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JOURNAL ARTICLE

# Cryopreservation of a small number of fresh human testicular spermatozoa and testicular spermatozoa cultured in vitro for 3 days in an empty zona pellucida

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It is known that the motility of human testicular sperm can be improved when they are cultured in vitro for a few days. The purpose of this study was to determine whether it is better to freeze human testicular spermatozoa on the day of biopsy (fresh) or after they were cultured for 3 days. A modified, single-sperm freezing technique was used in this study. The study consisted of two parts: (1) ejaculated spermatozoa were used to examine the influence of different concentrations of glycerol and synthetic serum substitute (SSS) on the survival rate after cryopreservation, and (2) the survival rates between cryopreserved fresh testicular spermatozoa (Group 1) and testicular spermatozoa that were cultured for 3 days before freezing (Group 2) were compared. Empty zonae pellucidae were obtained from mouse eggs. Five to 10 motile spermatozoa were selected and injected into an empty zona pellucida. For freezing, the zona pellucida with spermatozoa was transferred into a HEPES-buffered human tubal fluid containing different concentrations of glycerol and kept at room temperature for 10 to 15 minutes, and then loaded into a 0.25-ml-plastic straw. The straws were exposed to liquid nitrogen vapor for 2 hours and then plunged into liquid nitrogen. For thawing, the straws were taken out of liquid nitrogen and placed into a 37 degrees C waterbath for 25 to 30 seconds. There was no statistically significant difference in survival rates between 3% and 10% SSS with different glycerol concentrations. There was no statistically significant difference in the survival rates of spermatozoa between Group 1 and Group 2 after cryopreservation. It appears that in vitro culture of testicular spermatozoa before freezing does not increase survival rate.



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