ANDROLOGY

ABSTRACTS

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ORAL COMMUNICATIONS
GOLDEN COMMUNICATIONS

OC01

Risk of prostate cancer in men undergoing assisted reproduction

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Background: Register-studies have reported lower risk of incident prostate cancer for childless men than biological fathers. Other studies have indicated that men with impaired fertility are at higher risk for prostate cancer than fertile men. In order to investigate if this discrepancy is due to the hormonal influence on spermatogenesis, men undergoing intracytoplasmic sperm injection (ICSI) treatment, often due to severely impaired spermatogenesis were compared with those treated with in vitro fertilization (IVF) and fathers by natural conception. The former having insufficient spermatogenesis to fertilize an oocyte ex vivo; therefore their subfertility is likely related to hypogonadism.

Methods: This register-based study sourced data from the Medical Birth Register, the Cancer Registry, and the Quality Register for Assisted Reproduction. All fathers and their first child born 1994–2014 were identified. ICSI fathers were compared to those who had become fathers by natural conception (controls) and in vitro fertilization (IVF) fathers regarding incident prostate cancer during a follow up until 2016, in total 51990101 person-years). Sensitivity analysis stratified upon age at diagnosis of prostate cancer.

Among all fathers (n = 1181490), 20618 and 14882 had undergone IVF and ICSI, respectively; and 3211 were diagnosed with prostate cancer. Associations between mode of conception (ICSI/IVF/natural) and subsequent prostate cancer were investigated using Cox regression models, adjusted for age and education level. Early and late-onset prostate cancer was defined according to age at diagnosis: ≤50 and >50 years.

Results: Fathers who had undergone ICSI had a higher risk of prostate cancer (at any age) as compared to controls (HR = 1.47, CI 95% 1.15–1.89; p = 0.002). Conversely, IVF-men did not have an increase in prostate cancer risk when compared to controls (HR = 1.14, CI 95% 0.91–1.43; p = 0.25). When stratified into age groups at cancer, the fathers who had conceived through ICSI had higher risk for early-onset prostate cancer (HR = 2.94, 95% CI = 1.84–4.71; $p < 0.001$) i.e. diagnosed before 50 years of age. However, ICSI-men did not have an increased risk for late-onset prostate cancer compared to controls. No increased risk of early onset PCa was detected for IVF-fathers (HR = 1.06, 95% CI = 0.57–1.98; p = 0.86).

Conclusion: The results show immense risk for early-onset prostate cancer, generally considered more aggressive, in men referred for ICSI. These men may already have a latent tumor at the time of ICSI, why the possible benefits of targeted screening could be considered.

OC02

Impact of cancer therapy on risk of congenital malformations in children fathered by men treated for germ cell testicular cancer

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Background: There is concern for increased risk of congenital malformations among children of fathers treated for cancer, due to the potential mutagenic effects of chemo- and radiotherapy, as indicated in animal studies. Register studies indicate a slight excess of malformations for children conceived after paternal cancer diagnosis, but these registries lack information on given anti-cancer treatment. Furthermore, we recently reported increased malformation risk in children born prior to paternal germ cell testicular cancer (TC) diagnosis.

Objective: The aim of this study was, with detailed treatment data from the Swedish Norwegian Testicular Cancer Group (SWENOTECA), investigate whether anti-neoplastic treatment implies additional malformation risk.

Methods: All children born in Sweden 1994–2014 (n = 2027997) were included. Paternal TC diagnoses (n = 2380) and treatment data were gathered from SWENOTECA. Offspring malformation diagnoses were sourced from the Swedish Medical Birth Register. Among children born to fathers with TC (n = 4337), 122 had a major malformation. These children were grouped according to the paternal treatment regimen: chemotherapy, or radiotherapy; and according to if the child was conceived pre- (n = 2770) or post-treatment (n = 1437). Logistic regression was applied to calculate odds ratio (OR) for congenital malformation. Adjustments for parental age, maternal smoking and body mass index were made.

Results: Children to fathers with TC had a higher risk for major malformations as compared to children born to fathers without TC (OR = 1.36, 95% CI = 1.24 to 1.49, $p < 0.001$, 2.9% vs. 2.2) However, when comparing children conceived prior to and after treatment, no risk increase associated with chemotherapy (OR = 0.93, 95% CI = 0.57 to 1.51, p = 0.77, 3.0% vs. 3.1%) or with radiotherapy (OR = 1.21, 95% CI = 0.23 to 6.33, p = 0.82, 2.4% vs. 2.0%) could be detected. With all malformations as end point, the risk estimates were similar to those for major malformations.

Conclusion: No statistically significantly increased risk of congenital malformations was seen in children of TC men treated with radio- or chemotherapy. However, paternal TC was associated with a higher risk for malformations.

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OC03

In vitro culture of Klinefelter Spermatogonial stem cells: from mouse to human

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Background: Klinefelter Syndrome (KS) is characterized by masculine phenotype and supernumerary sex chromosomes (47 XXY). Starting at the onset of puberty, KS patients develop progressive testicular fibrosis and impaired fertility due to loss of spermatogonial stem cells (SSC). It's been hypothesized that early SSC cryopreservation could be an option for future fertility treatments in these patients including SSC transplantation or in vitro spermatogenesis. However, in order to do so it is critically important to adapt current in vitro SSC propagation systems for KS patients. Initial proof of concept was conducted on 3 day old KS mouse model testicular tissue and then the system was translated into KS human tissue.

Methods: 3 day old KS mouse testicular tissue was used from the model that has been developed and characterized by UCLA researchers. KS human testicular tissue was donated by three patients ages 13, 15 and 17 years old enrolled in experimental testicular tissue bank at Wake Forest Baptist Health. All three selected patients were non-mosaic 47, XXY according to peripheral blood karyotype. Testicular cells were isolated from cryopreserved tissue and propagated in long term culture adapting our previously established method on normal human testes. Propagated testicular cells were characterized using q-PCR, digital PCR, Flow Cytometry and Magnetic Activated Cell Sorting, next generation sequencing (NGS) based molecular karyotype and X/Y chromosome FISH staining.

