
SPERM PREPARATION

P-582 Wednesday, October 10, 2018 6:30 AM


OBJECTIVE: We tested a novel approach for treating couples with complete and persistent embryo aneuploidy. Using a microfluidic device, we selected spermatozoa with the highest progressive motility and genomic integrity, capable of generating euploid embryos.

DESIGN: In a 19-month period, seven couples with a history of high sperm chromatin fragmentation (SCF) and persistent embryo aneuploidy underwent a cycle of ICSI in which semen specimens were processed in a standard fashion or by microfluidics. SCF was assessed by TUNEL. Fertilization and clinical pregnancy rates were assessed and compared between the two preparation methods, and preimplantation genetic testing for aneuploidy (PGT-A) was performed on the resulting embryos.

MATERIALS AND METHODS: Consenting men had their ejaculates screened by standard semen analysis according to WHO 2010 criteria. Specimens were processed by density gradient and microfluidic sperm selection (MFSS). SCF was measured by TUNEL utilizing a commercial kit (In Situ Cell Death Detection Kit, Roche). At least 500 spermatozoa were counted under fluorescent microscopy, with an established threshold of 15%.

RESULTS: Seven couples (average maternal age, 38.3±3.6 years; average paternal age, 44.2±11 years) underwent 19 ICSI cycles. An average semen concentration of 11.5±16x10⁶/mL, 18.5±16% motility, 2.0±0% normal morphology, and an SCF of 29.2±10% were found. After selection by density gradient, the total motility of the sperm samples was 34.2±26%, resulting in a 60.4% fertilization rate. These cycles only generated 5 euploid embryos out of 23, which yielded two pregnancies, both resulting in miscarriage. Couples subsequently underwent 7 ICSI cycles in which the spermatozoa were processed by MFSS, which generated 98%±4% (P=0.0001) motility and an increased 4% morphology, while the SCF dropped to only 1.6±1% (P<0.0001). Although the fertilization rate was 67.1%, 7 euploid blastocysts out of 14 (50%) were obtained, yielding 5 out of 7 ongoing clinical pregnancies (71.4%; P=0.001).

CONCLUSIONS: Selecting a genomically competent male gamete may enhance the chances of obtaining an euploid conceptus for transfer. Couples with a persistent number of aneuploid embryos that cannot be solely attributed to the female partner may benefit from the selection of spermatozoa with intact chromatin to increase the chances of conceiving a child.

P-583 Wednesday, October 10, 2018 6:30 AM

OPTIMIZING SPERM CRYOPRESERVATION AND RECOVERY (OSCAR), R. Sabouni, K. J. Fresa, L. Stadtmauer. Obstetrics and Gynecology, Jones Institute for Reproductive Medicine, Norfolk, VA.

OBJECTIVE: The objective of this study is to compare three common sperm freezing methods and two common thawing methods to one another to determine if there is a superior method to enhance post-thaw sperm survival.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: Twelve discarded fresh semen samples were obtained with patient consent. Each fresh sample was washed with mHTF (LifeGlobal, Guilford, CT) and centrifuged for 15 minutes at 400G. The pellet was resuspended in a 3:1 ratio of mHTF to Artic™ Sperm Cryopreservation Medium (Irvine Scientific, Santa Ana, CA). Motility was evaluated manually and each participant’s sample was divided into six aliquots containing 0.5mL of specimen. Two aliquots of each specimen underwent one of 3 different freezing methods: (a) plunge into liquid nitrogen (LN2), (b) suspension in LN2 vapor for 15 minutes followed by plunge into LN2 or (c) suspension in LN2 vapor for 1 hour followed by plunge into liquid nitrogen. Each of the freezing methods was subject to two different thaw methods: (a) 37°C dry bath for 20 minutes followed by 40 minutes at room temperature or (b) room temperature (RT) for 1 hour. Following recovery, a second evaluation of motility was performed for each aliquot as a measure of sperm viability (percent motility versus initial). Analysis by two way repeated measures ANOVA was utilized with a p-value of 0.05 to determine significance.

RESULTS: The mean motility for plunge sperm thawed at RT and 37°C were 22.9±0.04% and 20.3±0.04%, respectively. The mean motility at RT and 37°C were 43.8±0.07% and 48.1±0.06%, respectively. Survival rates between all freezing methods varied significantly, with greater recovery noted in the 1 hour vapor phase when compared both plunge (p<0.001) and 15 minutes in vapor (p=0.005). The shorter vapor phase of 15 minutes also demonstrated statistically greater viability compared to plunge (p = 0.027). Thawing methods did not significantly affect sperm recovery.

CONCLUSIONS: The recovery of motile sperm varied directly with the length of cooling prior to plunge in LN2 with an hour suspension in vapor resulting in greatest motility compared to the initial sample. Recovery was not dependent on the thaw method used, with either of the two methods producing similar results for a given cryopreservation method. Interestingly, it was also noted that samples plunged directly from room temperature into LN2 retained appreciable, if diminished viability.