International Network for Young Researchers in Male Fertility

Winter edition - 2017

INYRMF Newsletter

In this issue:

INYRMF Board:

Main responsible for this issue:

Judit Castillo
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Greetings

Dear INYRMF member,

Although the new year 2017 started with unusual low temperatures, that was not enough to freeze the research advances in male fertility. As a prove, we bring you a new edition of the INYRMF Newsletter with plenty of hot topics to endure the last weeks of the coldest season of the year.
We start this edition with the announcement of remarkable changes in the network. Furthermore, we highlight the identification of regulatory elements in differentiating Sertoli cells, and the role of mRNA splicing in spermatogenesis. You cannot miss the interview with the former INYRMF board member Jan-Bernd Stukenborg either, an outstanding scientist who has recently made the transition from young to senior researcher. And finally, you will also find an interesting historical tale about *in vitro* fertilization, the suggestion of an hilarious website, and a lot of important dates to mark in your calendar. Enjoy your reading!

The INYRMF board

INYRMF Bulletin

Be on guard for exciting times in 2017!

In the upcoming weeks you will notice changes to our website, social media and Newsletters. We are happy to announce that the "International Network for Young Researchers in Male Fertility" (INYRMF) will be called **"Network for Young Researchers in Andrology" (NYRA)** in the near future. A more simple and easily pronounceable name for a network that will maintain the same essence and aim: to assist young scientist in andrology in achieving their highest potential as researchers.

Also, we will soon give more details about the 10th INYRMF meeting which will take place on **September 11th – 13th 2017 in Brussels, Belgium**. Registration will be open shortly and abstracts from young researchers will be selected for oral presentation. Pay attention to our website!

10th INYRMF Meeting
Brussels, Belgium – September 11th-13th, 2017

And last, but not least, we would like to thank **Pierre and Chiara** for their excellent work as board members of INYRMF and main organizers of the last two meetings. They are leaving the board, but they will always be part of us! We wish them all the best!

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Press Highlights

Identification of regulatory element in differentiating Sertoli cells

Numerous transcriptomic assays focused on identifying the genetic program underlying sex determination. In males, sex determination starts with the transient peak of expression of *Sry* that triggers Sertoli cell differentiation. In absence of the Y chromosome, the supporting cell lineage differentiate as pre-Granulosa cells with the activation of the β-catenin pathway controlled by *Foxl2*, *Wnt4* and *Rspo1*. As we know from cell lineage tracing and knock-out experiments, Sertoli cells and Granulosa cells come from the same precursor cells and keep the ability to trans-differentiate to their opposite sex counterpart. Although many transcription factors controlling the differentiation of the Sertoli cells have been identified, their to control cell fate decision remains poorly understood.

In this study, the team of Blanche Capel conducted and integrated three whole genome sequencing approaches to study gene expression regulatory elements in Sertoli cells isolated from mouse testes at 13.5 and 15.5 days *post-coitum* (*dpc*). They performed DNaseI-seq to identify DNaseI hypersensitive sites (DHSs), ChIP-seq for H3K27ac to target active enhancer elements, and RNA-seq to quantify the gene expression.

DNAseI-seq allows the identification of accessible chromatin zones that are functionally related to transcriptional activity, comprising promoters, enhancers, silencers and locus control regions. By comparing Sertoli cell DNaseI-seq data to other tissues, they identified around 25000 DHSs specifically present in the Sertoli cells, including TESC, the *Sox9* regulatory region.

Among the Sertoli cell-specific DHSs, they identified which of them were enhancer regions by performing a chromatin profiling for H3K27ac, a strong predictor of active enhancer activity. They found that Sertoli- and pregranulosa cell-specific genes present an enrichment of accessible chromatin zones, reflecting their shared developmental origin of the supporting cell lineages. However, H3K27ac-positive DHSs were only significantly enriched in neighboring Sertoli cell expressed genes.

Further, they looked at the DNA motifs in the identified enhancer regions and predicted the transcription factors binding to these regions. They found a significant enrichment of transcription factors known to be important for supporting cell development, such as SF1, SOX8 and SOX9, and GATA4.

Finally, Capel and colleagues identified a novel distant *Wt1* enhancer with putative binding sites for SF1, SOX and FOX. They generated a transgenic β-galactosidase reporter and validated that the region is only effectively active in Sertoli cells.

