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Redundant function of RALDH1 and RALDH2 in the seminiferous epithelium

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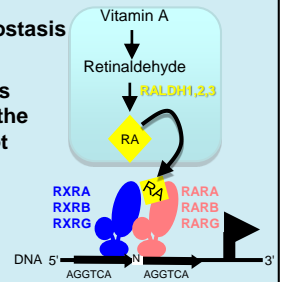
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Introduction/background

➤ The fact that retinoic acid (RA), the active metabolite of vitamin A (or retinol; ROL) is important in testis homeostasis is well documented since decades, but its precise role at different steps of spermatogenesis is still debated¹.

➤ RA, synthesized by the retinaldehyde dehydrogenases (RALDH1 to 3), binds to and activates nuclear receptors (RAR) either within the RA-synthesizing cells themselves or within neighboring cells. RALDH1 and RALDH2 are the main RA-synthesizing enzymes expressed in Sertoli cells and germ cells (GC), respectively, while RALDH3 is not detected in the seminiferous epithelium².

➤ In the past we showed that RALDH-dependent synthesis of RA in the Sertoli cells is instrumental to initiate spermatogonia differentiation and therefore to launch the 1st spermatogenic wave at the onset of puberty³. Here we evaluated the role of RALDHs in the GC (*Raldh*^{Germ-/-} mutants).



Methodologies

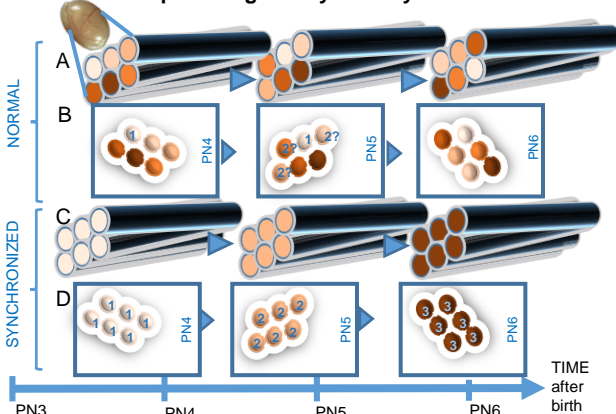
I Generation of mutant mice lacking all Raldhs selectively in GC.

To generate *Raldh*^{Germ-/-} mutants, in which the RALDH genes were excised only in GC, mice carrying loxP-flanked alleles of the *Raldh1*, *Raldh2*, and *Raldh3* genes were crossed with mice bearing the Stra8-Cre transgene⁴, which is active only in germ cells from postnatal day 3 onward.

II Quantitation of retinoic acid and retinol in testis.

Retinoids were determined in the whole testis from 8-10 week-old *Raldh*^{Germ-/-} mutants and wildtype using validated methodology⁵.

III Induction of spermatogenic synchrony.



To analyse the chronology of GC differentiation in the prepubertal testis, 3-days old (PN3) *Raldh*^{Germ-/-} mutant and control mice were treated with RA (subcutaneous injection of 16mg/Kg all trans retinoic acid). This treatment induces differentiation of spermatogonia/gonocytes in a synchronized manner.

A and C : Seminiferous cords within the testes;
B and D : Histological sections showing heterogeneous states (B) or homogenous states (D) of cell differentiation at each time point.

IV Histology and immunohistochemistry.

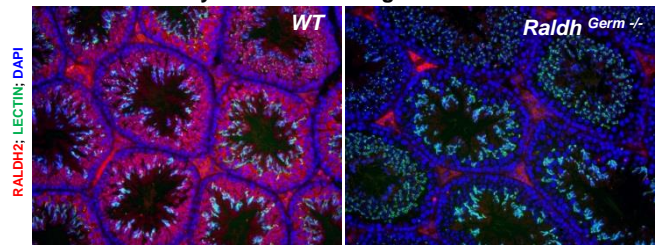
For histology, testis samples were fixed in Bouin's fluid for 16 hours and embedded in paraffin. Histological sections were stained with hematoxylin and eosin.

For immunodetection of RALDH2, testes were fixed by intracardiac perfusion of icecold 4% paraformaldehyde in PBS, then kept in the same fixative overnight at 4°C, washed in PBS, dehydrated and embedded in paraffin. 5µm sections were immunolabeled with RALDH2 antibody (Abcam, ab75674) and with Alexa Fluor 488-conjugated peanut agglutinin (lectin) to label acrosomal systems.

For co-immunodetection of STRA8/ZBTB16 or KIT/ZBTB16, testes were fixed in icecold 4% paraformaldehyde in PBS, then kept in the same fixative overnight at 4°C, washed in PBS, cryopreserved using a sucrose gradient and embedded in the matrix for cryostat sectioning. 10µm sections were immunolabeled with antibodies KIT (Cell signaling, 3074), ZBTB16 (R&D systems, AF2944) and or STRA8⁶.

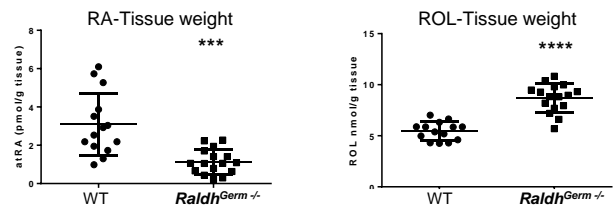
Results

I Stra8-Cre efficiently excise *Raldh* in germ cells of adult testis.

