The Effect of Temperature and the Duration of Cryopreservation on Human Sperm Chromatin

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Objective: The present study was carried out to assess the effect of different freezing temperatures and duration of freezing on human sperm chromatin integrity.

Design: Prospective study

Materials and Methods: Semen samples were obtained from 93 randomly selected men attending the University Clinic, specimens from 5 proven fertile volunteers served as the controls. After preliminary semen analysis, each sample was divided in five aliquots and mixed with commercially available cryopreservation medium (Tardigrade, IMV, France). One aliquot (A) was used for the pre-freeze study of chromatin integrity, two other aliquots (B1, B2) were frozen in a mechanical freezer at -80ºC and the last two aliquots (C1, C2) were frozen in liquid nitrogen (-196ºC). The duration of cryopreservation for aliquots B1 and C1 was 1 week, and 3 months for aliquots B2 and C2. Chromatin cryoinjury was examined under fluorescent microscope using acridine orange.

Results: The mean percentage of spermatozoa with intact chromatin in pre-freeze samples (aliquot A) was 90.1 ± 5.9%, it showed no difference from that of whole semen without a cryopreservative. After 1 week of freezing, chromatin integrity reduced to 76.5 ± 7.7% and 81.6 ± 8.1% in aliquots B1 & C1, respectively. It dropped to 69.6 ± 8.6% and 76.3 ± 7.1% for aliquots B2 & C2 respectively upon prolonged freezing for 3 months.

Conclusion: The process of freeze-thawing has an adverse effect on human sperm chromatin. The loss of chromatin integrity is more prominent in case of mechanical freezing at -80ºC than with liquid nitrogen, both at 1 week as well as after prolonged preservation for three months. Therefore, cryopreservation in liquid nitrogen should be recommended for freezing of semen specimens used for assisted reproduction.

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