Oral #1

EXTRACELLULAR VESICLES AS COMMUNICATORS OF STRESS-MEDIATED ALLOSTASIS AT THE EPIDIDYMIS

Nickole Moon[1,2], Jennifer Chan[1,2], Christopher Morgan[1,2], Tracy Bale[1,2]
[1]University of Colorado Anschutz Medical Campus, Aurora, CO, USA, [2]University of Maryland, Baltimore, Baltimore, MD, USA
Presented By: Nickole Moon, BA

Introduction & Objective: In males, mechanistic studies have identified prolonged effects of stress on somatic cells along the reproductive tract that causally influence offspring development. Within the epididymis, sperm receive essential maturation signals from epididymal epithelial cells (EECs) and previous studies found lasting changes in EECs following chronic stress. While these changes reflect allostatic reprogramming at the cellular level, the specific molecular mechanisms shifting the allostatic set point to influence sperm maturation are unclear. A key molecular target regulating allostasis is the glucocorticoid receptor (GR), a critical molecular sensor for chronic stress. Therefore, we examined GR-regulated epigenetic and mitochondrial processes hypothesized to shift the allostatic set point.

Methods: To identify GR- and stress-dependent processes, we chronically stressed mice with genetically reduced EEC GR, and analyzed RNA from ribosomes using the RiboTag line. To assess the distribution of H3K27me3, a stress-responsive ubiquitous transcriptional repressor in EECs, we used the high-efficiency epigenetic profiling approach, CUT&RUN sequencing, in our *in vitro* EEC model of chronic stress. Additionally, we studied the effect of GR-knockdown by RNAi on EEC mitochondrial respiration using whole cell respirometry following stress. Finally, our previous studies found that EEC secreted extracellular vesicles (EVs) act as vehicles for cargo necessary for sperm maturation, and that changes in EEC allostatic set point following chronic stress alter EV content. As mitochondria are necessary for sperm maturation, we assessed the role of EVs in regulating sperm mitochondria using whole cell respirometry.

Results: At the EEC active translatome, we detected two clusters of differentially expressed co-regulated genes related to mitochondria and chromatin-modifying processes, represented by over 400 and 1200 genes, respectively. Furthermore, CUT&RUN revealed dramatically increased binding by H3K27me3 at over 7000 regions of the EEC genome. Gene set enrichment analysis of aligned genes again revealed associations with mitochondrial processes. As stress-sensitive modulators of cellular energy, it is not surprising that mitochondria likely regulate allostasis. As expected, prior stress decreased basal mitochondrial respiration and GR-knockdown protected against this effect. Finally, we found increased sperm mitochondrial respiration following exposure to EVs from stress-EECs.

Conclusion: These studies identify GR as an allostatic mediator regulating energy requirements and signals of paternal experience to sperm. Resolving these regulatory mechanisms of allostasis in the male reproductive tract is critical to understanding the enduring contribution of paternal trauma and developing potential interventions.

Table 1. Summary of Experiments & Results

Sequencing Experiments	**n**	**Significance**	**Major Finding**				
EEC GR-Knockdown RNA-seq	4-6	adj. $p < 0.05$	EEC GR regulates 2 clusters of DEGs associated with mitochondria and chromatin regulation following prior stress exposure.				
H3K27me3 CUTNRUN seq	4-5	FDR < 0.05	H3K27me3 is enriched at 7135 regions of the genome but depleted at only 147 following prior stress exposure.				
EEC Whole Cell Respirometry Experiments Two-way Analysis of Variance	**n**	**Interaction**	**Stress Main Effect**	**shRNA Main Effect**	**Empty Vector (Control v Stress)**	**Non-targeted shRNA (Control v Stress)**	**GR-KD (Control v Stress)**
Basal Mitochondrial Oxygen Consumption Rate	10-12	F(2,60)=23.38 $p < 0.0001$	F(2,60)=275.1 $p < 0.0001$	F(1,60)=61.23 $p < 0.0001$	$p < 0.0001$	$p < 0.0005$	$p = 0.9997$
Control Predicted Mean (LS means)					7.782	8.427	3.845
Stress Predicted Mean (LS means)					5.44	7.318	3.917
Sperm Whole Cell Respirometry One-way Analysis of Variance	**n**	**Control Sperm**		**Sperm after incubation with Vehicle EVs**		**Sperm after incubation with Stress Evs**	
Mean Mitochondrial Oxygen Consumption Rate	6-7	4.500		5.757		11.69 ; $*p < 0.05$	
Std. Error		1.221		1.368		2.055	
ANOVA summary	F(2,17) = 5.593, p = 0+A1:H14.0136						

Summary of Statistics and Results

Oral #2

UNRAVELLING THE DISTRIBUTION AND ROLE OF THE PRIMARY CILIUM IN THE PROSTATE GLAND

Gabriel Campolina-Silva, Camille Lafront, Camille Lavoie-Ouellet, Louis Lacombe, Frédéric Pouliot, Étienne Audet-Walsh, Clémence Belleannée
Université Laval, Québec, QC, Canada
Presented By: Gabriel Campolina-Silva, MS, PhD

Introduction & Objective: The primary cilium is a sensory organelle that inhibits ill-timed cell proliferation and maintains tissue homeostasis through the control of signaling pathways, including Hedgehog. While aberrant formation/functioning of the primary

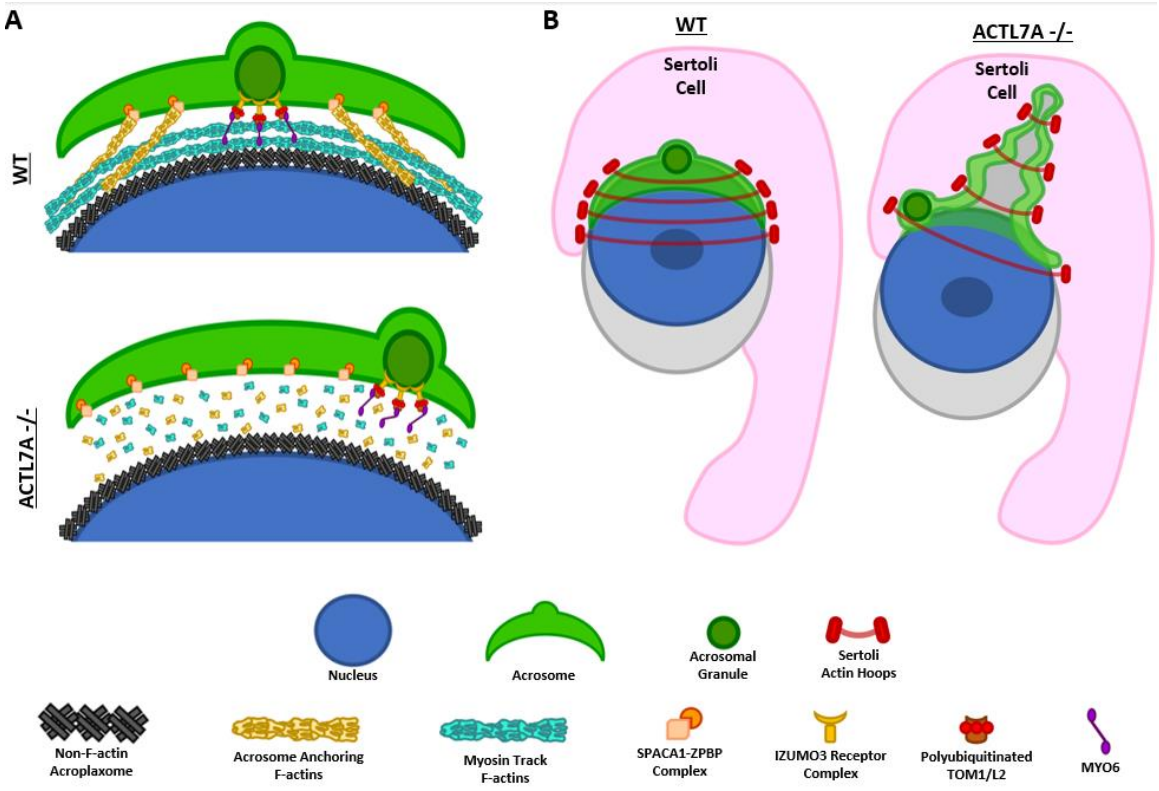
cilium is thought to influence the development of prostate cancer, the most commonly diagnosed malignancy in men, its distribution and role in the prostate gland remain largely unexplored. Here, we aimed at determining (i) the distribution of prostate primary cilia under normal and cancer conditions, and (ii) the causality between disturbed ciliogenesis and carcinogenesis.
Methods: Confocal microscopy was employed to characterize primary cilia distribution in the prostate of wild-type and $Pbsn^{Cre}$;$Pten^{fl/fl}$ adult mice, a model of prostate cancer. Similarly, the features (e.g. frequency and length) of the primary cilium were determined in primary and immortalized cell lines of human prostate cancer. To decipher the importance of the primary cilium for prostate homeostasis, we developed the $Ck5^{Cre}$;$Arl13^{fl/fl}$ mouse model (Arl13b cKO) in which loss of primary cilia function is observed in the prostatic epithelium.
Results: Our results revealed that, in normal acini and ducts of the prostate, the primary cilium protrudes as a single, short extension (2-5 µm length) from the surface of $CK5^+$ basal epithelial cells. As expected, this organelle is involved in signals transduction within the gland, as the G protein-coupled Hedgehog receptor Smoothened was found enriched at the ciliary axoneme/membrane. However, both the frequency and length of the primary cilium were found drastically reduced ($p<0.001$) in cancer epithelial cells from mouse and human prostates. We therefore used the Arl13b cKO mice to assess the impact of dysfunctional primary cilia (shorter and with no accumulation of Smoothened) in the glandular epithelium. Our analysis revealed that the epithelial cell organization was impaired in prostate tissues and basal cell-derived organoids from Arl13b cKO mice. Remarkably, the prostatic intraepithelial neoplasia (PIN) – the precursor lesion of prostate cancer – was observed arising in 60% (3/5, n=5) of 6-month-old Arl13b cKO but not in the age-matched control group (0/5, n=5), underscoring a causal relationship between defects in primary cilia function and prostate tumorigenesis.
Conclusion: Our findings show that primary cilia are predominantly associated to basal cells and play a tumor-suppressive role in the prostate gland through mechanisms that remain to be determined.

Oral #3
RBFOX2 COPY NUMBER VARIATION CAUSES HYPOSPADIAS BY DISRUPTING MESENCHYMAL TO EPITHELIAL TRANSITION IN PENIS DEVELOPMENT
Victor Ruthig[1], Fredi Ruiz Rojano[1,2], Andrew Yarilin[1,3], Marisol O'Neill[4], Jeffrey White[4], Dolores Lamb[1,4]
[1]Weill Cornell Medicine, New York, NY, USA, [2]New York Bioforce, New York, NY, USA, [3]Brooklyn Technical High School, New York, NY, USA, [4]Center for Reproductive Medicine, Houston, TX, USA
Presented By: Dolores Lamb, PhD

Introduction & Objective: Despite hypospadias frequency (1:125 live male births) the etiology is often not known. The Lamb Laboratory previously determined that RNA Binding Fox-1 Homolog 2 (*RBFOX2*) copy number variation (CNV) is associated with clinical cases of upper and lower tract genitourinary anomalies, including hypospadias. *RBFOX2* encodes a RNA binding protein that regulates mRNA splicing. The hypothesis was tested that altered *RBFOX2* gene dosage impacts the developing penis transcriptome by disrupting mRNA splicing. Specifically vitiating the mesenchymal to epithelial transition critical for penile urethra formation, resulting in hypospadias.
Methods: Immunofluorescenet and *in situ* hybridization imaging along a fetal time course of penis development defined anatomical regions of *Rbfox2* transcript and protein expression. To identify developmental pathways regulated by RBFOX2 in the genitourinary system, *Rbfox2*-KO (null) mice were developed to model the clinical phenotype. Penises were collected for RNA-seq analysis. Differential analysis comparing P1 penises of *Rbfox2*-KO and wild type siblings defined the transcriptome and splice-ome changes caused by *Rbfox2* loss. Significant differentially used genes were anatomically mapped in the developing fetal penis (E14.5-E16.5) using published scRNA-seq datasets [Amato & Yao 2021, Armfield & Cohn 2021]. RNA immunoprecipation (RIP) was used to distinguish between direct RBFOX2 transcript targets and downstream affected genes.
Results: Imaging analysis on fetal mouse penises revealed, after penile sex determination (E15.5), *Rbfox2* expression becomes restricted to the urethral mesenchyme and distal glans. Differential analysis on the *Rbfox2*-KO mouse penis transcriptome and splice-ome identified changes associated with *Rbfox2* loss in epithelial differentiation, pluripotency, keritizaton, hormone, and Tgf-beta signalling. Annotation of these affected genes, to regions of the developing penis using scRNA-seq data, localized some genes as regionally restricted to penis epithelium or mesenchyme. RIP agianst RBFOX2 in human kidney cells validated mRNA transcipt targets previously identified in cardiac cells [Verma 2016].
Conclusion: *RBFOX2* gene dosage alterations, clinically correlate with upper and lower tract genitourinary anomalies, specifically hypospadias. Data from human cell lines and mouse models seemingly indicate the phenotype is partially the product RBFOX2 directing the mesenchymal to epithelial transition. In addition to directing this transition there may be mechanistic differences between RBFOX2 action in epithlial versus mesenchymal regions, RBFOX2 is a bi-modal RNA binding protein. These findings have implications for other regions of the genitourinary tract such as gonad and kidney.

Oral #4
TESTIS-SPECIFIC ACTIN-LIKE 7A (ACTL7A) IS AN INDISPENSABLE PROTEIN FOR SUBACROSOMAL ASSOCIATED F-ACTIN FORMATION, ACROSOMAL ANCHORING, AND MALE FERTILITY
Pierre Ferrer[1], Srijana Upadhyay[1], Masahito Ikawa[2], Tracy Clement[1]
[1]Texas A&M University, College Station, TX, USA, [2]Osaka University, Osaka, Japan
Presented By: Pierre Ferrer, BS

Introduction & Objective: Formation of the acrosome during spermiogenesis is an essential process for creating fertilization competent sperm. Of the numerous aspects required for acrosome biogenesis, adherence of the acrosomal outer membrane to the nuclear surface is mediated by the subacrosomal perinuclear theca. However, the cellular dynamics and congruent functions pertaining to these acrosomal anchoring factors are not well understood – many of which have been implicated as potential causes for human male infertility. Actin-like protein 7A (ACTL7A) is one such factor for which deleterious polymorphisms have recently been shown to cause human male infertility. As such, it is thought that acrosomal attachment is coordinated by cytoskeletal associations between the acrosome and nucleus via the acroplaxome.
In this study we seek to further illuminate the mechanistic underpinnings of ACTL7A for essential acrosome associations. Here, we investigate the dynamic intracellular localization of ACTL7A in the developing germline, its molecular associations with other cytoskeletal components, and the cellular consequences of its ablation.
Methods: In order to study the functionality of ACTL7A in the male germline and its association with acrosome biogenesis we generated a knock-out C57BL/6n mouse model using CRISPR/Cas9 as to identify any observable phenotypic deviancies. Guided by in silico predictive models of protein-protein interactions we further investigated the functionality of ACTL7A through Co-IP to identify putative binding partners as well as traced its molecular association with specific intracellular compartments and cytoskeletal suprastructures in the developing germline via indirect fluorescent microscopy.
Results: Our intracellular localization data shows ACTL7A to be dynamically present within the nucleus and subacrosomal space and later associated with postacrosomal regions of developing spermatids. By examining the generated Actl7a knock-out mouse model we constantly observed disruption of acrosomal biogenesis with abnormal migration of the acrosomal granule, and peeling acrosomes during spermatid elongation. Significantly, we find a complete loss of subacrosomal F-actin structures in knock-out spermatids. Furthermore, using ACTL7A as bait for Co-IP assays we have been able to validate its interaction with canonical actin regulating and transport protein further demarcating its role as a potential subacrosomal F-actin coordinator.
Conclusion: In conclusion, considering our reported data together with the existing literature, herein we propose a mechanistic model explaining the essential role for ACTL7A in acroplaxome F-actin formation, acrosomal attachment integrity, and male fertility.



Proposed phenotypic model of ACTL7A ablation. (A) Intracellular model of the subacrosomal space depicting the attachment of the granule-anchoring MYO6 motor protein complex to ACTL7A dependent F-actins and their organizational disruption in the absence of ACTL7A. (B) Cellular diagram portraying a model as to why acrosomal fragments detached from spermatids and retained in Sertoli cell crypts in *Actl7a* KO mice.

Proposed phenotypic model of ACTL7A ablation.

Oral #5

THE MECHANISTIC BASIS FOR THE ISOFORM-SPECIFIC REQUIREMENT OF PROTEIN PHOSPHATASE 1 GAMMA 2 FOR NORMAL SPERM FUNCTION AND MALE FERTILITY.

Marwan Shalih Maraicar, Wesam Nofal, James Mawolo, Mustafa Kabi, Srinivasan Vijayaraghavan
Kent State University, Kent, OH, USA
Presented By: Marwan Shalih Maraicar, MS

Introduction & Objective: The serine threonine phosphatase, PP1g2, one of the two splice variants encoded by Ppp1cc is specifically expressed only in the testis and sperm of placental mammals. The PP1g1 and PP1g2 isoforms are identical except at their 6 – 21 amino acid C-termini. Targeted disruption of *Ppp1cc* results in male infertility. However, replacement of PP1g2 with PP1g1 in mouse testis and sperm using CRISPR/Cas9 gene editing impairs sperm function and male fertility. The evolutionarily ancient regulatory interactors of protein phosphatase 1 (RIPPO's), PPP1R2/I2, PPP1R7/SDS22, and PPP1R3/I3, are present in sperm and regulate PP1g2 during sperm motility initiation and maturation in the epididymis. Reversible phosphorylation of these RIPPOs results in PP1g2 activation or inhibition. The spatio-temporal expression of I2 and I3 match the expression of PP1g2 in developing testicular spermatogenic cells. The PP1g1 knock-in mouse model should shed light on the basis for the requirement of PP1g2 in mammals and provide insights into the biochemical basis for normal sperm function and fertility.
Methods: Western Blot, Immunocytochemistry, Immunohistochemistry, Co-Immunoprecipitation, Pulldown Assays and Phosphoprotein enrichment followed by Mass Spectrometry approaches were used in this study.
Results: In wild type mice, the expression levels of the RIPPOs, I2 and I3 correlated with PP1g2 expression in maturing spermatogenic cells within testis but interestingly, we found I2 and I3 to be absent in the testis of the mice bearing PP1g1. This results in drastically altered intra-sperm localization of PP1g1 compared PP1g2 and consequently impairs sperm motility and fertility. We anticipate that altered phosphorylation of enzymes and proteins involved in energy metabolism is responsible for the low levels of ATP in PP1g1 bearing sperm. A transcription factor called spermatogenic zip protein 1 (SPZ1) that is testis specific and present only in mammals and is known to bind to PP1g2 in isoform specific manner. The E-box promotor binding sites for this transcription factor is present in the genes for I2 and I3.
Conclusion: Our findings suggest that PP1g2, regulates the expression levels of I2 and I3 in testis through its interaction with the transcription factor SPZ1. It is likely that the isoform specific interaction of PP1g2 to SPZ1 is required for the optimal levels of expression of RIPPO's during spermatogenesis and providing a basis for the mammal specific role of PP1g2 in male fertility.

Oral #6

SPERM-ASSOCIATED ANTIGEN 6-LIKE (SPAG6L) IS ESSENTIAL FOR SPERMATOGENESIS AND MALE FERTILITY

Wei Li[1], Yi Tian Yap[1], Alivia Kearney[1], Jerome Strauss 3rd[2], Shizhen Song[3], Ling Zhang[3], Aminata Toure[4], Zhibing Zhang[1,5]
[1]1. Department of Physiology, Wayne State University, Detroit, MI, USA, [2]Center for Research on Reproduction and Women's Health, Department of Obstetrics and Gynecology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA, [3]Department of Occupational and Environmental Health, School of Public Health, Wuhan University of Science and Technology, Wuhan, China, [4]CHU Grenoble Alpes, UM GI-DPI, Grenoble, France, [5]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA
Presented By: Wei Li, MD

Introduction & Objective: Sperm-associated antigen 6 (SPAG6) is the mammalian orthologue of *Chlamydomonas PF16*, an axonemal central pair protein involved in flagellar motility. In mice, two *Spag6* genes have been identified. The ancestral gene, on mouse chromosome 2, is named *Spag6*. A related gene originally called *Spag6*, localized on mouse chromosome 16, evolved from the ancient *Spag6* gene, and has been renamed *Spag6-like* (*Spag6l*). Global *Spag6* knockout mice were grossly normal, and fertility was not affected in both males and females. The homozygous males had normal sperm parameters, including sperm number, motility, and morphology. Examination of testis histology revealed normal spermatogenesis.
Methods: A global *Spag6l* knockout mouse model was generated previously. In addition to a role in modulating ciliary beat, SPAG6L has many unexpected functions, including roles in the regulation of ciliogenesis/spermatogenesis, hearing, and the immunological synapse, among others. Even though some *Spag6l* knockout mice survived to sexual maturity in the early generations, all homozygous *Spag6l* knockout mice died before 4-weeks of age over the years, which impeded further investigation of function of the gene *in vivo*. To overcome the short survival of the conventional knockout mice, we developed a conditional allele by inserting two loxP sites in the genome flanking exon 3 of the *Spag6l* gene.
Results: By crossing the floxed *Spag6l* mice to a *Stra8-iCre* line which expresses Cre recombinase only in male germ cells, we obtained mutant mice that are missing SPAG6L in male germ cells. All *Spag6l*$^{flox/flox}$*;Stra8-iCre* mice had a normal appearance, no hydrocephalus was observed. However, all males were infertile. Compared to the control mice, these cKO mice had significantly reduced sperm number, and the sperm that were formed were morphologically abnormal and immotile. Histologic examination of the testis by light microscopy revealed impaired spermiogenesis. Abnormal sperm ultrastructure, including both head/chromatin and tail formation was discovered by electronic microscopy.
Conclusion: The newly established floxed *Spag6l* model provides a powerful tool for further investigation of the role of *Spag6l* in spermatogenesis, as well as the role of this gene in other cells and tissues *in vivo*.

Oral #7

PREVALENCE AND PREDICTORS OF TESTOSTERONE DEFICIENCY AMONG A NATIONAL, CONTEMPORARY COHORT OF ADULT MEN IN THE UNITED STATES

John Ernandez[1], Khalid Alkhatib[2], Brittany Berk[3], Catherine Gu[3], Phillip Pierorazio[2], Steven Chang[3], Alexandra Berger[3], Martin Kathrins[3]
[1]Harvard Medical School, Boston, MA, USA, [2]University of Pennsylvania, Philadelphia, PA, USA, [3]Brigham and Women's Hospital, Boston, MA, USA
Presented By: John Ernandez, BA

Introduction & Objective: Testosterone deficiency is associated with increased morbidity and mortality, impacting quality of life and placing an economic burden on health systems. Previous prevalence estimates have been restricted to particular care settings and may not reflect the impact of the aging population and increased prevalence of adult obesity. Herein, we evaluate the prevalence of testosterone deficiency and its predictors among a national, contemporary cohort of adult men.
Methods: We included men ≥ 18 years old who underwent serum testosterone testing using the Continuous National Health and Nutrition Examination Survey (NHANES) from 2011 to 2016. Testosterone deficiency was defined as total testosterone < 300 ng/dL. Prevalence values were weighted to reflect a nationally representative estimation. Multivariable Poisson regression adjusted for baseline covariates, including age, race and ethnicity, body mass index (BMI), presence of comorbid chronic diseases, smoking history, and weekly aerobic exercise via metabolic equivalents (MET score), was used to assess predictors of hypogonadism. A subgroup of men who had undergone morning testosterone testing was also defined. Two-way interactions between BMI and metabolic syndrome were determined to assess for effect modification.
Results: The cohort consisted of 7,805 men, of whom 2,146 (27%) had testosterone deficiency. A subset of 3,781 (49%) men had exclusively morning total testosterone recorded, of whom 787 (21%) had testosterone deficiency. The median total testosterone of the cohort of was 384.9 (377.5 – 392.3) ng/dL. Men with testosterone deficiency were significantly more likely to be older, married, have higher BMI, and have a history of chronic kidney disease (CKD) and metabolic syndrome. After adjusting for baseline covariates, predictors of testosterone deficiency included marital status, increasing BMI, and history of CKD and metabolic syndrome (Table 1). BMI was found to have significant effect modification on metabolic syndrome among men in the morning testosterone subgroup.
Conclusion: Testosterone deficiency may impact over one quarter of adult men in the U.S., and a history of metabolic syndrome and increasing BMI are independent predictors of deficiency. BMI demonstrates a near direct relationship with deficiency, which is mediated by metabolic syndrome, though increasing aerobic exercise is not associated with lower risk of deficiency.

Table 1. Predictors of testosterone deficiency on adjusted Poisson multivariable analysis.

	Total Cohort (*N* = 7,805) Adjusted Risk Ratio (95% CI)*	AM Testosterone Cohort (*N* = 2,146) Adjusted Risk Ratio (95% CI)*
Age	1.004 (0.999 – 1.010)	1.006 (0.997 – 1.014)
Time of Testosterone Measurement		
Morning	-	-
Afternoon	1.636 (1.465 – 1.828)	-
Evening	1.819 (1.564 – 2.11)	-
Race		
Non-Hispanic White	-	-
Non-Hispanic Black	0.845 (0.743 – 0.973)**	0.918 (0.754 - 1.118)
Hispanic	0.967 (0.842 – 1.110)	0.884 (0.673 - 1.162)
Other	1.103 (0.948 – 1.284)	1.066 (0.755 - 1.504)
Marital Status		
Married	-	-
Single, widowed, divorced, separated	1.295 (1.155 - 1.452)**	1.037 (0.839 - 1.280)
Level of education		
< High school	-	-
High school or GED	0.977 (0.847 - 1.127)	1.018 (0.743 - 1.395)
>High school	0.979 (0.834 - 1.150)	0.978 (0.725 - 1.320)
Household income		
$0-$14,999	-	-
$15k-34,999	0.968 (0.819 - 1.144)	0.902 (0.671 - 1.214)
$35k-54,999	0.999 (0.828 - 1.208)	0.993 (0.702 - 1.406)
$55k-74,999	0.954 (0.775 - 1.175)	0.957 (0.704 - 1.301)
>$75,000	0.953 (0.776 - 1.170)	0.970 (0.726 - 1.300)
Health insurance		
None	-	-
Private	1.099 (0.936 - 1.290)	0.964 (0.737 - 1.262)
Medicare	1.145 (0.912 - 1.438)	1.065 (0.682 - 1.665)
Medicaid or SCHIP	1.133 (0.848 - 1.514)	1.289 (0.852 - 1.951)
Other	1.232 (1.025 - 1.480)	1.289 (0.881 - 1.888)
BMI category		
<18.5	0.318 (0.102 - 0.997)**	0.266 (0.030 – 2.390)**
18.5 to <25	-	-
25 to <30	1.861 (1.581 - 2.192)**	1.685 (1.166 - 2.435)**
30 to <35	2.532 (2.138 - 3.000)**	2.201 (1.457 - 3.324)**
35 to <40	4.025 (3.340 - 4.851)**	3.851 (2.670 - 5.555)**
>40	4.701 (3.778 - 5.851)**	5.389 (3.595 - 8.078)**
Chronic kidney disease (i.e. eGFR < 60)		
No	-	-
Yes	1.242 (1.038 - 1.487)**	0.959 (0.690 - 1.332)
Chronic pulmonary disease		
No	-	-
Yes	1.002 (0.901 - 1.114)	0.942 (0.777 - 1.143)
Chronic liver disease		
No	-	-
Yes	1.246 (0.989 - 1.569)	1.424 (0.973 - 2.083)
Smoking history (pack-per-year)		
<1	-	-
1 to <20	0.995 (0.888 - 1.114)	1.132 (0.937 - 1.368)
>20	1.041 (0.869 - 1.247)	1.101 (0.841 - 1.441)
Alcohol use/week		
<1 unit	-	-
1 to <15 units	0.885 (0.783 - 1.001)	0.856 (0.692 - 1.059)
>15 units	0.945 (0.828 - 1.086)	0.954 (0.715 - 1.271)
MET category		
<500	-	-
>500	0.818 (0.551 - 1.216)	1.284 (0.551 - 2.991)
Metabolic syndrome		
No	-	-
Yes	***	1.572 (1.254 - 1.970)**

*Adjusted risk ratio with 95% confidence interval
**Statistically significant, $p < 0.05$
***Cannot estimate as defining metabolic syndrome within NHANES requires fasting blood glucose value, which is only available in AM cohort.

Baseline sociodemographic characteristics cohorts.

Oral #8

INTERFERON EPSILON: A NOVEL ANTI-VIRAL AGENT IN THE MALE REPRODUCTIVE TRACT

Rukmali Wijayarathna[1,2], Eveline de Geus[1,2], Rosemary Genovese[1], Michelle Tate[1,2], Paul Hertzog[1,2], Mark Hedger[1,2]

[1]Hudson Institute of Medical Research, Mebourne, Australia, [2]Department of Molecular and Translational Sciences, School of Clinical Sciences, Monash University, Melbourne, Australia
Presented By: Rukmali Wijayarathna, PhD

Introduction & Objective: The testis is a reservoir for viruses, including HIV, mumps, Zika and SARS, which can cause persistent infection and infertility. Increased susceptibility of the testis to viruses has been attributed to an inability of spermatogenic cells to produce interferons (IFN) or IFN-induced proteins, required for viral resistance. Challenging this dogma, we discovered that interferon-epsilon (IFNe), a type-I IFN first discovered in female reproductive epithelia, is constitutively expressed by meiotic and post-meiotic spermatogenic cells and testicular macrophages in mice and humans. IFNe was also present in human testicular interstitial fluid. The anti-viral role of IFNe in the reproductive tract was investigated using the Zika virus infection model.
Methods: To investigate IFNe in the mouse, adult wildtype mice (WT), *Ifne*$^{-/-}$ mice lacking IFNe, and *Ifnar1*$^{-/-}$ mice lacking the IFNAR1 receptor subunit required for IFN-signalling, received a single intraperitoneal injection of Zika virus (PRVABC59, $5x10^5$ pfu in saline). Controls received saline only. Reproductive organs were collected 7 days post-infection (peak illness). To investigate IFNe in the human, a human Sertoli cell-line (HSerc, ScienCell™) was used. Cells were treated with 100 IU recombinant human IFNe (rhIFNe), and 12 hours later were infected with 5 or 10 MOI Zika virus to assess a prophylactic role of IFNe. To investigate a therapeutic role, cells were treated with rhIFNe one hour after Zika infection. Cells and media were collected 24 and 48 hours post Zika infection.
Results: Infected WT mice lacked histological evidence of orchitis or epididymitis 7 days post-infection, but infected *Ifne*$^{-/-}$ and *Ifnar1*$^{-/-}$ mice displayed testicular hyperaemia, oedema and immune cell infiltrates. The epididymis of infected *Ifnar1*$^{-/-}$ and *Ifne*$^{-/-}$ mice displayed immune cell infiltrates, epithelial damage, luminal obstruction and fibrosis. Expression of critical Leydig cell (*Cyp11a1*, *Cyp17a1*) and spermatid genes (*Tnp1*) was also reduced in infected *Ifne*$^{-/-}$ and *Ifnar1*$^{-/-}$ mice. Prophylactic IFNe treatment reduced the viral load by ~98%, measured by qPCR for viral RNA and plaque assays for infectious virus at 24 and 48h post-infection. Therapeutic IFNe treatment reduced viral RNA by ~70% and infectious virus by 97%.
Conclusion: These data indicate that IFNe has a key role in protecting the testis against Zika virus, shifting the paradigm of testicular anti-viral defences, and identifying IFNe as a potential therapeutic and diagnostic target for reproductive tract infections.

Oral #9
REGULATORY T-CELL DEPLETION RESULTS IN SEVERE MALE SUBFERTILITY
Ferran Barrachina[1], Kiera Ottino[1], Maia Lina Elizagaray[1], Maria Gracia Gervasi[2,3], Leona Tu[1], Maria Agustina Battistone[1]
[1]Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA, [2]Bedford Research Foundation, Bedford, MA, USA, [3]University of Massachusetts Amherst, Amherst, MA, USA
Presented By: Ferran Barrachina, MS, PhD

Introduction & Objective: The male reproductive function relies on tolerance mechanisms towards sperm antigens, which avoids autoimmune reactions such as the production of anti-sperm antibodies (AsAb) that cause infertility. Here, we characterized the epididymal regulatory Foxp3$^+$ T cells (Tregs), key players for maintaining peripheral tolerance, and studied the consequences of the disruption of tolerance in the epididymis.
Methods: WT and transgenic mice expressing the diphtheria toxin (DT) receptor (DTR) under the Foxp3 promoter (Foxp3-DTR mice) were used to characterize epididymal Treg function. Confocal microscopy and flow cytometry were performed to study immune cell infiltration. The presence of immunoglobulins (Ig) was assessed by ELISA and confocal microscopy. Computer-assisted sperm analysis and *in vitro* fertilization assay were used to evaluate sperm function. Fertility was assessed by counting litter size.
Results: We showed that the abundance of Tregs decreases with age in the proximal and distal epididymides ($p<0.01$). To examine the consequence of the immune tolerance breakdown, we ablated Tregs by injecting DT into Foxp3-DTR mice. Two weeks after DT treatment, we observed a widespread infiltration of monocytes, neutrophils, F4/80$^+$ mononuclear phagocytes (MPs), and MHCII$^+$ MPs in all epididymal segments of Foxp3-DTR mice ($p<0.01$). Interestingly, we observed infiltration of MPs into the epididymal lumen. Despite this aberrant immune response, the epididymal epithelium was not damaged, and low levels of apoptosis were observed in the epithelium of WT and Foxp3-DTR mice. However, epididymal clear cells in Foxp3-DTR mice displayed an increase in apical bleb formation ($p<0.0001$). We revealed increased levels of serum Ig against sperm and epididymal antigens ($p<0.0001$) in the Foxp3-DTR mice. Moreover, confocal microscopy showed IgG accumulation in the interstitium of the epididymis, and AsAb were detected in sperm of Foxp3-DTR mice. All this inflammatory response resulted in reduced sperm counts ($p<0.01$), impaired sperm motility ($p<0.01$), low *in vitro* fertilization rate ($p<0.05$), and fertility was severely impacted, as shown by a decreased litter size ($p<0.001$) in Foxp3-DTR mice.
Conclusion: Tregs play a key role in maintaining a tolerogenic environment that protects sperm in the epididymis. Our results showed that immune tolerance disruption induces an aberrant pro-inflammatory response in the epididymis that ultimately results in severe male subfertility. Deciphering immunoregulatory mechanisms in the epididymis may contribute to developing new strategies to treat male infertility and identify potential targets for immuno-contraception.

Oral #10

AN APPROACH TO ADDRESS IATROGENIC MALE INFERTILITY AND DISEASE TRANSMISSION IN MOUSE AND HUMAN MODELS OF SICKLE CELL DISEASE

Mitchell Harancher[1,2], Yi Sheng[2], Amanda M. Colvin Zielen[2], Kyle E. Orwig[1,2]
[1]Molecular Genetics and Developmental Biology Program, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA,
[2]Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Women's Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
Presented By: Mitchell Harancher, BS

Introduction & Objective: Sickle cell disease (SCD) is a crippling disease that significantly shortens lifespan and decreases quality of life. The only curative option for SCD is hematopoietic stem cell transplantation (HSCT). However, myeloablative conditioning prior to HSCT can cause infertility. SCD patients desire to have children, but also have concerns about passing the sickle cell trait to their offspring. The UPMC Fertility Preservation Program is actively cryopreserving gonadal tissues for young sickle cell patients to safeguard their future fertility. Spermatogonial stem cells (SSCs) in those tissues may be transplanted in the future to restore spermatogenesis in patient survivors. This creates an opportunity for targeted gene correction of the sickle cell mutation in patient SSCs, ex vivo, prior to transplantation. Alternatively, the sickle cell mutation could be corrected in patient-derived induced pluripotent stem cells (iPSCs) that could be differentiated in vitro into transplantable stem cells or to sperm.
Methods: Three sgRNAs to target the human β-globin gene at the SCD locus were generated and tested in HEK293T cells. sgRNAs were co-transfected into HEK293T cells to delete a region of the locus. The locus was PCR amplified, excised from an agarose gel, and Sanger sequenced.
SSC cultures were generated from 6–8-day old Towne's mice pups. Thy1+ testis cells were isolated by MACS and cultured on STO feeder cells in α-MEM media supplemented with GFRα1, GDNF, and FGF2.
SCD iPSCs were generated by reprogramming primary fibroblasts with CytoTune-2.0™. Fibroblasts were derived from cryopreserved SCD patient testicular tissues.
Results: Sanger sequencing confirmed that all three candidate sgRNAs correctly targeted the SCD locus in the b-globin gene. Sanger sequencing also confirmed the SCD genotype in Towne's mice and human iPSCs. Histology of Towne's mice organs revealed the expected pathologies in the liver, spleen, and kidney. We established SSCs cultures from Towne's mice. TRA-1-60 expression was confirmed in SCD patient-derived iPSCs.
Conclusion: To conclude, future experiments will test the spermatogenic potential of Towne's SSCs by transplantation into infertile recipient male mice. Teratoma analysis and karyotyping will be used to validate SCD patient iPSCs. CRISPR/Cas9 gene editing with the validated sgRNAs will be used to correct the SCD mutation in Towne's SSCs and SCD patient-derived iPSCs.
Supported by the University of Pittsburgh Medical Center and Magee-Women's Research Institute.

Oral #11

AN INVESTIGATION OF GENOMIC AND EPIGENOMIC FACTORS IN IDIOPATHIC HYPOSPERMATOGENESIS

Nisha Sharma, Mili Kaur, Vivek Kumar, Ashutosh Halder, Manoj Kumar, Seema Kaushal, Manish Jain
All India Institute of Medical Sciences, New Delhi, India
Presented By: Nisha Sharma, PhD

Introduction & Objective: Hypospermatogenesis (HS) is most frequent histopathological subtype of primary testicular failure (PTF) characterized by overall reduction in number of germ cell within the seminiferous tubules resulting in to azoospermia or oligospermia. More than half of such severe phenotype of male infertility remain idiopathic despite molecular cytogenetic evaluations. More than 2000 genes participate in development of testis and around 800 of them regulate spermatogenesis. The current work is an effort to predict diagnostically relevant genes to answer and classify the causes of idiopathic HS. Since the etiological factors of primary testicular failure (HS) could be genomic as well as epigenomic origin Therefore; it is important to determine common genes with deleterious variants as well as targets of miRNA in HS cases.
Objectives: 1) To find out deleterious genomic variants associated with idiopathic HS cases, 2) To find out differentially expressing miRNAs targeting candidate and potential candidate genes in idiopathic HS cases.
Methods: A total of 130 patients attending department of Urology with the chief complaint of infertility who were evaluated for intratesticular cause by FNA have been prospectively recruited for the study. Clinical and pathological characterization found 26 of them to be HS. Yq microdeletion and XY-FISH has been performed for genetic exclusion. Whole exome sequencing has been done on thirteen idiopathic HS cases. CNV analysis and variant interpretation has been done and four HS cases has been excluded. Small RNA sequencing and n-counter gene expression analysis has been done on five idiopathic HS and five normospermatogenesis (NS).
Results: Variant interpretation predicted two novel Likely pathogenic, frameshift variants (c.1817delT) in CDC27, (c.4751_4752ins(34)) in FAM186A, Benign missense variant in EP300 and a missenses variant of unknown significance (c.1048C>T) associated with ZNF717. Differential expression profile of miRNAs (hsa-miR-935, hsa-miR-501-5p, hsa-miR-664a-5p, hsa-miR-15b-5p, hsa-miR-223-3p, hsa-miR-224-5p, hsa-miR-7-1-3p, hsa-miR-374a-3p, hsa-miR-10399-3p, hsa-miR-3148, hsa-miR-29a-5p, hsa-miR-520a-5p, hsa-miR-525-5p, hsa-miR-27a-5p and hsa-miR-584-3p etc.) shows high target score for above

mentioned genes in HS cases. GO annotation of genes shows cell cycle, proliferation, apoptosis and regulation of transcription as key processes involved.

Conclusion: The current findings support the possibility that CDC27, KMT2C, EP300 and ZNF717 genes are associated with genomic (deleterious variants) and epigenomic (aberrant miRNAs) factors in idiopathic hypospermatogenesis cases. Further investigations on larger cohort is needed to provide these genes a place in to gene panel for male infertility.

Potential candidate Gene in HS	Expression	GO Process of gene	miRNA in HS cases	log2FC	Target score
Cell division cycle 27 (CDC27)	Ubiquitus and High in testis (RPKM 11.6)	Involved in regulation of mitotic and meiotic cell cycle, involved in anaphase-promoting complex-dependent catabolic process	hsa-miR-935	2.85	61
			hsa-miR-501-5p	2.85	89
			hsa-miR-664a-5p	2.21	81
			hsa-miR-15a-5p	3.26	82
			hsa-miR-16-5p	6.52	87
			hsa-miR-223-3p	7.7	57
			hsa-miR-514a-5p	3.8	91
			hsa-miR-223-3p	-4.03	57
			hsa-miR-224-5p	-2	57
			hsa-miR-7-1-3p	-2.33	84
			hsa-miR-205-5p	-11.33	89
			hsa-miR-374a-3p	-5.43	61
			hsa-miR-548ar-3p	-6.23	65
			hsa-miR-548i	-8.85	62
			hsa-miR-605-5p	-9.57	59
			hsa-miR-617	-6.33	52
Zinc finger protein 717 (ZNF717)	Ubiquitus and High in testis (RPKM 1.7)	Involved in regulation of transcription by RNA polymerase II	hsa-miR-525-5p	2.59	52
			hsa-miR-584-3p	-3.67	77
E1A binding protein p300 (EP300)	High in testis (RPKM 17.5)	Cell proliferation and differentiation	hsa-miR-26b-5p	4.02	94
			hsa-miR-133a-5p	-10.3	81
			hsa-miR-200a-3p	-6.93	61
			hsa-miR-330-3p	-9.43	71
			hsa-miR-374a-3p	-5.43	79
			hsa-miR-548k	-9.25	56
Lysine methyltransferase 2C (KMT2C)	Testis (RPKM 4.4)	Transcriptional coactivation	hsa-miR-223-3p	7.7	57
			hsa-miR-26b-5p	4.02	73
			hsa-miR-27a-3p	-4.06	91
			hsa-miR-371b-5p	-6.68	79
			hsa-miR-934	-3.32	97

Genes list and differentially expressing miRNA in HS

Oral #12

SEARCHING FOR GENETIC VARIANTS RELATED TO GONADAL DEVELOPMENT AND MAINTENANCE IN MALES WITH LATE TESTICULAR REGRESSION SYNDROME

Lucas Ferreira, Nicolle Neves, Karen Ferreira, Marina Kizys, Magnus Silva
Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo, Brazil
Presented By: Lucas Ferreira

Introduction & Objective: The Testicular Regression Syndrome (TRS) is the failure of testis maintenance in 46,XY individuals. The embryonic TRS results in Differences of Sex Development, within the clinical spectrum of 46,XY Gonadal Dysgenesis (GD). We classified as late TRS 46,XY those subjects presenting typical penoscrotal genitalia, spontaneous/partial puberty, reduced testicular volume or anorchia, hypergonadotropic hypogonadism and infertility. Although the molecular etiology of embryonal GD is known, in whom pathogenic *DHX37* variants were identified, the genetic basis of the late TRS is still unknown. Thus, our objective was to investigate the genetic etiology in late TRS using a sequencing-based strategy to explore the spectrum of variants in genes related to human gonadal development, maintenance and function.

Methods: Genomic DNA was extracted from 20 subjects with late TRS. Sanger sequencing was used to investigate variants in *DHX37* exons. Whole Exome Sequencing (WES) was performed in 6 cases with late TRS and in eugonadal male and female

controls. We applied a pipeline of investigation based in 4 gene panels: 1) Testis development and descent (88 genes), 2) *DHX37* paralogs with testicular expression (27), 3) Maintenance and self-renewal of testicular cells (19), and 4) Primary Ovarian Insufficiency (POI, 60). We filtered for variants absent in controls, MAF<1% and deleterious effect (*in silico* tools).
Results: No pathogenic variants in *DHX37* were observed. The WES analysis identified the variants p.Ser18Asn in *SRY* (panel 1), p.Thr562Met in *DDX24* (panel 2) and p.Tyr87Cys in *SYCP1* (panel 4) in three different subjects presenting typical penoscrotal genitalia and absence of mullerian remnants. Two of them presented late TRS followed by testicular torsion. The subject with *DDX24* variant also presented cryptorchidism and seminoma. *DDX24* was reported to be one of the most downregulated genes in seminoma tissues. *SRY* was previously implicated in complete GD. *SYCP1* mutant mice have reduced testicular volume. The variants identified were classified as pathogenic or likely pathogenic according to the ACMG guidelines and confirmed by Sanger sequencing. Two other subjects presented variants in the genes *ATRX*, *SPRY4*, *DHX57* and *DNMTL3*, which are under investigation.
Conclusion: Our data suggest that genetic variants in *DHX37* are not related to the molecular etiology in late TRS. Cases of TRS and torsion during adulthood may have a different genetic etiology. Further investigation is necessary to understand the pathogenesis of late TRS.

Poster 1
MELATONIN IMPROVES THE ABILITY OF MICE SPERM TO BIND WITH EGGS
YaNan Liu[1], Zi Teng[2]
[1]Weifang medical university, Weifang, China, [2]Yantai Yuhuangding hospital, Yantai, China
Presented By: Yanan Liu

Introduction & Objective: Melatonin is a powerful antioxidant regulating various biological functions, including alleviating male reproductive damage under pathological conditions. Here, we aim to analyze the effect of melatonin on normal male reproduction in mice.
Methods: Male mice were received an intraperitoneal injection of melatonin (10mg/kg body weight) for consecutive 35 days. The testis and epididymis morphology, epididymal sperm parameters were examined. PCNA, SYCP3, ZO-1 and CYP11A1 expressions in epididymis or testis were detected by Western blotting. Male fertility was determined by *in vivo* and *in vitro* fertilization (IVF) experiments. The differentially expressed sperm proteins were identified by proteomics.
Results: No visible structural changes in testis and epididymis and no significant side effects on testis weight, testosterone levels, sperm motility, and sperm morphology were observed melatonin-treatment. Spermatogenesis-related molecules of PCNA, SYCP3, ZO-1, and CYP11A1 showed no significant differences in melatonin-treated testis. However, PCNA and HSPA2 increased their expressions in the epididymal initial segments by melatonin-treatment, but no significance. Normal two-cell and blastocyst development were observed in melatonin-treated, but melatonin significantly enhanced the sperm binding ability characterized as more sperm binding to one oocyte (control:7.2±1.3 versus melatonin:11.8±1.5). Sperm proteomics demonstrated that melatonin treatment enhanced the biological process of cell adhesion in sperm.
Conclusion: This study suggests that melatonin can promote sperm maturation and sperm function, providing important information for further research on the physiological function and protective effect of melatonin in male reproduction.

Poster 2
TARGETING KCTD13 AND B1PIX ENHANCE ANDROGEN RECEPTOR UBIQUITINATION AND DEGRADATION
Ahmed Chahdi[1], Carolina Jorgez[1,2], Abhishek Seth[1]
[1]Nemours Children's Health, Orlando, FL, USA, [2]Baylor College of Medicine, Houston, TX, USA
Presented By: Carolina Jorgez, PhD

Introduction & Objective: Congenital anomalies of the genitourinary (GU) tract are a common occurrence that arise from disturbances during embryonic development. These GU anomalies include hypospadias and cryptorchidism. Hypospadias is the improper positioning of the urethral opening on the ventral side of the penis. Cryptorchidism, or undescended testis (UDT), is the most common GU birth defect. We previously demonstrated the importance of KCTD13 in the GU tract development and in androgen receptor (AR) signaling. KCTD13 is a substrate-specific adapter of the CUL3-based ubiquitin ligase complex. The underlying molecular mechanisms of these congenital GU defects are not well understood. The objective of the present study is to determine the role of KCTD13 and β_1Pix (p21-activated kinase (PAK)-interacting exchange factor β) in AR ubiquitination and degradation. β_1Pix is a Rac/Cdc42 guanine nucleotide exchange factor (GEF) responsible for the recruitment and activation of Rho GTPases at the cell membrane.
Methods: Cell culture, immunoblotting, co-immunoprecipitation, immunohistochemistry, cell fractionation and luciferase assays were used to study the role of KCTD13 in AR ubiquitination/degradation.
Results: Ablation of the Kctd13 gene reduced the protein levels of AR and β_1Pix, and increased AR ubiquitination in mouse testicular tissues. Using co-immunoprecipitation studies, we showed that stimulation of AR increased β_1Pix binding to AR and nuclear β_1Pix/KCTD13/AR association leading to enhanced AR transcriptional activity. Rac1 inhibition blocked β_1Pix association with AR and reduced nuclear β_1Pix/KCTD13/AR association. AR was unable to bind to β_1Pix mutants lacking the GDP/GTP exchange activity, βPixΔDH and β_1Pix DHm (L238R, L239S) indicating that β_1Pix/AR association requires β_1Pix-GEF activity. Moreover, β_1Pix GEF deficient mutant was unable to reduce AR ubiquitination. Ectopic β_1Pix expression enhanced

β_1Pix/KCTD13/AR association, AR-dependent transcriptional activity, and decreased AR ubiquitination. Overexpression of KCTD13 increased the half-life of AR compared to control cells. KCTD13 and β_1Pix synergistically reduced AR ubiquitination and increased AR stability.
Conclusion: Collectively, our data reveal a novel signaling axis AR-β_1Pix-Rac-KCTD13 through which β_1Pix and KCTD13 are critical in maintaining AR stability and signaling by preventing AR ubiquitination and proteasomal degradation.

Poster 3

EFFECT OF TESTOSTERONE REPLACEMENT THERAPY ON GLYCEMIC CONTROL AND INSULIN SENSITIVITY IN HYPOGONADAL MEN WITH TYPE 2 DIABETES MELLITUS AND METABOLIC SYNDROME: A META-ANALYSIS.

John Ernandez[1], Khalid Alkhatib[2], Diego Ramonfaur[1], Phillip Pierorazio[2], Alexandra Berger[3], Martin Kathrins[3], Catherine Gu[3], Brittany Berk[3]
[1]Harvard Medical School, Boston, MA, USA, [2]University of Pennsylvania, Philadelphia, PA, USA, [3]Brigham and Women's Hospital, Boston, MA, USA
Presented By: John Ernandez, BA

Introduction & Objective: Hypogonadism is prevalent among men with type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetS). Testosterone replacement therapy (TRT) may be offered to men to address symptoms of hypogonadism, yet the role of testosterone in regulation of T2DM and MetS is complex, with mixed evidence suggesting TRT may benefit glycemic control. Herein, we performed a meta-analysis of randomized controlled trials (RCTs) evaluating the effect of TRT on glycemic control among men with T2DM and MetS.
Methods: We performed a literature review of electronic databases including Embase, Medline, Cochrane, Web of Science, and Google Scholar, including all placebo-controlled RCTs including use of subcutaneous and intramuscular TRT among men with T2DM or MetS. Case reports and series, systematic reviews, studies that did not report primary outcomes, and studies that used other modes of delivery of TRT were excluded. We assessed the effect of TRT on HbA1C, as a measure of glycemic control, and the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), as a measure of insulin sensitivity using a random-effects model. Study heterogeneity was evaluated using the I^2 test. Publication bias, included bias related to randomization, subject allocation, blinding, and reporting, was assessed via funnel plot. Our meta-analysis followed PRISMA guidelines.
Results: Our review yielded a total of 2,817 articles, of which 7 ultimately met inclusion criteria. With the exception of two studies which had high risk of attrition bias and detection bias, respectively, studies had otherwise similarly low or unclear risk of publication bias. However, substantial heterogeneity for both outcomes was observed between studies ($I^2 > 90$). TRT was not associated with a reduction in HbA1c ($p = 0.82$), though it was associated with a significant reduction in HOMA-IR ($p < 0.01$) (Figure 1). Cumulative analysis accounting for the duration of TRT demonstrated a non-significant reduction in HbA1c ($p = 0.282$) and HOMA-IR ($p = 0.114$).
Conclusion: Our meta-analysis of relevant RCTs did not demonstrate an effect of TRT on glycemic control among men with T2DM or MetS, though the included trials suffered from considerable heterogeneity in outcomes. Higher quality RCTs evaluating the effects of TRT on glycemic control and insulin sensitivity are needed to guide treatment.

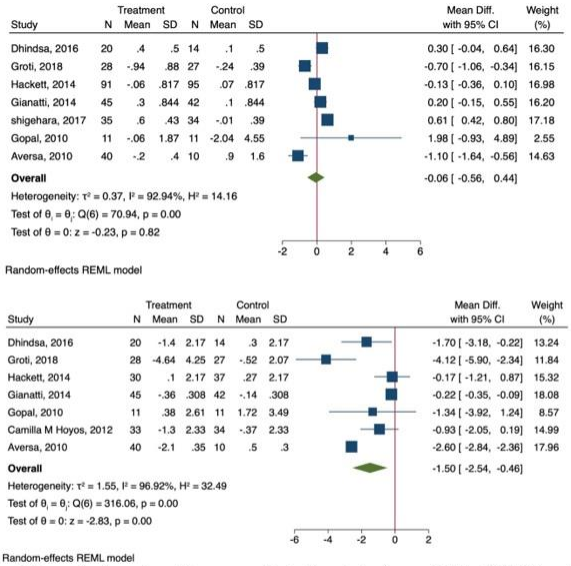


Figure 1. Forest plot for random-effects meta-analysis of association between TRT and a) HbA1c and b) HOMA-IR.

