Title: Routine use of blastocele aspiration of expanded blastocysts and assisted hatching of non-expanded blastocysts before vitrification

Amr Kader, MD, agarwaa@ccf.org, Ashok Agarwal, Ph.D, HCLD, agarwaa@ccf.org, Hussein Abdelrazik, MD, husseina2@ccf.org, Tommaso Falcone, MD, falcont@ccf.org, Jeffrey Goldberg, MD, goldbej@ccf.org and Rakesh Sharma,
Objective: Hatching allows better exposure of the expanded blastocele to the cryoprotectant and better dehydration of the blastocele. Blastocele aspiration provides total removal of the blastocele fluid content, thereby minimizing the chances of inadequate dehydration and ice formation. Our objective was to assess the added value of implementing these techniques on the post-warming integrity of blastocysts after vitrification.

Design: Prospective in vitro study

Materials and Methods: 58 expanded and 46 non-expanded blastocysts were used for the study. Blastocysts were randomly assigned to 9 groups as shown in the table. Vitrification was done using Irvine Scientific vitrification media and cryotip loading devices. After warming, blastocysts were incubated for 4 hours in albumin enriched HTF media at 37°C with 5% CO₂. All blastocysts were then fixed in 3% formaldehyde and were incubated with TUNEL staining for detecting DNA damaged nuclei. Blastocysts were mounted in Vectashield containing DAPI. The percentage of TUNEL positive and TUNEL negative blastomeres was assessed in each group by confocal microscopy.

Results: 1) The intervention by blastocele aspiration of expanded blastocysts or assisted hatching of non-expanded blastocysts resulted in significant improvement of the post-warming results and 2) application of intervention significantly reduces the post-warming DNA damage in the vitrified blastocysts, and 3) DNA damage in the vitrified blastocysts was comparable to the fresh blastocysts. The percentage of DNA integrity in each group is shown in the table.

Conclusions: We recommend blastocele aspiration of expanded blastocysts and assisted hatching of non-expanded blastocysts before vitrification to minimize the post-warming DNA damage to the blastomeres.

Support: None