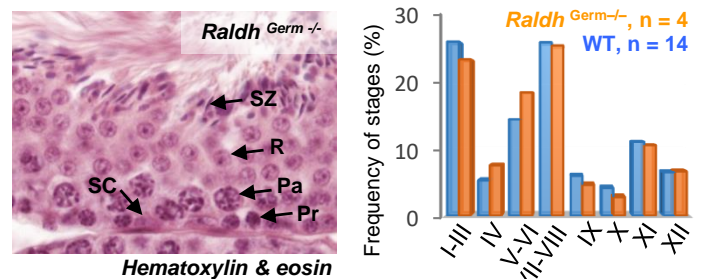


Immunohistochemistry

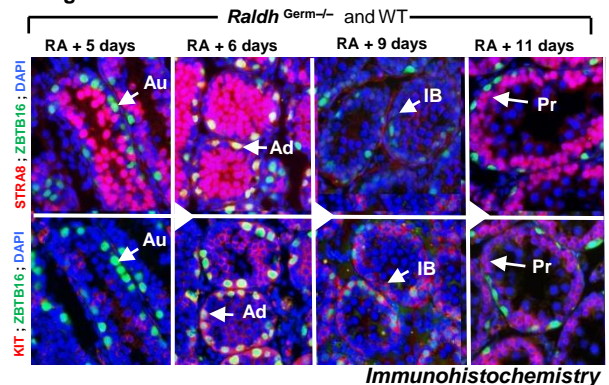
II *Raldh*^{Germ-/-} mutants testis has disrupted retinoid homeostasis in adult testis.



III Raldhs of germ cell origin are dispensable in the adult testis.



IV Absence of RA synthesis by GC does not alter the kinetics of spermatogonia differentiation.



Immunohistochemistry

Conclusion

Here we have used a combination of cell-type specific genetic ablations and pharmacological approaches *in vivo* to show that RA synthesis in postpubertal testis relies on both somatic and GC, two sources that are functionally redundant.

References

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ETW2018



**20TH
EUROPEAN
TESTIS
WORKSHOP
PORTUGAL**

PROGRAMME | MINIPOSTERS

May 23 - 27, 2018 / Óbidos Portugal

PROGRAMME

Wednesday, 23rd May 2018

14.00 - 19.00 – Registration

16.00 - 16.15 – Welcome

João Ramalho-Santos & Sandra Amaral

16.15 – Symposium 1

ENDOCRINOLOGY, SEX DETERMINATION & DEVELOPMENT

Chairpersons: Serge Nef, Anne Jørgensen & Ana Paula Sousa

16.15 - 16.45 – *Anu Bashamboo* – Mechanisms of sex determination

16.45 - 17.15 – *Robin Lovell-Badge* – Sox 9 and sex determination

17.15 – Coffee Break

Miniposter Session 1

17.30 – Selected miniposters

17.30 - 17.45 – Pedro Prudêncio, Leonardo Guilgur, João Sobral, Jörg Becker, Rui Martinho and Paulo Navarro-Costa – The unpacking of the sperm genome after fertilization requires the dMLL3/4 chromatin regulator

17.45 - 18.00 – Aurélie Lardenois, Bertrand Evrard, Séverine Mazaud-Guittot, Laurianne Lesné, Bernard Jégou, Frédéric Chalmel and Antoine Rolland – Characterization of the expression program underlying rat testis differentiation at the single cell level

18.00 - 19.00 – Short talks

Luliia Savchuk, Olle Söder and Konstantin Svechnikov – Steroidogenesis in human fetal testes in the first trimester of pregnancy

Stefan Bagheri-Fam, Katie Ayers, Raphael Rastetter, Paul Thomas, Andrew Sinclair, Juan Pié, Serge Nef and Dagmar Wilhelm – New pathway involved in 46,XY disorders of sex development

Deidre Mattiske and Andrew Pask – Oestrogen regulates key developmental pathways in somatic cells of the gonad

Melanie Stewart, Deidre Mattiske and Andrew Pask – Oestrogen blocks the nuclear translocation of SOX9 in the human Sertoli-like cell line NT2/D1

Cecilie Melau, John Nielsen, Hanne Frederiksen, Anna-Maria Andersson, Rod Mitchell, Anders Juul and Anne Jørgensen – Steroidogenic activity of the human fetal adrenal gland

Isabelle Stévant, Yasmine Neirijnck, Emmanouil Dermitzakis and Serge Nef – Reconstructing gonadal sex fate decision using single-cell RNA-seq

Isabelle Stévant, Pauline Sararols, Yasmine Neirijnck, Diane Rebourcet, Lee Smith and Serge Nef – Identification of discriminant marker genes between fetal and adult Leydig cells

Camilla Schwartz, Anne Vingaard, Sofie Christiansen, Frederic Chalmel and Terje Svingen – Characterizing novel molecular mechanisms for short AGD - A biomarker of fetal testicular dysfunction

Jitske Eliveld, Eva Van Den Berg, Fulco Van Der Veen, Sjoerd Repping, Katja Teerds and Ans Van Pelt – Human testicular PDGFRa+ cells can differentiate into cells with Leydig cell characteristics

Sirine Souali-Crespo, Nedege Vernet, Manuel Mark and Norbert Ghyselinck – Role of SF1 in embryonic Sertoli cells in mice

Jinfei Yang – A new strategy for the synthesis of reproductive medicine

Ahmed Buldan, Georgios Scheiner-Bobis and Lutz Konrad – Physiological and pathophysiological relevance of ZIP9, a zinc transporter, androgen receptor, and G-protein-coupled signal transducer

