The importance of number of blastomeres when embryos are transferred in the absence of controlled ovarian hyperstimulation

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Summary

Purpose: To determine if the previous findings that transferring embryos with a higher number of blastomeres results in higher pregnancy rates following fresh but not frozen embryo transfer (ET) was related to the use of controlled ovarian hyperstimulation (COH) in the former but not in the latter.

Methods: Retrospective review of pregnancy and implantation rates following fresh embryo transfer of donor egg recipient cycles (where no COH is used) vs frozen ETs during the same time period according to whether there was at least one embryo with eight blastomeres transferred or not.

Results: Significantly higher pregnancy rates with an 8-cell ET in donor oocyte recipient cycles but not frozen ETs.

Conclusions: A less favorable uterine environment caused by the use of high dose gonadotropin is not responsible for the once again observed difference in higher pregnancy rates with higher blastomere number in fresh vs frozen ET. However, an effect of the gonadotropin releasing hormone analogue was not ruled out by this study.

Key words: Controlled ovarian hyperstimulation; Blastomere number; Oocyte recipient; Frozen embryo transfer.

Introduction

A previous study found that the majority of embryos that attain blastocysts on day 5 reached an 8-cell stage by day 3 [1]. A study by Jones et al also found a positive correlation with the number of 8-cell embryos formed and subsequent blastocyst formation [2]. Another study found a significantly higher pregnancy rate and implantation rate in the cycle of egg retrieval when there was at least one embryo with eight blastomeres transferred compared to a maximum of only 5-7 blastomeres [3]. However there was much less difference when evaluating frozen embryos according to blastomere number [3].

There are data strongly suggesting that controlled ovarian hyperstimulation can create a hostile uterine environment which could lower the chances of embryos to successfully implant [4-9]. The possibility exists that successful pregnancy can be established in this hostile environment by a hearty embryo but not by a less robust one. Thus the study presented here evaluated whether the association of higher pregnancy rates with an increased number of blastomeres in the transferred embryos is less evident in the absence of controlled ovarian hyperstimulation (COH).

Material and Methods

All transfers, fresh or frozen, using exclusively embryos that resulted from fertilization of donor oocytes over a three-year period were evaluated. Clinical PRs, delivery/viable PRs, and implantation rates were determined according to the transfer of at least one eight-cell embryo or not. All ETs used three-day old embryos.

Recipients without ovarian function were treated with oral micronized estradiol, 2 mg x 5 days, 4 mg x 4 days, then 6 mg x 5 days, beginning on the sixth day of the donor's leuprolide acetate treatment. Recipients with ovarian function were suppressed with leuprolide acetate or oral contraceptives before starting the estradiol. Recipients were given P vaginal suppositories, 200 mg twice daily, and frequently 100 mg IM P beginning the day after the donor takes hCG, and transfer occurred on the fourth day of P supplementation.

Generally, twice as many embryos intended for transfer were allowed to cleave for three days and the best graded embryos were transferred and the remaining ones were cryopreserved if deemed adequate. Similarly twice as many frozen embryos intended for transfer were allowed to cleave and the best half were transferred and the remainder refrozen for future transfers [10]. Embryos were frozen using a simplified freezing technique with a one-step thawing protocol [11]. Frozen embryos were hatched prior to transfer as previously described [12]. The remaining two pronuclear embryos not intended for fresh transfer were also cryopreserved.

Results

The outcome of fresh ETs is seen in Table 1. Significantly higher clinical PRs, delivery rates, and implantation rates were seen in the group receiving at least one 8-cell embryo.

Interestingly, when evaluating frozen ET, there was no significant difference or even a trend for higher PRs with

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Table 1. — *Outcomes of fresh embryo transfers in donor-oocyte recipients according to blastomere number.*

	Maximum cell size <8	Maximum cell size ≥8
# of transfers	61	135
Embryos transferred	3.4 ± 1.1	3.4 ± 7
Clinical PR	37.7% (23)	67.4% (91)
Singleton	12	46
Multiple	11	45
Implantation rate	17.2% (36/209)	33.9% (154/454)
Spontaneous abortion	2	14
Delivery rate	34.4% (21/61)	57.0% (77/135)

Table 2. — *Outcomes of frozen embryo transfers according to blastomere number.*

	Maximum cell size <8	Maximum cell size ≥8
# of transfers	92	54
Embryos transferred	3.4 ± 1.1	3.8 ± 1.0
Clinical PR	43.5% (40)	37.0% (20)
Singleton	26	10
Multiple	14	10
Implantation rate	19.4% (61/315)	15.7% (31/197)
Spontaneous abortion	6	4
Delivery rate	37.0% (34)	29.60% (16/54)

transfer of an 8-cell embryo versus no 8-cell embryo as seen in Table 2.

The presence of one 8-cell embryo was 68.9% for fresh ETs vs 37% for frozen ETs. Leuprolide acetate was used by 59.8% of recipients with fresh ETs versus only 14.6% of those having frozen ETs.

Discussion

The results of this study found higher pregnancy rates whenever an 8-cell embryo was included in fresh transfers but no such advantage was found for frozen ETs. Thus these results were similar to the aforementioned study [3]. However, the difference here was by studying embryo transfer into recipients, thus one cannot conclude that the explanation for the discrepancy seen between fresh and frozen transfers is related to the less favorable environment created by the COH regimen.

However, the fact that leuprolide acetate was used by four times as many recipients receiving fresh embryos than the group receiving frozen ETs could still be consistent, and maybe the data could still be explained on the basis that fresh embryos were faced with a less favorable environment so that only the heartier embryos could implant. This hypothesis, however, would suggest it is the gonadotropin releasing hormone analogue rather than the gonadotropin per se that creates the hostile environment. There are data though that even the use of follicular maturation drugs without the purpose to hyperstimulate and without gonadotropin releasing hormone analogues can also have a negative effect on implantation [4, 13].

Another explanation, favored by the author, is that the process of cryopreservation can damage some blastomeres without effecting embryo viability. Thus a frozen-thawed embryo with fewer blastomeres might still have been one of the more heartier embryos that would have made it to an 8-cell embryo if there had not been injury to some specific blastomeres.

Future studies need to evaluate only fresh transfers without the use of gonadotropin releasing hormone analogues to better answer this question.

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