

# A study to determine if embryo cryopreservation influences the potential of rapidly growing embryos to successfully implant in uterine environments not influenced by controlled ovarian hyperstimulation

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## Summary

**Purpose:** To determine if transferring at least one embryo with eight blastomeres at 72 hours improves prognosis of donor oocyte recipients. The study aim was to verify if cryopreservation increases or decreases the advantage of rapidly growing embryos. The study could exclude the influence of controlled ovarian hyperstimulation (COH) on the uterine environment.

**Methods:** All transfers, fresh or frozen, using exclusively embryos that resulted from fertilization of donor oocytes over a three-year period were evaluated.

**Results:** Significantly higher pregnancy rates (PRs) and delivery rates were found in donor oocyte recipients receiving at least one eight-cell embryo compared to transfers without any eight-cell embryos. These differences were not found when comparing frozen embryo transfers (ETs). The data could not be explained by confounding variables.

**Conclusions:** The inclusion of at least one 8-cell embryo on day 3 fresh ET resulted in higher PRs even without the influence of COH. However, higher blastomere number did not influence frozen ET outcome.

**Key words:** Blastomere number; Cryopreservation; Ovarian hyperstimulation; Donor oocyte.

## Introduction

A positive correlation has been observed with the number of 8-cell embryos formed and subsequent blastocyst formation [1]. Also clinical pregnancy rates (PRs) and implantation rates were higher in in vitro fertilization (IVF)-embryo transfer (ET) cycles where there was at least one 8-cell embryo transferred on day 3 [2]. However, there was less of a difference in PRs and implantation rates when there was at least one 8-cell embryo vs none for frozen-thawed day 3 ETs [2].

One possible explanation for these differences for fresh vs frozen ET may relate to the fact that the former transfer occurred under conditions of controlled ovarian hyperstimulation (COH) whereas the latter transfers were with ovulation suppression and estrogen/progesterone (P) therapy. There are several studies suggesting that COH may adversely affect the uterine environment [3-6]. Thus the possibility exists that a more rapidly growing embryo is a heartier embryo and it has a greater capacity to implant into a hostile environment.

To test this hypothesis, the study presented here evaluated whether the presence of at least one 8-cell embryo improves PRs in oocyte recipients where COH is not used.

## Materials 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 8-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 before starting the estradiol. Recipients were given P vaginal suppositories, 200 mg twice daily, and frequently 50 mg IM P beginning the day after the donor took hCG, and transfer occurred on the fourth day of P supplementation.

Generally, twice as many embryos intended for transfer were allowed to cleave to day 3 and the best graded embryos were transferred, and the remaining ones were cryopreserved if deemed adequate. Embryos were frozen using a simplified freezing technique with a one-step thawing protocol [7]. Frozen embryos were hatched as previously described [8]. 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.

Table 1. — Outcomes of fresh cycles according to blastomere number.

	Maximum Blastomeres < 8	Maximum Blastomeres ≥ 8
# of transfers	61	135
Embryos transferred	3.4±1.1	3.4±.7
Clinical PR	37.7% (23)	67.4% (91)
Singleton	12 (52.1%)	46 (50.5%)
Multiple	11 (47.9%)	45 (49.5%)
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 1. — Outcomes of frozen embryo cycles according to blastomere number.

	Maximum Blastomeres < 8	Maximum Blastomeres ≥ 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 (65.0%)	10 (50.0%)
Multiple	14 (35.0%)	10 (50.0%)
Implantation rate	19.4% (61/315)	15.7% (31/197)
Spontaneous abortion	6	4
Delivery rate	37.0% (34)	29.6% (16/54)

Interestingly, when evaluating frozen ET, there was no significant difference or even a trend for higher PRs with transfer of an 8-cell embryo versus no 8-cell embryo as seen in Table 2.

The presence of at least one 8-cell embryo was found in 68.9% of 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 embryonic genome is fully activated after the 8-cell stage [9]. Therefore it does not seem likely that an 8-cell embryo is less likely to have a chromosomal anomaly as compared to an embryo with fewer blastomeres. Thus, the intrinsic capacity of a faster growing embryo to successfully implant does not seem to be because of better genetic selection.

The data presented here show that the discrepancy of higher PRs with 8-cell fresh embryos vs frozen embryos does not appear to be related to a heartier embryo being able to implant better in a hostile environment because 8-

cell embryos from oocyte donors resulted in higher PRs and implantation rates even in recipients not receiving COH.

These data, using a different type of patient, reconfirm previous conclusions that embryos with fewer blastomeres still result in respectable PRs following transfer on day 3 albeit lower than if an 8-cell embryo were present.

Further studies are thus needed to answer the question why blastomere number on day 3 embryos positively correlates with PRs following fresh ET but not frozen ET. From a practical standpoint though, one might compensate for the lower implantation rates of embryos with less blastomeres by adding an extra embryo for fresh ET. However, such an adjustment does not seem necessary for frozen ETs.

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