Association between TRT and a) HbA1c and b) HOMA-IR

Poster 4

GENERATION OF THE FIRST LEYDIG CELL-EXCLUSIVE MOUSE LINE EXPRESSING A CONSTITUTIVELY ACTIVE IMPROVED CRE RECOMBINASE

Shirley Ferrier-Tarin Chazot, Nicholas Robert, Solène Régis, Jacques Tremblay
Université Laval, Québec, QC, Canada
Presented By: Shirley Ferrier-Tarin Chazot, BS

Introduction & Objective: Leydig cells produce the hormones testosterone and INSL3 that are essential for male sex differentiation, fertility, and bone health. There are at least two distinct populations of Leydig cells that are responsible for producing these hormones: fetal (FLCs) and adult (ALCs) Leydig cells. FLCs atrophy shortly after birth. ALCs derive from undifferentiated precursors that are believed to be present during fetal life but start to differentiate prior to puberty. Whether the different Leydig cell populations share a common origin and whether they contribute to the development of each other, however, remain unknown. These important questions, along with the study of Leydig cell gene function, have long been hampered by the lack of suitable mouse models that can express the Cre recombinase exclusively in Leydig cells. Our goal was to generate a Leydig cell-exclusive Cre driver mouse line.
Methods: Since INSL3 is uniquely expressed in both FLCs and ALCs, we used CRISPR/Cas9 to knock-in the improved Cre recombinase (iCre) into the *Insl3* locus. The *Insl3*^{iCre} mice were crossed with the *Rosa26*$^{LSL\text{-}TdTomato}$ reporter mouse line that expresses the TdTomato fluorescent protein only when the preceding STOP cassette flanked by loxP sites (LSL) is removed by a Cre recombinase. Male offspring resulting from these crosses were euthanized at different embryonic and postnatal ages and the testes harvested, frozen and cut into 6 µm sections.
Results: TdTomato red fluorescence was detected in both FLCs and ALCs as confirmed by immunofluorescence for CYP17A1, a marker of Leydig cells, at all fetal and postnatal ages examined. TdTomato was observed in FLCs as early as E13.0 while it was detected as early as P5 in ALCs, establishing the earliest time of FLC and ALC differentiation. No fluorescence was observed in any other fetal or adult tissue examined.
Conclusion: The Leydig cell-exclusive iCre line will be invaluable for generating Leydig cell-exclusive gene knockouts. These new genetic tools represent a major advance to the field of andrology that will allow us to address fundamental questions about the biology and gene function of Leydig cells that have remained unanswered for decades. (Supported by CIHR)

Poster 5

MAPPING THE MEF2D GENOME-WIDE REGULATORY NETWORK IN MA-10 LEYDIG CELLS

Karine de Mattos, Julien Prunier, Claude Robert, Robert Viger, Jacques Tremblay
Université Laval, Québec, QC, Canada
Presented By: Karine De Mattos, DVM, MS

Introduction & Objective: Leydig cells are the source of testosterone, a hormone essential for male sex differentiation, reproductive function, and general health in men. Although testosterone synthesis requires the concerted expression of several genes, the molecular mechanisms regulating Leydig cell gene expression remain to be fully elucidated. We previously reported that members of the MEF2 family of transcription factors are present in the fetal and postnatal testis (Leydig and Sertoli cells), but not the ovary, suggesting a role in testis development and function. Leydig cells express MEF2A, MEF2C, and MEF2D, which contribute to the expression of steroidogenesis-related genes. However, the genome-wide DNA binding of MEF2 factors has not been defined in Leydig cells. The objective of this study was to determine the genome-wide binding signature of MEF2 factors in resting and cAMP-stimulated Leydig cells.
Methods: Chromatin immunoprecipitation followed by next-generation sequencing (ChIP-seq) was performed. Briefly, chromatin from resting and 8Br-cAMP-stimulated MA-10 Leydig cells was immunoprecipitated with a specific and validated MEF2D antibody. Samples were subjected to next-generation sequencing, using ENCODE project guidelines. Chromatin input samples were used as a negative control. Data was analysed using Galaxy platform and Gene Ontology Resource.
Results: Several peaks overlapped in resting and stimulated cells. However, differential peak intensity revealed a 1.7-fold increase in MEF2D-dependent DNA binding in stimulated cells compared to resting cells, indicating that MEF2D recruitment is enhanced in stimulated cells. Peak annotation analysis revealed several potential MEF2D targets, including *Stat5b, Foxa3, Nr2f2, Camk1, Nrf2, Mapk7, Mef2a, Mef2d,* and *Jun*. All these genes code for transcription factors and kinases known to activate the expression of genes essential for steroidogenesis in Leydig cells. Our results also revealed that MEF2 factors autoregulate their own expression, a hallmark of critical developmental regulators. Gene ontology analysis defined MEF2D targets as involved in the regulation of transcription. Motif analysis is being performed to identify unique MEF2 DNA binding motifs and potential MEF2 co-factors in Leydig cells.
Conclusion: Our results identify MEF2D as an upstream regulator of several transcription factor-encoding genes in a regulatory network controlling Leydig cell differentiation and function. Consistent with this, MEF2 autoregulation suggests key developmental and physiological roles of this master regulator in maintaining Leydig cell fate, differentiation, and function. (Supported by CIHR)

Poster 6

CAMKI, AN AMPLIFIER OF INSL3 GENE EXPRESSION IN LEYDIG CELLS

Kenley Joule Pierre, Jacques J. Tremblay
Université Laval, Québec, QC, Canada
Presented By: Kenley Joule Pierre, MS

Introduction & Objective: The hormone Insulin-like 3 (INSL3) is produced by Leydig cells. INSL3 is responsible for testis descent during fetal life and regulates bone metabolism in adults. To date, only a handful of transcription factors, including the orphan nuclear receptors SF1 and COUP-TFII, have been shown to regulate *Insl3* expression in Leydig cells. The regulatory network responsible for specifying *Insl3* expression exclusively in Leydig cells remains to be fully characterized. CAMKI kinase is expressed in Leydig cells where it was recently shown to contribute to *Star* gene expression. Our objective was to determine whether CAMKI cooperates with either COUP-TFII and/or SF1 in the regulation of *Insl3* gene transcription in Leydig cells.
Methods: Transient transfections were performed in Leydig (MA-10) and fibroblast (CV-1) cell lines using rat, mouse, and human *INSL3* promoter sequences fused to a luciferase reporter along with expression vectors for SF1, COUP-TFII, and constitutively active CAMKI (CAMKI CA) either alone or in combination. Protein sequence analysis revealed the presence of three potential CAMKI phosphorylation sites in COUP-TFII and two in SF1. Phosphorylation mutations have been introduced at these sites and are being analyzed.
Results: As previously reported, we found that COUP-TFII and SF1 can individually activate, and functionally cooperate on the mouse, rat, and human *INSL3* promoter. Interestingly, CAMKI CA alone led to an activation of the *Insl3 promoter*, indicating that it cooperates with endogenously expressed transcription factors. CAMKI CA also enhanced the activation mediated by COUP-TFII and by SF1. In MA-10 Leydig cells, the combination of all three proteins (COUP-TFII, SF1, and CAMKI CA) resulted in a synergistic activation of the *Insl3* promoter reaching >100-fold for the rat promoter, >20-fold for the human promoter, and 15-fold for the mouse promoter. Similar results were obtained in heterologous CV-1 fibroblast cells.
Conclusion: Although the mechanisms of CAMKI action remain to be fully deciphered, our data revealed the importance of CAMKI in the activation of the nuclear receptors COUP-TFII and SF1 for the regulation of *Insl3* gene expression in Leydig cells. Since COUP-TFII and SF1 regulate the expression of several genes in Leydig cells, the CAMKI amplifier effect might represent a new mechanism to ensure high gene expression levels when needed. (Supported by CIHR)

Poster 7

A HIGH-THROUGHPUT METHOD FOR ACCURATE MEASUREMENT OF FREE TESTOSTERONE IN SERUM USING EQUILIBRIUM DIALYSIS COUPLED WITH ID-UHPLC-MS/MS

Hui Zhou[1], Ashley Ribera[2], Uliana Danilenko[1], Hubert Vesper[1]
[1]Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA, [2]Battelle Memorial Institute, Atlanta, GA, USA
Presented By: Hui Zhou, PhD

Introduction & Objective: Testosterone is the most abundant circulating androgen in both men and women and serves as a key biomarker in diagnosis and monitoring of many diseases. Measurement of the metabolically active form, free testosterone (FT), has been part of the clinical work up to assess and manage patients with androgenic abnormalities. FT is currently measured using direct commercial immunoassays, assays using equilibrium dialysis (ED) or other separation techniques. Though the ED approach is recognized as the recommended approach, it is laborious and technically challenging for routine clinical use. The CDC Clinical Standardization Program is developing a high throughput method using the ED procedure coupled with isotope dilution ultra-high-performance liquid chromatography tandem mass spectrometry (ID-UHPLC-MS/MS) that is suitable for routine patient care and large-scale epidemiologic studies.
Methods: Serum samples are dialyzed in a custom-designed multi-well plate against a protein-free HEPES buffer (pH 7.4) at 37 °C until equilibrium. After isolating endogenous FT from protein-bound testosterone by ED, isotope-labeled internal standard ($^{13}C_3$-testosterone) was added to the dialysate for quantification. Certified pure primary reference material (National Measurement Institute-M914) was used to prepare calibrators, enabling traceability and ensuring measurement trueness. FT was further isolated from the dialysate matrix using supported liquid extraction and a chromatographic separation from interfering compounds and quantitation by tandem MS.
Results: The measurement ranges covered 0.2 – 1000 ng/dL for testosterone, with the bias within ±5% and precision less than 10% CV. A total of 45 samples with a wide range of total testosterone (TT, 21-912 ng/dL) and SHBG (11-129 nmol/L) were analyzed and showed the suitability of this assay to measure the serum free testosterone levels in normal, hypogonadal males as well as in the majority of normal females and females with androgen excess. The application of 48-well format plate and automated liquid handler system significantly improves the throughput of sample preparation. In addition, comparison of the TT results measured with the presented TT method with calculated TT concentrations, suggest that calculation overestimates TT concentration by 34% on average.
Conclusion: The described high throughput method for FT allows for sufficiently accurate and precise measurement for routine applications including large epidemiologic studies to help with establishing reference intervals.

Poster 8

CHARACTERIZATION OF TWO NOVEL MOUSE LINES EXPRESSING THE TDTOMATO FLUORESCENT PROTEIN EXCLUSIVELY IN LEYDIG CELLS

Nicholas Robert[1], Shirley Chazot[1], Solène Régis[1], Jacques Tremblay[1,2]
[1]Reproduction, Mother and Child Health, Centre de recherche du centre hospitalier universitaire de Québec - Université Laval, Quebec City, QC, Canada, [2]Centre de recherche en Reproduction, Développement et Santé Intergénérationnelle, Department of Obstetrics, Gynecology, and Reproduction, Faculty of Medicine, Université Laval, Quebec City, QC, Canada
Presented By: Nicholas Robert, BS, MS

Introduction & Objective: In the testis, Leydig cells produce insulin-like 3 (INSL3) and testosterone (T), two hormones essential for male sex differentiation and reproductive function. At least two distinct populations of Leydig cells are responsible for the production of these two hormones during fetal and postnatal life: fetal Leydig cells (FLCs) and adult Leydig cells (ALCs). Whether the different Leydig cell populations share a common origin and whether they contribute to the development of each other, however, remain unknown. Answering these important questions have long been hampered by the lack of suitable mouse models that can exclusively label Leydig cells for tracing purposes.
Methods: Since the *Insl3* gene is expressed exclusively in FLCs and ALCs, we used CRISPR/Cas9 gene editing to knock-in the red fluorescent protein TdTomato into the *Insl3* locus, with or without the native *Insl3* 3'UTR. Testes from both lines ($Insl3^{TdTomato\text{-}3'UTR}$ and $Insl3^{TdTomato}$) were harvested at different developmental timepoints, snap frozen, and cut into 6 μm sections.
Results: For both mouse lines, red fluorescence was restricted to Leydig cells; no fluorescence was observed in any other tissue examined. In the $Insl3^{TdTomato}$ (without the 3'UTR) mouse line, red fluorescence was only observed in FLCs both in the fetal and adult testis; ALCs were unlabelled. However, in the $Insl3^{TdTomato\text{-}3'UTR}$ mouse line, red fluorescence was detected in both FLCs and ALCs at all fetal and postnatal ages analyzed.
Conclusion: Expression of *Insl3* in FLCs and ALCs requires different sets of regulatory elements. Our data support the concept that the two Leydig cell populations are distinct, with population-specific regulatory gene expression mechanisms. Our novel mouse lines expressing the TdTomato fluorescent protein exclusively in FLCs or in FLCs + ALCs will be invaluable for studying the two Leydig cell populations in addition to allowing the purification of primary Leydig cells at different times during development by simple FACS sorting for primary cultures, and especially for generating an immortalized FLC line for which none currently exist. (Supported by CIHR MOP-81387)

Poster 9

ZINC MITIGATES LEAD-INDUCED SEXUAL DYSFUNCTION BY INHIBITING PENILE OXIDATIVE INJURY AND UPREGULATING CIRCULATING TESTOSTERONE

Elizabeth Besong[1], Tunmise Akhigbe[2,3], Precious Ashonibare[3,4], Ayoola Oladipo[3,4], J Obimma[1], Moses Hamed[3,5,6], Damilare Adeyemi[2], Roland Akhigbe[3,4]
[1]Ebonyi State University, Abakaliki, Nigeria, Abakaliki, Nigeria, [2]Osun State University, Osun State, Osogbo, Nigeria, [3]Reproductive Biology and Toxicology Research Laboratory, Oasis of Grace Hospital, Osogbo, Osun State, Nigeria, Osogbo, Nigeria, [4]Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, Ogbomoso, Nigeria, [5]Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria, Ado-Ekiti, Nigeria, [6]The Brainwill Laboratory, Osogbo, Osun State, Nigeria, Osogbo, Nigeria
Presented By: Roland Akhigbe, MD

Introduction & Objective: Lead exposure has been implicated in the aetiopathogenesis of male infertility via an oxidative stress-dependent pathway. However, till date, there is a dearth of data on the effect of lead and the associated mechanism of action on male sexual and erectile function. Conversely, zinc exerts antioxidant effects; nevertheless, the protective role of zinc in lead-induced sexual and erectile dysfunction is yet to be reported. The present study evaluated the impact of lead exposure with and without zinc therapy on male sexual and erectile function.
Methods: Twenty male Wistar rats were randomly assigned into four groups (n= 5 rats/ group). The control was vehicle-treated with 0.5 ml/day of distilled water, the zinc-treated group had 3 mg/kg/day of elemental zinc, lead-exposed rats received 20 mg/kg of lead acetate, and lead + zinc-treated group received a combination of the lead acetate and zinc as in the lead-treated and zinc-treated groups *per os* daily for 28 days.
Results: Zinc co-administration significantly improved absolute and relative penile weights, and the latencies and frequencies of mount, intromission, and ejaculation in lead-exposed rats. Also, zinc ameliorated lead-induced reduction in the motivation to mate and penile reflex/erection. These findings were accompanied by attenuation of lead-induced suppression of circulating NO, penile cGMP, dopamine, and serum LH, FSH, testosterone. In addition, zinc alleviated lead-induced upregulation of penile activities of acetylcholinesterase and xanthine oxidase, and uric acid and MDA levels. Furthermore, zinc ameliorated lead-induced decline in penile Nrf2 and GSH levels, and catalase, SOD, GPx and GST activities.
Conclusion: This study revealed that co-administration of zinc improves lead-induced induced sexual and erectile dysfunction by suppressing XO/UA-driven oxidative stress and upregulating testosterone via Nrf2-mediated signaling.

Poster 10

SPECTRUM OF CLINICAL PRESENTATIONS AND Y CHROMOSOME ABERRATIONS IN PATIENTS WITH X/XY MOSAICISM: A 35-YEAR MULTICENTRAL STUDY

Mahmoud Aarabi[1,2], Ebba Alkhunaizi[3,4], Jenna Plamondon[5], Selma Witchel[1,6], Diane Wherrett[3], Riyana Babul-Hirji[3], Annie Dupuis[7], Lyne Chiniara[3,8], Mary Ann George[3], Cheryl Shuman[3], Aleksandar Rajkovic[9], David Chitayat[3,4], Svetlana Yatsenko[1,2,10]
[1]University of Pittsburgh School of Medicine, Pittsburgh, PA, USA, [2]UPMC Magee-Womens Hospital, Pittsburgh, PA, USA, [3]The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada, [4]Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada, [5]University of Maryland-Baltimore, Baltimore, MD, USA, [6]UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA, [7]University of Toronto, Toronto, ON, Canada, [8]CHU Sainte-Justine, University of Montreal, Montreal, QC, Canada, [9]University of California San Francisco, San Francisco, CA, USA, [10]Magee-Womens Research Institute, Pittsburgh, PA, USA
Presented By: Mahmoud Aarabi, MD, PhD

Introduction & Objective: X/XY sex chromosome mosaicism is a difference in sex development (DSD) posing challenges in clinical management, gender assignment, and prediction of reproductive potential. Genital phenotypes range from apparently normal male external genitalia through fetuses/newborns with genital abnormalities to female external genitalia with an increased risk for gonadoblastoma. The full extent of the clinical manifestations and the long-term outcomes are unknown as previous reports included individual cases or small cohorts with limited follow-up.
Methods: We reviewed the clinical manifestations, Y chromosome aberrations and long-term follow up information on patients with pre/postnatal diagnosis of X/XY mosaicism at the Hospital for Sick Children (Toronto, Canada), Mount Sinai Hospital (Toronto, Canada), and hospitals affiliated with the University of Pittsburgh Medical Center (UPMC-USA). Retrospective chart review of patients ascertained during 1984-2019 was performed to collect clinical, pathological and genetics findings.
Results: A total of 100 individuals with a confirmed karyotype of X/XY mosaicism met the inclusion criteria for long-term retrospective analysis. Abnormal genitalia (47%), short stature (36%), cardiac (29%) and renal (19%) anomalies were among the common clinical presentations. In 53 individuals the sex of rearing was male (53%) and 47 were reared as females (47%). The level of sex chromosome mosaicism in peripheral blood samples had no direct correlation with the phenotype and thus gender of rearing or other health problems. Females were significantly shorter than males (P=0.04) and height Z-score was significantly decreased with age for both genders (P=0.02). There were 52 patients (52%) with a 45,X/46,XY karyotype and 43 patients (43%) with a structurally abnormal Y chromosome, including 28 with a rearranged Y chromosome comprising the *SRY* gene, 8 with the Y chromosome material presumably negative for the presence of *SRY* gene, and 7 with a complex Y chromosome rearrangement. Five patients had a 45,X/47,XYY karyotype. A total of 52 patients (17 reared as males, 35 reared as females) had either biopsy or gonadectomy of at least one of their gonads; streak gonad and testis were the most observed histological patterns. Five patients, reared as female, were diagnosed with gonadal tumor after gonadectomy.
Conclusion: This multicenter study represents the largest cohort of patients with X/XY sex chromosome mosaicism to date. We provide a comprehensive overview of the spectrum of Y chromosome anomalies, clinical presentations and malignancy risks in these patients.

Poster 11

PROMETHAZINE DISRUPTS TESTICULAR FUNCTIONS BY DOWN-REGULATING THE ACTIVITIES OF STEROIDOGENIC ENZYMES AND UPREGULATION OF XANTHINE OXIDASE/URIC ACID SIGNALING

Moses Hamed[1,2], Roland Akhigbe[3], Victor Ekundina[1]
[1]Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria, [2]The Brainwill Laboratory, Osogbo, Nigeria, [3]Ladoke Akintola University of Technology, Ogbomoso, Nigeria
Presented By: Moses Hamed

Introduction & Objective: Promethazine is a first-generation drug used to treat allergies, insomnia, nausea, sleep problems, and the common cold. Promethazine is a psychotropic medication, a class of drugs that disrupt testicular function. Whether promethazine, like other psychotropic medications, impairs testicular function is unclear since there is a paucity of data on its impact on testicular function. In light of this, we decided to look into how well promethazine affected testicular function and possible associated mechanisms in promethazine users.
Methods: Twenty adult male Wistar rats were randomized into two groups (n = 10 rats per group): the vehicle-treated control (0.5 ml of distilled water) and the promethazine-treated group (50 mg/kg). The administration was via gavage and lasted for 56 days. The doses are established as relevant human doses for rats.
Results: The administration of promethazine significantly decreased testosterone levels, which was accompanied by a decrease in the activity of the enzymes 3-beta-hydroxysteroid dehydrogenase and 17-beta-hydroxysteroid dehydrogenase in the testes. There was also a significant rise in levels of ALP, GGT, and LDH, all of which are indications of testicular damage. Promethazine-induced inflammation (as evidenced by elevated levels of NO, G6PD, MPO, TNF-α, and IL-6) was accompanied by increased xanthine oxidase activity and uric acid concentration, a redox imbalance (as evidenced by downregulation of testicular antioxidants such as GSH, SOD, and CAT and increased levels of MDA), and apoptosis (as evidenced by an elevated DNA fragmentation index).
Conclusion: This study revealed that Promethazine disrupts testicular activities by suppressing steroidogenic enzymes and elevating xanthine oxidase/uric acid signaling.

Poster 12

KCTD13 MODULATES GENE EXPRESSION IN DIFFERENT PENILE CELL POPULATIONS

Carolina Jorgez[1,2], Hunter Flores[1], Ahmed Chahdi[2], Marisol Oneil[1], Abhishek Seth[2]
[1]Baylor College of Medicine, Houston, TX, USA, [2]Nemours Children's Hospital, Orlando, FL, USA
Presented By: Carolina Jorgez, PhD

Introduction & Objective: KCTD13 is an important regulator of gonadal development as mice lacking *Kctd13* (*Kctd13*$^{-/-}$) have micropenis and small undescended testes that express significantly decreased levels of androgen receptor (AR) and Sox9. KCTD13 acts as a substrate-specific adapter of an E3-ubiquitin-protein ligase complex BTB-CUL3-RBX1. SOX9 and AR activity, expression, and localization are regulated by posttranslational modifications including ubiquitination, in which CUL3 plays an important role. We hypothesized that KCTD13 positively regulates the stability of AR and SOX9 in the penis, and that restoration of AR and SOX9 levels in the penis of *Kctd13*$^{-/-}$ mice could rescue the abnormal phenotype.
Methods: We generated a rescue mouse model that lacks *Kctd13* and conditionally expresses AR in the urethral mesenchyme, upon cre activation with *Twist2*^{cre} (*Kctd13*$^{-/-}$;CMV-AR;Twist2^{cre}), and SOX9 in the urethral epithelium, upon cre activation with *Shh*^{cre} (*Kctd13*$^{-/-}$;CAG-SOX9;Shh^{cre}). Penis morphology was evaluated using microcomputed tomography (mCT) to perform 3D penile reconstructions for comparing the genitourinary phenotype of control, Kctd13-KO, and rescue mice. Fertility was assessed by breeding male mice from each genotypic group to a wild-type (WT) female for 6 months, and then comparing litter size, number, and frequency between the groups.
Results: Loss of *Kctd13* results in micropenis. While the average penile length in WT mice was 5.88±0.16mm, the length of *Kctd13*$^{-/-}$ penises ranged from 2.90 - 5.54mm (13 of 15 characterized as micropenis (<5.48mm)) and 60% with a length below 4mm (11.5 SD). When compared to WT mice, the male urogenital mating protuberance (MUMP) length was significantly shorter in null mice. The length and base of the baculum (bone in the murine penis) were significantly shorter and narrower in null mice compared to those of WT mice. When the rescue mice were compared to *Kctd13*$^{-/-}$mice, we observed increased penile and MUMP lengths. No difference in the urethral meatus location was observed. Additionally, fertility of the double-KO mice improved, indicating AR and SOX9 rescue have an effect on penile development and fertility.
Conclusion: Restoration of AR and SOX9 in penile mesoderm and epithelium, respectively, improves penile development in *Kctd13*$^{-/-}$ mice, providing a better understanding of the contributions of epithelial and mesenchymal cell types and the role of Kctd13 in penile development.

Poster 13

MITOCHONDRIAL DYNAMICS: A PROMISING THERAPEUTIC TARGET FOR AGING LEYDIG CELL DYSFUNCTION

Samuel Garza, Chantal Sottas, Yuchang Li, Vassilios Papadopoulos
University of Southern California, Los Angeles, CA, USA
Presented By: Samuel Garza, MS

Introduction & Objective: Introduction and Objectives: Testicular Leydig cells, the main producers of testosterone in males, functionally decline with aging. Decreased expression of mitochondrial steroidogenic interactome (SITE) proteins and diminished mitochondrial function in aging Leydig cells suggest that mitochondrial dynamics play a role in maintaining adequate testosterone levels. Optic atrophy 1 (OPA1) protein regulates mitochondrial dynamics and cristae formation, and its expression influences androgen biosynthesis. Increasing OPA1 expression in dysfunctional Leydig cell models restored mitochondrial function, increased SITE protein levels and recovered androgen production to levels similarly seen in healthy Leydig cells, suggesting a tight relationship between cristae formation and steroidogenic capacity. This relationship suggests that mitochondrial dynamics may be a promising target to ameliorate diminished testosterone levels in aging males.
Methods: Methods: We used rats aged twelve months to explore the relationship between mitochondrial dynamics and Leydig cell function. Isolated Leydig cells from rats were treated with the cell-permeable mitochondrial fusion promoter 4-Chloro-2- (1- (2- (2, 4, 6-trichlorophenyl) hydrazono) ethyl) phenol (M1) which enhances mitochondrial tubular network formation. Rats were treated with 2 mg/kg/day M1 for six weeks. Body weight was measured daily, and blood serum was taken weekly. At the end of the six weeks, Leydig cells were isolated using a magnet-associated cell separation (MACS) method employing antibodies targeting the Leydig cell-specific prolactin receptor. Leydig cell purity was evaluated using flow cytometry and 3-beta-hydroxysteroid dehydrogenase staining. Steroid production was measured by ELISA, protein and gene expression were evaluated with immunoblot and quantitative polymerase chain reaction (qPCR) respectively, and mitochondrial morphology was assessed via transmission electron microscopy (TEM).
Results: Results: Flow cytometry analysis of MACS-isolated Leydig cells and 3-beta-hydroxysteroid dehydrogenase staining determined a purity of > 90%. Leydig cells isolated from M1-treated rats showed enhanced mitochondrial tubular network formation, improved mitochondrial function and produced higher testosterone levels, compared to controls.
Conclusion: Conclusions: Testosterone levels in aged rats are significantly lower when compared to young rats. Decreased expression of SITE and mitochondrial dynamic proteins suggest dysregulation of mitochondrial integrity leading to reduced steroidogenic capacity. Promotion of mitochondrial fusion using M1 enhanced Leydig cell mitochondrial integrity and androgen formation, suggesting that mitochondrial dynamics may provide a promising therapeutic target for Leydig cell dysfunction.

Poster 14

FETAL EXPOSURE TO GENISTEIN AND DEHP MIXTURES ALTERS THE EXPRESSION OF GENES INVOLVED IN CRITICAL TESTICULAR FUNCTIONS IN ADULT RATS

Priyanka Malusare, Martine Culty, Casandra Walker
University of Southern California, Alfred E. Mann School of Pharmacy and Pharmaceutical Sciences, Los Angeles, CA, USA
Presented By: Priyanka Malusare, MS

Introduction & Objective:
Approximately 50 million people worldwide suffer from infertility with male factors accounting for nearly half of all cases. Exposure to endocrine disrupting chemicals (EDCs) have been shown to play a role in the etiology of male reproductive diseases in animal models, and strong associations have been reported in human studies between exposures to EDCs and male reproductive diseases. Humans are exposed to multiple EDCs throughout their life. While exposure to the phytoestrogen genistein is dietary via soy products, exposure to the ubiquitous antiandrogenic plasticizer DEHP comes from various consumer products and medical devices. In previous studies, we found that in-utero exposure of rats to genistein and DEHP mixtures (Gen+DEHP) altered testis development and transcriptome, and increased infertility rates. My goal is to identify genes and pathways altered in rat testes after in-utero exposure to low doses of Gen+DEHP.
Methods:
Pregnant SD rats were gavaged from gestation day 14 to birth, with corn oil (vehicle) or Gen+DEHP mixtures at 0.1 or 10 mg/kg/day, doses mimicking human exposure. Pathway-analysis software (Partek Genomics Suite, Ingenuity, KEGG) were used to identify candidate genes and functional pathways to be validated.
Results:
Microarray and RNA-seq analysis revealed that the transcription factors FOXA1 and FOXA3 were downregulated in adult F1 rats exposed as fetuses to Gen+DEHP mixtures. While FOXA3 plays a role in Leydig cell differentiation and function, FOXA1 was shown to bind the promoter region of DNMT3A, a DNA methyltransferase regulating epigenetic processes in male germ cells. SULT1E1, a gene involved in protecting Leydig cells from excessive stimulation by estrogens was also downregulated in F1 Gen+DEHP exposed rats. STAT3 was among the genes downregulated in F2 descendants of Gen+DEHP exposed rats. Jak/STAT pathway regulates stem cell maintenance and differentiation in various organs, including testis. Therefore, the downregulation of these genes by Gen+DEHP mixtures in F1 and F2 generations could be involved in the adverse reproductive phenotypes observed in our studies.
Conclusion: These data suggest that fetal exposure to environmentally relevant doses of Genistein and DEHP mixtures can affect genes involved in signaling pathways and epigenetic processes in adult testis. Elucidating the mechanisms leading to the disruption of these genes should help understanding the link between their downregulation, changes in testicular function, and adverse reproductive effects of EDCs.

Poster 15

URETHRAL LENGTH (UL) AS A PREDICTOR FOR INTRACORPORAL LENGTH (ICL) PRIOR TO INFLATABLE PENILE PROSTHESIS (IPP) PLACEMENT: IMPLICATIONS FOR OPERATING ROOM PROCEDURE.

Nicholas Deebel, Justin Refugia, Collette O'Connor, Raymond Xu, Ryan Terlecki
Wake Forest School of Medicine, Winston-Salem, NC, USA
Presented By: Nicholas Deebel, MD

Introduction & Objective: Measurement of ICL dictates IPP size with reservoir sizing determined by cylinder length and/or surgeon preference. Stretched penile length helps set expectations for postoperative penile appearance but does not adequately predict total ICL. No prior research has determined if UL correlates to ICL.
Methods: Our IRB-approved database was reviewed for cases of IPP (penoscrotal) with urethral assessment. Initially, a 14 French silicone Foley catheter was placed with 10 mL in the balloon. Exposed catheter length (ECL) was measured (cm) with penis on stretch as distance between urethral meatus and catheter hub. This reflects UL given a fixed catheter length between hub and balloon.
Results: 116 patients (mean age 64.7 0.8 years) were included. History of intracavernosal injection or Peyronie's disease did not impact ECL ($p > 0.05$). Mean ECL were calculated per IPP size range: 16-19.9, 20-22.9, 23-26 cm; 8.8 ± 0.3, 7.0 ± 0.3, 3.8 ± 0.5 cm, respectively ($p < 0.0001$). Exclusion of patients with prior radical prostatectomy RALP ($n = 34$) were similar: 8.1 ± 0.3, 6.3 ± 0.3, 3.0 ± 0.4 cm respectively ($p < 0.0001$). ECL negatively correlated with total IPP size ($R^2 = 0.48$; slope -0.53). Exclusion of RALP patients yielded a stronger correlation ($R^2 = 0.66$; slope -0.67). After excluding RALP patients and significant simple linear regression, multivariate linear regression with BMI and ECL maintained a significance for ECL ($R^2 = 0.66$; slope -0.65; $p < 0.0001$). When stratified by race (African American, AA) mean ECL was less in AA men (4.4 ± 0.6 vs. 6.4 ± 0.3 cm)($p < 0.05$). ECL was examined as a predictive factor for 100cc reservoir size. A threshold of </= 7cm carried a sensitivity of 81.1% for all patients and 92.1% for those without RALP. When stratified by race, a threshold of </= 7 and </= 5.5 cm carried a sensitivity of 93.3% and 80% respectively for AA men and 90.5% and 60% respectively for Caucasian men.
Conclusion: ECL, a surrogate for UL, may predict ICL and dimensions of IPP components, especially in patients with intact prostates. This may allow for preemptive device preparation, increased operating room efficiency, and offer insight into adequacy of corporal dilation.

A.

	African American	Caucasian	p-value
Exposed catheter length (ECL) (cm)	4.4 ± 0.6	6.4 ± 0.3	**< 0.05**
Total device length (cm)	22.7 ± 1.8	20.7 ± 0.2	**< 0.05**
Rear tip extension length (cm)	1.3 ± 0.4	1.8 ± 0.2	> 0.05

B.

	African American	Caucasian	p-value
ECL vs total length (slope, R^2)	-0.56, 0.63	-0.64, 0.60	> 0.05
ECL vs. cylinder/total length ratio (slope, R^2)	-0.01, 0.1	-0.01, 0.04	> 0.05

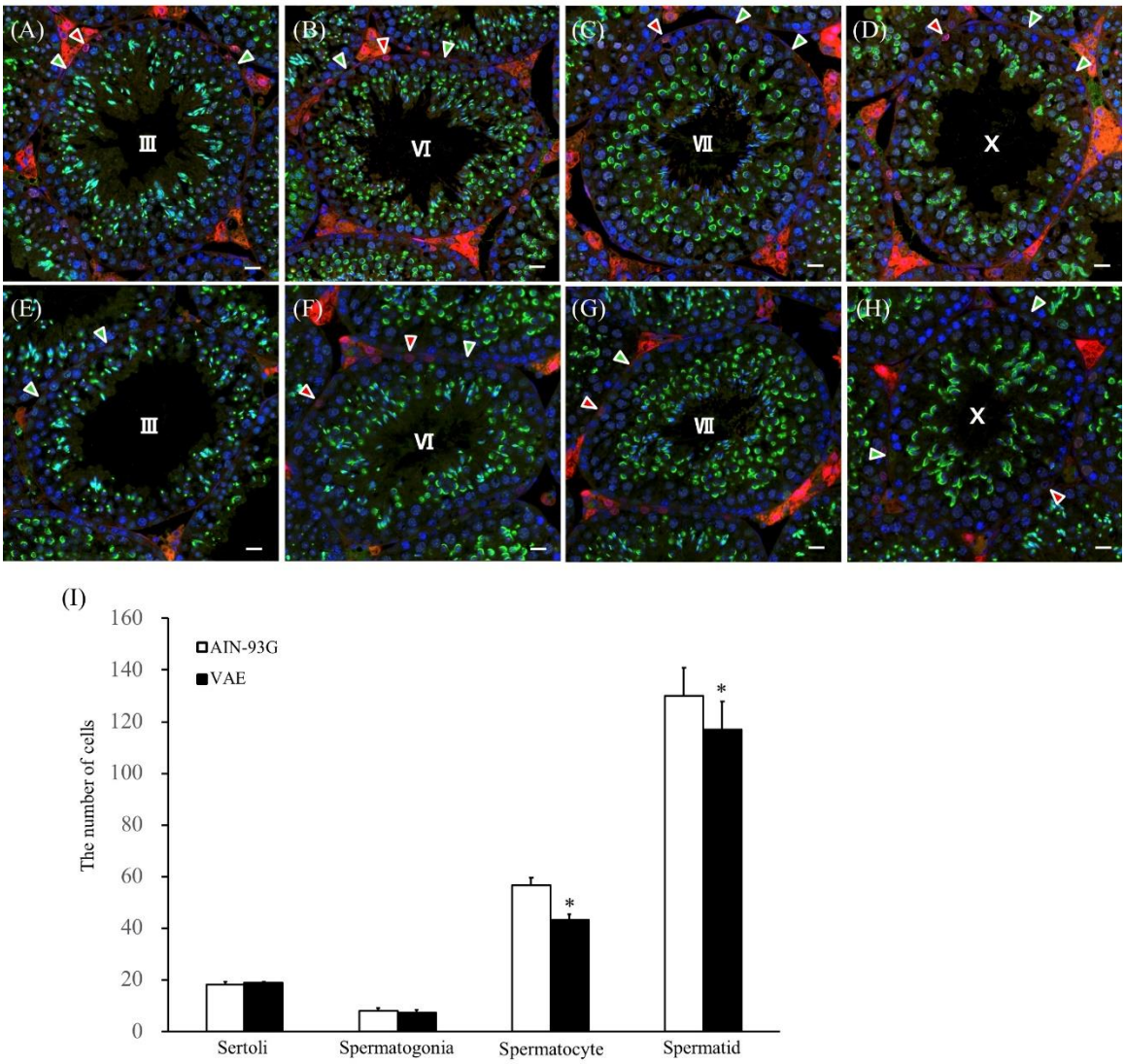
Stratification of IPP recipients without RALP by race.

Poster 16

DEVELOPMENT OF A NOVEL HISTOLOGICAL EVALUATION TO PREDICT THE EARLY SIGNS OF SPERM TOXICITY USING VITAMIN A EXCESS MOUSE MODEL

Satoshi Yokota[1,2], Tomohiko Wakayama[2], Hirokatsu Saito[1], Satoshi Kitajima[1]
[1]National Institute of Health Sciences, Kawasaki, Japan, [2]Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan
Presented By: Satoshi Yokota, PhD

Introduction & Objective: The increasing availability of fortified foods and supplements have been concerned an overconsumption of vitamin A (VA), above the recommended level. However, to date, the effects of chronic VA excess (VAE) on spermatogenesis remains unclear. Our previous study demonstrated that abnormality of sperm head morphology was significantly impaired when C57BL/6J mice were fed an AIN-93G semi-purified diet containing a VAE diet (250 IU/g) (Yokota et al. *Andrology*. 2021). However, classical histopathological analysis could not detect testicular toxicity caused by VAE. The aim of the present study was to develop a novel evaluation method of testicular toxicity as an early signs of sperm toxicity.
Methods: Dams were initially fed a control diet (4 IU/g) or a VAE diet (250 IU/g), 4 weeks prior to matting and during pregnancy. Dams and their male pups continued this diet regimen until the offspring reached 12 weeks of age. At 12 weeks of age, testes were collected and fixed in modified Davidson's fluid fixative. For the combination of lectin histochemistry and immunohistochemistry (IHC), sections were first treated with IHC for germ cell marker (i.e. ZBTB16, SCP3) and then with PNA lectin histochemistry for acrosomes. The sections were then counterstained with DAPI. Cell quantifications were performed to obtain the absolute numbers of different cell types per section of seminiferous tubule at each stage.
Results: The percentage of seminiferous tubules in stages VII and VIII was significantly lower in the VAE mice than in the control using PNA-lectin histochemistry. We measured a progress loss in initiation of meiosis, with significantly lower number of preleptotene spermatocyte in the VAE mice as compared with that in the control. The number of round and elongated spermatids were also decreased by chronically VAE ingestion.
Conclusion: To the best of our knowledge, few studies after that by Oakberg (1956) have performed a quantitative analysis of the cellular composition in mouse seminiferous tubules by counting the numbers of different types of spermatogenic cells in testis sections. We succeeded in demonstrating that lectin histochemistry and IHC using fluorescent dye-conjugated molecules appears to achieve a rapid and quantitative evaluation of testicular toxicity. These results suggest that this evaluation method is usefulness to predict the early signs of sperm toxicity and led us know how the seminiferous epithelium cycle is regulated by VA signals.



Determination of stages and identification of cell types.

Poster 17

DEVELOPMENT OF A NON-INVASIVE METHOD FOR TESTICULAR TOXICITY EVALUATION USING A NOVEL COMPACT MAGNETIC RESONANCE IMAGING SYSTEM

Satoshi Yokota, Kousuke Suga, Yuhji Taquahashi, Satoshi Kitajima
National Institute of Health Sciences, Kawasaki, Japan
Presented By: Satoshi Yokota, PhD

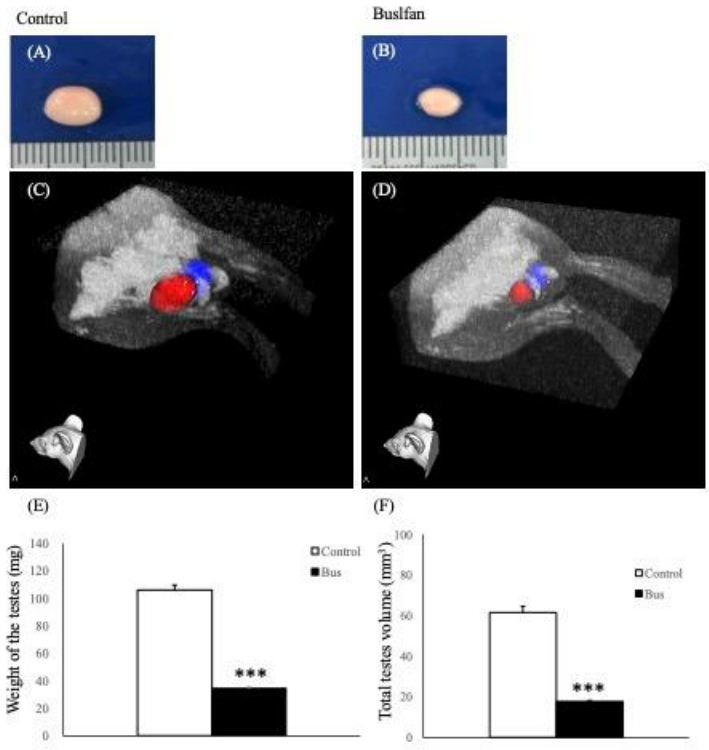
Introduction & Objective: In non-clinical animal studies for drug discovery, histopathological evaluation is the most powerful tool to assess testicular toxicity. However, histological analysis is extremely invasive; many experimental animals are needed to evaluate changes in the pathology and anatomy of the testes over time. As an alternative, small animal magnetic resonance imaging (MRI) offers a non-invasive methodology to examine testicular toxicity without radiation. The present study demonstrated the suitability of a new, ready-to-use compact MRI platform using a high-field permanent magnet to assist with the evaluation of testicular toxicity.

Methods: Twelve male C57BL/6J mice aged 6 weeks were randomly divided into two groups: the vehicle control and busulfan-treated groups. The busulfan-treated group received two i.p. of busulfan at a total dose of 40 mg/kg body weight with a 3-h interval between injections. After 28 days, the mice were subjected to MRI analysis under isoflurane anesthesia. High-resolution datasets were obtained using a T1-weighted 3D gradient-echo MRI sequence (echo time/repetition time, 2.0 msec/20.0 msec; field of view, 27.04 × 27.04 mm; matrix, 117 × 117; voxel size, 0.23 × 0.23 × 0.50 mm; scan time, 119 sec). For quantitative evaluation, the signal-to-noise ratios of selected testis structures were determined. Estimation of testicular volume were obtained by drawing 3D regions of interest in a sophisticated image processing and analysis software package that is fully integrated into the M7 imaging system. After MRI data were obtained, the reproductive organs of male mice were collected under isoflurane anesthesia. The testes were weighed and then rapidly fixed for histopathological analysis.

Results: On a T1-weighted 3D gradient-echo MRI sequences, the total testicular volume in busulfan-treated mice was significantly smaller than in controls. On T1-weighted images, the signal intensity of the testes was significantly higher in busulfan-treated mice than in controls. The mice were sacrificed, and the testes were isolated for histopathological analysis. The weight of the testes in busulfan-treated mice significantly decreased, similar to the results of MRI analysis. Additionally, periodic acid-Schiff stain–positive effusions were observed in the interstitium of the busulfan-treated mouse testes, potentially explaining T1 shortening due to a high concentration of glycoproteinaceous content.

Conclusion: The present study demonstrated a rapid evaluation of testicular toxicity in vivo by compact MRI.

Fig.1



T1-weighted, 3D gradient-echo MR images of the testes

Poster 18

AUTOLOGOUS TESTICULAR GRAFTING IN RHESUS MONKEYS MODELING PREPUBERTAL INDIVIDUALS UNDERGOING CANCER THERAPY

Gunapala Shetty[1], Jennifer Mitchell[1], Lesly Gil[1], Ramesh Tailor[1], Carol Hanna[2], Marvin Meistrich[1], Kyle Orwig[3]
[1]University of Texas MD Anderson Cancer Center, Houston, TX, USA, [2]Oregon National Primate Research Center, Beaverton, OR, USA, [3]Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA, USA
Presented By: Gunapala Shetty, PhD

Introduction & Objective: Testicular tissue cryopreservation is the only fertility preservation option for prepubertal patients who are at risk of infertility due to medical treatments for cancer or other conditions. Recently Fayomi et al. 2019 demonstrated that frozen/thawed prepubertal testicular tissues could be autologously grafted under the skin of castrated peri-pubertal rhesus macaques and matured to produce sperm that were competent to fertilize rhesus oocytes and resulted in the birth of a healthy offspring. To our knowledge, all successful reports of autologous, allogeneic or xenogeneic testicular tissue grafting have been performed on castrated recipients. Castration prior to grafting is not an option for adult survivors of childhood cancers. Therefore, it is necessary to demonstrate that this approach will work in adult recipients with testes.
Methods: Testicular tissues were collected from two prepubertal rhesus monkeys (44-45 months of age) by unilateral castration (#1) or biopsy of one testis (#2) and cryopreserved. Animals were then treated with busulfan chemotherapy (3 x 5 mg/kg) followed by 7-Gy of radiation. Increased testosterone levels indicated puberty at ages of 48 (#1) and 54 months (#2). Four to five months later, cryopreserved, prepubertal testicular tissues were grafted under the back skin and in the scrotum.
Results: The remaining testis and grafts were harvested from #1 at 11 months after grafting. In the testis, only 2% of the tubules showed differentiated germ cells confirming almost complete ablation of spermatogenesis. But sperm were observed in all grafts with the highest yield being 4×10^6 normal sperm from the right scrotal graft, placed adjacent to the remaining testis; 28% of these sperm were twitching indicating that they were viable. The graft-derived sperm were cryopreserved and used to fertilize rhesus oocytes by ICSI. The fertilization rate was 64% (54/85); the cleavage rate was 85% (46/54); and 26% of cleaved embryos (12/46) developed into blastocysts. Four blastocysts were implanted into recipient females. The grafts from #2 will be harvested shortly.
Conclusion: Testicular tissue harvested from a prepubertal individual before cytotoxic therapy and autologously grafted back into the radio- and chemotherapy-treated postpubertal male with intact testes can produce large numbers of viable testicular sperm capable of fertilization.

This work was supported by the *Eunice Kennedy Shriver* National Institute for Child Health and Human Development grant HD100197.

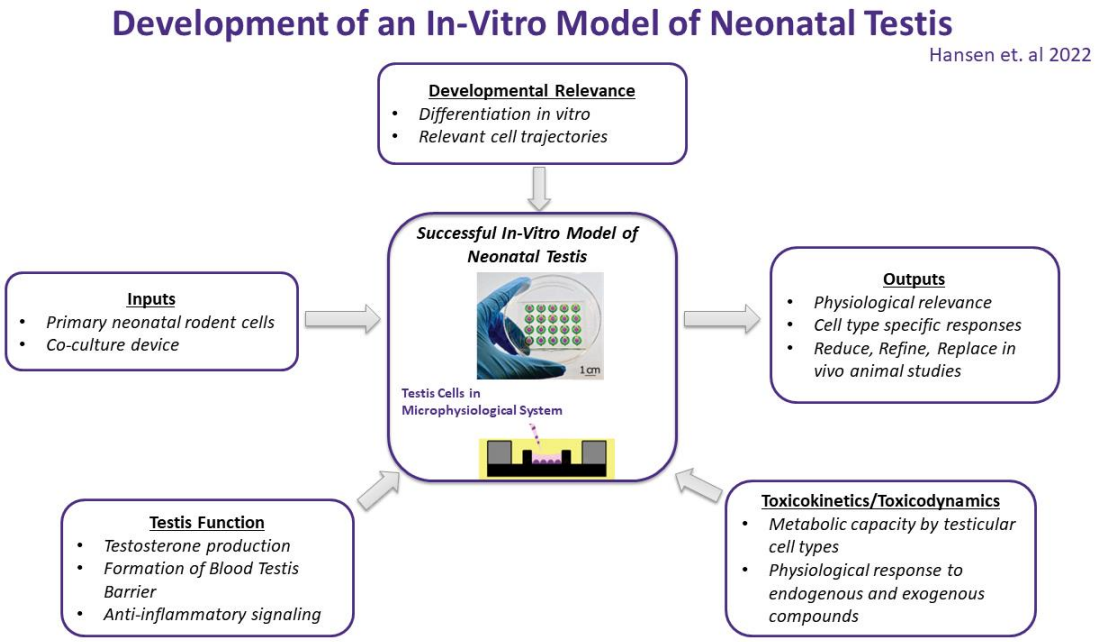
Poster 19

DEVELOPMENT OF AN IN-VITRO MODEL OF NEONATAL TESTIS

Brad Hansen, Edward Kelly, Elaine Faustman
University of Washington, Seattle, WA, USA
Presented By: Brad Hansen, BS

Introduction & Objective: A limitation in predictive toxicology is inadequate availability of in vitro systems for many critical organs, including the reproductive systems which are particularly susceptible during post-natal development. Successful in vitro models of toxicity in male reproductive development need to recapitulate cell types and functional activities in neonatal testis. To address this need, we developed a novel 3-D primary neonatal testicular cell culture system (TCS) using postnatal day five rat cells to model testicular toxicity during neonatal development.
Methods: The TCS incorporates 3-D co-culture of primary postnatal day five rat testis cells in a novel microculture plate. The microculture plate contains an array of 20 wells, each with a central culture area (16uL volume). Each culture is seeded with 25k cells in media with 4.4 mg/mL Matrigel protein, sufficient for formation of 3D structure. The cultures acclimated for 48 hours after seeding and maintained for seven experimental days after acclimation. Samples for testosterone were taken on experimental days 1, 3, 5, and 7. RNA-seq samples were collected on days 3, 5, and 7. The TCS was exposed to follicle stimulating hormone (FSH) and luteinizing hormone (LH) at low (5mIU/mL FSH and 50mIU/mL LH) and high doses (20mIU/mL FSH and 500mIU/mL LH) to determine capacity for hormone driven testosterone production.
Results: Testosterone production in the culture decreased over seven experimental days without hormone exposure ($p<0.001$). Transcriptomic data showed consistent expression of testis marker genes: Sox9 (Sertoli Cells), Cyp11a1 (Leydig cells) and Dazl (Germ cells). Gene expression related to testicular development increased over time: Igf1 [log fold-change (logFC) = 1.8, limma-voom adjusted p-value (adj.p) = 0.001), Nrg1 [logFC = 2.4, adj.p = 0.049) and Gas6 [logFC = 1.05, adj.p = 0.01). Enriched pathways included extracellular matrix organization [GO:0030198, adj.p < 0.0001] and protein kinase B signaling [GO:0043491, adj.p = 0.0002]. Hormone exposure increased testosterone production in the high [mean= 23.0 ng/mL, 95% CI: 15.3-30.7 ng/mL, p-value <0.001] and low [mean = 8.2 ng/mL, 95% CI: 2.4-13.9 ng/mL, p-value <0.01) experimental groups.

Conclusion: The TCS recapitulates testosterone production in response to hormone exposure and maintains major testis cell types. The TCS addresses a critical limitation in predicting reproductive toxicology during the post-natal period and will be further characterized to benchmark the temporal relevance and functional dynamics compared to in vivo testicular development.



Development of an In-Vitro Model of Neonatal Testis

Poster 20

EXPOSURE TO BENZO(A)PYRENE OF HUMAN SPERMATOZOA IMPAIRS THEIR FUNCTIONS: AN IN VITRO STUDY

Sara Marchiani, Giulia Traini, Lara Tamburrino, Giulia Guarnieri, Annamaria Morelli, Linda Vignozzi, Elisabetta Baldi
University of Florence-Florence-Italy, Florence, Italy
Presented By: Sara Marchiani, PhD

Introduction & Objective: Benzo(a)pyrene (BaP) is the most widely investigated polycyclic aromatic hydrocarbon and it has been classified as carcinogenetic for humans. Previous studies reported that BaP exposure leads to deleterious effects on male reproductive health.
The aim of the study was to evaluate BaP *in vitro* effects on human sperm functions.
Methods: Both washed semen and swim-up selected spermatozoa were *in vitro* incubated with different concentrations of BaP (1, 5 and 10 µM) for 3 and 24 hours at 37°C, 5% CO_2. The following parameters were analysed: progressive and total motility (by light microscopy), kinetic parameters and hyperactivation (by C.A.S.A. system), viability (by eosin/nigrosine staining), acrosome reaction (by FITC-labelled Lectin staining), penetration of artificial viscous medium (by capillary tubes filled with methylcellulose), caspases 3-7 activity (by FLICA Kit), sperm DNA fragmentation (sDF, by TUNEL/PI method) and reactive oxygen species (by CellROX®Orange Reagent and Dihydroethidium, DHE).
Results: In washed semen samples, a significant decrease of sperm progressive and total motility and viability was observed after 3 and 24 hours of incubation with 3 doses of BaP. In addition, an alteration of kinetic parameters and a reduction of sperm hyperactivated motility were evidenced after 3 and 24 hours of incubation. Similar results were found for swim-up selected samples. In selected samples, also a significant increase of sDF (after incubation with 5 and 10 µM of BaP) and an increase of ROS production, detected by DHE, in unviable spermatozoa (with all BaP doses) were observed. Oxidation of viable spermatozoa, revealed both by DHE and CellROX Orange, did not increase after BaP incubation. Finally, exposure to BaP significantly reduced acrosome reacted spermatozoa in response to progesterone (only at the higher BaP dose) and the mean number of spermatozoa able to penetrate an artificial viscous medium (with all BaP doses).
Conclusion: This study suggests that exposure to BaP could compromise the male fertility status and impairing important sperm functions. Moreover, the fact that BaP is present in female genital tract fluids indicates that spermatozoa could be hindered during their journey to reach the oocyte, thus affecting the fertilization process.

Poster 21

EFFECTS OF MEHP EXPOSURE ON STEM LEYDIG CELL DIFFERENTIATION AND TESTOSTERONE PRODUCTION

Connor Crull[1], Alanna McFail[1], Spencer Winspear[1], Vassilios Papadopoulos[2], Barry Zirkin[3], Kassim Traore[1]
[1]Campbell University Jerry M. Wallace School of Osteopathic Medicine, Lillington, NC, USA, [2]School of Pharmacy, University of Southern California, Los Angeles, CA, USA, [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
Presented By: Connor Crull

Introduction & Objective: The methodology is now available by which Leydig cells can be derived from stem cells on the surfaces of cultured seminiferous tubules. The Leydig cells produced in this way can produce high levels of testosterone for months. The primary objective of this study was to examine the effect of mono-(2-Ethylhexyl) phthalate (MEHP) exposure on testosterone production by the Leydig cells derived from tubule-associated stem Leydig cells.
Methods: Adult Brown Norway rats received a single intraperitoneal injection of ethane dimethanesulfonate (EDS) to eliminate the existing Leydig cells from the testes. Seminiferous tubules were isolated and cultured in a culture medium containing Insulin-Transferrin-Selenium, ITS (10 μg/ml), Smoothened Agonist, SAG (0.5 μM), and Luteinizing hormone, LH (100 ng/ml) for 8 weeks or longer. The formation of mature Leydig cells on the surfaces of seminiferous tubules was monitored by CYP11A1 staining and testosterone production. The effect of MEHP exposure was determined by culturing seminiferous tubules in differentiating medium in the presence of MEHP (0-5 μM) for 60 days.
Results: Incubation of seminiferous tubules isolated from EDS-treated rats in differentiating medium for 8 weeks resulted in the appearance of CYP11A1-positive, testosterone-producing Leydig cells on the surfaces of the tubules. The Leydig cells retained their ability to produce high levels of testosterone for months. With the use of this long-term culture methodology, we found that there was a suppressive effect on Leydig cell testosterone production by environmentally relevant doses of MEHP. Interestingly, in experiments in which there was a cessation of MEHP exposure after 4 weeks, testosterone production at 8 weeks was comparable to control levels.
Conclusion: Together, the current study and previously published studies indicate that stem Leydig cells on the surfaces of seminiferous tubules can be induced to differentiate to mature Leydig cells that can produce high testosterone levels. Exposure to an environmental toxicant such as MEHP during their formation can result in reduced testosterone production by the Leydig cells produced from the stem cells. Reduced testosterone can result from the effects of MEHP on either Leydig cell differentiation or the mature Leydig cells, or both. Although the mechanism by which reduced testosterone occurs is yet to be determined, preliminary data suggests that it may involve inhibition of the conversion of cholesterol to pregnenolone by CYP11A1.

Poster 22

EXPOSURE TO PERFLUOROALKYL OR POLYFLUOROALKYL SUBSTANCES ALTER SPERM METHYLOME IN MICE.

DruAnne Maxwell, Oladele Oluwayiose, Wanqing Liu, Michael Petriello, Richard Pilsner
Wayne State University, Detroit, MI, USA
Presented By: Druanne Maxwell, BS

Introduction & Objective: *Introduction:* Perfluoroalkyl or polyfluoroalkyl substances (PFAS) are a widely diverse group of synthetic chemicals that have relatively long half-lives and are widespread environmental contaminants found in water, air, animals, soil, and food products. PFAS breaks down slowly over time which is of public health concern as there is an increased susceptibility for biological accumulation. As such, there is compelling evidence supporting associations between PFAS exposure and adverse health conditions. However, research on the effects of PFAS exposure on male reproductive health and its potential mechanism is limited and results are inconsistent. Thus, our study aimed to examine the effect of PFAS exposure on sperm DNA methylation of adult mice.
Methods: *Methods:* Adult 9-week-old C57BL/6J male mice were exposed to 5 PFAS substances (0.02 mg/L) for 18-weeks or vivarium water as a control. Mice were sacrificed, epididymal sperm was collected and genome-wide methylation was assessed using reduced representation bisulfite sequencing (RRBS) method.

Differential methylation analysis at regional level (width = 100 bp, CpGs ≥ 3) was performed using the Methyl kit pipeline. Metascape was used to determine the functional relevance of PFAS-induced methylation change in sperm related to known biological processes. Fishers exact was used to perform enrichment analysis of our DMRs relative to all clusters and CpG features and genic regions.
Results: *Results:* PFAS exposure resulted in 2,861 DMRs ($q<0.05$, ≥ 10% methylation change), most (69%) of which were hypermethylated. For CpG features, PFAS associated DMRs were enriched in CpG open sea (83.6% vs. 43.3%, $p= 2.2 \times 10^{-16}$) and depleted in CpG islands and CpG shores. For genic regions, DMRs were depleted in exons (26.7% vs. 46.1%, $p= 2.2 \times 10^{-16}$), introns (35.5% vs. 51%, $p= 7.1 \times 10^{-13}$), and promoter regions (1.5% vs. 9.5%, $p= 1.1 \times 10^{-13}$) compared to all clusters. Additionally, gene ontology analyses of DMRs and differentially expressed genes showed enrichment of multiple developmental processes including lung epithelial cell differentiation and morphogenesis.
Conclusion: *Conclusion:* Our results show that PFAS exposure results in aberrant methylation in sperm. Further studies are needed to assess the role of the PFAS-associated sperm methylation on reproductive outcomes, the health and development of subsequent generations.

Poster 23

IN VITRO MODEL TO STUDY CANNABINOID EFFECTS ON DIFFERENT HUMAN TESTICULAR CELL TYPES.