Results: Both mouse and human KS cells were successfully isolated and propagated in culture for 80 days or longer. Cell specific gene expression confirmed the presence of all 4 main cell types expected in testes: Spermatogonia, Sertoli, Leydig and Peritubular cells. A population of ZBTB16 + undifferentiated spermatogonia was identified all along culture using digital PCR. Flow Cytometry analysis detected a HLA-/CD9 + /CD49f+ population suggesting a stem cell subpopulation all along culture. NGS testing showed all cells being 47, XXY up to 90% confidence interval. FISH staining for chromosomes X and Y showed most of cells in culture containing XXY combination but also small population of XY and XX cells. Both XY and XX populations were enriched by MACS sorting for CD as SSC enriched marker.

Conclusion: To the best of our knowledge this is the first study showing successful isolation and propagation of testicular cells from mouse and human KS testes. We believe these findings have the potential to impact fertility therapeutic options for azoospermia KS patients either in vitro or in vivo.

OC04

Mouse in-vitro spermatogenesis on alginate-based 3D bioprinted scaffolds

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Background: In-vitro spermatogenesis has already been successfully achieved in rodents by an organ culture system and a soft agarose culture system. However, the former has proven difficult to extrapolate to the human and the latter irreversibly encapsulates all cells limiting its translation and application in the clinic.

Objective: We aim to explore a new culture system in a mouse model using 3D bioprinting which gives control over cell deposition and scaffold design. Specifically, the goals were: Firstly, to culture testicular cells in easy-to-access macropores of printed cell-free scaffolds. Secondly, to culture tubular cells in the pores of printed interstitial cell-laden scaffolds.

Methods: Testicular tissue from prepubertal (max 6 dpp) C57BL/6NCrl (Acr3-EGFP: green acrosome) mice and adult (21 dpp) SV129/C57BL mice were used. Dissociation of the prepubertal tissues into single cells by enzymatic digestion was followed by separation of the tubular and interstitial cells through Magnetic-Activated Cell Sorting (MACS) for CD49f. Per cell-free scaffold, 2.7x105 CD49f+ and 0.2x105 CD49f- cells were consecutively seeded with 2 h interval in the macropores and cultured at the gas-liquid phase (= testicular organoids). In order to print cell-laden scaffolds, the bioink was first diluted 1/10 with adult interstitial cells (± 107/mL). These cells were obtained by separating the seminiferous tubules and digesting the interstitium of adult tissues using collagenase IA. Per cell-laden scaffold, 3.08x105 CD49f+ cells were seeded and cultured (= testicular constructs). Organ culture of prepubertal tissue served as positive control for IVS. The organ culture, organoids and testicular constructs were cultured for 40, 48 and 41 days, respectively. Periodic Acid-Schiff (PAS)/hematoxylin staining, selective peanut agglutinin (PNA) staining of the acrosome and double immunofluorescence (EGFP/CREM) were used to detect postmeiotic cells.

Results: PAS/hematoxylin staining indicated the presence of postmeiotic cells in the organ cultures, organoids and testicular constructs. Immunofluorescence confirmed round spermatids with EGFP/CREM expression and PNA binding to the acrosome in nearly all cultured testicular tissues (n = 4/5). Occasionally, even elongating spermatids with EGFP+ and PNA+ acrosome were observed. Organoid formation was observed in the weeks following cell seeding on cell-free scaffolds. Immunofluorescence showed CREM+ round spermatids in the Acr3-EGFP- organoids of one scaffold (n = 1/3), while two repeats with Acr3-EGFP+ organoids (n = 2/3) even showed elongated spermatids with PNA binding to the acrosome. Round spermatids with EGFP/CREM expression were also

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observed in printed testicular constructs (n = 2/3), of which one testicular construct showed elongating spermatids with PNA staining.
Conclusion: We confirmed previous reports by showing differentiation towards postmeiotic germ cells in the organ culture system. In addition, we showed germ cell differentiation in testicular organoids on cell-free printed scaffolds and in testicular constructs using cell-laden scaffolds. So far, this is the first report applying a 3D bioprinting approach for in-vitro spermatogenesis. It remains to be tested whether the germ cells generated on the alginate-based scaffolds are functional.

OC05

Withdrawn.

OC06

Comparative analyses of AZF microdeletions in leukocytes and testis tissue of TESE patients reveal genetic mosaicism in germ line impairing sperm prognosis

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Background: Three distinct microdeletions in the Azoospermia Factor (AZF) locus of the human Y chromosome cause male infertility with distinct testicular pathologies; they were therefore designated AZFa, AZFb, and AZFc, respectively. Their analysis is frequently used when patients suffering from non-obstructive azoospermia decided for testicular sperm extraction (TESE) before application of ICSI for reproduction in the IVF clinic. It is believed that complete AZFa or AZFb deletion in their leukocyte Y-DNA suggests, that no mature sperms are present in their testicular tubules, whereas patients with complete AZFc deletion do have a very good prognosis for presence of sperms in at least some of their testis tubules. Partial AZF deletions including deletions of only single AZF genes can cause the same testicular pathology as the corresponding complete deletion (e.g. DDX3Y gene deletions in AZFa), or might not be associated with male infertility at all (e.g. some DAZ gene deletions in AZFc). One assumes thereby that rate and extension of the AZF deletion identified in the patient leukocyte Y-DNA corresponds to that present in the patient's germ cells in the testis tissue. However, experimental proof of this prediction is still lacking.
Methods: We therefore collected a number of patients diagnosed with nonobstructive azoospermia and presence of a classical AZFb or AZFc deletion in their leukocyte Y-DNA which decided for TESE, to analyse for comparison the rate and extension of these AZFb/AZFc deletion in genomic Y-DNA samples extracted from their left and right testis tissue. For this purpose we performed PCR multiplex assays according to Vogt & Bender (in: Meth. Mol. Biol. vol. 927: 187–204, 2012.). This protocol is able to distinguish classical and partial AZFa, AZFb, AZFc deletions including deletions of only one of the 14 protein coding AZF genes. It can be certified as robust standard protocol because it includes the basic principles of quality control essential for each molecular genetic diagnostic deletion assay according to the strict guidelines of the "European Molecular Genetics Quality Network" (EMQN: www.emqn.org).
Results: We found seven samples with some heterogeneity in the genomic extensions of their AZFb/AZFc deletion in leukocytes and testis tissue. One patient presents a partial AZFb + complete AZFc deletion but only in leukocytes; 6 patients with complete AZFc deletions in leukocytes displayed only partial AZFc deletions in testis tissue. We conclude that the divergent AZF Y deletions observed in the testis samples of these patients suggest some genetic mosaicism of these AZF deletions in the patients germ line. It points to dynamic rearrangement(s) of the long Y arm in these patients testis DNA, probably due to the high frequency of recombination hot spots found in the repetitive sequence blocks concentrated in the distal AZFb and all AZFc amplicons.
Conclusion: Our results can probably help to explain the large heterogeneity of testicular pathologies found in patients with complete AZFb/AZFc deletions in their leukocytes and are important for clinical counselling when they ask for their sperm prognosis before performing testis biopsy.

