

these two populations of NOA men and what men may benefit from surgical correction.

**DESIGN:** Tissues and blood were obtained from men with NOA (n=16) and subdivided into those with varicoceles (n=9) and those without (n=7).

**MATERIALS AND METHODS:** Gene-expression microarray (Agilent Sureprint G3 8x60K) screened for gene expression with candidate genes identified using Ingenuity Pathway Analysis (IPA) software. Results were validated using qPCR.

**RESULTS:** NOA men with, and without varicoceles had similar ages (34±0.4 vs. 32±2 years) and testicular volumes (Left, 15±2 vs. 13±1 mL; Right, 14±2 vs. 13±1 mL). Serum levels for FSH (20±7 vs. 22±4 mIU/L), LH (7±1 vs. 8±1 mIU/L) and testosterone (313±43 vs. 296±30 ng/dL) were not different. IPA revealed 39 genes preferentially expressed in men with varicoceles (IPA threshold=12 fold) while network plotting identified 'Cellular' and 'Reproductive System Development' as the most perturbed bio-function in NOA men with varicoceles. Expression of top candidates was verified using qPCR with fold change (FC; relative to control) for genes involved in apoptosis (PLAT, FC=2.1±1.1; CAV1, FC=1.5±0.2; VASN, FC=0.3±0.7), hypoxic angiogenesis (ANGPTL4, FC=-2.4±0.7; UXT, FC=-0.6±0.5) and spermatogenesis (MEA1; FC=0.2±0.5). When sub-categorized by histological subtype (i.e. hypospermatogenesis, maturation arrest, Sertoli Cell Only), distinct patient-specific expression patterns were observed suggesting unique gene expression contributes to varicocele pathogenesis. IPA data-filtration revealed the serum biomarkers CAV1 and PLAT could be important in differentiating these patient populations.

**CONCLUSION:** The current study has identified numerous genes associated with the presence of varicoceles in men with NOA. Unique patient-specific expression patterns suggest varicocele pathogenesis is typically multifactorial.

**Supported by:** Supported by a Male Reproductive Health Research Career (MRH) Development Physician Scientist Award (K12) (HD073917-01) from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Program and by NIH grants P01HD36289 from the Eunice Kennedy Shriver NICHD and 1R01DK078121 from the National Institute of Kidney and Digestive Diseases.

**P-159** Tuesday, October 21, 2014

**THE POTENTIAL APPLICATION OF URINE DERIVED STEM CELLS IN MALE INFERTILITY.** G. Liu,<sup>a</sup> T. Li,<sup>a</sup> J. Zhang,<sup>a</sup> X. Yang,<sup>a</sup> X. Liang,<sup>a</sup> Y. Zhang.<sup>b</sup> <sup>a</sup>Center for Reproductive Medicine, Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China; <sup>b</sup>Wake Forest Institution for Regenerative Medicine, Winston Salem, NC.

**OBJECTIVE:** Urine derived cells can be obtained non-invasively and may represent a potentially significant source of autologous cells for tissue engineering. One goal of this study was to test the hypothesis that the cell population obtained from urine contains cells that meet the defining criteria of stem cells (self-renewal and multipotency). In particular, the ability of these cells to give rise to induced pluripotent stem cells (iPS) for the potential sperm progenitor cells generation was tested.

**DESIGN:** Human urine derived cells were characterized and induced into iPS.

**MATERIALS AND METHODS:** Human urine derived cells from 9 individual donors (ages 5 to 40 years) were plated on multi-well plates. Single cell growth was monitored using time-lapse microcinematography. The cells were analyzed for expression of canonical reprogramming factors and pericytes/mesenchymal stem cell (MSC) markers. Expression of telomerase in the isolated cells was assessed by ELISA. Next, urine derived cells were cultured in various induction media for 21 days and assessed for evidence of differentiation into various cell types, including adipocytes, osteocytes, chondrocytes, SMC, and UC. USC was induced into iPS by polycistronic lentiviral vector encoding human Oct-3/4, Sox2, Klf4 and c-Myc (OSKM).

**RESULTS:** Some urine derived cells grew rapidly from a single cell clone for over 25 population doublings. Six of seven independent clones of urine derived cells expressed detectable levels of telomerase. USCs express the canonical reprogramming factors c-myc and klf4, and positive for pericytes/MSC markers such as CD146, NG2 and PDGF-receptor beta. When placed

in appropriate induction media, these cells differentiated towards adipogenic, osteogenic and chondrogenic lineages. Pluripotency of urine-derived iPSC clones was confirmed by immunocytochemistry, RT-PCR and teratoma formation.