19.00 - 19.45 – Plenary session

Ilpo Huhtaniemi – Unravelling secrets of the pituitary-testicular axis

20.30 – Dinner

Thursday, 24th May 2018

07.00 - 08.45 – Breakfast

09.00 - 19.00 – Registration

09.00 – Symposium 2 SPERMATOGONIAL STEM CELLS

Chairpersons: *Jon Oatley, Elena Vicini & Stefan Schlatt*

09.00 - 09.30 – *Shosei Yoshida* – Flexible and stochastic behavior of spermatogenic stem cells

09.30 - 10.00 – *Miles Wilkinson* – Spermatogonial Stem Cell Establishment

Miniposter Session 2

10.00 – Selected miniposters

10.00 - 10.15 – *Geoffrey Maher, Hannah Ralph, Zhihao Ding, Nils Koelling, Hana Mlcochova, Pawan Dhama, Eleni Giannoulatou, Gilean McVean, Andrew Wilkie and Anne Goriely* – Assessing the landscape of selfish *de novo* mutations in human testes

10.15 - 10.30 – *Juho-Antti Mäkelä, Hue La, Ai-Leen Chan, Fernando Rossello, Christian Nefzger, Mia De Seram, Jose Polo and Robin Hobbs* – Genetic control of male germline stem and progenitor cell heterogeneity and fate

10.30 - 10.45 – *Michela Ciccarelli, Mariana Giassetti and Jon Oatley* – Regeneration of spermatogenesis in Sertoli-cell-only NANOS2 knockout pigs by homologous spermatogonial stem cell transplantation

10.45 – Coffee Break

11.15 - 11.45 – Short talks

Tessa Lord, Melissa Oatley, Deqiang Miao and Jon Oatley – Identification of novel testis-specific transcription factors that regulate spermatogonial stem cell maintenance using a large-scale siRNA screen

Alaa Sawaied, Natalie Geller, Eitan Lunenfeld and Mahmoud Huleihel – Interleukin-34 is a novel testicular factor that induced proliferation, meiotic and postmeiotic stages of immature mouse spermatogonial cells *in vitro*

Noy Bagdadi, Eitan Lunenfeld and Mahmoud Huleihel – Possible involvement of pigment epithelium derived factor (PEDF) in the proliferation and differentiation of immature mouse spermatogonial cells *in vitro*

Eva Wolff and Rüdiger Behr – Primordial germ cell culture of the common marmoset monkey

Maria Sousa, Ana Rodrigues, Bibiana Correia, João Ramalho-Santos – A novel pluripotent-paused state

Stephen Frankenberg, Micaela Sood and Andrew Pask – Isolation and characterisation of spermatogonial stem cells from a marsupial, the fat-tailed dunnart

12.00 – Lunch

13.15 – Symposium 3 SOMATIC CELLS & SPERMATOGENESIS

Chairpersons: *Lee Smith, Katja Teerds & Paula Mota*

13.15 - 13.45 – *Mario Ascoli* – The roles of the ERK1/2 cascade on the Proliferation and differentiation of Leydig cells

13.45 - 14.15 – *Luiz França* – Sertoli cells (plasticity, function, interaction with germ cells, differences among species)

Miniposter Session 3

14.15 – Selected miniposters

14.15 - 14.30 – *Eva Pohl, Joachim Wistuba, Sabine Kliesch and Jörg Gromoll* – Effects of ageing on the testis - Constant spermatogenic output appears to be maintained by enhanced spermatogonial proliferation in elderly men

14.30 - 14.45 – *Yasmine Neirijnck, Pierre Calvel, Karen Kilcoyne, Françoise Kühne, Isabelle Stévant, Richard Griffeth, Jean-Luc Pitetti, Silvana Andric, Meng-Chun Hu, François Pralong, Lee Smith and Serge Nef* – Insulin and IGF1 receptors are essential for the development and steroidogenic function of adult Leydig cells

14.45 - 15.40 – Short talks I

Shishir Patel and Shio Singh – Pyroglutamylated RFamide peptide (QRFP): Role in regulation of spermatogenesis in relation to energy metabolism

Tao Lei, Nancy Freitag, Yahia Almousa, Martin Bergmann, Sudhanshu Bhushan, Annie Huang, Hung-Lin Chen, Fu-Tong Liu, Sandra Blois, Andreas Meinhardt and Monika Fijak – Mice deficient for galectin 3 display disturbed maturation of Sertoli cells, delay of the first wave of spermatogenesis and increased apoptosis of germ cells

Michael Curley, Laura Milne, Sarah Smith, Nina Atanassova, Diane Rebourcet, Annalucia Darbey, Patrick Hadoke, Sara Wells and Lee Smith – Leukemia inhibitory factor receptor is required in Sertoli Cells but not germ cells to maintain normal spermatogenic function in mice

Agnieszka Milon, Piotr Pawlicki, Jerzy Galas, Alicja Kaminska, Laura Pardyak, Klaudia Lesniak, Anna Pacwa, Barbara Bilinska, Ewelina Gorowska-Wojtowicz and Malgorzata Kotula-Balak – Role of estrogen-related receptors in bank vole Leydig cell function

Malgorzata Kotula-Balak, Barbara Bilinska, Piotr Pawlicki, Agnieszka Milon, Patrycja Dutka, Maja Kudrycka, Malgorzata Sekula, Anna Hejmej and Ewelina Gorowska-Wojtowicz – Do estrogen-related receptors regulate tumor mouse Leydig cells?