SELECTED ORAL COMMUNICATIONS

OC07

Long-term effect of testicular germ cell tumor treatments on sperm DNA fragmentation

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Background: Testicular Germ Cell Tumor (TGCT) is the most frequent malignant disease in young men. In the large majority of cases, spermatogenesis is recovered after cancer-treatment hence the future welfare of children conceived by a father treated with cytotoxic therapy is of concern. Information are scarce, regarding the long-term effect of cytotoxic treatments on sperm DNA, with a maximum observation period of up to 2 years. In some studies, high DNA fragmentation and sperm aneuploidy rate were reported in a proportion of patient after 24 months, raising questions about the right timing for natural conception.

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Objective: To evaluate the effect of cytotoxic therapies on Sperm DNA Fragmentation (SDF) after 2 (T2) and 3 years (T3) from the end of treatment in TGCT patients.
Methods: Cross-sectional study. Total and brighter SDF (SDFtot and SDFbr, respectively) were analyzed in 2 TGCT-patients groups: 2 years post-therapy (T2 group, $n = 76$) and 3 years post-therapy (T3 group, $n = 54$). The analysis based on TUNEL-assay, was performed on 10x10^6 sperm cells. In each group: (i) data were compared with those of 58 healthy men (control group); (ii) the proportion of patients with %SDFbr>75th percentile of "normality" (i.e. >25%) was calculated.
Results: Patients were divided according to the type of treatment for each time-category (T2 and T3): Carboplatine ($n = 22$ and 14, respectively), PEB with ≤2 cycles ($n = 18$ and 14, respectively) or with ≥3 cycles ($n = 20$ and 11, respectively), radiotherapy ($n = 14$ and 12, respectively) and chemotherapy+radiotherapy ($n = 2$ and 3, respectively). The mean %SDFtot and %SDFbr of the control group were 29.11% ± 11.11% and 19.53% ± 9.48%, respectively.
i) T2 group: both %SDFtot and %SDFbr were significantly higher in patients vs fertile men in the following treatment's groups: (i) PEB with ≥3 cycles: %SDFtot = 39.98% ± 15.32%, $p = 0.001$; %SDFbr = 25.61%±10.37%, $p < 0.05$; (ii) radiotherapy: %SDFtot = 43.60%±17.35%, $p < 0.001$; %SDFbr = 32.75%±16.54%, $p < 0.001$; (iii) chemotherapy+radiotherapy: %SDFtot = 68.72% ± 38.13%, $p < 0.001$; %SDFbr = 39.88% ± 30.33%, $p < 0.01$. in the Carboplatine therapy group only %SDFtot was significantly higher compared to controls (36.34% ± 16.86%, $p < 0.05$). T3 group: both %SDFtot and %SDFbr were significantly higher in subjects treated with chemotherapy+radiotherapy vs fertile controls (% SDFtot = 54.45% ± 24.01%, $p = 0.001$; %SDFbr = 37.80% ± 14.15%, $p < 0.01$). in PEB with ≥3 cycles group only SDFtot was significantly higher compared to controls (38.64% ± 19.86%, $p < 0.05$).
ii) The proportion of patients with clearly pathological SDFbr value (i.e. >75th percentile) was above 30% in the T2 group for all type of treatments. In T3 time-category, the proportion was lower than this percentage in the less aggressive treatments-groups (i.e. Carboplatine therapy, PEB with ≤2 cycles) whereas it was above 30% in the most aggressive ones (i.e. PEB with ≥3 cycles, radiotherapy, chemotherapy+radiotherapy).
Conclusion: Currently, spontaneous pregnancy is not recommended during the first two years after cytotoxic treatments; in light of our finding of a relatively high incidence of patients with pathological SDF values after 3 years post-therapy, it would be advisable to re-evaluate the waiting period beyond this time-limit. In order to provide greater certainty to cancer patients (regarding most appropriate timing for the search of a natural conception), sperm DNA fragmentation study could be introduced in the clinical practice to monitor the genomic damage.

OC08

TEKT5 is a new candidate gene for male infertility

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Background: Diagnostics of male infertility includes semen and hormone analyses which often result in descriptive classifications but do not explain underlying causes. Genetic causes of male infertility include chromosomal aberrations, AZF deletions, CBAVD and CHH but overall only explain 4% of cases. The large majority of ~70% remains unresolved (1). Here we report on a new candidate gene for male infertility, namely TEKT5. TEKT5 is the youngest member of the Tektin protein family. Tektins are filament-forming proteins in the male germ cell-lineage present in centrioles, basal bodies and within ciliary and flagellar doublet microtubules. TEKT5 was initially identified in the rat. It is present in sperm flagella, plays an important role in flagella formation during spermatogenesis, and has also been implicated in sperm motility. The expression of TEKT5 mRNA is restricted to the testis in normal adult tissues (2, 3).
Methods: First, a specifically selected group of 36 men with complete bilateral Sertoli-Cell-Only syndrome (SCOS) were analysed by whole-exome sequencing (WES). In the next step, a larger cohort of well characterised infertile men ($n = 250$) with different phenotypes (SCOS $n = 109$, meiotic arrest $n = 24$, mixed atrophy $n = 22$, non-obstructive azoospermia [no biopsy] $n = 62$, severe oligozoospermia $n = 33$) was analysed by WES. Variants with MAF<1% in public genome databases (dbSNP, ESP, gnomAd, ExAc) were assessed by in silico algorithms (PolyPhen-2, SIFT, MutationTaster) and were modelled using Phyre-2 homology prediction and visualized by Pymol. IHC staining of TEKT5 was performed in fetal and adult testicular tissue from mouse and human and from patients with TEKT5 alterations. BeWo cells, which endogenously express TEKT5, where stained by immunofluorescence.
Results: In the initial patient cohort ($n = 36$) we prioritized TEKT5 as highest ranked candidate gene due to the identification of three heterozygous variants (1 stop-gained, 2 missense variants) and described testicular expression. In the entire patient cohort ($n = 286$) we identified 19 novel or rare (MAF<1%) heterozygous variants (2 stop-gained, 1 frameshift, 16 missense variants) and one deletion of the complete TEKT5 gene from our previous array-CGH analyses (4). IHC staining of TEKT5 was similar in adult mouse and human testes (late spermatocytes, round spermatids and spermatids). In addition, in fetal mouse and fetal human testes, TEKT5 is also expressed in germ cells. No TEKT5 expression could be detected by IHC in testicular biopsies of the patient showing a complete deletion.

