conducted a post-hoc power analysis and the study had a sufficient sample size to provide 80% power at α=0.05 for detecting at least change of 1% in sperm concentration for a change in distance of 500 meter.

CONCLUSIONS: Residential distance to major roadways as an indicator of traffic related air pollution exposure was not related to semen parameters among men attending a fertility clinic. This is the largest prospective cohort we are aware of that explored this association.

O-8 Monday, October 30, 2017 11:15 AM

HIGH DEGREE OF HETEROGENICITY IN SSEA POSITIVE HUMAN SPERMATOGENIA. R. Flannigan, a A. Mielnik, a B. Bolyakov, b F. Khani, c B. D. Robinson, c P. N. Schlegel, d W. Wright, e D. Paduch, e Urology, Weill Cornell Medicine, New York, NY; eUrology, Weill Cornell Medical College, New York, NY; Weill Cornell Medicine, New York, NY; Pathology, Weill Cornell Medicine, New York, NY; Biochemistry & Molecular Biology, John Hopkins University, Baltimore, MD; eDept of Urology, Weill Cornell Medical College, New York, NY.

OBJECTIVE: To characterize spermatogonial (SPG) cells and determine if heterogeneity exists among SSEA4 enriched SPG populations.

DESIGN: We used human cadaveric testis tissue. SGPs were isolated using SSEA4 based magnetic-activated cell sorting (MACS), and characterized using flow cytometry (FC) and fluorescence-activated cell sorting (FACS) using the WEKA algorithm.

MATERIALS AND METHODS: 6 human cadaveric testes were harvested and 200mg of tissue was mechanically disaggregated. SGPs were isolated from the cellular suspension using antibodies to SSEA4 and MACS. FC gates were established using NTERA (SSEA4+; EPCAM-) and LNCAP (SSEA4-; EPCAM+) cells as the positive and negative controls respectively. We then characterized the MACS sorted SSEA4+ cells by FC. SSEA4 MACS isolated testis cells were then double stained with a combination of either antibody against GFRA1 and vincidmint (Vim) or UTF1 and SOX-9 to detect SSC and Sertoli cells respectively. IF with antibodies for ZBTB16, UTF-1, GFRA1, and Vim were performed on normal testis biopsies to confirm FC findings. IF images were segmented and analyzed using the WEKA algorithm to measure cell heterogeneity.

RESULTS: Out of all SSEA4+ MACS isolated cells, 19% were GFRA1+, and 0.49% were UTF1+. Low numbers of UTF1+ cells indicate that SSEA4+ isolates are predominantly SPGA, dark, as reported by others. Upon further analysis of all GFRA1+/Vim- cells, we distinguished 4 different populations of GFRA1+ cells at 40:20:20:20 ratio based on the width and fluorescence intensity of GFRA1. Among UTF1+/SOX-9+ SGPs, two distinct populations with different size (width) but similar fluorescence intensity were identified at 50:50 ratio. IF analysis confirmed existence of two populations of UTF1 cells with different nuclear size but similar UTF1 staining intensity (p=0.001). Similar heterogenicity of SPG cells were identified within the testsis using anti-GFRA1 and ZBTB16 antibodies. WEKA identified 8 distinct classes of cells along the seminiferous tubule membrane indicating significant heterogeneity of SSCs/SPG in humans.

CONCLUSIONS: Using a commonly employed method of human SPG/SGS isolation (MACS with anti-SSEA4), we identified significant heterogeneity among isolated SSEA4+ SGPs. These findings suggest several sub-populations of SPG/SGS exist, and is essential to recognize when MACS isolated cells are used for downstream applications such as RNAseq among isolated testis cell populations. Future directions will be to determine if observed heterogeneity within SSEA4+ cells translate to differences in mRNA and miRNA expression profiles among these SPG/SGS sub-populations using the 10X platform.

Supported by: NIH grant P50HD076210 (Project II and outreach core); Howard & Irena Lans Foundation and Robert Dow Foundation; RF is supported by Urology Care Foundation Scholar Award Program New York Section.

O-9 Monday, October 30, 2017 11:30 AM

SUBCUTANEOUS LEYDIG STEM CELL AUTOGRAT IN MICE: A NOVEL APPROACH TO INCREASE SERUM TESTOSTERONE. H. Arora, a J. M. Hare, a R. Ramasamy, a Urology, University of Miami, Miami, FL; aISC, University of Miami, Miami, FL; University of Miami, Miami, FL.

