Effect of the length of time that donated embryos are frozen on pregnancy outcome

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Summary

Purpose: To determine if the longer length of time that embryos donated to an anonymous couple have been frozen has a negative effect of subsequent successful pregnancy following thawing and transfer to the recipients. *Methods:* Retrospective determination of pregnancy rates according to the length of cryopreservation time has on pregnancy outcome following transfer of embryos designated for donation. *Results:* Longer time of freezing did not adversely affect subsequent pregnancy rates following frozen embryo transfer. *Conclusions:* Donated embryos frozen for over five years (the time when some countries demand that the embryos be discarded) contributed to one-fourth of the donor embryo pool and one-third of the live deliveries.

Key words: Cryopreservation; Donated embryos; Length of freezing; Pregnancy rates.

Introduction

When couples undergoing in vitro fertilization (IVF) complete their families, some choose to donate their remaining cryopreserved embryos to other couples. The donation is anonymous and patients receive no financial compensation for their generous donation.

However, the donation may not occur for many years, due to many factors. One is the length of time it takes the donor couple to complete their family, plus the time needed to ultimately decide to donate the remaining embryos. Added to this is the entire process of "adopting" the embryos, including selection of a desirable batch by the recipient patient or couple, paperwork and preparation, and eventually cycling through for a frozen embryo transfer (ET). By this time, the embryos may have been in storage for over ten years.

The following data were collected retrospectively to determine whether duration of storage was detrimental to pregnancy outcomes.

Materials and Methods

Embryos were cryopreserved in 1.5 M 1,2-propanediol (PrOH) either at the 2-pronuclear (2PN) or multicell stage using a simplified freezing protocol in a BioCool alcohol-bath freezer [1]. Within 20 minutes of being placed in PrOH, embryos were loaded into 0.25 ml straws, heat-sealed at both ends, and manually seeded in the alcohol-bath freezer at -6.0°C. The straws were cooled at -0.4°C/min until -40°C was reached; after a 15 minute hold the embryos were plunged into liquid nitrogen for storage.

Thawing involved submersion in a 37°C waterbath and a simple one-step removal of cryoprotectant. Embryos were cultured for one to two days in either HTF (Irvine Scientific) supplemented with 10% synthetic serum substitute (SSS, Irvine Scientific) or in Quinn's cleavage medium containing 10% synthetic protein substitute (Sage BioPharma) under sterile mineral oil (Squibb or Irvine Scientific).

Patients were prepared for the frozen ET using either oral estradiol or leuprolide acetate-estradiol protocols for endometrial preparation. Progesterone supplementation was begun when the endometrial lining was approximately 10 mm thick and embryos were thawed and transferred accordingly. Transfers were performed on day 3 and were preceded by assisted embryo hatching using acid Tyrode's solution [2].

A retrospective evaluation of pregnancy rates following thawing of frozen donated embryos was made according to six time periods.

Results

In the group of patients whose embryos were stored longer than ten years there were two pregnancies and two live deliveries achieved in three transfers (Table 1).

One pregnancy resulted from embryos stored for 11.8 years. A total of six embryos were available for thawing, three at the 2PN stage, two at the 4-cell stage, and one at the 6-cell stage. All of the embryos survived the thaw. Three embryos were transferred into the recipient, and all three had reached the 8-cell stage and were good quality at the time of transfer. The patient conceived twins and delivered a healthy full-term boy and girl. The age of the donor at the time of retrieval was 38.0 years old.

Another delivery resulted from embryos which had been stored for 10.8 years. A total of four embryos were available for thawing, three at the 4-cell stage and one at the 7-cell stage. Three embryos survived, and two were transferred into the recipient. Both had good morphology, and had reached 8-9 cells at the time of transfer. The patient conceived and delivered a healthy full-term boy. The donor's age at time of cryopreservation was 27.9 years old.

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Table 1. — *Pregnancy and implantation rates following transfer of donated frozen embryos according to the length of time frozen.*

Years cryopreserved	≤ 1.9	2.0-3.9	4.0-5.9	6.0-7.9	8.0-9.9	≥ 10
No. of transfers	32	54	50	23	20	3
% clinical pregnancy/transfers	40.6	40.7	42.0	60.9	30.0	66.7
% pregnancies delivered/transfer	28.1	35.2	36.0	60.9	30.0	66.7
Average # embryos transferred	3.7	3.5	3.5	3.3	3.0	2.7
Implantation rate	13.7%	16.0%	20.1%	26.3%	18.6%	37.5%

There did not appear to be any decrease in pregnancy or implantation rates with longer storage duration. In contrast, there seemed to be a trend for the older embryos to do slightly better with a higher pregnancy rate and lower spontaneous abortion rate, though this was not significant. No birth defects were recorded in the deliveries of any of the groups, except one male with a hernia, which technically is not a birth defect but was reported by the patient.

Discussion and Conclusion

A careful literature review revealed that the longest time multicell embryos have been kept frozen before transfer resulting in a delivery was 12 years [3], approximately the same as our recorded delivery after 11.8 years. The longest duration of storage prior to a donated embryo transfer prior to this study was nine years [4]. Information was also presented on the website IVF.net regarding a pregnancy achieved after 13 years of storage, but we could not find any citations in peer-reviewed journals about this anecdotal case report.

These data are important since legislation in some countries allows (or requires) embryos to be destroyed after two to five years of storage. Indeed one-fourth of the donated embryos came from ones stored ≥ 6 years as did about one-third of the live pregnancies. Unequivocally, cryopreserved embryos can produce viable pregnancies and deliveries far beyond this arbitrary cut-off time. When considered in combination with a voluntary embryo donation program, it seems wise to allow IVF patients the option of cryopreserving their supernumerary embryos and donating them when no longer needed. Both pregnancies mentioned in our results were from donated embryos transferred into anonymous recipients, and neither would have been possible if the embryos had to had be destroyed after five years.

It cannot be assumed that embryos stored for increasing lengths of time will have suboptimal quality or reduced survival rates. Machtinger *et al*. [5] did not see a drop in either of these parameters. Original cell stage and quality seem to be more important than duration of storage when predicting survival and implantation [6]. Although we did not include any data on cell stage and quality in this study, the duration of freezing did not seem to have detrimental effects on the implantation or pregnancy potential of thawed embryos, as evidenced by the similar pregnancy and implantation rates in all categories.

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