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Title: Vitrification versus slow cryopreservation of expanded and non expanded blastocysts - Effect on DNA damage

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Objective: The objective of our study was to compare the effect of vitrification and slow cryopreservation on both expanded & non-expanded blastocysts by assessing the extent of DNA damage to the individual blastomeres.

Design: Prospective in vitro study

Materials and Methods: Forty-six non expanded and 51 expanded blastocysts were used in the study. We vitrified 22 expanded and 17 non-expanded blastocysts using vitrification media and cryotip loading devices. After warming, blastocysts were incubated for 4 hours in 5% albumin enriched HTF media at 37° C with 5% CO₂. In addition, 15 expanded and 16 non-expanded blastocysts were slowly cryopreserved using slow freezing media in a cryoplanner. We used 14 expanded and 13 non-expanded blastocysts as fresh controls. All blastocysts were fixed in 3% formaldehyde. Blastocysts were incubated with TUNEL staining for 1 hour at 37°C to stain DNA damaged nuclei. Blastomeres were mounted in Vectashield containing DAPI to stain blastomere nuclei. The percentage of TUNEL positive blastomeres were assessed in each group after imaging by confocal microscopy.

Results: 1) Both vitrification and slow cryopreservation techniques showed significant DNA damage compared with fresh control, 2) significantly lower post-warming DNA damage was seen after vitrification in both embryo stages as compared to the slow cryopreservation, 3) significantly higher DNA damage was seen in the expanded than the non-expanded frozen-thawed blastocysts regardless of the freezing protocol.

The percentage DNA integrity in the different study groups is shown in the table.

Conclusions: Vitrification results in lower DNA damage compared to the slow freezing technique. Cryopreservation of non-expanded blastocysts provides better results by either slow cryopreservation or vitrification.

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