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International Society of Andrology

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2nd Meeting of the
International Network for Young Researchers in Male Fertility

Thursday 17th - Saturday 19th September 2009

Münster, Germany
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Münster   Germany
Stockholm Sweden
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Meeting Venue
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Bismarckallee 47
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Programme
Thursday, September 17th
15.00-18.30  Registration
18:30        Meeting at Hotel Reception and Transfer
19.00        Welcome: Fürstenberghaus
             Domplatz 20-22, 48143 Münster
             Frank Tüttelmann, Germany
             & Jan-Bernd Stukenborg, Sweden
19.15-20.00  Plenary Lecture
             Chairs: Aida Wahlgren & Frank Tüttelmann
             Medical history of testosterone and the testes
             Eberhard Nieschlag, Münster, Germany
19.15-21.15  Dinner
21.30        City Tour of Münster
Friday, September 18th

7.30-9.00  Breakfast

9.00-9.45  Plenary Lecture  
Chairs: Claudia Rössig & Frank Tüttelmann  
Prevention and treatment of long-term adverse effects on reproductive function in childhood cancer survivors  
Olle Söder, Stockholm, Sweden

9.45-10.30  Plenary Lecture  
Chairs: Jörg Gromoll & Andreas Poplinski  
Post-meiotic reprogramming of the male genome: molecular actors and medical implications  
Sophie Pison-Rousseaux, Grenoble, France

10.30-11.00  Coffee Break

11.00-12.00  Session 1: Spermiogenesis/post-meiotic processes  
Chairs: Aida Wahlgren & Andreas Poplinski  
TAP-tagging of the testicular variant of histone H2B (tH2B) in mouse  
Emilie Montellier, Grenoble, France  
Isolation and characterization of chromatoid bodies of mouse round spermatids  
Oliver Meikar, Turku, Finland

Participants

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Androgen action in Sertoli cells is already evident from day 4 on
A Willems, K De Gendt, J Allemeersch, LB Smith, M Welsh, JV Swinnen, G Verhoeven

Earlier transcription profiling studies on testes of mice with a Sertoli cell (SC) selective knockout of the androgen receptor (SCARKO) and control mice suggested an effect of androgens on SC gene expression from day 8 (d8) or earlier. Recently we demonstrated that the androgen receptor (AR) in mouse SC already becomes immunologically detectable around d5. Hence we tried to identify the earliest responses to androgen action in SC with a new microarray study on testes of 6-day-old SCARKO and control mice. Unfortunately no obvious and new early response genes could be identified. However, several genes differentially expressed on a d10 microarray study already displayed a response to AR ablation on d6. An extensive time study from d4 to d50 using quantitative RT-PCR even revealed significant differences in expression level from d4 on for Eppin, Pci and Cldn11 and from d6 on for Rhox5. For several of these genes there is evidence for (in)direct regulation by androgens. From these studies we conclude that androgens already influence SC gene expression from d4 on.

Friday, September 18th

continued

Abnormal localization patterns and a mutant isoform of the oocyte activation factor, phospholipase C zeta, in spermatozoa from infertile men
Junaid Kashir, Oxford, United Kingdom

12.00-13.00 Lunch

13.00-13.45 Plenary Lecture
Chairs: Stefan Schlatt & Jan-Bernd Stukenborg
Spermatogonial stem cells
Dirk De Rooij, Amsterdam, Netherlands

13.45-15.30 Session 2: Stem cells/pre-meiotic processes
Chairs: Nina Kossack & Jan-Bernd Stukenborg

Hedgehog signaling in rat testis
Juho-Antti Mäkelä, Turku, Finland

Comparison of testicular expression patterns of eGFP and CD1 mice
Jennifer Kuhlen, Münster, Germany

Characterization of spermatogonial stem cells from juvenile marmoset testes
Silvia Albert, Münster, Germany

Karyotyping spermatozoa after spermatogonial stem cell transplantation by array-CGH
Ellen Goossens, Brussels, Belgium
Steroidogenesis in postnatal fetal Leydig cells in vitro

J Weisser J, O Söder, K Svechnikov

Department of Woman and Child Health, Pediatric Endocrinology Unit,
Astrid Lindgren Children’s Hospital, Karolinska Institutet, Stockholm, Sweden

