Comparable implantation rates with fresh vs frozen embryo transfer suggests that controlled ovarian hyperstimulation has an adverse effect on conception outcome

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Summary

Purpose: A retrospective comparison of fresh vs frozen embryo pregnancy rates.

Methods: All frozen embryos transferred used in the analysis including desellected embryos from the oocyte retrieval cycle, and twice-frozen embryos.

Results: Pregnancy and implantation rates following fresh or frozen embryo transfers were similar.

Conclusion: The similar outcomes despite the obvious disadvantages for the frozen-thawed embryo suggests that some other factor reduces the chance of embryo implantation on oocyte-retrieval cycles. An adverse affect of controlled ovarian hyperstimulation on the uterine environment is a strong possibility.

Key Words: Frozen Embryo Transfer; Deselection; Ovarian hyperstimulation.

Introduction

There are data suggesting that controlled ovarian hyperstimulation (COH) with the stimulation of multiple mature follicles for oocyte retrieval may exert an adverse influence on subsequent implantation. One study matched in vitro fertilization (IVF) patient characteristics to those of oocyte donors with regard to age and previous conception [1]. Despite the transfer of similar numbers of embryos and findings of no difference in embryo morphology for standard IVF patients and recipients, the clinical and ongoing pregnancy rates (PRs) and implantation rates were significantly higher in recipients [1]. Another study, using shared oocytes for donors and recipients, found twice the PR in recipients versus donors despite the recipient group averaging ten years older [2]. The same group subsequently repeated this study to be sure that the previous one was not influenced by the presence of hydrosalpinges in a much higher percentage of donors than recipients, but found, once again, significantly higher implantation rates in the recipients [3]. Thus these two latter studies support the one by Paulson et al. that COH may adversely affect subsequent PRs and implantation rates [1].

Though transferring frozen-thawed embryos would escape the potential adverse effect of COH, the consequences of freeze-thawing may negatively affect implantation rates. However, if the adverse effect of freeze-thawing was less than the effects of COH, it may be better to freeze all embryos and defer fresh ET. Before embarking on a prospective randomized trial to determine if it may be advisable to defer transfer on a stimulated cycle, we retrospectively compared PRs and implantation rates in two age groups following fresh or frozen ET.

Materials and Methods

A retrospective comparison of fresh versus frozen embryo PRs and implantation rates according to age (<39 vs >40) for 1997 and 1998 were made. Normally, the intention was to transfer embryos in a cycle with COH. Sometimes, because of a risk of ovarian hyperstimulation syndrome, in the opinion of the clinicians, or because of an inadequate endometrial thickness (<8 mm), or a homogeneous hyperechogenic pattern by sonography, all embryos were cryopreserved [4-10].

Embryo selection was used when transferring either fresh or frozen embryos by allowing twice as many embryos as intended to cleave to the 72 hour stage, and the best half, based on number of blastomeres and fragmentation, were transferred; those remaining were either frozen or refrozen (or discarded if very poor quality). Fragmentation scores were based on assigning 1 to those with no fragmentation; 2 for those with 25% or less (but not zero); 3 for 26-50%; and 4 for those with >50% fragmentation. The extra embryos not in the group used for the selection of the embryos for fresh transfer were cryopreserved at the 2 pronuclear (2PN) stage.

Whenever 2PN cryopreserved embryos were available they were used for thawing for frozen ET because their quality was unknown as opposed to the multi-cell embryos where they were frequently of lesser quality because of the deseletion process. If there were insufficient 2PN embryos to transfer, they were mixed with multi-cellular embryos. There were many retrieval cycles where all embryos were allowed to go to multi-cell stage because the number of embryos formed were not so large, and thus only frozen multi-cell desellected embryos were available for transfer.

Thus frozen ETs could exist of only those cryopreserved at the 2PN stage, or exclusively those at the multi-cell stage or could be mixtures of both. Multi-cell embryos used for transfer

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could be once-frozen or twice-frozen. Refrozen embryos were the last ones used [11].

All ETs occurred three days after oocyte retrieval. Assisted
hatching using acidic Tyrode’s solution was performed on all
frozen-thawed embryos prior to transfer [12]. Hatching was
performed also on fresh embryos especially in older patients or
those younger ones with thickened zona pellucida [13].

The embryos were frozen using a simplified method in which a
slow cooling program is started at the seedling temperature of
-6°C in an alcohol-bath controlled-rate freezer. 1.2 propanediol
was used as the cryoprotectant [14]. A one-step fast thawing pro-
cedure at room temperature was used and the cryoprotectant
was removed from the embryos in one step with a sucrose solution [14].

