Motion characteristics of frozen-thawed human spermatozoa processed by different methods: A comparative study

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Objective: Despite many refinements in cryopreservation techniques, the recovery of post thaw sperm remains sub-optimal. The most commonly reported detrimental effect of freeze-thaw of human sperm is substantial reduction in motility and velocity, which may be associated with extensive damage that occurs during sperm processing methods employed to remove cryoprotectants and seminal plasma. The aim of our study was to evaluate the motility parameters of cryopreserved human sperm following separation by the different sperm preparation methods such as 1) swim-up with and 2) without reduced glutathione (GSH) as an antioxidant, 3) hyaluronate swim-up and 4) density gradient.

Design: Prospective in vitro study

Materials and Methods: Twenty-five normozoospermic semen samples were cryopreserved with glycerol-based cryoprotectant. Thawed semen of each volunteer (n=12) was divided into two equal aliquots. Both underwent wash and swim-up procedure using Ham’s F-10 (HF-10) alone or HF-10 with GSH (5mMol/L) respectively. In the other group (n=13), one aliquot was processed by neat swim-up using hyaluronic acid (2mg/ml of HF-10) and the other by density gradient centrifugation (DGC, Puresperm™ 80/40) followed by a swim up. Post-processing samples were analyzed using a computer assisted semen analyzer (CASA; Hamilton-Thorn, CEROS) for sperm motion parameters. Results: In comparison to HF-10 wash and pellet swim-up, processing with GSH in HF-10 showed significantly higher percentage (%) motility (25.0 ± 6.2 vs. 12.0 ± 5.3; p = 0.003) and % rapid forms (24.5 ± 5.9 vs. 11.5 ± 5.4; p = 0.002). However, there were no significant differences in other CASA parameters. Both hyaluronate swim-up and DGC methods were equally good in the recovery of % motile (34.3 ± 3.4 vs. 47.3 ± 24.2; p = 0.380) and rapid forms (27.0 ± 6.4 vs. 40.5 ± 24.4; p = 0.391). Average path velocity and VCL were higher in DGC group (p = 0.037 and 0.041). In contrast ALH showed significantly higher values for hyaluronate group (2.2±1.3 vs. 4.8±0.5; p = 0.038). Conclusion: The selection procedure for post thaw semen samples should be carried out using optimized techniques so as to avoid further damage to fragile sperm population resulting from freeze-thaw process. Inclusion of GSH in processing
media can confer protection against cell damage by oxidants, electrophiles and free radicals. Density gradient centrifugation (DGC) or hyaluronate swim-up offer better alternatives for processing cryopreserved semen. Support: None

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