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TEKT5 immunofluorescence staining of BeWo cells showed a signal within the nucleus until confluency, implicating a so far undescribed nuclear role of TEKT5.
Conclusion: We propose for the first time that the testicular expressed TEKT5 gene is a new candidate gene for male infertility. We showed the expression of TEKT5 in germ cells and suspect a to date unknown nuclear role.
This work was carried out within the frame of the DFG Clinical Research Unit 'Male Germ Cells: from Genes to Function' (CRU 326).
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OC09

Metabolomic profiling by 1H-NMR of human seminal plasma and database-driven analysis reveal new features for glycerophosphocholine

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Background: Seminal plasma (SP) is a complex mixture that dilutes and transports spermatozoa and is recognized as an anatomical and functional reflect of the male genital tract. Therefore, SP exploration is informative for the etiological diagnosis of male infertility. Recently, 1H NMR studies of human biofluids have attracted much attention as a powerful tool to understand pathophysiological processes at metabolites level.
Objective: To perform and to compare untargeted metabolomic profiling by 1H-NMR of seminal plasma from normozoospermic (NZ) and azoospermic (AZ) patients.
Methods: SP samples from 47 consecutive NZ (according to WHO 2010 criteria) and 38 consecutive AZ were stored after routine investigations and analyzed by batches on a Bruker DRX 600 MHz NMR Spectrometer. A manual signal assignment of metabolites was performed and spectrum-shaped metabolomics data from NZ and AZ patients were transformed by the wavelet method before a Sparse-PLS-DA-based comparison. For further statistical analysis, data of 993 NZ patients and 461 AZ patients referred to our Andrological Center between 2005 and 2015 were extracted from the database.
Results: In preliminary experiments, 1H- and 13C–RMN spectra (1D, 2D COSY, 2D HSQC) were obtained and manual signal assignment retrieved 30 unambiguous metabolites. Sparse PLS-DA on wavelets-transformed spectra identified several intervals of chemical shift with significant differences between NZ and AZ. In the 2,9–3,9 ppm interval, 3 metabolites could be identified : choline, fructose and glycerophosphocholine (GPC). Enzymatic assays confirmed a difference for total seminal GPC between NZ and AZ (respectively 6.89 ± 4.06 and 3.11 ± 3.32 μmol, $p < 0.0001$) but not for total choline or fructose. Comparing NZ and AZ from the center database confirmed this difference in total GPC (respectively 7.9 ± 7.6 and 3.1 ± 3.0 μmol, $p < 0.001$). A detailed analysis of the NZ dataset revealed that total GPC is negatively correlated to patients' age ($p = 0.0003$) and BMI ($p = 0.003$), positively correlated to spermatozoa concentration ($p < 0.0001$) but not to sperm motility or viability.
Conclusion: Metabolomic profiling by 1H-NMR of SP and statistical analysis of 1454 patients shed light on GPC and revealed its link with spermatozoa concentration, opening new research perspectives.

OC10

Presentation, clinical features, and long term follow up of Leydig cell tumors (LCTs) of the testis: a single centre experience

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Background: With the improved imaging techniques, Leydig cell tumors (LCTs) are frequently found. Natural history of LCTs is relatively unknown, because of the small size and heterogeneity of available studies. Long-term follow-up is missing.
Objective: Our aim was to report the experience with a large cohort of prospectively collected LCTs.
Methods: Patients with LCTs were enrolled from 2005 to 2017. Clinical and biochemical features of LCTs were compared with two cohorts: patients with seminomas and patients without testicular lesions (NoL) randomly selected among patients referred in the same period.
Results: 83 patients had LCTs, 90 had seminomas, and 2683 had no lesion (NoL). Testicular volumes ($p = 0.001$), sperm concentration ($p = 0.001$) and morphology ($p < 0.001$) were lower in LCTs compared to NoL; gonadotropins ($p < 0.001$) and SHBG ($p < 0.001$) were higher and testosterone/LH ratio was lower ($p < 0.001$) in LCTs vs NoL. No differences were found in gonadal steroid after hCG test, between groups.
When compared to seminomas, LCTs did not show differences except for higher SHBG levels ($p = 0.001$), lower sperm concentration ($p = 0.029$) and motility ($p = 0.001$). Cryptorchidism (chi square=28.272, $p < 0.01$), gynaecomastia (chi square = 54.223, $p < 0.001$) and low testicular volume (chi square = 11.133, $p = 0.001$) were associated with a higher risk of LCTs. After a median follow-up of 66 months, no metastases have been detected.
Conclusion: LCTs have good prognosis when correctly recognized. Based on the largest existing series, we showed that infertility, gynecomastia, low testicular volume, and cryptorchidism are frequently associated with LCts, supporting the hypothesis that testicular dysgenesis syndrome could play a role. Active surveillance appears to be a safe option, but monitoring of Leydig cell failure remains necessary.

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OC11

Establishing a SNP-panel (on single nucleotide polymorphisms) associated with FSH action – an approach for personalized FSH treatment in men with unexplained infertility

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Background: 30–40% of infertile men suffer from unexplained infertility. Since there is no causal treatment, these patients are often referred to assisted reproductive techniques.