Christiane Pleuger, Madeleine Cooper, Daniela Fietz, Mark Baker, Hans-Christian Schuppe, Wolfgang Weidner, Sabine Kliesch, Moira O'Bryan and Martin Bergmann – The role of the cilia-related CBE1 (ciliated bronchial epithelium 1) in spermatogenesis

David Fleck, Justine Fiscoeder, Naofumi Uesaka and Marc Spehr – Visualization of spontaneous Ca²⁺ signals in intact mouse Sertoli cells

Marie-Justine Guerquin, Justine Galissant, Aurélie Chaulot-Talmon, Michele Darmon, Justine Masson, Jorge Diaz, Virginie Rouiller-Fabre and Gabriel Livera – Yif1b is essential for male fertility in mouse

Alberto De La Iglesia, Ferran Barrachina, Judit Castillo, Afsaneh Goudarzi, Sophie Rousseaux, Saadi Kochbin, Carme Mallofré, Leonardo Rodríguez-Carunchio, Josep Ballescà and Rafael Oliva – Characterizing H4K5bu in human spermatogenesis and testicular pathologies as a new acylation modulating the chromatin state

Valentina Mularoni, Valentina Esposito, Flaminia Fanelli, Sara Di Persio, Rossana Saracino, Elena Vicini and Carla Boitani – Changes in Leydig cell number and function during aging in men

Nadege Vernet, Marius Teletin, Betty Feret, Muriel Klopfenstein, Sirine Souali-Crespo, Maureen Kane, Norbert Ghyselinck and Manuel Mark – Redundant function of RALDH1 and RALDH2 in the seminiferous epithelium

15.40 – Coffee Break

16.10 - 17.15 – Short talks II

Sheyla Cisneros-Montalvo, Emmi Lokka, Juho-Antti Mäkelä, Mirja Nurmio, Jorma Toppari, Marko Salmi and Pia Rantakari – The role of PLVAP in testis development and spermatogenesis

Igor Tomczyk, Bernadeta Pawlicka, Agneta Auer, Katarzyna Rożek and Paweł Grzmił – PXT1 and BAG6 interplay in the control of sperm quality in mouse

Sara Di Persio, Rossana Saracino, Stefania Fera and Elena Vicini – Retinoic acid regulation of human spermatogonial genes

Karen Kilcoyne, Cornelia Roesl, Annalucia Darbey, Hazel Stewart, Laura Milne, Rod Mitchell, Richard Melanby, Pamela Brown and Lee Smith – Development of a single injection non-surgical sterilant via androgen receptor targeting

Lucía Martínez-Fresneda, Dawit Tesfaye, Karl Schellander, Francisco García-Vázquez and Julian Santiago-Moreno – Seasonal variations of the seminiferous tubules morphometry in domestic rams (*Ovis aries*)

Marion Delessard, Côme Ialy-Radio, Mélina Blanco, Charlotte Moretti and Julie Cocquet – Male germ cell knockout of the histone methyl transferase Dot1l leads to multiple defects during spermatogenesis

Katja Hartmann, Sabine Kliesch, Florian Wagenlehner, Martin Bergmann and Daniela Fietz – Membrane-bound steroid hormone-receptors and their expression pattern in the testis of humans and different domesticated species

Judit Castillo, Orleigh Bogle, Carme Mallofré, Josep Ballescà and Rafael Oliva – Characterization of the histone lysine demethylase PHF8 during human spermatogenesis

Margareeta Mäkelä, Tiina Lehtiniemi, Fuping Zhang and Noora Kotaja – SMG6 endonuclease is essential for spermatogenesis in mice

Fei Sun – Nano contraception

Rossana Saracino, Sara Di Persio, Stefania Fera and Elena Vicini – Retinoic acid down-regulates GDNF expression in immature Sertoli cells

Bushra Dohai, Amphun Chaiboonchoe, Erwin Goldberg and Kourosh Salehi-Ashtiani – Metabolic properties of mouse spermatogenesis defined through constraint-based modeling

Matteo Da Ros, Tiina Lehtiniemi, Oliver Meikar, Opeyemi Olotu and Noora Kotaja – Simple protocol to enrich spermatocytes and spermatids from mouse testes using standard laboratory equipment

17.15 – Break

17.45 – Symposium 4

MALE REPRODUCTIVE TRACT: DUCTS AND ACCESSORY GLANDS

Chairpersons: *Andreas Meinhardt, Ralf Middendorff & Alexandra Amaral*

17.45 - 18.15 – *Sylvie Breton* – Extracellular communication networks in the epididymis: impact on optimal environment for sperm maturation

18.15 - 18.45 – *Petra Sipilä* – Epididymal gene expression: impact on sperm maturation

Miniposter Session 4

18.45 – Selected miniposters

18.45 - 19.00 – Ferran Barrachina, Meritxell Jodar, Ada Soler-Ventura, Josep Estanyol, Juan Corral, Josep Ballescà and Rafael Oliva – The protein abundance from the male reproductive tract secretions may be regulated by the sperm presence

19.00 - 19.15 – Giulia Matusali, Anne-Pascale Satie, Florence Aubry, Dominique Mahé, Laurent Houzet, Julie Frouard, Salomé Bourgeau, Karim Bensalah, Christian Platel, Anna Le Tortorec and Nathalie Dejucc-Rainsford – Zika virus replicates within the human testis and infects somatic cells and germ cells

20.00 – Dinner

Friday, 25th May 2018

07.00 - 08.30 – Breakfast

08.45 – Miniposter Session 4 (cont.)

