spermatozoa were identified in their testicular sperm extraction (TESE) specimens.

DESIGN: TESE samples were collected from 12 men (10 OA, 2 NOA) from August 2016 to May 2017. Each cell type was characterized using immunofluorescence (IF). IF intensity and cell localization (nucleus vs cytoplasm) of the stained protein in addition to cell diameter and state of the nucleus was used to identify GC. Gene expression was investigated.

MATERIALS AND METHODS: TESE specimen from men aged 42.8 ± 11 years old were smeared on slides and air dried. The slides were stained for spermatogenesis stage-specific markers using antibodies to DAZL (all germ cells), SYCP3 (spermatocytes), and the lectin peanut agglutinin (PNA; acrosomal membrane). TESE samples in which spermatozoa were identified were considered positive controls for the markers. Once validated, these markers were used to identify GC presence in NOA samples with failed TESE. Differences in IF and cell density were compared. Epigenetic assay was carried out by next generation sequencing (NGS).

RESULTS: To validate our assay, we evaluated 10 TESE samples (9 OA and 1 NOA) with successful sperm extraction yielded an average of $1.9\pm2x10^6$/mL spermatozoa with 3±2% motility. A positive staining for DAZL signified the presence of immature GC. While spermatogonial cells stained positive for DAZL in the nucleus, the more advanced stages (spermatocytes and spermatids) displayed the DAZL marker only in the cytoplasm (69%). Nuclear characteristics, nuclear-cytoplasmic ratio, cell diameter and SYCP3 positivity helped to identify spermatocytes at the pachytene stage with an average of 10% in all control specimen examined. In addition, staining for the acrosome in late spermatids and spermatozoa yielded 33% positive cells. RNA-seq for gene expression confirmed the maturational stages.

In 5 couples undergoing TESE and ICSI, an extensive search by several embryologists of 48-64 drops under oil failed to identify any sperm cells. To address the failed expectation, oocytes were cryopreserved and we investigated with the above mentioned assay to identify eventual presence of GC. In two samples, no IF markers were visualized, suggesting either GC aplasia or sertoli-cell-only syndrome. In 1 specimen, count of DAZL positive cells yielded 3%, however with an irregular nuclear shape, indicating spermatogonial stem cells. In 2 men, we identified approximately 1% positive for DAZL in the nucleus with scant cells positive for DAZL (in the cytoplasm) and SYCP3.

CONCLUSIONS: Staining for GC and specific maturational stages help to characterize TESE specimens that fail to yield spermatozoa. While IF assays are helpful to expeditely screen specimens, RNA-seq would confirm presence of the scant post-meiotic stages. Profiling testicular tissue that fail to yield spermatozoa at a TESE attempt may be invaluable for diagnostic and patient counseling purposes.

P-548 Wednesday, November 1, 2017

WHOLE-EXOME SEQUENCING IDENTIFIES NOVEL HETEROZYGOUS MUTATION IN RAF1 IN FAMILY WITH CRYPTORCHIDISM AND NEONATAL TESTICULAR TORSION. T. P. Kohn,[a] D. M. Lopategui,[b] A. J. Griswold,[c] R. Ramasamy.[b] [a]Baylor College of Medicine, Houston, TX; [b]University of Miami, Miami, FL; [c]John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL.

OBJECTIVE: To investigate the genetic cause of neonatal bilateral testicular torsion in two siblings and paternal cryptorchidism in an American family without history of consanguinity.

DESIGN: Homozygosity mapping followed by targeted exon/whole-exome sequencing to identify genetic variations.

MATERIALS AND METHODS: We sequenced the exomes of two brothers with neonatal bilateral testicular torsion and their father with a history of cryptorchidism. All variants passing filter criteria were validated with confirming absence in the control population. DNA was isolated from the blood samples using the commercial kit QIAamp blood Maxi. WES was performed with the Agilent SureSelect xt 60 mb v6 and Illumina Hiseq PE 100 technologies.