Janmejay Hingu[1,2], Salome Ambokadze[2], Bita Nickkholgh[1,2], Allyn Howlett[1,2], Hooman Sadri-Ardekani[1,2]
[1]Wake Forest University School of Medicine, Winston-Salem, NC, USA, [2]Wake Forest Institute of Regenerative Medicine, Winston-Salem, NC, USA
Presented By: Janmejay Hingu, MD

Introduction & Objective: The endocannabinoid system (ECS) is a well-regulated network of endogenous cannabinoids (eCB), transporter, and metabolic enzymes involved in various organ systems, including male reproduction. The pharmacological properties of eCBs are mediated by their binding to CB1 and CB2 cannabinoid receptors. This study aims to assess the effect of selective CB1 and CB2 agonism and inverse agonism on human testicular cell cultures to study the *in vitro* effects on cellular profile and modulation of male reproductive function.
Methods: Human adult testicular biopsy specimens for cell culture were cryopreserved in 8% dimethyl sulfoxide and 20% fetal calf serum in minimum essential medium and stored at –196°C. Cells were isolated and cultured at 24,000 cells/cm^2 in StemPro-34 medium at 37°C supplemented with growth factors. After reaching at least 80% confluency, six experimental conditions were applied to the cells: (1) CP55,940 (cannabinoid agonist, 100nM, 2 hours), (2) SR141716 (CB1 inverse agonist, 100nM, 2 hours), (3) SR144528 (CB2 inverse agonist, 100nM, 2 hours), (4) initially SR141716 (100nM, 5 min), followed by CP55,940 (100nM, 2 hours), (5) initially SR144528 (100nM, 5 min), followed by CP55,940 (100nM, 2 hours), and (6) no treatment as control. RNA was isolated using the RNeasy Kit (Qiagen), and cDNA was synthesized using the high-capacity cDNA reverse transcription kit (Applied Biosystems). RT-qPCR determined relative gene expression for cellular markers of undifferentiated spermatogonial cells (ZBTB16, UCHL1, THY1), Sertoli cells (SOX9, Clusterin), Leydig cells (STAR, TSPO), and peritubular cells (ACTA2, CD34), as well as FAAH (a target gene involved in spermatogenesis regulation) using TaqMan gene expression assay with technical triplicates.
Results: RT-qPCR Ct values were normalized to control using POLR2A housekeeping gene as the endogenous control. ANOVA showed a significant difference between conditions for FAAH *($p=0.0488$)*, Clusterin *($p=0.0444$)*, and TSPO *($p<0.0001$)*. TSPO expression showed a significant difference for SR141716, SR144528, CP55,940+SR141716, and CP55,940+SR144528 when compared to control (*$p<0.0001$)*.
Conclusion: FAAH and Clusterin expressions appear to be downregulated with cannabinoid agonist, CP55,940. TSPO expression was significantly upregulated for all treatment groups except CP55,940. Spermatogonial and peritubular markers did not show any significant changes for evaluated markers. To verify comparable gene expression data, more biological replicates, varying cannabinoid concentrations, and different exposure durations are needed.

Poster 24

DIFFERENTIAL MIRNA BIOMARKER EXPRESSION IN THALLIUM-EXPOSED 3D HUMAN TESTICULAR ORGANOIDS.

Janmejay Hingu[1,2], Adam Cohen[1,2], Nima Zarandi[3], Anthony Atala[1,2], Hooman Sadri-Ardekani[1,2]
[1]Wake Forest Institute of Regenerative Medicine, Winston-Salem, NC, USA, [2]Wake Forest University School of Medicine, Winston-Salem, NC, USA, [3]University of Pittsburgh Medical Center - Central Pa., Pittsburgh, PA, USA
Presented By: Janmejay Hingu, MD

Introduction & Objective: Previous studies have demonstrated that heavy metal exposure can negatively impact male infertility and testicular carcinogenesis due to increased oxidative stress. This can lead to molecular dysfunction, part of which is mediated by small non-coding RNA known as miRNA, which may serve as potential biomarkers of disease. We present a miRNA analysis following thallium exposure to three-dimensional human testicular organoids (HTOs).
Methods: 3D HTOs were created from 2D testicular cell cultures derived from three brain-dead adult patients. HTOs were exposed to either thallium or ultrapure water. The miRNA analysis was performed using the Nanostring nCounter Human v3b miRNA panel, representing 827 unique miRNA probes. Nanostring nSolver 4.0 was used to execute target normalization with the geometric mean of the top 100 miRNAs detected, followed by background subtraction of 57 counts, which was the average of exogenous negative controls in the panel plus two standard deviations. All miRNAs with average counts <5 within a sample group were excluded to ensure robust expression. Qiagen Ingenuity Pathway Analysis (IPA) miRNA target filter was used to select for experimentally observed mRNAs associated with reproductive pathology and testis cells.
Results: 9 miRNAs were uniquely expressed within the thallium HTOs compared to 3 in the control. Seven of the 107 miRNAs expressed in both were significantly differentially expressed (miR-574-3p, miR-374a-5p, miR-423-5p, miR-106a-5p+miR-17-5p, miR-29b-3p, and miR-26b-5p were upregulated; miR-145-5p was downregulated). However, none survived the application of the false discovery rate. IPA miRNA target filter showed that these miRNAs regulated 161 mRNAs, including BCL2, MYC, CDKN1A, CCNA2, CCND1, and E2F1.
Conclusion: Certain miRNAs have previously been linked to male infertility (miR-145-5p, miR-574-3p, miR-26b-5p), asthenozoospermia (miR-423-5p), and testicular diffuse large B-cell lymphoma (miR-17-5p). Previous studies have also demonstrated that oxidative stress from thallium-containing compounds can induce apoptosis by affecting Bcl-2 (BCL2) expression and activate nucleolar stress by altering c-Myc (MYC) and p21 (CDKN1A) expression. Thallium has also been shown to modify cell cycle progression through cyclin A (CCNA2), cyclin D1 (CCND1), and E2F-1 (E2F1). Although only 4 of these miRNAs have

been implicated in male infertility and testicular cancer, further studies may elucidate other miRNAs and their downstream regulation of mRNA.

Poster 25

CAN THE MALE REPRODUCTIVE MORPHOPHYSIOLOGY BE ALTERED BY THE BIRTH ROUTE DUE TO CHANGES IN THE GUT MICROBIOTA AND ITS SYSTEMIC IMPACTS IN THE LONG TERM?

Marcella Santiago[1], Lais da Silva[1], Maria Christina Avellar[2], Juliana Perobelli[1]
[1]Universidade Federal de Sao Paulo - Instituto do Mar, Santos, Brazil, [2]Universidade Federal de Sao Paulo - Escola Paulista de Medicina, Sao Paulo, Brazil
Presented By: Marcella Santiago, MS

Introduction & Objective: The gastrointestinal tract hosts about 10^{13} microorganisms that characterize the gut microbiota (GM). This community performs many important functions for the host, such as modulating the physiology of organs, including the reproductive organs, the immune system, and the metabolism of xenobiotics. It is known that the GM matures during the first years of the child's life and that the birth route (BR), whether vaginal (VD) or by cesarean section (CS), is an important factor that can disrupt this. Our group has recently standardized a preclinical model in rats to assess the early- and long-term impact of the BR on the GM composition and physiopathology of reproductive tissues, in regular conditions and in response to toxicants. Here we report data on early effects of the BR on GM and testicular morphophysiology.
Methods: Male Wistar rats were born via VD or CS and then euthanized at postnatal day 1 (PND1; n=5) and PND21 (n=6). Colon were collected and processed for GM composition analysis by bacterial 16S rRNA gene high-throughput sequencing (Illumina MiSeq/HiSeq). Body mass, reproductive organs mass, serum testosterone level, testicular histopathology and immunohistochemistry for connexin 43 (Sertoli cell function) and 8-OHDG (DNA/RNA oxidative damage) were evaluated in samples from rats at PND21 (n=10).
Results: At PND1, the GM composition of animals born via VD and CS differed in bacterial diversity and relative abundance, whereas at PND21 it differed particularly in relative abundance. At least at PND21, the biometric, hormonal, testicular histomorphometric and immunostaining profile (Connexin-43/8-OHDG) were similar compairing rats born via VD and CS.
Conclusion: Despite the BR altered the GM composition of rats at PND1 and PND21, testosterone and testicular morphophysiology were not altered at PND21. We hypothesize that the dysbiosis induced by the CS may modulate the host's immunologic and physiologic profile, which may only become apparent in the long term. These changes would, in turn, lead to greater susceptibility of the individual to environmental pollutants, particularly in light of the toxic effects of these compounds on the morphological and functional integrity of male reproductive organs, which are important targets of pollutants, especially endocrine disruptors. The next steps of the present study will allow us to test this hypothesis. Financial support: FAPESP 2021/08127-6. Ethics Committee approval #2428081021/CEUA-Unifesp.

Poster 26

THE IMPACT OF SOCIOECONOMIC STATUS AND EDUCATION LEVEL ON THE SPERM EPIGENOME

Chad Polard[1], Neil Perkins[2], Elizabeth DeVilbiss[2], Kelaney Stalker[1], Joemy Ramsay[3], Joshua Horns[3], Jim Hotaling[3], Tim Jenkins[1]
[1]Department of Cell Biology and Physiology, Brigham Young University, Provo, UT, USA, [2]Eunice Kennedy Shriver National Institute of Child Health and Human Development, Rockville, MD, USA, [3]Department of Surgery, University of Utah, Salt Lake City, UT, USA
Presented By: Chad Polard, BS

Introduction & Objective: Cigarette smoking, obesity, and aging have been associated with differences in epigenetic signatures, such as DNA methylation. As it has been suggested that perturbations can impact fertility, we sought to assess relations between socioeconomic status and the sperm methylome.
Methods: Sperm DNA methylation data were obtained from the previously published FAZST study. The samples were divided into groups based on education level: high school or less (n=167), some college (n=482), Bachelor's degree (n=485), and Master's degree or higher (n=317) or income level: >$100k/yr (n=326), $75-100k/yr (n=315), $40-70k/yr (n=526), and $<40k/yr (n=173). Differential methylation analyses, epigenetic age calculations, and epigenetic variability analyses were performed to determine the relations between education, income, and DNA methylation. A constrained analysis was utilized to maximize power for the hypothesized protective effect of increasing income and education. Adjustment for various potential confounders was performed to understand the true drivers of signals identified.
Results: When adjusting for race, age, and education, four different regions of the genome (chr1:6085799-6086484; chr19:1752160-1752803; chr19:11649122-11650345; chr6:170449417-170449885) were significantly differentially methylated among different income levels. Patterns of epigenetic age or epigenetic variability were not significantly different between categories of income ($p>0.05$). Following adjustment for race, age, and income level, seven genomic regions were significantly differentially methylated as a function of education (chr5:1594330-1595049; chr17:79183386-79183967; chr19:9271193-9272146, chr4:1201315-1203050; chr11:65374263-65375204; chr2:54086854-54087742; chr10:685962-686763). No difference in epigenetic aging was identified, however, epigenetic variability was found to be significantly increased with decreasing levels of education ($p=0.0079$).

Conclusion: Both income and education displayed modest but significant methylation alterations at distinct regions of the genome. Further, lower education level was found to be associated with increased epigenetic variability in the genome. This pattern has been associated with disease states in various tissues, including sperm. As such alterations have been purported to arise as a result of various exposures (including occupational), it will be important to follow up this work with more direct assessments of specific exposures and their relations with epigenetic variability.

Poster 27

EFFECTS OF EARLY LIFE EXPOSURE TO ACETAMINOPHEN AND IBUPROFEN ON SPERMATOGENESIS AND SIGNALING MECHANISMS IN RAT TESTIS

Amina Khan, Amy Tran-Guzman, Martine Culty
Alfred E. Mann School of Pharmacy and Pharmaceutical Sciences, Los Angeles, CA, USA
Presented By: Amina Khan, MS

Introduction & Objective: Spermatogonial stem cells (SSCs) are the precursors to spermatozoa. Disrupting SSCs could hinder spermatogenesis and lead to infertility or cancer. We have previously shown that SSCs express eicosanoid pathway proteins cyclooxygenase (COX), produce prostaglandins (PGs), and that COX inhibitors upregulated Notch3, a gene regulating neonatal germ cells. Therefore, Acetaminophen (Ace) and Ibuprofen (Ibu) - which inhibit COXs, may disturb SSCs, which develop in infancy. Thus, we hypothesized that treating infants with Ace and Ibu could alter spermatogenesis. Our objective was to examine the in vivo effects of Ace and Ibu on testis development in rats.
Methods: Rats treated with vehicle, low or high doses of Ace (0.7 or 1.4 mg/day) or Ibu (0.4 or 0.7 mg/day) (equivalent to doses given to infants) from postnatal day (PND) 1 up to PND7 were euthanized at PND4, 8 and 90, and their testes processed for histological and immunofluorescence (IF) analysis.
Results: H&E staining showed changes in testicular morphology, with all treatment groups at PND4 having pronounced vacuolation compared to controls. Vacuolation was seen at PND8 for low doses, but less so with high Ace and Ibu doses. IF staining of PND8 testes showed decreased expression of COX2 for high Ace and Ibu doses compared to controls, while Notch3 expression was concomitantly increased. At PND90, COX2 expression was decreased in rats exposed as neonates to low Ace within the tubules. COX2 was decreased in the interstitium at both doses. COX2 expression was overall reduced after neonatal treatment with Ibu. The expression of germ cell marker VASA was increased with Ace and the low dose of Ibu. Notch3 expression was higher in PND90 rats exposed to Ace and Ibu, more strongly by low doses, compared to controls. Overall COX1 expression was decreased with both Ace and Ibu.
Conclusion: Neonatal treatments with Ace and Ibu at doses comparable to those used in babies altered the expression of Notch3, eicosanoid markers, testicular morphology and spermatogenic proteins in juvenile and adult rats. This suggests that neonatal exposure to drugs affecting the eicosanoid pathway disrupts testis and spermatogonial development, potentially impeding spermatogenesis. Thus, caution should be taken when treating baby boys with these drugs.

Poster 28

EFFECTS OF FILGOTINIB, A SELECTIVE JAK-1 INHIBITOR, ON MURINE SPERMATOGENESIS

Sarai Milliron, Pierre Ferrer, Tracy Clement
Texas A&M University, College Station, TX, USA
Presented By: Sarai Milliron, BS, DVM

Introduction & Objective: Filgotinib (GLPG0634, Jyseleca), a highly selective inhibitor of Janus kinase 1 (JAK1) with significantly less specificity for JAK2, is intended to treat adults with moderate to severe rheumatoid arthritis (RA). The JAKs are commonly associated with signal transducer and activator of transcription (STAT) proteins in JAK/STAT signaling pathways. The JAK/STAT signaling pathways are involved in important processes throughout the body including regulation of energy balance and immune function, hematopoiesis, and thrombopoiesis via regulating cell proliferation, differentiation, and apoptosis. The involvement of JAK/STAT signaling in spermatogenesis has been investigated in a few species including *Drosophila melanogaster*, *Xenopus laevis*, and *Mus musculus*. In *Drosophila* and *Xenopus*, JAK/STAT signaling has been shown to be involved in self-renewal of spermatogonial stem cells (SSC), whereas in mice JAK/STAT signaling is involved in SSC differentiation. Specifically, STAT3 plays a key role in the differentiation of murine SSC via JAK2 activation. During preclinical trials with Filgotinib, evidence of testicular toxicity consisting of germ cell depletion and degeneration were reported in mice, rats, and dogs, as well as decreased sperm production in humans. To date, the pathogenesis of the observed testicular toxicity is poorly understood.
Methods: To investigate the testicular effects of Filgotinib, we divided 25 male 11-week-old C57bl/6 mice into five dose groups and administered Filgotinib or water orally once daily for seven days. Following the seventh day of dosing, mice were euthanized, tissues, blood, and sperm were collected, and tissues were weighed and fixed accordingly. Testes and epididymides were fixed in Bouin's fixative, embedded in paraffin, sectioned, and stained using hematoxylin and eosin for microscopic evaluation.
Results: Histologic examination of testes showed moderate, multifocal secondary spermatocyte apoptosis and degeneration with fewer spermatogonia effected, primarily in stages I-VI, in Filgotinib treated versus control mice. Additionally, round spermatids primarily in stages I-VI lacked appropriate morphology, appearing to resemble secondary spermatocytes.

Conclusion: Oral administration of Filgotinib causes post-meiotic retention of secondary spermatocytes presumably by inducing a meiosis II block preventing progression from a secondary spermatocyte to a round spermatid. Analyses are ongoing to further elucidate the underlying mechanisms of results observed thus far.

Poster 29

CONVENTIONAL AND SUPPLEMENTED SPERM CRYOPRESERVATION IN REACTIVE OXYGEN SPECIES, GLUTATHIONE PEROXIDASE, AND INTERACTION OOCYTE-SPERM PARAMETERS IN NORMOZOOSPERMIC SAMPLES

Carla Silva[1], Ana Florêncio[1], Carolina Sanches[1], Mayara Rodrigues[2], Larissa Chiba[2,3], Jorge Hallak[2,3,4,5], Juliana Pariz[1,2]
[1]Methodist University of Sao Paulo, SBC, Brazil, [2]Urology Division of the University of Sao Paulo Medical School, São Paulo, Brazil, [3]Androscience, Science and Innovation Center in Andrology and High-Complex Clinical and Andrology Research Laboratory, Sao Paulo, Brazil, [4]Department of Pathology, Reproductive Toxicology Unit, University of São Paulo Medical School, São Paulo, Sao Paulo, Brazil, [5]University of Sao Paulo Innovation Center, Sao Paulo, Brazil
Presented By: Carla Silva, BS

Introduction & Objective: Previously studies have demonstrated that caffeine and melatonin addition in sperm samples forwarded has proven to be a very effective and simple way to improve semen motility in post-thaw sperm. We aimed to verify the effect of conventional and supplemented sperm cryopreservation in reactive oxygen species, glutathione peroxidase, and interaction oocyte-sperm parameters in normozoospermic samples.
Methods: This prospective study evaluated 46 semen samples (progressive motility ≥32%) from male voluntaries from Methodist University (Sao Paulo, Brail) between August 2021 and November 2022. Basic semen analysis was performed in the fresh sample and two aliquots (1ml after sperm processing) were cryopreserved by slow-freezing. One aliquot was cryopreserved with supplement substances (SUP group): an antioxidant substance (2 mM melatonin) was added to the pre-cryopreservation solution, and a stimulant substance (2 mM caffeine) was added in post-thaw samples. All aliquots were submitted before cryopreservation and after thawing to seminal analysis, reactive oxygen species levels (ROS) by chemiluminescence and glutathione peroxidase (GPx) levels by colorimetric test, and oocyte-sperm interaction test by hyaluronan binding assay. The sperm cryosurvival rate was calculated after slow thawing. The Student's T-Test was used to compare means.
Results: Initial samples presented total motility (%) = 69.90±9.68, progressive motility (%) = 45.82±9.22, sperm vitality (%) = 80.82±7.20, ROS ($x10^4$ CPM) = 1.86±3.33, GPx ($x10^6$ GSH-Px/mL) = 0.11±0.01, HBA (%) = 63.81±19.62. Both non-supplemented (Control group) and SUP group demonstrated significant statistic reduction in total motility ($p<0.001$), progressive motility ($p<0.001$), sperm vitality ($p<0.001$), GPx levels ($p<0.001$). In addition, was observed %HBA reduction in SUP samples when compared with fresh samples ($p=0.019$). Comparing CONT and SUP groups, was observed %HBA reduction on SUP group ($p=0.002$). No differences were observed in ROS levels between groups.
Conclusion: The benefits of supplementation of melatonin and caffeine in normozoospermic sperm cryopreserved samples were published previously, however, the present study demonstrated that this supplementation decreases sperm-oocyte interaction using supplementation, but preserves all other parameters. Therefore, supplemented samples can be reduced fertilization success when used in classic in vitro fertilization.

Poster 30

MRI BASED PREDICTIVE MODEL FOR SPERM RETRIEVAL FROM TESTICULAR TISSUE OF MEN WITH AZOOSPERMIA

Ana Planinić[1,2], Siniša Škokić[3], Davor Ježek[1,2]
[1]Department of Histology and Embryology, School of Medicine, University of Zagreb, Zagreb, Croatia, [2]Scientific Centre of Excellence for Reproductive and Regenerative Medicine, Zagreb, Croatia, [3]Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia
Presented By: Ana Planinić, MD

Introduction & Objective: 15% of infertile men have azoospermia, a condition where there are no spermatozoa in the ejaculate. Those men undergo testicular biopsy and half of each tissue sample is cryopreserved while the other half is histologically processed and analyzed. In around 50% of patients, sperm is detected. The other half of that sample is then thawed, sperm extraction is attempted, and used for IVF if successful. The shortcomings are that histology can be heterogeneous and the method is invasive. Magnetic resonance imaging (MRI), on the other hand, is a non-invasive method that can provide whole testis imaging and parameter mapping. The objective of this study was to construct a predictive model for successful sperm retrieval based on ex-vivo magnetic resonance imaging and spectroscopy data as well as patient history data obtained through the standard diagnostic evaluation of azoospermia.
Methods: Samples of testicular tissue (n=48) were obtained via testicular biopsy and underwent 7T MRI, specifically T2 mapping, diffusion tensor imaging (DTI), magnetization transfer imaging (MTI), and magnetic resonance spectroscopy (MRS). Images were analyzed in ImageJ and T2, ADC, FA, and MTR were determined. Spectra obtained with MRS were analyzed with the jMRUI software and the concentrations of choline, creatine, glutamate, and myo-inositol were determined using the QUEST algorithm. Samples were then histologically processed and analyzed. Multivariate logistic regression was performed to evaluate the relationship between 11 independent variables and sperm retrieval.

Results: Sperm was present in 47.92% (n=23) of samples based on histological analysis and sperm retrieval data from fertility clinics. The independent variables used for the construction of the predictive model were MRI parameters (T2, ADC, FA, and MTR), concentrations of metabolites determined with MRS (choline, creatine, glutamate, and myo-inositol), and patient history data (FSH and LH levels, patient history of cryptorchidism). The predicted area under the ROC curve was 0.9339 (95% confidence interval, 0.8647-1.000; $p<0.0001$). The negative predictive power of the model was 91.67%, while the positive predicting power was 87.5%.
Conclusion: Predicting successful sperm retrieval is possible with parameters from ex-vivo MRI, MRS, and patient history. To construct a non-invasive predictive model for successful sperm retrieval from testicular tissue that could precede testicular biopsy the model needs to be validated using clinical MRI and MRS data obtained in-vivo.

Poster 31

DIFFERENTIAL HISTONE RETENTION IN SPERM CHROMATIN OF NORMOZOOSPERMIC EJACULATES INFLUENCES THE PRE-IMPLANTATION EMBRYO DEVELOPMENTAL OUTCOMES

Riddhi Pandya[1], Ameya Jijo[1], Aswathi Cheredath[1], Shubhashree Uppangala[1], Sujith Salian[1], Vani R[1], Guruprasad Kalthur[1], Sanjay Gupta[2], Satish Adiga[1]
[1]Manipal Academy of Higher Education, Manipal, India, [2]Advanced Centre for Treatment, Research and Education in Cancer, Navi-Mumbai, India
Presented By: Riddhi Pandya

Introduction & Objective: During spermiogenesis, sperm cells develop a unique epigenetic architecture by retaining < 15 % of either canonical or variant histone proteins in the genome which is essential for normal embryogenesis. Recent studies have shown the heterogenous levels of retained histones in morphologically normal spermatozoa. However, the effect of differentially retained histones on embryogenesis is not entirely elucidated. Therefore, the study aims to understand the influence of paternal retained histone PTMs on the developmental competence of the early embryos, embryo metabolism, and pregnancy outcome.
Methods: This study included 62 normozoospermic ejaculates from patients attending a routine semen analysis or infertility treatment. 27 out of 62 study subjects were enrolled in the donor oocyte ICSI program. Processed spermatozoa were tested for DNA integrity by sperm chromatin dispersion assay and extracted retained histones were subjected to western-blot analysis. Correlation analysis was performed between levels of retained histone PTMs and post-wash sperm characteristics. Further, the retained histone PTM levels of 27 normozoospermic ejaculates were correlated with pre-implantation embryo development and pregnancy outcome. Post embryo transfer, spent culture media (SCM) was analyzed for levels of metabolites using nuclear magnetic resonance (NMR) spectroscopy.
Results: Western blot analysis showed heterogeneous levels of retained histones in the genome of normozoospermic ejaculates. Post-wash sperm yield was affected by the increase in H3K27Me3 and H4K20Me3 levels in the sperm chromatin. Also, spermatozoa with higher histone H3 retention showed increased DNA damage. These spermatozoa when injected into the donor oocyte, the fertilization rate decreased with an increase in sperm histone H3 and H3K27Me3. Interestingly, the quality of the resultant embryos was affected due to an increase in histone H3 in spermatozoa. Furthermore, the level of sperm histone H3 was higher in couples who failed to demonstrate clinical pregnancy and post-embryo transfer. NMR signature has shown an increased intensity of the amino acid methionine in the non-pregnant group compared to the pregnant group. Moreover, a negative correlation between sperm histone H3 and methionine levels in the pregnant group was observed.
Conclusion: Overall, altered epigenetic signatures in the spermatozoa can be one of the factors for the development of idiopathic male infertility, Such spermatozoa may influence embryonic behavior and thereby affect the success rate of ART procedures. To understand the mechanisms underlying these observations, more research is needed in this direction.

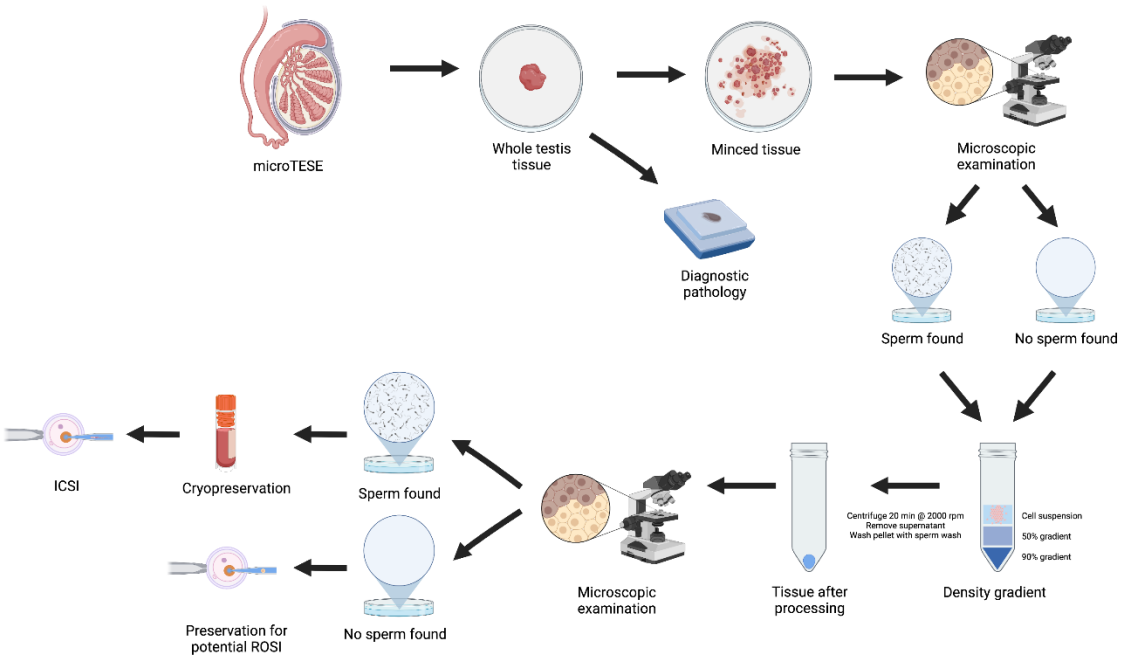
Poster 32

COMBINATION OF MICROSCOPIC TESTICULAR SPERM EXTRACTION (MTESE) AND DENSITY GRADIENT PROCESSING TO OPTIMIZE SPERM RETRIEVAL AND PREGNANCY OUTCOMES IN MEN WITH NONOBSTRUCTIVE AZOOSPERMIA (NOA)

Nicholas Deebel[1], Banafsheh Nikmehr[2,3], Janmejay Hingu[1,2], Collette O'Connor[1], Rachel Ruble[3], Yueqiang Song[3], Ayse Vuruskan[3], Anuradha Devenini[3], Cihan Halicigil[4], Stuart Howards[1], Tamer Yalcinkaya[3], Hooman Sadri-Ardekani[1,2,3]
[1]Wake Forest School of Medicine, Winston-Salem, NC, USA, [2]Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, USA, [3]Carolinas Fertility Institute, Winston-Salem, NC, USA, [4]Yale School of Medicine, New Haven, CT, USA
Presented By: Nicholas Deebel, MD

Introduction & Objective: mTESE with Intracytoplasmic sperm injection (ICSI) is the gold standard for treating infertile couples when NOA in the male is present. However, using conventional tissue processing techniques, many patients will remain negative for sperm on mTESE. Standard practice in assisted reproduction technology (ART) does not utilize the density gradient centrifugation process. We hypothesize that gradient utilization can enrich sperm retrieval following mTESE.
Methods: A cohort of patients with non-obstructive azoospermia underwent mTESE from 2019-2022 by a single surgeon. The biopsy specimens underwent mechanical dissection. Subsequent tissue processing and gradient wash were carried out through a gradient column including 90% and 50% gradient solution diluted in standard sperm wash medium. Upon verification of sperm, the post-gradient sperm and the remaining un-dissociated tissue were cryopreserved separately. On the day of ICSI, the frozen

gradient sperm were first thawed to recover viable sperm. If needed, frozen tissue was thawed, subjected to mechanical dissociation, and a subsequent gradient to obtain enough viable sperm. Fertilization and embryo development were assessed morphologically with the Embryoscope and genetically with preimplantation genetic testing for aneuploidy (PGT-A). Following embryo transfer, biochemical (ß-HCG) and clinical (gestational sac) pregnancy were assessed.
Results: Overall, 24 male patients (36 ± 1.5 years old) with NOA underwent mTESE for an average operating time of 110.6 ± 6.9 minutes (range: 37-177). The primary sperm identification rate after the mechanical dissociation of tissue was 37.5% (n=9). The use of the density gradient led to an overall sperm retrieval rate of 70.8% (n=17) ($p < 0.05$) and secondary sperm retrieval rate (sperm identification in initially negative patients) of 53.3% (n=8). Retrieved sperm were cryopreserved to be used later for ICSI. Thirteen couples (average female age and AMH 32.1 ± 1.6 and 3.2 ± 0.8 ng/ml respectively) underwent ICSI using extracted sperm. The overall fertilization, per-cycle pregnancy, and per-transfer pregnancy rates were 66%, 76%, and 100% respectively. Amongst these couples, 6 underwent PGT-A, and 70.0% of tested blastocysts were euploid.
Conclusion: Utilization of mTESE with subsequent gradient technology may serve as a highly sensitive sperm detection and enrichment method for NOA patients in order to obtain superior ART outcomes.



Proposed integration of gradient technology following mTESE

Poster 33
ADMINISTRATION OF A MICRONUTRIENT SUPPLEMENTATION COMBINED WITH A PHOSPHODIESTERASE TYPE 5 INHIBITOR: EFFECTS ON STANDARD SPERM PARAMETERS, SPERM FUNCTIONAL PARAMETERS, AND SPERM DNA INTEGRITY: A PROSPECTIVE, BLIND,RANDOMIZED, CONTROLLED TRIAL
Nikolaos Sofikitis[1], Aris Kaltsas[1], Athanasios Zachariou[1], Chara Tsiampali[1], Ioannis Giannakis[1], Athanasios Papatsoris[2], Andreas Karagiannis[1], Athanasios Zikopoulos[1], Panagiota Tsounapi[3], Atsushi Takenaka[3], Charalampos Mamoulakis[4], Fotios Dimitriadis[5]
[1]Department of Urology, University of Ioannina, Ioannina, Greece, [2]2nd Department of Urology, Sismanogleion General Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, [3]Department of Urology, Faculty of Medicine, Tottori University, Yonago, Japan, [4]Department of Urology, Medical School, University of Crete, Heraklion, Greece, [5]Department of Urology, Aristotle University of Thessaloniki, Thessaloniki, Greece
Presented By: Nikolaos Sofikitis

Introduction & Objective: The effects of a micronutrient supplementation (MSUP) combined with avanafil (AV) on sperm function were evaluated.

Methods: Oligoasthenospermic men (n = 261) were treated daily for 90 days with either an MSUP (BrudyFertil; n=54, 3 caps/day; Group A), L-carnitine (n=52, 1,000 mg/day Group B), BrudyFertil plus AV (50 mg X2/day; n=51, Group C) or AV (51 men, 50 mg X2 /day; Group D); another subgroup of 53 men (Group E) received no treatment. Sperm parameters were evaluated before and after the end of the experimental period (EXP) within each Group A, B, C, D, and E. Wilcoxon-test for paired observations or Chi-square test was employed to evaluate, within each group, differences in the mean values for each parameter. A probability P smaller than 0.05 was considered as statistically significant.
Results: The % motile spermatozoa (%MS), the hypoosmotic swelling test outcome (%HOST; J. Reprod Fertil, 70, 219), the % hyperactivated sperms (%HS; Fertil Steril, 55, 363) after incubation under conditions known to induce sperm capacitation, and the % sperms with integral DNA (% sperm- normal DNA; Fertil and Steril, 95, 110) were significantly greater after BrudyFertil or BrudyFertil plus AV treatment or AV treatment alone than before the respective treatment, within the Group A (mean ±SD for % sperm-normal DNA: 53.18±9.34 vs 35.20±14.59, respectively), C, or D. In contrast, differences in each of the above parameters between the beginning and the end of the EXP, within groups B or E, were not significant.
Conclusion: The antioxidant properties of docosahexaenoic acid (known to play a role in the intracellular production of glutathione), vitamin C, vitamin E, selenio, glutathione included in BrudyFertil improve the sperm membrane permeability resulting in an increase in %MS, %HOST, % HS, and the % sperm-normal DNA. The effects of the inhibition of the testicular PDE5 by AV (Andrologia. 2018;50:e13071) may result in the generation of sperms with better structure and function. It appears that BrudyFertil or BrudyFertil combined with AV or AV alone may have a positive and significant role in the decrease in the sperm DNA oxidative damage with a subsequent improvement in the overall sperm function.

Poster 34

PENILE IMPLANT INSIDE ACQUIRED INTRA-CAVAL EPIDERMOID CYST (CASE REPORT)

Alaa Aglan
Alaa Clinic Hospital, Giza, Egypt
Presented By: Alaa Aglan, PhD

Introduction & Objective: : Epidermal inclusion cyst is a benign lesion. To our knowledge there have been no previous reports of malignancy developing in cystic disease of penis or due to skin grafts. It may developed after circumcision, repair of hypospadias, or other types of penile surgery when islands of epithelium are left behind in the subcutaneous tissues. Congenital epidermoid cyst may form a long the median raphe of the penis or penile shaft or glans usually treated by excision.
Methods: peno-scrotal approach, extraction of implant (mealiable), irrigation, and re-implantation were done. In the right side the implant was inserted into the formed skin envelope (in sub-tunical plane) around previous implant. In left side the implant was put in sub-tunical plane also then closure in layers.
Results: patient had done penile implant 5 years ago. He complained that it is was too short, too thin, and penis always curved can`t be stable at 180 degree. He reported that the wound was infected and gaped for two months. By inspection we noticed that skin at previous incision was depressed. By exploration we found a cyst lined by skin envelopes the right implant. A hole was seen in the implant and the core was exposed with oily discharge. The implant was divided into two pieces and gathered beside each other but completely separated it seems that surgeon underestimated the length of implant and cut it too short then he put the two parts beside each other to compensate the shortage in length Which was 23 cm in old implant and girth 11 mm. The new was 26 cm and girth 13 mm. Due to infection and wound gaping islands of epidermis creeped inside the tunica and formed epidermoid cyst around the implant. Pathology revealed cyst lined by stratified squamous epithelium, culture revealed no growth. We inserted the implant in the tunnel formed by epidermoid cyst that enveloped the old implant as its excision will damage the caval tissues which was not atrophied due to small girth of implant. Urine culture two weeks post –operative revealed no growth, patient was satisfied, no complication reported.
Conclusion: Intra-caval epidermoid cyst shouldn’t be excised to preserve caval tissues intact.

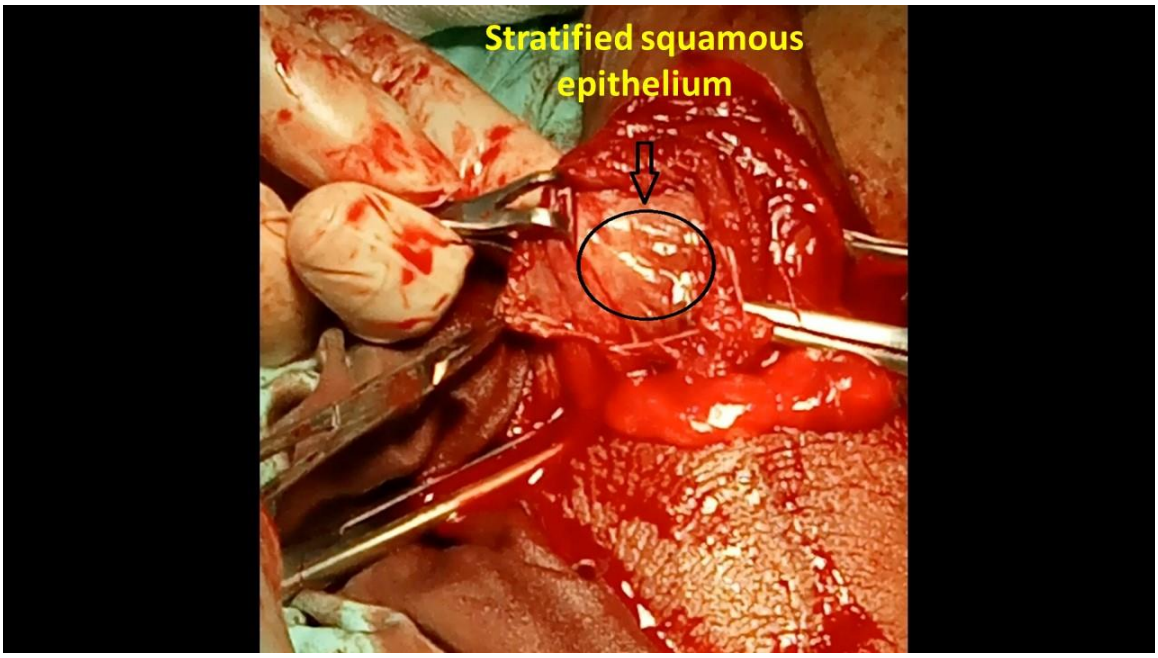


FIGURE1

Poster 35

SUCCESSFUL FERTILITY PRESERVATION USING SINGLE-SPERM VITRIFICATION AFTER 18 YEARS OF ESTROGEN THERAPY IN A TRANSGENDER FEMALE

Chaya Rothschild[1], Bobby Najari[2], Rachel Bluebond-Lagner[2], Lee Zhao[2], Michael Werner[1]
[1]Maze Sexual and Reproductive Health, New York, NY, USA, [2]New York University School of Medicine, New York, NY, USA
Presented By: Chaya Rothschild, BS

Introduction & Objective: Sperm banking prior to starting gender affirming hormones for transwomen is the standard of care, but is often not implemented. Little information is available on the likelihood, timing, and quantity of return of spermatogenesis after pausing hormone administration for the purpose of reproduction. Often if any sperm are found, they will be very low in number.

Cryptozoospermia represents a challenge for conventional sperm freezing, with often no sperm able to be frozen in advance or found fresh on the day of egg retrieval. Extended Sperm Search and Microfreeze (ESSM) is a technique previously published for use in cismen with both presumed azoospermia and cryptozoospermia. The entire processed specimen is plated in droplets under oil, and individual sperm placed into wells with the use of a micromanipulator, allowing for vitrification of individual live sperm with high survival rates.

We present the results of an ESSM performed on a semen specimen from a transwoman prior to her undergoing vaginoplasty.

Methods: A 40-year-old transwoman with a history of 18 years of estrogen therapy, spironolactone, and finasteride presented for fertility preservation. She was counseled to stop her gender affirming medications. After 10 months, standard semen analysis performed at an IVF center continued to demonstrate azoospermia. She was referred for ESSM on a fresh semen specimen the day before undergoing vaginoplasty and bilateral orchiectomy. If no sperm were found, the orchiectomy specimens from the next day's surgery would be brought to the lab for ESSM.

Results: In preparation for semen sample collection, the patient was advised to abstain from ejaculation for 7-10 days. After extended search of the sample, a total of 278 motile sperm were recovered from the sample, 195 of which were graded as "progressive'", 83 of them graded "non-progressive". A total of 13 cryo-devices (SpermVD, MFC Global) were vitrified, containing 19-26 sperm per device. The patient elected to defer testicular sperm extraction.

Conclusion: ESSM was successful in recovering and freezing small numbers of sperm in the semen specimen of a transwoman 18 years after starting hormone therapy. No sperm had been detected previously in several standard semen analyses. The implementation of this protocol, with ESSM of orchiectomy specimens as backup, may allow for the recovery of sperm in many transwomen who began hormone treatment prior to fertility preservation.

Poster 36

FLUOXETINE ADVERSELY AFFECTS ERECTILE FUNCTION BY CAUSING OXIDATIVE INJURY, INFLAMMATION AND APOPTOTIC DAMAGE IN PENILE TISSUES OF RESERPINE-INDUCED DEPRESSED RABBITS

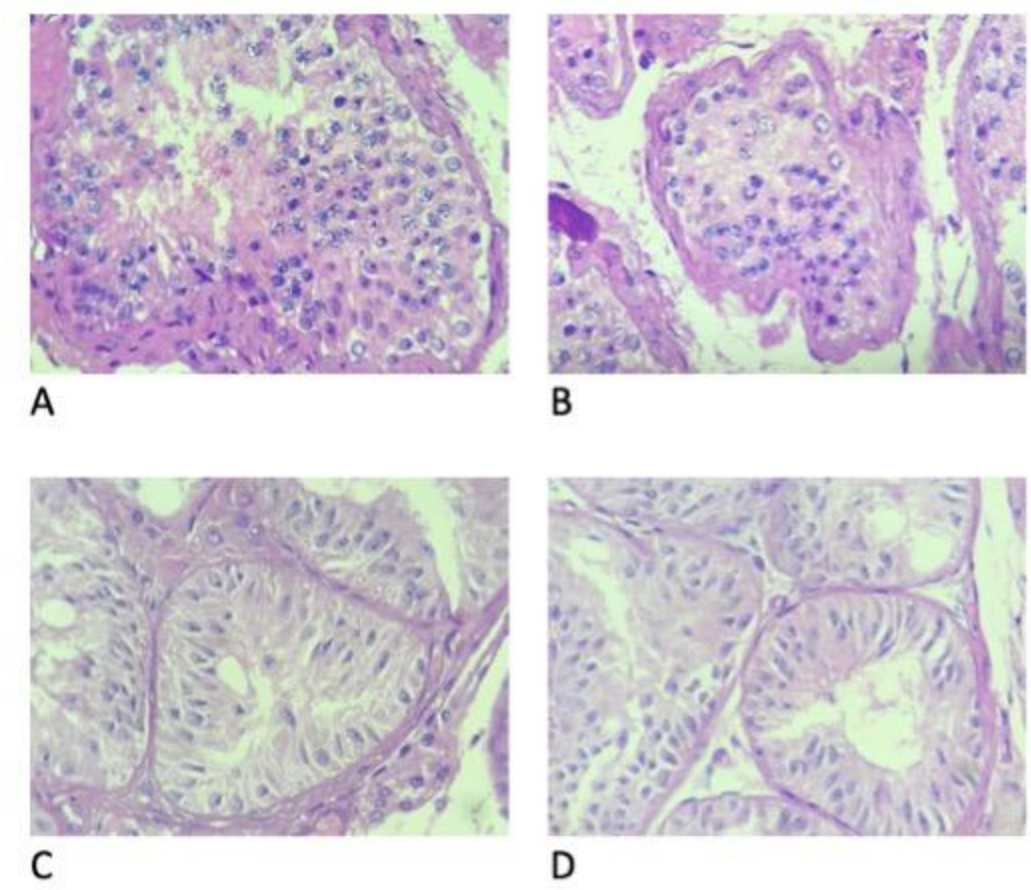
Damilare Adeyemi[1,2], Yinusa Raji[2]

[1]Osun State University, Osogbo, Nigeria, [2]University of Ibadan, Ibadan, Nigeria
Presented By: Damilare Adeyemi, MS

Introduction & Objective: Depression is on the rise globally, hence the increase in the use of antidepressants such as fluoxetine. Both depression and antidepressants have been implicated in the aetiopathogenesis of erectile dysfunction; however, the associated mechanism involved is still obscure. This study therefore, investigated the effects of reserpine-induced depression and fluoxetine treatment on erectile functions in new Zealand rabbits.
Methods: Twenty adult male rabbits of comparable weight were randomly allotted into four groups (n=5) and thereafter acclimatized for two weeks before the commencement of drug administrations. Group 1 (control) received distilled water at 1 ml/kg, group 2 was induced with depression using reserpine (1 mg/kg), group 3 was induced with reserpine and treated with fluoxetine (10 mg/kg), and group 4 received 10 mg/kg of fluoxetine only. All administrations were via oral gavage and lasted 28 days.
Results: Reserpine significantly reduced sucrose preference with a concomitant decline in movement towards open arm of the elevated pluz maze platform thereby suggesting the onset of depression. This was attenuated significantly with fluoxetine intervention. Despite fluoxetine effectively improved indices of depression, the indices of sexual behaviour were significantly altered in both depressed rabbits and those that received fluoxetine evidenced by the decline in mount and intromission frequencies and a concomitant increase in mount and intromission latencies. This was accompanied by a significant decline in serum testosterone and penile NO levels. In addition, penile levels of malondialdehyde, TNF-α, and IL-6 were significantly elevated while glutathione concentration and catalase activity declined significantly. The penile expression of pro-apoptotic protein, p63, was significantly increased while Bcl-2,, an anti-apoptotic protein, decreased significantly.
Conclusion:
Findings from this study revealed that both fluoxetine and reserpine-induced depression impaired sexual activity via androgen dysregulation, NO suppression and penile tissue apoptotic damage triggered by oxidative stress and inflammation.

Poster 37
FIRST REPORT OF MICROSCOPIC TESTICULAR SPERM EXTRACTION FOR ASSISTED REPRODUCTION IN 46,XY DIFFERENCES OF SEX DEVELOPMENT (DDS) CAUSED BY 5-ALPHA REDUCTASE TYPE 2 DEFICIENCY
Joao Cardoso, Marcello Cocuzza, Berenice Mendonça, Guilherme Wood
Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil
Presented By: Joao Cardoso, MD, MS

Introduction & Objective: The 46,XY differences of sex development (DDS) caused by 5-alpha reductase type 2 (5ARD2) presents with often bilateral undescended testes, otherwise normal internal reproductive structures, prostate hypoplasia and undervirilized male genitalia. It is one of the few DDS where fertility is possible, in a diverse clinical presentation, with scarce reported cases on assisted reproduction. Fertility potential, reproductive counseling and treatment depend on the clinical presentation of this DDS, especially testicular position and urethral anatomy. Potential influence of timing and modality of surgeries for hypospadias and cryptorchidism should be considered. We aimed to describe two cases where microscopic testicular sperm extraction (microTESE) was used in this population.
Methods: First report of assisted reproduction for two azoospermic 5ARD2 deficiency patients through microTESE.
Results: A 33-year-old male with bilateral orchidopexy, phalloplasty, and urethroplasty at age 9, with successful sperm retrieval but failed embryo development after intracytoplasmatic sperm injection. Testicular histology demonstrated late spermatogenic arrest. A 28-year-old male with bilateral orchidopexy, phalloplasty, and urethroplasty at age 25 with unsuccessful sperm retrieval. Testicular histology demonstrated Sertoli cell-only pattern.
Conclusion: The potential impact of the time between atypical genitalia procedures and orchidopexy on fertility should be highly considered. Nevertheless, microTESE is a technique that may be used to assist azoospermic patients in this population. Early orchidopexy and penile and urethral corrections should be considered key strategies to preserve fertility potential on 5ARD2 patients.



A/B Late maturation arrest, C/D Sertoli cell-only ; HE 400x

Poster 38

HOW TO ESTIMATE POST-THAWING SEMEN QUALITY? AN INITIAL REPORT

Ana Beatriz da Silva[1], Carla da Silva[1], Carolina Sanches[1], Mayara Rodrigues[2], Larissa Chiba[2,3], Jorge Hallak, Sr.[2,3,4,5], Joel Drevet[6], Juliana Pariz[1,2]

[1]Methodist University of Sao Paulo, SBC, Brazil, [2]Urology Division of the University of Sao Paulo Medical School, Sao Paulo, Brazil, [3]Androscience, Science and Innovation Center in Andrology and High-Complex Clinical and Andrology Research Laboratory, Sao Paulo, Brazil, [4]Department of Pathology, Reproductive Toxicology Unit, University of São Paulo Medical School, Sao Paulo, Brazil, [5]University of Sao Paulo Innovation Center, Sao Paulo, Brazil, [6]Université Clermont Auvergne, GReD Institute, Faculty of Medicine, Clermont-Ferrand, France

Presented By: Ana Beatriz Da Silva

Introduction & Objective: Sperm cryopreservation is a widely used method to store spermatozoa in liquid nitrogen. However, storage procedures still have low post-thawing recovery rates. Oxidative stress is the main cause of sperm damage, resulting in spermatozoa with low motility or inability to fertilize, even in samples considered normal before cryopreservation. Describe how the semen will behave in fertilization after cryopreservation is a challenge. Therefore, the objective of this study was to correlate the pre-cryopreservation parameters with the post-thawing results, especially in parameters that are predictors of successful fertilization.

Methods: This study evaluated 35 semen samples from voluntaries men from Methodist University of Sao Paulo. The initial analysis was performed on a fresh sample according to the World Health Organization, performing the seminal processing and was separated in aliquots to perform the slow-freezing cryopreservation. Reactive oxygen species (ROS) levels by chemiluminescence, glutathione peroxidase (GPx) levels by colorimetric test, and oocyte-sperm interaction test (hyaluronan binding test – HBA) were performed pre-cryopreservation and post-thaw. The cryosurvival rate was calculated using motility evaluation. The correlation was calculated by Pearson test (r), and $p>0.05$ was considered statistical significance.

Results: Subjects have mean of age = 26, 74 years-old, and standard deviation (SD)=7.78. Initial analysis in fresh samples presented total motility (%) = 47.71±26.26, progressive motility (%) = 8.83±12.78, ROS ($x10^4$ CPM) = 0.394±0.472, GPx ($x10^6$ GSH-Px/mL) = 0.032±0.021, HBA (%) = 49.97±30.29. Pre-cryopreservation sperm concentration demonstrated positive correlation with post-

thaw total motility (r=0.569, p<0.001), progressive motility (r= 0.418, p<0.012), cryosurvival (r=0.642, p<0.001), HBA (0.454, p=0.006) and ROS (r=0.549, p=0.003). Pre-cryopreservation progressive motility demonstrated positive correlation with post-thaw total motility (r=0.569, p<0.001), cryosurvival (r=0.677, p<0.001), and HBA (r=0.512, p=0.002). Pre-cryopreservation oocyte-sperm interaction (HBA) demonstrated positive correlation with post-thaw total motility (r=494, p=0.003), and GPx(r=0.358, p=0.035).
Conclusion: Pre-cryopreservation parameters determine sperm quality post-thaw. In addition, the antioxidant role of GPx demonstrates that initial samples with oxidative stress can present reduced sperm-oocyte interaction post-thaw. This study can help to decide the quantity of samples necessary for cryopreservation in diverse andrological situations.

Financial support: Fapesp 2019/18571-0

Poster 39

EFFECT OF CONVENTIONAL AND SUPPLEMENTED SPERM CRYOPRESERVATION IN REACTIVE OXYGEN SPECIES, GLUTATHIONE PEROXIDASE, AND INTERACTION OOCYTE-SPERM PARAMETERS OF ASTHENOZOOSPERMIC SPERM SAMPLES.

Carolina Sanches[1], Carla Pereira[1], Ana Beatriz da Silva[1], Mayara Rodrigues[2], Larissa Chiba[2,3], Jorge Hallak[2,3,4,5], Juliana Pariz[1,2]
[1]Methodist University of Sao Paulo, SBC, Brazil, [2]Urology Division of the University of Sao Paulo Medical School, Sao Paulo, Brazil, [3]Androscience, Science and Innovation Center in Andrology and High-Complex Clinical and AndrologyResearch Laboratory, Sao Paulo, Brazil, [4]Department of Pathology, Reproductive Toxicology Unit, University of São Paulo Medical School, Sao Paulo, Brazil, [5]University of Sao Paulo Innovation Center, Sao Paulo, Brazil
Presented By: Carolina Sanches, BS

Introduction & Objective: Asthenozoospermia is a seminal diagnostic of progressive motility <32%, but that suggest severe sperm alterations, such as reduced DNA, membrane and mitochondrial integrity, increased reactive oxygen species in seminal plasma, or even alterations in the cytoskeleton. These defects must be considered in cryopreservation processes. The aim of this study was to verify the effect of conventional and supplemented sperm cryopreservation in reactive oxygen species (ROS), glutathione peroxidase (GPx), and interaction oocyte-sperm parameters of asthenozoospermic sperm samples.
Methods: This prospective study included fourteen asthenozoospermic semen samples (progressive motility <32%) from male voluntaries between August 2021 and November 2022. Basic semen analysis was performed in the fresh sample and two aliquots (1ml after sperm processing) were cryopreserved by slow-freezing. One aliquot was cryopreserved with supplement substances: an antioxidant substance (2 mM melatonin) was added in the pre-cryopreservation solution, and stimulant substance (2 mM caffeine) was added in post-thaw samples. All aliquots were submitted before cryopreservation and after thawing to seminal analysis, ROS and GPx levels, and oocyte-sperm interaction test by hyaluronan binding assay. Sperm cryosurvival rate was calculated after slow-thawing. The Student's T-Test was used to compare means.
Results: The mean of age = 32.19 years-old; Standard Deviation (SD) = ±7.28. Initial samples presented total motility (%) = 71.71±8.57, progressive motility (%) = 18.36±6.73, ROS (%) = 0.810±0.70, GPx (GSH-Px/mL) = 0.107±0.01, HBA (%) = 59.50±15.94. Both non-supplemented (Control group) and SUP group demonstrated significant statistic reduction in total motility (p<0.001), progressive motility (p<0.001), GPx levels (p<0.001), HBA (p<0.001). In addition, was observed ROS reduction in CONT samples when compared with fresh samples (p=0.036). Comparing CONT and SUP groups, was observed progressive motility reduction on SUP group (p=0.011).
Conclusion: Previously studies have demonstrated that caffeine and melatonin addition in sperm samples forwarded to cryopreservation has proven to be a very effective and simple way to improve semen motility in post-thaw sperm, however, this effect did not be observed in asthenozoospermic semen samples because there was reduction in progressive motility in supplemented samples. We also suggest the inclusion of the sperm-oocyte interaction test in the pre-cryopreservation samples, so that the fertilization potential of the stored spermatozoa can be estimated and the number of cryopreserved samples can be determined.

Poster 40

DIAGNOSIS AND TREATMENT OF EXTREME FORMS OF MALE FACTOR INFERTILITY

Philip Xie, Stephanie Cheung, Zev Rosenwaks, Gianpiero Palermo
Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY, USA
Presented By: Philip Xie, BS

Introduction & Objective: Conventional semen analysis provides only limited information on male gametes and is insufficient in cases of rare sperm defects such as globozoospermia, stumped tail–caused dysplasia of fibrous sheath, or primary ciliary dyskinesia. Therefore, we aim to tailor ART treatment based on precise characterization of extreme forms of sperm defects using ultrastructural analysis with concurrent genomic sequencing.
Methods: In the past 3 years, we included 25 consenting men with a history of ART failure due to severe astheno-/teratozoospermia. To evaluate sperm ultrastructures, transmission electron microscopy (TEM, JEOL-1400) was performed on resin-embedded spermatozoa at 300,000X magnification. Sperm acrosome, nucleus, fibrous sheath, and axoneme were assessed.

