Variations of Human Semen Nemaspermic Parameters after Long Term Cryopreservation in Liquid Nitrogen

D. Tagliasacchi, M. Bruschi, T. Marsella, F. Bastai
Mother Infant Department, Policlinico of Modena, Italy

Introduction
Human semen samples, cryopreserved for more than 9 years at the Centre for Reproductive Medicine of Policlinico di Modena, were thawed and analyzed to determine the effect of a long term cryopreservation on nemaspermic parameters.

Materials and Methods
Semen samples of 38 patients, stored between 1995 and 2003, were thawed and analysed. The ages of patients at freezing were between 19 and 47 years. The mean cryopreservation time is 11.8 years (9-18 years).
The semen had been slow-frozen with the addition of the cryoprotectant "Freezing medium – test yolk buffer with gentamicin sulphate" (Irvine Scientific) and kept in tanks with liquid nitrogen at -196°C. An analysis of nemaspermic parameters had been done at freezing.
Samples were thawed at room-temperature and washed with the culture medium "Syn Vitro Flush" (Origio) to eliminate the cryoprotectant. An optical microscopic analysis was done to determine the variations in sperm concentration, motility and morphology according to the WHO 2010.
According to their characteristics at freezing, samples belong to classes of normospermia (A) and asthenozoospermia (B) regarding sperm motility and to classes of normospermia (C) and teratozoospermia (D) regarding sperm morphology.

Results
Sperm concentration at freezing and at thawing resulted unchanged in all samples.
The mean total motility loss is of 32.6 % in samples A, with an increase of 3.2% per year of cryopreservation, and of 21.85% in samples B.
The mean loss of sperm with a normal morphology is of 16% in samples C, with an increase of 5.36% per year of cryopreservation, and of 5.43% in samples D.

Conclusion
The total motility loss is positively influenced by sperm concentration and negatively influenced by cryopreservation time. However, from data and trends analysis, we predict the sperm motility is less affected by cryopreservation time than via the freezing/thawing procedure. This hypothesis should be verified with additional studies regarding the cryoconservation procedure. Moreover, cryopreservation time leads to a progressive increase in sperm with head defects and amorphous sperm. Cryopreservation time affects the loss of sperm with normal morphology more than the total motility loss.