MALE FACTOR INFERTILITY CRYOPRESERVATION OF SPERM AT -86°C

Preservation of sperm in liquid nitrogen (LN_2) is an effective technique, but the use of LN_2 for long-term storage requires significant cost and space allowances.

Sperm is frozen to preserve fertility in men diagnosed with cancer or for use in assisted reproduction techniques. However, freezing can result in the formation of ice crystals, which can impair cell function by increasing lipid peroxidation, reducing motility and inducing cell-death mechanisms. In a study published online in *Andrologia*, a team from Chile report the use of a novel rapid LN_2 freezing vitrification technique, which does not require the use of permeable cryoprotectants and can enable the cryopreservation of sperm samples at -86°C , eliminating the need for long-term storage in LN_2 .

The team describes an aseptic vitrification technique that takes advantage of the viscosity of the spermatozoal intracellular matrix—which contains proteins and sugars—and uses the intracellular matrix itself as a cryoprotectant, eliminating the need for permeable cryoprotectants. Rapidly freezing sperm in a sucrose-based medium means that the freezing process does not require the addition of seminal plasma or permeable cryoprotectants, which have been shown to be potentially mutagenic on sperm in previous studies, and reduces damage caused by cryopreservation itself.

Semen samples were collected from healthy men and, after centrifugation, were suspended in a vitrification solution of 0.25 M sucrose in HTF medium with human serum albumin. Samples were hermetically sealed and plunged into LN_2 , after which half remained in LN_2 storage at -196°C for 60 days and half were removed and stored at -86°C . Motility, DNA fragmentation and mitochondrial membrane potential were then compared between the two sets of samples. Analysis of the two sets of samples showed no significant difference between the two freezing techniques in any of the measured sperm parameters, demonstrating that LN_2 is not required for the storage of sperm. The vitrification technique does not require permeable cryoprotectants, is simple and quick, and reduces costs and space associated with LN_2 storage.

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Original article Sánchez, R. *et al.* Vitrified sperm banks: the new aseptic technique for human spermatozoa allows cryopreservation at -86°C . *Andrologia* doi:10.1111/j.1439-0272.2012.01314.x