A comparison of efficacy of freezing embryos at the 2 pronuclear (2PN) stage vs multi-cell when using a simplified freezing protocol with one-step removal of cryoprotectant

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Summary

Purpose: To compare the efficacy of freezing embryos at the 2 pronuclear stage vs multi-cell stage using a simplified freezing protocol with a one-step removal of the cryoprotectant. *Methods:* A retrospective analysis was performed. Survival, delivered pregnancy and implantation rates were compared in transfers of all embryos frozen at 2 pronuclear stage (2PN) or all embryos frozen at multi-cell stage. The results were further stratified and compared according to the number of high quality embryos transferred. *Results:* In all categories despite comparing similar numbers and quality of embryos transferred there was a significantly higher survival rate of 2PN embryos. Significantly higher delivered pregnancy and implantation rates were seen with 2PN vs multi-cell embryos when there was only one or two embryos with ≥ 6 blastomeres and < 25% fragmentation, and a trend for higher delivered pregnancy rates when there were three top quality embryos transferred. *Conclusions:* When given the option it is preferable when using this simplified freezing and thawing protocol to freeze at the 2PN stage.

Key words: Simplified freezing protocol; 2 pronuclear stage; Multi-cell embryos.

Introduction

An embryo cryopreservation technique is described using a single-step addition of the cryoprotectant 1,2 propanediol in freezing straws preloaded with sucrose, and then plunged into an alcohol bath, then placed in a control rate freezer (Biocool) and then finally plunged into liquid nitrogen [1]. This technique was found to be very effective for freezing at the 2 pronuclear (2PN) stage [1]. The purpose of this study was to determine the relative effectiveness of using this freezing protocol for day 3 multi-cell embryos as compared to the 2PN stage.

Materials and Methods

Embryos were cryopreserved using a simplified protocol with a single step removal of the cryoprotectant 1,2 propanediol upon thawing [1]. All transfers were on day 3 and were preceded by assisted embryo hatching [2].

Frozen embryos were derived as follows: 1) Fresh embryo transfer because of risk of ovarian hyperstimulation or endometrial factor, e.g., inadequate endometrial thickness or homogenous hyperechogenic pattern on the day of hCG [3, 4]. Thus all the embryos were frozen at the 2PN stage. 2) Generally twice as many embryos as intended for transfer are allowed to cleave to day 3; the least quality embryos based on blastomere number and fragmentation are frozen at the multi-cell stage and the best ones transferred fresh. 3) Twice as many frozen embryos intended for transfer are thawed on day 3 and the lesser quality ones are re-frozen at the multi-cell stage [5]. 4) Germinal vesicle stage or metaphase I eggs are fertilized after another day in culture and then frozen at the 2PN stage [6].

Pregnancy outcome was compared for embryo transfer where all embryos thawed were at the 2PN stage vs all embryos thawed at the multi-cell stage. Pregnancy outcome was compared according to three groups: 1) At least one embryo in each group had an embryo with six blastomeres and ≤ 25% fragmentation. 2) At least two embryos had six blastomeres and ≤ 25% fragmentation, and 3) At least three embryos had six blastomeres and ≤ 25% fragmentation.

Results

Table 1 summarizes the survival, implantation and delivered pregnancy rates. All three of these parameters were significantly higher for thawed 2PN vs multi-cell embryos when there was at least one or two good grade embryos transferred. Survival rates after thawing were also significantly higher for 2PN vs multi-cell embryos when there were three top quality embryos.

However, there was no significant difference in either implantation or viable pregnancy rates when three top quality embryos were transferred (but a trend for lower delivered pregnancy rates was seen even when three good quality embryos were frozen at the multi-cell stage).

Discussion

If the data had shown equal chances of achieving a pregnancy with embryos thawed at the multi-cell stage compared to the 2PN stage then we would have to ques-

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Table 1. — *Survival, implantation and viable (ongoing past 16 weeks or delivered) pregnancy rates according to the stage of embryo freezing.*

	Rate	All 2PN	All multi-cell
At least one 6-cell B embryo	Survival*	97.6% (5557/5691)	90.6% (722/797)
with ≤ 25% fragmentation	Implantation*	20.9% (665/3186)	12.3% (73/595)
	Delivered pregnancy rate*	39.9% (386/967)	19.8% (41/207)
At least two 6-cell B embryos	Survival*	97.8% (4018/4107)	92.5% (481/520)
with ≤ 25% fragmentation	Implantation*	21.5% (489/2277)	14.9% (58/390)
	Delivered pregnancy rate*	41.7% (283/679)	24.0% (31/129)
At least three 6-cell B embryos	Survival*	97.1% (2249/2316)	93.5% (243/260)
with ≤ 25% fragmentation	Implantation**	21.6% (272/1258)	19.4% (36/186)
	Delivered pregnancy rate**	43.9% (155/353)	33.3% (18/54)

*$p < 0.05$ comparing all 2PN to all multi-cell stage, **p = NS comparing all 2PN to all multi-cell stage.

tion our policy of allowing only twice as many embryos to cleave as intended to transfer and then freeze the rest at the 2PN stage.

The pregnancy rate per oocyte harvest has been defined as the chance of a pregnancy from a given egg retrieval cycle without the need for performing another egg retrieval [7, 8]. Based on these data we will continue our policy of allowing only twice as many embryos as intended to transfer to cleave and then freeze the rest at the 2PN stage.

It is possible that a prospective study comparing the present policy described above vs allowing all embryos to cleave on day 3; choose the best ones, and freeze the remaining ones at the multi-cell stage could prove that a higher pregnancy rate on the fresh transfer could be achieved by having a larger number of embryos from which to select the best ones. However, the critical question from this prospective study is even if a higher fresh embryo pregnancy rate is found, if the fresh embryo transfer fails, will the less quality frozen multi-cell embryo lead to an overall lower pregnancy rate per oocyte harvest? Even if changing the policy to allow all embryos to cleave did not result in a lower pregnancy rate per harvest, would it lead to less chance of a second pregnancy for the first retrieval with subsequent frozen ETs?

One should note that possibly other freezing techniques might not show any advantage of freezing at the 2PN stage. Hopefully this study will encourage other IVF centers using a different technique for freezing to perform similar studies.

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