International Network for Young Researchers in Male Fertility

Spring edition - 2016

INYRMF Newsletter

In this issue:

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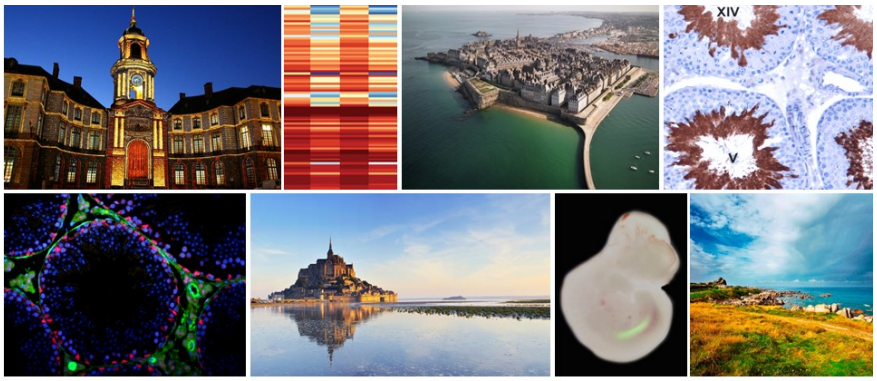
Greetings

Dear INYRMF member,
The days are becoming longer, it is getting warmer outside and the first blossoms are flourishing. Spring has finally arrived! And for our Spring edition, we offer you a controversial discussion about *in vitro* generation of mouse sperm, information about the paternal contribution to the offspring, the experience of a real spermatogonial stem cell expert about his career, a story about the father of *the mother cell* and useful information about colloquia, careers and funding. Enjoy your reading!

Cheers,
The INYRMF board

INYRMF Bulletin

We are about 2 months away from our next INYRMF meeting!
It will be held in Rennes, France, on the 9th and 10th of June 2016 preceding the ETW2016. As we will celebrate the 10th anniversary of our network, we will combine a series of presentations from experts in our field with more general issues and round table discussions. We are happy to announce that we will not only select six abstracts for short oral presentations, but will also give free registration for the ETW to 3 PhD students out of the selected talks. Furthermore, the best speaker will receive a prize sponsored by the International Society of Andrology. This is really exciting and we are looking forward to see you all in June! For more information about the program, the registration and abstract submission deadlines, please see our website: www.youngresearch.eu



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Press Highlights

In vitro generation of mouse spermatid-like cells able to produce viable offspring

Scientists have long struggled to produce functional sperm cells from pluripotent stem cells. Reproducing germ cell development *in vitro* could not only help couples suffering from infertility but also be used to study the genetic, epigenetic and environmental factors involved in spermatogenesis. In a recent *Cell Stem Cell* article, the groups of Xiao-Yang Zhao, at the Southern Medical University in Guangzhou, and Qi Zhou, at the Institute of Zoology in Beijing, together with colleagues from Nanjing Medical University show results of a successful production of mouse spermatid-like cells that are able to produce viable and fertile offspring when injected into oocytes. This is the first study to show the occurrence of meiosis, including chromosome synapsis and recombination, in developing sperm cells *in vitro*.

To generate primordial germ cell-like cells (PGCLCs) the groups utilized a method previously published by Hayashi *et al.* (2011) where incubation of epiblast-like cells in a medium supporting differentiation led to the production of PGCLCs. Thereafter, they cocultured the cells with mouse neonatal testicular cells that stimulated initiation of meiosis by allowing retinoic acid signaling. Successful generation of haploid spermatids was determined according to "the golden standards" of meiosis, including erasure of imprints, chromosome synapsis and recombination. Postmeiotic germ cell differentiation was achieved by combined exposure to testosterone, follicle-stimulating hormone (FSH), and bovine pituitary extract (BPE). This resulted in production of haploid small round spermatid-like cells that contained a cap-shaped acrosome structure but lacked the elongated tail of spermatozoa. Viable offspring was produced from the spermatid-like cells by intracytoplasmic sperm injection. The number of pups generated from these cells were lower than that of control animal sperm. However, the adult offspring were fertile and able to produce pups of their own.