Chairpersons: *Andreas Meinhardt & Alexandra Amaral*

08.45 - 09.05 – Short talks

Ida Boisen, Lieve Verlinden, Mette Lorenzen, Geert Carmeliet, Anders Juul and Martin Jensen – Vitamin D and transcellular calcium transport in the male reproductive tract

Daniela Beyer, Andrea Mietens, Dieter Müller, Davor Jezek, Gerhard Schuler and Ralf Middendorff – Spontaneous contractions of the immature epididymal duct drive directional movement of exfoliated epithelial cells
Ansgar Reckmann, Claudia Tomczyk, Daniela Beyer, Michail Davidoff, Tatyana Michurina, S Arnhold, Dieter Müller, Andrea Mietens and Ralf Middendorff – Nestin indicates proliferating vascular wall cells in the epididymis during postnatal development and in case of testosterone deficiency

Andrea Mietens, Sabine Tasch, Daniela Beyer, Gerrit Eichner and Ralf Middendorff – Wait and see: different functions for superimposed contraction patterns in seminiferous tubules?

09.05 – Symposium 5

SPERM & ART

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Chairpersons: *João Ramalho-Santos, Rafael Oliva & Sandra Amaral*

09.05 - 09.35 – Christophe Arnoult – New actors of acrosome and flagella biogenesis revealed from infertile male patients

09.35 - 10.05 – Timo Strünker – The role of CatSper channels in sperm navigation: swimming against the current!

10.05 - 10.35 – Mariana Medina Sánchez – Sperm micromotors and its potential to improve ART success and reproductive health

10.35 – Coffee Break

Miniposter Session 5

11.00 – Selected miniposters

11.00 - 11.15 – Janice Jeschke, Verena Nordhoff, Andreas Schüring, Timo Strünker and Christoph Brenker – The action of follicular fluid on human sperm: exploring a steroid-binding site

11.15 - 11.30 – Sandra Laurentino, Eva Pohl, Christopher Schröder, Klaus Redmann, Claudia Krallmann, Jann-Frederik Cremers, Sabine Kliesch, Bernhard Horsthemke and Jörg Gromoll – Age-related epigenetic changes in human sperm

11.30 - 12.25 – Short talks

Marco Ragusa, Teresa Chioccarelli, Gilda Cobellis, Cinzia Di Pietro, Dulia Brex, Davide Barbagallo, Silvia Fasano, Rosaria Meccariello, Bruno Ferraro, Michele Purrello, Riccardo Pierantoni and Rosanna Chianese – CircRNA-associated-competitive endogenous RNA network (ceRNET) in mammalian reproduction: role of NAPEPLDiso1 and iso2
Sandra Amaral, Raul Da Costa, Frank Wübbeling, Klaus Redmann and Stefan Schlatt – Raman micro-spectroscopy analysis of different sperm regions: a species comparison

Alexandra Amaral, Hermann Bauer, Jürgen Willert and Bernhard Herrmann – Rac1 signalling regulates sperm motility and is involved in mouse t-haplotype non-Mendelian inheritance

Simon Schneider, Melanie Balbach, Jan Jikeli, Daniela Fietz, Daniel Nettersheim, Sina Jostes, Rovenna Schmidt, Monika Kressin, Martin Bergmann, Christian Trötschel, Dagmar Wachten, Klaus Steger and Hubert Schorle – From fertility to sterility: The different flavors of Prm2 haploinsufficiency in mice

Manon Oud, Linda Hendricks, Petra De Vries, Brendan Houston, Özlem Okutman, Nicolas Jouve, Brigitte Benzacken, Michiel Noordam, Lisenka Vissers, Moira O'Bryan, Liliana Ramos, Stéphane Viville and Joris Veltman – Whole exome sequencing reveals two new genes involved in human globozoospermia

Marie Nygaard, Amy Herlihy, Charlotte Jeanneau, John Nielsen, Irina Gaukasian, Eric Bennett, Niels Jørgensen, Henrik Clausen, Ulla Mandel, Ewa Rajpert-De Meyts and Kristian Almstrup – Polypeptide N-acetylgalactosaminyltransferase 3 (GALNT3) is expressed specifically in the equatorial segment and marks good quality spermatozoa
Merixell Jodar, Ada Soler-Ventura, Steven Barrett, Julia Salzman, Josep Ballescà, and Rafael Oliva – Spermatozoa retain circular RNAs

Sara Escada-Rebelo, Ana Sousa, Teresa Almeida-Santos, Artur Paiva and João Ramalho-Santos – On the hunt for quality: reactive oxygen species – can we use them in our favor?