Foetal Leydig cells (FLCs) function during the prenatal and early postnatal period and are the primary source of androgens required for the proper masculinisation of internal and external genitalia. At present, little is known about steroidogenesis in FLCs due to lack of an appropriate model system to explore these cells in vitro. We have established primary cultures of FLCs using MiniMACS isolation system. By using the method highly purified (about 98%) LH receptor (LHR) positive FLC were isolated from 8-day-old rats. Isolated FLC were cultured with or without human chorionic gonadotropin (hCG), cholera toxin and (Bu)2cAMP for 72 hours and steroid levels were further explored in culture media. Stimulation of purified FLCs with the activators for 72 hours showed a time-dependent decline in their capacity to produce testosterone (T). However, suppression of T biosynthesis was accompanied by an accumulation of progesterone in the culture media of stimulated FLCs, suggesting an attenuation of their P450c17 activities. However, the major steroid produced by FLCs in steady high values was 5α-androstane-3α,17β-diol (3α-ADIOL), indicating high activities of 5α-reductase and 3β-HSD in FLCs in vitro.

All together, our findings indicate that postnatal FLCs display steroidogenic profile similar to that observed in adult-type immature Leydig cells.
Chorionic gonadotropin (CG) - An insight into gene evolution

C Adams, A Henke, J Gromoll

Centre of Reproductive Medicine and Andrology, Münster, Germany

The glycoprotein hormones luteinizing hormone (LH) and chorionic gonadotropin (CG) are crucial for reproduction, as LH induces sex hormone production and ovulation, and CG is essential for the establishment of pregnancy and foetal male sexual differentiation. Both consist of two heterodimeric peptides of which the α-subunit is common to both hormones whereas the β-subunit is hormone-specific. The CGB gene was derived from LHB by gene duplication and frame shift mutation that led to a read-through into the formerly 3'-untranslated region, giving rise to the carboxyl-terminal peptide. Owing to nucleotide changes within the 5'-region of CGB, a new transcriptional start site and regulatory region was gained. These changes led to the specific expression of CGB in the placenta and its decrease in the pituitary. The tissues specific regulation of the CGB in the placenta is driven by a short (100 bp) sequence. Within this region there are transcription factor binding sites like SP1 and EGR1. Additionally an analysis of the methylation pattern revealed a huge CpG Island within the promoter. Recent findings on gonadotropins led to an extended model for the sequence of events in the evolution of the CGB gene in primates and its tissue-specific expression via transcription factors and methylation status.

Saturday, September 19th

7.30-9.00 Breakfast
9:00 Meeting at Hotel Reception and Transfer
9.30-12.30 Workshop 1
Sperm evaluation
Barbara Hellenkemper
& Andreas Poplinski, Münster, Germany

Workshop 2
Histology & Microscopy
Jutta Salzig, Münster, Germany
& Jan-Bernd Stukenborg, Stockholm, Sweden

Workshop 3
Genetics
Frank Tüttelmann, Münster, Germany

12.45-13.00 Closing Remarks
13.00-14.00 Lunch
Testicular peritubular cells as putative progenitors for adult Leydig cell lineage

L Landreh, K Svechnikov, O Söder

Peritubular cells have been ascribed stem cell potential for Leydig cells. Those cells named putative stem Leydig cells (PSLC) are situated in the peritubular region in the testis and processes controlling their differentiation into adult Leydig cell lineage are still remain unclear. The aim of this study was to isolate and characterize PSLC biology.

To purify PSLC a magnetic cell separation for PDGFR-α positive cells was used. About 98% of the isolated cells expressed PDGFR and LIFR detected by immunostaining. PSLC were shown to express α-actin, stem cell marker OCT-4 and several steroidogenic enzymes (e.g., P450scc, 3β-HSD, P45017c, 5α-RI) but not Insl3. The major steroid produced by PSLC in high values was 5α-androstane-3α,17β-diol (3α-ADIOL), while testosterone biosynthesis was not detected in the cells. Stimulation of PSLC with (Bu)2cAMP resulted in accumulation of progesterone in culture media in a time dependent manner, a process associated with activation of 3β-HSD and suppression of P45017c expression. Moreover, (Bu)2cAMP suppressed OCT-4 expression.