FSH is the key player in initiation and maintenance of spermatogenesis. General unclassified hormone treatment with FSH in infertile men only improves semen quality and pregnancy rates in an undefined subgroup. Previously it was shown that the single nucleotide polymorphism (SNP) within the FSHB gene (c.-211G>T) negatively impacts endocrine feedback, testicular size and spermatogenesis. However, polymorphisms in FSHB and FSHR only explain ~5% of FSH variation. Further polymorphisms, which have to be identified, will most probably contribute to FSH action. Taking the hypergonadotropic Klinefelter syndrome (KS) (47,XXY) as a model for FSH action we found the impact of the FSHB SNP on serum FSH levels in KS to be 23-fold increased compared to our infertile male cohort.

Methods: In a discovery study on 104 KS patients (no mosaicism, no prior treatment) SNP analysis was performed using HumanOmniExpressExome arrays. We used Illumina®GenomeStudio and plink (connection tool) to perform a quantitative association analysis based on FSH phenotype values. The SNPs were annotated using dbSNP, the Human Protein Atlas and expression data from GTEx Consortium datasets. Pathways related to the SNPs were analyzed using Ingenuity® Pathway Analysis and Reactome. SNPs were then manually prioritized based on the statistical and biological properties of the annotated dataset.

Results: Using a combination of statistical and biological properties (*p* value, expression in endocrine related genes/pathways, expression in hypothalamus/pituitary gland/testis) we identified $n = 252$ relevant SNPs for validation.

Conclusion: Here, the KS is used as model to identify further SNPs/genes affecting FSH action. In the array analysis, we found 252 candidate SNPs in testis, hypothalamus or pituitary genes showing associations with FSH levels. This presents the first step towards a targeted SNP panel, which will be validated in a large cohort of patients with unexplained infertility and be compared to fertile men. Further, functional analyses on the highest ranked genes will reveal further information on FSH signalling. Altogether, we aim to identify a subset of infertile men with specific SNPs, which render them eligible to FSH treatment.

OC12

A chromosomal scan of single sperm cell by combining. Fluorescence-activated cell sorting and Next-generation sequencing

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Background: Reciprocal translocations are the most frequent structural chromosomal rearrangements in humans that may produce unbalanced gametes by meiotic segregation.

Methods: In this study, we developed an experimental approach combining fluorescence-activated cell sorting (FACS), whole genome amplification, and next-generation sequencing that allows detecting the full aneuploidies and structural chromosomal alterations from single sperm cells.

Results: By applying this protocol, six sequenced single sperms from a normozoospermic man showed a normal 23-chromosome profile with two and four of the cells bearing X and Y chromosome, respectively. Furthermore, the analysis of 31 single sperms from one carrier of a reciprocal translocation 46, XY,t(7;13)(p10;q10) revealed that 35.6% of sperms had normal haploid chromosomal composition; and 64.5% of analyzed sperms showed several variants of aneuploidies. The sperms with partial or full aneuploidies of chromosomes 7 and 13 represented the meiotic products of adjacent I and II segregation and one sperm showed a gain of chromosome 9.

Conclusion: The application of this method enables comprehensive chromosomal aberration screening of a large number of sperms and provides an effective tool for studying the production of gametes from patients carrying chromosomal diseases. Moreover, it could be implemented in clinics to support the personalized family planning in several patients' groups, such as men with chromosomal aberrations and/or male infertility, and in couples with recurrent miscarriages.

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OC13

Testicular ultrasound inhomogeneity is more informative than testicular volume in fertility evaluation

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Background: Testicular volume (TV) is proposed as a positive predictor of male fertility status considering its known relation to the seminiferous tubules content. Although the testicular size assessment remains in clinical practice the first approach to indirectly evaluate spermatogenesis, its real predictive significance remains not clearly detected.
Objective: To assess TV and ultrasound (US) characteristics of patients undergoing single operator-testis US, matching results with seminal and hormonal parameters.
Methods: All consequent out-patients undergoing testis US from March 2012 to March 2018 for any reason were considered eligible. TV was calculated using the ellipsoid formula with the 0.71-coefficient. Testis US inhomogeneity was defined as the absence of the uniform hypoechoic structure characterizing normal testicular parenchyma evaluable at ultrasound. Analyses were performed dividing patients according to clinical reason for attending. Patients examined for fertility workup were further subdivided according to the seminal status, whereas hypogonadal patients were subdivided considering the administration of replacement androgenic therapy. Correlations among TV and hormonal and semen parameters were evaluated.
Results: 302 men were enrolled (mean age 39.8 ± 15.2 years). Reasons for US evaluation were gynecomastia (12.3%, $n = 37$), hypogonadism (33.4%, $n = 101$), couple infertility (CI) (39.1%, $n = 118$) and sexual dysfunctions (15.2%, $n = 46$). CI patients presented normozoospermia in 25.8%, impaired semen quality (oligo- and/or astheno- and/or teratozoospermia) in 55.9% and azoospermia in 18.3% of cases. In CI group, the mean TV value was 14.96 ± 7.45 ml (right testis) and 13.83 ± 6.80 ml (left), significantly higher compared to hypogonadal patients ($p < 0.001$). A significant direct correlation between TV and testosterone levels was observed in not-treated hypogonadal patients ($R = 0.911$, $p < 0.001$), whereas this correlation was absent in CI group. Normozoospermic patients presented significantly higher TV (19.16 ± 8.51 ml and 18.24 ± 6.57 ml) compared to impaired semen quality (13.48 ± 5.69 ml and 12.69 ± 5.55 ml) ($p = 0.003$) and azoospermia (11.71 ± 6.67 ml and 9.15 ± 4.38 ml) ($p = 0.003$) groups. TV was directly related to sperm number only in normozoospermic patients ($R = 0.577$, $p = 0.005$). Testis US inhomogeneity was more frequent in patients with impaired sperm quality (55.0%) ($p = 0.007$), compared to azoospermic (40%) and normozoospermic (5%), whereas the US finding of microcalcifications did not differ ($p = 0.090$).
Conclusion: In our cohort, although testicular size is significantly higher in normozoospermic patients, TV does not appear informative of the fertility status. Indeed, TV correlates with sperm number in normozoospermic men, but not in patients with altered semen quality. Moreover, in our population, the direct correlation between TV and testosterone observed in hypogonadal not-treated patients suggests that testicular sizes could be related with the testosterone-secreting rather than the spermatogenic compartment. Conversely, since different distribution of testis US inhomogeneity is highlighted comparing normozoospermic and patients with poor seminal quality, the sonographic pattern could better relate with the fertility status. With this in mind, in the CI workup the US evaluation seems to be more informative rather than the simple TV assessment.

