Effect of Percoll-Wash on Cryopreserved Human Spermatozoa

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Objectives: Fresh sperm remain viable for up to 24 hrs in the female reproductive tract. This period is critical for sperm-egg fusion which may result in a successful pregnancy. Methods that improve sperm quality after freezing and thawing at least 24 hrs or more would be beneficial in assisted reproductive technology programs. The aim of this study was to study the effect of Percoll-wash on cryopreserved human spermatozoa due to conflicting reports in the literature on pregnancy outcome.

Design: Sperm characteristics were examined in spermatozoa cryopreserved after density-gradient filtration (Percoll-wash) and samples without treatment (raw). The changes at different time periods were compared with respect to 0 hr. The two methods were then compared to determine if the wash method improved cryopreserved sperm quality.

Materials and Methods: Samples from normal donors (n=10) were evaluated for sperm motion characteristics (percent motility, VCL, VSL, VAP, LIN, and ALH) with a computer-assisted semen analyzer; viability and membrane integrity were assessed with the cosin-nigrosin and the hypoosmotic swelling (HOS) tests respectively. The semen samples were divided into two aliquots. The first aliquot was filtered on a Percoll gradient. The raw aliquot was not subjected to any treatment. Both aliquots were diluted with freezing medium (Test yolk buffer, 1:1 v/v), and then stored in liquid nitrogen. Sperm characteristics, viability and membrane integrity were assessed before freezing and 0, 1, 6, and 24 hrs post-thaw.

Results: Percent sperm motility and other motion parameters decreased significantly (P<.0001) after thawing in both samples at 0 hr. In the raw sample, there was a significant decrease in the following parameters: percent motility (P<.0001); VSL (P<.01), VAP (P<.02), and LIN (P<.03) after 24 hrs. Percent viability decreased significantly at 6 and 24 hrs (P<.01 and .001); similarly, significant decrease in HOS score (P<.02 and .03) was seen. In the wash sample the motility decrease at 24 hrs was 30.1% (P<.001) compared to 0 hr, VCL and VAP significantly improved in the wash sample (P<.03) after 6 and 24 hrs. A significant decrease in VSL (P<.001), VAP (P<.01), and LIN (P<.001) was seen. At 6, and 24 hrs, the decrease in the percent viability was significant (P<.05 and .01), HOS score was not significantly different from time 0. When these changes were compared between the two methods using a paired 't' test the wash sample retained motility for 24 hrs (P<.03).

Conclusion: 1) Compared to time 0, a higher percentage of motility, motion characteristics, viability and membrane integrity is retained in Percoll-wash cryopreserved sperm samples 24 hrs after thawing, suggesting that Percoll washing helps preserve sperm quality. 2) When the above changes are compared between the raw and wash samples, the later retains motility for a longer period. However, Percoll-treatment did not improve other sperm characteristics. We feel that Percoll-wash results depend on the method of interpretation and this may influence the pregnancy outcome.