Our findings suggest that the PSLC have potential for pluripotency and the cAMP-PKA signaling pathway may play a role in triggering differentiation of PSLC into the adult Leydig cell lineage.
Abnormal localization patterns and a mutant isoform of the oocyte activation factor, phospholipase C zeta, in spermatozoa from infertile men

J Kashir, C Young, E Heytens, P Grasa, C Jones, M Ruas, SY Yoon, RA Fissore, CM Deane, D Nikiforaki, ST Tee, P de Sutter, J Parrington, K Coward

In mammals, oocyte activation at fertilization is thought to be induced by a sperm-specific phospholipase C, PLCzeta. However, it still remains to be conclusively demonstrated that PLCzeta is the endogenous agent of oocyte activation. Recent immunofluorescent studies in our laboratory have shown PLCzeta to be localized in the equatorial region of sperm from healthy fertile men. However, we failed to detect PLCzeta in this region in sperm from a number of patients exhibiting defective oocyte activation. Some types of human infertility appear to be caused by failure of the sperm to activate the oocyte. It remains unclear as to whether this is due to defects in PLCzeta. Molecular analysis of genomic DNA from infertile patients focusing on each PLCzeta exon, and associated flanking sequences, successfully identified a number of changes to the PLCzeta gene sequence. In one case, analysis identified a point mutation in the PLCzeta gene in an infertile male exhibiting normal sperm morphology but defective oocyte activation capability, which leads to a significant change in a conserved amino acid in the catalytic region of the protein that may inhibit normal function. Our findings suggest that PLCzeta may play an important role in male factor infertility, specifically linking infertility with defective forms of PLCzeta, and may provide important information about PLCzeta’s structure-function relationships. Our current studies aim to investigate the functional properties of this mutant form of PLCzeta, as well as identifying further mutations in the protein in a wider population of infertile men. Our studies may lead to the possibility of using analysis of PLCzeta as a diagnostic tool for certain types of male factor infertility.

Hedgehog signaling in rat testis

JA Mäkelä, S Bourguiba, V Saario, S Ventelä, M Nurmio, M Parvinen, J Toppari

Departments of Physiology and Paediatrics, University of Turku, Finland

Hedgehog signaling pathway regulates cellular differentiation and development of multiple different tissues and organs. It takes part in maintenance of regenerative tissues in adult life, too. The testis-specific member of mammalian Hedgehog (Hh) protein family, Desert hedgehog (Dhh), is one of the first genes to be expressed in developing mouse testis at 11.5 dpc. The significance of Hh signaling for testicular development is highlighted by smaller testes with irregular and anastomotic cord histology leading to infertility in Dhh-/- mutants. In mouse Dhh is expressed in Sertoli cells. Interstitial and myoid cells express Hh receptor Patched 1 whereas Patched 2 is expressed in spermatogonia and spermatocytes. Besides regulating fetal Leydig cell differentiation, Hh signaling is important for germ cell survival and differentiation. The aim of this study is to elucidate the role of Hh signaling in rat testis. Based on immunohistochemical staining we have identified primary spermatocytes as cells where Hh signaling is most active during rat spermatogenesis. In addition, postmeiotic cells, i.e. round and elongating spermatids, strongly express the negative regulator of the pathway, Suppressor of Fused. Hedgehog signaling has a stage-specific pattern of activity during spermatogenesis. In rat both the ligand and receptors are most highly expressed in stages II-VI of seminiferous epithelial cycle. In seminiferous tubule culture setting we have studied the regulation of Hedgehog signaling. Tumor necrosis factor alpha and follicle-stimulating hormone strongly activate the pathway, whereas sex hormones testosterone and estradiol do not affect it. Interestingly, imatinib mesylate, a tyrosine kinase inhibiting anticancer drug, activates the pathway also.
Comparison of testicular expression patterns of eGFP and CD1 mice
J Kuhlen¹, J Gromoll¹, N Kossack², J Ehmcke¹, S Koschmieder², J Wistuba²

¹Centre of Reproductive Medicine and Andrology and ²Department of Molecular Haematology/Oncology of the University, Münster, Germany