Katerina Dvorakova-Hortova, Natasa Sebkova, Lukas Ded, Audrey Lumeau and Michaela Frolíková – Protein-protein interactions in the sperm membrane prior to fusion with the egg

Joana Serrano, Callista Mulder, Lisa Catsburg, Tessa Roseboom, Sjoerd Repping and Ans Van Pelt – A practical blueprint to systematically study life-long health consequences of novel medically assisted reproductive treatments
Dharmaraj Palanisamy, Mohamed Ashraf, Chinmayi Kulkarni and M Shylaja – Microsurgical Testicular Sperm Extraction in 750 cases non-obstructive azoospermia: sperm retrieval and Success in a single centre: an Indian study

12.30 – Lunch

13.45 – Convivium – Excursion to the Óbidos village

18.00 - 19.30 – Round-Table Discussion – “TestEthics”

Moderator: *João Ramalho-Santos* (PhD, Head of Center for Neuroscience and Cell Biology, University of Coimbra, Portugal)

Teresa Almeida-Santos (MD, PhD, Head of Human Reproduction Department of the University Hospital of Coimbra, Portugal)

Stefan Schlatt (PhD, Head of the Centre of Reproductive Medicine and Andrology, University of Münster, Germany)

Teresa Firmino (Science Editor, Newspaper Público, Lisbon, Portugal)

Paula Mota (DVM, PhD, Head of the Animal Facility of Center for Neuroscience and Cell Biology, University of Coimbra, Portugal)

Jens Ehmcke (PhD, Head of Central Animal Facility of the University of Münster, Germany)

20.00 – Dinner

Saturday, 26th May 2018

07.00 - 08.30 – Breakfast

08.45 – Symposium 6 **MEIOSIS, GENETICS & EVOLUTION**

Chairpersons: *Jörg Gromoll, Anne Goriely & Frank Tüttelmann*

08.45 - 09.15 – *Frederic Chalmel* – Spermatogenesis in modern humans: How did we succeed our hybrid speciation?

09.15 - 09.45 – *James Turner* – Fertile offspring from sterile sex chromosome trisomic mice

Miniposter Session 6

09.45 – Selected miniposters

09.45 - 10.00 – *Thomas Darde, Estelle Lecluze, Nathan Alary, Aurélie Lardenois, Olivier Collin, Frank Tüttelmann, Serge Nef, Antoine Rolland and Frédéric Chalmel* – The ReproGenomics Viewer 2.0: a major update integrating features for single-cell data

10.00 - 10.15 – *Sabrina Jan, Aldo Jongejan, Cindy Korver, Saskia Van Daalen, Ans Van Pelt, Sjoerd Repping and Geert Hamer* – Distinct prophase arrest mechanisms in human male meiosis

10.15 – Coffee Break

10.45 - 11.30 – Short talks I

Daniela Fietz, Josefine Bennien, Katharina Bakhaus, Sabine Kliesch, Florian Wagenlehner, Joachim Geyer and Martin Bergmann – Biological significance of sodium-dependent organic anion transporter SOAT in human spermatogenesis

Joachim Weitzel, Alexander Sobczak, Dirk Koczan, Martina Langhammer, Norbert Reinsch and Marten Michaelis – Testicular transcriptional signatures associated with high fertility

Sofia Winge, Marlene Dalgaard, Jacob Jensen, Kirstine Belling, John Nielsen, Niels Graem, Lise Aksglaede, Søren Brunak, Mikkel Schierup, Niels Skakkebaek, Anders Juul, Ewa Rajpert-De Meyts and Kristian Almstrup – Transcriptome profiling of Klinefelter testis tissue reveals crucial roles of somatic differentiation and long non-coding RNAs in testis development

Laura Bellutti, Leila Demini, Sophie Tourpin, Delphine Moison and Gabriel Livera – MeioD, a new conserved meiotic gene dispensable for mouse fertility

Nihan Öztürk, Sabrina Gies, Sina Kürschner, Klaus Steger, Hans Schuppe, Kai Ni, Nina Rogenhofer and Undraga Schagdarsurengin – Epigenetic regulation of LINE1 and its impact on male subfertility

Nils Van Der Bijl, Albrecht Röpke, Laura Hankamp, Sabine Kliesch and Frank Tüttelmann – Mutations in STAG3 cause autosomal-recessive male infertility due to meiotic arrest

Annalucia Darbey, Laura Milne and Lee Smith – Developing techniques to target the adult testicular somatic cells with deliverable transgenics

Agnieszka Malcher, Tomasz Stokowy, Soroosh Monem, Karolina Nowicka-Bauer, Adam Czyzyk, Piotr Jedrzejczak, Blazej Meczekalski, Natalia Rozwadowska, and Maciej Kurpisz – Genetic characterization of patients with complete androgen insensitivity syndrome (CAIS)

Nina Neuhaus, Jörg Gromoll, Sabine Kliesch, Stefan Schlatt and Sandra Laurentino – Methylation profile of human spermatogonia compared to sperm and somatic cells

11.30 – Break

11.50 - 12.40 – Short talks II

Herta Ademi, Beatrice Conne, Isabelle Stevant and Serge Nef – A Wnt5aTetOn-3G knockin mouse model for steroidogenic cell lineage tracing

Jonathan Ribeiro, Cynthia Petrillo, Pauline Dupaigne, Clotilde Duquenne, Xavier Veaute, Carole Saintomé, Orestis Faklaris, Didier Busso, Raphael Guerois, Emmanuelle Martini and Gabriel Livera – MEIOB and SPATA22 resemble RPA subunits and interact with the RPA complex to ensure meiotic recombination

Arka Baksi, Ruchi Jain, Vasan Ss, Paturu Kondaiah and Rajan Dighe – Correlation between the aberrant testicular germ cell gene expression and human male infertility

Rüdiger Behr, Drummer Charis, Vogt Edgar and Berit Neumann – Frequent silencing of a transgene in marmoset monkeys after male and female germline transmission

Tiina Lehtiniemi, Fuping Zhang, Frederic Chalmel, Jennifer Dumdie, Miles Wilkinson and Noora Kotaja – The pivotal role of the endonuclease SMG6 in the control of male germ cell transcriptome