To conclude, Sertoli cells present both pro-Sertoli and pro-granulosa accessible regulatory regions but only the the Sertoli specific gene neighboring regions are active, consistent with the common origin of the two cell type. Pro-Sertoli and pro-granulosa genes present cis-regulatory regions available for competitive occupancy by male- or female-promoting transcription factors. The pro-granulosa genes with adjacent DHSs might be repressed by Sertoli cell transcription factors right after sex determination to restrict their cell fate as Sertoli cells. A similar approach conducted on Granulosa cells should validate this hypothesis.

Curiosity spot!

Dax1 (or *Nr0b1*) is a gene acting as a pro-testis and an anti-testis factor at the same time. Too much DAX1 causes male-to-female sex reversal, too few disrupts testis development.

Reference:

Maatouk DM, Natarajan A, Shibata Y, Song L, Crawford GE, Ohler U, & Capel B (2017). **Genome-wide identification of regulatory elements in Sertoli cells.** *Development*, 144(4).

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Regulation of meiosis initiation during spermatogenesis by mRNA splicing

Knowing the ins and outs of the regulation of germ cell development is one of the main objectives of numerous research groups nowadays. Spermatogenesis is a highly complex process, and the identification of the main actors orchestrating its control would lead not only to increase the molecular knowledge of the testis, but also to identify new causes of male infertility, discover new targets for drug development, and find out potential critical steps for the generation of male germ cells *in vivo* and *in vitro*.

In a recent study published in *Nature Communications*, Liu and colleagues showed evidences that alternative splicing machinery regulates meiosis initiation in mouse spermatogenesis through the role of BCAS2 (Breast cancer amplified sequence 2). mRNA splicing is an important regulatory mechanism that increases the diversity of proteins transcribed from a single gene. To date, eight types of alternative splicing have been reported: cassette exon, alternative 5' splice site, alternative 3' splice site, mutually exclusive exon, coordinates cassette exons, alternative first exon, alternative last exon and intron retention. In the testes, alternative splicing of genes is involved in signal transduction at every stage of sperm production, and it is important for both testis development and spermatogenesis.

The group of Dr. Lei Li, from the Chinese Academy of Science, generated specifically *Bcas2* conditional knock-out by crossing Bcas2 *Floxed/Floxed* mice and *Vasa-Cre* transgenic mice with the recombinase specifically active in germ cells. BCAS2 is a pre-mRNA splicing factor highly expressed in mouse spermatogonia, which is involved in the conformation and activation of the spliceosome. Interestingly, disruption of BCAS2 in murine germ cells led to infertile males with reduced testis size and weight, and no presence of post-meiotic cells. Further analyses of the meiotic stage of the knock-out testes showed reduced expression of γH2AX (meiotic recombination marker) and SCP3 (synapsis marker). In addition, no leptotene, zygotene and pachytene spermatocytes were detected in BCAS2-depleted seminiferous tubules, while the effect in the proliferative phase of sperm development was almost undetectable.

By a smart combination of RNA sequencing and analysis with the Alternative Splicing Detector (ASD) software, the authors performed an in-depth examination of the splicing profile of the conditional knock-out testes. A total of 279 alternative splicing events were identified as significantly deregulated, the major part of them corresponding to exon-skipping. Interestingly, alternative splicing of 11 transcripts from genes involved in sexual reproduction was affected in BCAS2-depleted mice, which remarkably includes the meiosis-promoting factor *Dazl.* In particular, the expression of the isoform DAZL-FL (full length) was found dramatically down-regulated in knockout mice, while the levels of DAZL-Δ8 variant (short form, lacking exon 8) were increased in presence of low levels of BCAS2.

With this research, Liu and colleagues showed that pre-mRNA splicing control by BCAS2 is essential for the initiation of meiosis prophase I in male mouse germ cells.

Curiosity spot!

The organs with the highest levels of alternative splicing in the body are testes, liver and brain.

Reference: Liu W, Wang F, Xu Q, Shi J, Xhang X, Lu X, Zhao Z, Gao Z, Ma H, Duan E, Gao F, Gao S, Yi Z, Li L. (2017) **BCAS2 is involved in alternative mRNA splicing in spermatogonia and transition to meiosis.** *Nature communications*, 8:14182

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Tête-à-tête with Jan-Bernd Stukenborg

Dr Jan-Bernd Stukenborg is senior research specialist and associate professor at the Department of Women's and Children's Health of the Karolinska Institutet and University Hospital of Stockholm, Sweden. Since he was a PhD student at the Centre of Reproductive Medicine and Andrology in Münster (Germany), his research has been mainly focussed on male germ cell differentiation. Nowadays, he is also the scientific coordinator of The Nordfertil project, a network including Nordic and Baltic countries with the aim of preserving future fertility in young boys with treatments affecting the testicular function. Jan-Bernd was one of the founders of the INYRMF and together with other young researchers he was responsible for the growing of INYRMF and for the establishment of most of activities that are still on today.