OC14

Antisperm-antibodies prevalence and relationship of autoimmunisation degree with semen parameters and post-coital test outcome. A retrospective analysis on over 10,000 men

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Background: Although the IgG-MAR test has been recommended by WHO as an integral part of semen analysis for the screening of anti-sperm antibodies (ASA), their prevalence and relationship with semen parameters, as well as the cut-off values of clinical relevance are controversial.
Methods: We retrospectively analyzed semen analysis from 12,296 consecutive men seeking semen evaluation, representing the largest series ever reported. Immunological screening was performed to all ejaculates by IgG-MAR-test. Positive samples (≥10%) were tested also for IgA-ASA. The prevalence of positive IgG-MAR tests and the relationship of both degree and isotype of sperm-autoimmunization with semen parameters were evaluated. Outcomes of post-coital test (PCT) performed in couples where the male partner exhibited a positive MAR-test were also analyzed.
Results: Excluding from analyses samples with not-executable MAR-test (2,271 out 12,296 samples), due to azoospermia or severe oligo- and/or astheno-zoospermia, the prevalence of MAR-test results ≥10%, ≥50% and 100% was 4.0%, 3.4% and 2.0%, respectively. Samples with 100% positive IgG-MAR-tests exhibited a significantly higher prevalence of both mixed positivity pattern and concomitant IgA-ASA and a significantly lower prevalence of tail-tip pattern. Both total sperm count (TSC) and progressive motility (PM) were significantly lower in samples with 100% positive IgG-MAR-test. At the bivariable regression models, TSC was independently associated to both IgG-MAR-test and IgA-MAR-test positivity, whereas, PM was independently associated with IgG-MAR-test but not with IgA-MAR-test positivity. Analyzing the PCT in 120 couples where the male partner exhibited a positive IgG-MAR-test, the number of forward-moving spermatozoa/HPF was negatively correlated with positivity % of both IgG-MAR-test ($r = -0.54$; $p < 0.0001$) and IgA-MAR-test ($r = -0.28$; $p = 0.001$).

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However, at the bivariable regression analysis, an independent negative association was found for IgG-MAR-test (β: −1.7; $p < 0.0001$), but not for IgA-MAR-test positivity (β: −0.1; $p = 0.1$). The percentage of couples with negative PCT outcome significantly increased with the percentage of IgG-MAR-test positivity. At the multiple logistic regression analyses, in the case of 100% positive MAR-test, neither cervical mucus score nor seminal total motile sperm count significantly contributed to negative PCT outcome. Whereas, in the case of 50–99% positive MAR-test, total motile sperm count significantly contributed, besides the presence of ASA, to negative PCT outcome.

Conclusion: This study, the largest so far reported, provides a reliable estimate of ASA prevalence. Although 50% positive MAR-test represents the cut-off suggested by the WHO with possible clinical relevance, only 100% positive MAR-tests were significantly associated with a "mixed" pattern of link, concomitant occurrence of IgA-ASA, lower sperm quality and poor outcome of PCT, a surrogate infertility-related end-point, as the impairment of sperm penetration through the cervical mucus represents the primary mechanism of ASA interference with fertility.

OC15

Testosterone replacement therapy is able to reduce prostate inflammation in men with BPH, metabolic syndrome and hypogonadism: preliminary results from a randomized placebo-controlled clinical trial

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Objective: BPH results from prostate tissue inflammation, which frequently occurs in men with metabolic syndrome (MetS). MetS is often associated with low testosterone (T). Recent evidence shows that low, rather than high, T levels are associated with BPH/lower urinary tract symptoms (LUTS). The aim of the study was to evaluate if T replacement therapy (TRT) for 6 months in BPH men with MetS and low T, is able to improve LUTS and prostate inflammation (as assessed by ultrasound and gene expression in prostate tissue).

Methods: 120 men in waiting list for BPH surgery and diagnosed with MetS were enrolled in the clinical trial. According to total T (TT) and calculated free T (cFT), they were categorized into eugonadal (TT≥12 nmol/L and cFT≥225 pmol/L; $n = 48$) and hypogonadal men (TT<12 nmol/L and/or cFT<225 pmol/L; $n = 72$). Hypogonadal men were randomly assigned to receive T gel 2% (5 g/daily) or placebo for 6 months. At baseline and follow-up visit (after 6 months), all men filled out the International Prostatic Symptoms Score (IPSS) and NIH-Chronic Prostatitis Symptom Index (NIH-CSPI) questionnaires and underwent a trans-rectal prostate ultrasound. After surgery, prostate tissue was collected.

Results: After adjusting for the baseline value, together with age, TT and waist circumference, NIH-CSPI total score significantly decreased in both the groups ($p < 0.001$ vs. baseline), whereas IPSS total score did not change in any of the groups. IPSS bother score significantly decreased only in T-treated ($p = 0.042$ vs. baseline value). Although a significant increase in total prostate and adenoma volume occurred in T-treated (both $p < 0.05$ vs. the baseline value), T arm was characterised by a significant decrease in ultrasound markers of prostate inflammation, including arterial velocity and acceleration (both $p < 0.01$ vs. baseline value). In a subset of patients (9 eugonadal, 11 placebo and 9 T-treated), the expression by prostate tissue of inflammatory markers was evaluated. COX2, MCP1 and RORC were found significantly decreased in T-treated as compared with placebo arm (all $p < 0.01$) and for COX2 and MCP1 even in comparison with eugonadal men (both $p < 0.05$).

Conclusion: Six-month treatment with T gel 2% in hypogonadal men with BPH and MetS is able to improve several clinical, ultrasound and molecular proxies of prostate inflammation. This results into a moderate improvement in symptoms, particularly prostatitis-like symptoms and bother for LUTS.

OC16

Effects of different follicle-stimulating hormone preparations on pre-pubertal porcine Sertoli cell cultures: preliminary results

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Background: At present, there are no "in vitro" studies on the effects of different preparations of follicle-stimulating hormone (FSH) on pre-pubertal Sertoli cells, which could provide important information in reproductive medicine. The different preparations of FSH available in the market, obtained both by recombinant technology (α and β follitropin) and post-menopausal urine (urofollitropin), are characterized by structural and functional heterogeneity lies on their different content in sialic acid C-terminus residue of the oligosaccharide chain. The unique testis receptor of FSH (FSH-r) is exclusively localized on Sertoli cells. The use of FSH in oligozoospermic males, a useful therapy in selected patients, is affected by conflictual findings.

