

Evaluation of the Effects of Different In Vitro Incubation Conditions on Sperm DNA Fragmentation

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Background: Prolonged in vitro incubation of spermatozoa has been shown to have negative effects on sperm motility, vitality as well as on DNA integrity. Knowledge regarding how shorter incubation periods prior to the assisted reproductive technique (ART) procedure influence semen quality is, however, limited. The aim of the study was to examine if sperm DNA integrity was affected during incubation in three different conditions for 2 h after sperm preparation prior to the ART procedure.

Materials and Methods: Density gradient prepared samples from two hundred men undergoing infertility work-up were included in the study. Following preparation the samples were divided into three aliquots; 0) frozen immediately after preparation (reference sample); or incubated for two hours in: 1) room temperature (23-24 °C) ; 2) In a 37°C humidified incubator with 6%CO₂ and 5%O₂. and 3) In a 37°C humidified without 6%CO₂ and 5%O₂. (atmospheric air). The Sperm Chromatin Structure Assay (SCSA) was used to assess the extent of DNA strand damage. Sperm DNA fragmentation rate was expressed as DNA fragmentation index (DFI).

Results: A statistically significant increase in DFI was seen in density gradient prepared samples incubated for 2 h at 37°C, 6%CO₂ and 5%O₂ compared to the reference sample taken immediately after preparation. This was the case also for samples incubated at 37°C in atmospheric air. Moreover, statistically significant lower DFI levels were seen in the group incubated at room temperature compared to those incubated at 37°C, 6%CO₂ and 5%O₂ or at 37°C in atmospheric air.

Conclusion: In order to prevent against further sperm DNA damage after density gradient preparation, samples should be stored at room temperature prior to the ART procedure.