Concurrent whole exome sequencing (WES) was performed on spermatozoal DNA to identify mutations contributing to the specific sperm ultrastructural defects.
Results: Three types of sperm ultrastructural defects were identified: globozoospermia, dysplasia of fibrous sheath (DFS), and primary ciliary dyskinesia (PCD). In 15 globozoospermic cases, 97–100% of the spermatozoa exhibited complete round heads, absence of acrosomes, and perinuclear theca deformities. Axonemal structures appeared normal. WES identified mutations on genes (*DPY19L*, *PICK1*, *SPATA16*, and *PIWIL1*) known for their role in spermatid elongation and spermiogenesis. Based on these findings, assisted oocyte activation was subsequently performed for 7 couples, which improved fertilization rate from 5.4% to 37.9% (P<0.00001), resulting in 6 clinical pregnancies of which 5 are ongoing or deliveries. In men with DFS (n=8), most spermatozoa (90%) were characterized by dysplastic fibrous sheaths and disorganized axonemal structures. WES identified mutations on genes (*AKAP4*, *SPAG16*, and *CATSPER1*) involved in flagellar development/function. Four patients underwent 5 subsequent ICSI cycles using the scarce motile spermatozoa identified, obtaining a 52.2% fertilization rate that yielded 3 pregnancies, all resulting in singleton births. In 2 patients with PCD, chaotic microtubular configuration and absence of outer dynein arms were observed in 90% of the axonemal cross-sections, confirmed by a *DNAH5* deletion. These two patients underwent 3 ICSI cycles using twitching spermatozoa identified after pentoxifylline treatment, yielding a 45.3% fertilization rate that generated 3 pregnancies, leading to 2 singleton and 1 twin births.
Conclusion: Ultrastructural analysis and diagnostic DNA sequencing can help pinpoint cellular abnormalities linked to sperm dysfunction corroborated by the identification of the responsible gene. Based on both phenotypical and genotypical findings, tailored treatment can yield successful outcomes for couples with extreme male infertility.

Poster 41
PROTEOMIC PROFILING OF EXTRACELLULAR VESICLES IN SEMINAL PLASMA WITH DIVERGENT QUALITY STATUS
Notsile Dlamini, Jean Feugang, Tina Nguyen, Olga Pechonova, Tibor Pechan
Mississippi State University, Starkville, MS, USA
Presented By: Notsile Dlamini, BS

Introduction & Objective: Infertility is a global health problem affecting 10% of couples, with half of these cases linked to males. Male infertility is a result of various intrinsic and extrinsic factors related to the male reproductive system affecting semen quality. However, current diagnostic tools are still unsatisfactory in identifying male infertility disorders. Therefore, we hypothesized that seminal plasma composition might reflect the divergent quality of the corresponding spermatozoa. We used the pig as the research model to address this issue by focusing on the extracellular vesicles (EVs) contents of boar semen, whose effects on sperm quality remains unfolded. This study aimed to investigate the proteomic content of seminal plasma-derived EVs (SP-EVs) isolated from accepted (good) or rejected (poor) boar semen.
Methods: Raw semen was collected (n=75) from sexually mature Duroc boars at a commercial stud (Prestage Farms, MS) for eight weeks. Raw semen samples were subjected to sperm motility and morphology analyses and classified as poor or good quality based on assessment criteria cut-offs (<70% and ≥70%, respectively). Semen samples were subjected to serial centrifugation to separately collect spermatozoa and seminal plasma (SP). Thereafter, SP was ultracentrifuged to isolate EVs (SP-EVs), followed by confirmations through transmission electron microscopy (TEM), dynamic light scattering (DLS), and immuno-western blotting. Six pools of extreme individual SP-EV samples (sixteen individual boar collections) were selected based on motility and morphology parameters (mean±2SEM). Samples were subjected to total protein extraction, trypsin digestion, and nanoLC-MS/MS and bioinformatic analyses. Significant differences were set for P<0.05.
Results: The isolated SP-EVs had round and spherical morphology, with 30 to 400nm diameter size distribution. They expressed specific protein markers, CD9, CD63, and CD81. A total of 364 and 325 SP-EV proteins were detected in poor and good semen, respectively. Differentially detected proteins belonged to cell surface and extracellular structures. They were associated with peptidase and heparin-binding and maintained structural molecules and endopeptidase activities. In addition, unique proteins found in good-quality SP-EVs were associated with sperm functionality, capacitation, and fertilization.
Conclusion: Our study identified proteomic variations between good-quality and poor-quality semen. Hence, these SP-EV proteins may contribute to the development of potential biomarkers of sperm fertility and therapeutic strategies for male infertility.

Poster 42
INCIDENCE AND ALLELIC HETEROGENEITY OF TEX15 VARIANTS IN MEN WITH SPERMATOGENIC FAILURE
Sidra Qureshi[1], Jimmaline Hardy[2], Christopher Pombar[2], Andrea Berman[3], Agnieszka Malcher[4], Tara Gringrich[2], Jannah Kuong[2], Sarah Munyoki[2], Kyle Orwig[2], Jawad Ahmed[1], Marta Olszewska[4], Maciej Kurpisz[4], Donald Conrad[5], Muhammed Jaseem Kahn[1], Alexander Yatsenko[2], Kathleen Hwang[6]
[1]Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan, [2]Magee-Womens Research Institute, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA, [3]Dietrich School of Arts and Sciences, University of Pittsburgh, Pittsburgh, PA, USA, [4]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland, [5]Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, USA, [6]School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA
Presented By: Christopher Pombar, MS

Introduction & Objective: Spermatogenesis is a complex process, requiring numerous testis-specific genes. Issues at any stage of the process could result in defects in sperm production and/or viability. Specifically, the function of many meiotic proteins encoded by germ cell specific genes are required for the maturation of haploid spermatids and normal spermatozoa that is necessary for fertilization. These proteins are also sensitive to small changes in the coding DNA. We have focused on a comprehensive genomic study of testis-expressed gene 15 (*TEX15*) in a NOA cohort. The TEX15 protein is believed to mediate double strand break repair during meiosis. Previously documented pathogenic *TEX15* variants have caused spermatogenic failure (SPGF) that ranged from oligozoospermia (low sperm) to nonobstructive azoospermia (no sperm) with meiotic arrest.
Methods: Using a whole genome approach, we examined a cohort of 1151 unrelated individuals with NOA and idiopathic SPGF. We performed *in silico* analysis of any variants in *TEX15* within this cohort, selecting individuals that had homozygous and/or compound/complex heterozygous variants that had a mean allele frequency of less than 1%.
Results: We identified 7 out of 1151 (0.6%) unrelated individuals who had *TEX15* variants. Six of these patients had multiple heterozygous variants. These variants included splice site, insertions/deletions (indels), and missense substitutions which some resulting in loss-of-function (LOF) effects. There was one homozygous missense substitution c.6835A>G (p.Ala2279Thr) which co-segregated with cryptozoospermia within a family case.
Conclusion: We performed an extensive study of sporadic and familial SPGF, identifying potentially damaging variants in *TEX15* in 0.6% of patients. This supports the notion of increased frequency of the TEX15 gene variants in SPGF. Such a rate is anticipated considering its genetic and allelic heterogeneity of such complex diseases such as male infertility.

Poster 43

A RETROSPECTIVE STUDY COMPARING THE OUTCOMES OF IN VITRO FERTILIZATION (IVF) CYCLES UTILIZING DONOR SPERM FROM VARIOUS SPERM BANKS.

Justin Bopp[1], Jennifer Matucha[1], Bradford Bopp[2], Matthew Will[2], Erica Anspach Will[2], Kathleen O'Leary[2], Glen Adaniya[1]
[1]Ovation Fertility Indianapolis, Carmel, IN, USA, [2]Midwest Fertility Specialists, Carmel, IN, USA
Presented By: Justin Bopp, BS

Introduction & Objective: The objective of this study was to compare fertilization and subsequent embryo development and ploidy rates from IVF cycles with preimplantation genetic testing (PGT) that utilized donor sperm from four sperm banks.
Methods: Cycle data was collected from 85 IVF cycles with PGT between January 2019 and August 2022. Oocytes underwent intracytoplasmic sperm injection (ICSI) with sperm from four donor sperm banks: A (N = 299), B (N = 213), C (N = 369) and D (N = 132), and 440 embryos were biopsied from these cycles. Cycle characteristics, including age, anti-muellerian hormone (AMH), number of oocytes retrieved per cycle, and post-sperm-thaw concentration and motility were analyzed by ANOVA, and differences were further examined by independent t-tests. Rates of fertilization, day 3 embryo development, blastocyst utilization and ploidy were analyzed by z-proportional tests to compare banks. A p-value less than 0.05 was defined as statistically significant, with a 95% confidence interval.
Results: No significant difference in age, AMH, number of oocytes retrieved, and post-thaw motility was observed between the different sperm bank cycles. However, there was a significantly higher post-sperm-thaw concentration from bank A compared to C (74.7 ± 35.8 vs. 45.2 ± 16.8; p<0.001). Fertilization was significantly lower in oocytes inseminated with sperm from bank A compared to banks B, C and D (75% vs. 82%; p=0.046, 75% vs. 84%; p=0.004, 75% vs. 87%; p=0.002, respectively). Day 3 embryo development and blastocyst utilization rates from bank A cycles were significantly lower than C (79% vs. 88%; p=0.041 and 52% vs. 61%; p=0.041, respectively). Embryo euploidy and aneuploidy rates were not significantly different between the donor banks. However, the rate of embryo mosaicism was significantly higher in cycles from bank C compared to D (17% vs. 7%; p=0.02).
Conclusion: Although sperm bank A had a significantly higher post-thaw sperm concentration, these cycles resulted in significantly lower fertilization, embryo progression and blastocyst development compared to bank C. These results indicate that sperm bank selection may impact IVF cycle outcomes and result in a lower number of usable embryos. Clinicians should consider monitoring donor sperm cycle outcomes to better advise patients.

Poster 44

RETROSPECTIVE LOOK INTO AN EIGHT-YEAR, SINGLE CENTER FERTILITY PRESERVATION PROGRAM INCORPORATING SPERMATOGONIAL STEM CELL AND ROUND SPERMATID TECHNOLOGIES.

Adam Cohen[1,2], Janmejay Hingu[1,2], Nima Zarandi[3], Guillermo Galdon[4], Omar Abdelaal[5], Banafsheh Nikmehr[6], Kimberly Stogner-Underwood[1], Stanley Kogan[1,2], Steve Hodges[1], Stuart Howards[1], Thomas McLean[1], Anthony Atala[1,2], Hooman Sadri-Ardekani[1,2]
[1]Wake Forest University School of Medicine, Winston-Salem, NC, USA, [2]Wake Forest Institute of Regenerative Medicine, Winston-Salem, NC, USA, [3]University of Pittsburgh Medical Center - Central Pa., Pittsburgh, PA, USA, [4]Universidad de Barcelona, Barcelona, Spain, [5]Zagazig University, Zagazig, Egypt, [6]Carolinas Fertility Institute, Winston-Salem, NC, USA
Presented By: Adam Cohen, MD

Introduction & Objective: Male infertility has been associated with many disease processes, including chemotherapy and radiotherapy for cancer, undescended testis (UDT), and genetic conditions such as Klinefelter Syndrome (KS). Preservation of human spermatogonial stem cells (SSC) may be a suitable option for young male patients at risk of losing fertility. With optimal

cryopreservation, cell viability can be retained to reestablish spermatogenesis in the future through in vitro germ cell differentiation or round spermatid injection.
Methods: Urology and hematology-oncology patients with malignancy, UDT, or KS were approached for consent and assent (if ≥ seven years old) for the fertility preservation program. If participation was accepted, patients were given the option to undergo a fertility-preserving testicular biopsy, preferably during general anesthesia for other procedures such as Port-a-Cath placement, orchidopexy, or lymph node biopsy. They were also allowed to enroll as a registry participant (non-biopsy control). Histological and microbiological analyses were performed on biopsies before being cryopreserved. Further data were obtained retrospectively via chart review.
Results: Over eight years of banking, a total of 202 patients were approached for the study, with 125 (61.8%) undergoing a biopsy, 39 (19.3%) enrolling as non-biopsy control, and 38 (18.8%) declining participation. The mean age at which biopsy was performed across all diagnoses was 8.39 years (range 0.61 to 37.39). Of the 125 biopsy patients, 73% opted to donate a portion (up to 20%) of the tissue for basic research with no direct benefit to the donor. Of the biopsied patients, 76 (60.8%) were performed for cancer, 38 (30.4%) for unilateral or bilateral UDT, and 11 (8.8%) for KS. Based on PRM1 immunofluorescence, 48.3% of specimens had round spermatids. Short-term complications for testicular biopsy, primarily pain and swelling, were typically mild to moderate.
Conclusion: A multidisciplinary approach can accommodate patients at risk for reduced future fertility due to various pathologies. The preservation of testicular tissue at present can aid in future fertility restoration through techniques such as in vitro SSC differentiation or round cell spermatid injection (ROSI).

Poster 45
THE INFLUENCE OF SILDENAFIL ON THE ENZYMES REGULATING TESTICULAR TESTOSTERONE BIOSYNTHESIS
Fotios Dimitriadis[1], Aris Kaltsas[2], Athanasios Zachariou[2], Charalampos Mamoulakis[3], Athanasios Papatsoris[4], Andreas Karagiannis[2], Evlalia Vlachopoulou[2], Athanasios Zikopoulos[2], Panagiota Tsounapi[5], Atsushi Takenaka[5], Nikolaos Sofikitis[2]
[1]Department of Urology, Aristotle University of Thessaloniki, Thessaloniki, Greece, [2]Laboratory of Spermatology, Department of Urology, University of Ioannina, Ioannina, Greece, [3]Department of Urology, Medical School, University of Crete, Heraklion, Greece, [4]2nd Department of Urology, Sismanogleion General Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, [5]Department of Urology, Faculty of Medicine, Tottori University, Yonago, Japan
Presented By: Fotios Dimitriadis

Introduction & Objective: We evaluated the effect of sildenafil on the activity of the enzymes playing a role in testicular testosterone biosynthesis in rats with primary testicular damage.
Methods: Group A included eight Wistar rats (8-week-old). Groups B and C included another 10 and 10 rats, respectively, of the same age. Left cryptorchidism had been surgically induced in each rat of groups B and C at the age of 4-week- old. A group D of rats (n=10) of the same age did not receive any pharmaceutical treatment. From the age of 8-week-old, all rats of groups A and B were administered sildenafil (30mg/Kg/day) for six weeks in the drinking water. In contrast rats of group C and D did not receive any pharmaceutical treatment during the subsequent six-week-period. At the end of the experimental period, each rat of all groups was 14-week-old and was killed. Left testes were collected and intratesticular testosterone content (ITC) (Biol Reprod 1987, 36: 933) and androgen-binding protein (ABP) (J Urol. 1996;156:267) activity in testicular cytosols were assessed. In addition, the enzymatic activity of 17α-hydroxylase, 17,20-desmolase, and 17β-hydroxysteroid dehydrogenase playing a role in the Δ4 pathway of testicular testosterone biosynthesis was assessed in the left testis of each rat (Biol Reprod 1987, 36: 933). Analysis of variance plus Duncan's test was used for statistical analysis. A probability $P<0.05$ was considered to be statistically significant.
Results: Mean left ITC (ng/g testis; mean±SD) was significantly smaller in group C (56±16) than in group A (156±22), in group B (90±18) and in group D (142±26). Left ABP activity (pmol/mg protein) was significantly larger in group A (0.36±0.05), in group B (0.27±0.05), and in group D (0.31±0.07) than in group C (0.18±0.06). Mean activity of 17,20-desmolase (nmol of substrate/time/testicular weight) and mean activity of 17β-hydroxysteroid dehydrogenase (nmol of substrate/time/testicular weight) in the left testis was not significantly different among groups A, B, C, and D. In contrast mean activity of 17α-hydroxylase (nmol of substrate/time/testicular weight) was significantly larger in groups A (106±9), B (92±10) and D (98±11) than in group C (73±15).
Conclusion: Sildenafil administration in rats with left cryptorchidism enhances the activity of 17α-hydroxylase stimulating Leydig and Sertoli cellular function. The guanylate cyclase/cGMP second messenger plays a role in testicular testosterone biosynthesis.

Poster 46
COMPARISON OF MICROSURGICAL TESE AND CONVENTIONAL TESE IN MEN WITH NON-OBSTRUCTIVE AZOOSPERMIA AND SERTOLI CELL-ONLY SYNDROME
Aris Kaltsas[1], Fotios Dimitriadis[2], Athanasios Zachariou[1], Charalampos Mamoulakis[3], Athanasios Zikopoulos[1], Andreas Karagiannis[1], Evlalia Vlachopoulou[1], Athanasios Papatsoris[4], Panagiota Tsounapi[5], Atsushi Takenaka[5], Nikolaos Sofikitis[1]
[1]Laboratory of Spermatology, Department of Urology, University of Ioannina, Ioannina, Greece, [2]Department of Urology, Aristotle University of Thessaloniki, Thessaloniki, Greece, [3]Department of Urology, Medical School, University of Crete, Heraklion, Greece, [4]2nd Department of Urology, Sismanogleion General Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, [5]Department of Urology, Faculty of Medicine, Tottori University, Yonago, Japan
Presented By: Fotios Dimitriadis

Introduction & Objective: The objective the current study was to compare two methods of testicular sperm retrieval (SR) in men with non-obstructive azoospermia (NOA) and a histological pattern of Sertoli cell-only syndrome (SCOS). The histological pattern influences the SR rate. Individuals diagnosed with SCOS have a lower SR rate than those diagnosed with maturation arrest and hypospermatogenesis. Microsurgical testicular sperm extraction (mTESE) and non-magnified conventional testicular sperm extraction (cTESE) were evaluated and compared in this study.
Methods: A retrospective analysis was performed in 452 male patients diagnosed with NOA who underwent surgery for SR at the Department of Urology of the University of Ioannina between January 1, 2013, and December 31, 2021. Two semen samples from each participant were centrifugated before surgery. The sediment was microscopically observed to confirm the presence of NOA. A number of men (n=338) underwent mTESE and an additional number of 114 underwent cTESE. A part of the testicular tissue biopsy material was processed for cryopreservation (therapeutic testicular biopsy). The remaining part of the recovered testicular tissue was processed for hematoxyline-eosin stain (diagnostic testicular biopsy).
Results: Spermatozoa were found in 207 out the 452 males operated (45.8%). Furthermore, spermatozoa were demonstrated in 165 men who underwent mTESE (SR rate=48.8%). In addition, spermatozoa were found in 42 men (SR rate=36.8%) who underwent cTESE. SCOS was demonstrated in 62 males out the 114 NOA males who underwent cTESE and in 203 males out the 338 who underwent mTESE. The SR rate was 35.9% in NOA-men with SCOS who underwent mTESE but it was 12.9% in NOA-men with SCOS who underwent cTESE. This difference is statistically significant (chi-square test; $P<0.05$).
Conclusion: In males with NOA and the histological diagnosis of SCOS, mTESE has been shown to result in superior SR rates than cTESE.

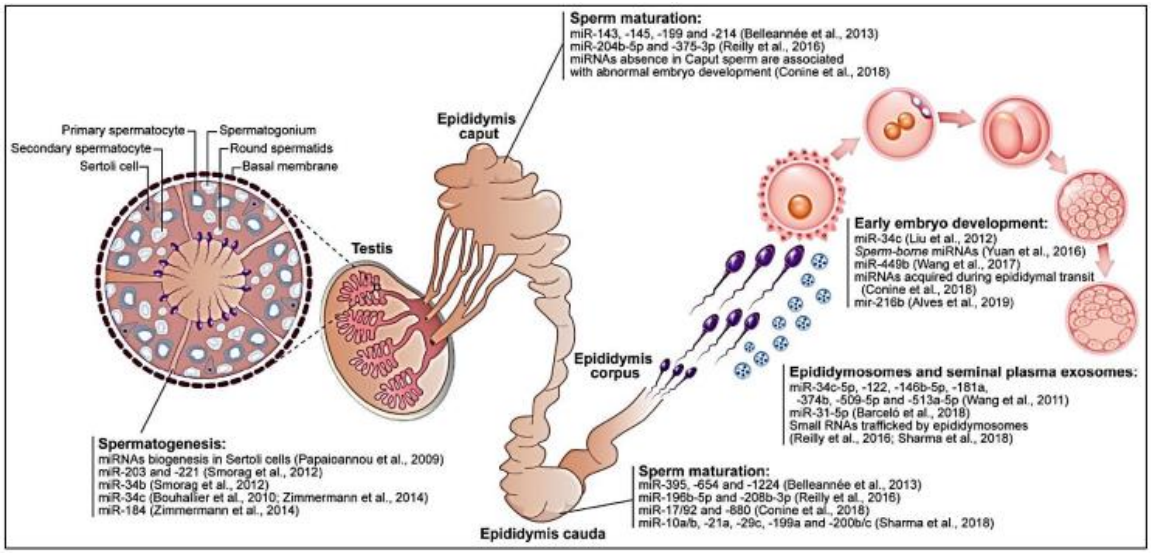
Poster 47
PRE-FERTILIZATION GENETIC TESTING FOR HEALTHY GAMETE SELECTION IN A MURINE MODEL OF MARFAN SYNDROME
Ashley Aluko[1,2], Philip Xie[1,2], Gianpiero Palermo[1,2], Zev Rosenwaks[1,2]
[1]Weill Cornell Medicine, New York, NY, USA, [2]Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, NY, USA
Presented By: Ashley Aluko, MD

Introduction & Objective: For patients with heritable genetic conditions seeking parenthood, preimplantation genetic testing for monogenic disorders (PGT-M) allows us to select non-affected conceptuses for transfer. However, PGT-M is inherently inefficient and can lead to embryo wastage. We propose a protocol for gamete pre-fertilization genetic testing to prevent vertical transmission of disease alleles in the paternal germline. Using a Marfan syndrome mouse model, we aim to generate haploid androgenotes and prioritize those with the unaffected gene (*Fbn1*) to be used as pseudo-gametes for IVF.
Methods: A PCR primer for the *Fbn1*^{tm1Hcd} mutation in B6 mice was developed and validated using tail clippings and embryo biopsy. Spermatozoa from heterozygous Marfan syndrome mice were used to inseminate enucleated wildtype mouse oocytes, yielding haploid androgenetic embryos. These haploid androgenetic embryos were allowed to progress to the 8-cell stage. Subsequently, a single blastomere was removed for genotyping, while the remainder were individually grafted into activated recipient wildtype oocytes and fused using inactivated Sendai virus to produce biparental conceptuses. Resulting diploid conceptuses were cultured in a time-lapse system then genotyped to confirm the desired paternal haplotype.
Results: 140 oocytes were enucleated (survival rate of 95.7%) and inseminated with mutant spermatozoa, yielding 114 (85.1%) monopronucleated male haploid constructs. In total, 82 (71.9%) haploid androgenetic embryos progressed to the 8-cell stage, of which 46% carried the *Fbn1* wildtype healthy allele. Individual haploid blastomeres from five *Fbn1* wildtype androgenetic embryos were isolated and grafted into recipient oocytes to generate biparental conceptuses with the desired paternal haplotype. A total of 20 biparental conceptuses were produced; 18 underwent successful syngamy and embryo cleavage. After 96 hours in culture, 55.6% (10/18) reached the blastocyst stage. We confirmed that these blastocysts carried the healthy genotype and matched the original paternal haplotype.
Conclusion: We were able to generate 8-cell embryos containing haploid androgenotes in a murine model of Marfan syndrome. These pseudo-gametes were not only capable of supporting full preimplantation development, but were also genotyped for healthy gamete selection. This ultimately led to the production of blastocysts containing the *Fbn1* ^{tm1Hcd} wildtype allele. In summary, our protocol for pre-fertilization genetic testing identifies healthy male gametes, void of specific transmissible disease mutations, for use in IVF.

Poster 48
ROLE OF MICRO RNA-145 IN ASSESSMENT OF NON-OBSTRUCTIVE AZOOSPERMIA
Mohamed Abdelwahed, Rashad Mostafa, Mohamed Mohamed, Hoda Abdallah
Faculty of medicine, Suez Canal University, Ismailia, Egypt
Presented By: Mohamed Abdelwahed, MBBS, MS

Introduction & Objective: MicroRNAs (miRNAs) are a major area of interest within the field of non-invasive diagnostic biomarkers of diseases. They are small non-coding single stranded RNA molecules. miRNA expression patterns have been related to different spermatogenic impairments in patients with azoospermia. In this study we assessed the role of miRNA-145 in non-obstructive azoospermia (NOA).

Methods: This prospective case-controlled study included 48 infertile men with NOA (as a case group) and 48 men with normal semen (as a control group). Semen samples were collected, and then quantitative real-time PCR was used to identify miRNA-145 and assess its expression levels in seminal plasma of both groups.
Results: There was a significant increase in the expression levels of miRNA-145 in NOA cases regarding fold change (FC) than controls (p=0.024), and in cycle threshold (Ct) (p= 0.003). There was also a significant relationship between miRNA-145 FC and TESE results in NOA cases (p=0.0479), with a high expression level of miRNA-145 in TESE -ve NOA cases which strongly support the role of miRNA-145 in sperm maturation.
Conclusion: miRNA-145 expression level in semen was increased in NOA cases group. Being 1.38 and their expression levels in the control group was 1.0 which may provide a new approach for diagnosing male infertility.



contribution of miRNAs in the reproductive events

Poster 49
IMPACT OF YOGA ON HARMONIZATION OF SPERM GENOME AND EPIGENOME IN INFERTILE MEN
Rajesh Kumar, Dibakar Borthakur, Rima Dada
All India Institute of Medical Sciences New Delhi, New Delhi, India
Presented By: Rajesh Kumar, MBBS, MD

Introduction & Objective: Male infertility is a common and complex lifestyle disorder. Half of the cases of male infertility are of unknown etiology. The idiopathic and unexplained male infertility is attributed to environmental factors, oxidative stress (OS) due to elevated reactive oxygen species (ROS), or genetic-epigenetic abnormalities etc. Unchecked and supraphysiological levels of OS can cause seminal oxidative DNA damage and aberrant sperm DNA methylation with the end result of decline in semen quality. *MTHFR* being a key regulatory enzyme of one carbon metabolism pathway, ensures steady supply of universal methyl donor the S-adenosyl methionine (SAM) for methylation reactions. It also replenishes the master antioxidant glutathione having a pivotal role in rescuing sperm in stress. OS damages all biomolecules including mitochondrial and nuclear genome. The most common type of endogenous DNA damage is base modification which is handled by the base excision repair (BER) pathways in sperms. Yoga based lifestyle intervention (YBLI) has proven benefits of reducing OS and positive modulation of gene expression profile. We aimed to study the impact of 3 weeks of supervised YBLI on key genes of the one carbon and BER pathways.
Methods: The present study represents a portion of a prospective exploratory longitudinal project and 75 infertile men were recruited. The participants had undergone a supervised yoga session of 1 hour per day. Obtained blood and semen samples were analyzed at baseline and at the end of yoga intervention (21 days). Analysis of gene expression was performed by quantitative polymerase chain reactions on spermatozoal *MTHFR, OGG1, APE1, XRCC1* and *PARP*. The seminal OS was assessed indirectly by measuring ROS by chemiluminescence.
Results: After 3 weeks of adoption of YBLI, the genes *MTHFR, OGG1, PARP1, XRCC1, SOX3* exhibited up-regulation and *APE1* exhibited down-regulation (Table1). There is significant reduction of OS ($p<0.05$) in the infertile men post intervention (Table2).
Conclusion: YBLI in infertile men up-regulates *MTHFR* expression, up-regulates BER pathway genes, decreases OS and can improve prognosis. Adoption of this simple lifestyle intervention - can improve sperm DNA quality and may lower incidence of idiopathic male factor infertility. YBLI may be beneficial and can be used as adjunctive therapeutic intervention in infertile men.

Genes	ΔCt (0 Day)	ΔCt (21 Day)	$2^{-\Delta\Delta Ct}$	Axis fold change(AFC)
OGG1	4.8 ± 1.18	3.9 ± 1.34	3.4	1.67
PARP1	-0.431319	-0.460888	1.020707148	1.020707148
XRCC1	1.54697	0.689534	1.811815436	1.811815436
APE1	3.167391	3.753089	0.66632688	-1.500764909
SOX3	7.1 ± 2.68	6.6 ± 2.6	3.67	1.33
MTHFR	1.57120	0.617115	-0.95409	5.517974

Table 1: Relative expression (average ΔCt) of OGG1, PARP1, XRCC1, APE1, SOX3 & *MTHFR* with respect to β-*ACTIN* and *GAPDH* in blood samples of infertile men and AFC in the gene expression post-yoga with respect to pre-yoga. Values are expressed as mean (n=75). GOI, genes of interest; AFC, axisfold change.

Characteristics	Baseline	3 wks	P Value
Oxidative Stress			
ROS(RLU/min/10^4 neutrophils)	36.47 (4.97 to 151.37)	21.19 (0.92 to 109.14)	<0.05

Table 2: Change in Reactive oxygen species (ROS) in infertile men participating in YBLI (n=75). NOTE: Values of the Control Group not shown

Impact of yoga on sperm genome and epigenome

Poster 50

A NOVEL SPERM SELECTION TECHNIQUE TO YIELD EMBRYOS OF THE DESIRED SEX

Stephanie Cheung, Philip Xie, Zev Rosenwaks, Gianpiero Palermo
Weill Cornell Medicine, New York, NY, USA
Presented By: Stephanie Cheung, MS

Introduction & Objective: To use a novel sperm selection technique to increase rates of embryos and offspring of the desired sex for couples undergoing ICSI with preimplantation genetic testing for aneuploidy (PGT-A).

Methods: Over 6 years, 1,370 couples undergoing ICSI with PGT-A (n=1,261) and sex selection (PGT-A/GS, n=109) were included in this study. Standard sperm processing was performed for the PGT-A cohort, while a proprietary sex selection technique (SST) was used for the PGT-A/GS group to enrich spermatozoa for the desired sex (IRB 1306014043, NCT 05500573). To confirm sex enrichment, ≥1,000 spermatozoa were screened by fluorescent in situ hybridization. The proportion of male and female spermatozoa, PGT-A results, and ICSI outcomes were compared between the two cohorts.

Results: Couples (n=1,261) in the PGT-A cohort (maternal age, 37.1±4yrs; paternal age, 39.1±6yrs) underwent 2,356 ICSI cycles, yielding an 80.9%(14,830/18,321) fertilization. PGT-A results confirmed that 46.6% (n=760) of embryos were female and 53.4% (n=872) were male. After transfer of a frozen/thawed euploid embryo (FET), couples achieved a 76.3%(725/950) implantation and 64.9%(617/950) clinical pregnancy rate resulting in 569 healthy deliveries (48% female, 52% male).

From the study cohort (n=109), those (n=60) who desired female offspring (maternal age, 37.9±4yrs; paternal age, 40.8±6yrs) obtained an 81.6% sperm sex enrichment. They underwent 74 ICSI cycles and achieved a 77.6%(592/763) fertilization rate, resulting in a greater proportion of female embryos than the control (78.1%, 235/301, P<0.05). Of those, 78.7%(185/235) were euploid. Thus far, after FET, couples (n=29) obtained a 79.3%(23/29) implantation rate, with 16 healthy female singletons, all developing normally.

Couples (n=49) (maternal age, 37.6±3yrs; paternal age, 40.8±5yrs) preferring male offspring obtained an 80.8% sperm sex

enrichment. They underwent 53 ICSI cycles and achieved a 74.7%(481/644) fertilization rate with a greater proportion of male embryos (231/292, 79.1%) compared to the control (P<0.05). Of those, 66.2%(153/231) were euploid. Currently, the couples' (n=22) implantation rate is 90.9%(20/22), yielding 14 healthy male singletons, all developing normally.
Conclusion: SST was able to yield, through the selection of spermatozoa, a statistically significant higher proportion of conceptuses of the aspired sex without affecting embryo euploidy rate. For couples who underwent FET, embryo developmental competence, delivery rates, and offspring health were not impaired by using spermatozoa processed by SST. These encouraging findings support SST efficacy and safety, rendering it effective as well as ethically palatable.

Poster 51
MALE INFERTILITY AN EARLY BIOMARKER OF CANCER: THE POSSIBLE CAUSES AND IMPACT OF YOGA
Anjali Yadav, Manoj Kumar, Kalpana Luthra, Rajeev Kumar, Richa Mishra, Rima Dada
All India Institute of Medical Sciences (New Delhi, India, 110029), New Delhi, India
Presented By: Anjali Yadav, PhD

Introduction & Objective: Infertility affects about 15% of couples and ~50% of cases are due to the male factor. The incidence of gonadal & extra-gonadal tumors in infertile men is associated with poor semen quality, oxidative stress (OS), oxidative DNA damage (ODD), shortened telomere length (TL) & OS-induced genome-wide hypo-methylation. This exerts genome instability & hyper-mutability by the accumulation of DNA adduct (8-OHdG) and dysregulation of genomic integrity, tumor suppressor genes & oncogenes that enhance the risk of cancer. As infertility is a psychosomatic disease, induced by lifestyle factors, psychological stress, and unhealthy social habits, an alternative approach like mind-body intervention i.e. yoga is needed. This study evaluates the impact of yoga on male infertility-associated cancer biomarkers (OS, sperm DNA damage, genome-wide methylation and sperm TL).
Methods: We enrolled an equal number (25) of cases and controls (age: 25-45yrs). Seminal reactive oxygen species (ROS) level was measured using Luminometer, 8-OHdG, global methylation (5mC) & hydroxyl-methylation (5hmC) levels by ELISA and ODD by SCSA. The sperm TL and selected genes expression analysis was done using Q-PCR after the Yoga intervention (45 days).
Results: ROS levels were significantly higher ($p<0.001$) in infertile men (45.3RLU/sec/million sperm) as compared to controls (18.9RLU/sec/million sperm), however, post-yoga group (16.8RLU/sec/million sperm) showed significant reduction. The DFI in the infertile group was higher (37.2%) than in controls (26.1%) ($p<0.001$) however, it reduced in the Post-Yoga group (36.8%) ($p<0.071$). The TL of infertile men was found to be shortened (T/S ratio=0.59) as compared to HC (T/S ratio=0.68) ($p<0.004$), which increases after Yoga intervention (0.65±0.09). The levels of 8-OHdG were higher in the infertile group (86.02pg/ml) as compared to HC (34.18pg/ml) while, it was significantly decreased (66.47pg/ml; $p>0.05$) post-yoga. Yoga intervention increases the percentage of 5mC & decreases the levels of 5-hmC. The expression level of various genes maintaining genomic integrity is being done to confirm the expression levels.
Conclusion: Yoga reduced OS, ODD, increases global methylation and maintains sperm TL which improves genomic integrity and overall health. In our ongoing studies, we planned to evaluate the effect of yoga on cell proliferation of cancer cell lines and would delineate the risk of cancer in infertile males.

Poster 52
TRANSCRIPTOMIC PROFILING OF SEMINAL PLASMA AS A POSSIBLE BIOMARKER TO PREDICT SUCCESSFUL TESTICULAR SPERM RETRIEVALS IN NOA MEN
Lily Ng, Philip Xie, Stephanie Cheung, Zev Rosenwaks, Gianpiero Palermo
Reproductive Medicine, Weill Cornell Medicine, New York, NY, USA
Presented By: Lily Ng, BS

Introduction & Objective: To predict successful testicular sperm retrieval using epigenetic profiling of seminal plasma in men with idiopathic non-obstructive azoospermia (iNOA).
Methods: Twenty three men with iNOA were deemed azoospermic after repeated extensive semen analyses and were categorized according to whether spermatozoa were retrieved (+Sperm) or not (-Sperm) at micro-TESE. Gene expression profiling by RNAseq using Illumina HiSeq platform at 2x150bp was carried out on the cell-free seminal plasma from these men in comparison to a fertile donor control and among the two cohorts. An absolute log2fold change of >1 and a P-value of <0.0005 were considered significant.
Results: The clinical outcome of our iNOA study population was assessed. The +Sperm cohort (n=11) underwent 23 ICSI cycles with their female partner (36.6±8yrs) that yielded a fertilization rate of 33.3% (72/216) and a clinical pregnancy rate of 33.3% (6/18). Some couples from the +Sperm group (n=3) and all from the -Sperm cohort (n=12) that failed to achieve a pregnancy, considered alternative forms of reproductive treatment.
All 11 individuals in the +Sperm cohort (39.0±12yrs) had DEG profiles that revealed 13 imbalanced genes involved in spermatogenesis (n=8) and sperm function (n=5).
For the 12 men in the -Sperm cohort (34.3±5yrs), RNAseq identified 12 gene imbalances involved in spermatogenesis (n=5), sperm function (n=3), sperm maturation (n=1), and cell cycle regulation (n=3).
When DEG profiles were compared between the two cohorts, eight imbalanced genes were identified that were common to both iNOA groups. IGSF11-AS1, expressed in the testis and implicated in spermatid development, was consistently underexpressed in

all men in the -Sperm group. TPTE2, a testis-specific gene regulating spermatogenesis, was overexpressed in 81.8% (9/11) of the individuals in the +Sperm cohort and conversely underexpressed in the –Sperm group.
Most interestingly, we identified one gene, NEU1, involved in acrosome development and fertilization, that was clearly overexpressed in all individuals of the +Sperm group, yet consistently underexpressed in the entire -Sperm group.
Conclusion: Transcriptomic profiling of the semen ejaculate of azoospermic men serve as a non-invasive biomarker to predict the residual spermatogenesis in iNOA men. Once verified, our findings may help to identify those men to be exposed to anesthesia and surgical risks. This would help to mitigate patient emotional and financial distress and not least, implications for third party payers.

Poster 53
USING SPERM EPIGENETICS (SPERMQT) FOR QUALIFICATION OF NEW SPERM DONORS FOR IUI
Kristin Brogaard[1], Fredik Andreasson[2], Angelo Allard[2], Ryan Miller[1]
[1]Inherent Biosciences, Salt Lake City, UT, USA, [2]Seattle Sperm Bank, Seattle, WA, USA
Presented By: Kristin Brogaard, PhD

Introduction & Objective: Currently there is a severe shortage of sperm donors driving increased prices and use of unregulated sperm donations . The shortage is the result of multiple factors including globally declining semen parameters and strict FDA regulations. For use of donated sperm in an intrauterine insemination (IUI), sperm banks are largely restricted to utilize donors that have ≥10 million total motile count (TMC) post thaw. This cutoff reduces the number of sperm donors, and is not predictive of actual live births . This study is an initial analysis of using the Sperm Quality Test (SpermQT) to identify quality sperm in combination with lower TMC, with the ultimate objective of qualifying more sperm donors for use in IUI.
Methods: Seattle Sperm Bank provided InherentBio 44 semen samples from 32 men for epigenetic analysis using SpermQT. SpermQT measures epigenetic stability within 1233 gene promoters that have been shown to be crucial for sperm quality. 20 semen samples were from potential donors, and 20 samples from 10 qualified donors. For the 10 qualified donors, the two samples were collected on different dates, where the first and second samples could be an IUI (≥10M TMC) or ICSI (< 10M TMC) quality sample. 4 semen samples were from 2 donors and were from the same collection date to assess assay replicability. SpermQT results and the number of unstable promoters were assessed.
Results: For the potential donors, 5% had an Excellent SpermQT, 84% had a Normal, and 11% had a Poor result. There was no correlation with the number of epigenetically unstable promoters and the post-thaw TMC, R2=0.003. All potential donor samples with a TMC <10M had a normal SpermQT result. In the IUI donor group (N=10), 0% had an excellent result, 90% had a normal result, and 10% had a poor result. In the ICSI donor group (N=12) 8.3% had an excellent result 91.7% had normal results, and 0% had a poor result. 100% of the ICSI donors were considered epigenetically normal. 20% of donors had significant changes in the number of unstable promoters between collections, with 1 donor having lifestyle changes.
Conclusion: Initial analysis of epigenetic sperm quality of qualified and potential donors from Seattle Sperm Bank suggests a new method to qualify sperm donors and address the global shortage of sperm donors.

Poster 54
UNILATERAL VERSUS BILATERAL VASO-EPIDIDYMAL ANASTOMOSIS FOR IDIOPATHIC OBSTRUCTIVE AZOOSPERMIA: A RANDOMIZED CONTROLLED TRIAL
Manoj Kumar, Nishant Gurnani, Ritesh Goel, Rima Dada, Rajeev Kumar
All India Institute of Medical Sciences (AIIMS), New Delhi, India
Presented By: Manoj Kumar, MBBS, MS

Introduction & Objective: Men with idiopathic obstructive azoospermia (OA) are candidates for surgical reconstruction with a vasoepididymal anastomosis (VEA) performed on one or both testis. There are no randomised trials comparing the success of unilateral versus bilateral VEA. We conducted a randomised trial comparing the two surgical options.
Methods: Between April 2017 and March 2022, men with infertility due to idiopathic OA were randomised to unilateral (Group 1) or bilateral (Group 2) VEA in an ethics committee approved clinical trial registered with the Clinical Trials Registry. The primary outcome was successful surgery defined as appearance of sperms in the ejaculate, evaluated at 3 monthly intervals after surgery. Additional outcomes were pregnancy rates and complications between the two groups. Men with successful surgery were compared with those without patency to identify predictors of success.
Results: 54 men fulfilled the criteria and 52 who completed follow-up were included in the analysis. Accrual did not reach the planned number of 70 subjects due to the COVID-19 pandemic. Success rate and spontaneous pregnancies were higher in men with bilateral surgery (46%, 3) than unilateral surgery (27%, 0) but were not statistically significant. Both groups had similar complication rates. Although bilateral surgery and presence of sperms in epididymal fluid were higher in men with patency, these were not statistically significant.
Conclusion: Bilateral VEA was associated with higher patency and spontaneous pregnancy rates compared to unilateral surgery. Although the results were not statistically significant due to the underpowered study, they suggest a clinically significant outcome.

Poster 55

UTILIZATION OF ZYMŌT WITH BACKUP EXTENDED SPERM SEARCH AND MICROFREEZE (ESSM) IN SEVERE OLIGOSPERMIA- RELIABLY AND QUICKLY FINDS ADEQUATE, PROGRESSIVELY MOTILE SPERM

Chaya Rothschild, Tristan Charran, Michael Werner
Maze Sexual and Reproductive Health, New York, NY, USA
Presented By: Chaya Rothschild, BS

Introduction & Objective: Severe oligozoospermia and cryptozoospermia are characterized by sperm count fluctuations that may result in the inability to detect viable sperm for intracytoplasmic sperm injection (ICSI) on day of oocyte retrieval. The ability to reliably find sperm in these specimens, including freezing in advance, would prevent the need for egg freezing or cycle cancellation. It would also dramatically decrease the time spent searching for sperm on the day of retrieval. We evaluated whether using Zymōt in place of density gradient centrifugation for specimen preparation would consistently yield sufficient high-quality sperm to proceed with ICSI without the need for extended search.

Methods: Any patient that presented with a sperm count that was detectable by standard semen analysis was considered for inclusion. Samples with motility >0 (the observation of at least one motile sperm in the neat specimen) but with a total sperm concentration below 1 M/mL (0.0002M/mL - 0.7M/mL, n=20) or motility below 1% (n=2) were processed by ZyMōt Multi 850µL using the standard protocol. The remainder of the ejaculate was processed by density gradient. If the sperm concentration after ZyMōt was <1 sperm per µL, the sample was centrifuged at 300g for 5 minutes and resuspended in 100µL. If the final concentration was still <1 sperm per µL, the sample was subjected to ESSM (plating the pellet in microdroplets and systematically searching.) The majority of these samples were vitrified on SpermVD to be available in the event that the fresh sample on day of retrieval did not yield sufficient sperm. A total of 22 samples were analyzed.

Results: Every sample yielded progressive sperm through the ZyMōt. 17 samples had observable progressively motile sperm in the first 5µL droplet evaluated and were vitrified as suspensions (200-600 sperm). The remaining 5 samples yielded 6-65 progressively motile sperm after extended search.

Conclusion: The use of ZyMōt allowed for more samples with severely limited sperm concentrations to be usable for ICSI without extensive searching. It also significantly reduced the time spent per sample in isolating individual sperm from sample debris and immotile sperm. When combined with ESSM, motile sperm were found in all cases despite extremely low initial concentrations. In our experience this protocol dramatically increases the availability of sperm in these challenging cases, while simultaneously significantly decreasing the time spent per specimen.

Poster 56

PATERNAL CONTRIBUTION TO EMBRYO PLOIDY

Olena Kocur, Philip Xie, Stephanie Cheung, Gianpiero Palermo, Zev Rosenwaks
The Ronald O. Perelman & Claudia Cohen Center for Reproductive Medicine of Weill Cornell Medicine, New York, NY, USA
Presented By: Olena Kocur, BA

Introduction & Objective: To identify and ameliorate a paternal contribution to embryo aneuploidy.

Methods: From 2018-2022, we identified couples who underwent Intracytoplasmic Sperm Injection (ICSI) cycles utilizing spermatozoa processed by density gradient (DG), that resulted in a high incidence of embryo aneuploidy at preimplantation genetic testing-aneuploidy (PGT-A) and poor clinical outcome. Male partners consented to sperm chromatin fragmentation (SCF) assessment by TUNEL (≥500 spermatozoa, ≤15% normal threshold). Neutral comet was used to measure specifically double-stranded DNA (dsDNA) breaks (≥200 spermatozoa, ≤3% normal threshold). Based on an elevated SCF, these couples underwent subsequent ICSI/PGT-A cycles with microfluidic sperm selection (MFSS). Total SCF, dsDNA breaks, embryo aneuploidy, implantation, clinical pregnancy, delivery, and pregnancy loss were compared between DG and MFSS processing.

Results: A total of 57 couples (maternal age: 36.5±5, paternal age: 40.9±6) underwent 71 ICSI/PGT-A cycles, with sperm parameters of 2.0±1 mL volume, 36.7±32x10^6/mL concentration, 34.5±15% total motility, and 2.0±1% normal morphology, and once processed by DG, became 0.5±0 mL volume (P<0.001), 12.8±11x10^6/mL concentration (P<0.001), 71.3±34% total motility (P<0.001), and 2.0±1% normal morphology. Total SCF decreased to 18.0±6% after DG, compared to 26.2±8% in the raw (P<0.001), and the dsDNA break remained unaffected after DG at 3.1±1%, compared 3.6±2% in the raw. These couples resulted in a fertilization of 68.8% (594/863) and a blastocyst euploidy rate of only 25.3% (71/281). Of 19 FET cycles performed, a 6.7% implantation (2/30) yielded only two clinical pregnancies that resulted in miscarriage. All 57 couples underwent subsequent cycles, utilizing MFSS. Compared to DG, MFSS resulted in the following parameters: 0.6±0 mL volume, 8.0±13x10^6/mL concentration, 96.9±9% total motility (P<0.001), and 3.3±1% normal morphology (P<0.05). The total SCF and dsDNA breaks remarkably decreased to 1.9±1% and 0.3±0.2%, respectively, following MFSS processing (P<0.001). Most importantly, with MFSS, the embryo euploidy rate rose to 42.9% (167/389) *(P<0.001)*. Consequently, the implantation increased to 65.5% (38/58), and the clinical pregnancy rate became 64.6% (31/48), resulting in a 62.5% delivery rate (30/48) *(P<0.001)*.

Conclusion: In couples with a young and reproductively healthy female partner undergoing ICSI, a subtle male factor may be present. Elevated SCF may contribute to a higher proportion of embryonic structural chromosomal abnormalities. Relying on the inverse relationship of SCF and progressive motility, MFSS remarkably enhanced the proportion of euploid embryos and clinical outcome.

Poster 57

COMBINING SPERM MAPPING AND SPERM VITRIFICATION TO MINIMIZE REPETITIVE TESTICULAR BIOPSY

Chaya Rothschild, Tristan Charran, Michael Werner
Maze Sexual and Reproductive Health, New York, NY, USA
Presented By: Chaya Rothschild, BS

Introduction & Objective: Men with azoospermia present both a diagnostic and therapeutic challenge. The vast majority of these cases are managed with micro-TESE combined with IVF/ICSI. However, mTESE is invasive, expensive, difficult to schedule concurrently with egg retrieval, and inevitably causes some damage to the testes. Sperm mapping is less expensive, less invasive, easy to schedule, causes virtually no damage to the testes, and if negative prevents the partner from going through an unnecessary IVF cycle.
Methods: When the sperm mapping reveals areas with spermatogenesis, it is almost invariably followed by mTESE. However, since it by definition yields a "map" of where the sperm should be, we have begun to do a percutaneous needle biopsy (PNB) in those areas. We then searched for and froze excess sperm using Extended Sperm Search and Microfreeze, involving individual sperm vitrification. We hypothesized the combination of sperm mapping, PNB, and ESSM would yield adequate sperm for both a fresh cycle and future frozen cycles. We present two case reports managed with the above protocol. Case #1 is a 33-year-old male with history of testicular torsion, b2/b3 Y-chromosome microdeletion, and mildly atrophied testicles. Case #2 is a 43-year-old male with mildly atrophied testicles with history of diagnostic biopsy that demonstrated rare spermatids.
Results: In Case #1, 8 of 36 sperm mapping slides demonstrated sperm. Using the map, a PNB targeting those areas with sperm was performed. 65 sperm were identified for ICSI. This cycle resulted in 3 euploid embryos. 136 additional sperm were isolated and vitrified, divided between 7 SpermVDs for future use. In Case #2, 10 of 36 sperm mapping slides demonstrated sperm. Using this information, an appropriately targeted PNB was performed, and the sample was divided between the IVF lab and our andrology lab. The IVF lab recovered 6 intact sperm, which were used for ICSI with 3 retrieved oocytes (female partner 43-years-old). The andrology lab recovered an additional 23 sperm which were vitrified for future use, divided between 4 SpermVDs.
Conclusion: The combination of sperm mapping followed by targeted PNB and sperm vitrification allows men to be screened for sperm, and then biopsied when appropriated, without ever undergoing an open and damaging mTESE. This protocol simultaneously provides the patient with enough sperm for multiple egg retrieval cycles without the need for future procedures.

Poster 58

TARGETING THE MEIG1/PACRG INTERACTION FOR MALE CONTRACEPTION

Yi Tian Yap[1], Timothy Hasse[1], Wei Li[1], Ljiljana Mladenovic-Lucac[1], Qian Huang[1,2], Shuo Yuan[1,2], Wei Qu[1,2], James Granneman[1], Mindy Huang[1], Zhibing Zhang[1]
[1]Wayne State University, Detroit, MI, USA, [2]Wuhan University of Science and Technology, Wuhan, China
Presented By: Yi Tian Yap, BS

Introduction & Objective: A precise and coordinated interaction between specified proteins is required for correct mammalian spermatogenesis. In particular, interaction between mouse meiosis-expressed gene 1 (MEIG1) and Parkin co-regulated gene (PACRG) is essential for sperm formation and male fertility. PACRG recruits meiosis-expressed gene 1 (MEIG1) to the manchette for normal spermiogenesis, and the key amino acid on mouse MEIG1 that mediates interaction with PACRG has been identified. Given that MEIG1-PACRG interaction is specific to male germ cells and conserved in humans, this protein complex presents as an excellent target for male contraceptive development.
Methods: Availability of the MEIG1/PACRG structure makes it possible to in silico virtual and artificial intelligence (AI) screens for small molecules that block MEIG1/PACRG interaction, with biochemical validation hits. To increase specificity of compound design, we evaluated how the known mutations contribute to the loss of interaction by performing Gaussian accelerated molecular dynamics (GaMD) on apo-MEIG1, apo-PACRG and MEIG1-PACRG complex.
Results: This analysis revealed that mutations of W50 and Y68 on MEIG1 greatly destabilizes the interaction between these amino acids with nearby residues at the MEIG1-PACRG interface. According to MD trajectories, 10 potential ligand binding pockets were identified. Specifically, a pocket involving W50 and Y68 was identified, presenting as a potential target for drug design for the inhibition of MEIG1-PACRG interaction. 29 compounds identified by in silico virtual screen based on human PACRG structure and 81 compounds designed through Atomwise artificial intelligence (AI) drug design platform based on MEIG1 structure were evaluated for their efficiency in the inhibition of MEIG1-PACRG interaction in vitro by luciferase-based complementation assay. Preliminary screening revealed several compounds that inhibited MEIG1-PACRG interaction.
Conclusion: In light of these findings, targeting the key factors contributing to the tight interaction between MEIG1-PACRG serves as an optimistic strategy for the development of male contraceptive.

Poster 59

SPERM CONCENTRATION REMAINS CONSISTENT FOR FIVE DAYS AFTER EJACULATION WHEN STORED IN THE LABORATORY

Yanhe Lue[1], Ronald Swerdloff[1], Laura Hull[1], William Bremner[2], Christina Wang[1]

[1]Division of Endocrinology, The Lundquist Institute at Harbor-UCLA Medical Center, Torrance, CA, USA, [2]Department of Medicine, University of Washington, Seattle, WA, USA
Presented By: Yanhe Lue, MD

Introduction & Objective: Male contraceptive methods that rely on suppression of spermatogenesis require frequent semen analysis to confirm that sperm concentration is below a threshold that offers contraceptive efficacy. The WHO laboratory manual for the examination and processing of human semen (6th Edition) recommends sperm concentration should be determined within the same day. This precludes weekend clinic visits unless a trained technologist is available. During the COVID-19 pandemic, timely measurement of sperm concentration during male contraceptive efficacy studies were not possible when institutions/laboratories are closed to research participants. Samples collected at home and mailed to the laboratory (with refrigerant gel packs) would circumvent this problem. We hypothesized that sperm concentration from semen stored in a collection cup may not be significantly changed over a short period of time of 5 days.
Methods: To test this hypothesis, a total of 57 ejaculates were collected from 57 participants in hormonal male contraceptive studies. The ejaculate was collected in 3 different types of collection containers (118 or 80ml specimen container or 30ml self-standing conical tube) and stored at 4ºC for 5 days after the first standard semen analysis performed according to the WHO Semen Manual. The sperm concentration was determined using hemocytometer method by 3 technologists at day of collection day 0, then day 1, 2, 3, 4 and 5 after ejaculation. After warming sample up at 37ºC for 15 min and mixing well with transfer pipet, we counted sperm number in ejaculates with sperm concentration ranging from 0.7 to 155 million/ml.
Results: We found the sperm concentration from semen in different collection containers were not significantly different within 5 days of collection irrespective of which type of containers. Regression analysis showed sperm concentration assessed at day 0 exhibited very high correlation with those examined at day 1 (r^2=0.99), day 2 (r^2=0.97), day 3 (r^2=0.95), day 4 (r^2=1.0) and day 5 (r^2=0.97) after ejaculation.
Conclusion: We conclude that sperm concentration from semen stored in collection containers remains consistent within 5 days after ejaculation. These results are important in the context of an andrology laboratory operations in emergent situations such the COVID-19 pandemic, over weekends, and on occasions when participant cannot come to clinic. It should be noted that sperm motility, morphology and vitality essential elements should not be assessed in stored sample.

Poster 60
PROTEIN-PROTEIN INTERACTIONS IN MOUSE SPERMATOZOA: IDENTIFYING EPPIN BINDING SITE ON SEMINAL VESICLE-SECRETORY PROTEIN 2 (SVS2)
Noemia Aparecida Mariani, Alexandre Andrade, Hélio Kushima, Erick Silva
Institute of Biosciences, São Paulo State University (UNESP), Botucatu, Brazil
Presented By: Noemia Aparecida Mariani, MS

Introduction & Objective: Seminal plasma proteins members of the Rapidly-evolving seminal vesicle transcribed (REST) family are involved in the formation of the semen coagulum and regulation of sperm function upon ejaculation. Semenogelin 1 (SEMG1), the major human REST protein, acts as an endogenous regulator of sperm motility and capacitation. The effects of SEMG1 are triggered by its interaction with epididymal protease inhibitor (EPPIN) on the sperm surface. EPPIN's SEMG1 binding site is a promising strategy for male contraception targeting spermatozoa. The mouse equivalent to SEMG1 is the seminal vesicle-secretory protein 2 (SVS2). Our previous studies indicated that SVS2 is an EPPIN-binding partner in mouse spermatozoa, suggesting a common event by which REST proteins modulate sperm function in humans and mice. Here, we investigated the interaction profile between mouse EPPIN and SVS2.
Methods: We employed AlphaScreen, a bead-based interaction assay, to study the interaction between recombinant mouse EPPIN and SVS2. We used full-length GST-tagged mouse EPPIN (mEPPIN; P22-T134) conjugated to anti-GST acceptor beads and full-length recombinant 6xHis-tagged mouse SVS2 (mSVS2; G35-G375) and its truncated constructs: i) Q32-V118 ($mSVS2^{Q32\text{-}V118}$, C-terminal truncation containing SVS2's unique Cys residue); ii) R98-G375 ($mSVS2^{R98\text{-}G375}$, N-terminal truncation containing SVS2's two 31-aminoacid long repeats), and iii) Y221-G375 ($mSVS2^{Y221\text{-}G375}$, N-terminal truncation lacking both the SVS2's Cys residue and the 31-aminoacid long repeats) conjugated to Ni^{2+}-chelate donor beads. Experiments were conducted in triplicates, and negative controls were performed in the absence of mEPPIN or mSVS2.
Results: mEPPIN-mSVS2 binding was concentration-dependent and saturable, with a calculated EC_{50} (95% confidence interval) 24 nM (20.6-27.9) when 0.3-100 nM mSVS2 was titrated in the presence of 10 nM mEPPIN, and 0.6 nM (0.4-0.8) when 0.1-10 nM mEPPIN was titrated in the presence of 30 nM mSVS2. $mSVS2^{R98\text{-}G375}$ truncation retained EPPIN-binding capacity similar to full-length mSVS2 (89.5 ± 9.4%; mean ± SEM). Conversely, $mSVS2^{Q32\text{-}V118}$ and $mSVS2^{Y221\text{-}G375}$ truncations showed reduced EPPIN binding (20.1 ± 4.8% and 31.1 ± 5.7%, respectively, $P < 0.05$).
Conclusion: We confirmed that EPPIN and SVS2 are binding partners in mouse spermatozoa and showed that the SVS2 sequence R98-S220, spanning its two 31-amino acid long repeats, contains the major EPPIN binding site. Our results highlight that EPPIN is a conserved sperm surface binding site for REST proteins and may open novel roads for male contraceptive development. Support: FAPESP 2021/04746-3; 2020/04841-3 and CAPES.