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

I started my research on spermatogonial stem cells and *in vitro* male germ cell development during a student internship in spring 2004 at the Institute of Reproductive Medicine in Münster (now Centre of Reproductive Medicine and Andrology), under the supervision of Joachim Wistuba and Craig Marc Luetjens. Some weeks later, I met for the first time Stefan Schlatt, who was working at the University of Pittsburgh at that time. He became later my main supervisor. The research performed at the Institute and especially working together with Joachim, Marc and Stefan and the other colleagues in Münster influenced my research life in a positive way. Actually, I never thought about leaving the field of "Male Fertility". Definitely one of the best decisions I made.

You were one of the "Young Testis Club" (now INYRMF) founders. Can you tell us what was your main motivation to be part of that project?

If I remember correctly, it was rather a spontaneous decision than a planned one. As already mentioned by Frank Tüttelmann in your last newsletter, the INYRMF was initiated by Jörg Gromoll, who can be considered as "the Godfather" of the INYRMF. My personal motivation at that time, was to join a network meant to support the exchange of ideas, and to establish contacts between young researchers in reproductive biology and medicine. I was and I am still convinced that collaboration is more important than competition. Collaboration is essential to get new ideas, and most of the time you will meet a lot of wonderful people, too.

Can you briefly tell us your experience as board member of INYRMF?

This question can be answered quickly: we have had a really great and productive time, and we really became close friends. Thank you, Frank, Mirja, Aida, Karel, Eddy, Michelle, and Mona-Lisa!

You have recently made the transition from "young-to-senior" researcher. Could you share with us how did you do this path?

I think, the most important aspect for me becoming more "senior" in research, was that my supervisors allowed me to take initiatives and responsibilities. Especially, when I started my time as a Post-Doc in the lab of Olle Söder at Karolinska Institutet, I was encouraged to find my own way of doing things. Thanks to Olle, I learned during this time a lot on how to network and to set up an own research profile, and how to finance it. In addition to writing new research projects, publishing and receiving financial support, a crucial aspect to stay in research is to be present and "visible" in research societies and networks. A good working network can really "boost" your career and the INYRMF was the ideal network for me to make the first steps

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Do you have any advices for young researchers wanting to reach the same goal?

A good advice is to collaborate in an open and honest way. Another extremely important aspect is not to forget your private life, while trying to generate the next high impact factor journal article.

Jan-Bernd Stukenborg and Aida Wahlgren during the 4th INYRMF meeting in Edinburgh (2011)

The history of ART: *in vitro* fertilization

In 1876, more than a century after the discovery of sperm by van Leeuwenhoek, and 30 years after the first description of the mammalian egg, Oscar Hertwig, while he studied sea urchin development, was the first person to prove that fertilization occurs by the fusion of a spermatozoa and an egg cell. This formidable advance was the beginning of a long course to develop human *in vitro* fertilization (IVF).

Between 1878 and 1953, many scientists published reports of successful IVF of rabbit eggs, but in the light of modern knowledge, we know now it was more misinterpretations of the results than a real achievement. The probable cause of the actual lack of success of the early IVF experiments was revealed with a major discovery by Austin and Chang in 1951. They observed a significant delay before spermatozoa became able to penetrate an egg *in vivo*, and called this phenomenon "capacitation". Few years after this discovery, rabbit eggs were fertilized *in vitro* for the first time with sperm recovered from the uterus (Dauzier *et al*, 1954). However, we had to wait another 20 years before this was repeated with *in vitro*-capacitated spermatozoa (Ogawa *et al*, 1972). Following the discovery of capacitation and the first IVF in rabbit, Chang proved in 1959 that rabbit eggs fertilized *in vitro* were capable of developing and produce live progeny when transferred into a uterus. After that, technical aspects of IVF were thoroughly evaluated and tested in many mammalian species including hamsters, mice, rats, but also sheep, cats, and dogs.