**CONCLUSION:** Urine-derived cells expressed the phenotypic features of pericytes/ MSC, including self-renewal and multipotency. One urine-derived cell clone can differentiate to multiple cell lineages. These results demonstrate the feasibility of generating iPS from a urine sample and that urine-derived iPS might be further exploited for potential sperm progenitor cells generation study.

**P-160** Tuesday, October 21, 2014

**ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) IN METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) AND MALE INFERTILITY.** R. Ng, K. Louie, K. Poon, V. Chow, S. Ma. Department of Obstetrics and Gynaecology, University of British Columbia, Vancouver, BC, Canada.

**OBJECTIVE:** To determine whether C677T SNPs in the *MTHFR* gene are associated with an increased risk of infertility in a Canadian population.

**DESIGN:** We compared the frequencies of the SNPs obtained from genotyping the *MTHFR* gene in men with oligospermia, azospermia, and fertile men. We also investigated the possible risk of infertility in different ethnic populations.

**MATERIALS AND METHODS:** Peripheral blood samples were obtained from oligospermic (N=17) and azospermic (N=22) patients. Blood samples for controls (N=19) were from men who had fathered a child within the previous year and from men with proven fertility undergoing vasectomy reversals. DNA was extracted from blood using the Genra Puregene Blood Kit (Qiagen) according to their protocol. Polymerase chain reaction (PCR) was used to amplify the region of interest at *MTHFR*. Restriction fragment length polymorphism (RFLP) with HinfI enzyme (New England Biolabs) was used on the amplified DNA to digest the DNA according to its genotype. Following RFLP, the samples were electrophoresed on a 3% gel stained with SYBR safe (Invitrogen) to separate the fragments and the bands were visualized using a UV illuminator. Fisher's exact test was used to determine significance between groups.

**RESULTS:** Frequencies of the wild type 677CC genotype and the 677CT/677TT genotypes associated with infertility in fertile controls were 42.1%, 26.3%, and 31.6%; 70.6%, 23.5%, and 5.9% in oligospermic men; and 45.5%, 45.5%, and 9.1% in azospermic men, respectively. Comparing 677CT polymorphism, controls vs. azospermia (P=0.330) and controls vs. oligospermia (P=1.000) were not significant. Comparing 677TT polymorphism, controls vs. azospermia (P=0.115) and controls vs. oligospermia (P=0.092) were not significant. Ethnicity data were available for 15 oligospermic (11 Asian, 4 Caucasian) and 12 azospermic men (4 Asian, 8 Caucasian). Comparing Asian vs. Caucasian populations, the 677CT polymorphism (P=1.000) and the 677TT polymorphism (P=1.000) were not significant.

**CONCLUSION:** There does not seem to be a correlation between 677CT and 677TT polymorphisms and an increased risk of oligospermia or azospermia. Furthermore, these polymorphisms are not significantly different in infertile Asian vs. infertile Caucasian populations, contrary to current literature. More cases in all the groups are necessary to further the investigation.

**Supported by:** This study was Supported by the Canadian Institutes of Health Research (CIHR, grant to Sai Ma).

**P-161** Tuesday, October 21, 2014

**ASSOCIATION OF RS3129878 AND RS498422 IN THE HLA REGION WITH NON-OBSTRUCTIVE AZOOSPERMIA IN THE HAN CHINESE POPULATION.** S. Zou,<sup>a</sup> P. Song,<sup>a</sup> T. Chen,<sup>a</sup> J. Chen,<sup>b</sup> X. He,<sup>c</sup> P. Xu,<sup>d</sup> M. Liang,<sup>e</sup> K. Luo,<sup>f</sup> X. Zhu,<sup>g</sup> E. Tian,<sup>h</sup> Q. Du,<sup>h</sup> Z. Wen,<sup>b</sup> Z. Li,<sup>b</sup> M. Wang,<sup>b</sup> Y. Sha,<sup>b</sup> Y. Cao,<sup>c</sup> Y. Shi,<sup>b</sup> Z. Li,<sup>a</sup> H. Hu.<sup>a</sup> <sup>a</sup>Department of Urology, Shanghai, China; <sup>b</sup>BIO-X Center, Shanghai, China; <sup>c</sup>Reproduction Center, Hefei, Anhui, China; <sup>d</sup>Reproduction Center, Shenyang, Liaoning, China; <sup>e</sup>Second Affiliated Hospital of Shandong, Jinan, Shandong, China; <sup>f</sup>Reproduction Center, Nanning, Guangxi, China; <sup>g</sup>Reproduction Center, Nanchang, Jiangxi, China; <sup>h</sup>Sheng Jing Hospital of China Medical University, Shenyang, Liaoning, China; <sup>i</sup>Xiamen Women and Children Health Care Hospital, Xiamen, Fujian, China.