# Vitrification of Human ICSI/IVF Spermatozoa Without Cryoprotectants: New Capillary Technology

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**ABSTRACT:** The aim of this study was to develop and to test the standardized aseptic technology of permeable cryoprotectant-free vitrification of human spermatozoa in capillaries (for intracytoplasmic sperm injection [ICSI] or in vitro fertilization [IVF]). To test the effect of vitrification on basic sperm parameters, each of 68 swim-up–prepared ejaculates from oligo-, astheno-, and teratozoospermic patients were aliquoted and distributed into 3 groups: 1) nontreated control, 2) 10 μL of spermatozoa cryopreserved by slow conventional freezing with glycerol-contented medium, and 3) 10 μL of spermatozoa vitrified in 50-μL plastic capillaries in culture medium with 0.25 M sucrose. Spermatozoa motility (1, 24, and 48 hours after warming), plasma membrane integrity, acrosomal integrity, and spontaneous capacitation-like changes were determined after warming. Aseptic cryoprotectant-free vitrification showed a significantly stronger cryoprotective effect compared with conventional freezing. One hour after warming, motility, plasma membrane integrity, and acrosomal integrity were significantly higher than is observed for conventionally frozen spermatozoa (28% vs 18%, 56% vs 22%, and 55% vs 21%, respectively; $P < .05$), although lower than in fresh spermatozoa (35%, 96%, and 84%, respectively; $P < .05$). Capacitation-like changes did not differ significantly between vitrified and conventionally frozen samples (8% vs 9%, respectively; $P > .1$) (2% in fresh spermatozoa). The newly developed technology of aseptic vitrification of human spermatozoa in capillaries can effectively preserve these cells from cryo-injures. Spermatozoa, vitrified by this technology, are free from seminal plasma owing to swim-up preceding vitrification and are free from permeable cryoprotectants. They are ready for further use immediately after warming without any additional treatment. Therefore, the reported technology has a great potential for use in ICSI/IVF.

Key words: Cryoprotectant-free, motility, membrane, acrosome.
**J Androl 2012;33:000–000**