Although scientists in the field of male reproduction find these results encouraging, there are still some scepticism as to how similar the artificially produced spermatids are to those produced in the mouse testis. In a recent article published in *Nature*, some scientists claim that the short time of development (14 days compared with 4 weeks in mice) would not be enough for the primordial germ cells to go through all steps required for proper development. Others question the health of the offspring; although viable and fertile at 15 months of age, more tests are needed to exclude any defects that could arise later in life.

The current objective is to produce human spermatids in a similar manner. However, there may still be some obstacles to overcome before this goal can be achieved, not only the ethical aspects but also regarding differences in spermatogenesis between human and mouse.

References:

1) Zhou Q, Wang M, Yuan Y, Wang X, Fu R, Wan H, Xie M, Liu M, Guo X, Zheng Y, Feng G, Shi Q, Zhao XY, Sha J, Zhou Q (2016). **Complete Meiosis from Embryonic Stem Cell-Derived Germ Cells In Vitro.** *Cell Stem Cell*. Mar 3;18(3):330-340

2) Cyranoski D (2016). **Researchers claim to have made artificial mouse sperm in a dish.** *Nature*. 25th of February

Paternal inheritance through sperm tRNA fragments

In recent years, an increasing number of studies have shown a link between paternal nutrition and the health of offspring. Two independent studies, published earlier this year in *Science*, report that this inheritance may be imparted by short tRNA fragments (tRFs). Mice subjected to a low-protein or high-fat diet displayed changed amounts of tRFs in their sperm. Furthermore, injection of sperm tRFs into zygotes caused an altered gene expression of embryos.

Generation of tRFs from mature tRNAs is often observed in cells after oxidative stress or starvation. No longer thought to be just by-products of tRNAs, tRFs are now known to affect gene expression and translation in a similar manner to other small RNAs, by binding to target DNA or mRNA sequences. Oliver Rando's group at the University of Massachusetts, together with collaborators, showed that male mice produced altered tRF levels in their reproductive tract after being fed a low-protein diet. This was also reflected in sperm, as deep sequencing of small RNAs showed a strong correlation between up-regulation of specific 5' tRFs in the epididymis and the levels of tRFs in sperm, indicating incorporation of tRFs during sperm maturation. To study how this would affect the offspring, they utilized antisense oligonucleotides to inhibit tRFs in embryonic stem cells. Interestingly, they only observed a change in gene expression after inhibition of one specific tRF, 5' tRF-Gly-GCC. The ~70 genes which displayed increased expression after tRF-Gly-GCC inhibition were regulated by the long terminal repeat of the endogenous retroelement MERVL. It is estimated that around 3% of all mRNAs produced in the two-cell stage embryo are regulated by MERVL elements that function as gene promoters. In blastocysts, expression of MERVL regulated genes subsides as the embryo starts to differentiate. Rando's group further showed that IVF using sperm from low-protein fed males led to reduced expression of MERVL regulated genes in preimplantation embryos when compared with sperm from control males. Similar results were also achieved by injection of sperm small RNAs or tRF Gly-GCC into zygotes.

In the second study, groups from the Chinese Academy of Sciences in Beijing and Shanghai showed changed levels of tRFs in sperm from mice fed a high-fat diet. These tRFs were also found to bind to promoter regions of target genes rather than coding regions. Furthermore, injection of sperm tRFs from high-fat diet males into zygotes produced offspring which developed glucose intolerance. The altered gene expression observed in the pancreatic islets of the F_1 generation was attributed to a down-regulation in the expression of genes involved in metabolic pathways already present in the preimplantation embryo. These results further emphasize the long term effect of paternally transferred small RNAs.

Although some of the paternal diet effects in offspring could not be explained by tRFs alone, the studies show a clear link between the sperm epigenome and metabolic defects in offspring. As tRFs have also been found in sperm of other species, these results could have broader implications.

