

**COMPARISON OF SPERM QUALITY OBTAINED FROM TRADITIONAL SPERM SELECTION METHODS WITH NOVEL SPERM SELECTION APPROACHES.** L. Simon,<sup>a</sup> S. Ge,<sup>a</sup> D. Carrell.<sup>a,b,c</sup> <sup>a</sup>Department of Surgery, University of Utah School of Medicine, Salt Lake City, UT; <sup>b</sup>Department of Obstetrics and Gynecology, University of Utah School of Medicine, Salt Lake City, UT; <sup>c</sup>Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT.

**OBJECTIVE:** This study investigates the quality of sperm selected using traditional sperm preparation methods for ART and novel sperm selection techniques.

**DESIGN:** Fifty men attending the andrology lab for semen analysis were recruited for this study. Each semen sample was divided into seven aliquots and subjected to density gradient centrifugation (DGC), swim-up, morphologically selected sperm post-DGC as performed for ICSI (ICSI), zeta potential, hyaluronic acid binding assay (HBA) and magnetic activated cell sorting (MACS) sperm preparation methods, while one fraction was used as control.

**MATERIALS AND METHODS:** Sperm processed by each method were analyzed for sperm DNA damage by the TUNEL assay and histone retention using aniline blue staining. Data were analyzed using paired sample t-test and non-parametric test.

**RESULTS:** The percentage of sperm with DNA damage was higher in control ( $5.95 \pm 0.57$ ) compared with DGC ( $4.55 \pm 0.44$ ,  $p < 0.001$ ), swim-up ( $4.23 \pm 0.42$ ,  $p < 0.001$ ), ICSI ( $3.43 \pm 0.41$ ,  $p < 0.001$ ), zeta ( $3.34 \pm 0.37$ ,  $p < 0.001$ ) and MACS ( $4.06 \pm 0.38$ ,  $p < 0.001$ ). There was no significant reduction in DNA damage after sperm selection by HBA method. However, selection of morphologically normal sperm after HBA sperm preparation showed a significant reduction in DNA damage ( $3.03 \pm 0.32$ ,  $p < 0.001$ ). There was a reduction in the percentage of sperm with abnormal histone retention using the swim-up ( $8.44 \pm 1.14$ ,  $p = 0.001$ ), zeta ( $8.64 \pm 1.37$ ,  $p = 0.001$ ), MACS ( $5.38 \pm 0.67$ ,  $p < 0.001$ ) and HBA ( $3.76 \pm 0.61$ ,  $p < 0.001$ ) separation procedures compared to the control ( $13.28 \pm 1.52$ ).

**CONCLUSION:** These data indicate that careful morphological selection of sperm for ICSI results in sperm with decreased DNA damage. The new approaches resulted in the isolation of sperm with low histone retention. To isolate physiologically normal and genetically fit sperm, we recommend new approaches should include morphological selection as an additional criterion.

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**ADDITION OF B VITAMINS TO GRADIENT WASHED SPERM SAMPLES IMPROVES LONG TERM HYPERACTIVITY.** M. A. Delaney,<sup>a</sup> J. E. Adams,<sup>a</sup> H. Sangi-Haghighi,<sup>a</sup> R. C. Dunn,<sup>b</sup> C. T. Valdes,<sup>a</sup> W.-S. A. Wun.<sup>b</sup> <sup>a</sup>Department of Reproductive Endocrinology and Infertility, Baylor College of Medicine, Houston, TX; <sup>b</sup>Fertility Specialists of Houston, Houston, TX.

**OBJECTIVE:** To identify a benefit of the addition of B vitamins added on standard sperm kinematical parameters in a population of men with normal or mildly abnormal sperm parameters.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** Fresh sperm samples were obtained from anonymous, discarded samples during 12/2012 to 4/2013, with exempt IRB approval. Samples collected were pooled to achieve a minimum of 50 million sperm per milliliter ( $n = 7$ ). Samples were processed in a 40% and 80% isolate gradient, washed and divided. B vitamins (Biotin B7, Riboflavin B2, Thiamine B1, and Inositol B3, all at  $10^{-8}M$ ) were added to aliquots of prepared sperm. Pentoxifylline 1mM was used as a control. 1000 spermatozoa per sample were counted and analyzed on the Hamilton Thorne CASA system for the following spermatozoa kinematical parameters: Motility, Hyperactivation (HA), Rapid motility, curve linear velocity (VCL), amplitude of lateral head displacement (ALH), and linearity (LIN). Samples were analyzed at 0, 1, 2, 4 and 20 hours after addition of B vitamins. The data was analyzed in SAS (v9.3) using a mixed effect model. Capacitation was measured using Chlortetracycline staining patterns according to the method of Perry (1995; 64:150-9).

**RESULTS:** At 1 hour, B1+B2 and B1+B3 samples had increased HA compared to control ( $p < 0.02$ ). At 4 hours, B1+B7, B3+B7 and B1+B2+B3+B7 samples also had increased HA ( $p < 0.01$ ). There was no uniform statistical improvement between other kinematical parameters analyzed. There was no difference between uncapacitated, capacitated and

acrosome reacted spermatozoa in controls versus B1+B2+B3+B7 supplementation at 2.5 hours.