Peter Vogt, Jutta Zimmer, Ulrike Bender, Frank Dechend, Notker Graf and Thomas Strowitzki – AZFa deletion extension in proximal Yq11 including UTY + VCY causes complete loss of all premeiotic germ cells in human male germ line

Matthias Vockel, Carolin Dreier, Sabine Kliesch, Peter Wieacker and Frank Tüttelmann – Mutational spectrum and functional relevance of the adhesion receptor ADGRG2 in X-linked CBAVD

Liina Nagirnaja, Jannette Rusch, Alexandra Lopes, Kristian Almstrup, Brendan Houston, Ewa Rajpert-De Meyts, Maris Laan, Moira O'Bryan, Kenneth Aston and Donald Conrad – GEMINI study: understanding the genetics and genomics of severe male infertility

Vyacheslav Chernykh, Maria Shtaut, Tatiana Yamandi, Natalia Safina, Aleksandr Polyakov, Liubov Kurilo, Anna Kashintsova, Victor Spangenberg and Oksana Kolomiets – Complex study of spermatogenesis abnormalities in infertile men with AZF deletions

Alexandra Lopes, Liina Nagirnaja, Filipa Carvalho, João Gonçalves, Susana Fernandes, Iris Pereira-Caetano, Kristian Almstrup, Ewa Rajpert-De Meyts, Susana Seixas, Brendan Houston, Alberto Barros, Moira O'Bryan, Kenneth Aston and Donald Conrad – Assessing the impact of copy number variation on severe spermatogenic impairment with exome data

13.00 – Lunch

14.15 – Symposium 7

LIFESTYLE, ENVIRONMENT, INFECTION & IMMUNITY

Chairpersons: *Bernard Jégou, Jorma Toppari & Renata Tavares*

14.15 - 14.45 – *Kristian Almstrup* – Pubertal development - crosstalk between environment and the epigenome

14.45 - 15.15 – *Romain Barrès* – Sperm-mediated epigenetic inheritance in metabolic disorders

Miniposter Session 7

15.15 – Selected miniposters

15.15 - 15.30 – Pablo Hurtado-Gonzalez, Joni Macdonald, Anne Jorgensen, Richard Anderson, Karen Kilcoyne, Richard Sharpe and Rod Mitchell - Exposure to therapeutic levels of paracetamol or ibuprofen affects germ cell development and epigenetic regulators in human fetal testis and ovary

15.30 - 15.45 – David Kristensen, Christèle Desdoits-Lethimonier, Abigail Mackey, Marlene Dalgaard, Federico De Masi, Cecilie Munkbøl, Bjarne Styrisshave, Christian Platel, Anders Hay-Schmidt, Tina Jensen, Laurianne Lesné, Séverine Mazaud-Guittot, Karsten Kristiansen, Søren Brunak, Michael Kjaer, Anders Juul and Bernard Jégou – Ibuprofen alters human testicular physiology to produce a state of compensated hypogonadism

15.45 – Coffee Break

16.15 - 16.50 – Short talks I

Anna Hejmej, Alicja Kaminska, Laura Paradyak, Sylwia Marek, Malgorzata Kotula-Balak and Barbara Bilinska – Notch signaling pathway in adult rat testis as a target for bisphenol A

Andrew Pask, Deidre Mattiske, Shawna Zhao and Melanie Stewart – Transgenerational effects of diethylstilbestrol on male germ cells

Sonia Abdallah, Delphine Moison, Juliette Borderies, Jean-Philippe Antignac, René Habert, Gabriel Livera, Virginie Rouiller-Fabre and Marie-Justine Guerquin – Fetal exposure to bisphenol A-related substitutes impair germ cell differentiation in mice

Aurore Gely-Pernot, Chunxiang Hao, Louis Legoff, Luc Multignier, Shereen D’Cruz, Christine Kervarrec, Bernard Jégou, Sergei Tevosian and Fatima Smagulova – Transgenerational effects promoted by chlordecone are mediated via histone trimethylation in a limited regions in mouse genome

Hanna Johansson, Lene Nielsen, Anne Vinggaard, Martin Bahl and Terje Svingen – Glyphosate alone does not adversely affect testicular androgen function in mature rats

Anna Rudnicka, Evdochia Adoamnei, Carrie Nielson, José Noguera-Velasco, Jaime Olivares and Alberto Torres-Cantero – Associations of vitamin D intake and vitamin D serum levels with semen quality and reproductive hormone levels in young men from southern Spain

Eliska Valaskova, Eva Zatecka, Hasmik Margaryan, Romana Bohuslavova, Katerina Dvorakova-Hortova, Gabriela Pavlinkova and Jana Peknicova – Alterations in expression of selected testicular genes and protein level in mice model of diabetes mellitus 2

16.50 – Break

17.15 - 17.55 – Short talks II

Rita Rahban, Laerke Priskorn, Alfred Senn, Eric Stettler, Niels Jørgensen and Serge Nef – Poor semen quality with geographical variations in Switzerland

Katja Eubler, Carola Herrmann, Frank-Michael Köhn, Ulrich Schwarzer, Lars Kunz and Artur Mayerhofer – Ca²⁺ signaling and interleukin-8 secretion in human testicular peritubular cells involves the cation channel TRPV2

Nina Schmid, Lena Walenta, Astrid Tiefenbacher, Ullrich Schwarzer, Frank-Michael Koehn, Leena Strauss, Matti Poutanen and Artur Mayerhofer – NLRP3 is expressed by somatic cells of the human testis