References:

1) Sharma U, Conine CC, Shea JM, Boskovic A, Derr AG, Bing XY, Belleannee C, Kucukural A, Serra RW, Sun F, Song L, Carone BR, Ricci EP, Li XZ, Fauquier L, Moore MJ, Sullivan R, Mello CC, Garber M, Rando OJ (2016). **Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals.** *Science.* Jan 22;351(6271):391-6.

2) Chen Q, Yan M, Cao Z, Li X, Zhang Y, Shi J, Feng GH, Peng H, Zhang X, Zhang Y, Qian J, Duan E, Zhai Q, Zhou Q (2016). **Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder.** *Science* Jan 22;351(6271):397-400.

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Tête-à-tête with experts: Stefan Schlatt

Professor **Stefan Schlatt**, PhD, has gained experience in male reproductive research for more than 25 years exploring the genetic, cellular and physiological mechanisms of testicular development and function. Since 2008, he is the Director of the Center of Reproductive Medicine and Andrology (CeRA), the largest Andrology centre in Germany. His research focus is on preclinical research towards male fertility including disturbances in spermatogenesis, development of male contraceptive approaches, *in vitro* spermatogenesis and the physiology and application of spermatogonial stem cells.

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

In 1988 when I was looking for a place to do my diploma thesis, I spontaneously decided to knock on the door of Prof. Nieschlag at the Institute for Reproductive Medicine. My reason for choosing this field was the scientific courses on ethical responsibility in reproductive medicine I had taken during my former studies in Catholic theology. As I had already worked on this subject from the theological/ethic point of view, I hoped to find something on this line in Prof. Nieschlag's group. So I actually ended up on ethics in reproductive medicine.

During my diploma thesis I examined the reproductive cycles of female hamsters, which was a largely unexplored subject at that time. Afterwards, I got offered a PhD thesis position to investigate the newly discovered hormone inhibin in the reproductive organs of male monkeys. As I had always been quite critical of working with primates I really had to think it through and at first I found it very difficult. Luckily I did not have to work directly with the monkeys themselves during my PhD studies, but could make use of a large collection of histological materials. In addition, I also participated in studies on male contraception, which were always carried out on monkeys. And so I ended up in primate research despite my initial skepticism and reservations. After my PhD, I was already infected by the 'virus' of male fertility and I started to develop my own research interests in the field. Therefore, I applied for a scholarship and went for 2 ½ years to Monash, Australia to study human testicular spermatogonia.

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

The first thing that comes to mind is maybe the high impact factor papers we have published. We once had a Nature paper on Grafting published in 2002, which was quite exciting because it was just a technological breakthrough. However, I am even more proud of my former doctoral candidates and what they have achieved. I have met many a great young person with whom I have been able to share my enthusiasm for research. Of course it is nice to see those that continue doing research, but I am also proud if my former students end up as successful directors of IVF laboratories or lead departments in the industry. The important thing is that they can say that they have enjoyed a good education and are happy with what they are doing now.

It is always difficult to highlight only one or two things. Every place where I have been (Münster, Monash, Philadelphia or Pittsburgh) has had some particular line of expertise that has been outstanding. Perhaps I was quite good at bringing together the available expertise at each place. That is why the greatest successes I have achieved scientifically have always been collaborations. It was only in this way that we managed to write a Nature paper in Philadelphia within one year. We just connected people who all pulled together, and that is something I can be proud of.

Can you name a moment of failure (and which lesson did you learn with it)?

Not only one and there are still moments of failure. One thing is important, especially for young people: those who want to start a scientific career must be willing to accept uncertainty. The road from a PhD to a reliable career is highly competitive and thus incredibly challenging. For example, I had a great time in Monash but after 2 years it was over and I had nothing new. I called the Wellcome Trust scholarship agency at 3 am and asked them to make a decision whether or not we would get a renewed scholarship in Australia or if we were out of work the next week. That was really difficult especially since reproductive medicine is not a discipline like oncology where hundreds of jobs are available. When you have three kids and a wife these are not great moments, but that's part of it. You just do not get a permanent position immediately but transition from scholarships to a 3-year or 1-year financial stability, and therefore you must have faith. Anyone who is not willing to live this way is not made for science. My family was certainly not happy to do the transcontinental move 6 times, but that was the price you had to pay to get a reliable career at the end. And from today's perspective it's ok.