Poster 61

THE SLOPE BETWEEN TWO POST-VASECTOMY SEMEN ANALYSIS RESULTS COULD HELP CONFIRM VASECTOMY SUCCESS.

Lyne Massicotte, Mathieu Boilard
Nasci Biologie Médicale Inc, Longueuil, QC, Canada
Presented By: Lyne Massicotte, PhD

Introduction & Objective: Introduction: Post-vasectomy semen analysis (PVSA) is required to confirm the success of the vasectomy. Two flow cytometry procedures developed by our company have revealed themselves of unprecedented sensibility and precision. The first multi-parametric method (PVSA1) is performed on a fresh sample. With this method, vasectomy success (VS) is confirmed by the absence of viable spermatozoa. The second method is performed on less than 48 hours-old samples dropped at local service points (PVSA48). It confirms VS when sperm concentrations are below 5000 per ml. The problem of the more popular PVSA48 is that many men require up to three PVSA or more to confirm VS because each PVSA48 has about an 80% chance of yielding results below 5000/ml.
Objectives: We wanted to verify if analyzing the slope of the concentration between the two PVSA48 results could allow us to confirm vasectomy success without further analysis and, if so, for how many patients.
Methods: Method: A retrospective analysis of more than a thousand patients who had two PVSA48 done was performed.
Results: Results: The slope was negative (reduction in sperm concentration between the two analyses) in 83% of cases. Also, in 68 cases, the patient showed up to the lab for a third analysis on a fresh sample with the PVSA1 method after his two PVSA48 failed to confirm VS (showed concentrations less than 1000 000/ml but above 5000/ml). In 52 (74.5%) of those 68 cases, the slope was negative and in 51 cases out of 52 (98%) the patient was confirmed sterile by the absence of viable spermatozoa using the PVSA1 method on a fresh sample.
Conclusion: Conclusion: These data suggest that patients with two PVSA48 results between 5000 and 100 000 spermatozoa/ml and a negative slope could be considered sterile with a very minor risk of conception. This could help reduce the number of PVSA required to confirm sterility while maintaining the advantages associated with the incredible sensibility of the flow cytometry method over any other method including microscopy. More data will be collected in the future to evaluate the possibility to validate this method for clinical use.

Poster 62

SURGICAL RECONSTRUCTION FOR PENILE FRACTURE: SHORT-TERM OUTCOME AND COMPLICATIONS

Myagmarsuren Purevsuren, Bayarbaatar Lamid-Ochir, Munkhjargal Batgerel, Bayan-Undur Dagvadorj, Nyamsuren Davaajav
The First Central Hospital of Mongolia, Ulaanbaatar, Mongolia
Presented By: Myagmarsuren Purevsuren, MD, MS

Introduction & Objective: Penile fracture is an emergency surgery case. They result from trauma to the erect penis leading to rupture of the tunica albuginea of the penile corpora. Prompt diagnosis and early surgical repair are essential to ensure a successful outcome. We evaluate the clinical perform and characterize the changes in erectile function findings in men with penile fracture before and after surgery.
Methods: We retrospectively assessed the case series data of 9 patients with a clinical diagnosis of penile fracture who had been admitted to our facility between March 2019 and May 2021. An IIEF-5 questionnaire was sent to the 9 patients who were treated by means of surgery penis recovery.
Results: The patients' age ranged from 25 to 57 years (mean 40 years). The time elapsed between trauma and hospital admission ranged from 12 h to 14 days. The clinical presentations were hematoma (100%), penile edema (100%), a snapping sound (n-6), pain (n-4), and acute urinary retention (n-2)
The one subcutaneous hematoma and wound infection were reported as a post-surgical early complication. As a late complication penile deformity one case was reported. Pre and post-surgery IIEF-5 score is statistically irrelevant /p-0.94/
Conclusion: In this study, sexual activity, using alcohol before intercourse, using PDE5 sexual intercourse, was the most common cause of penile fracture, with injuries most often caused by the 'woman-on-top' positions. Doggy-style positions showed more associations with bilateral fractures of the corpus cavernosal and urethral lesions. The post-surgical erectile dysfunction was not noted.

Picture 1. Swollen and deformed fractured penis

Poster 64

HYPERPOLARIZATION INDUCED ACROSOME REACTION IS MEDIATED THROUGH THE ACTIVATION OF GSK3 ALPHA IN SPERM HEAD

Gayatri Mohanty[1], Bidur Paudel[1], Claudia-Sanchez Cardenas[2], Darya Tourzani[1], Alberto Darszon[2], Maria Gracia Gervasi[1], Pablo Visconti[1]

[1]University of Massachusetts, Amherst, MA, USA, [2]Universidad Nacional Autónoma de México (UNAM), Cuernavaca, Mexico

Presented By: Gayatri Mohanty, PhD

Introduction & Objective: The multifunctional protein glycogen synthase kinase (GSK3) has two isoform alpha and beta plays an essential role during sperm capacitation and fertilization. No sooner the spermatozoa get exposed to the capacitating environment there is an efflux of cholesterol with albumin as acceptor and an influx of bicarbonate ions that activates the cAMP-PKA signaling pathway. This causes hyperpolarization mediated by SLO3 K+ channels within the sperm flagellum sufficient enough to prepare the sperm for an agonist-stimulated acrosome reaction. Previously, Nixon's group has shown that GSK3 activity is required for the progesterone-induced acrosome reaction. This kinase is down regulated by phosphorylation of Ser 21 in their N-terminal domain. The objective of the current study is to investigate the regulation of GSK3 isoforms during capacitation.

Methods: Mouse epididymal spermatozoa were incubated in media that support or not capacitation. Both isoforms of GSK3 were visualized and its phospho-regulation was studied using key components of capacitation media – bovine serum albumin (BSA) and bicarbonate. Hyperpolarized state was induced through 1uM of Valinomycin while cAMP-PKA pathway was modulated through 50uM TDI10299- inhibitor of soluble adenyl cyclase (SAC) or exogenous addition of dbcAMP (100mM) and IBMX (100uM) and GSK3 phosphorylation status was studied using specific antibodies.

Results: We observed that capacitation was associated with activation (dephosphorylation) of GSK3 alpha located predominantly in head region. While the capacitation media caused dephosphorylation, BSA increased phosphorylation, with bicarbonate causing dephosphorylation. Modulation of cAMP-PKA axis through absence of bicarbonate in media or SAC inhibition with exogenous

addition of dbCAMP and IBMX caused an activation of GSK3a. Considering the role of hyperpolarization in the preparation of acrosome reaction, we explored the extent membrane potential regulates GSK3 alpha Ser 21 phosphorylation status. On the other hand, lithium and Chir99021 block the acrosome reaction. Overall, our results suggest that GSK3 alpha dephosphorylation during capacitation is mediated by changes in membrane potential and it is an essential step to prepare the sperm for acrosomal exocytosis. **Conclusion:** These results indicate that capacitation process is isoform specific with GSK3a playing active role mediated by hyperpolarization. This proves that cAMP pathway can be bypassed with these key events and is not a necessity for GSK3 activation while its inhibition blocks acrosome reaction indicating that this kinase plays an active role in acrosomal exocytosis.

Poster 65

DOES THE USE OF MELATONIN IN SEMEN CRYOPRESERVATION INFLUENCE THE OOCYTE-SPERM INTERACTION, THE OXIDATION-REDUCTION CAPACITY AND THE FUNCTIONAL TESTS?

Larissa Chiba[1,2], Juliana Pariz[3], Heloisa Faquineti[1], Raul Sanchez[4], Joel Drevet[5], Mabel Rubilar[1,2,6], Jorge Hallak[4]
[1]Androscience – Science and Innovation Center and High Complexity Clinical and Research Andrology Laboratory, São Paulo, Brazil, [2]Department of Urology, University of São Paulo (USP), São Paulo, Brazil, [3]Methodist University of São Paulo, São Bernardo Campo, Brazil, [4]Universidad de La Frontera, Temuco, Chile, [5]Université Clermont Auvergne, Clermont-Ferrand, France, [6]Institute for Advanced Studies, University of Sao Paulo (IEA-USP), São Paulo, Brazil
Presented By: Larissa Chiba, BS

Introduction & Objective: Fatherhood is one of the most desired goals in an adult's life. However, in many clinical situations, sperm cryopreservation is necessary to preserve the man's fertility. Although slow freezing of male gametes allows for reproductive success, it is clear that it is accompanied by cell damage. To correct this pitfall and improve the quality of frozen sperm cells, the addition of molecules such as melatonin, which has several beneficial functions such as antioxidant, anti-apoptotic, anti-inflammatory, are studied. The aim of this study was to determine what concentration of melatonin during slow freezing would improve the functional qualities of spermatozoa.
Methods: Sixty-eight seminal samples from male volunteers (18-45 years) with semen concentration ≥16 million/ml were included in this study. Samples were cryopreserved by the slow freezing method with TEST- YOLK (TYB) buffer as cryoprotectant, supplemented or not (CONT) with 0.01mM, 2mM, or 3mM melatonin. Seminal parameters including: reactive oxygen species level (by luminol chemiluminescence), sperm mitochondrial activity (by DAB staining), sperm membrane lipid peroxidation (by TBARS determination), measurement of glutathione content, sperm DNA fragmentation (by SCSA®) and determination of sperm binding to hyaluronan were assessed before and after cryopreservation. Cryosurvival rates were calculated after thawing. Comparisons between groups were analyzed by one-way analysis of variance (ANOVA).
Results: The results are presented in Table 1. The p-value was not statistically significant between groups. However, looking at the means in more detail, ROS levels and cryo-survival rates were improved in samples with 0.01mM melatonin, whereas samples supplemented with 3mM melatonin showed improvement in DNA fragmentation, mitochondrial activity, and lipid peroxidation compared with the negative control. The hyaluronan binding assay was improved in samples cryopreserved with 2mM melatonin.
Conclusion: Although statistical significance was not reached between groups, melatonin supplementation at different concentrations during the slow freezing procedure showed beneficial effects on different functional sperm parameters. At 0.01 mM, melatonin tended to decrease sperm ROS levels and improve cryo-survival, whereas at 3 mM, melatonin tended to improve sperm DNA fragmentation, sperm mitochondrial activity and sperm membrane lipid peroxidation. Further research is needed to find an optimal concentration of melatonin to use.

Table 1. Results post-thawing with and without melatonin.

	Negative control	0.01mM	2mM	3mM	p-value
Total motile sperm number (million/mL)					
Mean; SD	**2.09; 2.41**	1.71; 2.50	1.76; 3.17	1.75; 2.79	0.834
Min-Max	0.00-10.75	0.00-13.46	0.00-17.00	0.00-14.70	
Progressive motility (%)					
Mean; SD	**7.91; 8.30**	6.96; 8.73	6.93; 8.11	6.88; 7.96	0.864
Min-Max	0.00-30.16	0.00-38.30	0.00-39.24	0.00-29.03	
Total motility (%)					
Mean; SD	**16.08; 11.24**	13.12; 11.25	13.00; 11.36	13.00; 12.81	0.325
Min-Max	0.00-42.42	0.00-48.30	0.00-53.17	0.00-69.00	
ROS (10^4cpm/million spermatozoa)					
Mean; SD	2.92; 9.26	**2.09; 6.48**	3.56; 9.23	3.67; 12.19	0.782
Min-Max	0.00-60.10	0.00-44.62	0.00-41.16	0.00-74.02	
DNA fragmentation (DFI%)					
Mean; SD	56.00; 23.63	58.17; 25.94	54.65; 23.93	**52.94; 25.72**	0.692
Min-Max	2.00-99.00	11.00-99.00	16.00-98.00	16.00-98.00	
Mitochondrial activity (DAB I+II%)					
Mean; SD	56.11; 16.64	57.24; 15.04	56.77; 16.37	**58.41; 12.80**	0.975
Min-Max	22.00-77.00	22.00; 76.00	16.00-75.00	33.00-78.00	
Cryosurvival (%)					
Mean; SD	32.27; 35.93	**35.06; 43.76**	23.12; 20.04	23.18; 22.68	0.059
Min-Max	0.00-266.00	0.00-252.50	0.00-90.12	0.00-100.97	
Lipid Peroxidation (TBARS/ml nanograms of spermatozoa)					
Mean; SD	488.61; 593.34	445.35; 634.61	328.39; 562.46	**197.56; 159.98**	0.489
Min-Max	0.00-1824.06	0.00-2321.83	0.08-2013.42	0.00-506.41	
Glutathione (x10^6 GSH-Px/mL)					
Mean; SD	0.02; 0.00	0.02; 0.00	0.02; 0.00	0.02; 0.00	0.925
Min-Max	0.02-0.03	0.02-0.03	0.02-0.03	0.02-0.03	
Hyaluronan binding assay (%)					
Mean; SD	81.25; 12.09	74.00; 29.44	**86.33; 11.72**	83.00; 13.88	0.844
Min-Max	64.00-91.00	40.00-91.00	73.00-95.00	70.00-95.00	

Mean; Standard Deviation (SD); Min-Max: Minimum and maximum values; significant p-value <0.05.

Table 1. Results post-thawing with and without melatonin.

Poster 66

EFFECT OF YOGA ON SPERM MOLECULAR CONTRIBUTIONS AND PERCEIVED QUALITY OF LIFE IN EARLY PREGNANCY LOSS

Vidhu Dhawan, Neena Malhotra, Neeta Singh, Vatsla Dadhwal, Rima Dada
All India Institute of Medical Sciences, New Delhi, India, New Delhi, India
Presented By: Vidhu Dhawan, MBBS, MD, PhD

Introduction & Objective: A significant increase in complex lifestyle diseases witnessed in last decade has exerted a dramatic impact on the reproductive potential. The contribution of sperm molecular factors have been found to play a critical role in health and disease of offspring. Early pregnancy loss is a psychologically devastating condition affecting the quality of life (QOL). Thus it needs to be managed by a mind body integrated approach like yoga, which can act as an adjunct in management. The present prospective single arm exploratory study was designed to analyze the expression profile of spermatozoal genes critical for early embryogenesis, oxidative DNA damage, telomere dynamics and QOL.

Methods: Semen sample was obtained from male partners of couples experiencing recurrent pregnancy loss (RPL) (n=30), recurrent implantation failure (RIF) (n=30) patients and healthy fertile controls (n=30). Semen samples were obtained at the beginning and at completion of yoga for 6 weeks. q-PCR analysis was done by $2^{-\Delta\Delta Ct}$ method for the relative quantification of *FOXG1, SOX3, STAT4, RPS6, RBM9, RPL10A, RPS17, RPL29, WNT5A, HSP90, TOMM7, EIF5A, OGG1 and PARP1* after normalization to *GAPDH* and β-actin. The levels of seminal ROS (RLU/sec/million sperm), DNA damage (%) and relative sperm telomere length (STL) were assessed by chemiluminescence, sperm chromatin structure assay (SCSA) and qPCR. QOL was assessed by WHOQOL-BREF questionnaire.

Results: A significant increase in the sperm concentration ($p<0.0001$**** & $p=0.007$**) and progressive motility ($p<0.001$***), a decline in ROS ($p<0.001$***) and DFI (0.0002*** & $p=0.071$) was observed, while a significant increase in STL ($p<0.001$*** & $p=0.008$**) was found at the end of the yoga intervention in the RPL and RIF groups respectively. The relative expression of the genes showed normalization towards the levels of controls. WHOQOL-BREF questionnaire scores showed an improvement in all the 4 domains (physical, psychological, social and environmental).
Conclusion: The significant and positive impact on the QOL by yoga has witnessed an improvement in seminal parameters. This is the first study to assess the impact of adoption of yoga on the seminal biomarkers, telomere length, DNA integrity and gene expression. Integration of yoga as an adjunct in the management of early pregnancy loss exerts beneficial effects in improving seminal oxdative DNA damage, normalization of sperm transcripts and QOL highlights the need to use a holistic approach targeting the mind & body.

Poster 67
TIMING OF TESTICULAR SPERM RETRIEVAL IN NOA PATIENTS DOES NOT APPEAR TO AFFECT ICSI CLINICAL OUTCOMES
Mary McKnight, Philip Xie, Peter Schlegel, Gianpiero Palermo, Zev Rosenwaks
Weill Cornell Medicine, New York, NY, USA
Presented By: Mary Mcknight, BS

Introduction & Objective: In men with non-obstructive azoospermia (NOA) and residual spermatogenic foci, microscopic testicular sperm extraction (micro-TESE) is able to retrieve competent spermatozoa. Because of the inconsistency of sperm retrieval and for scheduling purposes, it is preferable to perform the biopsy antecedently to (D-1) or day of (D0) oocyte retrieval. We wonder if the interval between sperm retrieval and ICSI has any effect on clinical outcome.
Methods: In the last 2 decades, couples with NOA who underwent ICSI using freshly retrieved spermatozoa by micro-TESE were grouped according to whether the surgery was done at least one day before (D-1 TESE) or the day of (D0 TESE) oocyte retrieval. Embryology findings including fertilization and embryo development were compared between the 2 cohorts. Clinical outcomes such as implantation, clinical pregnancy and deliveries were also assessed and compared.
Results: Of 703 patients who underwent a total of 841 successful micro-TESE cycles, 246 cycles (223 patients, paternal age=35.1±6) were carried out on at least one day before oocyte retrieval. The D-1 testicular sample yielded a concentration of $0.1x10^6$/ml, motility of 1.3%, and were injected in 2904 oocyte (maternal age=32.2±5). These yielded 47.0% (1366/2904) fertilization rate resulting 470 (82.7%) embryos that were transferred in 246 embryos replacement procedures mostly on day 3 (82.5%) yielding a clinical pregnancy rate of 42.7% (105/246) and 99 deliveries (40.2%).
A total of 595 micro-TESE (480 patients, paternal age=35.4±6) were carried out on the same day of the oocyte retrieval. Testicular spermatozoa with $0.2x10^6$/ml concentration and 1.8% motility from these D0 samples were injected in 6994 oocytes (maternal age=32.2±5), yielding a 50.2% (3513/6994; $P<0.05$) fertilization. There were 1179 embryo replaced in 595 cycles mostly performed at day 3 (80.3%) resulting in 43.4% (258/595) clinical pregnancy rate, and yielding 240 deliveries at 40.3%.
Conclusion: It appear that the timing of testicular biopsy, whether on the same day of oocyte retrieval or at least 24 hours earlier, does not influence fertilization, embryo development and implantation. Early timing of the procedure maybe preferable due to unpredictability of sperm retrieval and for scheduling purposes.

Poster 68
IDENTIFICATION OF A NEW CLASS OF EXTRACELLULAR VESICLES : EFFERENT DUCTULES CILIARY EVS
Ludovic Vinay, Camille Lavoie-Ouellet, Céline Augière, Clémence Belleannée
The CHU de Québec-Université Laval Research Center, Québec, QC, Canada
Presented By: Ludovic Vinay

Introduction & Objective: The extracellular vesicles (EVs) of the male reproductive system participate in a unique communication system between somatic cells and spermatozoa, via the transfer of bioactive molecules (*e.g.* proteins, sncRNAs). Our laboratory has identified the primary cilia, a sensory organelle present at the surface of efferent ductules, as a new source of EVs: the ciliary EVs (cEVs). To ultimately evaluate the contribution of cEVs in the control of sperm maturation, our objective was to develop a method to isolate cEVs and identify their molecular features.
Methods: After digestion of mouse efferent ductules, stromal cells are removed and isolated epithelial cells are cultured on Matrigel. cEVs were then isolated by ultracentrifugation from the supernatants of these primary cultures. Potential cEV markers were validated by dedicated particle analysis flow cytometry, immunofluorescence, and Western-blot prior to mass spectrometry analysis of cEVs protein composition. Spinning disk confocal imaging strategy is used on ciliated cells presenting an endogenous fluorescence (Cetn2-GFP; Arl13b-mCherry) to determine the kinetic and mode of release of cEV *in vitro*.
Results: A protocol was optimized to isolate and culture epithelial cells from mice efferent ductules. Our primary culture conditions (including stromal cells depletion and culture on Matrigel coat) allowed the enrichment of 52 % of epithelial cells that express villin (efferent ductules epithelial cells marker), and that expose a primary cilium. The presence of EVs in the supernatant was confirmed by the detection of the EV marker phosphatidylserine by flow cytometry and the use of suitable controls (*e.g.* Triton X-100, EDTA). The detection of Arl13b and CD151 cEVs markers confirmed the presence of cEVs, and the analysis of their protein

signature is in progress. In addition, live imaging performed on ciliated cells of the efferent ductules allowed us to detect the dynamic of the primary cilium and will be instrumental to determine the mechanism by which cEVs are released into the extracellular environment.
Conclusion: Primary cell culture of efferent ductules epithelial cells allowed us to isolate a unique sub-population of EVs harboring conventional Arl13b and CD151 cEVs markers. The exhaustive analysis of cEVs protein composition and future *in vitro* functional assays will allow us to evaluate the possible involvement of cEVs in intercellular communication and sperm maturation.

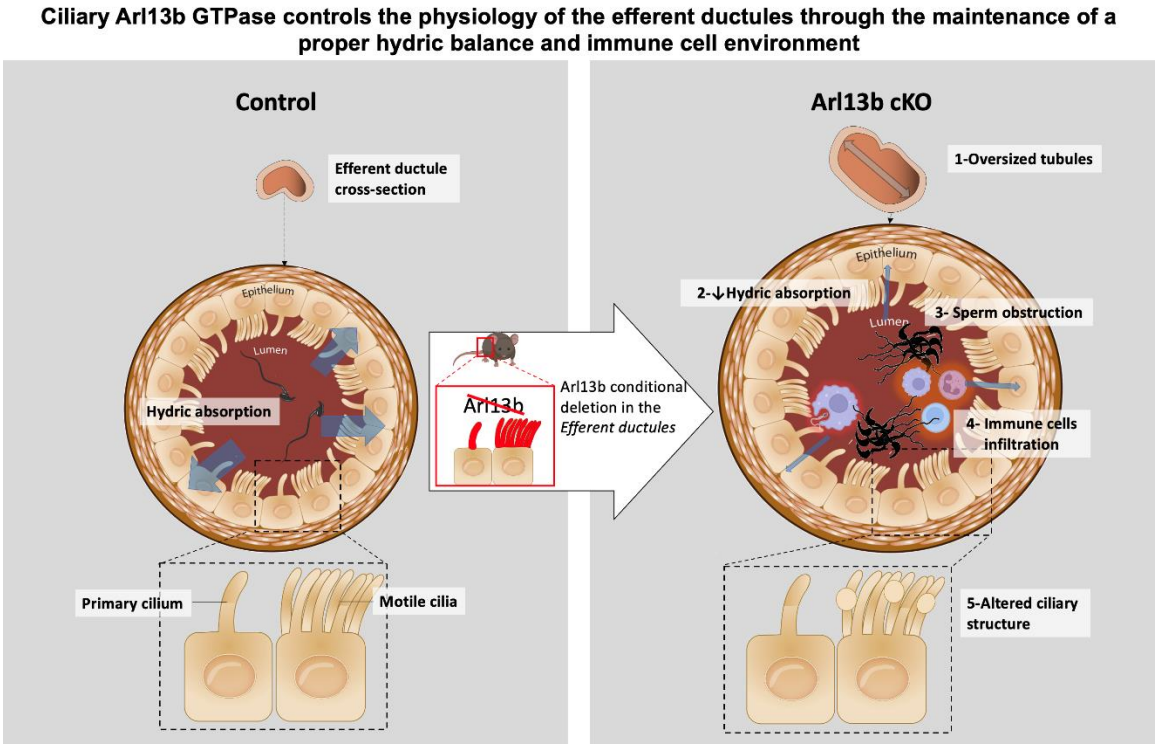
Supported by a NSERC grant to CB and a Fondation Desjardin fellowship to LV.

Poster 69

ARL13B CILIARY GTPASE CONTROLS THE PHYSIOLOGY OF EFFERENT DUCTULES THROUGH THE MAINTENANCE OF A PROPER HYDRIC BALANCE AND IMMUNE CELL ENVIRONMENT.

Celine Augiere[1], Gabriel Campolina[1], Camille Lavoie-Ouellet[1], Ferran Barrachina[2], Kiera Ottino[2], Maria A. Battistone[2], Charles Joly Beauparent[1], Arnaud Droit[1], Ezequiel Calvo[1], Rex Hess[3], Clemence Belleannee[1]
[1]Department of Obstetrics, Gynecology and Reproduction, Université Laval, CHU de Québec Research Center (CHUL), Quebec City, QC, Canada, [2]Program in Membrane Biology, Nephrology Division, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA, [3]Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois, Urbana, IL, USA
Presented By: Celine Augiere, PhD

Introduction & Objective: Efferent ductules (EDs) are tubules connecting the rete testis to the head of the epididymis whose reabsorptive capacity controls spermatocrit (ratio sperm/fluid) and male reproductive potential. Proper fluid reabsorption is ensured by two epithelial cell populations that function in a well-concerted manner: the multiciliated cells that maintain sperm in suspension through the stirring activity of their motile cilia, and the adjacent cells that reabsorb almost 90% of the luminal fluid and expose a long solitary primary cilium. While the contribution of motile cilia to ED physiology is well described, the combinatory function of motile and primary cilia remains unknown. We hypothesized that motile and primary cilia are both important to the luminal sensory perception associated with fluid reabsorption and male fertility, so we investigated the consequences of their dual functional impairment *in vivo*.
Methods: A conditional mouse model was generated to invalidate Arl13b, a ciliary GTPase controlling both motile and primary cilia sensory function, in the epithelial cells of the EDs (Villin-cre; Arl13b-flox, cKO). A combination of Computer-Assisted Semen Analysis (CASA), ciliary beating high-speed video microscopy, confocal imaging, electron microscopy, and RNA sequencing approaches were performed to determine the role of Arl13b in the control of EDs physiology and male fertility.
Results: In male cKO mice, a correlation between EDs obstruction and male infertility was observed ($p<0,006$; Speaman's Rho -0,75), causing a heterogenous infertility phenotype with 22% of reported infertile cKO males. This phenotype was associated with the presence of enlarged dilated tubules and the alteration of *ion channel-transporters gene* expression (i.e. AQP2, AQP5, Slc9c1), characteristic of an impaired reabsorptive function. The detection of sperm phagocytosis by $F4/80^{+}$ macrophages and the expression of inflammatory markers in epithelial cells (i.e., MHCII and Lcn6), suggests an imbalanced immune environment possibly caused by the obstruction. Moreover, while the structure of both motile and primary cilia was altered following the loss of Arl13b, the motility of cilia was maintained in cKO mice compared to controls.
Conclusion: Our study underscores the Arl13b ciliary component's contribution to the physiology of the EDs by maintaining a proper hydric balance and immune cell environment. Our data suggest that, beyond their role in controlling fluid dynamics through their motility properties, motile and primary cilia organelles also play sensory functions that regulate male fertility potential.



Arl13b controls the physiology of efferent ductules

Poster 70

NON-SPERM CELL CONCENTRATION IN POST-VASECTOMY SEMEN SPECIMENS CORRELATE WITH RESIDUAL SPERM COUNTS EVEN IN VASECTOMY SUCCESSES: AN ANATOMICAL MYSTERY

Alexander Hauser[1], Ryan Kiessling[1], Katherine Bertolini[1], Jeffrey Steinberg[2], Ann Kiessling[1]
[1]Bedford Research Foundation, Bedford, MA, USA, [2]Milton Hospital, Milton, MA, USA
Presented By: Alexander Hauser, BS

Introduction & Objective: Non-sperm cells (NSC) in semen have been the subject of numerous studies. A marked decrease in NSCs following vasectomy has been reported suggesting the epididymis is the source of the NSCs. We have analyzed a cohort of post-vasectomy semen specimens to quantify and characterize the cells contributed to semen specifically by the tissues proximal to the vasectomy site.

Methods: Approximately 12 weeks post vasectomy, patients mail to the laboratory for sperm count a semen specimen added to a fixative that contains red blood cells as an internal control. Aliquots of the fixed specimen are Hoechst 33258 stained and scanned in an EVOS M7000 in bright field to quantify internal control cells and in fluorescence mode to quantify DNA-containing sperm heads and NSCs, which were further characterized by peroxidase staining and immunostaining.

Results: Forty-four (5%) of 874 post vasectomy semen specimens contained greater than 100,000 sperm/ml, deemed vasectomy failures, with average sperm count of 6.2 x 10^6, range of 10^5 to 10^7; the NSC count averaged 1.8 x 10^6, range of 10^4 to 10^7. One hundred twenty-seven (14.5%) specimens were markedly acellular, containing fewer than 10,000 sperm and NSCs/ml. A subset of 377 specimens (43%) with sperm counts greater than 10^3 and less than 10^5 had average sperm counts of 1.2 x 10^4, range of 10^3 to 10^5; the NSC counts averaged 4x10^5, range of 10^3 to 10^7, a statistically significant ($p<0.001$) order of magnitude fewer than the vasectomy failure group. The subset of 453 specimens (52%) with fewer than 10^3 sperm/ml contained an average of 8.3 x 10^4 NSCs, range of 10^3 to 10^6, a statistically significant ($p<0.001$) order of magnitude fewer than the other two groups. Histochemical analyses reveal most NSCs stain positive for leukocyte markers with some staining positive for prostate antigen.

Conclusion: These results support previous reports that most non-sperm cells in ejaculated semen arise distal to the vasectomy site, principally the epididymis. Characterization of the NSCs post vasectomy will provide important clues about the anatomical locations of residual sperm and possibly overall health of the ejaculatory ducts and accessory glands.

Poster 71

COMPARISON OF EPIDIDYMAL MORPHOLOGY AND SPERM NUMBERS BETWEEN THREE STRAINS OF MICE

Christopher Pearl, Hailey Plata

Grand Valley State University, Allendale, MI, USA
Presented By: Christopher Pearl, PhD

Introduction & Objective: One of the most widely used models for human physiology studies are mice. However, there is limited information directly assessing potential similarities and differences of male reproductive traits in mice, specifically related to the epididymis. The goal of this study was to compare epididymal sperm numbers and morphology between three commonly used research mice strains: C57, Balb/c, and Swiss.
Methods: Three strains of mice were used for this study: C57BL/6J (Jax code: 664), Balb/cJ (Jax code: 651), and Swiss (Jax code: 034608). Twelve mice from each strain were obtained from Jackson Laboratories at ten weeks of age; at twelve weeks of age animals were euthanized for collection of blood and reproductive tissues. One epididymis from each mouse was fixed in Bouin's fixative for histological/morphological analysis while the other was frozen for sperm counts. Testes were also collected for assessment of daily sperm production.
Results: Swiss mice had the highest body weight with correspondingly larger testes and epididymides by weight. Epididymal tubule diameter increased and cell height decreased from initial segment to cauda as expected. Tubule diameter of the cauda was significantly different between all three strains with Swiss > Balb/c > C57. Initial segment cell height was also significantly different between the three strains with C57 > Balb/c > Swiss. Total epididymal sperm number was different between all three strains with Swiss mice having the highest sperm number. When separated into proximal (IS/caput/corpus) and distal (cauda) regions and adjusted for tissue weight, sperm/mg cauda was higher in the distal regions in all three strains. Interestingly, the sperm/mg in the proximal region was similar amongst all three strains, while sperm/mg cauda was different between the three strains. Epididymal transit time was similar between C57 and Balb/c mice with both being significantly less than Swiss mice. Serum testosterone and estradiol concentrations were similar between all three strains.
Conclusion: These results identify similarities and differences related to epididymal morphology and sperm numbers in three commonly used mice models. Additionally, these results may provide an improved baseline for choosing an appropriate mouse model to address questions and hypotheses related to male fertility/infertility.

Poster 72
PHARMACOLOGICAL INHIBITION OF NFKB SIGNALING DIFFERENTIALLY MODULATES TOLL-LIKE RECEPTOR SIGNALING CASCADE TRANSCRIPTS IN MOUSE MODELS OF EPIDIDYMITIS
Alexandre Andrade[1], Hélio Kushima[1], Maria Christina Avellar[2], Christiane Pleuger[3], Erick Silva[1]
[1]São Paulo State University, Botucatu, Brazil, [2]Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo, Brazil, [3]Justus-Liebig-University Giessen, Giessen, Germany
Presented By: Erick Silva, PhD

Introduction & Objective: Bacterial epididymitis is a condition that affects men's well-being and fertility. The initial response of the epididymis to infection relies on the recognition of pathogen- and damage-associated molecular patterns by members of the Toll-like receptor (TLR) family. TLR4 and TLR2/TLR6, which recognize lipopolysaccharide (LPS) and lipoteichoic acid (LTA) from Gram-negative and Gram-positive bacteria, respectively, are constitutively expressed along the epididymis. Activation of TLR4 and TLR2/TLR6 leads to signaling pathways culminating in the activation of transcription factors, such as NFKB, and the upregulation of inflammatory mediators. Here, we investigated the role of NFKB on the responses of TLR pathway-related genes in LPS- and LTA-induced epididymitis in mice.
Methods: Male C57BL/6 mice (90 days, n=5-6/group) were anesthetized (ketamine/xylazine). Epididymitis was then induced by ultrapure LPS from *E. coli* (50 ug) or LTA (125 ug) from *S. aureus* after (1) interstitial injection into the epididymal initial segment (IS) or (2) retrograde intravasal injection into the vas deferens toward the cauda epididymidis (CD) of mice that were pre-treated or not with PDCT (100 mg/kg, i.p.; NFKB inhibitor) 1 h before induction. Control groups were treated with sterile saline (vehicle). After 24 h, mice were euthanized, and the IS and CD were collected for RT-qPCR studies to evaluate *Tlr2*, *Tlr4*, *Tlr6*, *MyD88*, *Trif*, *Cd14*, *Cd36*, *Traf3*, and *Traf6* transcript levels (endogenous control: *Hprt*). Ethics Approval: 6272050320-IBB/CEUA.
Results: In the IS, interstitial LPS and LTA injection increased *Tlr2*, *Tlr4*, *Myd88*, and *Trif* transcripts (fold-change ~4, ~4, ~2, ~2 for LPS, and ~4, ~4, 1.4, ~2 for LTA, respectively). LPS upregulated *Cd14* (~8), *Traf6* (~4) and *Traf3* (~4), and downregulated *Cd36* (~0.1), whereas LTA upregulated *Tlr6* (~3). In the CD, intravasal LPS injection upregulated *Tlr2* (~3) and *Cd14* (~4) and downregulated *Cd36* (~0.5), whereas LTA showed no effect. In the IS, NFKB inhibition with PDCT prevented LTA-induced upregulation of *Tlr2*, *Tlr4*, *Myd88*, and *Trif* but did not affect the LPS-induced changes. Conversely, in the CD, PDTC prevented LPS-induced upregulation of *Tlr2* and *Tlr4*.
Conclusion: The activation of TLR4 and TLR2/TLR6 by LPS and LTA triggers region-specific pathways modulating the expression of key TLR-associated signaling cascade genes in the epididymis. The responses of NFKB inhibition to LPS and LTA suggest that targeting NFKB could generate novel adjuvant therapies to dampen bacterial epididymitis negative outcomes. Support: FAPESP/CAPES.

Poster 73
REGULATORY T-CELL DEPLETION RESULTS IN SEVERE MALE SUBFERTILITY
Ferran Barrachina[1], Kiera Ottino[1], Maia Lina Elizagaray[1], Maria Gracia Gervasi[2,3], Leona Tu[1], Maria Agustina Battistone[1]

[1]Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA, [2]Bedford Research Foundation, Bedford, MA, USA, [3]University of Massachusetts Amherst, Amherst, MA, USA
Presented By: Ferran Barrachina, MS, PhD

Introduction & Objective: The male reproductive function relies on tolerance mechanisms towards sperm antigens, which avoids autoimmune reactions such as the production of anti-sperm antibodies (AsAb) that cause infertility. Here, we characterized the epididymal regulatory Foxp3$^+$ T cells (Tregs), key players for maintaining peripheral tolerance, and studied the consequences of the disruption of tolerance in the epididymis.
Methods: WT and transgenic mice expressing the diphtheria toxin (DT) receptor (DTR) under the Foxp3 promoter (Foxp3-DTR mice) were used to characterize epididymal Treg function. Confocal microscopy and flow cytometry were performed to study immune cell infiltration. The presence of immunoglobulins (Ig) was assessed by ELISA and confocal microscopy. Computer-assisted sperm analysis and *in vitro* fertilization assay were used to evaluate sperm function. Fertility was assessed by counting litter size.
Results: We showed that the abundance of Tregs decreases with age in the proximal and distal epididymides ($p<0.01$). To examine the consequence of the immune tolerance breakdown, we ablated Tregs by injecting DT into Foxp3-DTR mice. Two weeks after DT treatment, we observed a widespread infiltration of monocytes, neutrophils, F4/80$^+$ mononuclear phagocytes (MPs), and MHCII$^+$ MPs in all epididymal segments of Foxp3-DTR mice ($p<0.01$). Interestingly, we observed infiltration of MPs into the epididymal lumen. Despite this aberrant immune response, the epididymal epithelium was not damaged, and low levels of apoptosis were observed in the epithelium of WT and Foxp3-DTR mice. However, epididymal clear cells in Foxp3-DTR mice displayed an increase in apical bleb formation ($p<0.0001$). We revealed increased levels of serum Ig against sperm and epididymal antigens ($p<0.0001$) in the Foxp3-DTR mice. Moreover, confocal microscopy showed IgG accumulation in the interstitium of the epididymis, and AsAb were detected in sperm of Foxp3-DTR mice. All this inflammatory response resulted in reduced sperm counts ($p<0.01$), impaired sperm motility ($p<0.01$), low *in vitro* fertilization rate ($p<0.05$), and fertility was severely impacted, as shown by a decreased litter size ($p<0.001$) in Foxp3-DTR mice.
Conclusion: Tregs play a key role in maintaining a tolerogenic environment that protects sperm in the epididymis. Our results showed that immune tolerance disruption induces an aberrant pro-inflammatory response in the epididymis that ultimately results in severe male subfertility. Deciphering immunoregulatory mechanisms in the epididymis may contribute to developing new strategies to treat male infertility and identify potential targets for immuno-contraception.

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UNRAVELLING THE CONTRIBUTION OF BASAL CELL PRIMARY CILIA IN EPIDIDYMIS (RE)GENERATION FROM ORGANOID MODELS
Camille Lavoie-Ouellet[1,2], Laura Girardet[3], Gabriel Campolina[1,2], Clémence Belleannée[1,2]
[1]Faculty of Medicine, Department of Obstetrics, Gynecology and Reproduction, CHU de Québec Research Center (CHUL), Québec, QC, Canada, [2]Centre de Recherche en Reproduction, Développement et Santé Intergénérationnelle (CRDSI) - Université Laval, Québec, QC, Canada, [3]Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada., Montréal, QC, Canada
Presented By: Camille Lavoie-Ouellet, MS

Introduction & Objective: The adult epididymis is composed of a pseudostratified epithelium whose maintenance in response to tissue damage or inflammation is essential to male fertility. Some epididymal basal cells have the capacity to self-renew and differentiate *in vitro (Pinel and Cyr, BOR, 2021)* through unknown signaling pathways. Acknowledging that basal cells form primary cilia that control post-damage epididymis regeneration *in vivo (Girardet et al, 2022)*, we hypothesized that primary cilia-dependent pathways control basal cell self-renewal and differentiation capacities needed for epididymis regeneration.
Methods: To verify this hypothesis, we assessed the capacity of basal cells to form 3D organoids in normal condition compared to primary cilia impaired condition by using control and cKO mouse models in which primary ciliogenesis and ciliary function are conditionally impaired in keratin5 positive basal cells (Krt5^{Cre}; IFT88$^{fl/fl}$; Krt5^{Cre}; Arl13b$^{fl/fl}$, respectively). Mouse models were validated through genotyping and immunodetection of the ciliary components IFT88 and ARL13B by confocal microscopy and western blotting. Basal cells populations were isolated by FACS from controls and cKO mice by using CD49f-BV421 and Epcam-APC-Cy7 markers.
Results: From wild type mice, the FACS-sorted basal cell (BC) enriched fraction was composed of 86% of keratin5 positive cells and generated 16 times more 3D organoids compared to the non-basal cell (NBC) fraction (130 *vs.* 18 organoids in average per experiment). In addition, organoids formed from BC fraction were larger (35 *vs.* 27 pixels) with a higher number of cells (18 *vs.* 10 cells/organoid) compared to the NBC fraction. The same experimental strategy is currently conducted in parallel on BC and NBC fractions isolated from control and cKO mice to assess the contribution of primary cilia to 3D organoids formation.
Conclusion: Further downstream analyses, including pharmacological treatments and RNAscope *in situ* hybridization strategies will be performed to identify what are the primary cilia-dependent pathways controlling basal cell differentiation during 3D organoid formation. Ultimately, our model will help us determine the role of primary cilia in tissue proliferation/regeneration response observed following epididymal tissue damage/insult occurring in a context of epididymitis.

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ADOLESCENT ERECTILE DYSFUNCTION: PRESENTING CHARACTERISTICS AND COMORBIDITIES

Amelia Pantazis[1], Israel Franco[2], Spencer Shain[1], Alexander Fang[3], Steven Friedman[4], Ronnie Fine[1,5], Mark Horowitz[6], Richard Schlussel[7], Lori Dyer[8], Paul Zelkovic[8], Jaime Freyle[4], Richard Shoor[9], Jordan Gitlin[1,5]
[1]NYU Langone Hospital - Long Island, Mineola, NY, USA, [2]Yale School of Medicine, New Haven, CT, USA, [3]SUNY Downstate Medical Center, Brooklyn, NY, USA, [4]Pediatric Urology Associates P.C. - Brooklyn, Brooklyn, NY, USA, [5]Pediatric Urology Associates P.C. - New Hyde Park, New Hyde Park, NY, USA, [6]Pediatric Urology Associates P.C. - Staten Island, Staten Island, NY, USA, [7]Pediatric Urology Associates P.C. - Manhattan, Manhattan, NY, USA, [8]Pediatric Urology Associates P.C. - Tarrytown, Tarrytown, NY, USA, [9]NYU Langone Urology Associates, Smithtown, NY, USA
Presented By: Amelia Pantazis, BS

Introduction & Objective: Traditionally, erectile dysfunction (ED) has been considered a disease affecting older men. Recently, pediatric urologists have seen an increase in adolescent male patients presenting with ED. An extensive literature review reveals a paucity of publications, making management a significant challenge. Our aim was to better understand this population and collate presenting characteristics.
Methods: A retrospective, single practice chart review from 2014-2022 was conducted in adolescent males aged 14 - 21 presenting with a primary complaint of ED. We evaluated mental health status, laboratory values, setting and severity of ED, seasonal timing, age of presentation, BMI, and the number of visits. Statistics were performed using SPSS, including descriptives of the cohort.
Results: 129 males with a mean age of 16.89 ± 1.41 years were evaluated. Mean BMI was 23.5 ± 3.90 kg/m^2. Patients presented with a mean number of 1.2 ± 0.53 visits. New patients increased from 1 in 2014 to 32 in 2020, with an increase during winter months (61%) compared to summer (38%). Quality of erections was reported in 100 (77%) patients. Delayed onset of erection was noted in 9 patients (9%), difficulty of maintenance in 26 (26%), partial in 50 (50%), and failure to achieve in 15 (15%). 84 of 129 patients reported ED setting, noting during intercourse in 33 (39%), masturbation in 29 (35%), and both in 22 (26%). In 25 of 33 patients asked about mental health (77%), a diagnosis of neuropsychiatric conditions included anxiety in 10 (40%), depression in 8 (32%), psychosis in 1 (4%), and combination in 6 (24%). 33 of the 129 patients had an evaluation of total testosterone and prolactin levels. Normal age-range of total testosterone was observed in 31 of 33 (94%). Normal age-range values of prolactin were noted in 25 of 33 (76%) with 8 (24%) having abnormally elevated values.
Conclusion: There has been a recent increase in adolescent males presenting with ED. The majority presented with partial erections. Comorbidity with mental health issues ranged from 19% to 77% when questions about neuropsychiatric conditions were asked. ED presentations increased in winter months, peaking at the start of the COVID-19 pandemic. Laboratory analysis revealed abnormally elevated prolactin levels in 24% of those with values. This characterization may aid in the future clinical management of adolescent patients presenting with ED.

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SCROTAL TRAUMA TREATMENT AND OUTCOMES

Moshe Wald, Tomas Lence
University of Iowa, Iowa City, IA, USA
Presented By: Moshe Wald, MD

Introduction & Objective: Genitourinary tract injuries were reported to account for 3 to 10% of trauma patients, and scrotal trauma is particularly prevalent in males 10 to 30 years of age, thus posing a potential threat to fertility. Scrotal trauma could be blunt or penetrating in nature. The mechanism of scrotal trauma could have an impact on the management and outcomes of this type of injury. Surgical intervention in the setting of scrotal trauma is indicated in cases of disruption of the tunica albuginea with extrusion of testicular tissue, and when doppler study suggests testicular devascularization. While milder cases of tunica albuginea disruption may be surgically repaired with salvage of the testicle, more severe cases of such disruption and cases of testicular devascularization would require an orchiectomy. This study assessed the relative occurrence of blunt and penetrating scrotal trauma, as well as the differences in the management, hospital stay, and need for orchiectomy between these two types of scrotal injury.
Methods: A retrospective chart review was conducted. Charts of adult patients who presented to our institution for scrotal trauma between 1/1/2000 and 6/1/2022 were reviewed. The cohort had 102 patients. Each chart was examined to determine whether the scrotal trauma was blunt or penetrating, the duration of hospital stay, and if an orchiectomy was required.
Results: The average age of patients in the study was 39.5 years (18.7-77.2 years). Of the 102 patients included in the study, 56 had blunt scrotal trauma, and 46 suffered a penetrating scrotal injury. Average hospital stay was 15.6 days (11.1 and 19.6 days in the blunt and penetrating trauma groups, respectively). Total of 61 patients were treated conservatively (44 and 17 patients in the blunt and penetrating trauma groups, respectively). Of the 41 patients who required surgical intervention, 12 had blunt trauma and 29 suffered penetrating injury. 11 patients underwent orchiectomy – 4 from the blunt trauma group, and 7 from the penetrating trauma group.
Conclusion: The occurrence of scrotal trauma in younger males might jeopardize fertility. In our study, blunt scrotal trauma was slightly more common than penetrating injury. Blunt scrotal trauma was associated with a higher rate of conservative management, and fewer patients with blunt trauma required orchiectomy. Further study is needed to better understand the impact of scrotal trauma on future fertility.

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ROLE OF BULBOSPONGIOSUS MUSCLE CUTTING BILATERALLY AND FRENULAR DELTA EXCISION (ALAA AGLAN OPERATION) WITH PENILE IMPLANTS

Alaa Aglan
Alaa Clinic Hospital, cairo, Egypt
Presented By: Alaa Aglan, PhD

Introduction & Objective: In patients with implants the erection time is unlimited but pleasure time is limited till ejaculation . After ejaculation patient enters in sexual death period and complete the intercourse like a robot in a mechanical manner-without sensation just to satisfy the partner so we should elongate the ejaculation time in patients with implants to increase the value and benefits of implantation. So Alaa Aglan operation was done routinely - with few exceptions - with penile implants in our protocol. Alaa Aglan operation is a new procedure consists of two parts. First part is cutting bulbospongiosus muscles bilaterally , And 2nd part is cutting of elliptical part of frenular delta) . it is the most sensitive area in penis and excision of its central part decreases penis sensitivity. The operation has its own prediction test, it is easy just spraying local anesthesia (procomail ®) at coronal ridge (inhibits glans-bulbospongiosus reflex) and at frenular delta (temporary excision). Due to in ability to do prediction test with implant we depended on questionnaire and history of good response to local anesthesia.
Methods: 154 patients were operated between 8/8/2011 and 7/6/2013 with 3 years follow up. Peno-scrotal approach, muscle cutting, penile implantation and frenular delta excision were done.
Results: 153 patients were satisfied by ejaculation time, 6 patients complained from rejection, 5 patients re-implanted , 1 patient refused and discontinued the study.
Conclusion: Alaa Aglan operation is a permanent treatment for premature ejaculation and increases the value of penile implant.

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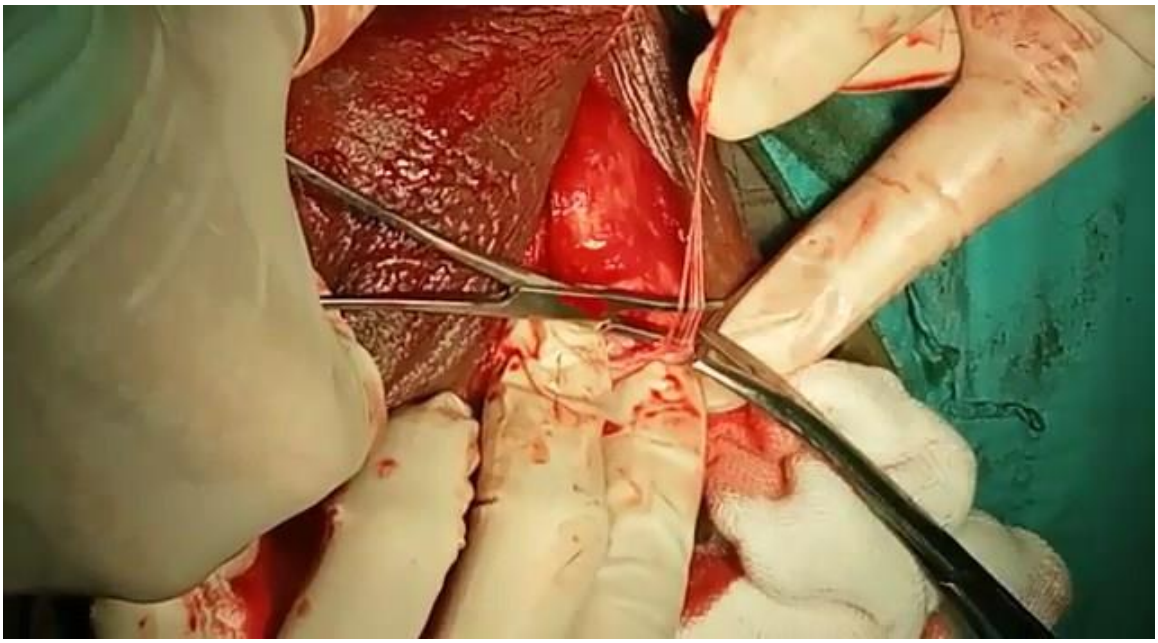
EFFECT OF BULBOSPONGIOSUS MUSCLES CUTTING WITH FRENULAR DELTA EXCISION AND SELECTIVE DORSAL NEURECTOMY FOR TREATMENT OF PREMATURE EJACULATION (ALAA AGLAN3 OPERATION)

Alaa Aglan
Alaa Clinic Hospital, cairo, Egypt
Presented By: Alaa Aglan, PhD

Introduction & Objective: : Alaa Aglan3 operation is a combination of selective dorsal neurectomy,figure1, plus Alaa Aglan operation (bulbospongiosus muscles cutting bilaterally+ frenular delta excision),figure2,3 its prediction test consists of spraying local anesthesia at coronal ridge (inhibits glans bulbospongiosus reflex) and frenular delta area (temporary excision) plus injection of local anesthesia xylocaine 10%® around right or left main dorsal nerve/s (temporary neurectomy).We developed two techniques for permanent neurectomy. In first technique we enveloped the proximal end of nerve by a primary skin graft (cap) with the epithelized area towards the nerve to prevent regrowth (better than silicone cap which we used before. In second technique we cut the nerve and dissect proximal end till penis root and put the whole nerve at penis root with proline suture beside it as a mark. The cutting of nerves in both techniques is near glans as if we need to reverse the technique we can remove the skin cap and dissect the nerve and elongate it to reach glans immediately and in second technique we can identify the nerve at penis root by the proline and dissect it and reattach it to glans area immediately .
Methods: 229 patients were operated between 12/11/2011 and 4/6/2013 with 5 years follow up . Bulbospongiosus cutting ,Frenular delta excision and selective neurectomy (right or left) main dorsal branch were done.
Results: 3 patients reported neuroma treated by trimming, 16 patients reported temporary numbness disappeared within three months and 5 patients were not satisfied .
Conclusion: Alaaa Aglan 3 operation has obvious, quick, and permanent results.



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ACETATE MAINTAINS PENILE HOMEOSTASIS AND ERECTILE FUNCTION IN CYCLOPHOSPHAMIDE-TREATED MALE WISTAR RATS BY TARGETING OXIDATIVE STRESS, INFLAMMATION, AND APOPTOSIS

Damilare Adeyemi[1], Moses Hamed[2], Tunmise Akhigbe[1], Roland Akhigbe[3]
[1]Osun State University, Osogbo, Nigeria, [2]Afe Babalola, Ado-Ekiti, Ekiti, Ado-Ekiti, Nigeria, [3]Ladoke Akintola University, Ogbomoso, Nigeria
Presented By: Damilare Adeyemi

Introduction & Objective: Cyclophosphamide is an alkylating agent that is effective in cancer and immunosuppression therapies. A major shortcoming of this chemotherapeutic drug is its toxic effects on the testis and germ cells via the induction of oxidative stress. However, there is paucity of data on the effect and associated mechanism of cyclophosphamide on penile homeostasis and erectile function. Acetate, on the other hand, has been shown to confer cytoprotection by alleviating oxidative stress. Therefore, this study investigated the effect of acetate in cyclophosphamide-induced penile dyshomeostasis and erectile-dysfunction.
Methods: Forty adult male Wistar rats of comparable weight were acclimatized for two weeks, then randomly assigned into four groups (n = 10). The control rats received 0.5mL of distilled water as vehicle, acetate-treated animals received 200 mg/kg of sodium acetate orally daily for 14 days, cyclophosphamide-treated rats received 150 mg/kg of cyclophosphamide *ip* on day 8, while the acetate + cyclophosphamide-treated rats received treatment as acetate-treated as well as cyclophosphamide-treated. The doses of drugs used were as previously reported for rats.
Results: Acetate treatment significantly attenuated cyclophosphamide-induced reductions in body weight gain and absolute and relative penile weight. Also, acetate prevented cyclophosphamide-induced sexual dysfunction evidenced by improved motivation to mate, post-ejaculatory interval, and mount, intromission, and ejaculation latencies and frequencies. In addition, acetate ameliorated cyclophosphamide-induced reductions in penile reflexes, serum NO and penile cGMP, and suppressed cyclophosphamide-induced increase in penile phosphodiesterase-5, arginase, and acetylcholinesterase activities. The protective activity of acetate on penile homeostasis and erectile function was associated with improved cyclophosphamide-induced reduction in penile activities of superoxide dismutase and catalase, glutathione concentration, and increase in malondialdehyde, TNF-α, IL-1β, MPO and caspase 3 activities.
Conclusion: These findings demonstrated that acetate maintains penile homeostasis and erectile function by preventing oxidative stress, inflammation, and caspase 3-mediated apoptosis.

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ACETATE AMELIORATES DOXORUBICIN-INDUCED SEXUAL AND ERECTILE DYSFUNCTION VIA THE MAINTENANCE OF PENILE HOMEOSTASIS AND ERECTOGENIC ENZYMES

Roland Akhigbe[1], Damilare Adeyemi[2], Moses Hamed[3], Maryanne Akhigbe[2]
[1]Ladoke Akintola University of Technology, Ogbomoso, Nigeria, [2]Osun State University, Osogbo, Nigeria, [3]Afe Babalola University, Ado-Ekiti, Nigeria
Presented By: Roland Akhigbe

Introduction & Objective: Doxorubicin is an anthracycline antibiotic and a chemotherapeutic agent that is effective in the management of hematological and solid tumors. Despite its effectiveness, it is also associated with toxicities of various organs, including the testis through increased oxidative stress and apoptosis. However, there is a dearth of information on the effect and associated mechanism of doxorubicin on penile homeostasis and erectile function. On the other hand, acetate confers cytoprotection by alleviating oxidative stress. Hence, the present study investigated the effect of acetate on doxorubicin-induced sexual and erectile-dysfunction.
Methods: Forty adult male Wistar rats of comparable weight were acclimatized for two weeks, then randomly assigned into four groups (n = 10). The control rats received 0.5mL of distilled water as vehicle, acetate-treated animals received 200 mg/kg of sodium acetate orally daily for 14 days, doxorubicin-treated rats received 7 mg/kg of doxorubicin *ip* on day 8, while the acetate + doxorubicin-treated rats received treatment as acetate-treated as well as doxorubicin-treated. The doses of drugs used were as previously reported for rats.
Results: Acetate treatment significantly attenuated doxorubicin-induced reductions in body weight gain, and absolute and relative penile weight. Acetate treatment also significantly attenuated doxorubicin-induced sexual dysfunction evidenced by enhanced motivation to mate, post-ejaculation interval, and mount, intromission, and ejaculation latencies and frequencies. In addition, acetate inhibited doxorubicin-induced decline in penile reflexes and brain dopamine and serotonin concentrations. More so, acetate blunted doxorubicin-driven increase in penile phosphodiesterase-5, arginase, and acetylcholinesterase activities. Furthermore, acetate improved doxorubicin-induced reduction in penile activities of superoxide dismutase and catalase, glutathione content, and increase in malondialdehyde, TNF-α, IL-1β, IL-6, and NF-kB. The protective activity of acetate on penile homeostasis and erectile function was associated with upregulation of doxorubicin-induced suppression of NO/cGMP signaling.
Conclusion:
The current study revealed that acetate ameliorates doxorubicin-induced sexual and erectile dysfunction via the maintenance of penile homeostasis and erectogenic enzymes.