The first successful IVF of human eggs was achieved in 1969 (sperm penetration and formation of pronuclei), while the first pregnancy, although it lasted only few days, was reported in 1973. In 1977, Patrick Steptoe and Robert Edwards successfully carried out a conception which resulted in the very first birth from IVF, Louise Brown, on July 25 1978 in Great Manchester, UK.


Evening News

Meet Louise, the world's first test-tube arrival

SUPERBABE

Wide-eyed Louise Brown pictured in hospital 18 hours after she was born. Today she's doing well. See Page Three

In October 1978, the Indian physician Subash Mukhopadyay, while performing experiments on his own with rudimentary instruments, created the second so-called "test-tube baby". However, for a long time his work was not recognized by the international scientific community due to state authorities preventing him to present evidences of his achievement.

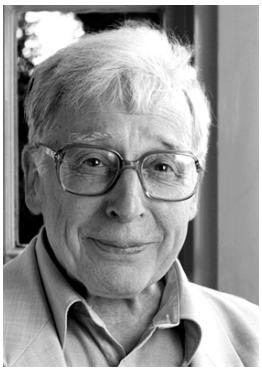
Advances in the technique, such as the use of hormones to stimulate cycles and control oocyte maturation, and the ability to freeze, thaw and transfer embryos, have significantly contributed to the spread of IVF as assisted reproductive technique.

In 2010, Robert Edwards was awarded the Nobel Prize in Physiology or Medicine for the development of IVF, and Carl Wood was ascribed the father of IVF for having pioneered the use of frozen embryos.

So far, it is estimated that more than a million babies were born from IVF worldwide.

References:

1) Bavister BD. Early history of in vitro fertilization. *Reproduction* 2002; **124**:181–96.
2) https://en.wikipedia.org/wiki/History_of_in_vitro_fertilisation



Robert G Edwards
www.nobelprize.org

Highlighted websites

http://tylervigen.com/spurious-correlations

Spurious correlations is a hilarious website presenting plenty of graphs showing very strong correlation between completely unrelated things. It is a very good reminder that correlation does not imply causation.

Scientific colloquia: mark your calendar!

17th World Congress of Academy of Human Reproduction

Rome (Italy), 15th-18th March 2017

http://hr2017.humanrepacademy.org/

Registration OPEN Abstracts for poster

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16th International conference on Preimplantation Genetics Valencia (Spain), 26th-29th March 2017 ***http://www.pgdis2017.com/***	**Registration deadline 24th March 2017**
11th International Congress of Andrology Copenhagen (Denmark), 6th-9th May 2017 ***http://www.ica2017.dk/***	**Registration OPEN**
ISSCR 2017 Annual meeting (International Society for Stem Cell Research) Boston (Massachusetts, US), 14th-17th June 2017 ***http://www.isscr.org/home/annual-meeting/isscr-2017-boston***	**Abstract submission deadline 12th April 2017**
SSR 2017 50th Annual meeting (Society for the Study of Reproduction) Boston (Massachusetts, US), 14th-17th June 2017 ***http://www.ssr.org/17Meeting***	**Abstract submission deadline 7th March 2017**
Gordon Research Conference in Germinal Stem Cell Biology Hong Kong (China), 18th-23rd June 2017 ***https://www.grc.org/programs.aspx?id=15863***	**Abstract submission deadline 21st May 2017**
Annual meeting of the Canadian Fertility and Andrology Society Vancouver (Canada), 14th-16th September 2017 ***https://cfas.ca/events/vancouver-2017/***	**Abstract submission deadline 30th April 2017**

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4th World Congress in Reproductive Biology Okinawa (Japan), 27th-29th September 2017 ***http://www.wcrb2017.jp/index.html***	**Abstract submission deadline 24th March 2017**
5th World Congress of the International Society for Fertility Preservation Vienna (Austria), 16th-18th November 2017 ***http://www.isfp2017.cme-congresses.com/***	**Abstract submission deadline 1st October 2017**

Jobs Ads & Funding Prospects

MSCA-IF-2017 Individual fellowships

Marie Sklodowska-Curie actions

MSCA-IF-EF-CAR Career Restart panel, MSCA-IF-EF-RI Reintegration panel, MSCA-IF-EF-SE Society and Enterprise panel, MSCA-IF-EF-ST Standard EF, MSCA-IF-GF Global Fellowships
Opening: 11th April 2017

http://ec.europa.eu/research/participants/portal/desktop/en/opportunities/h2020/topics/msca-if-2017.html

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