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

For whom safety is essential, the academic career is not the right thing. But those who can engage in risk and are creative enough to handle difficult situations can make it. I think it is currently easier than a few years ago. More biologists are needed again, more people are needed in the pharmaceutical industry, and there are simply more opportunities. But those who want to end up in academic research must make an effort. You need to be determined and flexible. There is not only one path, but there are 20 and you have to watch for new opportunities without switching randomly. I can only recommend seeking a mentor who you can trust. That helps tremendously. Of course, one can have several mentors or change mentors. If possible, you should talk to the mentors about your career options or exchange ideas with like-minded people. It always makes sense to hear how someone else has made it. All careers are individual careers and everyone must follow their individual way to reach its destination. It may help to avoid becoming too specialized in the beginning. There are so many options and you have to realize what you are good at, what you enjoy, what you can imagine yourself pursuing and then qualify yourself specifically in that area. And especially leave roads you do not like and do not keep all options open. Do not be too specialized, but also not too little.

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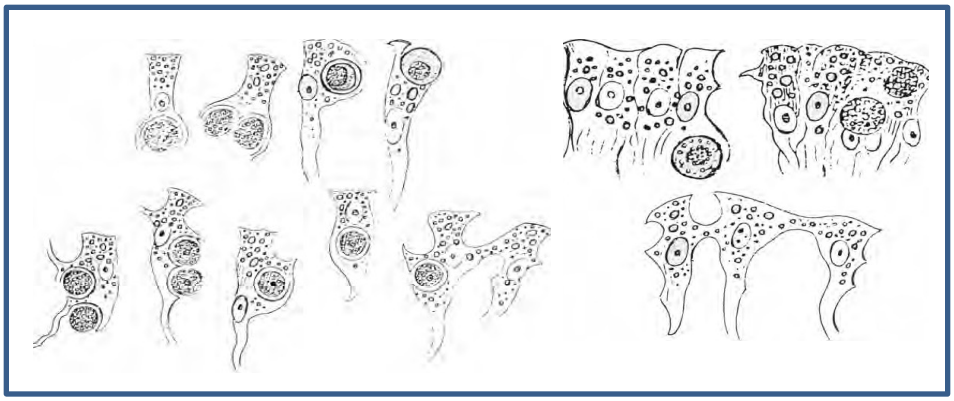
Memoirs: Enrico Sertoli – and the "mother cell" of the testis

Enrico Sertoli (source: reference 3)



Drawings taken from the original paper of Sertoli (reference 1: Figs. III a–d, II a–e, V and V a–b)

Enrico Sertoli (1842 – 1910) was born in Sondrio and began his study of medicine at the University of Pavia. There he worked alongside such individuals as Giulio Bizzozzero, the discoverer of platelets, and Camillo Golgi, a future Nobel Prize winner. After graduating at the age of 23, Sertoli continued his studies of physiology at the University of Vienna (Austria) before he returned to Italy for military service 1 year later. After leaving the military in 1867, Sertoli obtained an appointment as an assistant in the Laboratory of Physiology in Tubingen, Germany. From 1870, when only 28 years old, he worked as a Professor of Physiology in the Department of Anatomy and Physiology of the Secondary School of Veterinary Medicine in Milan. Shortly thereafter, Sertoli became the director of the Institute of Physiology where he worked on the duration of the excitability and heat sensibility of muscular fibers.