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SOUTH ASIAN COCKTAIL IMPAIRS SEXUAL AND ERECTILE FUNCTION BY SUPPRESSING CIRCULATING TESTOSTERONE AND CGMP/NO SIGNALING IN MALE WISTAR RATS
Moses Hamed[1], V Ekundina[1], Roland Akhigbe[2]
[1]Afe Babalola University, Ado-Ekiti, Nigeria, [2]Ladoke Akintola University of Technology, Ogbomoso, Nigeria
Presented By: Moses Hamed

Introduction & Objective: The South Asian Cocktail (SAC) is a substance of abuse that is gradually gaining popularity globally. It has been linked with adverse effects such as higher risk behaviour and increased HIV transmission among users. Although its constituent pharmaceuticals (such as codeine, rohypnol, and promethazine) have been associated with increased risk of male infertility, the available data on these constituents are scanty, and there is yet a paucity of data on the impact of SAC on sexual and erectile function. Therefore, we evaluated the effects of SAC and its constituent pharmaceuticals on sexual and erectile function. This is in an attempt to assess the impact and possible associated mechanism of SAC on male sexual function among SAC users.
Methods: Fifty adult male Wistar rats were randomized into five groups (n= 10/group), vehicle-treated (0.5 ml of distilled water), codeine-treated (5 mg/kg of codeine), rohypnol-treated (4 mg/kg of rohypnol), promethazine-treated (50 mg/kg of promethazine), and SAC-treated (5 mg/kg of codeine, 4 mg/kg of rohypnol, and 50 mg/kg of promethazine). The doses are established relevant human doses for rats. Administration was via gavage for 56 days.
Results: SAC and its constituent pharmaceuticals induced sexual dysfunction evidenced by significant reduction in the motivation to mate and mount, intromission, and ejaculation latencies and frequencies, and increased post-ejaculatory interval. Also, SAC and its constituent pharmaceuticals induced erectile dysfunction evidenced by significantly reduced penile reflexes. Furthermore, SAC and its constituent pharmaceuticals reduced penile activities of superoxide dismutase and catalase, and glutathione concentration, and increased xanthine oxidase activity, and uric acid, malondialdehyde, TNF-α, IL-1β, IL-6, and NF-kB concentrations. These findings were associated with marked reduction in brain dopamine and serotonin levels as well as penile cGMP and circulating testosterone and NO, and increased penile phosphodiesterase-5 and arginase activities. These findings were significantly more pronounced in SAC-treated rats when compared to codeine-treated, rohypnol-treated, or promethazine-treated rats.
Conclusion: Summing up, this study revealed that SAC exposure impairs sexual and erectile function via suppression of circulating testosterone and downregulation of NO/cGMP.

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ALTERATION OF NEURAL ANDROGEN RECEPTOR EXPRESSION AFTER CONTUSIVE SPINAL CORD INJURY: ANALYSING THE EFFECT OF MUCUNA PRURIENS (LINN.) USING MALE WISTAR RATS
Seppan Prakash, Khayinmi Wungpam Shimray, Mohammad Zafar Iqbal Khan, Girishya Bollyamoni, Sumi Borah, Sankar Venkatachalam
Department of Anatomy, University of Madras, Taramani Campus, Chennai, India
Presented By: Seppan Prakash, PhD

Introduction & Objective: Introduction: Spinal cord injury (SCI) can lead to various sexual and reproductive disorders in males. Androgens exert a profound organizational effect on the central and peripheral nervous systems. Androgen action on target is

mediated through the androgen receptor (AR), a ligand-dependent transcription factor. Diminished AR expression in neural tissue has been associated with neurodegenerative pathogenesis. *Mucuna pruriens* (*M.pruriens*), is a leguminous plant identified for its aphrodisiac, spermatogenic, androgenic properties and is also used to mitigate neural disorders.
Objective: To analyze the distribution of neural AR in the spinal cord and the dorsal nerve of the penis (DNP) of normal and SCI rats and to evaluate the effect of *M.pruriens* in AR regulation and its functional significance.
Methods: Methods: Male Wistar albino rats (3-4 months & weighing 250 ± 50 g) were used for the study. Following animal, groups were used i.e. Control, SCI, Control+ *M. pruriens* and Control+ *M. pruriens*. SCI at T10-11 junction was created using MACSIS impactor. Animals were subjected to hormonal and mating behavioural analyses under various time points up to 90 days of POP. Animals were euthanized and tissues were processed for routine histology and immune-staining of androgen receptor and S100.
Results: Results: AR expression is predominantly expressed in the grey column of the spinal cord in control and control+ *M. pruriens* groups. AR expression in the grey column was reduced after SCI. No obvious AR expression in white matter in all the groups. Similarly, there was a diminished expression of AR in DNP of SCI rats when compared to control and SCI+ *M. pruriens*. Reduced expression of AR in neuronal tissue suggests a potential pathological sign of neurodegeneration. Concomitantly, the mating behavioural evaluation showed poor sexual function in SCI rats when compared to other groups. Hormone analyses showed reduced testosterone levels in SCI rats when compared to SCI+ *M. pruriens*.
Conclusion: Conclusion: Neural tissue alteration after SCI was dynamic over the period. Administration of *M. pruriens* to SCI rats showed the potential of neural tissue protection, predominately through androgen and AR participation. Consequently, suggesting their active role in neural tissue integrity in normal and pathology.

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SPATIAL TRANSCRIPTOMICS TO DELINEATE CELL-CELL COMMUNICATION IN TESTICULAR GERM CELL TUMOURS

Sarah Moody[1,2], Benedict Nathaniel[1], Daniela Fietz[3], Hans-Christian Schuppe[3], Mark Frydenberg[2,4], Ben Tran[5,6], Kate Loveland[1,2]
[1]Hudson Institute of Medical Research, Clayton, Australia, [2]Monash University, Clayton, Australia, [3]Justus-Liebig University Giessen, Giessen, Germany, [4]Cabrini Institute, Melbourne, Australia, [5]Peter MacCallum Cancer Centre, Melbourne, Australia, [6]Walter and Eliza Hall Institute, Melbourne, Australia
Presented By: Sarah Moody, PhD

Introduction & Objective: The incidence of testicular germ cell tumours (TGCTs), the most common tumour in men aged 19-44, is increasing. Precursor germ cell neoplasia *in situ* (GCNIS) cells arise during fetal life following a failure of germ cells differentiate; GCNIS eventually form seminoma or non-seminoma tumours. What controls GCNIS formation and tumour progression is not understood, though both genetic and environmental factors contribute. We hypothesise that activin A and TGFβ superfamily signaling contribute to tumour development and progression. This study used spatial transcriptomics to identify relevant transcript profiles over different tumour regions, and offers an approach to identifying key cell populations and signalling pathways governing TGCT behaviours.
Methods: Fresh testis tissue from orchidectomies were obtained by consent (three non-seminoma and one seminoma, including tumour-free regions). Tissues were 4% PFA-fixed, paraffin-embedded and sectioned. Nanostring GeoMx Spatial Whole Transcriptome analysis was performed, with 95 regions of interest (ROI) selected following immunodetection of CD45, PanCK, and a nuclear stain. ROIs included normo- and hypospermatogenic tubules, interstitium, immune cell infiltrates, GCNIS, non-seminoma and seminoma. After data QC and normalisation, we mapped the expression profile of key markers and signalling pathway components, and undertook preliminary differential gene expression analyses.
Results: ROIs were identified based on histology and confirmed by identifying transcripts robustly linked with specific phenotypes, e.g. seminoma ROIs expressed *SOX17*. Activin/TGFβ/BMP signaling components were selectively expressed, with *INHA* in interstitium and Sertoli cell only tubules, and *ACVR2B* and *ALK4* (encoding activin A receptors) expressed in seminoma. Interestingly, individual patients had distinct ligand transcript profiles; Patient 1 expressed *BMPs 1*, *4*, *5* and *7*, while Patient 3 expressed *GDF3* and *GDF15* across various ROIs. Two seminoma regions from a single patient were compared to investigate intra-patient heterogeneity, and these exhibited distinct transcript profiles, demonstrated by identification of 352 differentially expressed genes (FDR<0.05, LogFC>1). Strikingly, *KIT*, a common seminoma marker, was differentially expressed, while other markers such as *SOX17* and *POU5F1* were consistent across both regions.
Conclusion: Spatial transcriptomics provides a powerful platform for unbiased transcript analysis, allowing targeted interrogation of multiple signalling pathways. Validation studies are underway in conjunction with tissue culture of fresh testis and tumour tissue from additional samples. These approaches will identify cell communication networks within TGCTs and allow analyses of mechanisms governing tumour phenotypes.

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MTOR MEDIATED REGULATION OF THE BTB PERMEABILITY REGULATES EPIGENETIC AGING OF SPERM

Alexander Suvorov[1], Saira Amir[2], Ekaterina Mironova[3], J. Richard Pilsner[4], Oladele Oluwayiose[5], Joseph McGaunn[1], Olatunbosun Arowolo[1]

[1]University of Massachusetts - Amherst, Amherst, MA, USA, [2]COMSATS University, Islamabad, Pakistan, [3]Moscow State University, Moscow, Russian Federation, [4]Wayne State University, Detroit, MI, USA, [5]NIAID Collaborative Bioinformatics Research (NIH), Bethesda, MD, USA
Presented By: Alexander Suvorov, PhD

Introduction & Objective: Introduction: Advanced paternal age at conception is associated with increased susceptibility to early development of cancer and neurodevelopmental disorders such as schizophrenia and autism in offspring. Emerging evidence links altered offspring phenotype with an age-related accumulation of epigenetic changes in the sperm of fathers. However, the understanding of the molecular mechanisms responsible for the epigenetic aging of sperm remains a critical knowledge gap. We hypothesize, that age-dependent changes in sperm epigenome result from age-dependent deterioration of the blood-testes barrier (BTB) resulting in a biochemically "noisy" environment in the apical compartment of the seminiferous epithelium, where fundamental epigenetic changes occur during spermatogenesis.

Objectives: To test if a decrease or increase of the BTB permeability may slow down or accelerate epigenetic aging of sperm respectively.
Methods: Methods: It was demonstrated recently that BTB permeability is regulated by the balance of the serine/threonine protein kinase mammalian target of rapamycin (mTOR) complexes, whereby mTOR complex 1 (mTORC1) promotes BTB disassembly and mTOR complex 2 (mTORC2) promotes its integrity. To achieve a permanent decrease or increase in the BTB permeability we created transgenic mice with Sertoli-specific inactivation of mTORC1 or mTORC2 respectively. Male reproductive parameters were assessed in KO and WT animals at different time points. Sperm DNA methylation was analyzed using reduced representation bisulfite sequencing (RRBS).
Results: Results: Inactivation of mTORC2 results in increased permeability of the BTB and changes in sperm parameters and mitochondria DNA copy number concordant with accelerated aging phenotype. Most important, physiological age-dependent changes in genome-wide DNA methylation were reversed by mTORC1 inactivation, while mTORC2 inactivation resulted in accelerated epigenetic aging of sperm.
Conclusion: Conclusions: For the first time we describe age-dependent changes in the BTB permeability as a mechanism responsible for age-dependent changes in sperm DNA methylation. Manipulation of the balance between mTOR complexes activity may be used to slow down epigenetic aging of sperm.

Poster 86
ROLES OF CD38 AND LOW NAD IN THE DECLINE OF SPERM DNA INTEGRITY IN AGING MALES
Alexie Zwerdling[1], Mirella Meyer-Ficca[1], Abby Tucker[1], Sierra Lopez[1], Miles Wandersee[1], Ralph Meyer[1,2]
[1]Utah State University, Logan, UT, USA, [2]Utah Agricultural Experiment Station, Logan, UT, USA
Presented By: Alexie Zwerdling, BS, MPH

Introduction & Objective: As the paternal age increases, the genetic integrity of sperm cells declines, along with their ability to support successful pregnancy. Concomitantly, other hallmark signs of aging are decreased plasma levels of nicotinamide adenine dinucleotide (NAD) and increased tissue inflammatory processes. Therefore, we hypothesize that low NAD levels and inflammation are causally linked to poor sperm quality and DNA integrity in aging men. The ecto-enzyme CD38 promotes proinflammatory events by producing cyclic ADP-ribose, and it is considered a major consumer of NAD body-wide due to its NADase activity through the salvage pathway.
Methods: To test our hypothesis, a group of transgenic mouse models (CD38 knockout ($CD38^{-/-}$) mice; acquired niacin dependency (ANDY) mice, in which NAD can be lowered by dietary niacin restriction; as well as $CD38^{-/-}$ /ANDY double transgenic mice) was generated. These animals and appropriate controls were subjected to dietary niacin restriction to create low levels of NAD in adult chronologically young males, in the presence and absence of CD38 expression. Flow cytometry-based terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays were used to quantify DNA strand breaks in sperm of these mice.
Results: Statistical analysis (one-way ANOVA, Tukey's multiple comparison, where $n=4$-5 and $p<0.05$ was considered significant) was performed using GraphPad Prism software version 9.4.1. The results demonstrated that $CD38^{-/-}$ /ANDY double transgenic mice were protected from lowered NAD levels on niacin deficient diets and had significantly less sperm DNA damage compared to $CD38^{+/+}$/ANDY mice under conditions of niacin deficiency ($p = 0.0309$).
Conclusion: The data support the hypothesis that low NAD levels and increased inflammation mediated by the ecto-enzyme CD38 are involved in the deterioration of sperm quality and DNA integrity in the aging male. Further investigation is needed to determine how the CD38 enzyme influences NAD levels in the testis tissues.

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INTERFERON EPSILON: A NOVEL ANTI-VIRAL AGENT IN THE MALE REPRODUCTIVE TRACT
Rukmali Wijayarathna[1,2], Eveline de Geus[1,2], Rosemary Genovese[1], Michelle Tate[1,2], Paul Hertzog[1,2], Mark Hedger[1,2]
[1]Hudson Institute of Medical Research, Mebourne, Australia, [2]Department of Molecular and Translational Sciences, School of Clinical Sciences, Monash University, Melbourne, Australia
Presented By: Rukmali Wijayarathna, PhD

Introduction & Objective: The testis is a reservoir for viruses, including HIV, mumps, Zika and SARS, which can cause persistent infection and infertility. Increased susceptibility of the testis to viruses has been attributed to an inability of spermatogenic cells to produce interferons (IFN) or IFN-induced proteins, required for viral resistance. Challenging this dogma, we discovered that interferon-epsilon (IFNe), a type-I IFN first discovered in female reproductive epithelia, is constitutively expressed by meiotic and post-meiotic spermatogenic cells and testicular macrophages in mice and humans. IFNe was also present in human testicular interstitial fluid. The anti-viral role of IFNe in the reproductive tract was investigated using the Zika virus infection model.
Methods: To investigate IFNe in the mouse, adult wildtype mice (WT), *Ifne*$^{-/-}$ mice lacking IFNe, and *Ifnar1*$^{-/-}$ mice lacking the IFNAR1 receptor subunit required for IFN-signalling, received a single intraperitoneal injection of Zika virus (PRVABC59, $5x10^5$ pfu in saline). Controls received saline only. Reproductive organs were collected 7 days post-infection (peak illness). To investigate IFNe in the human, a human Sertoli cell-line (HSerc, ScienCell™) was used. Cells were treated with 100 IU recombinant human IFNe (rhIFNe), and 12 hours later were infected with 5 or 10 MOI Zika virus to assess a prophylactic role of IFNe. To investigate a therapeutic role, cells were treated with rhIFNe one hour after Zika infection. Cells and media were collected 24 and 48 hours post Zika infection.
Results: Infected WT mice lacked histological evidence of orchitis or epididymitis 7 days post-infection, but infected *Ifne*$^{-/-}$ and *Ifnar1*$^{-/-}$ mice displayed testicular hyperaemia, oedema and immune cell infiltrates. The epididymis of infected *Ifnar1*$^{-/-}$ and *Ifne*$^{-/-}$ mice displayed immune cell infiltrates, epithelial damage, luminal obstruction and fibrosis. Expression of critical Leydig cell (*Cyp11a1, Cyp17a1*) and spermatid genes (*Tnp1*) was also reduced in infected *Ifne*$^{-/-}$ and *Ifnar1*$^{-/-}$ mice. Prophylactic IFNe treatment reduced the viral load by ~98%, measured by qPCR for viral RNA and plaque assays for infectious virus at 24 and 48h post-infection. Therapeutic IFNe treatment reduced viral RNA by ~70% and infectious virus by 97%.
Conclusion: These data indicate that IFNe has a key role in protecting the testis against Zika virus, shifting the paradigm of testicular anti-viral defences, and identifying IFNe as a potential therapeutic and diagnostic target for reproductive tract infections.

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SINGLE-CELL RNA SEQUENCING OF TESTICULAR CELLS FROM NAD+-DEFICIENT MICE REVEALS POTENTIAL MOLECULAR CHANGES ASSOCIATED WITH IMPAIRED SPERMATOGENESIS

Morgan Feuz[1], Alexie E. Zwerdling[1,2], Laura Welch[1], Miles Wandersee[1,2], Mirella Meyer-Ficca[1,2], Ralph Meyer[1,2,3]
[1]Dept. of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT, USA, [2]College of Veterinary Medicine, Utah State University, Logan, UT, USA, [3]Utah Agricultural Experiment Station, Logan, UT, USA
Presented By: Morgan Feuz, MS

Introduction & Objective: Nicotinamide adenine dinucleotide (NAD^+) and $NADP^+$, along with their reduced forms NADH and NADPH, are essential cofactors for many cellular redox reactions as well as for a variety of detoxifying pathways. Research into the functions of NAD^+ has intensified in recent years due to the insight that abnormally low levels of NAD^+ are involved in many human pathologies including metabolic disorders, neurodegeneration, reproductive dysfunction, cancer, and, importantly, aging. Using our transgenic mouse model of acquired niacin dependency (ANDY) and a chemically defined diet, we previously reported that a body-wide decrease in NAD^+ levels of young adult male mice to levels similar to or lower than in old mice resulted in profound phenotypes of aging. Notably, spermatogenesis was significantly disrupted, leading to small testis sizes and reduced sperm counts, reminiscent of age-associated testicular decline.
Methods: Here, we performed 10x Genomics single-cell RNA sequencing (scRNA-seq), coupled with ongoing analysis using CellRanger and Loupe Browser workflows, on testicular cells from either NAD^+-deficient or NAD^+-replete young adult ANDY mice (6-7 months of age) along with old (32 month) mice to investigate the potential underlying mechanisms involved in NAD^+-associated and in aging-associated testicular decline.
Results: Differential gene expression between NAD^+-deficient and old mice compared to the NAD^+-replete controls mostly affected Sertoli cells, and Leydig cells were the second cell type most commonly affected by changes in gene expression. The groups of differentially expressed genes (DEG) in NAD^+-deficient and old mice compared to NAD^+-replete mice shared some similarities, which suggests that aging and NAD deficiency may have a similar impact on the transcriptomes of these cell types.
Conclusion: Our results support our hypothesis that a decline in NAD^+ levels is one of the drivers of the aging-related process of testicular decline.

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RARE HETEROZYGOUS DMRT1 VARIANTS IN SPERMATOGENIC FAILURE AND PRIMARY OVARIAN INSUFFICIENCY (POI) CASES

Helen Madeen[1], Kristiina Lillepea[2], Tihana Marić[3], Anna-Grete Juchnewitsch[2], Margus Punab[2,4], Kristjan Pomm[4], Avirup Dutta[2], Maja Barbalić[5], Daniel Moreno-Mendoza[6], João Gonçalves[7], Alexandra Lopes[8], Davor Ježek[3], Kenneth Aston[9], Kristian Almstrup[10], Ana Katušić Bojanac[3], Maris Laan[2], Donald Conrad[1]
[1]Division of Genetics, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, USA, [2]Chair of Human Genetics, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia, [3]Scientific Centre of Excellence for Reproductive and Regenerative Medicine, University of Zagreb School of Medicine, Zagreb, Croatia, [4]Andrology Clinic, Tartu University Hospital, Tartu, Estonia, [5]Department of Medical Biology, University of Split, School of Medicine, Split, Croatia, [6]Andrology Department, Universitat Autònoma de Barcelona, Barcelona, Spain, [7]Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon, Portugal, [8]Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal, [9]Andrology and IVF Laboratory, Department of Surgery (Urology), University of

Utah School of Medicine, Salt Lake City, UT, USA, [10]Department of Growth and Reproduction, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark
Presented By: Helen Madeen, BA, MS

Introduction & Objective: Non-obstructive azoospermia (NOA) is one of the more severe forms of male infertility, however many cases remain unexplained. DMRT1 is a transcription factor that is important in regulating development and sex determination. Disease-causing variants with autosomal dominant inheritance in *DMRT1* have been linked to many human infertility phenotypes, including disorders of sexual development and NOA. The connection between *DMRT1* variation and primary ovarian insufficiency (POI) in humans has not been determined. This study aimed to understand the effect of rare heterozygous *DMRT1* mutations in patients with spermatogenic failure (SPGF) and POI, and the genetic burden of carrying these mutations.
Methods: We utilized whole exome sequencing from the GEnetics of Male INfertility Initiative (GEMINI) and Estonian Andrology (ESTAND) cohorts; n=1,940 SPGF, n=105 POI, and n=644 normozoospermic controls. To analyze causative *DMRT1* variants, a computational pipeline was developed to filter variants and ACMG guidelines were followed to determine variant pathogenicity. Gene-based burden testing was performed using the GEMINI+ESTAND cases and population-based controls from publicly available data in gnomADv2.1.
Results: We identified an excess of rare potentially pathogenic *DMRT1* variants in the combined male infertility cases (n=14), compared to normozoospermic controls (n=0, Fisher's Exact test $p<0.05$). Burden testing with the larger set of population controls from gnomAD with damaging *DMRT1* variants (n=519/141,456) compared to GEMINI+ESTAND was significant ($p<0.05$). Nine variants, including two in POI cases, clustered in the DNA binding (DM domain) of the DMRT1 protein. Six variants clustered together in two hotspot positions, both of which were highly conserved across species and previously reported as functionally important in the DM domain. We identified three rare *DMRT1* variants in POI cases, a significant enrichment compared to gnomAD (p=7.26E-03). One POI case had a substitution in the DM domain which was also detected in two NOA cases. Phenotypes observed in patients with DM domain variants included smaller testes, spermatogonial arrest, and germ cell neoplasia *in situ* (GCNIS).
Conclusion: These findings increase the understanding of the role of rare heterozygous *DMRT1* variants in male infertility, revealing how missense substitutions in the DM domain could be a dominant cause of male infertility. The connection between *DMRT1* mutations and POI in humans has not been determined and the shared positions between NOA and POI cases is a further avenue for investigation on *DMRT*1 and POI.

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A NOVEL HOMOZYGOUS TOPAZ1 MISSENSE VARIANT HAS BEEN IDENTIFIED IN THREE CONSANGUINEOUS SIBLINGS DIAGNOSED WITH NON-OBSTRUCTIVE AZOOSPERMIA

Rachel Hvasta[1], Christopher Pombar[1], Michelle Malizio[1], Jimmaline Hardy[1], Marta Olszewska[2], Agnieszka Malcher[2], Maciej Kurpisz[2], Kathleen Hwang[3], Kyle Orwig[1], Sidra Qureshi[4], Muhammad Jaseem Khan[4], Alexander Yatsenko[1]
[1]Magee-Womens Research Institute, University of Pittsburgh - School of Medicine, Pittsburgh, PA, USA, [2]Polish Academy of Sciences, Poznan, Poland, [3]Magee-Womens Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA, USA, [4]Khyber Medical University, Peshawar, Pakistan
Presented By: Rachel Hvasta, BS

Introduction & Objective: Non-Obstructive Azoospermia (NOA), hallmarked by the complete absence of spermatozoa in ejaculate, affects approximately 1-2% of males globally with 40% or more cases originating from idiopathic etiology. Underlying genetic factors are often causal in cases without accepted, clinically standard diagnoses for NOA. In three consanguineous Pakistani siblings (proband F_{roh}=0.0373, sibling 1 F_{roh}=0.0303, sibling 2 F_{roh}=0.0562) diagnosed with NOA, Whole Exome Sequencing (WES) analysis was performed to evaluate potential genetic causal factors. A novel *TOPAZ1* homozygous missense variant (p.Ala1560Val; position highly conserved in vertebrates [Fig. 1]) was identified as the most likely causal variant. First identified via Suppression Subtractive Hybridization (SSH), TOPAZ1, which is exclusively expressed in germline cells, may downregulate testis-specific long non-coding RNAs when inhibited, potentially impacting fertility. Following identification via SSH, a *Topaz1* $^{-/-}$ mouse model was created, resulting in azoospermic mice due to meiotic arrest at the diplotene-metaphase I transition. Compound heterozygous variants, including a *De Novo* missense variant, have been previously associated with NOA in one human male.
Methods: DNA was extracted and purified from whole blood of 5 family members (father, mother, proband, and two affected siblings). WES was performed by SOPHiA GENETICS and genotypes were analyzed via Fabric Genomics/SOPHiA DDM softwares. Variants were assessed by *in silico* analysis of protein/RNA tissue expression, RNA single cell expression, predicted protein models, species conservation, existing mouse models/human research, predictive metrics, and minor allelic frequencies (MAFs).
Results: WES analysis revealed 970 variants shared in the family with MAFs <1%. Of these 970 variants, 121 variants were analyzed due to shared genotypes among the 3 siblings; genotypically similar variants in the father were excluded. Analysis of the 121 variants determined the *TOPAZ1* homozygous missense variant in the protein's functional, conserved domain was most likely causal for NOA.
Conclusion: This novel *TOPAZ1* variant would be the first reported homozygous likely pathogenic variant inherited through a familial line. Future implications for this finding include gene therapy efforts to attempt to recover meiotic progression/treat NOA and the creation of models to elucidate the mechanism behind meiotic inhibition. Previously, it was believed that TOPAZ1

contained a PAZ domain; however, this domain is currently in dispute and through homology, it is believed that TOPAZ1 may be a putative aspartate racemase; isolated TOPAZ1 in the presence of aspartate could support aspartate racemase classification.

Figure 1: *TOPAZ1* p.Ala1560 conservation in vertebrates. Complete position conservation observed across 23 species (top to bottom: human, mouse, rat, cow, Rhesus macaque, White-tuffed-ear marmoset, Western lowland gorilla, Cynomolgus monkey, African clawed frog, ghost shark, Cape Golden mole, Tasmanian devil, Atlantic Salmon, Ma's night monkey, Central bearded dragon, Canada lynx, African elephant, Kakapo, pig, dog, cat, horse, and giant panda) created from canonical FASTA sequences aligned through Clustal Omega v1.2.4.

Figure 1: TOPAZ1 p.Ala1560 Conservation in Vertebrates

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INTRACELLULAR SARS-COV-2 IN SPERMATOZOA LEADING TO ETOSIS-LIKE CELL-FREE DNA EXTRACELLULAR TRAPS REVEALED BY TRANSMISSION ELECTRON MICROSCOPY

Jorge Hallak[1], Elia Caldini[1], Thiago Teixeira[1], Maria Correa[1], Amaro Duarte-Neto[1], Fabiola Zambrano[2], Anja Taubert[3], Carlos Hermosila[3], Jöel Drevet[4], Marisa Dolhnikoff[1], Raul Sanchez[2], Paulo Saldiva[1]
[1]Faculdade de Medicina da Universidade de São Paulo, Sao Paulo, Brazil, [2]Universidad de La Frontera, Temuco, Chile, [3]Justus Liebig University Giessen, Giessen, Germany, [4]CNRS-INSERM-Université Clermont Auvergne, Clermont-Ferrand, France
Presented By: Jorge Hallak, MD, PhD

Introduction & Objective: In the human testis, somatic ACE2 receptors, which constitute the docking agent allowing SARS-CoV-2 entry, are found in Leydig and Sertoli Cells, while spermatogonia, spermatids, and ejaculated spermatozoa express germline ACE. Although SARS-CoV-2 is rarely reported in human semen PCR assays, autopsy studies confirm the presence of the virus in all testicular cell types and structures, including Sertoli and Leydig cells, basement membrane, fibroblasts, and vascular endothelium, eliciting a local inflammatory response and generating extensive damage. Our study aims to detail the electron microscopy results of ejaculated spermatozoa from convalescent COVID-19 men.
Methods: This cross-sectional study included eleven confirmed patients with moderate to severe COVID-19 and two patients with mild disease symptoms in an overall cohort of twenty-six men (mean 34.3 ± 6.5 years; range: 21-50 years). The infected patients' clinical scenario required some medical intervention, not severe enough to host them in an intensive care unit. These patients were recruited from the largest tertiary hospital in Latin America. Patients were initially recruited in the acute phase during hospital care and were prospectively followed 30-90 days after diagnosis. Semen samples were obtained by masturbation and processed within one hour following the guidelines of the World Health Organization (WHO) manual (6th edition). All patients tested negative for the presence of SARS-CoV-2 in ejaculate via RT-PCR during hospitalization and recovery. Micrographs were obtained with a Jeol JEM 1010 electron microscope (Tokyo, Japan, 80 kV). The HC-FMUSP Research Ethics Committee approved the study (CAAE registration number 30135720.8.0000.0068), and each participant gave and signed an informed consent form.
Results: In moderate-to-severe scenarios, using EM, we identified SARS-CoV-2 within spermatozoa in eight (61.5%) patients up to 90 days after hospital discharge and in one of two patients with mild COVID-19 disease, comprising nine positive findings out of thirteen patients (69.3%). Moreover, DNA-based extracellular traps (ETs) were reported in eleven (84.6%), including two in which the virus was not identified on EM examination. Decondensed chromatin filaments from a sperm nucleus trapping clusters of virus particles were observed.
Conclusion: intracellular presence of SARS-CoV-2 in germ cells and ejaculated spermatozoa of men with mild to severe COVID-19 up to one hundred and ten days after the initial infection is detected. Suicidal ETosis is one of the mechanisms of cellular damage caused by the virus.

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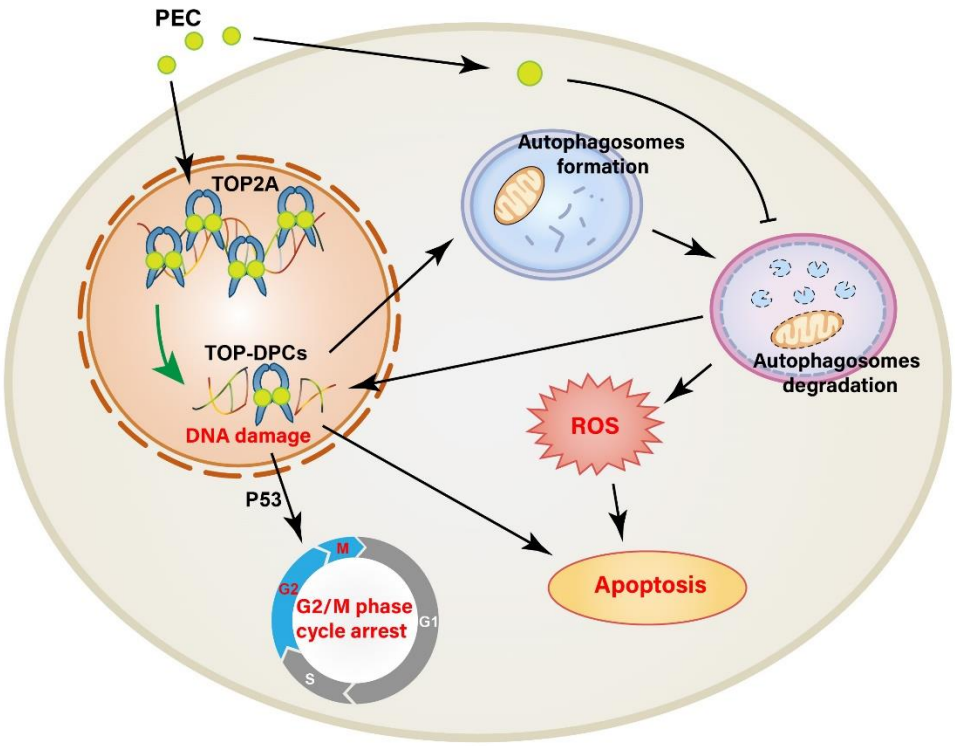
PECTOLINARIGENIN INHIBITS PROLIFERATION OF HUMAN BLADDER UROTHELIAL CARCINOMA PROLIFERATION REGULATING DNA DAMAGE/AUTOPHAGY PATHWAYS

Mengxue Yu, Zhao Deng, Lingao Ju, Xinyue Cao
Zhongnan Hospital of Wuhan University, Wuhan, China
Presented By: Mengxue Yu, MS

Introduction & Objective: Pectolinarigenin (PEC), a natural product isolated from traditional herbal medicine, has shown potential anti-tumor abilities against several types of cancer cells. However, the biological function of PEC in bladder cancer (BLCA) remains obscure.
Methods: MTT and clone formation assay were used to examine cell proliferation. Wound healing and transwell migration assay were used to explore the cell migration ability. Cell apoptosis rates, cell cycle, and reactive oxygen species (ROS) levels were detected by flow cytometry analysis. The gene expression was detected by qRT-PCR. The protein level was detected by western blots, immunofluorescence, and immunohistochemistry (IHC) to explore the mechanism of PEC. Comet assay was used to show the DNA damage. Transmission Electron Microscopy (TEM) was used to observe cellular ultrastructure. Lastly, we used BALB/c-nude mice to construct xenograft mouse models and pulmonary metastasis models.
Results: We first revealed the anti-cancer mechanisms of PEC, which can significantly induce cell apoptosis, G2/M phase cycle arrest, and inhibit late autophagic flux in BLCA. Moreover, we discovered that PEC was one of the TOP2A (DNA topoisomerase II alpha) poisons inducing DNA damage. Simultaneously, the inhibition of autophagic flux further enhanced the DNA damage effect of PEC and led to the accumulation of ROS. In addition, we proved that PEC could intensify the cytotoxic effect of gemcitabine (GEM) on BLCA cells *in vivo* and *in vitro*.
Conclusion: The current findings should be considered the first systematic study to interpret the novel target and mechanism of PEC in treating BLCA and presented a promising prospect for future BLCA treatment.



Schematic illustration of the study

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AN EPIGENETIC PERSPECTIVE ON COVID-19 INFECTION AND MALE REPRODUCTIVE HEALTH

Isaac Stirland, Lily Millar, Berlin Barnett, Timothy Jenkins
Brigham Young University, Provo, UT, USA
Presented By: Isaac Stirland, BS

Introduction & Objective: The rate of COVID-19 infection has driven concerns of potential sequel in various areas of human health. While many symptoms of infection have been characterized, much remains unknown about the lingering effects of COVID-19, especially in relation to male reproductive health. This study analyzes the sperm epigenetic profiles of 32 COVID-19-infected and 32 normal seminal DNA samples. Through this assessment we analyzed the impact of COVID-19 specifically on the male gamete to explore the potential for both the direct and transgenerational impacts of infection.
Methods: Human DNA was extracted from lysed spermatozoa, bisulfite converted, and analyzed on Illumina's Human MethylationEPIC array. In each sample more than 850,000 CpG sites were probed to produce an equal number of fraction methylation (beta) values. Global methylation profiles for each sample were then constructed using these generated beta values. A series of bioinformatic analyses, including differential methylation analysis, the Horvath and Jenkins age prediction models, as well as epigenetic instability analyses were conducted to comprehensively explore differences in methylation profiles between groups.
Results: While differences were observed between groups at a global level, all regional tests comparing epigenetic age, instability, global, and regional characteristics between COVID-19-infected and normal groups failed to achieve significance. These results suggest that while methylation patterns differ slightly between groups there is no associated impact on cell health or epigenetic vitality. Of importance is the persistence of epigenetic similarity between groups in samples collected as early as 0.5 and as late as 15.5 months post infection.
Conclusion: The combined results of this study strongly suggest that COVID-19 infection has a negligible effect on the methylation patterns and thus the epigenetic vitality of human sperm cells. Methylation patterns at multiple genomic loci remain unchanged in COVID-19-infected spermatozoa when compared to healthy controls. Our data are limited to an assessment of covid infection alone and should not be used to make further inference that COVID-19 infection has no impact on male reproductive health. From an epigenetic perspective, human spermatozoa from COVID-19-infected individuals are indifferentiable from a normal cohort and display substantial resilience to genetic alterations throughout the first 15.5 months directly following infection.

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MICROBIOLOGICAL FLORA AND ANTIBIOTIC RESISTANCE IN GENITOURINARY TRACT INFECTION OF MEN WHO PRACTICE ANAL INTERCOURSE

Igor Coimbra, Pedro Zandona, Thiago Teixeira, Adriana Stelati, Eduarda Goes, Lucas De Lima, Pedro Rodrigues, Jorge Hallak
Hospital das Clínicas FM USP, São Paulo, Brazil
Presented By: Igor Coimbra, MD

Introduction & Objective: Anal intercourse is a usual sexual practice with divergent prevalence in different countries, cultures, and religions. Because of the intestinal flora, men who engage anal sexual activity are at risk of genitourinary tract infection caused by a broader spectrum of bacteria, especially *Enterobacteriaceae.* Most of the current prevalence studies derive from samples of men who have sex with men, excluding heterosexual couples. We aim to report microbiological flora and antibiotic resistance findings in cultures of men with genitourinary tract infection between men who do and do not practice penetrative anal intercourse.
Methods: We have analyzed 901 culture samples, including urinary midstream culture, seminal culture, urethral swab, and urethral discharge belonging to 438 men, tested in the last 11 years in a specialized andrology lab. We then calculated the odds ratio of each microorganism infection and the sensibility to various types of antibiotics between men with and without anal sexual intercourse in different age groups.
Results: In our study we have not found a significant difference between etiology agents of genitourinary tract and male accessory gland infection in men who do not have anal intercourse and those who do with or without the use of preservatives. Respectively, in these groups, the most prevalent bacteria were Staphylococcus sp (38,1% x 43,3% x 40,1%) Enterobactariaceae (34,2% x 32,8% x 38,0) E. Coli (7,9% x 0% 6,3%%) and Klebsiella sp (6,2% x 4,4% x 4,9%). Multiple antibiotics were tested. Cloranfenicol was shown to have a lower resistance in the non-anal intercourse groups, when compared to men who have intercourse without condoms (OR 0,418 IC 0,244-0,716 $p<0,05$). Gencitabin and Cefalotin also show a trend towards lower resistance in the non-anal intercourse group (OR 0,545 IC 0,289-1,027 $p=0,06$ and OR 0,143 IC 0,016-1,265 $p=0,08$).
Conclusion: In our retrospective study we have not found a significant difference between the prevalence of different bacteria in the groups analyzed and only Cloranfenicol was seen to have a higher resistance in the anal sex groups.

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ASSISTED REPRODUCTION PROGRAM FOR HIV-INFECTED MEN DURING THE COVID-19 PANDEMIC

Ann Kiessling[1], Alexander Hauser[1], Matthew Antonellis[1], Typhonie Oun[1], Maureen Kearnan[1], Jeffrey Steinberg[2], Ryan Kiessling[1]
[1]Bedford Research Foundation, Bedford, MA, USA, [2]Milton Hospital, Milton, MA, USA
Presented By: Ann Kiessling, PhD

Introduction & Objective: The Special Program of Assisted Reproduction (SPAR) screens semen specimens from HIV-infected men for the presence of HIV and cytomegalovirus (CMV) to eliminate infectious specimens from cycles of assisted reproduction. If gestational carriers (GC) are needed for the pregnancy, FDA-required blood and urine tests are obtained within seven days of the semen specimens, and the GCs are counseled independent of the intended parents, the fertility clinic and the surrogacy

agency. Many SPAR clients are international. We have analyzed SPAR data during the 23 months of COVID-19 pandemic "lockdown" era, April 1, 2020 through March 1, 2022.
Methods: HIV-infected men and female partners or GCs are counseled that approximately 15% of semen specimens from men on successful anti-viral therapy with an undetectable burden of virus in blood plasma test RT-PCR positive for HIV. Since half the semen specimen is used for the PCR analysis, the men produce two to three specimens for testing. Men using a GC for pregnancy travel to the SPAR lab in person to ensure compliance with FDA paired testing requirements. During the COVID19 travel restrictions, two Andrology labs in Europe established programs of semen collection and blood/urine collection, with an aliquot of semen over-night couriered to the SPAR lab for RT-PCR testing; those SPAR consults were conducted by Zoom. GC counseling was unchanged, described the FDA requirements, and the SPAR program requirements for HIV-antibody testing following the embryo transfer.
Results: Twenty three Zoom consults were completed with international clients, a total of 399 semen specimens (95 international) from 147 HIV+ men (approximately 40% of the pre-covid 23 months) on successful anti-viral therapy (33 international) were RT-PCR tested for HIV and CMV; of those 46 (12%) tested positive for HIV and 124 (31%) tested positive for CMV. Testing results were comparable between the domestic and international specimens During the same time frame, 43 babies were born to SPAR clients, 101 surrogates were counseled, 82 surrogates and female partners underwent embryo transfers, none resulting in either HIV or CMV infection and 32 pregnancies were reported.
Conclusion: Travel restrictions and lockdowns decreased the number of assisted reproduction cycles for HIV-infected men, but that population was not more affected by COVID-19 disease than the non-HIV-infected population.

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ADVERSE HISTOLOGICAL CHANGES IN THE TESTIS OF ADULT MALE MICE EXPOSED TO INCREASING PERIODS OF SOCIAL ISOLATION
Luiz Gustavo Fressatti[1], Diego Ribeiro[1], Sanseray Cruz-Machado[1], Marcella Santiago[2], Joel Gabrili[1], Juliana Perobelli[2], Maria Christina Avellar[1]
[1]Universidade Federal de Sao Paulo - Escola Paulista de Medicina, Sao Paulo, Brazil, [2]Universidade Federal de Sao Paulo - Instituto do Mar, Santos, Brazil
Presented By: Diego Ribeiro

Introduction & Objective: Social isolation (SI) in experimental animal models is an important tool for deciphering various neuropsychiatric disorders. The effects of SI on male reproductive function are still largely unknown. Recently, our group has shown that SI of adult mice for 30 days places the epididymis in an inflammatory state that delays epididymal sperm transit time and decreases caudal sperm motility. Given the importance of this issue in andrology, the aim of this study was to investigate the effect of increasingly prolonged SI on testicular and epididymal morphology using the same preclinical model in mice.
Methods: Male Swiss mice (60 days) were kept in groups (N=4/cage; control) or individually (N=1/cage; social isolation) for 2, 15 or 30 days. Body mass was monitored during the experiments. After euthanasia, testes and epididymides were collected, weighed and processed for histopathology (Bouin's fluid - 24 h). Histopathological and stereological analyses were performed on HE-stained tissue sections (5 μm). Testicular and epididymal photomicrographs were taken and blind assessments performed.
Results: Mice housed individually and in groups had comparable body mass and testicular, epididymal and adrenal mass at all time points of SI examined. Cross sections of testicular tubules from group-housed and socially isolated mice showed no marked difference in the number of normal seminiferous tubules (presence of concentric and normally organized germ cell layers and Sertoli cells) nor in the frequency of abnormalities commonly found in seminiferous tubules/epithelia (presence of cell debris in the lumen, multi-nucleated formation, vacuole formation, epithelial degeneration, cell death). Morphometric and stereological analysis of testes from 15- and 30-day isolated mice showed a smaller tubular diameter and a larger area occupied by the interstitium, displaying vascular changes (greater number of blood vessels) compared with corresponding controls. Epididymal histopathology showed signs of inflammatory infiltrate and other epithelial changes (cell vacuolization, clear cell numbers, luminal cell debris) as early as 2 days of SI, with changes becoming more evident as SI progressed.
Conclusion: Our data suggest differential histopathological changes in testicular and epididymal architecture when adult male mice are increasingly exposed to SI. Using this preclinical experimental model, our group is unraveling how this prolonged stress can compromise testicular and epididymal function and thus male fertility. Ethics Committee approval #3228110520/CEUA-Unifesp. Financial support: CNPq, CAPES, CAPES-Print, and FAPESP (2020/06364-8).

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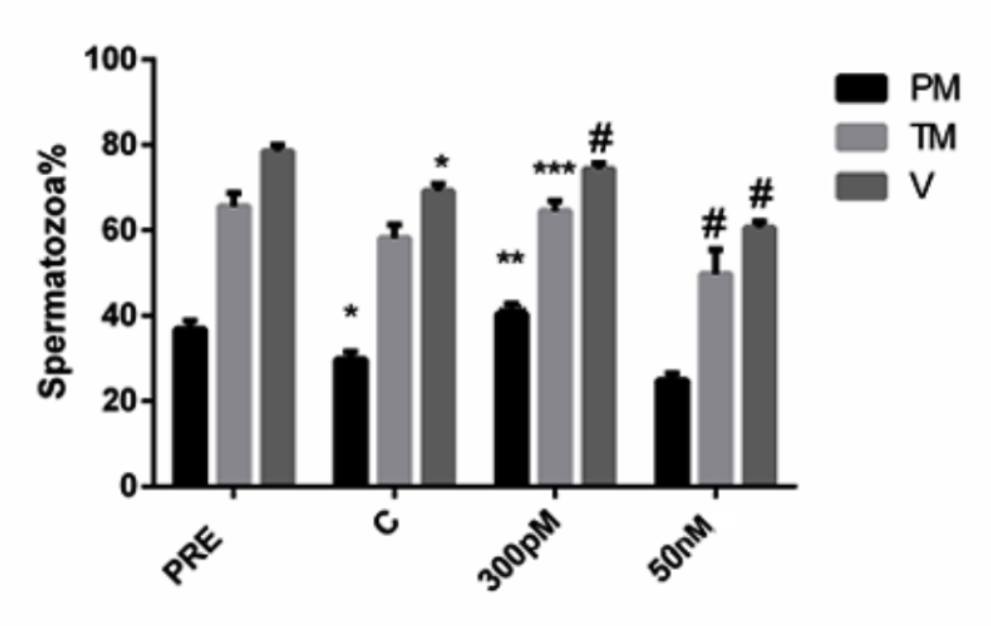
THE CONCENTRATION OF GLUCOSE IN THE MEDIA INFLUENCES THE SUSCEPTIBILITY OF STALLION SPERMATOZOA TO FERROPTOSIS
Francisco MARTIN CANO, Gemma Gaitskell-Phillips, Cruz Gil-Anaya, Cristina Ortega Ferrusola, Fernando Peña
UNIVERSIDAD DE EXTREMADURA, CACERES, Spain
Presented By: Fernando Peña, DVM, PhD

Introduction & Objective: Stallion spermatozoa depend on oxidative phosphorylation as the major source of ATP. The composition of the media to which the spermatozoa are extended or exposed in the mare's reproductive tract exerts a profound effect on sperm function and even may accelerate cell demise. Recent research indicates that high concentrations of glucose in the

media, although common in commercial extenders, may be detrimental. To determine if supraphysiological glucose concentration may induce or predispose to ferroptosis, aliquots of stallion ejaculates were incubated under different concentrations of glucose, 67mM (HG) or 1mM plus 10mM pyruvate (LG-HP), in the presence or not of known inductors of ferroptosis.
Methods: Semen was collected from 3 fertile Andalusian stallions routinely used as semen donors in our center Stallions were maintained according to the European Directive 2010/63/EU.To test the hypothesis that the composition of the media influences the susceptibility of spermatozoa to ferroptosis, aliquots of the same ejaculate we extended in HG and LG-HP media in presence of two inductors of ferroptosis: Erastin (40microM), an inhibitor of the SLC7A11-xCT antiporter, and RSL3 (10microM) and inhibitor of the glutathione peroxidase 4, GPX4. After 3 hours of incubation at 37 ºC samples were analyzed by flow cytometry. The percentage of necrotic/ferroptotic spermatozoa was assessed using Viakrom 808, considering ferroptotic/necrotic spermatozoa those dead spermatozoa negative for caspase 3.
Results: Storage and incubation of spermatozoa under high glucose concentration led to an increase in the percentage of necrotic spermatozoa ($P<0.001$). Moreover, ferroptosis was more intensely induced in sperm in media with high glucose concentrations. Finally, we observed that induction of ferroptosis modified two proteins (oxoglutarate dehydrogenase and superoxide dismutase-2) in spermatozoa incubated in media containing 67mM glucose, but not in media containing 1mM glucose and 10 mM pyruvate. It is concluded that the composition of the media exerts a major impact on the functionality and lifespan of the spermatozoa, and the results reported here may pave the way for the improvement of current semen extenders.
Conclusion: We provide evidence of ferroptosis in the spermatozoa, supporting the hypothesis that different forms of cell death may coexist in the ejaculate. Also, the concentration of glucose may affect the susceptibility of spermatozoa to ferroptosis induced cell death; strategies to improve the efficiency of glycolysis may expand the lifespan of the spermatozoa

Poster 98
EFFECT OF GLP-1 (GLUCAGON -LIKE-PEPTIDE-1) ON MODULATE HUMAN SPERM CELL SIGNALING CASCADES
Roberto Castiglione[1], Saveria Aquila[2], Rosario D'Agata[1]
[1]University of Catania, Catania, Italy, [2]University of Calabria, Cosenza, Italy
Presented By: Roberto Castiglione, PhD

Introduction & Objective: Glucagon-like peptide-1 (GLP-1) is an incretin hormone released by enteroendocrine L cells in the intestine in response to food ingestion, and GLP-1 effects first include higher insulin release in response to glucose (*Drucker, 2015*). However, the presence of this receptor is not limited to this organ, as it is expressed in the heart, lungs, kidneys, and adipocytes (*Pyke et al., 2014; Waser et al., 2015; Abu-Hamdah et al., 2009*). Recent evidence suggests that the male gonad is a potential target of GLP-1 action). Recently, we detected the receptor for GLP-1 in human sperm and demonstrated that activation of this receptor is important in the regulation of sperm homeostasis by acting on different metabolic branches (*Rago et al., 2020). In vitro* findings strengthen the important role of insulin in sperm physiology: insulin is expressed and secreted by human spermatozoa, provides autocrine regulation of sperm metabolism (*Shokri and Aitken, 2012).*
Methods: We collected sperm from normozoospermic and pathological subjects with grade III varicocele of the left testis. Semen samples were incubated for different times in the presence of a dose of a GLP-1 mimetic analog, exendin-4 (Exe). Normozoospermic and pathological semen was used for immunohistochemical localization of insulin receptor substrate-1 (IRS-1) and Jun N-terminal protein kinase (JNK). Sperm parameters and three kinases, phosphorylated-Akt (p-Akt) (as Protein kinase B), phosphorylated IRS-1 (p-IRS-1 Ser 312) (as Insulin receptor substrate 1 Serin 312), and phosphorylated JNK (p-JNK Thr183/Tyr185) isoforms, were considered and evaluated.
Results: Sperm parameters declined progressively with incubation time. The maximum decline was associated with a significant decrease in phosphorylated p-Akt concomitantly to an increase in p-IRS-1 (Ser312) phosphorylation and p-JNK isoforms (Thr183/Tyr185). Pre-incubation with Exe prevented this decline and maintained sperm motility.
Conclusion: A decline in sperm parameters with time was associated with a decrease in p-Akt and an increase in p-IRS-1 Ser312 and p-JNK isoforms. In contrast, increased phosphorylation of p-IRS-1 (Ser312) and p-JNK(Thr183/Tyr185) are important elements involved in sperm performance alteration. GLP-1 also acts by reducing levels of the negative kinases IRS-1 phosphorylated at Ser312 and p-JNK isoforms.



Total motility (TM), progressive motility (PM) and vitality (V) are plotted after Exe exposure. All data presented are mean ± SEM of multiple experiments. The results were analyzed by one-way ANOVA ($p<0.05$) and post hoc comparison of means was determined using Fisher's PLSD test. Sperm parameters: *$p<0,005$ vs PRE; **$p<0,0006$ vs C; ***$p<0,004$ vs C; # $p<0.01$ vs C. Kinases *$p<0.05$ vs PRE; **$p<0.002$ vs C; ***$p<0.01$ vs C; a $p< 0.05$ vs C. PRE parameters before incubation; C untreated samples.

Sperm motility plotted after Exe exposure

Poster 99

A SPERM ASSAY PREDICTIVE OF IVF (WITHOUT ICSI) CYCLE FAILURE

Zimu Chen[1], Barb Cohen[1], John Walsh[1], Erin Schnellinger[2], Paul Turek[3]
[1]Arex Life Sciences, Watertown, MA, USA, [2]United Network for Organ Sharing, Richmond, VA, USA, [3]The Turek Clinic, Beverly Hills, CA, USA
Presented By: John Walsh, PhD

Introduction & Objective: Freshly ejaculated mammalian sperm have poor fertilizing ability; fertility is gained only after sperm undergo capacitation and the acrosome reaction. We have discovered that these maturation processes occur in sequential waves and among synchronized cohorts of sperm and not randomly among individual sperm leading to egg fertilization by vanguard sperm. This has been termed the "sperm fertility cycle." We designed an assay to visualize sperm maturation waves by identifying exposed molecules (Fc receptor) in the acrosomal matrix and on the inner membrane. We sought to understand whether knowledge of individual sperm fertility cycles could predict the success of in vitro fertilization (IVF) cycles among infertile couples.
Methods: The Arex Assay of functional Fc receptor levels (FcR) was performed on inseminating semen samples among infertile couples undergoing IVF at a North American fertility center. Briefly, 5ul aliquots of semen were analyzed at 30-min intervals to determine the proportion of sperm that were expressing FcR and the temporal changes in FcR expression levels were analyzed. Semen samples were categorized as either "cycling" or "non-cycling," depending on FcR expression patterns. Knowledge of Arex Assay findings was not used to alter the timing of IVF procedures. IVF success was defined as the development of embryos to a stage suitable for freezing and transfer. Failure was defined as an IVF procedure resulting in no frozen or transferable embryos. Notably, ICSI was not used for any of these IVF cycles.
Results: Among n=35 IVF cycles performed in 35 couples, 28/35 (80%) were successful while 7/35 (20%) had failed IVF, according to our criteria. Among successful IVF cases, 23/24 (96%) were associated with cycling sperm. Importantly, among IVF failures, 6/7 (86%) were associated with non-cycling sperm. The odds ratio for reproductive outcome among cycling vs non-cycling semen specimens was 27.6 (95% CI: 2.25, 1321.8). p= 0.0017; Fisher's exact test).
Conclusion: In this pilot study, IVF procedures performed with "cycling" semen specimens resulted in statistically significantly higher success rates than those performed with non-cycling specimens. The Arex Assay has the potential to characterize the quality of sperm maturation processes and can be used to classify semen samples as either "fertile" or "infertile" based on IVF (without ICSI) outcomes.

