Outcome of thaw embryo transfer after cryopreservation of all embryos in patients at risk of ovarian hyperstimulation syndrome

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Objective: To determine the incidence of ovarian hyperstimulation syndrome (OHSS) and subsequent pregnancy rates (PRs) if ET is delayed in patients at risk of OHSS by allocating all embryos to cryopreservation.

Design: Retrospective analysis of clinical and laboratory data from 724 consecutive stimulated cycles of IVF.

Setting: University hospital-based IVF program.


Main Outcome Measures: Fertilization rates, cryosurvival rates, subsequent PRs, and the occurrence of severe OHSS.

Results: Ten of the 564 patients (1.8%) who had ET in the stimulation cycle developed severe OHSS. Sixty-nine patients had all embryos frozen because of the risk of OHSS, of which one (1.4%) developed severe OHSS. The subsequent PR after thaw transfer was 25.2% per transfer, with a cumulative PR per patient after additional thaw transfers of 40.6%.

Conclusions: Cryopreservation of all embryos and delayed ET in patients at risk of OHSS results in a low incidence of severe OHSS. Oocyte quality, fertilization rates, and cryosurvival of frozen embryos are equal to those for patients who have a normal stimulation profile. Subsequent thaw embryo replacements result in a satisfactory PR. Fertil Steril 1994;62:1192–1196

Key Words: In vitro fertilization, human embryo cryopreservation, ovarian hyperstimulation syndrome, risk reduction

The most significant complications of infertility therapies that use controlled ovarian hyperstimulation (COH) are multiple pregnancy and ovarian hyperstimulation syndrome (OHSS). Although improved methods for monitoring follicular development and endocrine response in women treated with hMG have resulted in the virtual elimination of the risk of mortality, a significant risk of morbidity because of OHSS remains, particularly in patients who conceive in the treatment cycle (1). Severe OHSS may be associated with massive ovarian enlargement, intravascular volume depletion, ascites, pleural effusions, and electrolyte and coagulation disturbances (2). Since 1989, we have aimed to reduce the incidence of OHSS after IVF by allocating all suitable embryos to cryopreservation if the E2 profile and the degree of follicular development suggest a significant risk of OHSS. Previous studies have demonstrated an association between very high E2 values and a reduced pregnancy rate (PR) (3, 4). However, it is uncertain whether this relationship is due to decreased endometrial receptivity or poorer quality oocytes that are associated with reduced fertilization rates, inferior quality.
embryos, and reduced rates of cryosurvival and implantation.

The purpose of this retrospective study was to analyze outcomes for patients when all embryos were cryopreserved after the stimulated cycle for IVF because of the risk of OHSS. Records were also reviewed for patients who developed severe OHSS after ET in the stimulation cycle.

MATERIALS AND METHODS

Records were reviewed from all patients treated with IVF at this center between September 1989 and December 1992 to identify those subjects whose stimulation was canceled or ET delayed because of a perceived risk of OHSS. Criteria for cancellation of oocyte retrieval or proposed ET are indicated in Table 1.

Ovarian stimulation for IVF was performed using a long-acting GnRH agonist (GnRH-a) protocol with buserelin acetate (Hoechst Canada Inc., Montréal, Quebec, Canada), 200 μg IN administered five times per day from the 21st day of a menstrual cycle for a minimum of 16 days. A pelvic ultrasound (US) was then performed to document ovarian suppression, and hMG (Pergonal; Serono Canada Inc., Mississauga, Ontario, Canada) was commenced at a dose of 150 IU/d IM. Higher or lower initial doses were used if necessary as dictated by any previous response to ovarian stimulation. Monitoring of E₂ and follicular growth by US was commenced on the 7th day of hMG. Administration of hCG, 10,000 IU IM, was performed when there were at least two follicles of ≥1.8 cm mean diameter, with an E₂ level of ≥272 pg/mL (1,000 pmol/L) per large follicle. Buserelin acetate was discontinued when hCG was given. Transvaginal oocyte retrieval was performed 34 to 36 hours after administration of hCG under US control. A standard swim-up procedure was undertaken for spermatozoal preparation. Patients who had ET performed in the stimulation cycle were given hCG, 2,500 IU IM on the day of ET and repeated 3 and 6 days later. If symptoms suggestive of OHSS developed, no further hCG was administered. In patients who did not have ET in the stimulation cycle, no additional postretrieval measures were taken to reduce the risk of OHSS.