OBJECTIVE: Leydig cell loss or dysfunction is associated with impaired testosterone production. Exogenous testosterone supplementation can be used to treat low testosterone; however it has several adverse effects including infertility due to negative feedback on the hypothalamic-pituitary-gonadal axis. In the present study, we describe a novel approach to increase testosterone in mouse models by autografting Leydig stem cells isolated from testes in skin.

DESIGN: Adult Leydig cells presence was confirmed in autografts using H&E staining and Immunofluorescence (IF) for 3B8HSD and LH expression. Levels of testosterone, FSH and LH were checked in autografts and were compared to that in control animals.

MATERIALS AND METHODS: A total of 12 wild-type adult C57/B6 mice were included in the study. Orchietomy was conducted in nine mice (6 experimental and 3 negative controls) and the remaining three were used as positive controls. Leydig Stem cells (LSCs) were harvested from testis by collagenase/trypsin digestion. LSCs in combination with peritubular myoid cells and Sertoli cells from three mice were cultured and expanded in the proliferation medium for ten days after which the combination of the cells were autografted in the subcutaneous tissue with matrigel. Four weeks later, graft was harvested for structural evaluation. Testosterone pellets were placed subcutaneously for positive control and orchietomized mice were used as negative control.

RESULTS: We successfully isolated and cultured up to 1 X 106 million LSCs/testis from all three animals up to 14 days. When treated in media containing leutinizing hormone, LSCs differentiated into adult Leydig cells and expressed 3B8HSD. Stem cell property of LSCs was confirmed by 3B8HSD-negative, lutetinizing hormone (LH) receptor (LHR) -negative, and platelet-derived growth factor receptor Alpha (PDGFRα) -positive. When LSCs were implanted into subcutaneous tissue of mice, they expressed 3B8HSD, resulting in the increased levels of serum testosterone compared to mice that did not receive autograft (22.27+ 1.33 ng/dl vs 13.20+ 0.37 ng/dl). Importantly, mice following stem cell autograft maintained production of LH and FSH with levels higher than mice that underwent exogenous testosterone administration (LH 3.00 +0.36 ng/ml vs 0.016 +0.0 ng/ml, FSH 78.30 +15.34 ng/ml vs 51.7+ 7.4 ng/ml).

CONCLUSIONS: Our results demonstrate that Leydig stem cells in subcutaneous autograft can increase serum testosterone without inhibiting circulating LH and FSH. Leydig stem cell autograft is a novel therapeutic approach to increase serum testosterone while simultaneously preserving testicular function.

Reference: 1. Research Scholar Award from the SMSNA / AUA Urology Care Foundation

Supported by: This work was supported in part by the Research Scholar Award from the SMSNA / AUA Urology Care Foundation

O-10 Monday, October 30, 2017 11:45 AM

UNDERSTANDING SEMINAL PLASMA PROTEOMIC SHIFTS BROUGHT UPON BY DIVERSE BIOLOGICAL CONDITIONS. P. Intasqui, a M. P. Antoniassi, a M. Camargo, b V. Carvalho, b R. Bertolla, a aSao Paulo Federal University, Sao Paulo, Brazil; bFleurys Group, Sao Paulo, Brazil.

OBJECTIVE: Several studies have evaluated the seminal plasma proteome associated with male infertility factors. However, little is known regarding the molecular composition of normal seminal plasma, and how this deviates in diverse causes of male infertility. Therefore, a bioinformatics study was performed to evaluate the seminal plasma proteomic constitution of controls, and how different cellular effects (such as sperm DNA fragmentation) and biological conditions (such as varicocele) alter this proteomic map.

DESIGN: Basic research study with separate prospective cohorts.

MATERIALS AND METHODS: Two comprehensive quantitative shotgun proteomics seminal plasma maps were plotted: one in which proteins were flagged for DNA fragmentation, mitochondrial activity, acrosome integrity, or semen oxidative stress; and one in which proteins were flagged for varicocele, obesity or smoking. For both studies, controls were only patients without male infertility factors or alterations to sperm function (the three parameters were required), and with normal semen (WHO, 2010). Maps mapped data from separate studies (326 patients), and were analyzed for differential protein expression using the CytoScape suite for construction of protein-protein interaction networks, inferred from databases such as IntAct, String and BIOGRID.

RESULTS: In the sperm function map, a total of 799 proteins were identified, with 2,735 interactions among them. One protein was exclusively