Yoni Baert, Isabel Ruetschle, Ilka Maschmeyer, Alexandra Lorenz, Anika Oehme, Uwe Marx and Ellen Goossens – A Multi-organ-chip co-culture of human testis and liver equivalents for substance testing

Constanze Maresch, Dina Stute, Thomas Fleming, Hans-Peter Hammes and Thomas Linn – Hyperglycemia induces testicular DNA damage in type 1 diabetic Ins2Akita+/- mice via RAGE associated mechanisms

Britta Klein, Sudhanshu Bhushan, Rukmali Wijayarathna, Ralf Middendorff, Kate Loveland, Mark Hedger and Andreas Meinhardt – Bacteria themselves and/or subsequent, persistent inflammation – what may be the “crux”? Understanding the pathogenesis of epididymitis-associated subfertility

Cinthia Mendez, María Ferreira, Lucas González, Cristian Sobarzo, Livia Lustig, Patricia Jacobo and Maria Theas – Undifferentiated spermatogonia cell cycle arrest contributes to the impairment of spermatogenesis in the testis under a chronic inflammatory process

Yongning Lu, Amir Rafiq, Monika Fijak, Joerg Klug, Trinad Chakraborty, Martin Bergmann, Andreas Meinhardt and Sudhanshu Bhushan – Necrotic cell death of Sertoli cells: a mechanism evolved by uropathogenic Escherichia coli to impair testicular function

18.00 - 18.45 – Plenary session

Andreas Meinhardt – The immune system of the testis: 3 infectious stories

20.30 – Gala Dinner

Sunday, 27th May 2018

07.00 - 08.45 – Breakfast

09.00 – Symposium 8

FERTILITY THREATS & PRESERVATION

Sponsored by  **SPMR** SOCIEDADE PORTUGUESA DE MEDICINA DA REPRODUÇÃO

Chairpersons: *Rod Mitchell, Ewa Rajpert-De Meyts & Teresa Almeida-Santos*

Closing Lecture

09.00 - 09.30 – *Ans Van Pelt* – Preservation of fertility in oncological patients: lessons learned from Growsperm programme

Miniposter Session 8

09.30 – Selected miniposters

09.30 - 09.45 – *Caroline Allen, Rod Mitchell, Federica Lopes and Norah Spears* – Comparison of toxicity of chemotherapy drugs cisplatin and carboplatin on prepubertal mouse gonads

09.45 – Coffee Break

10.15 - 11.20 – Short talks

João Alves-Lopes, Magdalena Kurek, Halima Albalushi, Ahmed Reda, Olle Söder, Cecilia Petersen, Kirsi Jahnukainen and Jan-Bernd Stukenborg – Explant cultures to test functionality of immature testicular tissue *in vitro*

Christine Andreassen, Mette Lorenzen, Anders Juul and Martin Jensen – RANKL inhibition reduces proliferation and growth of testicular germ cell tumors

Mette Lorenzen, John Nielsen, Anders Juul and Martin Jensen – Direct effects of luteinizing hormone receptor on testicular cancer Cells *in vitro* and *in vivo*

Antoine Oblette, Ludovic Dumont, Cynthia Bichara, Marion Delessard, Justine Saulnier, Nathalie Rives and Christine Rondanino – Assessment of epigenetic modifications of embryos obtained with mouse spermatozoa after *in vitro* maturation of prepubertal fresh or frozen/thawed testicular tissue

Federica Lopes, Prathima Tholeti, Richard Anderson, Rod Mitchell and Norah Spears – Effect of cisplatin and doxorubicin on prepubertal mouse testicular germ cells

Ali AbuMadighem, Ronnie Solomon, Eitan Lunenfeld and Mahmoud Huleihel – Distinct effect of busulfan on subpopulations of spermatogonial cells from immature-treated mice

Joana Portela, Cindy Korver, Saskia Van Daalen, Andreas Meißner, Annemieke De Melker, Sjoerd Repping and Ans Van Pelt – Organotypic culture of fresh and cryopreserved-thawed human prepubertal and pubertal testis

Laura Heckmann, Joachim Wistuba, Stefan Schlatt, Sabine Kliesch and Nina Neuhaus – Altered marker expression of somatic and germ cell populations in immature Androprotect patients at risk for germ cell loss

Ieva Masliukaite, Elissavet Ntemou, Marianne Van De Wetering, Andreas Meißner, Leontien Kremer, Sjoerd Repping, Kirsi Jahnukainen, Ellen Goossens and Ans Van Pelt – Spermatogonial quantities in untreated prepubertal cancer patients are comparable to normative prepubertal spermatogonial values

Teresa Almeida-Santos, Rita Cardoso, Alexandra Carvalho and Ana Sousa – Fertility preservation in an adolescent with an OT-DSD

Melissa Tharmalingam, Joni MacDonald, Federica Lopes, Norah Spears and Rod Mitchell – Potential effects of cisplatin on germ cells in the immature human testis

Jaime Onofre-Meza, Prashant Kadam, Yoni Baert and Ellen Goossens – Effects of the cryopreservation of testicular tissues and testicular cell suspensions on the efficiency of spermatogonial stem cell transplantation

Lihua Dong, Stine Kristensen, Simone Hildorf, Jorgen Thorup and Yding Andersen – Propagation of human spermatogonial stem cells *in vitro* from infant boys

11.30 – Closing Remarks

12.00 – The end

13.00 – Transfer bus to Lisbon Airport