Embryos were cryopreserved at the early cleavage stage (usually 2 to 8 cells) approximately 48 hours after oocyte retrieval. Blastomere number and physical characteristics were used as selection criteria to determine suitability of embryos for cryopreservation (5). Cryopreservation was performed using a 1,2-propanediol and sucrose solution in phosphate-buffered saline (PBS) as the cryoprotectant (6). Embryos were placed in a 1.5 M 1,2-propanediol solution for 10 to 15 minutes at room temperature, transferred to a 1.5 M:0.1 M sucrose solution, and then loaded immediately into a plastic 0.25-mL straw and frozen in a Planar Kryo-10 freezer (Diamed, Mississauga, Ontario, Canada). Freezing curves were −2°C/min to −8°C, −0.3°C/min to −30°C, and −50°C/min to −150°C. Straws were then plunged into liquid nitrogen.

For the management of subsequent thaw cycles, patients were given the option of a natural cycle with daily US and LH monitoring to time ET appropriately or a controlled cycle using exogenous E₂ as micronized 17-β E₂ (Estrace; Bristol-Myers Canada, Inc., Ottawa, Ontario, Canada), started on the 2nd to 5th day of the cycle at a dose of 2 mg/d. Estrogen dosage was not varied throughout the cycle. On the 13th day of E₂ administration, P was added in the form of 50-mg vaginal suppositories three times per day. Embryo transfer was performed on the 3rd day of P administration. We have previously demonstrated no difference in PRs between these two protocols (7).

Embryos were thawed by removing the straws from liquid nitrogen, which were held at room temperature for 30 seconds and then warmed in a 30°C water bath for 40 seconds. During rehydration, embryos were expelled into a 1 M 1,2-propanediol:0.2 M sucrose solution, left for 5 minutes, transferred into a 0.5 M 1,2-propanediol:0.2 M sucrose solution for 5 minutes and then into a 0.2 M sucrose for 10 minutes before being placed in PBS for 20 minutes (10 minutes at room temperature, 10 minutes at 37°C). All embryos were transferred into culture medium until uterine replacement. If one or more embryo(s) had not survived the cryopreservation, additional embryos were thawed if available until a

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Criteria for Cancellation of Cycle or Delay of ET</th>
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<tbody>
<tr>
<td>Cycle cancellation</td>
<td>Delayed transfer</td>
</tr>
<tr>
<td>E₂ on day 7*</td>
<td>≥1,089 pg/mL</td>
</tr>
<tr>
<td>E₂ on day of hCG</td>
<td>≥4,086 pg/mL</td>
</tr>
<tr>
<td>Number of follicles</td>
<td>≥50</td>
</tr>
<tr>
<td>on day of hCG (regardless of E₂)</td>
<td></td>
</tr>
</tbody>
</table>

* Conversion factor to SI unit, 3.671.
† NA, not available.
maximum of two embryos were prepared for transfer. Data were collected on the characteristics of the stimulation cycles, pregnancy outcomes, and outcomes of subsequent thaw transfers from those patients who did not have ET in the stimulation cycle and from all patients who developed severe OHSS (2) after ET in the stimulation cycle during the study period.

