result status were compared by chi squared analysis. ROS activity in the different populations was compared by ANOVA on ranks.

RESULTS: The median and interquartile ranges for ROS activities of 2,224 infertile men without a varicocele, and the 17 men that had undergone a vasectomy reversal, were 2 (1–5) and 1.3 (1–7.2), respectively. Sixty-four infertile males were identified as having undergone a varicocele repair with a median ROS activity before surgery of 3.7 (2–10.8). The differences in ROS activity among the 3 populations was statistically significant (P<0.01). In those patients that had a varicocele repair, semen samples were analyzed a median of 2 months before surgery (range 0–20). Postoperative semen analysis were performed a median of 6 months after varicocele repair (range 3–50). Quantitative ROS activity decreased from a median of 3.7 before surgery to a median of 2.00 after surgery (P=0.036). Sixteen percent of patients that were initially ROS positive, became ROS negative after varicocele repair (P=0.048).

CONCLUSIONS: Varicoceles significantly impair fertility through oxidative stress. Semen ROS activity was greater in infertile men with varicoceles when compared to infertile men without varicoceles and compared to fertile men. In our cohort, varicocele repair significantly reduced ROS activity in the seminal plasma of infertile males, but not to the level of fertile males.

Supported by: AUA Foundation (JA), NIH M01RR00188 (BN).

#### P-848

## WITHDRAWN

## P-849

MALE AGE INFLUENCES THE PREGNANCY RATES ON ICSI CYCLES WITH EPIDIDYMAL SPERMATOZOA. E. Borges, Jr, A. Iaconelli, Jr, T. C. S. Bonetti, P. Queiroz, L. G. L. Maldonado, F. F. Pasqualotto. Clinical, Fertility — Assisted Fertilization Center, Sao Paulo, Brazil; Scientific, Sapientiae Institute — Research Center, Sao Paulo, Brazil; Andrology Laboratory, Fertility — Assisted Fertilization Center, Sao Paulo, Brazil; Biological Sciences, University of Caxias do Sul, Caxias do Sul, Rio Grande do Sul, Brazil; Clinical, Conception — Human Reproduction Center, Caxias do Sul, Rio Grande do Sul, Brazil.

OBJECTIVE: Aging influence over female fertility has been shown by many epidemiological studies, but less is know about male. However, sperm DNA fragmentation increases with age, and male age constitute an important risk factor for conceiving failure by assisted reproduction. Intracytoplasmic sperm injection (ICSI) allows men with reduced sperm production to reproduce. Patients with obstructive azoospermia can also be benefited as percutaneous epididymal sperm aspiration (PESA). Men who underwent vasectomy are a suitable model for studying age effect, as spermatogenesis is usually preserved. The aim of this study was to determine male age effect on ICSI cycles carried out with epididymal sperm collected by PESA after elective vasectomy. DESIGN: Retrospective study.

MATERIALS AND METHODS: It was analyzed 49 ICSI cycles with PESA recovered sperm, after vasectomy. Female infertility factor was excluded by age (≤35 years old) and oocytes number after controlled ovarian stimulation (≥4 recovered oocytes). Cycles were arranged in 2 groups according to men's age: ≤45 years (G≤45); and >45 years old (G > 45). Correlation coefficients between male age, fertility rate and embryo development parameters (pronuclear morphology, early cleavage, and embryo morphology on day 2 and day 3) were calculated. Differences on fertility rate, pronuclear morphology, early cleavage, embryo morphology on day 2 and day 3 were compared between groups through Mann-Whitney test or student T-test.

RESULTS: Male age had no significant correlation to normal fertilization rate (P=0.321), good pronuclear morphology (P=0.289), early cleavage (P=0.375), and good morphology on day 2 (P=0.301) and day 3 (P=482). Normal fertilization rate didn't differ (P=0.792) between  $G \le 45$  (70.2%) and G > 45 (68.7%), neither on embryos development parameters, as percentage of embryos with good pronuclear morphology ( $G \le 45 = 33.3\%$  and G > 45 = 35.4%; P=0.690); early cleaved ( $G \le 45 = 65.6\%$  and G > 45 = 55.6%; P=0.192), good morphology on day 2 ( $G \le 45 = 84.3\%$  and G > 45 = 78.3%; P=0.164) and good morphology on day 3 ( $G \le 45 = 56.4\%$  and G > 45 = 47.7%; P=0.132). However, pregnancy rate was 39.1% for  $G \le 45$ , while it was 13.6% for those older than 45 years old (G > 45) (P=0.049).

