Re-expansion grading and optimal vitrification timing of biopsied blastocyst

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Abstract

This protocol offers a optimal timing of vitrification after TE biopsy. The findings are useful for further enhancing the clinical outcomes obtained from vitrified euploid embryos.

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Protocol

zona opening by laser at timing of TE biopsy

Step 1.

the hole is drilled on day 5, away from the inner cell mass to ensure that only trophectoderm cells herniate.

trophectoderm biopsy

Step 2.

The blastocysts for TE biopsy were loaded in culture dishes, which contained two to three microdroplets of a blastocyst medium (Sage BioPharma, Inc.) overlaid with paraffin oil (Vitrolife, Kungsbacka, Sweden). The blastocysts were held using a holding pipette (Humagen, Charlottesville, VA, USA), and laser pulses were used to punch a small hole in the ZP away from the inner cell mass to accommodate the passage of several TE cells. Approximately 5–10 TE cells detached from the ZP were aspirated into the biopsy pipette with smooth suction. The aspirated cells were detached from the blastocysts with several laser pulses combined with smooth suction. The detached cells were aspirated into the TE biopsy pipette and released into the biopsy drop.

Re-expansion grading at the time of vitrification

Step 3.

All intervals between TE biopsy and vitrification were clearly recorded at the time of vitrification.

The morphology of the biopsied blastocysts was immediately assessed at the time of vitrification, with the morphology defined according to the re-expansion grade.

Briefly, biopsied blastocysts were culturing to re-expansion status relative to the original blastocoel as follows an expanding blastocyst with a blastocoel that is at least 3/4 of the volume of the embryo (RE) or a fully expansion blastocyst with a blastocoel completely filling the embryo (FE).

vitrification and warming protocols

Step 4.

Vitrification and warming with Cryotech (Cryotech, Japan) was performed according to the protocol described by Gutnisky et al. (2013)

embryo transfer

Step 5.

One to two euploid blastocysts were selected for transfer, and warmed embryos were cultured in a blastocyst medium at 37°C (6% CO_2 and 5% O_2) for 1–2 h before the transfer. The warmed blastocyst survival was checked at the timing of embryo transfer and “survival” being regarded as either >80% of cells intact or full re-expansion.