RESULTS

Between September 1989 and December 1992, 724 stimulation cycles for IVF were conducted in this center. These resulted in 588 ETs and 152 clinical pregnancies (25.9% per ET). There were 80 (11.0%) cycles that resulted in a response considered to indicate an increased risk of OHSS. There were 11 (1.5%) in whom the risks were considered too high to administer hCG and proceed to oocyte retrieval. In the remaining 69 (9.5%), the risks were not believed to be sufficiently high to justify cycle cancellation, but delayed ET was recommended. These 69 study patients had an average age of 34 years (range, 25 to 39 years), with 48 patients (70%) presenting with primary infertility. The mean duration of infertility was 5.7 ± 2.7 (SE) years. The primary indications for IVF were tubal disease (70%) and unexplained infertility (23%), with the remainder being for male factor or endometriosis.

One patient with clomiphene citrate-resistant polycystic ovarian disease was converted to IVF from gonadotropin ovulation induction because of an excessive response to hMG. The distribution of these demographic data was not significantly different from other patients treated during the same time period. The mean peak $E_2$ concentration, number of follicles seen, number of oocytes retrieved, fertilization rates, and numbers of embryos cryopreserved are depicted in Table 2.

One of the 69 patients (1.4%) developed severe OHSS despite deferring ET in the initial cycle. In these 69 patients, a total of 123 thaw transfer cycles have been performed with one to six ETs being undertaken per patient. Of the 316 embryos thawed, 266 (84%) survived with at least one blastomere intact, which could be replaced. Thirty-one clinical pregnancies occurred in 28 patients, with a PR of 40.6% per patient and 25.2% per thaw transfer cycle. Of these pregnancies, 5 ended in spontaneous abortion and 1 in heterotopic pregnancy with a tubal pregnancy treated surgically and a subsequent vaginal delivery of a healthy infant. There were 3 twin pregnancies and 1 triplet pregnancy (despite replacement of only 2 embryos) in which one gestational sac resorbed spontaneously, and monozygotic twins were delivered. These figures compare to a PR of 14.9% per thaw transfer cycle in all other women undergoing IVF during the same time period ($P = 0.00023$). There were no significant differences in the characteristics of the stimulation cycles between those patients who did and did not conceive in a subsequent thaw transfer cycle (Table 2).

The PR in cycles controlled with exogenous $E_2$ and P was slightly, but not significantly, higher than in natural cycles (20 of 68 [29%] versus 11 of 55 [20%]). In comparison, 10 of the 564 patients (1.8%) during the study period who had an ET in the stimulation cycle developed severe OHSS after IVF-ET. Seven of the 10 were confirmed to be pregnant. One had a spontaneous abortion at 10 weeks, and one had a twin gestation. One individual (patient 2, Table 3) had cycle characteristics suggestive of a risk for OHSS but insisted on ET. She required hospital admission and did not become pregnant. The average length of stay in hospital for those patients with severe OHSS was 4.1 days (range, 1 to 11 days). Cycle characteristics for these patients and for the patient who developed OHSS despite delayed transfer are depicted in Table 3.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Characteristics of the Stimulation Cycles in Women Who Did Not Undergo ET in the Stimulation Cycle—a Comparison of Those Who Conceived With Those Who Did Not in a Subsequent Thaw Transfer Cycle*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampules of hMG used</td>
<td>Yes (n = 28)</td>
</tr>
<tr>
<td>Peak $E_2$ (pg/mL)†</td>
<td>18.2 ± 7.2</td>
</tr>
<tr>
<td>No. of follicles seen on US</td>
<td>3,275 ± 938</td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>28.6 ± 7.6</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>73.6 ± 18.7</td>
</tr>
<tr>
<td>No. of embryos frozen</td>
<td>12.1 ± 4.8</td>
</tr>
</tbody>
</table>

* Values are means ± SD.
† Conversion factor to SI unit, 3.671.