Objective: The aim of our study was to assess the effects of different FSH preparations on ultrapure, viable and functional porcine pre-pubertal Sertoli cells (pSC) by evaluating modulation of their specific markers.

Methods: We have evaluated pSC, obtained from 15–20 days old neonatal porcine testes, in terms of purity by immunofluorescence and cytofluorimetric analysis. Subsequently, purified pSC culture were treated with:

- α-follitropin, β-follitropin and urofollitropin at the same molar concentration (100 nM) for 48 hours;

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- Testosterone (T): 0.2 mg/ml;
- Combinations of different FSH preparations with T.

Both in basal and after 48 hours of FSH stimulation, we have performed:

a) Real Time PCR analysis of anti- Müllerian hormone (AMH), inhibin B and FSH-r;
b) Western blotting analysis (WB) of FSH-r, pospho-AKT, pospho-ERK1/2;
c) ELISA assay, both in cell extract and culture medium, for AMH and inhibin B.

Results: In our model, we observed, that:

- All three preparations of α-follitropin, β-follitropin and urofollitropin induced, as expected, a reduction of AMH in terms of mRNA, cell extract and secreted protein;
- All three preparations induced an increase of inhibin B in terms of mRNA and cell extract protein and, while interestingly, only α-follitropin induced an increase of inhibin B secreted in the culture medium;
- All three preparations induced, as expected, a reduction of FSH-r mRNA but only α-follitropin was associated with downregulation of FSH-r (WB).
- Only α-follitropin induced a downregulation of pospho-AKT (WB).
- All three preparations induced an increase of pospho-ERK1/2 (WB).

Conclusion: These results preliminarily showed, that the three FSH preparations were associated with different effects in terms of inhibin B secretion, arising the question if, in the treatment of the infertile male, should be preferred FSH preparations that increase inhibin B secretion or not. Our present study could help better understanding the effects of different FSH preparations, thus providing important information on both, the conflictual findings with regard to use of FSH in oligozoospermic males and, in general, reproductive medicine.

OC17

Molecular and functional characterization of a unique genotype in a man affected by congenital hypogonadotropic hypogonadism

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Background: Congenital hypogonadotropic hypogonadism (cHH) is a rare endocrine disease (incidence of 1:8000 males), caused by the deficient production, secretion or action of gonadotropin-releasing hormone (GnRH). Its typical clinical manifestation is delayed puberty and azoospermia. Homozygous or compound heterozygous inactivating mutations in the GnRH receptor gene are among the most frequent causes of normosmic CHH (nCHH), accounting for about 10% of cases. The human GNRHR gene maps to chromosome 4 (4q13.2).

Methods: Molecular characterization of a novel homozygous mutation (p.Gly99Glu) in the exon 1 of the GnRHR through: i) gene dosage (qPCR); ii) SNP array; iii) functional studies consisting of competition binding assay and inositol phosphate (IP) signaling assay.

Results: A novel GnRHR mutation (p.Gly99Glu) was identified in a 20-year-old man with eunuchoid body shape, Tanner Stage 1 and bilateral cryptorchidism with low total testosterone, LH and FSH levels. The same mutation was found in heterozygosity in the mother, whereas the father was wild type. After confirming biological paternity, real-time PCR analyses (qPCR) showed two GnRHR copies. In order to investigate about the origin of homozygosity in the proband, SNP array (CytoScan® 750K Affymetrix) was performed. It revealed that the patient has inherited two copies of chromosome 4 from the mother (maternal heterodisomy; hUPD) with 2 regions showing loss of heterozygosity (maternal isodisomy; iUPD) on the long arm of chromosome 4 (4q12-q21.21 and 4q33-q35.2), one of which contains the mutated gene. Since our patient has no paternal contribution from the whole chromosome 4, we sought to explore the presence of maternally imprinted genes on chromosome 4. The NAP1L5 gene has monoallelic paternal expression. Our proband does not show any dysmorphic features or congenital malformations but he is of short stature, opening questions on the role of this gene in growth retardation. Functional studies revealed that cells transfected with the Gly99Glu mutant GnRH receptor showed no measurable radioligand binding. Appending a carboxy-terminal tail to the Gly99Glu mutant receptor did not recover radioligand binding, suggesting that any increased expression was not sufficient to allow binding of radioligand at the low concentration (~0.1 nM) used in competition binding assays. GnRH stimulated IP production in cells transfected with the Gly99Glu mutant GnRH receptor, suggesting that the mutant receptor protein was well-expressed. However, GnRH potency was three orders of magnitude lower (EC50, 899.3 nM). Appending the carboxy-terminal tail to the Gly99Glu mutant GnRH receptor had small effects on GnRH-stimulated Emax and GnRH potency. The above data suggested that the mutation severely decreases GnRH binding affinity. Thus, based on its functional consequences, this novel GnRHR variant can be classified as a severe partial loss of function (pLOF) mutation.

Conclusion: In conclusion, we have demonstrated both a novel causative missense mutation of GnRHR (p.Gly99Glu) described as severe pLOF and a maternal hUPD/iUPD of chromosome 4 causing ncHH. Similar chromosomal rearrangements of chromosome 4 have been described only in 4 cases, and it is the first case of a patient affected by nCHH due to this rare chromosomal rearrangement.