RESULTS: A novel non-synonymous heterozygous mutation in RAF proto-oncogene serine/threonine-protein kinase (*RAF1*) was identified in a non-consanguineous family. This mutation in exon 7 (chr3: 12645786 G>C) of RAF1 is likely a disease-causing mutation as it is predicted to be damaging, is a novel variant, and is found in the two brothers with neonatal testicular torsion as well as in the father with cryptorchidism. The mutation is not found in the single nucleotide polymorphism (SNP) database, the 1000 Genomes Project, ExACT or esp6500. Other reported mutations in this gene are associated with Noonan Syndrome, in which 60-77% of males have bilaterally undescended testis. Additionally, several case reports have reported testicle torsion in boys with Noonan syndrome. One proposed mechanism of testicular torsion is abnormal testis descent during development. We hypothesize that variable penetrance of this gene accounts for the paternal cryptorchidism as well as the neonatal testicular torsion seen in the two brothers.

CONCLUSIONS: Using WES, we identified a novel heterozygous mutation in *RAF1* as a likely disease-causing variant in an American family diagnosed with neonatal testicular torsion and cryptorchidism. Our data reinforce the clinical role of WES in the molecular diagnosis of highly heterogeneous genetic diseases which conventional genetic approaches have previously failed to conclude a molecular diagnosis.

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ELECTROMAGNETIC FIELD RADIATION LEAD TO ALTERATION OF BLOOD-TESTIS BARRIER REGULATING PROTEIN LEVELS IN THE RAT TESTIS. N. Gungor-Ordueri,[a] H. Er,[b] C. Celik-Ozenci,[c] E. Erdem,[d] S. Ozen.[e] [a]Medicine Faculty, Biruni University, İstanbul, Turkey; [b]Biophysics, Akdeniz University, Antalya, Turkey; [c]Histology and Embryology, Akdeniz University, Antalya, Turkey; [d]Urology, Ota-Jinemed Hospital, Istanbul, Turkey; [e]Engeneering Faculty, Akdeniz University, Antalya, Turkey.

OBJECTIVE: The use of electromagnetic field (EMF) generating apparatuses such as cell phones is increasing, and has caused an interest in the investigations of its effects on human health. Spermatogenesis is a process, which is controled by blood-testis barrier (BTB) and its regulatory proteins between the adjacent Sertoli cells in the seminiferious tubules.

DESIGN: We analyzed proteins in preparations from the whole testis in the adult male Sprague-Dawley rats that were exposed to 900 MHz and 2150 Mhz EMF radiation for 2 h/day for 1 week and 10 weeks, simulating a range of possible human cell phone use. Blood testis barrier (BTB), which have an essential role for maintaining the spermatogonesis, is founded between two adjacent Sertoli cells. In this study we aimed to understand the relationship between the BTB and the radiation effects to the spermatogenesis by determining the BTB regulating proteins localizations.

MATERIALS AND METHODS: Subjects were sacrificed immediately after the end of the experiment and testes lysates were prepared for the confirmation N-cadherin levels in the different groups by immunoblotting and analyzed by Image J. N-cadherin, occludin, ezrin and fascin were identified by immunohistochemistry. The expression levels of the BTB regulating proteins were anlyzed by their expression levels at the BTB side of the seminiferious tubules at 900 and 2150 MHz radiation. We also scored the tubules at stage VIII by apoptosis.

RESULTS: We examined that the seminiferous tubule epithelia were disorganized after the 900 and 2150 MHz radiation. Apoptosis index was %40 and %55 in 900 and 2150 MHz, respectively. The expression levels of N-cadherin decreased in the 2150 MHz radiation groups at the BTB side. Protein levels of occludin didn't change, however ezrin and fascin proteins decreased and dislocalized at the BTB sides under both 900 and 2150 MHz radiation.

CONCLUSIONS: These results demonstrate significant effects of radio frequency modulated EMFs exposure on blood testis barrier, particularly in protein species in the rodent testis, and suggest that a 1 week and 10 weeks exposure to EMF radiation were maintain the stress in testicular tissue. Thus, BTB may not get back to its first physiological location because of the disorientation of the regulating proteins of BTB, which disturb the spermatogenesis process.