In 1865, his most important paper reported his histologic discoveries regarding the seminiferous tubules with the title: "Dell'esistenza di particolari cellule ramificate nei canalicoli seminiferi del testicolo umano" (About the existence of special, branched cells in the seminiferous tubules of the human testis). He demonstrated previously unseen, branching, tree-like cells with the bases of their "trunks" abutting the inner wall of the seminiferous tubules. Sertoli first described the non-spermatogenic cells of the seminiferous tubules as "cellule madri", or "mother cells" and drew with intricate detail what he observed through his microscope.

After 37 years of scientific study, Sertoli ended his career and retired to Sondrio, where he died in 1910. He never married and had no known children, but he is clearly known as the **father of *the mother cell* of the testis.**

References: 1) Sertoli E (1865) **Dell'esistenza di particolari cellule ramificate nei canalicoli seminiferi del testicolo umano**, *Morgagni* 7: 31–33. 2) Giorgio M. Baratelli, Alessandro Lanzani, And Russell N. Sacco (2002), **Biography Of Enrico Sertoli**, *Urology* 60: 196–198. 3) Wessel GM (2011) **Accessorizing the testis. Enrico Sertoli and the "mother cell" of the testis**. *Mol Reprod Dev*. 78. (3).

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Scientific colloquia: mark your calendar!

19th European Testis Workshop

Palais du Grand Large of Saint-Malo (France), 11– 15 June 2016

www.cfas.ca

Early bird rates extended!

9th European Congress of Andrology

Rotterdam (Netherlands), 21 – 23 September 2016

www.ecacongress2016.com

Four EAA travel grants available (2 of 700 € and 2 of 500 €) application deadline June 20th

Deadline for abstracts: 1 May 2016

American Society of Reproductive Medicine Scientific Congress and Expo

Salt Lake City (USA), 15-19 October 2016

www.asrmcongress.org

Registration opens April 2016

PRE-CONFERENCE YOUNG SCIENTIST SYMPOSIUM
13th Congress of the International Society for Immunology of Reproduction and the European Society for Reproductive Immunology

Erfurt (Germany), 22-25 June 2016

www.isir2016.de/young-scientist-symposium.html

Future fertility for the male child and adolescent with cancer: best practice, research breakthroughs and current dilemmas

Münster (Germany), 13-14 May 2016

www.eshre.eu/Education/Campus-events/

Course

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Jobs Ads & Funding Prospects

Three EAA travel grants for EAA accredited courses (of 500 € each) deadline June 20th and October 1st 2016
www.andrologyacademy.net/grants.aspx

Travel grant!

Postdoctoral Fellowship

A Postdoctoral position at Yale School of Medicine, USA.
Studies on ion channels and accessory subunits that regulate sperm motility and fertility in mammals.
http://www.jeanjuchunglab.org/news/postdoc-position

Research Fellow

University of Leeds, United Kingdom
Research fellow to perform electron microscopy studies on the extracellular filaments of the male accessory gland/prostate across the animal kingdom
Deadline: **May 13th 2016, *naturejobs.com***

Postdoctoral Fellowship

Two Postdoctoral fellowships at Linköping University, Sweden.
Studies on the epigenetic inheritance of metabolism in Drosophila.
Deadline: **May 22nd 2016, *naturejobs.com***

PhD positions

CELLS IN MOTION Cluster of Excellence Graduate School

International Max Planck Research School Molecular Biomedicine and Cells in Motion Graduate School

Joint PhD program of the University of Münster and the Max Planck Institute for Molecular Biomedicine

16 PhD Positions in Münster (Germany): Imaging Cellular Processes and Disease

The joint graduate program of the **Excellence Cluster Cells in Motion** (CiM) and the **International Max Planck Research School – Molecular Biomedicine** (IMPRS-MBM) offers positions to pursue PhD projects in the areas of **biology, chemistry, physics, mathematics or computer science**. We are looking for young scientists with a vivid interest in **interdisciplinary** projects to **image cell dynamics** from the **subcellular to the patient level**. PhD projects range from the analysis of basic cellular processes to clinical translation, from the application of novel biophysical approaches and the generation of mathematical models to the development of new imaging-related techniques and compounds.

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