Recent publications demonstrated the plasticity of isolated spermatogonial stem cells (SSCs) utilizing mouse models, in which undifferentiated germ cells express transgenes suitable as SSC markers. We aim at the characterization of those undifferentiated germ cells in mice without specific transgenic modifications and investigated the gene expression in testicular cells of two mouse strains establishing a marker panel combining markers for pluripotency, spermatogonia, meiosis, spermiogenesis, Sertoli, Leydig and peritubular cells. Testicular expression patterns were evaluated from juvenile (8 days old; n=5) as well as adult mice (> six weeks; n=5) of eGFP transgenic (ubiquitous expression under the β-actin promoter) and CD1 mice. SYBR green rtPCR was applied using an adult eGFP mouse as calibrator. Expression differences in juveniles were smaller among the strains than they were between adults and immature animals within one strain. Germ cell number was lower in juvenile animals because differentiated stages were absent. The relatively enriched SSCs were leading to a higher expression of pluripotency markers relative to the adult eGFP mouse. We conclude that the expression profile in both mouse strains is very similar. Our data will be used for further experiments aiming at isolation of eGFP positive SSCs for culture and transplantation assays.

Supported by the IZKF, University Münster, Project WI 2/023/07

Isolation and characterization of chromatoid bodies of mouse round spermatids
O Meikar, J Toppari, N Kotaja

Institute of Biomedicine, Department of Physiology, University of Turku, Turku, Finland

The chromatoid body (CB) is an intriguing perinuclear, cytoplasmic cloud-like structure in male germ cells, which appears first in late pachytene spermatocytes, condenses to its final shape in post-meiotic round spermatids and disappears in elongating spermatids. CB contains male germ cell specific components such as MVH and MIWI, but also components of both the microRNA pathway and the RNA decay pathway. CB is believed to be involved in the control and organization of RNA processing and/or storage in round spermatids. It has been shown by knock-out mouse models that the deletion of different CB components leads to the block of spermatogenesis, implying a crucial role for CB during male germ cell differentiation.

To better understand the role of CB in male germ cell biology, it is essential to get the comprehensive view of its composition. We have isolated and purified the CB by immunoaffinity approach. We are identifying its protein components, as well as studying the RNA species that are gathered to CBs. We believe that these studies will be important in our attempt to elucidate the complex regulatory mechanisms of male germ cell differentiation and fertility.
TAP- tagging of the testicular variant of histone H2B (tH2B) in mouse

E Montellier, Grenoble, France

One of the most dramatic chromatin remodeling and genome reorganization ever observed takes place during the post-meiotic maturation of male germ cells. Indeed, after meiosis, early male haploid cells, or spermatids, inherit a somatic-like chromatin-based genome organization. During the following stages of their maturation, histones are removed and replaced by testis-specific basic proteins, while their genome undergoes a dramatic compaction. To shed light on some of the factors involved in the post-meiotic re-organization of the male genome, and more specifically unravel the molecular mechanisms and the factors controlling the general histone removal and the assembly of new structures, we introduced by homologous recombination a TAP-tag in mouse embryonic stem cells of a testicular variant of histone H2B, namely tH2B. We have generated mice bearing the tH2B\textsuperscript{TAPtag} gene. These mice are viable and to our surprise, the expression of tH2B mRNA and protein was reduced to undetectable levels. Hence, this TAPtag model of tH2B resembles a knock down and should helps understand the molecular functions of this histone variant during mouse spermatogenesis. We are currently conducting two main approaches to identify the genomic location of tH2B (ChIPSeq), as well as their interacting partners in vivo (proteomics).

Characterization of spermatogonial stem cells from juvenile marmoset testes

S Albert\textsuperscript{1}, J Ehmcke\textsuperscript{2}, J Wistuba\textsuperscript{1}, HR Schöler\textsuperscript{2}, S Schlatt\textsuperscript{1}, J Gromoll\textsuperscript{1}

\textsuperscript{1}Centre of Reproductive Medicine and Andrology, University Münster, Germany
\textsuperscript{2}Max-Planck Institute for Molecular Biomedicine, Münster, Germany

