st of February 2016

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Frontiers in Reproduction (FIR) Course. Molecular and Cellular Concepts and Applications.

Woods Hole , Massachussets (USA), 30th April – 12th June 2016

www.mbl.edu/fir/

Deadline!

13th January

The *Frontiers in Reproduction* (FIR) course is "an intensive 6 week laboratory and lecture course for scientists-in-training who seek to improve their knowledge and experimental skills in order to pursue a career in the Reproductive Sciences." Since FIR is supported by funding agencies, attendees only have to pay a small part of tuition and accommodation costs.

FIRbees (alumni) testimonies:

I have taken this course in 2009 and I strongly recommend any young scientist wanting to pursue a career in Biology of Reproduction to apply for it!!! It is really worth it!!! During those 6 weeks I have not only learned novel concepts and new techniques, but I have also acquired new skills and amplified my contact network. And the best of it is that I did all that while having great fun! The environment is completely informal and all the staff involved (directors, teachers, secretary, etc.) is very friendly and keen to help you in whatever they can. This is a unique opportunity to spend some weeks in a "summer camp" learning science with experts and drinking beer with new friends. When I think about the time I spent in Woods Hole, loads of good memories come to my mind!
Alexandra Amaral (Firbee 2009)

I attended the Frontiers in Reproduction Course in 2014, just before finishing my PhD. During those 6 weeks I increased my knowledge in different aspects of reproduction biology, I learned techniques, I met tens of top scientists working in the field, and I shared hundreds of unforgettable moments with people from the world. It was a great experience which has certainly helped me in the next steps of my research career. Frontiers in Reproduction is a high-quality course, given by high-quality professionals, in a splendid place as it is the Marine Biological Laboratory in Woods Hole. I strongly recommend it to all those young researchers working in reproduction, especially for those that, as it was my case, are in the last year of their PhD. I am really proud to say: I am a Firbee!!
Judit Castillo (Firbee 2014)

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Jobs Ads & Funding Prospects

PhD position

Rep-Biotech Joint Doctoral project Marie-Skłodowska Curie Innovative Training Network funded by the European Commission under the Horizon 2020 Programme.

15 3-year PhD positions (2016-2019) in Biology and Technology of Reproductive Health (human and animal).
12 leading academic research groups and 3 companies from 9 different countries: Spain, France, Ireland, Italy, Belgium, Germany, USA, Japan and The Netherlands.

Deadline: 31st December, 2015

www.um.es/rep-biotech/

Post-doctoral positions

Personalized Medicine, Sperm Nuclear Structure & Computational Genetics

Laboratory of Dr. Stephen Krawetz
Wayne State University, Detroit, MI, USA

compbio.med.wayne.edu/positions.html

PhD/Post-doctoral positions

Influence of environmental exposures on the heritable information in the sperm epigenome

Laboratory of Dr. Sarah Kimmins
McGill University, Montreal, Canada.

www.mcgill.ca/csr-cer/about-us/available-positions

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DGA Deutsche Gesellschaft für Andrologie e.V.