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POSITIVE EFFECTS OF *IN*-VITRO *MYO*-INOSITOL SUPPLEMENTATION OF CRYOPRESERVED HUMAN SPERM ON THE OUTCOME OF CRYOPRESERVATION: A RANDOMIZED CONTROLLED TRIAL.

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OBJECTIVE: *Myo*-inositol is a naturally occurring component of vitamin B complex that is involved in cellular signaling pathways. There is a growing evidence on the potential beneficial effects of *Myo*-inositol supplementation on both natural and assisted fertility. The objective of this study was to investigate the effects of *in* vitro *Myo*-inositol supplementation of cryopreserved human semen on the post-thaw sperm quality.

DESIGN: A randomized controlled trial (RCT).

MATERIALS AND METHODS: The study had been approved by the institutional review board, and conducted in an infertility center between September 2016 and March 2017. The study included semen samples obtained from 25 infertile men during routine infertility work-up. *Myo*-inositol was supplied in a powder form (Sigma Aldrich Chemical Co, USA). Under complete aseptic condition, *Myo*-inositol was dissolved in sperm nourishment media (AllGrad wash®, Life global, Canada) to prepare aliquots of 10 μl stock solutions, each containing 1 mg *Myo*-inositol. Standard semen analysis was performed according to the WHO guidelines (fifth edition, 2010). Azoospermic and leukocytospermic samples were excluded. Each semen sample was divided into two equal aliquots (0.5 ml each) for cryopreservation using slow freezing technique. The two identical aliquots of each semen sample were randomized into two groups (A and B). Group A was treated with cryo-protectant plus 10 μl *Myo*-inositol solution, while group B was treated with cryo-protectant alone (control). Frozen samples were thawed at 37 °C, and examined for post-thaw total motility, progressive motility and progressive recovery rate (PRR). PRR = post-thaw progressive motility/pre-freeze progressive motility X100. Data were presented as median (25^{th} and 75^{th} percentiles). Paired sample *t* test was used for comparison of the pre and post-thaw results. P value < 0.05 was considered significant.

RESULTS: The median (25^{th}, 75^{th}) of pre-freeze sperm concentration was 25 (18, 41) $X10^6$ sperm, and percentage of normal sperm forms was 4 (2, 5). Comparisons of pre-freeze and post-thaw percentages of total motility, progressive motility and PRR between samples in group A and group B are shown in Table 1.

CONCLUSIONS: *In* vitro *Myo*-inositol supplementation of cryopreserved ejaculate sperm, from infertile men, resulted in a significant enhancement of post-thaw sperm quality. Such finding is interesting, and may have important implications on the future outcome of assisted reproductive techniques using cryopreserved sperm.

Comparisons of pre-freeze and post-thaw percentages of total motility, progressive motility and PRR

Parameter	Pre-freeze	Group A	Group B	P 1 (pre-freeze vs Group A)	P 2 (pre-freeze vs Group B)	P 3 (Group A vs Group B)
Total Motility (%)	40 (31, 45)	10 (4.5, 15)	10 (3.8, 10)	< 0.001	< 0.001	0.03
Progressive Motility (%)	25 (16, 25)	5 (1.5, 10)	5 (1.8, 5)	0.04	0.001	0.01
Progressive Recovery Rate (%)		12.5 (6.4, 25.0)	10 (4.3, 18.8)			0.01

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SPERM SOURCE INFLUENCES THE EXTENT OF DNA FRAGMENTATION AND SHAPES REPRODUCTIVE OUTCOME.

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OBJECTIVE: During the later stages of spermatogenesis, DNA breakage is physiologically required to allow tight chromatin compaction. While most spermatozoa undergo DNA repair, reactive oxygen species (ROS) are the main cause for DNA injury. We question whether sperm chromatin integrity differs among spermatozoa isolated from different sections of the male genital tract and how it may affect reproductive outcome.

DESIGN: Over 42 months, men with high SCF in their ejaculates (n=77) underwent surgical sampling, often bilateral, from vas deferens, epididymis, and testis. SCF was assessed by TUNEL, and clinical outcome was recorded for each sperm source for men undergoing ICSI treatment.

MATERIALS AND METHODS: Ejaculates processed in the standard fashion were assessed for SCF by TUNEL. Surgical samples were minced and prepared for SCF evaluation and were cryopreserved for later use with ICSI. DNA fragmentation was measured by TUNEL on specimens isolated from all sites. TUNEL was executed by utilizing a commercial kit (In Situ Cell Death Detection Kit, Roche). At least 500 spermatozoa were counted per site under fluorescent microscopy with an adopted threshold of 15%.

RESULTS: Of the original 77 patients, 54 were treated by ART with an average SCF of 23.7± 11.7% (11.8-42.3) in their ejaculate. In 10 men aspiration of the vas deferens resulted in 19.9± 6.4% SCF (range 35-5.8) while in 41 men epididymal sampling yielded 16.0 ± 7.6% SCF (range 38.4-5.3) and in 77 the SCF on testicular spermatozoa was 11.5± 5.7% (range 31.2-1.5). The SCF progressively decreased as TUNEL was measured proximally from the ejaculate toward the vas deferens (P=0.02), the epididymis (P=0.005), and testis (P<0.001). A fertilization of 68.3% (287/420), 79.4% (124/156) and 60.3% (251/416) was achieved by ICSI using ejaculated, epididymal, and testicular spermatozoa, respectively. While the clinical pregnancy rate in ejaculated spermatozoa was only 21.2%, ART outcome with ICSI utilizing these surgical sources yielded a clinical pregnancy of 35.8%. Based on these preliminary findings a subgroup of patients (n=28), with SCF of 30.5 ± 17.4 bypassed the prerequisite cycle with ejaculated spermatozoa and opted to undergo TESE with ICSI. The clinical pregnancy rate achieved was 35.0% per cycle that translated to 50% per couple treated.

CONCLUSIONS: DNA integrity assessment on the spermatozoa isolated at different levels of the male genital tract evidenced that oxidative stressors progressively alter DNA integrity toward the ejaculate. Couples unable to achieve a pregnancy using ejaculated spermatozoa with compromised DNA may benefit from undergoing testicular retrieval for diagnostic and therapeutic purposes.

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VARICOCELE-INDUCED MALE INFERTILITY - A MITOCHONDRIAL DISEASE.

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OBJECTIVE: Impaired respiratory chain, oxidative phosphorylation, mitochondrial protein import, alterations of the inner mitochondrial membrane composition and defects of mitochondrial dynamics are characteristics of mitochondrial disease. Our recently published proteomic data on comparative proteomic analysis of sperm proteins identified 22 differentially expressed proteins (DEP) of mitochondrial origin. Proteins involved in mitochondrial organization (LETM1, EFHC1 and MIC60), import receptor TOM22, 3 crucial subunits of electron transport chain (ETC) and the core enzymes of carbohydrate and lipid metabolism were under-expressed in the varicocele group. In varicocele, stagnation in the testicular microcirculation inducing hypoxic-ischemic degenerative changes in all cell types in the sperm production site. During hypoxia, superoxide production at low oxygen concentrations results in oxidative stress (OS). With this background, we hypothesize that hypoxia-mediated OS is involved in sperm dysfunction in varicocele due to impaired blood supply to the testis.

DESIGN: Validation of key DEP by Western blot (WB) analysis.

MATERIALS AND METHODS: Oxidation-reduction potential (ORP) as an index of OS was measured in infertile men with varicocele (n=13) and