Spermatogonial stem cells have the capacity to renew themselves and differentiate into daughter cells that maintain gametogenesis. Since the appearance of the spermatogenic epithelium in man is similar to that in the non-human primate Callithrix jacchus, this monkey has been used to illuminate human testicular stem cell physiology. Details of spermatogonial markers, their mRNA expression and the corresponding developmental cell dynamics are not known for the marmoset. We therefore studied the changes in spermatogonial and pluripotency markers during marmoset testicular development. Immunohistochemical and morphometric analyses of seminiferous cords and quantitative PCR analysis of testicular mRNA from newborn and 8-week-old monkeys for the germ and stem cell markers VASA, OCT3/4 and AP-2γ revealed that the number of VASA-positive cells doubled from the newborn to 8-weeks old whereas numbers of AP-2γ- and OCT4-positive cells remained constant. This difference in relative cell numbers indicates dynamic growth of the germ cell population while the population of gonocytes- and spermatogonia-expressing pluripotency markers remains constant. Characterization of markers identifying the pluripotent stem cell population within the non-human primate testis is crucial for opening novel perspectives in stem cell-based approaches to regenerative medicine. (Supported by DFG FOR 1041).
Karyotyping spermatozoa after spermatogonial stem cell transplantation by array-CGH.

E Goossens, P de Vos, H Tournaye

Thanks to advances in chemo- and radiotherapy, cure rates of childhood cancers are high. Long-term survivors face significant adverse effects such as spermatogenic failure and infertility. For adult men sperm banking before treatment may circumvent this adverse effect. However, pre-pubertal patients cannot benefit from this option. A possible solution for the preservation of their fertility is spermatogonial stem cell transplantation (SSCT). The aim of this study is to examine whether spermatozoa obtained after SSCT show genetic abnormalities.

Five to ten months after SSCT epididymal spermatozoa were isolated to perform array-CGH. Chromosomal aberrations, such as polyploidy or aneuploidy could not be detected in spermatozoa from transplanted males. The spermatozoa of two offspring showed a single deviation. The spermatozoa of one male offspring showed a deletion of the H2-M1 gene on chromosome 17. The aberration in the spermatozoa from the other male offspring showed a duplication of the Slc7a12 gene on chromosome 3. This needs to be confirmed.

Our findings show that SSCT can restore the fertility in an otherwise infertile individual without causing significant chromosomal aberrations. These results are important and reassuring for further implementation of SSCT in a clinical setting.

Murine spermatogonial stem cells have the potential to transdifferentiate into hematopoietic cells in vivo

N Liang, E Goossens, M Geens, D Van Saen, I Van Riet, H Dalin, H Tournaye

*Biology of Testis (BITE), Vrije Universiteit Brussel, Brussels, Belgium

Objective: To explore the plasticity and transdifferentiation potential of murine spermatogonial stem cell (SSCs) into hematopoietic cells. Methods: SSCs of GFP+ mice were isolated by magnetic activated cell sorting (MACS) using CD49f antibody and transplanted into the testis of Busulfan treated GFP- mice. Four months later, to exclude hematopoietic cells, GFP+CD49f H-2Kb cells from the transplanted testes were isolated by fluorescence-activated cell sorting (FACS) and injected into the bone marrow of Busulfan treated GFP- female mice. Mice transplanted with bone marrow cells were used as controls. Three months after the transplantation the mice were killed and their bone marrow, peripheral blood and spleen cells were collected. Fluorescence in situ hybridization (FISH) for the Y chromosome and immunohistochemistry (IHC) for the GFP protein were used to detect cells originated from the donors. Co-expression of hematopoietic markers (CD34, Sca-1, CD45, and CD8) and GFP was examined by FACS and fluorescent immunohistochemistry to confirm transdifferentiation from SSCs into hematopoietic cells. The in vitro and in vivo functionality of the isolated GFP+CD34+ H-2Kb cells was evaluated by the Colony-forming units (CFU) test and bone marrow transplantation (BMT). Results: Y chromosome positive cells co-expressing hematopoietic markers and GFP were observed in the bone marrow, peripheral blood and spleen of transplanted mice. GFP+CD34+ H-2Kb cells sorted from the bone marrow of recipients showed similar functionality with hematopoietic cells in vitro and in vivo. Conclusion: SSCs have the potential to transdifferentiate into hematopoietic cells in vivo.