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Ultrastructural features of human sperm cells cryopreserved by different methods

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Cryopreservation of human spermatozoa has been recognized as a key strategy for management of male fertility. Nevertheless, current protocols of sperm freezing are neither optimal nor standardized between different labs (1).

In this study we compare the ultrastructural features of human normospermic sperm samples (according to WHO parameters 2010) from 5 different freezing techniques in order to identify the best methods of cryopreservation. After informed consent, 21 normospermic patients (from the Medically Assisted Procreation PMA Center of the Fondazione IRCCS Policlinico San Matteo in Pavia) were recruited and both traditional and improved analysis of sperm quality were applied, in order to define critical steps of cryopreservation.

Cryopreservation of human spermatozoa has been related to decreased motility associated with impaired velocity and viability of sperm pre-freeze and post-thaw. For all applied methods there was a significant reduction of progressive and total motility (P) as a result of freezing. To investigate ultrastructural details, 5 additional cryopreserved samples by the best two freezing methods were analyzed with electron microscopy (TEM). Preliminary data showed the minimal differences between the protocols, with a large number of queues detached and large quantities of cytoplasmic debris after of the first protocol. Spermatozoa appear to be better preserved in the second analyzed method, despite both procedures induced deteriorations at ultrastructural level (2). Other non-routine analysis will be performed to determine whether the cooling time to +4°C may affect the procedure; Comet Assay (to assess the degree of sperm DNA fragmentation) (3) and flow cytometry (to study light scatters patterns and membrane integrity) (4) will be applied.

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