Poster 100

THE SPHINGOSINE 1-PHOSPHATE SIGNALING PROMOTES NITRIC OXIDE PRODUCTION AND CAPACITATION IN HUMAN SPERMATOZOA

Steven Serafini[1,2,3], Cristian O'Flaherty[1,2,3,4,5]
[1]Departments of Medicine (Experimental Medicine Division) - McGill University, Montréal, QC, Canada, [2]Departments of Surgery - McGill University, Montréal, QC, Canada, [3]The McGill University Research Institute, MUHC, Montréal, QC, Canada, [4]Anatomy and Cell Biology - McGill University, Montréal, QC, Canada, [5]Pharmacology and Therapeutics - McGill University, Montreal, QC, Canada
Presented By: Steven Serafini, BS

Introduction & Objective: ~15% of couples worldwide struggle with infertility, and 50% of these cases are due to a male-related factor. The causes of male infertility are unknown in 34% of the cases. Assisted reproductive techniques (ARTs) (e.g., IVF and ICSI) are available to treat infertility but are costly, have low efficiency (~30%) and are linked to complications in the offspring. Sperm capacitation is the process that the spermatozoon must undergo to fertilize the oocyte. The role of lipid signaling in sperm capacitation is unknown. Sphingosine (Sph), ceramide (Cer), and their phosphorylated forms S1P and C1P promote nitric oxide ($NO^{\bullet}$) production in endothelial cells. $NO^{\bullet}$ production is downstream of the PI3K/AKT pathway in sperm capacitation, but its regulation is unknown. We hypothesize that Sph, Cer, S1P and C1P regulate tyrosine (P-Tyr) and PI3K (P-PI3K) phosphorylations and $NO^{\bullet}$ production needed for sperm capacitation. Our objectives are: 1) To determine whether Sph, Cer, S1P and C1P promote sperm capacitation and its associated phosphorylations and $NO^{\bullet}$ production. 2) To determine the presence of S1P1 receptor (S1PR1) and SphK1 in spermatozoa and whether the S1PR1 signaling activates capacitation-associated phospho-proteins and $NO^{\bullet}$ production.
Methods: Highly motile human spermatozoa were incubated in BWW medium for 4h at 37°C, with or without our control capacitation inducer Fetal Cord Serum ultrafiltrate (FCSu), and with or without Sph or Cer and PF453, NVP231, and VCP23019, L-NAME, inhibitors of SPHK1, CERK, S1PR1, and nitric oxide synthase (NOS), respectively. P-Tyr and P-PI3K levels were assessed by immunoblotting. S1PR1 and phospho-SphK1 (P-SPHK1) were localized in spermatozoa using immunocytochemistry.
Results: Sph- and Cer-treated spermatozoa have increased P-Tyr compared to non-treated controls. Inhibition of SPHK1 and CERK decreased P-Tyr and P-PI3K in FCSu- or Sph- and Cer-treated spermatozoa compared to controls. S1PR1 and P-SPHK1 were localized in the post-acrosomal region in capacitated spermatozoa. P-SPHK1 was higher in capacitated compared to non-capacitated controls. Inhibition of S1PR1 decreased P-Tyr and P-PI3K compared to non-treated controls. Inhibition of NOS decreased P-Tyr in Sph- and Cer- treated compared to non-treated samples, indicating $NO^{\bullet}$ is produced downstream of the S1P pathway.
Conclusion: S1P signaling is necessary to activate PI3K and subsequent $NO^{\bullet}$ during capacitation, and its dysfunction could be a cause of male infertility. These studies can allow the development of novel diagnostic tools and treatments for male infertility.

Poster 101

EVALUATION OF SPERM CAPACITATION STATUS FROM SWIM-UP IN DIFFERENT MEDIA USING BOTH FRESH AND FROZEN SAMPLES

David Pening, Isabelle Demeestere, Pascale Lybaert
Laboratoire de Recherche en Reproduction Humaine - Université Libre de Bruxelles, Brussels, Belgium
Presented By: David Pening, MD

Introduction & Objective: Recent data from meta-regression analysis shows that sperm count has declined over the last 50 years and the prevalence of male infertility has risen. The WHO semen analysis remains a poor predicator of the sperm fertilizing ability. Additional sperm parameters need to be developed to refine the diagnosis of male infertility such as sperm capacitation, a physiological process characterized by membrane hyperpolarization and intracellular alkalinisation. The main objective of this study is to assess membrane potential from normozoospermia samples prepared in different swim-up media.
Methods: We included patients < 60 years old with normal 2021 WHO criteria for semen analysis. Exclusion criteria are male infertility of known cause, hypogonadism, cystic fibrosis, chromosomal aberrations and diseases treated by gonadotoxic drugs. We compared sperm prepared by swim-up in two different media (Sperm Medium® and Human Tubal Fluid) to assess capacitation status by measuring membrane potential using both spectrofluorimetry and flow cytometry (FACS) from fresh and frozen samples.
Results: We analyzed and compared the mean membrane potential of a sperm population from the same sperm donor after swim-up in Sperm Medium® (SM) or HTF media. The mean membrane potential (Em) was -80.1 ± 4.9mV using spectrofluorimetry for fresh sperm samples after swim-up in SM and -82.3 ± 0.18mV using FACS. Similarly, a mean Em of -74.3 ± 1.2mV was obtained for frozen samples after swim-up in SM, using spectrofluorimetry and -74.9 ± 4mV using FACS. After swim-up in HTF, a mean Em of -62.7 ± 4.4mV was obtained for fresh samples using spectrometry and -70.3 ± 4.4mV using FACS. Frozen samples in HTF gave a mean Em of -75 ± 3.6mV using spectrometry and -65.1 ± 5.8mV using FACS. The sperm membrane potential was statistically more hyperpolarized for samples prepared in SM versus HTF ($p=0.03$). No significant differences were observed between the measuring techniques (spectrometry versus flow cytometry).
Conclusion: Defining an appropriate media for sperm swim-up to promote capacitation is utterly important. Sperm Medium® appears to achieve a more hyperpolarized status than the HTF media for sperm swim-up. In this context, we will pursue the study to determine if the average membrane potential of the sperm sample can be associated with high or low fertilization rates in IVF/ICSI.

Poster 102

SERUM LIPID PROFILE LEVELS AND SEMEN QUALITY: NEW INSIGHTS AND CLINICAL PERSPECTIVES FOR MALE INFERTILITY AND MEN'S HEALTH

Gustavo Andrade[1], Ivan Iori[1], Mariana Hsieh[1,2], Giovanna Milani[1], Pedro Zandoná[1,3], Thiago Teixeira[1,3,4,5], Joël Drevet[6], Elaine Costa[3,4], Jorge Hallak[1,3,4]

[1]Androscience - Science and Innovation Center and High Complexity Clinical and Research Andrology Laboratory, São Paulo, Brazil, [2]Faculdade Israelita de Ciências da Saúde Albert Einstein School of Medicine, São Paulo, Brazil, [3]University of São Paulo, São Paulo, Brazil, [4]Men's Health Study Group, Institute for Advanced Studies, São Paulo, Brazil, [5]Federal University of Amapá, Macapá, Brazil, [6]GReD Institute, CNRS-INSERM-Université Clermont Auvergne, Clermont-Ferrand, France

Presented By: Jorge Hallak, MD, PhD

Introduction & Objective: Several clinical scenarios and sperm structural and functional variables determine the final ejaculated semen quality, which is a crucial element in the success of a pregnancy. Sperm motility and sperm plasma membrane fusogenic activity, which determines reproductive success, primarily rely on the peculiar sperm lipid composition, which is influenced by the patient's metabolism, genetics, nutritional, environmental status. This study aimed to determine whether there is a concrete relationship between serum lipid profile and semen quality and between serum lipid profile and total and free testosterone.

Methods: This retrospective study uses medical charts of 278 infertile men who attended andrological care between 2000 and 2019. Seminal analysis data, lipid profile, and total serum testosterone were collected. A multiple linear regression analysis was performed to evaluate the influence of the lipid parameters on the seminal variables. Statistical analyses were carried out with $p \leq 0.05$ considered statistically significant.

Results: Seminal creatine kinase activity (p=0.024) is negatively related to HDL (p=0.032) and triglycerides (p=0.037) while total testosterone (p< 0.0001) and seminal volume (p=0.046) appeared both to be negatively related to triglycerides (p = 0.030 and p=0.033, respectively). See Table 1.

Conclusion: Medical advice commonly advocated to prevent cardiovascular disease and improve HDL cholesterol and triglyceride levels in dyslipidemic patients should also be given to infertile men. Physicians should provide patients with a thorough assessment, including the blood lipid profile, hormonal status, and routine seminal examinations. We propose a more comprehensive men´s health checkup for the infertile male population, not limited to a simple evaluation of basic sperm parameters.

Table 1:

Predictors (constant): TG, LDL, HDL, VLDL

Dependent variable: each parameter evaluated.

Evaluated parameter	HDL	LDL	VLDL	TG
	P	*p*	*p*	*p*
semen volume	0.298	0.449	0.105	**0.033**
creatine kinase (CK) activity	0.032	0.289	0.208	**0.037**
Total testosterone	0.511	0.801	0.322	**0.030**

Table 1

Poster 103

SPERM DNA METHYLATION ALTERATIONS AS A FUNCTION OF DNA DAMAGE MEASURE BY BOTH COMET AND TUNEL

Hailey Johnson, Chad Polard, Tim Jenkins

Department of Cell Biology and Physiology, Brigham Young University, Provo, UT, USA

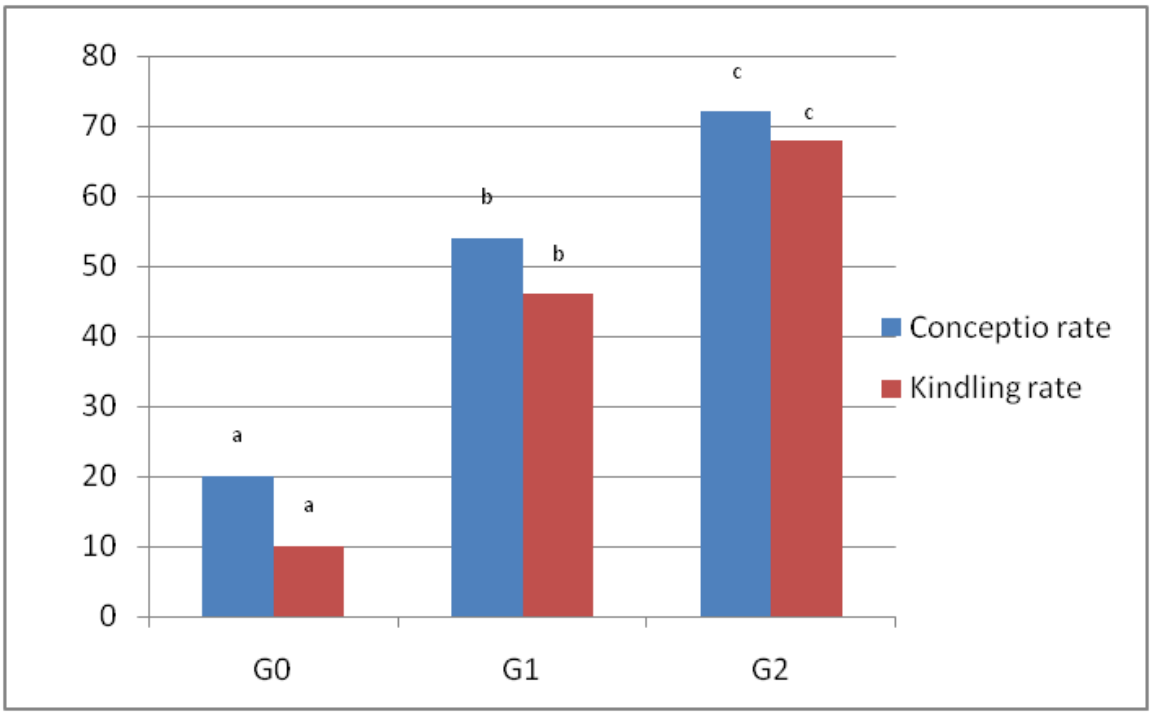
Presented By: Hailey Johnson

Introduction & Objective: It has been suggested that DNA damage correlates with global DNA methylation patterns in the sperm methylome. To more comprehensively assess this relationship, we assessed the relationship between DNA damage (as

measured by both Comet and TUNEL) and sperm DNA methylation with CpG resolution data (array) in the largest sperm DNA methylation dataset currently available (FAZST dataset).
Methods: Over 1,400 sperm DNA methylation array samples were obtained from the previously published FAZST study. Samples were stratified by both Comet and TUNEL (DFI) scores and groups were produced based on the highest and lowest values; high comet (n = 103), low comet (n = 148), high DFI (n = 104), and low DFI (n = 150). These groups were produced to facilitate various analyses assessing differential methylation, epigenetic age, and epigenetic instability as a function of DNA damage measured by TUNEL or Comet. Further, we were able to compare the TUNEL and Comet measures themselves and their correlations to DNA methylation alterations.
Results: Regional differential methylation analysis revealed 43 significantly altered regions (42 hypermethylated; 1 hypomethylated) between the high and low Comet categories and 17 significantly altered regions (12 hypermethylated; 6 hypomethylated) between high and low DFI values (p-values of <0.0001). Epigenetic instability at gene promoters was significantly elevated in samples in the high Comet group compared to the low Comet group ($p < 0.05$). Similarly, there was a significant increase in epigenetic variance in the high DFI group compared to the low DFI group ($p < 0.05$) though the difference in the DFI group was of lower magnitude than in the Comet group.
Conclusion: We found that DNA damage is correlated with DNA methylation based on many measures. High DNA damage was associated with significant regional alterations throughout the methylome in addition to increased epigenetic instability. Importantly, the Comet and TUNEL assay present a similar pattern between sperm DNA methylation and DNA damage (including a bias toward hypermethylation), however the differences between the high and low Comet scores were of far higher magnitude than the differences between high and low DFI scores. It is possible that these findings suggest that Comet scores offer a more relevant assessment of DNA damage and it's association to epigenetic changes though more work is required to confirm this finding.

Poster 104
"INJECTION OF VITAMIN E AND SELENIUM IMPROVED TESTICULAR SIZE, SEMEN QUALITY AND FREEZABILITY, AND FERTILITY OF SUBFERTILE RABBIT BUCKS"
Aya Fadl, II[1], Abdallah Mohamed, II[2]
[1]Faculty of Veterinary Medicine Cairo University, Cairo, Egypt, [2]Faculty of Veterinary Medicine University of Calgary, Calgray, AB, Canada
Presented By: Aya Fadl, II, PhD

Introduction & Objective: Introduction: Rabbit gene sequences are similar to human sequences, which makes the rabbit an excellent model for different aspects of research in reproduction. Last two decades, the semen quality has been progressively decreased, which has reflected very badly on the fertility. Oral supplementation of vitamin E and selenium (Se) has been used to mitigate the adverse effects of heat stress on reproduction; nonetheless, it is the first time to examine its effect on subfertile rabbit bucks.
Objective: Determine effects of vitamin E and Se on testicular size, semen quality and freezability, antioxidant activity, testosterone, and fertility of subfertile rabbit bucks
Methods: Twenty one New Zealand rabbit bucks were classified as subfertile bucks based on their semen characters and fertility test. The bucks were randomly allocated into 3 equal groups (G0:control; G1:injected with Vit E 100 IU/head +Se 0.1 mg/kg b.w.; G2: injected with Vit E 200 IU/head +Se 0.2 mg/kg b.w. once weekly for 8 weeks). Semen and blood samples were collected weekly for all bucks to assess fresh semen (semen volume, concentration, motility, viability, and acrosome integrity) and semen was frozen and the post-thaw semen quality were evaluated, and serum testosterone level and total antioxidant capacity (TAC) were measured. At the 8th week of the study, 150 multiparous does were artificially inseminated with fresh semen to assess the fertility of bucks after treatment; 50 does for each group. At the end of the study, bucks were slaughtered to assess testicular size. Fresh and post-thaw semen data, testosterone and TAC levels were compared using repeated measures while fertility and testicular size results were analyzed using one way ANOVA.
Results: Fresh and post-thaw semen quality parameters were significantly ($p <0.05$) higher in G2 in comparison with G1 and G0, respectively. Also, testosterone level was significantly ($p <0.05$) increased at 2nd week W2 in G2 in comparison with other groups. Conception and kindling rates were significantly ($p <0.05$) higher in does who inseminated with semen of G2 (Figure 1). Testicular size was significantly ($p <0.05$) increased in G2 in comparison with G1 and G0, respectively.
Conclusion: Injection of vitamin E and selenium improved the tested parameters and most importantly the fertility of subfertile rabbit bucks.



Effect of vit E and selenium on the fertility of bucks.

Poster 105

THE EFFECTS OF WHITE BLOOD CELLS IN THE SEMEN ON SPERM PARAMETERS, PROTAMINE AND DNA FRAGMENTATION

Bandar AlAbdulkareem, III[1], Amro Jad, IV[1], Faiza Boudouaou, V[1], Houda Amor, Jr.[2], Mohamed Mammadeh, Sr.[2], Ali Zeyad, II[1,2]

[1]Drsulaimanalhabib medical group (HMG), Riyadh, Saudi Arabia, [2]University Of Saarland, Saarland, Germany, Homburg,Saar, Germany

Presented By: Ali Zeyad, II, PhD

Introduction & Objective: High concentration of leukocytes (≥1 × 10^6/mL) in semen sample may be considered as a marker for infection (WHO, 1999). Leukocytes appear in semen at the second stage of the genital tract infection, and may stay present in semen for some time (Fraczek and Kurpisz, 2015, Zeyad et al 2017) and cause deterioration in sperm parameters (Domes *et al*., 2012; Fraczek *et al*., 2014 and Moretti *et al*., 2014; Djordjevic et al., 2018). The Negative effects refer to the ability of WBCs to produce some reactive oxygen species (ROS) (Henkel *et al*., 1997; Saleh *et al*., 2002; Lemkecher *et al*., 2005) which increase the apoptosis in spermatozoa (Sasikumar *et al*., 2013) and sperm DNA fragmentation index (Domes *et al*., 2012). This study aimed to evaluate the effects of high level of WBCs in the semen samples on sperm quality.

Methods: The present study performed at the Department of Obstetrics and Gynaecology, University of Saarland, Germany. 120 semen samples were included in this investigation. samples were collected from unselected male partners of couples consulting for infertility. Semen samples were analysed according to WHO guideline 2010. Sperm nuclear protamine were estimated using polyacrylamide acid urea gel electrophoresis. Chromomycin A3 was used for chromatin condensation evaluation, TUNEL assay for DNA fragmentation detection and the Endtz test for quantification of WBCs in the semen.

Results: Mean value of WBCs count showed a positive significant ($P<0.05$) correlation with abnormal form ($r= 0.197$) and protamine deficiency (CMA3) ($r= 0.366$). In addition, a negative significant ($P<0.010$) correlation with sperm concentration ($r= -0.317$), motility ($r= -0.318$) and progressive motility ($r= -0.191$) has been shown. WBCs concentration of both low (1.44 ± 2.337; %) and high (1.39 ± 2.831; %) protamine ratio P1/P2 groups was significantly ($P= 0.05$) higher than with normal P1/P2 ratio (0.53 ± 1.727; %).

Conclusion: Presence of WBCs in the semen samples may decrease the quality of human sperm subsequently cause infertility.

Poster 106

CAPACITATION ABILITY MAY HELP IN THE DIAGNOSIS AND TREATMENT OF IDIOPATHIC MALE FACTOR INFERTILITY

G. Charles Ostermeier[1], Cristina Cardona[1], Alexander Travis[1,2]

[1]Androvia LifeSciences, Mountainside, NJ, USA, [2]Cornell University, Ithica, NY, USA
Presented By: G. Charles Ostermeier, PhD

Introduction & Objective: Semen analysis (SA) fails to identify more than 50% of male infertility cases, leading to calls for tests that measure sperm fertilizing ability. Prior to fertilization, sperm must capacitate. Cap-Score™ reports the proportion of sperm that can capacitate and has been shown to prospectively predict pregnancy. Previously, we reported high prevalence of impaired capacitation ability in men questioning their fertility (MQF) even if they were normozoospermic. Here, the associations between SA, Cap-Score, and its associated probability of generating a pregnancy (PGP), were evaluated in MQF in comparison with fertile men, with over a doubling of MQF patient sample size since the prior report.
Methods: Cap-Score and SA measures (volume, concentration, and motility) were obtained from 5,992 MQF. Samples were either fixed and sent to Androvia (n=3,109) or diluted in TYB, cooled, and then shipped to Androvia (n=2,883). Samples diluted and cooled are known to have reduced motility, making the numbers of men who are normozoospermic or have $>10^6$ total motile cells (TMC) conservative. Fertile men were assessed previously (n=76). Table 1 contains even PGP bins and was evaluated by Chi-square and Fisher's exact test.
Results: Compared to fertile men, more MQF had Cap-Scores ≤31 (PGP bins ≤19, 20-29 & 30-39). Fewer than expected MQF had Cap-Scores ≥32 (PGP bins 40-49, 50-59 & ≥60). 47.2% (2,831/5,992) of MQF were normozoospermic and 81.5% (4,883/5,992) had $>10^6$ TMC. When compared to fertile men, sperm capacitation ability was reduced in MQF even if they were normozoospermic or had $>10^6$ TMC. Using the Cap-Score cut-off of >27.6%, which was shown to prospectively predict pregnancy, 43% (1,218/2,831) of normozoospermic MQF, 46% (2,265/4,883) of MQF having $>10^6$ TMC and 12% (9/76) of fertile men were identified with low capacitation ability.
Conclusion: When compared to known fertile men, capacitation ability reductions are more common in MQF, even if they are normozoospermic or have $>10^6$ TMC. Men presenting with capacitation ability reductions are likely to have difficulties in generating a pregnancy. These data strongly suggest that assessments of sperm capacitation/fertilizing ability should be performed as complements to traditional SA, so that optimal methods of assisted reproduction can be recommended, and/or treatments to improve a man's fertility can be attempted.

Cap-Score (%)	PGP (%)	% of MQF (n=5992)	% MQF with normozoospermia (n=2831)	% MQF with >10M TMC (n=4883)	% fertile men (n=76)
≤ 18	≤ 19	11.2 (673) p=0.003	6.5 (185) p=0.090	8.8 (428) p=0.013	1.3 (1)
19 - 25	20 - 29	28.8 (1727) p<0.001	26.8 (759) p<0.001	27.8 (1357) p<0.001	9.2 (7)
26 - 31	30 - 39	28.3 (1694) p=0.007	29.7 (840) p=0.003	29.1 (1420) p=0.005	14.5 (11)
32 - 36	40 - 49	16.9 (1010) p<0.001	18.7 (530) p=0.001	18.2 (889) p<0.001	35.5 (27)
37 - 42	50 - 59	9.1 (548) p<0.001	11.3 (320) p=0.003	9.9 (482) p<0.001	23.7 (18)
> 42	≥ 60	5.7 (340) p=0.001	7.0 (197) p=0.010	6.3 (307) p=0.003	15.8 (12)

p-values reflect Fisher's exact test comparing to the same row in the fertile men column.

Table 1. Data relating Cap-Score, PGP, and traditional SA

Poster 107

MALE FERTILITY AS A FUNCTION OF SPERM PROTEIN REDOX STATE

Kate Hansen[1], Caroline Catterton[1], Timothy Jenkins[1], Jason Hansen[1], Ammon Bayles[2], Adriana Burger[2]
[1]Brigham Young University, Provo, UT, USA, [2]Utah Fertility Center, Pleasant Grove, UT, USA
Presented By: Kate Hansen

Introduction & Objective: In the recent past, human reproductive capacity has declined though etiologies remain elusive. Oxidative stress could contribute to male infertility outcomes. The glutathione (GSH) and glutathione disulfide (GSSG) couple represent a predominant, robust redox couple that is indicative of cellular redox environments. Shifting GSH/GSSG redox states to abnormal, more oxidizing conditions can promote alterations to protein redox states, leading to cellular dysfunction. This study investigates sperm GSH/GSSG redox potential (Eh) and its correlation with sperm quality, namely progressive motility and count. Sperm samples were collected from 60+ from patients and run through well-established high performance liquid chromatography (HPLC) methods for GSH/GSSG analysis.
Methods: 64 Sperm samples were collected at Utah Fertility Center under an IRB approved protocol. High performance liquid chromatography (HPLC) methods were utilized to perform GSH/GSSG analyses. A BCA protein assay determined the protein concentration in each sample used to normalize total HPLC data to concentrations of both GSH and GSSG. The GSH/GSSG redox state (Eh) was then calculated by the Nernst equation using intracellular the obtained GSH and GSSG concentrations, where more positive values represent more oxidizing environments and more negative numbers represent more reducing ones.
Results: Extensive analyses with the intent of looking at the relationship between GSH/GSSG Eh and sperm quality were conducted. Based on a linear regression model, a statistically significant inverse correlation between GSH/GSSG Eh and motility was identified ($p=0.048$ and $R^2=0.046$). Correlation between GSH/GSSG Eh and total sperm count were found to be insignificant (with $p=0.077$ and $R^2=0.034$). Lastly, the analysis between GSH/GSSG Eh and total motile failed to yield any significance ($p=0.102$ and $R^2=0.027$).
Conclusion: This study suggests that motility decreases as redox potential increases. The inverse correlation between sperm motility and GSH/GSSG Eh in semen analyses indicate that more oxidized sperm, those with more positive redox potentials (more oxidizing), may contribute to male infertility. Despite this finding being statistically significant, this study suggests that GSH/GSSG Eh does not impact an individual's total sperm count of total motile value. Because this finding is only moderately significant, future studies are needed to fully establish this correlation. If this correlation proves to be significant with larger sample number, it provides a potential therapeutic target for intervention for patients who suffer from asthenozoospermia.

Poster 108
NOVEL COMPUTATIONAL TOOL TO ACCURATELY IDENTIFY SOMATIC CELL CONTAMINATION WITHIN PURIFIED SPERM SAMPLES
Chad Pollard[1], Ki Aston[2], Tim Jenkins[1]
[1]Brigham Young University, Provo, UT, USA, [2]University of Utah, Salt Lake City, UT, USA
Presented By: Chad Pollard, BS

Introduction & Objective: Understanding the sperm epigenetic profile and its relationship to male infertility has yielded important insights into improved diagnosis and clinical guidance of male infertility. It's no surprise that epigenetic profiles are distinct between cell types, and this is particularly true for sperm, which is distinct from all somatic cell types. Thus, much work has been performed to minimize the risk of somatic cell contamination by utilizing various washing, filtering, and somatic cell lysis techniques. Although powerful, there is no computational tool to confirm whether these techniques have indeed adequately removed somatic cells. This is particularly difficult when assessing regions that should be differentially methylated between sperm and somatic cells as small amounts of contamination in one group can significantly alter results. Thus, it has become increasingly important to confirm removal of somatic cells to provide the most accurate sperm derived epigenetic profile for the diagnosis and treatment of male infertility. Our novel approach acts as a high accuracy screen for somatic cell contamination within a sample to confirm the successful removal of somatic cells prior analysis.
Methods: Using a whole genome sliding window analysis, we identified 64 differentially methylated genomic regions (DMR) between blood and sperm DNA methylation patterns from Illumina Methylation Array data. A computational tool was built to calculate and compare mean methylation signatures at each of the 64 DMR's to identify contamination signals. These scores were compared to DLK1 signatures, as historically a mean beta value of 0.2 or higher at the DLK1 loci is used to identify somatic cell contamination. To confirm predictability of our tool, we tested 16 sperm samples intentionally spiked with blood DNA and predicted contamination with nearly 100 percent accuracy.
Results: Of 166 samples, we found that traditional DLK1 loci methods identified 15 contaminated samples. Our method identified 34 contaminated samples, including all 15 originally identified. Interestingly, 74 percent of the contaminated samples originated from men diagnosed with oligozoospermia. Indicating the need for extra caution when purifying sperm DNA from men with low sperm count.
Conclusion: Computational screens that can identify contamination signals with high accuracy are extremely important for the reliability and efficacy of epigenetic analyses. We show that our tool can be used as a high accuracy screen, to identify somatic cell contamination in sperm DNA methylation analyses.

Poster 109
IMPORTANCE OF STAT3 IN THE CONTROL OF SPERM MOTILITY
Ariane Lapointe-Belleau[1], Andrée-Anne Saindon[1], Mathieu Boilard[2], Pierre Leclerc[1]
[1]Obstetrics, Gynecology and Reproduction department, Université Laval - Reproduction, Mother and Youth Health, CHU de Québec Research Center, Québec, QC, Canada, [2]NASCI Biologie Médicale, Montreal, QC, Canada
Presented By: Ariane Lapointe-Belleau, BS

Introduction & Objective: Spermatozoa are haploid cells devoid of transcriptional and translational activities which only function is to fertilize the egg. They require good motility in order to make their way to the egg. We previously showed that the inhibition of STAT3 (Signal Transducer and Activator of Transcription 3) reduces motility, suggesting that STAT3 plays an important role in controlling sperm motility. This study aims to determine how STAT3 impacts sperm motility through its non-transcriptional activity.
Methods: This study is approved by the CHU de Quebec-Université Laval's Ethics committee for research on human subjects. Sperm samples were obtained from men consulting for couple infertility after written informed consent. The relative quantity of STAT3 was determined by Western blot in sperm proteins extracts from asthenozoospermic (<40% of total motility) and from normozoospermic patients with total motility higher than 60%. Motility parameters were analyzed by CASA, and motile and immotile spermatozoa were isolated by centrifugation through a discontinuous Percoll gradient. The presence and localization of STAT3 were analyzed by indirect immunofluorescence on fixed/permeabilized spermatozoa.
Results: STAT3 was detected at higher levels in spermatozoa from asthenozoospermic compared with normozoospermic men. In this latter group, higher STAT3 levels were detected in immotile than in motile spermatozoa, although, no difference between motile and immotile spermatozoa was measured in asthenozoospermic men. Furthermore, a negative correlation between STAT3 levels and sperm velocity parameters was observed. The proportion of cells expressing STAT3 might not be linked to the percentage of motility as no difference was observed between motile and immotile spermatozoa from astheno or normozoospermic men. Similarly, no difference was observed according to the localization of STAT3 which was detected in sperm neck and midpiece.
Conclusion: Our results show functional differences between spermatozoa from asthenozoospermic and normozoospermic men according to STAT3. Higher amounts of STAT3 were observed in asthenozoospermic than in normozoospermic men, although the respective percentage of STAT3-positive cells is similar among the two groups. Although STAT3 plays an important role in the regulation of cell functions through non-genomic activity, further studies are required to elucidate the involvement of STAT3 in the regulation of sperm motility. Supported by a grant from the Canadian Institutes of Health Research. The author is supported by a scholarship from FRQNT.

Poster 110

DEVELOPING AN ASSAY TO ASSESS HUMAN SPERM FERTILIZATION CAPACITY IN PATIENTS WITH NORMOSPERMIC INFERTILITY

Juan Ferreira, Caitlin Martin, Kelsey Anderson, Lis Puga-Molina, Celia Santi
Washington University in Saint Louis. School of Medicine. Obstetrics and Gynecology department., Saint Louis, MO, USA
Presented By: Juan Ferreira, PhD

Introduction & Objective: Introduction and objectives: Infertility affects 10-15% of couples and 50 % of these cases are due to a male-factor. The diagnosis of male infertility relies on semen analysis, an imperfect tool, that fails to predict sperm fertilizing capacity (25% of normal-appearing semen samples fail to fertilize). Therefore, we need a simple and reliable tool that allows us to predict sperm's ability to fertilize at the molecular level. After ejaculation, human sperm cannot fertilize the egg until they undergo a maturation process known as capacitation. Capacitation involves changes in membrane potential (Vm) where the membrane becomes more negative inside (hyperpolarization) and an increase in intracellular calcium concentration ($[Ca^{2+}]_i$). Both changes are driven by an increase in sperm's intracellular pHi. Using flow cytometry and a voltage-sensitive dye, we previously showed that membrane hyperpolarization correlates with high conventional in vitro fertilization rates (IVF) (Molina LCP. et al 2020). We now propose to develop a user-friendly system that tests both Vm and $[Ca^{2+}]$i in human normospermic samples and predicts which couples will fail conventional IVF and might need the use of intracytoplasmic sperm injection (ICSI).
Methods: Methods: This is an IRB-approved, laboratory study of normospermic men undergoing IVF at Washington University in St Louis. Fresh semen was collected on the day of oocyte retrieval from normospermic infertile men undergoing IVF. Vm was measured using a microplate reader or a single-sample-spectrophotometer after incubation with a Vm-sensitive dye ($DISC_3(5)$). $[Ca^{2+}]$i was measured with an epifluorescence system or a microplate reader using the calcium-sensitive dye Fluo-4AM. Results were obtained at pH 5.8, 7, and 8.1.
Results: Results: To validate our assay, we compared measurements of Vm at pHs 7 and 8 from Wild-type and SLO3-null mice sperm using a single-sample-spectrophotometer (Gold-Standard) and a microplate reader (Figures 1B and C). Sperm lacking the SLO3 K+ channel do not hyperpolarize and are infertile. In Figures 1D and E, we compared the results obtained in human sperm at pH 5.8 and 8.1 using both techniques. In figures 1F-H, we measured [Ca2+]i responses obtained in human sperm at two pHs using the epifluorescence (G) and the plate reader (H).
Conclusion: Conclusion: Correlating the Vm and [Ca2+]i measurements obtained with the microplate reader with IVF outcomes we will develop a clinically applicable assay with the potential to predict IVF success.

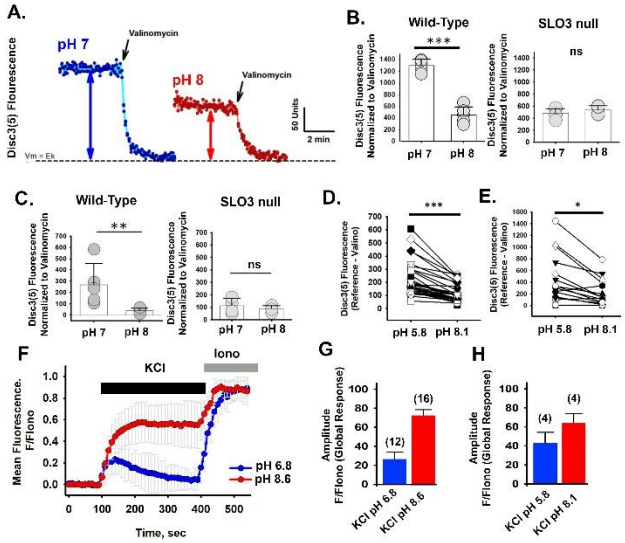


Figure 1. A. Representative recordings of the changes in Disc(3)5 fluorescence from human sperm sample measured by single-sample spectrophotometry at pH 7 and pH 8. B and C, changes in DiSC(3)5 fluorescence measured at pH 7 and 8, obtained from wild-type (left) and SLO3 null (right) mice using single sample spectrophotometry (B) and the microplate reader (C). D and E, changes in DiSC(3)5 fluorescence measured at pH 5.8 and 8.1 obtained from human semen samples using single sample spectrophotometry (D) and the microplate reader (E). F. Representative traces of calcium responses to 50 mM KCl in human sperm using an epifluorescence system (Gold-standard) at the indicated pHs. G and H, graphs showing the amplitude of the global responses to 50 mM KCl at the indicated external pH using a epifluorescence system (G) and the microplate reader (H). Values represent mean ± SD, *P < 0.050, **P < 0.010, ***P < 0.001, and ns, non-significant. Iono = 5 mM Ionomycin + 2 mM Ca2+.

Figure 1

Poster 111

EFFECTS OF EXTERNAL RADIATION THERAPY FOR PROSTATE CARCINOMA ON HUMAN SPERMATOGENESIS

Andre Caldeira-Brant[1,2], Reginaldo Martello[2], Augusto Reis[2], Edson Samesima[2], Fernando Reis[2], Fernanda Almeida[2], Kyle Orwig[1], Helio Chiarini-Garcia[2]

[1]University of Pittsburgh, Pittsburgh, PA, USA, [2]Federal University of Minas Gerais, Belo Horizonte, Brazil

Presented By: Andre Caldeira-Brant, PhD

Introduction & Objective: Prostate cancer (PC) is the most common cancer that affects men. While external radiation therapy (RT) is effective in terms of eliminating prostate cancer cells, scattered radiation may cause deleterious effects on patient's fertility. Little is known about how scattered radiation from RT impairs the spermatogenesis of men with prostate cancer. Therefore, we evaluated the germ cells and Sertoli cells of adult patients diagnosed with PC and that underwent RT.

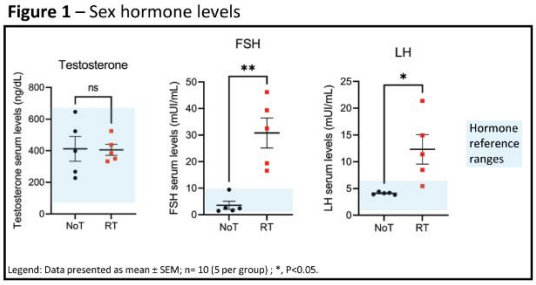
Methods: Five fertile men (69 ± 9 years) that had PC and received total external radiation doses ranging from 64-72 Gy, for 32-40 days, were castrated 15-24 months post-treatment due to cancer recurrence. Additionally, testes from five other patients (55 ± 7 years) with advanced PC that were not exposed to RT prior to castration were collected as the control group (NoT). Sex hormone levels, histological and immunohistochemistry (IHC) analysis were performed.

Results: While testosterone serum levels were similar between groups, FSH and LH were augmented in the RT group (Figure 1). VASA+ germ cells were observed in 100% of NoT seminiferous tubules compared to only 9% of tubules in RT patients in which 70% of tubules had a Sertoli cell (SOX9+) only phenotype and 21% were fibrotic (Fig. 2). The numbers of Adark, Apale and B spermatogonia, and preleptotene spermatocytes were lower in RT than NoT (Fig. 3A), which was confirmed by MAGEA-4 spermatogonial immunostaining (Fig. 3B). While the number of UTF1+ undifferentiated spermatogonia were reduced in the RT group (Fig. 4A), MCM7+ proliferating UTF1+ cells were higher in that group (Fig. 4B). Most (85%) MAGEA-4+ spermatogonia were undergoing apoptosis (CASP3+) in RT patients, whereas only 1% were apoptotic in NoT (Fig. 5). Similarly, SOX9+ Sertoli cells were reduced in RT group (Fig. 6A) but exhibited increased proliferation (Fig. 6B) and apoptosis (Fig. 6C). The Claudin-11 blood-testis barrier protein was present in RT Sertoli cells but was more evenly organized in NoT patients (Fig. 7).

Conclusion: Scattered radiation resulted from RT impairs spermatogenesis of adult men by affecting both germ and Sertoli cells. While germ cells and Sertoli cell numbers were significantly reduced by one-year post-treatment in RT patients, their proliferation and apoptosis were significantly increased compared to NoT patients.

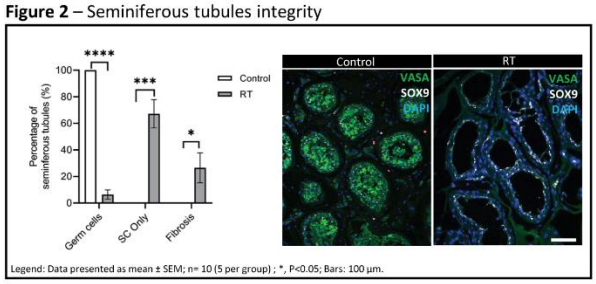
Financial support: CNPq, CAPES, FAPEMIG, NIH HD100197

Figure 1 – Sex hormone levels



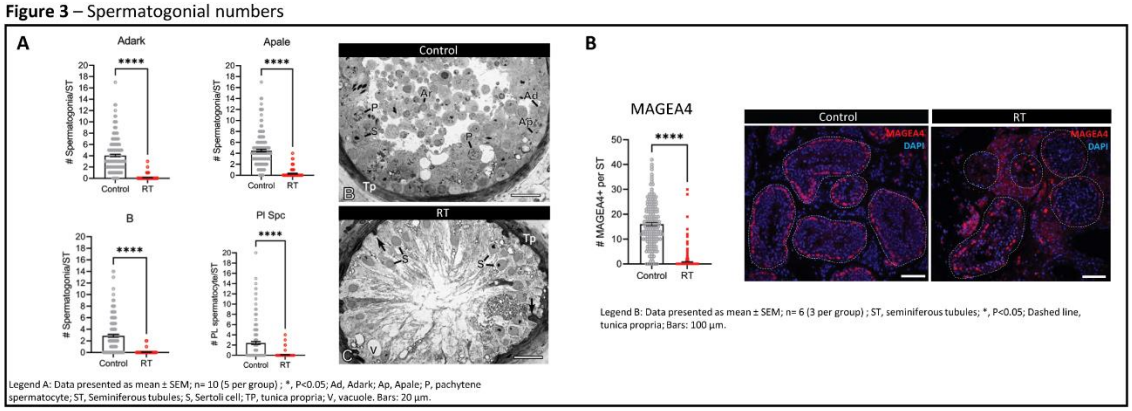
Legend: Data presented as mean ± SEM; n= 10 (5 per group) ; *, P<0.05.

Figure 2 – Seminiferous tubules integrity



Legend: Data presented as mean ± SEM; n= 10 (5 per group) ; *, P<0.05; Bars: 100 µm.

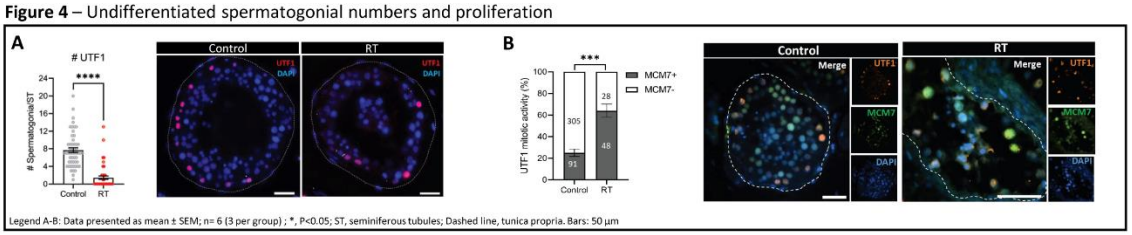
Figure 3 – Spermatogonial numbers



Legend A: Data presented as mean ± SEM; n= 10 (5 per group) ; *, P<0.05; Ad, Adark; Ap, Apale; P, pachytene spermatocyte; ST, Seminiferous tubules; S, Sertoli cell; TP, tunica propria; V, vacuole. Bars: 20 µm.

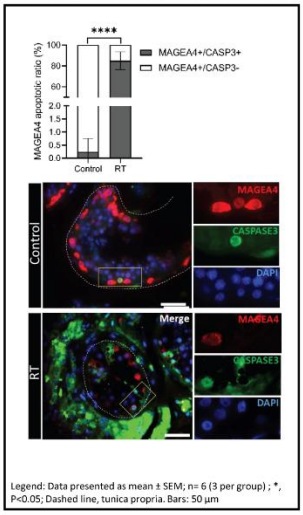
Legend B: Data presented as mean ± SEM; n= 6 (3 per group) ; ST, seminiferous tubules; *, P<0.05; Dashed line, tunica propria; Bars: 100 µm.

Figure 4 – Undifferentiated spermatogonial numbers and proliferation



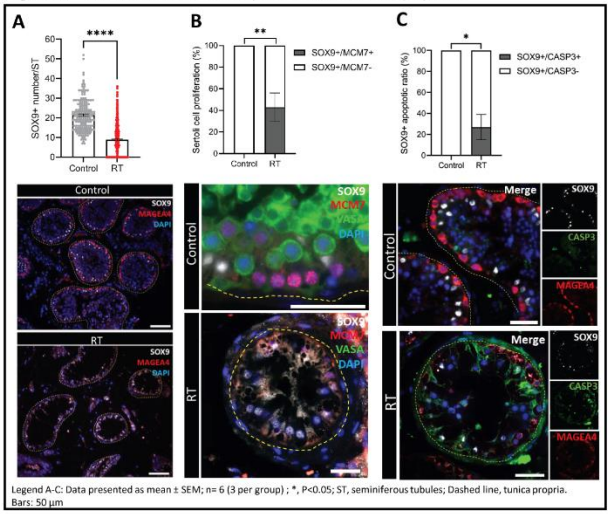
Legend A-B: Data presented as mean ± SEM; n= 6 (3 per group) ; *, P<0.05; ST, seminiferous tubules; Dashed line, tunica propria. Bars: 50 µm

Figure 5 – Spermatogonial apoptosis



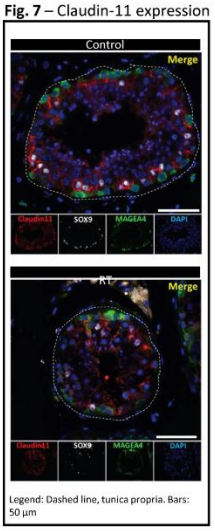
Legend: Data presented as mean ± SEM; n= 6 (3 per group) ; *, P<0.05; Dashed line, tunica propria. Bars: 50 µm

Figure 6 – Sertoli cell numbers, proliferation, and apoptosis



Legend A-C: Data presented as mean ± SEM; n= 6 (3 per group) ; *, P<0.05; ST, seminiferous tubules; Dashed line, tunica propria. Bars: 50 µm

Fig. 7 – Claudin-11 expression



Legend: Dashed line, tunica propria. Bars: 50 µm

Caldeira-Brant Abstract Figure

Poster 112

INVESTIGATE THE PROGRESSION OF TESTICULAR FIBROSIS INDUCED BY ACUTE AND CHRONIC INSULTS

Ya-Yi Yang, Pei-Shiue Tsai

National Taiwan University, Taipei, Taiwan
Presented By: Ya-Yi Yang, BS, DVM

Introduction & Objective: Oxidative stress is one of the common causes of male infertility. Excessive production of intracellular free radicals results in mitochondria and endoplasmic reticulum stresses. Repeated or constant oxidative damages can lead to the up-regulation of TGFβ expressions and promote the transition of fibroblast to myofibroblast. The activation of myofibroblasts allows secretion and accumulation of excessive extracellular matrix such as collagen type 1/4, which results in organ fibrosis. In our study, we aim to understand whether reactive oxygen species resulting from different insults would affect the progression of testicular fibrosis.
Methods: For acute testicular injury model, we performed ischemia-reperfusion (IR) surgery on mice by clamping the testicular arteries and nearby capillaries for two hours in order to mimic testicular torsion. For chronic testicular injury model, mice received three intraperinatal injections of cisplatin (10 mg/kg), a platinum anti-cancer drug, to mimic the course of chemotherapy. Mice from both groups were sacrificed at week 6 for further analyses.
Results: The size and weight of both IR and cisplatin-treated testes decreased with a pale and firm appearance when compared to control testes. Through histology examination and indirect immunostaining, significant loss of spermatogenic layers and disrupted testicular structure with the accumulation of collagen in the interstitial compartment of the testes were observed in both testicular injury models, which proved the formation of testicular fibrosis. In addition to fibrogenic genes, such as TGFβ, αSMA, and collagen, the expression of the thioredoxin domain-containing protein 5 (TXNDC5), a novel mediator of organ fibrosis, was also upregulated.
Conclusion: Based on current data, the TGFβ-TXNDC5-αSMA-collagen signaling pathway is highly associated with both IR- and cisplatin-induced testicular fibrosis. However, whether insult-specific molecules and signaling pathways are crucial to the progression of testicular fibrosis requires further study. In conclusion, IR surgery and cisplatin administration cause acute and chronic testicular damage, revealing oxidative stress-related molecules and signaling pathways would be beneficial for the understanding of the progression of testicular fibrosis.

Poster 113
THE EFFECTS OF PLATINUM-CONTAINING CHEMOTHERAPY COMPOUND ON THE TESTICULAR DAMAGE AND REGENERATION
Wei-Yun Lee, Pei-Shiue Tsai
National Taiwan University, Taipei, Taiwan
Presented By: Wei-Yun Lee

Introduction & Objective: Cisplatin is a platinum-based chemotherapy drug, which widely used in various solid tumors treatment. Cisplatin can form DNA adducts by crosslinking with nucleic acids and cause cell apoptosis. In the male reproductive organ, cisplatin-induced damage, which includes oxidative stress and DNA lesion in germ cells, may lead to infertility. Cisplatin-induced DNA double-strand break is the most severe damage that can lead to genome instability. Therefore, the DNA repair ability of spermatogenic cells is positively correlated to the quality of sperm.
Methods: In this study, 3 dosages of cisplatin (10mg/kg BW) were given to C57BL/6 mice intraperitoneally within 6 weeks to induce testicular damage. After sacrificing the mouse, a pair of testis and epididymis were weighed and freezed in liquid nitrogen for protein analysis. The other pair of reproductive tissue was fixed in formalin for immunofluorescence assay and histological analysis.
Results: In the cisplatin-treated group, the testicular weight was significantly decreased, and the spermatogenic layers in the seminiferous tubules were lost compared to the control group. The cisplatin-induced oxidative stress was evidenced by the elevation of lipid peroxidation in the testes. As for DNA damage response, γ-H2AX expression was elevated after cisplatin injury, but in contrast, the critical mediator of DNA damage checkpoint 1 (MDC1) protein expression was decreased.
Conclusion: Based on these results, cisplatin enhanced testicular lipid peroxidation and DNA damage; however, the testicular MDC1-associated DNA repair mechanism seemed to be compromised by cisplatin. However, downstream DNA repair proteins should also be elucidated in the future.

Poster 114
DOES MOUSE IFT43 GENE PLAY A ROLE IN GERM CELL DEVELOPMENT AND MALE FERTILITY?
Alivia Kearney[1], Wei Li[1], Yi Tian Yap[1], Gregory Pazour[2], Zhibing Zhang[1]
[1]Wayne State University, Detroit, MI, USA, [2]University of Massachusetts, Worchester, MA, USA
Presented By: Alivia Kearney, BS

Introduction & Objective: Intraflagellar transport (IFT) is the mechanism essential for the assembly and conservation of cilia and flagella in nearly all eukaryotic cells. The IFT complex has two subcomplexes known as IFT-A and IFT-B, each contains several IFT subunits that contribute to the formation and maintenance of flagella/cilia in different manners. Of the subunits in the IFT-A complex, the role of IFT43 has yet to be fully uncovered.
Methods: Mouse *Ift43* mRNA is present in the tissues that are known to have cilia, with a higher expression level in the lung and testis. The highest expression of mouse IFT43 protein is in the lung, but not in the testis, which is different from other mouse *Ift*

genes analyzed. “CpG” islands, that are present in other *Ift* gene studied, are not present in the proximal promoter region of mouse *Ift43* gene, suggestive of a unique mechanism of transcriptional regulation of this *Ift* gene.
Results: Like other IFT proteins, the IFT43 protein level is higher in spermiogenesis phase when sperm tails are formed, during the first wave of spermatogenesis. An IFT43/pEGFP-N2 plasmid was constructed, and the fusion IFT43/GFP protein was present as granules when expressed in HEK293 cells. Immunofluorescence staining on mouse testicular sections revealed that IFT43 was also present as granules in round spermatids.
Conclusion: To further understand the role of IFT43 in male germ cell development, the *Ift43* $^{flox/flox}$ females were crossed with the *Stra8-iCre* males to obtain *Stra8-iCre; Ift43*$^{flox/+}$ mice, and the *Stra8-iCre; Ift43*$^{flox/+}$ males were crossed back with *Ift43*$^{flox/flox}$ females again, and the *Stra8-iCre; Ift43*$^{flox/flox}$ are considered to be the homozygous mutant mice (KO). The effect of disruption of mouse *Ift43* gene in male germ cells on male fertility and spermatogenesis is being studied.

Poster 115
PROTEOMIC CHANGES AND MOLECULAR PATHWAYS DYSREGULATED IN THE TESTIS OF MOUSE INFECTED WITH SARS-COV-2
Ajaya Moharana, Manesh Kumar Panner Slevam, Saradha Baskaran, Suresh Sikka
Department of Urology, Tulane University School of Medicine, New Orleans, LA, USA
Presented By: Ajaya Moharana, MS

Introduction & Objective: Coronavirus disease (COVID-19) caused by SARS-CoV-2 affects the reproductive health of males. The Mouse Adapted SARS-CoV-2 (SARS-CoV-2 MA10) infection in C57BL/6 mice induces histopathological changes in the testis. However, there are no reports on the molecular changes in the testis during SARS-CoV-2 infection. Therefore, the main objective of this study was to compare the testicular proteome of SARS-CoV-2 MA10 infected C57BL/6 mice with the uninfected group.
Methods: Testicular tissue samples (n=3, pooled and two individual samples in each group) from SARS-CoV-2 MA10 infected and uninfected C57BL/6 mice were processed for global proteome profiling by using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Label-free quantitation (LFQ) intensities were generated using a targeted library-based search algorithm on Spectronaut then filtered to have at least two unique peptides for protein identification. Further, differentially expressed proteins (DEPs) were subjected to ingenuity pathway analysis (IPA) to detect the molecular networks affected.
Results: Of 1264 proteins identified, 413 were DEPs in the infected group compared to the uninfected group. Bioinformatic analysis revealed that proteins associated with sperm motility and morphology were compromised in SARS-CoV-2 MA10-infected mice (Figure 1). Furthermore, protein-protein interaction analysis revealed dysregulation of the telomere signaling pathway. Proteins such as E2F1, RB1, HETS, MYC, and SP1 were found to suppress the function of TERT, which is essential for telomerase activity. In addition, proteins (TERF1, TERF2, TINF2, and POT1) associated with telomere maintenance were underexpressed in the testis of SARS-CoV-2 MA10-infected mice.
Conclusion: We have demonstrated by proteomic analysis defective/ altered reproductive pathways in the testis of mice infected with SARS-CoV-2 MA10. Our data also suggest that telomere function in the testis is compromised during COVID-19, indicating that testicular DNA is highly vulnerable to damage.

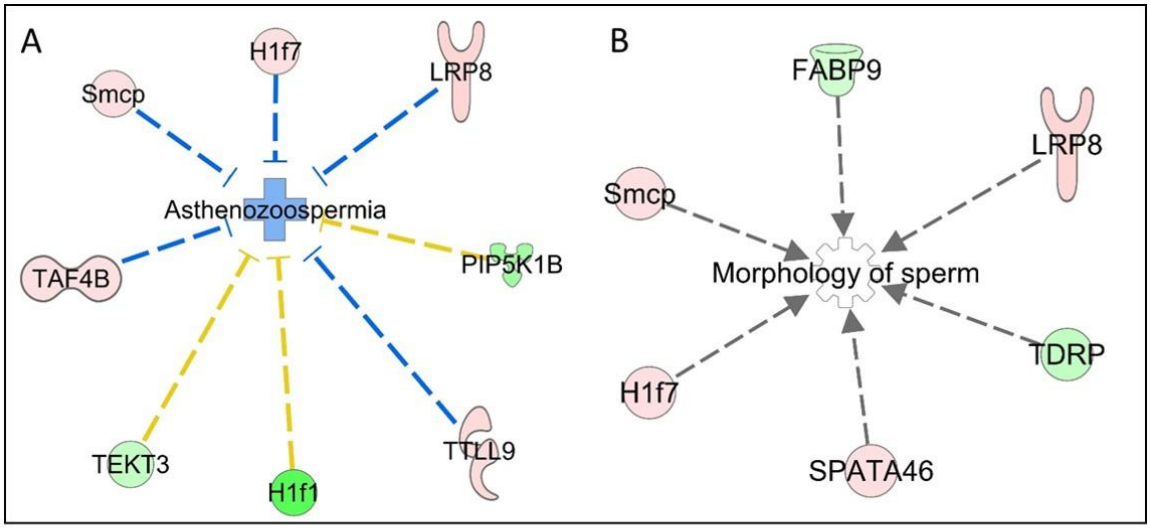


Figure 1: Differentially expressed testicular proteins regulating (A) sperm motility and (B) sperm morphology in C57BL/6 mice infected with SARS-CoV-2 MA10.

Figure 1

Poster 116

AUTOMATED ANALYSIS OF GERM CELL POPULATIONS IN THE DEVELOPING AND ADULT TESTIS

Chris Geyer[1,2], Bryan Niedenberger[1], Taylor Johnson[1], Matthew Thomas[1], Emma Gilbert[1]
[1]Brody School of Medicine at East Carolina University, Greenville, NC, USA, [2]East Carolina Diabetes and Obesity Institute at East Carolina University, Greenville, NC, USA
Presented By: Chris Geyer, PhD

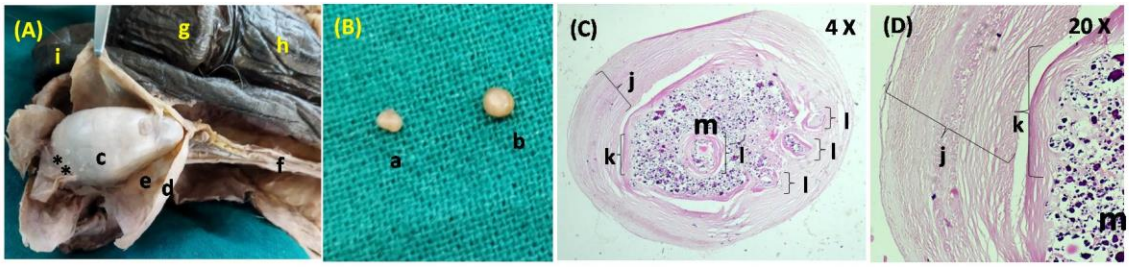
Introduction & Objective: The mammalian testis contains the seminiferous tubules, which house somatic Sertoli cells along with germ cells undergoing all phases of spermatogenesis. During spermatogenesis in postnatal mice, male germ cells undergo at least 17 nomenclature changes as they proceed through mitosis as spermatogonia (=8), meiosis as spermatocytes (=6), and spermiogenesis as spermatids (=3). Adding to this complexity, combinations of germ cells at each of these stages of development are distributed together along the seminiferous tubules. Due to this, considerable expertise is required to accurately analyze changes in spermatogenesis in animals that have been genetically modified (transgenic, mutant, or knockout (KO)), treated with pharmacologic agents, or injured.
Methods: Here, we combine our laboratory's expertise in spermatogenesis with the open source 'Quantitative Pathology & Bioimage Analysis' (QuPath) software platform to automate analyses of germ cell populations in both the developing and adult mammalian testis. Testis paraffin-embedded and cryosections were immunostained and imaged by either brightfield or fluorescence microscopy. These images were then entered into QuPath and an algorithm established to predict seminiferous epithelial cell identity based on shape, diameter, and threshold intensity values for various immunodetected protein cell fate markers. Concurrently, manual cell counts were done on the same tubules for comparison.
Results: The results obtained from QuPath were highly similar to manual cell counts, although the program significantly undercounted steps 10-16 elongated and condensing spermatids due to their small size and close proximity. Methods are being currently tested to overcome this limitation, and efforts are underway to automate quantitation of interstitial somatic cell populations.
Conclusion: Overall, we conclude that with the correct optimization, QuPath provides a rapid and reliable unbiased means to quantify both germ and somatic cell populations in the mammalian testis.

