Cryopreservation of blastocysts using a modification of a simplified freezing protocol with a one step removal of cryoprotectant successfully used previously to freeze 2 pronuclear or multi-cell embryos

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Summary

Purpose: To describe a modification of a simplified freezing protocol for the cryopreservation of blastocysts. *Methods:* 1.5 M glycerol was substituted as a cryoprotectant instead of propanediol. *Results:* There was a survival rate of 59.1% (13/22) with three live deliveries in seven transfers (42.9% per transfer). The implantation rate was 28.6% (4/14). *Conclusions:* This is the first description of a new technique for freezing blastocysts. A larger series is needed to determine if the good pregnancy rates will continue.

Key words: Blastocyst; Cryopreservation; Implantation rates.

Introduction

A minority of in vitro fertilization (IVF) centers use, for the cryopreservation of 2-pronuclear (2PN) or multicell embryos, a simplified freezing protocol with a one-step removal of the cryoprotectant 1,2-propanediol (PrOH) [1]. This simplified technique requires an alcohol-bath freezer rather than a liquid nitrogen (LN2) cooled freezer [1].

The present study describes a modification of the simplified freezing protocol, with a one-step removal of cryoprotectant that can be used to cryopreserve blastocysts.

Materials and Methods

The modification for blastocyst freezing involved substituting 1.5 M glycerol for the 1.5 M 1,2 propanediol that is routinely used for 2PN or multicell embryos.

Basically the blastocysts were placed in Hepes-buffered HTF (Sage BioPharma) supplemented with 10% serum protein substitute (SPS, Sage BioPharma) at room temperature for 10 min. Blastocysts were then transferred to 1.5 M glycerol (Sigma-Aldrich) solution for approximately 10 min, loaded into 0.25 ml straws with a separate sucrose column, and heat-sealed at both ends. Straws were placed vertically in the BioCool alcohol-bath freezer (FTS Kinetics) and manually seeded at -6°C with a chilled spatula. The embryos were cooled at -0.4°C/min until -40°C was reached; after a 15 min hold the straws were plunged into LN2 for storage.

All stages of blastocysts were frozen: early, expanded, and hatched. The groups were too small to analyze by growth stage. Thawing involved submersion in a 37°C waterbath and "shaking" the straw to mix the 1.0 M sucrose diluent column with the smaller 1.5 M glycerol column containing the embryo.

Blastocysts were considered to have survived if at least 50% of the inner cell mass and trophectoderm were intact after being placed in culture, even if the blastocyst was collapsed. All blastocysts frozen in this study were the extras not transferred fresh on day 5. In no instance did day 3 fresh embryo transfer occur but the rest of the embryos were allowed to cleave to blastocyst stage.

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Results

One couple only froze one embryo and it did not survive the thaw, so this cycle was included in the survival statistics but not the pregnancy rate/transfer data. A total of 22 embryos were thawed and 13 survived (59.1% survival). There were three live delivered pregnancies in seven transfers (42.9% per transfer). The implantation rate was 28.6% (4/14).

Discussion

These data show adequate survival and pregnancy rates with cryopreserved blastocysts with this modification of the one-step freezing procedure. The pregnancy rate and implantation rate both appear to be similar to that of Behr *et al*. [2] who had a pregnancy rate of 36% and an implantation rate of 16% when using Menezo's two-step protocol and a planar (LN2) freezer. Thus, those IVF centers having good success with the simplified freezing protocol for 2PN and multicell embryos do not have to purchase a LN2 freezer just to freeze blastocysts or switch to vitrification. Of course determination of the true efficacy of this new modified procedure will require a larger study group.

References

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