COMPARISON OF TWO CRYOPRESERVATION PROTOCOLS FOR FREEZING HUMAN SPERMATOZOA

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Objective:
Sperm quality decreases significantly following freezing and research on improving cryosurvival rates is crucial. We compared the effects of two cryopreservation protocols to determine which method allows better preservation of sperm characteristics.

Design:
Prospective study in an Andrology Laboratory.

Materials/Methods:
Each sample was divided into two aliquots after liquefaction. Each aliquot was cryopreserved using two freezing protocols [Cleveland Clinic Foundation method (gradual freezing) and the Irvine Scientific (rapid freezing) method] using TEST-yolk buffer as the freezing medium. In the Cleveland Clinic Foundation method (CCF method) a 5mL vial of freezing medium was thawed and an aliquot equal to 25% of the original specimen volume was added to the specimen. This process was repeated four times to give a final ratio of 1:1 (v/v) of freezing medium to ejaculate. These aliquots were placed in cryovials at -20°C for 8 minutes followed by nitrogen vapors for 2 hours before being immersed in liquid nitrogen. In the Irvine Scientific method (IS method), the entire volume of freezing medium was added at one time and the specimens were immersed in liquid nitrogen. The step that included placing of cryovials at -20°C for 8 minutes was omitted. Prefreeze and post-thaw total sperm count, percentage motility, sperm motion characteristics and morphology (Kruger and WHO) were evaluated. Motility was analyzed at 0, 60, 120, 180 minutes after thawing.

Results:
Percentage motility was significantly lower in postthaw samples compared to prefreeze values in samples cryopreserved by the two methods. Postthaw sperm motility was greater in specimens processed by IS method compared to the CCF method (15.94 ± 9.14 vs. 12.07 ± 7.31; P <0.006). In addition, percent cryosurvival was also greater in IS method compared to the CCF method (47.42 ± 17.44 vs. 35.76 ± 17.56; P <0.008). Morphology was similar with both methods.

Conclusions:
Specimens cryopreserved by the IS method had a smaller decrease in sperm motility over time when compared to the CCF method. The IS method for sperm cryopreservation is easy and gives good results. It can be used routinely for long-term storage of human spermatozoa. Rapid freezing of sperm in cryopreservation gives better survival rates compared to gradual freezing, although one may assume that gradual acclimatization to very low temperatures would maintain the functional integrity of the sperm to a greater extent. This as ascertained by morphology is the similar by both protocols thus refuting common belief.

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