**CONCLUSION:** Supplementation with combinations of B1, B2, B3 and B7, improves HA at 1 and 4 hours post-compared to untreated samples. Addition of B vitamins to gradient washed sperm could prove to be an important treatment option for sperm samples in those undergoing insemination cycles as prior studies have shown that HA is the single most important predictor of fertilization potential.

**TERATOZOOSPERMIC SPERM WITH HIGHLY FRAGMENTED DNA SUBJECTED TO DISCONTINUOUS GRADIENT CENTRIFUGATION-ANNEXIN V-MACS HAVE SIMILAR FERTILIZATION AND PREGNANCY RATES THAN NON-APOPTOTIC CONTROLS.** J. A. Notrica,<sup>a</sup> M. H. Vazquez-Levin,<sup>b</sup> N. M. Bossi,<sup>a</sup> D. E. Notrica,<sup>a</sup> E. Polak de Fried.<sup>a</sup> <sup>a</sup>Reproductive Medicine, CER Medical Institute, Buenos Aires, Argentina; <sup>b</sup>Reproductive Medicine, IBYME Institute of Biology and Experimental Medicine, Buenos Aires, Argentina.

**OBJECTIVE:** To evaluate fertilization (FR) and pregnancy (PR) rates in patients with sperm showing high levels of DNA fragmentation and treated with ICSI after Annexin V-Magnetic Activated Cell Sorting (AV-MACS) sperm selection.

**DESIGN:** Prospectively, all patients with high DNA fragmentation levels (TUNEL assay) in semen were subjected to ICSI treatment after AV-MACS sperm filtration. Controls (low % apoptotic sperm) were treated with ICSI in the same time period.

**MATERIALS AND METHODS:** Seventy four couples with male factor infertility (Kruger morphology  $\leq 14\%$ ) were included in the study. DNA fragmentation analysis was done using TUNEL assay (abnormal  $> 20\%$  sperm). Semen samples were processed by Discontinuous Gradient Centrifugation (DGC; Isolate®, Irvine, USA) and then filtered using AV-MACS® (Miltenyi, Germany) when TUNEL  $> 20\%$ . The eluted fraction was used for ICSI (ICSI-AV-MAC=42; Control=14) or OD/ICSI (OD/ICSI-AV-MAC=10; OD/Control=8). Statistics: Fisher Exact and Mann-Whitney tests ( $p < 0.05$ , significant).

**RESULTS:** Male/female patient age was similar between AV-MAC and Controls. Semen depicted normal sperm concentration and motility but abnormal morphology in all groups. TUNEL assay revealed differences in semen assigned to AV-MAC compared to controls (ICSI-AV-MAC=35.4 $\pm$ 2.1 vs Control=12.6 $\pm$ 1.5,  $p < 0.0001$ ; OD/ICSI-AV-MAC=32.3 $\pm$ 4.4 vs OD/Control=10.8 $\pm$ 0.7,  $p < 0.001$ ). After DGC+AV-MACS sperm depicted high FR (%) and PR (%) levels, and similar to those scored in controls, FR ICSI-AV-MAC=77.4 $\pm$ 3.0 vs Control=84.2 $\pm$ 4.4,  $p = 0.4$ ; PR ICSI-AV-MAC=39.0 (16/41) vs Control=42.9 (6/14);  $p = 1$ . FR OD/ICSI-AV-MAC=89.3 $\pm$ 4.5 vs OD/Control=85.4 $\pm$ 7.3,  $p = 0.8$ ; PR OD/ICSI-AV-MAC=37.5(3/8) vs OD/Control=37.5 (3/8),  $p = 0.7$ . All babies born were healthy.

**CONCLUSION:** Teratospermic sperm with high levels of DNA-fragmented subjected to DGC+AnnexinV-MACS have comparable FR and PR rates to those found with non-apoptotic controls, showing DGC+AnnexinV-MACS is a safe and efficient procedure to select highly functional sperm.

**IMPROVED SPERM HARVESTING FROM CRYPTOZOOSPERMIC SPECIMENS VIA OBSERVATION OF SEDIMENTS IN MICRODROPS CONTAINING PENTOXIFYLLINE (PXF): ALTERNATIVES FOR SPERM FREEZING AND ICSI.** J. R. Correa-Perez S. P. Marynick. Texas Center for Reproductive Health, Dallas, TX.

**OBJECTIVE:** Finding sperm in cryptozoospermic specimens is highly variable/ time consuming. Spermatozoa from these specimens tend to be immotile and surrounded by high concentrations of debris/WBCs. Stimulation of sperm motility via the use of PXF, coupled with microscopic examination in microdrops, has resulted in enhanced identification of viable sperm from cryptozoospermic specimens. The aim of this study was assess the role of microdrop/PXF for harvesting sperm from cryptozoospermic specimens for freezing and ICSI.

**DESIGN:** Prospective clinical study.

**MATERIALS AND METHODS:** Azoospermic status was established in 12 patients after two semen analyses. Patients underwent male factor work-up (e.g., hormonal profile, genetic evaluation, etc) and semen analysis, but with the additional step involving exposure of centrifuged