Various COH regimens were used including leuprolide
acetate (LA) started in mid-luteal phase and continued even
when gonadotropins (usually 300 IU daily) were started 11 days
later (preferred regimen for younger patients); or LA was
started in the early follicular phase and continued even when
300 IU gonadotropins were started usually around day five
(regimen used by many women in the late 30’s whose baseline
FSH was normal). Patients age 40 or over or those with elevated
gonadotropins on day three were frequently treated in mid-
luteal phase with a reduced amount of LA (0.5 mg) for ten days
only with gonadotropins started at 450 IU daily, or a microdose
protocol where they received only 0.1 mg LA from early folli-
cular phase with 450 IU gonadotropins started on day three and
this was usually preceded by one cycle of oral contraceptives.

Most frozen ET cycles used oral estradiol started on day two
at 2 mg with graduating doses after five days up to 4 mg x four
days, then 6 mg for five days, or sometimes 8 mg if the endo-
metrial lining was insufficient. If premature luteinization oc-
curred, then LA was given at 1 mg x ten days beginning in mid-
luteal phase and oral estradiol was started after ten days of LA.

All patients having ET in 1997 and 1998 were included
without exception. Though there have been some studies sug-
gesting that embryos fertilized by intracytoplasmic sperm injec-
tion do not fare well with freezing [15], that has not been our
experience [16] and these patients were not excluded.

Discussion

There are data suggesting that increasing the number of
blastomeres in the embryos transferred correlates posi-
tively with outcome [17]. The frozen embryos in both age
groups and in both years studied had a lower number of
blastomeres compared to fresh embryos.

The fact that no differences were found between PRs
or implantation rates following fresh or frozen ETs
despite the trauma of freeze-thaw (sometimes even twice)
and the adverse advantage of embryo deselection, sup-
ports previous data that the COH regimen adversely
affects implantation [1-3]. The possibility thus exists that
by deferring fresh ET with cryopreservation of all
embryos, the PRs and implantation rates of first ETs may
increase by allowing the best quality embryos to be trans-
ferred after freeze-thaw.

This retrospective comparative study, and one case
study [18] has provided us, and hopefully will provide
other centers, the encouragement to perform a prospec-
tive randomized study comparing PRs and implantation
rates following first transfers in which one group will
receive fresh embryos with a selection procedure (the
same as is presently being conducted), and the other
group will have all embryos cryopreserved at the 2PN
stage, and transfer with embryo selection will occur two
months later after thawing the frozen embryos on artifi-
cial estrogen and progesterone replacement. We plan on
initiating the study first in the 40 year and older group.

If an IVF center is not interested in deferring all fresh
ETs in favor of frozen ETs on non-hyperstimulated
cycles, they should certainly give some consideration to
this approach if the patient has failed to conceive fol-
lowing two or three fresh transfers.

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Table 1. — Pregnancy (clinical and viable) and implantation
rates - fresh vs frozen embryos.

<table>
<thead>
<tr>
<th></th>
<th>Fresh (539)</th>
<th>Frozen (539)</th>
<th>Fresh (240)</th>
<th>Frozen (240)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997 No. Transfers</td>
<td>292</td>
<td>212</td>
<td>93</td>
<td>43</td>
</tr>
<tr>
<td>Clinical PR</td>
<td>46.9%</td>
<td>47.6%</td>
<td>22.6%</td>
<td>27.9%</td>
</tr>
<tr>
<td>Delivered PR</td>
<td>42.9%</td>
<td>41.0%</td>
<td>19.4%</td>
<td>23.2%</td>
</tr>
<tr>
<td>Implantation rates</td>
<td>21.9%</td>
<td>20.6%</td>
<td>8.9%</td>
<td>11.6%</td>
</tr>
<tr>
<td>Avg. cell stage transferred</td>
<td>6.3</td>
<td>5.1</td>
<td>5.9</td>
<td>5.3</td>
</tr>
<tr>
<td>Avg. fragmentation score</td>
<td>2.0</td>
<td>2.1</td>
<td>2.0</td>
<td>2.1</td>
</tr>
</tbody>
</table>

| 1998 No. Transfers | 296         | 208          | 94          | 54           |
| Clinical PR       | 47.9%       | 42.8%        | 31.4%       | 29.6%        |
| Delivered PR      | 44.3%       | 34.6%        | 17.4%       | 18.5%        |
| Implantation rates | 21.6%     | 20.2%        | 11.5%       | 13.5%        |
| Avg. cell stage transferred | 6.4   | 5.6          | 6.5         | 5.1          |
| Avg. fragmentation score | 2.1  | 2.1          | 2.1         | 2.0          |


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