Effect of transferring frozen-thawed embryos resulting from fertilization of immature oocytes matured one day in culture prior to intracytoplasmic sperm injection (ICSI) on implantation rates

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Summary

Purpose: To evaluate whether it is efficacious to allow immature (metaphase I or germinal vesicle stage) oocytes to incubate one more day and then perform ICSI.

Method: A retrospective study of frozen embryo transfers (ET) of deselected frozen embryos was performed to see if inclusion of a higher percentage of embryos derived from in vitro maturation of oocytes resulted in lower implantation rates.

Results: The implantation rate following transfer of frozen-thawed embryos, where embryos derived from immature oocytes constituted ≤ 40% of the embryos (group 1) was similar to group 2 with > 40% (11.3% vs 9.8%). However, group 1 had a 10.2% implantation rate for viable (past first trimester) sacs vs only 4.3% for group 2.

Conclusions: Pregnancies from transfer of frozen-thawed embryos derived from in vitro cultured immature oocytes are as likely to implant as the other deselected frozen-thawed embryos.

Key words: Germinal vesicle; Cryopreservation; Metaphase I.

Introduction

One of the ways to train embryologists to do intracytoplasmic sperm injection (ICSI) without compromise to the patient is to practice on germinal vesicle stage or metaphase I oocytes that have been matured in culture. A problem that arises from this policy, however, is the dispensation of the embryos so formed.

There are case reports of live pregnancies that have resulted from the exclusive transfer of all in vitro matured metaphase I or germinal stage oocytes [1-3]. In in vitro fertilization (IVF) programs such as ours, where life is believed to start with conception, discarding these embryos is not pragmatic. Therefore, the policy is to cryopreserve them at the 2 pronuclear stage.

Empirically, these embryos are considered the least likely to implant. Thus, when a woman is coming for frozen embryo transfer (ET), these are the last embryos to be included in the transfer. It is rare that they are the only embryos available for transfer, but sometimes if there are insufficient embryos available for transferring the desired number by the couple, these embryos derived from immature oocytes will be included to make up the desired amount.

Thus, these embryos, when transferred, are most frequently included with the last of the remaining frozen embryos (which will include ones deselected for less blastomeres or increased fragmentation or twice-frozen twice-thawed embryos).

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This retrospective study was designed to evaluate the impact of these frozen-thawed embryos derived from one-day in vitro maturation of immature oocytes on clinical and viable pregnancy rates (PRs) and implantation rates.

Materials and Methods

Following controlled ovarian hyperstimulation (COH) with gonadotropins using various protocols including luteal phase leuprolide, follicular phase leuprolide (standard or microdose), or ganirelix at the time of a 14 mm follicle after gonadotropin stimulation, oocyte retrieval was performed 35-37 hours after 10,000 U of human chorionic gonadotropin (hCG). The hCG injection was given when there was adequate follicular size and number, and sufficient serum estradiol. Some couples had predetermined to have the oocytes fertilized by ICSI because of either male factor problems (oligo, asthenozoo, or teratozoospermia, low hypoosmotic swelling test scores, presence of antisperm antibody), history of poor fertilization, or risk of fertilization failure, e.g., unexplained infertility.

Only metaphase II oocytes were fertilized by ICSI on the day of retrieval. Metaphase I and germinal vesicle stage oocytes were matured one more day in culture to reach the metaphse II stage when ICSI would be performed by embryology trainees.

Some couples would proceed with fresh ET during the cycle of oocyte retrieval. The policy was to allow twice as many embryos as the patients desired to transfer (which usually was 3) to reach the 72-hour cleavage stage. The best half of the embryos, based on blastomere number, fragmentation scores and symmetry, were transferred and the lower quality embryos that progressed to day 3 were cryopreserved [4]. Those embryos

not allowed to cleave were frozen at the 2 pronuclear stage. The embryos formed from in vitro culturing of immature oocytes were always cryopreserved and not transferred during the cycle of oocyte retrieval. The embryos were cryropreserved using a simplified freezing protocol [5].

Patients who failed to conceive following fresh ET or those who had been previously successful and desired another child, would request frozen ET. Again twice as many embryos were allowed to thaw as the number of embryos requested for transfer. The best half was chosen for transfer, and the rest, assuming they did not have cleavage arrest, were refrozen [6, 7]. Three-day old embryos had assisted hatching performed before they were transferred to the uterus [8].

The decision as to which embryos to thaw was as follows: first preference – 2 pronuclear embryos; second choice – multicell embryos with the most blastomeres and the least fragmentation; third choice – twice-frozen embryos; last choice – embryos derived from in vitro maturation of immature oocytes.

Pregnancy outcome and implantation rates were compared according to the percentage of the embryos of the total transferred that were derived from immature oocytes. Having at least one embryo transferred from immature oocytes helped keep the type of embryos being transferred uniform, i.e., in general, the lower quality embryos.

Results

The implantation rate according to percentage of the embryos that were derived from immature oocytes is seen in Table 1. The implantation rates were similar whether there were ≤ 40% of the embryos (group 1) or > 40% (group 2) of the embryos derived from immature oocytes. However the miscarriage rate per implanted sac was significantly higher in group 2.

Table 1. — *Implantation rates according to percentage of embryos that were derived from immature oocytes.*

% of embryos that were from immature oocyte	Embryos transferred	Sacs (implantation rate)	Viable sacs (implantation rate)*	Miscarriage rate per sac
≤ 40	274	31 (11.31%)	28 (10.21%)	3/31 (9.7)*
> 40%	133	13 (9.8%)	5 (4.3%)	8/13 (61.5%)*
				*p < .05

* Viable refers to ongoing past the first trimester or delivered.

Discussion

The results suggest that frozen-thawed embryos derived from immature oocytes have about the same chance of implanting as deselected frozen-thawed embryos derived from fertilization of mature oocytes. However, for some reason, these embryos have a much greater chance of resulting in spontaneous abortion.

The fact that the embryos formed from continuing culture of immature oocytes and fertilization by ICSI leads to embryos with a much lower chance of a live baby justifies our policy of allowing less experienced embryologists to gain more experience by practicing ICSI on these oocytes. However, the fact that these embryos can result in a live pregnancy is assuring since those couples believing that life begins with conception, and therefore insisting on transfer of these embryos, will not be taking medication and undergoing ET for naught.

These data also serve the purpose of reporting the fourth pregnancy resulting in a live delivery where all of embryos used for transfer were derived from one extra day of in vitro maturation of germinal vesicle stage or metaphase I oocytes before ICSI was performed.

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