CONCLUSIONS: Although the patients' ages have no correlation to fertilization potential and embryo development, lower pregnancy rates after ICSI cycles with PESA retrieved spermatozoa are obtained for men older than 45 years, providing a strong evidence for a paternal age effect on clinical outcomes.

Supported by: None.

### P-850

# SURFACTANT PROTEINS A AND D IN HUMAN SPERMATOZOA.

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OBJECTIVE: Surfactant proteins A (SP-A) and D (SP-D) belong to the collectin family and play pivotal roles in the innate immunity of the lung. Pulmonary collectins directly bind with broad specificities to a variety of microorganism and possess anti-microbial effects. Today, both SP-A and SP-D are increasingly recognized as molecules involved in the host defense system of several organs, and their expression has been detected with RT-PCR in many mucosal surfaces in human tissues, including kidney, brain and testis.

DESIGN: Determination of SP-A and SP-D proteins in human sperm.

MATERIALS AND METHODS: In the present work, the presence of SP-A
nd SP-D proteins in human spermatozoa has been investigated in normozoo-

and SP-D proteins in human spermatozoa has been investigated in normozoo-spermic patients (N = 10; sperm conc.:50.4  $\pm$  6.4  $\times$   $10^6$  sperm/ml; motility: 58.0  $\pm$  5.3%). Western Blot analysis of human sperm proteins and indirect immunofluorescence assays of human sperm for SP-A and SP-D were performed.

RESULTS: Western Blot analysis and indirect immunofluorescence assays of human sperm indicated that both of SP-A and SP-D proteins were present in human spermatozoa. Indirect immunofluorescence stainings indicated SP-A presence in midpiece, tail, and sometimes at equatorial regions of human spermatozoa. A green brilliant light detected SP-D in the tails of some sperm. The immunostaining specificity was verified by the absence of immunoreaction in negative controls as well as in absorption controls. The anti-SP-A antibody detected a single band corresponding to the molecular weight values of 34 kDa in spermatozoa while no band was observed in negative control. The anti-SP-D antibody showed the expected band at 43 kDa in sperm. Positive control (Lung) showed both the SP-A and SP-D bands while no band has been observed in negative control. On the other hand, in order to confirm the results, western blotting was also performed with pooled sperm samples and washed sperm with isolate density gradients (Irvine Scientific) for leukocyte removal, both showed the presence of SP-A and SP-D proteins.

CONCLUSIONS: This is the first report and a novel finding about the presence of surfactant glycoproteins in human spermatozoa. Functional studies are needed to highlight the presence and localization of these proteins in human sperm. Moreover, it is important to investigate whether they are related to the defense system of human sperm while passing through the male and female genital tracts, or capacitation, or fertilization potential. The findings of SP-A and SP-D expressions in human sperm open a new area of study in male reproduction.

Supported by: This research was supported by the TUBITAK (The Scientific and Technological Research Council of Turkey).

## P-851

ANALYSIS OF THE LONG-TERM EFFECTS OF VARDENAFIL ON SEMEN CHARACTERISTICS IN HEALTHY MEN, AND MEN WITH ERECTILE DYSFUNCTION. K. Jarvi, E. Dula, M. Drehobl, J. Shapiro, M. Seger. Mount Sinai Hospital, Toronto, ON, Canada; West Coast Clinical Research, Tarzana, CA; Scripps Clinic, San Diego, CA; Bayer Corporation, West Haven, CT; Bayer Healthcare, Toronto, ON, Canada.

OBJECTIVE: Phosphodiesterase type 5 (PDE5) inhibitors are the first-line treatment for erectile dysfunction (ED). Many men in their reproductive years are now using PDE5 inhibitors. The objective of this study was to examine the long-term effects of 20 mg vardenafil and 100 mg sildenafil, vs. placebo, on sperm concentration and other semen characteristics.

DESIGN: This was a randomized, double-blind, placebo-controlled, parallel-arm, multicentre study.

MATERIALS AND METHODS: A total of 200 men without ED, or men with ED able to produce semen samples without ED therapy, aged 25–64 years, were randomized to treatment. Subjects underwent an unmedicated screening period of 1 month, followed by daily treatment with vardenafil, sildenafil or placebo for 6 months. Subjects with abnormal semen analysis also completed a 3 month post-treatment follow-up period. The primary variable was the proportion of vardenafil-treated subjects with a  $\geq$ 50% decrease in mean sperm concentration from baseline to 6-month last observation carried forward (LOCF), compared with placebo-treated subjects.

RESULTS: The difference (vardenafil minus placebo) in the proportion of subjects (intent-to-treat population) with  $\geq 50\%$  decrease in mean sperm