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OC18

X-chromosome exome sequencing in highly selected idiopathic azoospermic patients: identification of novel and recurrent genetic factors for early spermatogenic failure

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Background: The severest form of male factor infertility is non-obstructive azoospermia (NOA), which occurs in approximately 1% of all men in reproductive age and in the majority of patients the etiology remains unknown. Despite the well known enrichment of the X chromosome in genes specifically expressed in the testis, so far only two X-linked genes are diagnostic targets in NOA. The apparent paucity of X-linked NOA is likely to be the consequence of the lack of comprehensive, whole X chromosome targeting studies.
Methods: X-chromosome exome sequencing (a total of 836 protein-coding genes) in 50 idiopathic NOA patients with known testis histology. Variants were filtered and prioritized according to their minor allele frequency (MAF≤0.01), their predicted pathogenicity and their tissue expression profiling. Expression analyses was performed in human testis biopsies for the RBBP7 gene through RT-qPCR. RNA interference was used to determine the role of Caf1-55 (the human RBBP7 ortholog) in Drosophila spermatogenesis.
Results: We identified 74 rare and predicted as pathogenic variants in 38/50 NOA patients. 72 variants were private mutations whereas one was found in two unrelated patients. Nine genes (six of them with testis specific expression or overexpression in the testis) were recurrently mutated in 16 different patients. Two patients affected by spermatogonial arrest presented pathogenic mutations in the RBBP7 gene. Expression analysis in testis biopsies with different histology corroborates that RBBP7 is highly expressed in testis and shows an overexpression in spermatogonia Conditional Caf1-55 KO showed that male mutants had tiny testis, no spermatozoa and were sterile.
Conclusion: This is the first X chromosome exome analysis in highly selected NOA patients. Our approach was relatively successful in identifying candidate genes for the NOA phenotype. Up to now, we performed functional analysis only for the RBBP7 gene demonstrating that the protein is essential for Drosophila spermatogenesis hence we propose it as a novel genetic factor for early spermatogenic failure.
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OC19

Top-down proteomic approach to study the protamine post-translational modifications profile in the human spermatozoa

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Background: The protamine 1 (P1) and protamine 2 family (P2) comprise the most abundant basic proteins in human spermatozoa. P1 is synthesized as a mature form, whereas the P2 family is generated from the proteolysis of a precursor, giving rise to the mature forms HP2, HP3, and HP4. Protamines pack approximately 85–95% of the paternal genome, while the other 5–15% remain attached to histones, which together with the histone post-translational modifications (PTMs) code contribute to the paternal epigenetic signature during the preimplantation embryogenesis. However, the epigenetic function of a potential protamine PTMs code has been poorly studied, although protamines are much more abundant in sperm chromatin. Protamines are utterly basic proteins with particular physical-chemical properties due to their amino acid sequences. Because of this, protamine identification by the well-established bottom-up mass spectrometry (MS) strategy on trypsinized proteins is not as straightforward as for other basic proteins. A top-down MS approach is therefore proposed to identify human protamines and their PTMs. The intact protamine-enriched fraction from one normozoospermic individual was isolated from purified spermatozoa after histone removal and disulfide bonds reduction. Protamine enrichment was verified through acid-urea gel electrophoresis. The protamine-enriched fraction was analyzed by nano-liquid chromatography coupled to tandem MS (nanoLC-MS/MS) using a chip-based Advion nanoelectrospray source and an Orbitrap Fusion Lumos (Thermo Scientific) mass spectrometer. The latter was operated in data-dependent acquisition (DDA) mode, and the most abundant ions were selected for fragmentation by Electron Transfer Dissociation (ETD). Data analysis was performed using Proteome Discoverer 2.1 with Prosight PD 4.0 and Sequest HT nodes and TopPIC software. In addition, RNA was isolated and purified from one individual to confirm the presence of a new potential P2 isoform at RNA level by direct sequencing. The top-down proteomic MS approach mainly allowed the identification of the intact naïve P1, while HP2 and HP3 were detected with minor peak intensities. In

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contrast, HP4 was not detected, probably due to its physiological low abundance. A phosphorylation pattern in P1 and combinations of other PTMs among the different protamines were detected. Notably, hyperoxidation on cysteine residues irreversibly modified to sulfinic acid were found in almost 100% of HP2 and HP3 and in nearly 70% of HP1, most likely because of the presence of reactive oxygen species (ROS). However, the mass of the hyperoxidized HP2 totally matches with an alternative spliced variant of P2 identified at RNA level by our group and others. The incorporation of a middle-down MS strategy using a digestion with GluC would allow differentiating between the potential alternative spliced variant of P2 and the hyperoxidized HP2. The establishment of the normal protamine PTMs profile in fertile individuals and the identification of pattern alterations in different types of infertile patients, including those with abnormal elevated ROS, would provide insights into the role of protamine PTMs code to male fertility and its potential function as epigenetic mark during early stages of preimplantation embryogenesis.

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OC20

Functional characterization of Binder of SPerm homolog 1 in sperm-egg interaction and fertilization

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Background: In mice and humans, the Binder of SPerm homolog 1 (BSPH1) protein is exclusively expressed in the epididymis. BSPH1 proteins have been shown to be involved in the sperm membrane changes underlying capacitation. Findings from experiments with the recombinant mouse BSP homolog (rec-BSPH1) suggest that the protein initially resides on the surface of the sperm and then relocalizes over the head and mid-piece during capacitation, suggesting a potential role for BSPH1 in sperm-egg interaction.

Objective: In the current study, we investigated the role of mouse recombinant BSP homolog 1 (rec-BSPH1) in sperm-egg interaction using an in vitro fertilization (IVF) assay.

Methods: Mouse rec-BSPH1 was produced by transforming E. coli with a pET32a vector carrying BSPH1 cDNA and purified using immobilized metal (Ni2 +) affinity chromatography. Oocytes were pre-treated with rec-BSPH1, control proteins or media alone, and inseminated with capacitated sperm. In addition to IVF assay, the potential binding of rec-BSPH1 to the oocyte surface was investigated using immunofluorescence. Finally, sperm-bound native BSPH1 was immuno-neutralized by anti-rec-BSPH1 antibodies to indirectly verify implication of BSPH1 in sperm-egg interaction and fertilization.

Results: Our results showed that eggs pre-incubated with rec-BSPH1 protein exhibited a dose-dependent decrease in fertilization rate compared to those exposed to control proteins or media alone. Since BSPH1 binding sites were not identified on the egg, the observed inhibition in fertilization rate when eggs were pre-incubated with rec-BSPH1 suggested that an alternate mechanism was at play. Moreover, sperm immuno-neutralization with anti-rec-BSPH1 led to dramatic motility changes, followed by compromised fertilization.

Conclusion: Taken together, our results suggest that BSPH1 would be involved in the late sperm capacitation events. However, the mechanism through which egg pre-incubation with BSPH1 affects fertilization warrants further investigation. In view of these results, we conclude that BSPH1 could be a marker of sperm fertility and thus an eventual target for male contraceptive development. (Supported by the Canadian Institutes of Health Research)

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