Poster 117

SCROTOLITHS IN THE TUNICA VAGINALIS TESTIS IN AN ELDERLY MALE CADAVER: CLINICAL IMPLICATIONS.

Dibakar Borthakur, Rima Dada, Rajesh Kumar, Tony Jacob
All India Institute of Medical Sciences, New Delhi, New Delhi, India
Presented By: Dibakar Borthakur, MBBS, MD

Introduction & Objective: Scrotoliths or "Scrotal pearls" is a fibrinoid loose body found occasionally in the tunica vaginalis cavity that is commonly associated with hydrocele. It is usually smaller than 1 cm x 1 cm in dimension with a reported incidence of 3-4% across literatures. Scrotoliths are often detected as incidental findings during high frequency ultrasonography or surgery. Repeated low grade inflammation following chronic microtrauma in specific professionals such as mountain riders or those having parasitic infestation with filarial parasites are implicated in their etiopathogenesis. Some investigators have linked it with torsion and subsequent detachment of appendix testis or appendix epididymis. Though mostly benign, they are also found associated with mesothelioma of the testis. Scrotal pearls in a cadaver have not been reported to the best of our knowledge.
Methods: The institutional guidelines for use of human cadaver for teaching and research were followed. Consent from the kin of the deceased was obtained at the time of body donation. During routine cadaveric dissection of testes of an embalmed male cadaver we found small round, whitish masses in the tunica vaginalis of the testis. Gross measurements were taken with digital Vernier calliper and an electronic weighing machine. Thereafter, we processed the tissue for paraffin embedding and hematoxylin and eosin staining. We photographed the relevant macroscopic and microscopic features.
Results: The present case reports two masses in the lower lateral part of the tunica vaginalis cavity of the left testis. The left testis measured 3.4 cm x 2.6 x 1.8 cm with all tunics intact. The sizes and weights of the pyramidal and ovoid masses were 0.23 cm x 0.17 cm, 0.42 cm x 0.35 cm and 0.17 g and 0.29 g, respectively. Microscopy of stained sections revealed a central heterogeneous area of calcification surrounded by concentric acellular fibrous lamellae with the outermost cellular layer containing fibroblasts and fibrocytes. Several smaller similar lamellar structures were seen within the larger lamellae.
Conclusion: We report a cadaver with scrotoliths that appeared as calcified necrotic tissue surrounded by a lamellar fibrous capsule and showed no signs of any malignancy.



(A) The testicular concretions in the left tunica vaginalis cavity i.e. Pearls were present in the tunica vaginalis cavity [scrotoliths location marked with asterix] (B) Pearls seen as hard, glistening, whitish to pale yellow in colour, one mass pyramidal and the other mass ovoid shaped, measuring 0.23 cm x 0.17 cm & 0.42 cm x 0.35 cm and respectively. (C) & (D) Histologically the masses consist of several layers of lamellate fibrous material surrounding few smaller similar concentric lamellar bodies with the centre of the concretions harbouring calcified contents seen as scattered purplish black flakes. The peripheral laminae of the fibrous lamellae exhibit fibroblasts which probably synthesise the fibrous materials. a-pyramidal pearl, b-ovoid pearl, c- left testis tunica vaginalis visceral layer, d- tunica vaginalis parietal layer, e-cavity of the tunica vaginalis, f-spermatic cord, g- glans penis, h-shaft of the penis, i-scrotum, j- outer concentric fibrous lamellae, k-inner larger lamellar body, l- inner smaller lamellar bodies, m-calcified necrotic contents

Figure 1: Gross & histologic features of scrotal pearl

Poster 118

SECRETION OF EXTRACELLULAR VESICLES BY SERTOLI CELLS; THEIR POSSIBLE INVOLVEMENT IN THE DEVELOPMENT OF SPERMATOGENESIS IN VITRO.

Mahmoud Huleihel, Sivan Naor Margaliot, Saray Tabak, Eli Beit-Yannai, Eitan Lunenfeld
Ben-Gurion University of the Negev, Beer-Sheva, Israel, Beer-Sheva, Israel
Presented By: Mahmoud Huleihel, PhD

Introduction & Objective: Spermatogenesis is the process of sperm generation from spermatogonial stem cells (SSCs) that develop in the seminiferous tubules of the testes through meiotic and post-meiotic stages. The SSCs proliferation and differentiation to the different stages of spermatogenesis occur between Sertoli cells. Their development is affected by physical contact and secreted material from Sertoli cells. Aims of the study: To examine the capacity of mouse and human Sertoli cells to secrete extracellular vesicles (EVs) and to evaluate the capacity of the secretions of Sertoli cells to induce spermatogenesis in vitro.
Methods: Sertoli cells were enzymatically isolated from immature mice, pre-pubertal cancer patient boy or azoospermic patient. The cells were cultured in (media containing-sera)-free of exosomes. After 3-4 days, the cultures were treated with hypotonic shock media to eliminate spermatogonial cells. After overnight, the cultures were washed and a new fresh media-free of exosomes were added for 8 hours. The media were collected and examined for the presence of exosomes by differential ultracentrifugation, followed by passing the exosomes through 0.22 µm filters. The concentration and size distribution of the EVs were measured with a NanoSight NS500 instrument. The presence of exosomes was also confirmed by Cryo-Transmission Electron Microscope. The conditioned media were also used to examine their capacity to induced development of spermatogenesis in 3-dimensional (methylcellulose; MCS) in vitro culture system using isolated cells from seminiferous tubules of immature mice. The presence of pre-meiotic, meiotic and post-meiotic cells in the MCS after 4 weeks of culture were examined by immunofluorescence staining and or qPCR analysis using specific antibodies or primers, respectively for cells of each stage.
Results: In the present study, we show that Sertoli cells of mouse and human origin secrete extracellular vesicles (EVs) in vitro according to their size (60-123 nm) and identification by cryo-TEM. We also showed that the addition of conditioned media from mouse Sertoli cells induces the development of spermatogonial cells to pre-meiotic, meiotic, and post-meiotic stages in vitro using a 3-dimension methylcellulose culture system as examined by immunofluoresence staining and/or qPCR analyses.
Conclusion: Conclusions: Thus, it is possible to suggest that the effect of conditioned media from Sertoli cells on the development of spermatogenesis in vitro could be related, at least partially, to the secreted EVs in addition to soluble growth and differentiation factors.

Poster 119

EFFECT OF TESTICULAR GERM CELL DEPLETION IN VITRO OR FOLLOWING BUSULFAN TREATMENT IN VIVO ON THE FUNCTIONALITY OF SERTOLI CELLS IN-VITRO; POSSIBLE RESTORATION OF THIS FUNCTIONALITY BY HORMONES

Alaa Sawaied[1], Bat Ell Levi[1], Eden Arazi[1], Eitan Lunenfeld[2], Mahmoud Huleihel[1]
[1]Ben-Gurion University of the Negev, Beer-Sheva, Israel., Beer-Sheva, Israel, [2]2The Center of Advanced Research and Education in Reproduction (CARER), Faculty of Health Sciences, 3Faculty of Medicine, Ariel University, Israel, Ariel, Israel
Presented By: Alaa Sawaied, PhD

Introduction & Objective: Introduction: Spermatogenesis involves interactions between testicular somatic and germ cells. Sertoli cells produce factors which are crucial in the development of normal spermatogenesis. Depletion of spermatogonial cells affects the functionality of Sertoli cells. This development of normal spermatogenesis is regulated by hormones and autocrine/paracrine factors.
Aim: Evaluation the effect of testicular germ cell depletion in vitro or following busulfan treatment in vivo, on the physiological levels of factors produces by Sertoli cells that involved in normal spermatogenesis.
Methods: Material and methods: Seminiferous tubule cells were enzymatically isolated from testes of immature (7-day-old) mice (group 1) or immature mice that treated with a single injection of busulfan (45 mg/kg; group 2) (or DMSO as a control for busulfan group; group 3) and sacrificed after 10 days of treatment. These cells were cultured *in-vitro* for 3 days. Thereafter, cultures were of group 1 were treated with hypotonic-shock to remove germ cells. After over-night, fresh media without (control; CT) or with FSH, testosterone (T) or FSH+T were added to the hypotonic-treated or untreated (CONTRO) cultures and for cultures of group 2 and 3 for 8 or 24 hours. Expression of growth factors (CSF-1, LIF, SCF, GDNF) or other specific Sertoli cell factors [transferrin, inhibin, androgen receptor (AR), androgen binding protein (ABP), FSH receptor (FSHR)] were examined by qPCR.
Results: Results: The expression levels of the examined growth factors and other Sertoli cell factors were significantly changed in hypotonic-shock cultures compared to untreated cultures (CONTROL) in the control (CT) and following treatment with FSH or T. However, treatment of the hypotonic-shock cultures with both FSH+T restored the expression levels of all the growth factors, ABP and FSHR to those of CONTROL cultures, but not transferrin, inhibin and AR. Similar results were demonstrated also in group 2 that treated with busulfan.
Conclusion: Conclusion: Our results clearly demonstrate the crucial role of germ cell in physiological role of Sertoli cells, and the possible role of FSH+T to fix, at least partially, their abnormal activity following germ cell depletion under physiological and pathological conditions.

Poster 120

EFFECT OF TNFA ON THE DEVELOPMENT OF DIFFERENT STAGES OF SPERMATOGENESIS IN VITRO, USING SPERMATOGONIAL CELLS ISOLATED FROM NORMAL AND BUSULFAN-TREATED IMMATURE MICE.

Nagam Ali[1], Eitan Lunenfeld[2], Mahmoud Huleihel[1]
[1]Ben Gurion University, beer-sheva, Israel, [2]Ariel University, Ariel, Israel
Presented By: Nagam Ali, MA

Introduction & Objective: TNFα is a paracrine factor, secreted by germ and Sertoli cells. In the testis, TNFα is known to affect Leydig and Sertoli cell functions and steroidogenesis. Busulfan (BU) is a cytotoxic anti-cancer drug. It damages all types of proliferating cells. Therefore, it may lead to sub-fertility or even infertility. Aims: To evaluate the effect of TNFα on the capacity of spermatogonial cells isolated from normal and busulfan-treated immature mice, and to develop different stages of spermatogenesis in vitro.
Methods: Seven-days-old immature mice were used as normal or were intraperitoneally (i.p) injected with busulfan (45 mg/kg) and were sacrificed after 10 days. Cells from seminiferous tubules (STs) were enzymatically isolated, and cultured in methylcellulose (MCS) without (CT) or with TNFα (1,10,100 pg/ml). Fresh media without (CT) or with TNFα were added from the beginning, and after two weeks of culture. Developed cells were collected after 5 weeks, and examined for different stages of spermatogenesis by specific immunofluorescence staining (IF) or qPCR analysis.
Results: Addition of TNFα to MCS that contained isolated cells from STs of normal immature mice significantly increased the development of VASA, BOULE, and ACROSIN. It also differently affected Sertoli cell functionality markers (Androgen receptor, Androgen binding protein, Transferrin, GDNF, FSH-Receptor) expression levels. The effect of TNFα on the development of spermatogenesis from STs of busulfan-treated immature mice in MCS was lower compared to normal mice.
Conclusion: Our findings indicate the possible involvement of TNFα in the development of spermatogenesis in vitro. This effect could be directly on spermatogonia cells and/or through Sertoli cells. Thus, our results could suggest TNFα as a factor to be used in developing future in vitro therapeutic strategies for prepubertal male fertility preservation.

Poster 121

TESTICULAR TEMPERATURE SHOWED INCREASE IN THE DEVELOPMENT OF CELLS OF THE DIFFERENT STAGES OF SPERMATOGENESIS IN VITRO COMPARED TO BODY TEMPERATURE; POSSIBLE REGULATION THROUGH SERTOLI CELL FUNCTIONALITY.

Areej Jorban[1], Eitan Lunenfeld[2], Mahmoud Huleihel[1]
[1]Ben-Gurion University, Beer sheva, Israel, [2]Ariel University, Ariel, Israel
Presented By: Areej Jorban, MA

Introduction & Objective: Spermatogenesis is the process of proliferation and differentiation of spermatogonial cells to meiotic and post-meiotic stages. It occurs *in-vivo* at 34 ^{0}C-35 ^{0}C. Sertoli cells play a crucial role in the development of normal spermatogenesis.

Aim: to examine the effect of temperature on the development of spermatogenesis *in-vitro*, and evaluating the possible involvement of Sertoli cell functionality under these conditions.
Methods: Seminiferous tubules cells were enzymatically isolated from testes of 7-day-old sexually immature mice. The cells were cultured in methylcellulose [as a 3-dimension (3D) *in-vitro* culture system] and incubated in CO_2 incubator at 35 ^{0}C or 37 ^{0}C. After 2-6 weeks the development of different stages of spermatogenesis were evaluated by immunofluorescence staining (IF) or qPCR analyses using specific antibodies or primers, respectively, for cells at each stage. Factors that indicate functionality of Sertoli cells were examined by qPCR.
Results: Our results show that under 35^{0}C, the percentages and/or the expression levels of the developed pre-meiotic (VASA, PLZF, GFR-a), meiotic (BOULE, CREM) and post-meiotic cells (ACROSIN and PROTAMINE) were significantly higher compared to 37^{0}C. The expression levels of androgen receptor, FSH receptor, transferrin, androgen binding protein and glial-derived nerve growth factor were higher at 35 ^{0}C compared to 37 ^{0}C.
Conclusion: Our results show that optimal conditions for development of spermatogenesis *in-vitro* is 35 ^{0}C, similar to *in-vivo*. This could be related, at least partially, to Sertoli cells activity. These findings may deepen our understanding of the mechanisms behind optimal conditions of normal spermatogenesis *in-vivo* and *in-vitro*

Poster 122
GENERATION OF A IFT25/GFP KNOCK-IN MOUSE MODEL
Congxiao Cheng, Wei Li, Yi Tian Yap, Alivia Kearney, Moazzam Hossain, Patrick Mueller, Christopher Kelly, Zhibing Zhang
Wayne State University, Detroit, MI, USA
Presented By: Congxiao Cheng, MD

Introduction & Objective: Intraflagellar transport 25 (IFT25) is a component of the IFT-B complex. In mice, even though this IFT component is not required for cilia formation in the somatic cells, it is essential for sperm formation. However, the intracellular localization of this protein in male germ cells is not known given no antibodies are available for histologic studies.
Methods: To examine localization of the protein in male germ cells and further investigate the mechanism of IFT in sperm formation, particularly to look into the dynamic trafficking of the protein, we generated a mouse IFT25/GFP knock in mouse model using the CRISPR/cas9 system, with the mouse IFT25 protein fused with a GFP tag in the C-terminus. Three independent lines were analyzed.
Results: Western blotting using both anti-IFT25 and anti-GFP antibodies showed that the IFT25/GFP fusion protein has the same tissue distribution as the endogenous IFT25 protein, with the highest level in the testis; fluorescence signal was also observed in these tissues. Examination of localization of the IFT25/GFP in isolated germ cells revealed that the fusion protein was present in the cytoplasm of spermatocytes and round spermatids and a strong signal was present in the developing sperm flagellar. Diffusion analysis of IFT25 within the developing flagellar revealed the presence of both mobile and immobile fractions. Preliminary fluorescence recovery after photobleaching (FRAP) data revealed 40% of the IFT25 were mobile with a diffusion rate of 0.5 $\mu m^2/s$ while the remaining 60% of the IFT25 were immobile and did not recover after photobleaching. By tracking the fluorescence intensity along the tails versus time, dual-directional trafficking of IFT25/GFP was observed.
Conclusion: The model provides a tool to investigate the mechanisms of IFT25 in assembling mouse sperm flagella.

Poster 124
ROLE OF LSD1 IN MOUSE SERTOLI CELLS
Ali Dadbin, Elena Fujiwara, Pooja Gandhi, Marie-Claude Hofmann
University of Texas - MD Anderson Cancer Center, Houston, TX, USA
Presented By: Marie-Claude Hofmann, PhD

Introduction & Objective: In the seminiferous tubules of the testes, Sertoli cells produce proteins that are critical for germ cell development and differentiation. The RBPJ transcription factor is a mediator of NOTCH signaling in Sertoli cells that can inhibit or drive the expression of growth factors. For example, we have previously demonstrated that RBPJ and its target effectors are major inhibitors of *Gdnf* and *Cyp26b1* expression, therefore playing a role in the regulation of spermatogonial maintenance and differentiation[1]. To further understand the role of RBPJ in Sertoli cells, we investigated which nuclear proteins functionally associate with this transcription factor.
Methods: Using primary mouse Sertoli cells (21 day-old) we performed yeast two-hybrid assay, Western blots and co-immunoprecipitations to identify functional RBPJ-interacting proteins. As one of the identified proteins was LSD1 (KDM1A), we established and characterized a Sertoli cell-specific knockout model (*Amh-Cre*$^{+/+}$; *Kdm1a* $^{fl/fl}$) to get some insight into the function of KDM1A within the RBPJ repressor complex.
Results: *Results*: We found that RBPJ may associate with HDACs, BHC80 and LSD1. LSD1, or KDM1A, demethylates both mono- and di-methylated H3K4 and therefore acts as a transcriptional co-repressor. However, its functional role in Sertoli cells is so far unknown. In comparison with *Kdm1a*$^{fl/fl}$ control animals, the Sertoli cell-specific *Kdm1a* knockout testes showed disorganized seminiferous tubules and germ cell loss, in particular detachment/sloughing of pachytene spermatocytes and spermatids over time. We also observed clustering of spermatocytes as well as disruption of the structure of seminiferous tubules including the basement membrane.

Conclusion: *Conclusion*: Altogether, KDM1A might be part of a mechanism controlling the on/off switch of RBPJ/NOTCH signaling, as well as that of other Sertoli genes. It possibly regulates Sertoli cell genes/factors that maintain the seminiferous tubules architecture, including basement membrane and the blood-testis-barrier. Therefore, aside from germ cell loss, the Sertoli cell *KDM1a* LOF phenotype is different from the Sertoli cell *Rbpj* LOF phenotype[1] and the germ cell *Kdm1a* LOF phenotype[2,3] and therefore warrants further investigation. Elucidating KDM1A function in Sertoli cells is critical since its expression occurs only after puberty and its ablation in these cells impairs spermatogenesis over time.

Supported by NIH HD081244 and HD101650

1. Garcia TX et al, *Development*, 2014
2. Lambrot R et al, *FASEB J*, 2015
3. Myrick DA et al, *PLoS One*, 2017

Poster 125
SINGLE-CELL RNA SEQUENCING IMPLIES MAGE-A-REGULATED PROTEIN UBIQUITINATION IN GERMLINE RESPONSE TO CALORIC RESTRICTION OF AGED MICE
Maria Camila Hoyos Sanchez[1], Tadej Jeroncic[1], Robert E. Hammer[2], Klementina Fon Tacer[1]
[1]Texas Tech University, Amarillo, TX, USA, [2]UT Southwestern Medical Center, Dallas, TX, USA
Presented By: Maria Camila Hoyos Sanchez, MS

Introduction & Objective: Spermatogenesis is a lifelong process that persists in aging men. However, aging is associated with decreased fertility and an elevated risk of congenital disorders in progeny. Given the importance of germline integrity, several mechanisms must have evolved to protect germ cells in non-optimal conditions. We have recently shown that melanoma antigens-a (Mage-a) protect the male germline in times of food scarcity, but the underlying molecular mechanisms and the role of age are not understood. Here, we applied single-cell RNA sequencing (scRNA-Seq) to address the changes in germ cells during aging, understand the molecular mechanisms behind the response to caloric restriction and determine the molecular role of Mage-as in these processes.
Methods: 16 months old wild-type and Mage-a KO male mice were exposed to 3 months-long nutrient restriction. Mice were food restricted to keep them at 80% of the initial body weight (IBW). To address the testicular response on a molecular level, we performed a scRNA-Seq analysis. One testicle from each mouse was dissociated to obtain a single-cell sequencing library. Two libraries per group were generated. Data were analyzed with CellRanger7.1.0 and SeuratV4.
Results: In contrast to our previous findings in younger animals and due to higher IBW, 20% body weight loss had a lighter effect on body fat depots and consequent metabolic stress. We observed a slight decrease in seminal vesicle weight and sperm concentration in fasted animals, however, there was no difference between the genotypes on the whole testis level. Given the previously determined expression of Mage-a in spermatogonia, we focused our analysis on these cells. We compared the transcriptome of WT mice exposed to fasting with control animals to get insights into the mechanisms activated in response to food restriction. Intriguingly, in addition to the inhibition of growth factor pathways, several genes implicated in protein ubiquitination were upregulated, suggesting ubiquitination as important quality control in spermatogenesis. This is interesting as Mage proteins act as regulators of protein ubiquitination, however, their function in the germline is still not established. Interestingly, in Mage-a-KO animals caloric restriction resulted in the activation of different ubiquitin-protein ligase-related genes, suggesting protein ubiquitination might be deregulated.
Conclusion: Our data imply that protein ubiquitination may represent an important protective mechanism in the testis of fasted animals, which is regulated by Mage proteins upon stress.

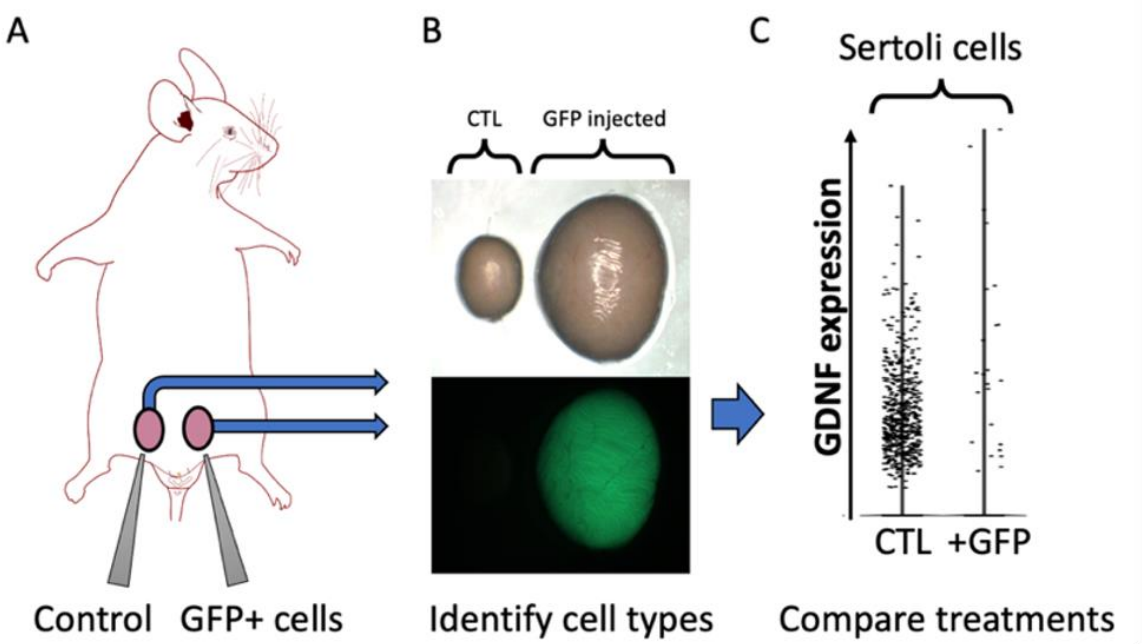
Poster 127
SPERM-ASSOCIATED ANTIGEN 6-LIKE (SPAG6L) IS ESSENTIAL FOR SPERMATOGENESIS AND MALE FERTILITY
Wei Li[1], Yi Tian Yap[1], Alivia Kearney[1], Jerome Strauss 3rd[2], Shizhen Song[3], Ling Zhang[3], Aminata Toure[4], Zhibing Zhang[1,5]
[1]1. Department of Physiology, Wayne State University, Detroit, MI, USA, [2]Center for Research on Reproduction and Women's Health, Department of Obstetrics and Gynecology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA, [3]Department of Occupational and Environmental Health, School of Public Health, Wuhan University of Science and Technology, Wuhan, China, [4]CHU Grenoble Alpes, UM GI-DPI, Grenoble, France, [5]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA
Presented By: Wei Li, MD

Introduction & Objective: Sperm-associated antigen 6 (SPAG6) is the mammalian orthologue of *Chlamydomonas PF16*, an axonemal central pair protein involved in flagellar motility. In mice, two *Spag6* genes have been identified. The ancestral gene, on mouse chromosome 2, is named *Spag6*. A related gene originally called *Spag6*, localized on mouse chromosome 16, evolved from the ancient *Spag6* gene, and has been renamed *Spag6-like* (*Spag6l*). Global *Spag6* knockout mice were grossly normal, and fertility was not affected in both males and females. The homozygous males had normal sperm parameters, including sperm number, motility, and morphology. Examination of testis histology revealed normal spermatogenesis.

Methods: A global *Spag6l* knockout mouse model was generated previously. In addition to a role in modulating ciliary beat, SPAG6L has many unexpected functions, including roles in the regulation of ciliogenesis/spermatogenesis, hearing, and the immunological synapse, among others. Even though some *Spag6l* knockout mice survived to sexual maturity in the early generations, all homozygous *Spag6l* knockout mice died before 4-weeks of age over the years, which impeded further investigation of function of the gene *in vivo*. To overcome the short survival of the conventional knockout mice, we developed a conditional allele by inserting two loxP sites in the genome flanking exon 3 of the *Spag6l* gene.
Results: By crossing the floxed *Spag6l* mice to a *Stra8-iCre* line which expresses Cre recombinase only in male germ cells, we obtained mutant mice that are missing SPAG6L in male germ cells. All *Spag6l*$^{flox/flox}$*;Stra8-iCre* mice had a normal appearance, no hydrocephalus was observed. However, all males were infertile. Compared to the control mice, these cKO mice had significantly reduced sperm number, and the sperm that were formed were morphologically abnormal and immotile. Histologic examination of the testis by light microscopy revealed impaired spermiogenesis. Abnormal sperm ultrastructure, including both head/chromatin and tail formation was discovered by electronic microscopy.
Conclusion: The newly established floxed *Spag6l* model provides a powerful tool for further investigation of the role of *Spag6l* in spermatogenesis, as well as the role of this gene in other cells and tissues *in vivo*.

Poster 128
COMPARISON OF FERTILE AND INFERTILE MICE TESTES BY SINGLE-CELL RNA SEQUENCING REVEALS SOMATIC CELL SIGNALING NETWORKS THAT SUPPORT GERM CELLS.
Eoin Whelan, John Swain, Mary Avarbock, Antonia Rotolo, Clara Malekshahi, Daniel Beiting, Ralph Brinster
University of Pennsylvania, Philadelphia, PA, USA
Presented By: Eoin Whelan, BS, PhD

Introduction & Objective: Spermatogenesis relies on a complex interplay between the differentiating germ cells and the surrounding somatic tissue, including Sertoli and Leydig cells that are vital for the normal development of sperm. Deficiencies in the normal behavior of these cells represents a cause of male infertility and many of these interactions and their clinical relevance remain to be elucidated. We employed single-cell RNA-sequencing (scRNA-seq) to simultaneously investigate the germ line-soma interactions across whole transcriptomes in all cell types.
Methods: *Kit*-mutant, infertile *Wv* mice almost entirely lacking germ cells were used as recipients for transplantation of wildtype GFP+ germ cells. For each animal, one testis was transplanted with sufficient cells to restore normal spermatogenesis and fertility, whereas the other testis was injected with vehicle only to evaluate somatic cell aberrations in the absence of germ cells. scRNA-seq was performed on cells from matched testis (n=4 pairs) and somatic cells from each treatment were compared.
Results: Compared to testes with normal spermatogenesis, we found increased expression of a wide range of transcripts in somatic cells from testes without germ cells. Sertoli cells showed the greatest changes, including a notable increase in the proportion of cells expressing *Gdnf* and *Egf*, which are known to be canonical factors promoting spermatogonial stem cells growth. Sertoli cells also displayed a considerable upregulation of genes related to cytoskeleton and involved in cell adhesion. Similarly, myoid cells also exhibited a significant increase in the expression of *Fgf2* and *Gdnf*. Lack of germ cells was also associated with *Fgf2* expression in Leydig cells, which was otherwise undetectable. A number of long non-coding RNAs implicated in the regulation of spermatogenesis were significantly upregulated, such as *Neat1* in Leydig cells and *Malat1* in Sertoli cells from the deficient testes. Collectively, our findings are consistent with the hypothesis that the lack of germ line-soma interactions triggers compensatory feedback networks that attempt to restore spermatogenesis. As these comparisons are made within individual animals, these effects were local and not systemic.
Conclusion: This work provides a systematic single-cell dissection of signaling initiated by somatic cells to maintain germ cells. It also highlights a role of regulatory non-coding RNA transcripts in promoting stem cell signaling, providing novel insights into potential therapeutic targets in the soma that support germ cells.



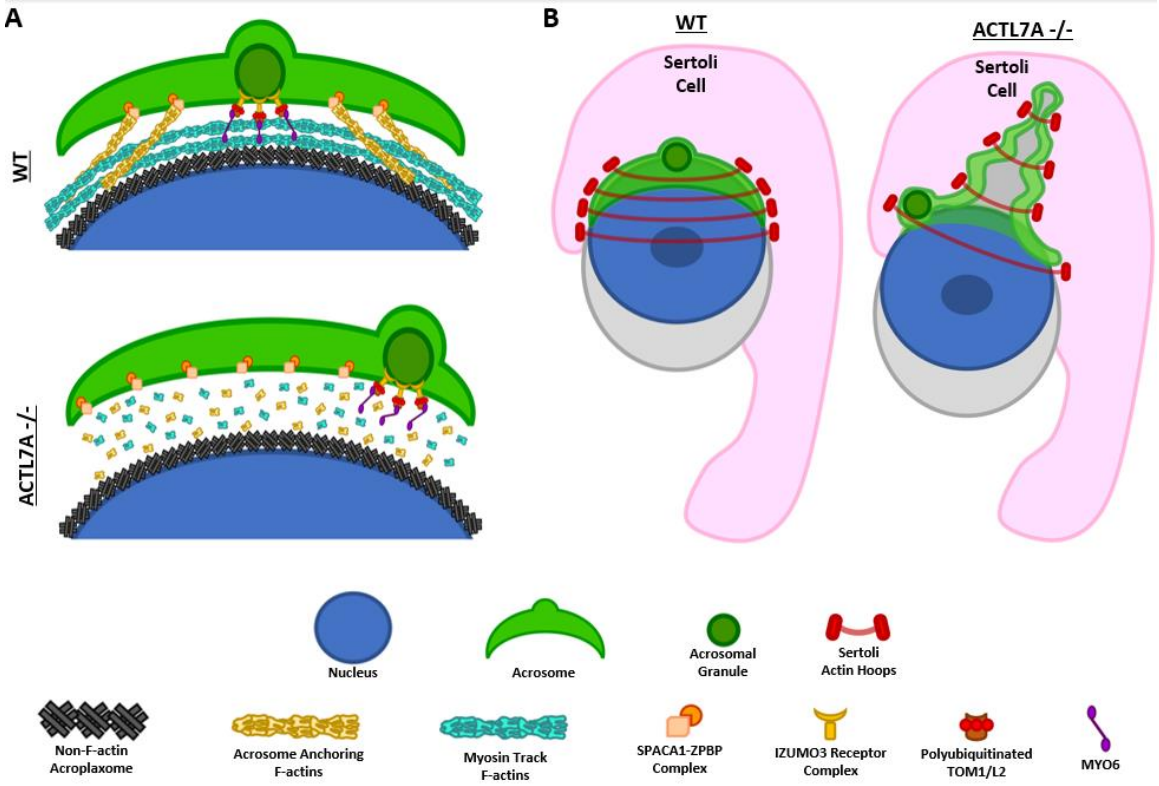
Somatic transcriptomes with/without presence of germ cells

Poster 129

TESTIS-SPECIFIC ACTIN-LIKE 7A (ACTL7A) IS AN INDISPENSABLE PROTEIN FOR SUBACROSOMAL ASSOCIATED F-ACTIN FORMATION, ACROSOMAL ANCHORING, AND MALE FERTILITY

Pierre Ferrer[1], Srijana Upadhyay[1], Masahito Ikawa[2], Tracy Clement[1]
[1]Texas A&M University, College Station, TX, USA, [2]Osaka University, Osaka, Japan
Presented By: Pierre Ferrer, BS

Introduction & Objective: Formation of the acrosome during spermiogenesis is an essential process for creating fertilization competent sperm. Of the numerous aspects required for acrosome biogenesis, adherence of the acrosomal outer membrane to the nuclear surface is mediated by the subacrosomal perinuclear theca. However, the cellular dynamics and congruent functions pertaining to these acrosomal anchoring factors are not well understood – many of which have been implicated as potential causes for human male infertility. Actin-like protein 7A (ACTL7A) is one such factor for which deleterious polymorphisms have recently been shown to cause human male infertility. As such, it is thought that acrosomal attachment is coordinated by cytoskeletal associations between the acrosome and nucleus via the acroplaxome.
In this study we seek to further illuminate the mechanistic underpinnings of ACTL7A for essential acrosome associations. Here, we investigate the dynamic intracellular localization of ACTL7A in the developing germline, its molecular associations with other cytoskeletal components, and the cellular consequences of its ablation.
Methods: In order to study the functionality of ACTL7A in the male germline and its association with acrosome biogenesis we generated a knock-out C57BL/6n mouse model using CRISPR/Cas9 as to identify any observable phenotypic deviancies. Guided by in silico predictive models of protein-protein interactions we further investigated the functionality of ACTL7A through Co-IP to identify putative binding partners as well as traced its molecular association with specific intracellular compartments and cytoskeletal suprastructures in the developing germline via indirect fluorescent microscopy.
Results: Our intracellular localization data shows ACTL7A to be dynamically present within the nucleus and subacrosomal space and later associated with postacrosomal regions of developing spermatids. By examining the generated Actl7a knock-out mouse model we constantly observed disruption of acrosomal biogenesis with abnormal migration of the acrosomal granule, and peeling acrosomes during spermatid elongation. Significantly, we find a complete loss of subacrosomal F-actin structures in knock-out spermatids. Furthermore, using ACTL7A as bait for Co-IP assays we have been able to validate its interaction with canonical actin regulating and transport protein further demarcating its role as a potential subacrosomal F-actin coordinator.
Conclusion: In conclusion, considering our reported data together with the existing literature, herein we propose a mechanistic model explaining the essential role for ACTL7A in acroplaxome F-actin formation, acrosomal attachment integrity, and male fertility.



Proposed phenotypic model of ACTL7A ablation. (A) Intracellular model of the subacrosomal space depicting the attachment of the granule-anchoring MYO6 motor protein complex to ACTL7A dependent F-actins and their organizational disruption in the absence of ACTL7A. (B) Cellular diagram portraying a model as to why acrosomal fragments detached from spermatids and retained in Sertoli cell crypts in *Actl7a* KO mice.

Proposed phenotypic model of ACTL7A ablation.

Poster 130

HISTOLOGICAL ASSESSMENT OF TESTICULAR FUNCTION IN MOUSE MUTANTS WITH SATINN – SOFTWARE FOR THE ANALYSIS OF TESTIS IMAGES WITH NEURAL NETWORKS

Ana Cristina Lima, Ran Yang, Alexandra Stendahl, Donald Conrad
Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, USA
Presented By: Ana Cristina Lima, MS, PhD

Introduction & Objective: To assess fertility of mouse mutants, testicular function is evaluated by estimating daily sperm production (DSP) and by testis histopathology. These assays are low-throughput in nature and challenging to perform in a quantitative and reproducible manner. We sought to develop a software – SATINN – to overcome these limitations and evaluated its performance in the detection of mild histopathologies in a new mouse line – *Crispy*- with a targeted deletion predicted to impact spermatogenesis.

Methods: Our computational workflow combines image processing and segmentation with neural network-based recognition to automatically analyze and classify whole mouse testis cross-sections. As training data, we annotated over 2,000 tubules and 7,800 individual cells stained with a set of immunofluorescent markers: Hoechst, Acta2, and Acrv1. We then evaluated testis function in the *Crispy* line using SATINN and compared it to manually generated pathology data: a) Periodic Acid-Schiff stained seminiferous tubules annotated for abnormalities (n=5,248); b) DSP values (n=38) and c) RNA *in situ* hybridization using probes against *Dmrtb1* and *Lrp8* (n=120).

Results: SATINN classifies nuclei into 7 cell types and tubules into 12 developmental stages, provides measurements for nuclei and tubule features and calculates spermatogenic index (ratio of elongated spermatids:spermatogonia counts). Using SATINN to evaluate *Crispy* mice, we found subtle but significant changes in the nuclear areas of most cell types and an apical shift in nuclei location of primary and secondary spermatocytes ($p<10^{-5}$), indicating a mild phenotype in mutants. Similarly, manual evaluation revealed an increase in the frequency of abnormal tubules in mutants when compared to wild-type ($p= 0.007$; Wald test). While tubule and lumen sizes are normal, *Crispy*$^{-/-}$ show a 35% reduction in spermatogenic index ($p<10^{-5}$), matching the 28% decrease

estimated by DSP (p=0.016; Wald test) from frozen testis of the same mice. These changes in testis function may in part result from the small, but significant, increase in expression of *Dmrtb1* ($p < 10^{-4}$; Wilcoxon test) and *Lrp8* ($p < 10^{-31}$; Wilcoxon test) detected in mutants.
Conclusion: Using SATINN, we confirmed the manual quantifications and improved on the identification of mild testicular changes detected in the *Crispy* mutants. Importantly, SATINN eliminates the need to perform the DSP assay and allows for a high-throughput analysis of hundreds of tubules, providing a fast, unbiased and skill-independent way of analyzing testicular histology.

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REGENERATIVE POTENTIAL OF H-UCMSC-DERIVED EXOSOMES IN AMELIORATING CHEMOTHERAPY-INDUCED DAMAGE IN HUMAN SERTOLI CELLS BY INCREASING CELL PROLIFERATION, REDUCING APOPTOSIS AND DNA DAMAGE

Omer Raheem, Farzana Liakath, Jin Seok, Hang-Soo Park, Mohammad Mousaei, Ayman Al-Hendy
The University of Chicago, Chicago, IL, USA
Presented By: Omer Raheem, MD

Introduction & Objective: Cytotoxic anticancer agents result in gonadal dysfunction in cancer survivors as the chemotherapeutic agents disrupt spermatogenesis in the germinal epithelium of Sertoli cells. Previous studies in rodents have shown the regenerative potential of MSCs in restoring testicular injury and spermatogenesis. As a cell-free therapeutic approach, we leveraged the regenerative potential of exosomes derived from the h-UCMSCs in restoring damaged Sertoli cells which are essential for spermatogenesis. This study aims to understand the molecular mechanisms underlying the regenerative effect of h-UCMSCs exosomes in an *in vitro* testicular dysfunction model using human Sertoli cells.
Methods: We used the human Sertoli cells (hSerC) to create a testicular dysfunction model by inducing apoptosis using 250 µg/ml of cyclophosphamide for 24 h and treated these damaged hSerC cells with a dosage of $1.50X10^9$ exosomes derived from in-house h-UCMSCs at day 24 hours post cytotoxicity. Cell proliferation and viability assays were performed, and relative gene expression was studied using four sets of genes which include i) markers of cell proliferation and survival (PCNA, AKT, Ki67, P21, CYC D1) ii) markers of apoptosis (Cas3, Bcl-2, and Bax) iii) makers of DNA damage (BRCA1 and RAD51) and iv) steroidogenesis (hFSHR).
Results: The exosome-treated hSerC group showed improved cell proliferation ($p<0.05$) compared to untreated damaged cells and increased cell viability was observed in exosome treated group (78%) compared to the damaged cells (56%). Among the genes tested, the relative gene expression of cell proliferative markers PCNA, AKT, Ki67, and P21, in the exosome, treated group was significantly upregulated ($p<0.001$) compared to untreated damaged cells with a concurrent downregulation of Cas3 and RAD51 which indicate anti-apoptotic and DNA repair mechanisms. Immunoblot analysis and RNA sequencing are undertaken currently to identify key miRNAs and molecular pathways responsible for Sertoli cell regeneration.
Conclusion: We showed that treatment of hSerC cells with exosomes derived from h-UCMSCs stimulates cellular proliferation and cell survival with the concurrent upregulation of cell proliferative markers, and downregulation of apoptotic and DNA damage at the molecular level. These preliminary findings encourage us to identify key miRNA molecules and downstream anti-apoptotic/DNA repair/cell proliferative pathways responsible for the regenerative potential of h-UCMSCs-derived exosomes. These exosomes can potentially be considered a novel cell-free therapeutic option for restoring fertility in cancer survivor patients.

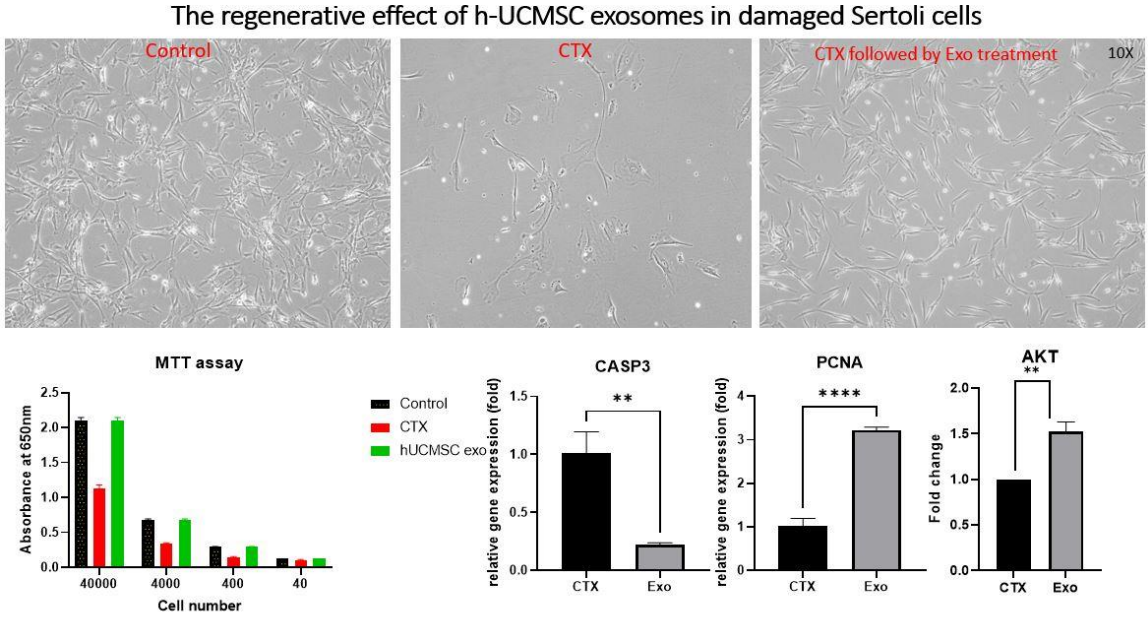

Table 1

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CHARACTERIZATION OF THE SPERMATOGENIC FUNCTION OF HUMAN DAZ FAMILY MEMBERS USING HUMANIZED TRANSGENIC FLIES

Liping Cheng[1], Eugene Xu[1,2]
[1]State Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing, China, [2]Cellular Screening Center, the University of Chicago, Chicago, IL, USA
Presented By: Eugene Xu, PhD

Introduction & Objective: Deletion of *Deleted in AZoospermia* (*DAZ*) genes is the most common molecular lesion of male infertility without sperm, yet the spermatogenic function of the DAZ gene remains unknown. DAZ genes, consisting of a cluster of four genes (*DAZ1*, *DAZ2*, *DAZ3*, and *DAZ4*) on the Y chromosome, also have two autosomal paralogs, *BOULE* and *DAZL*. Both *BOULE* and *DAZL* genes are restricted to germ cells and are required for fertility in diverse animals. Much progress has been made in our understanding of mechanisms by which BOULE and DAZL regulate spermatogenesis in animals, yet the spermatogenic function of human BOULE and DAZL as well as DAZ proteins remained difficult to study. Why humans need all three members for sperm development and what is the functional difference among the three members remain largely unexplored.
Methods: We established humanized transgenic animal models in Drosophila to directly examine the spermatogenic function of human DAZ gene family members (*DAZ3*, *DAZL*, and *BOULE*).
Results: Human BOULE and DAZL are able to regulate meiosis and rescue the meiotic defects of infertile fly *bol* mutant but diverge in their ability in rescuing the defects in spermatid differentiation. DAZ3, though containing similar conserved RBD domain and DAZ repeats, diverged significantly from BOULE and DAZL in its regulation of meiosis and spermiogenesis, and failed to rescue defects in either meiosis or spermatid differentiation. Swapping of RBD domains and DAZ repeat further defines the specific contribution of the conserved domains to their functional similarity/difference during spermatogenesis.
Conclusion: Our finding supports the hypothesis that human DAZ family members (DAZ, BOULE, and DAZL) have diverged in their spermatogenic function but BOULE and DAZL retain similar functions in the regulation of meiosis.

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HIGH-THROUGHPUT SINGLE-CELL ANALYSIS OF CYTOPLASMIC-NUCLEAR TRANSLOCATION OF PROTAMINES IN THE MALE GERM LINE

Eduardo Roldan[1,2], Clara Agudo[1,2], Julie Farnsworth[1], Larry Boyle[3], Maria Teves[1]
[1]Virginia Commonwealth University, Richmond, VA, USA, [2]Natural Sciences Museum, CSIC, Madrid, Spain, [3]Luminex Corporation, Austin, TX, USA
Presented By: Maria Teves, PhD

Introduction & Objective: Protamines (PRMs) are small, arginine-rich, nuclear proteins important for chromatin compaction, gene silencing and nuclear remodeling. During male germ cell differentiation, PRMs translocate from the cytoplasm to the nucleus. Alterations in this process may result in lower protamine levels in the nucleus and ensuing sperm DNA fragmentation, cell

abnormalities, altered sperm function and decreased male fertility. To gain insight into protamine transport and function we employed image flow cytometry to quantify nuclear translocation of PRM1 with simultaneous characterization of different spermatid subpopulations.
Methods: Male germ cells were isolated from 6-week-old mice by chemical and mechanical digestion. Cells were immunolabeled using anti-PRM1 primary and Alexa 594-labeled secondary antibodies and co-stained with the nuclear marker Hoechst 33342. The Amnis ImageStreamX Mark II™ image flow cytometer was used to acquire ~200,000 cells/sample using bright field 40X magnification and lasers of 405nm for Hoechst33342 and 592nm for Alexa 594. Analyses were performed using IDEAS® v6.3 software. In parallel, similarly prepared germ cells were mounted on a slide and images captured using a Zeiss LSM 700 confocal laser-scanning microscope. Analyses were carried out using ImageJ software.
Results: Single cells were first gated according to DNA content (lower or higher Hoechst33342 intensity). Second, haploid cells were selected and gated based on area and aspect ratio (width to height ratio). This feature was instrumental to separate different spermatid maturation steps. Thus, round spermatids were expected to have an aspect ratio closed to 1 and elongating spermatids <1. Third, several subpopulations were gated to identify elongating spermatids stages (R11, steps 9-10; R8, steps 11-12; R12, steps 13-14, R13, steps 14-15 and R7, step 16). Fourth, nuclear translocation was assessed using a mean similarity score. Results from this final analysis revealed that the percentage of nuclear localization of PRM1 in each subpopulation was R11=0.74%, R8=0.66%, R12=4.48%, R13=39.1% and R7=71.5%. These results indicate that nuclear localization of PRM1 initiates at spermatid step 12 during mouse spermiogenesis, which is consistent with earlier analyses.
Conclusion: This method provides a very sensitive and objective approach to measure nuclear translocation of PRM1 and other nuclear proteins at the single-cell level. In comparison to regular confocal microscopy, it represents a much more efficient approach since it allows for an assessment a larger number of cells in a shorter period of time.

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OPTIMIZATION OF HYDROSTATIC AND DIFFUSION PARAMETERS FOR TESTIS CULTURE AND GENERATION OF A NOVEL ORGANOID MODEL
Tracy Clement, Jacob Cabler, Pierre Ferrer
Texas A&M University, College Station, TX, USA
Presented By: Tracy Clement, PhD

Introduction & Objective: A functional testis organoid system allowing complete spermatogenesis in vitro is needed to advance basic research, animal toxicity alternatives, and clinical applications. Here we aim to distill limitations of current systems down to first-principles and address limitations in testis physiology/cell biology and bioengineering for development of organoids replicating key in vivo testis physiology including architecture and spermatogenic output. In this study we determine the effects of diffusion limitation, specific hydrogel materials, and hydrostatic pressure on in vitro testis cultures.
Methods: We developed spectrofluorometer based methods to test permeability and diffusion of relevant molecules and measured diffusion across hydrogel barriers in a modified transwell culture vessel. We compared diffusion limitation profiles of hydrogel types and layer thicknesses. We used our modified system to compare 7.5dpp mouse testis explant responses to hydrogels of various diffusion limitations cultured for up to 41 days. Tissue growth rates were recorded and compared over time. Qualitative and quantitative assessments of tissue responses were determined after tissue fixation using a combination of standard assessments, multiplexed IHC, and semi-automated image-J scripts assessing specific cell types and tissue responses. We also developed a novel system to test hydrostatic pressure responses of cultured testis. A third method was developed using a combination of bioengineering approaches to generate organoid constructs of various controlled diffusion profiles and a platform for culture with varied hydrostatic and hydrodynamic conditions while also replicating the morphology and physiology of the seminiferous epithelium.
Results: We determined the diffusion limitations profiles for hydrogel barriers and found that both hydrogel type and thickness impacted long term tissue growth, morphology, and cell type ratios. The best responses were material specific and favored a specific diffusion limitation range. We further developed and optimized testis organoids replicating the seminiferous epithelium through a novel production process. Sertoli-like cells seeded in these constructs produce coiled seminiferous tubule-like organoids.
Conclusion: Hydrostatic and hydrodynamic variables including diffusion limitation and hydrostatic pressure are important for in vitro testis model responses. A novel bioengineering process can allow for production of organoids with better control of these variables for further in vitro model development and testing.

Poster 135
IRON DEPOSITION IN EPIDIDYMIS LINKED TO FIBROSIS AND INFERTILITY IN AUTOIMMUNE REGULATOR DEFICIENT MALE MICE.
Geoffrey Grzesiak, Margaret Petroff, Thomas O'Halloran, Soo Hyun Ahn, Keith MacRenaris
Michigan State University, East Lansing, MI, USA
Presented By: Soo Hyun Ahn, PhD

Introduction & Objective: The epididymis is an elongated tubule where testicular sperm mature and gain fertilization competence. Inflammation of the epididymis - epididymitis - is a contributing factor to male infertility. Most cases of epididymitis are attributed to ascending bacterial infection through the vas deferens; a minority of cases can arise from an underlying autoimmune disorder

such as Autoimmune Polyglandular Syndrome Type 1 (APS-1). APS-1 is caused by a mutation in Autoimmune regulator (*Aire*) which is a transcription factor that is expressed in the thymus. By regulating the expression of peripheral tissue-specific antigens, Aire ensures generation of a T cell receptor repertoire that is tolerant to self-antigens but averse to foreign antigens. Among many symptoms, men with APS-1 can suffer from testicular insufficiency and infertility. The objective of this research is to determine the lesions in the male reproductive tract that cause male infertility in APS-1.

Methods: Mice with targeted mutations in *Aire* were generated by CRISPR/Cas9 recombination, resulting in a 32-bp deletion on the Balb/c genetic background (*32delAire*) in Exon 2 of *Aire*, as well as *32delAire.RAG1ko*, which lack both *Aire* and Recombinase Activating Gene 1 (*Rag1*), and therefore are T- and B cell deficient. Wild type mice served as controls. Immunohistochemistry and Masson's trichrome were used to characterize the immune cells and fibrosis in the epididymis, respectively. Laser Assisted Inductively Coupled Plasma Time Of Flight Mass Spectrometry (LA-ICP-TOF-MS) was used to spatially map and quantify iron concentration in the epididymis.

Results: *32delAire* males had severely reduced fertility compared to WT and *32delAire.RAG1ko* males. All *32delAire* males (n=10) had epididymitis, infiltration of CD8+ T and CD19+ B cells and fibrosis. LA-ICP-TOF-MS revealed increased iron deposition in the interstitial space of the epididymis. These changes were prominent in the corpus and cauda epididymis, but absent in the caput, and were observed in male mice as young as 9 weeks of age. No immune cell infiltration, fibrosis, or iron deposition were observed in *32delAire.RAG1ko* mice, suggesting that T and B cells target the epididymis and contribute to fibrosis.

Conclusion: WE show in the absence of *Aire*, T and B lymphocytes target the epididymis and contribute to fibrosis and iron deposition. Further studies will reveal the specific role of lymphocytes in causing these changes, and how iron deposition influence sperm quality.

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SEMEN ANALYSIS PARAMETERS IN TRANSGENDER PATIENTS COMPARED TO CISGENDER PATIENTS

Sai Allu[1], Stephen Schmit[1], Sreeya Komaravolu[2], Mark Sigman[2], Gabriella Avellino[2]
[1]Alpert Medical School, Providence, RI, USA, [2]Department of Urology, Alpert Medical School, Providence, RI, USA
Presented By: Sai Allu, BA

Introduction & Objective: Transgender and nonbinary people represent an important urological patient population with unique healthcare needs. There is limited literature assessing the viability of semen cryopreservation for this patient population. Prior research has demonstrated that transgender patients have reduced semen analysis (SA) parameters in comparison to cisgender populations, regardless of initiation of hormonal therapy (HT). This study aims to report differences in cryopreservation outcomes between transgender patients and cisgender patients, with a secondary analysis comparing transgender patients with a history of HT to transgender patients with no history of HT.

Methods: We retrospectively reviewed charts of donors at a single academic institution between January 2011 and July 2022. Collected data included patient demographics, past medical history, and SA data. SA parameters included volume, total sperm count, sperm concentration, and percent motility. Maximum values were extracted for patients with multiple SAs in order to compare differences between cisgender and transgender patients. For transgender patient subgroup analysis, multiple samples were treated as individual data points. HT was defined as androgen blocking-agents or estrogen replacement therapy within 3 months before semen collection. Statistical significance was determined with the independent one-tailed, two-sample t-test.

Results: Chart review yielded a final sample of 92 patients, with 58 cisgender and 34 transgender patients. Average ages were 37.1 and 22.0 for cisgender and transgender patients, respectively. Comparison of SA parameters yielded the following average maximum values for cisgender versus transgender patients, respectively: semen volume (3.24 mL vs. 3.00 mL, $p = 0.51$), total sperm count (139.43 million vs. 123.17 million, $p = 0.24$), sperm concentration (84.13 millions/mL vs. 71.3 millions/mL, $p = 0.14$), and percent motility (61.59% vs. 55.19%, $p = 0.036$). Among transgender patients, 8 individuals (16 samples) had been or were receiving HT within three months of providing a sample, and 25 individuals (42 samples) had no history of HT. There were no significant differences in SA parameters regardless of hormonal therapy (Table 1).

Conclusion: Transgender patients demonstrated inferior SA parameters, though only the difference in percent motility reached statistical significance. HT was not associated with significant differences in SA. Future studies with larger sample sizes are needed to allow detailed analysis of type, dose, and time effects of HT in this patient population. These findings may have important implications for transgender patients considering fertility preservation.

	Volume (mL)	Motility (%)	Concentration (millions/mL)	Sperm Count (millions)
Hormonal Treatment	3.12	50.94	70.56	124.81
No Hormonal Treatment	2.43	52.40	61.96	96.51
p value	0.08	0.39	0.25	0.17

Mean SA values in transgender patients with and without HT