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Morphology and viability of human spermatozoa vitrified with a new, cryoprotectant-free, artificial seminal fluid

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Cryopreservation is a process finalized to store tissues and cells at a very low temperature. The most common freezing protocols used for gamete preservation in Assisted Reproductive Technologies are slow freezing and vitrification (1). Vitrification combines ultrarapid cooling with high concentrations of cryoprotectants; it avoids, better than slow freezing, the formation of ice crystals. It has been demonstrated, however, that cryoprotectant addition may significantly reduce cell viability (2). This study was aimed to design a new, cryoprotectant-free, medium similar to normal human seminal fluid (SF) formulation (artificial seminal fluid; ASF), and to compare the cryoprotective potential of this medium with SF and Human tubal fluid (HTF) medium. Thirty normal ejaculates were processed with swim-up technique and sperm suspensions were divided in four groups: fresh (controls); vitrified in HTF (Vit HTF); vitrified in patients' SF (Vit SF); and vitrified in ASF (Vit ASF). To identify the effects of the different media we assessed sperm parameters of motility, viability and morphology after warming. Spermatozoa ultrastructure was also evaluated by scanning and transmission electron microscopy (SEM and TEM). The results showed that sperm motility, viability and normal morphology were significantly higher in Vit ASF than in Vit HTF. The same parameters were better in Vit ASF than in Vit SF, but only viability differed significantly. Deep cytoplasmic invaginations and folded tails were commonly observed by SEM in all vitrified sperms, but this alterations were more evident in Vit HTF and Vit SF than in Vit ASF. By TEM, acrosome damage, plasma membrane loss, chromatin vacuolation, disruption of mitochondria and adherence of several tail sections together were observed in all vitrified groups; the latter phenomenon, however, was more evident in Vit HTF and Vit SF than in Vit ASF. In conclusion, vitrification of human spermatozoa with ASF seems more effective in preserving sperm quality than Vit SF and, particularly, Vit HTF.

References

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Keywords
Spermatozoa; vitrification; artificial seminal fluid; human.