DISCUSSION

This is the first study to report the impact of delayed ET to reduce the risk of OHSS on the PR after IVF. Although review articles have recommended such an approach (8), only one previous
that minimize hCG exposure can be considered in
exogenous administration), a number of maneuvers
of a source of hCG in the luteal phase (pregnancy or
cols that employ GnRH-a. In this present study, no
taken in any patients to prevent
result in a lower risk of severe
OHSS (10), although controlled trials have
yet been reported. In a recent report, the use of
glucocorticoids failed to prevent the development
of OHSS (11). The use of GnRH to trigger the LH
surge rather than giving hCG has also been sug­
ggested, but in a controlled trial this was not shown
to result in a lower risk of severe OHSS (12). Fur­
thermore, such an approach cannot be used in the
more successful and higher risk stimulation proto­
cols that employ GnRH-a. In this present study, no
additional measures after oocyte retrieval were
taken in any patients to prevent OHSS.

Because severe OHSS is very rare in the absence
of a source of hCG in the luteal phase (pregnancy or
exogenous administration), a number of maneuvers
that minimize hCG exposure can be considered in
attempts to reduce the risk of OHSS occurring.
In the presence of an extremely high E₂, the hCG
trigger to ovulation can be withheld. Provided a
GnRH-a protocol is being used, ovulation will not
occur, and OHSS will not develop. Future stimula­
tions in such patients would use lower doses of go­
adotropins. Where the risk is not marked, P can
be used for luteal phase support rather than hCG.
For the intermediate situation, all embryos can be
cryopreserved for transfer in subsequent cycles.
Because no embryos are transferred in the stimula­
tion cycle, no luteal phase support is required, the
presence of exogenous hCG is kept to a minimum,
and the risk associated with endogenous hCG is
eliminated. In the present series, 8 of the 10 pa­
tients who developed severe OHSS had E₂ levels
well below the mean value attained in those who
underwent delayed ET. In the latter group, only 1 of
10 subjects developed severe OHSS, and that pa­
tient would not have received the hCG trigger to
ovulation had she been treated later in the series.
Otherwise, no severe cases of OHSS were encoun­
tered despite the excessive stimulations expe­
tenced in these patients.

Where the response to stimulation is normal,
PRs after frozen-thawed ETs are not as high as
after fresh transfers. Where there is an excessive
response to stimulation, there might be an addi­tional concern that the exposure of the oocytes to
the very high E₂ levels might adversely affect their
function. The clinical PR of 25.2% per transfer of
these frozen embryos is significantly superior to the
normal frozen transfer rate in this center and is
equivalent to the fresh transfer PR. This improve­
ment might be considered to result from the freez­ing of highest quality rather than the worst quality
embryos. However, in this center we use a policy of
freezing the better embryos in all cases and replac­ing
in the stimulation cycle those embryos consid­
ered unlikely to survive cryopreservation. There­
fore, the quality of the embryos selected for
cryopreservation should not be different. However,
there were more embryos from which to choose if no
transfer was performed in the initial cycle, although
the number of embryos frozen was similar whether
or not subsequent pregnancy resulted. Conse­
quently, these data suggest that oocytes aspirated
in cycles at risk of hyperstimulation resulted in em­
bryos more likely to implant in future cycles than
did oocytes aspirated in cycles in which the re­
sponse to stimulation was “normal.”

In summary, this retrospective study demon­
strates that the risk of OHSS may be minimized by

Table 3 Characteristics of the Stimulation Cycle in Patients
Who Subsequently Developed Severe OHSS

<table>
<thead>
<tr>
<th>Peak E₂ (pg/mL)</th>
<th>No. of follicles</th>
<th>No. of oocytes</th>
<th>Fertilization rate</th>
<th>ET performed</th>
<th>Pregnant</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3,642</td>
<td>40</td>
<td>37</td>
<td>44</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>3,552</td>
<td>22</td>
<td>20</td>
<td>85</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>1,782</td>
<td>17</td>
<td>14</td>
<td>79</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>2,984</td>
<td>18</td>
<td>11</td>
<td>91</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>1,974</td>
<td>30</td>
<td>8</td>
<td>88</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>1,769</td>
<td>26</td>
<td>18</td>
<td>78</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>1,970</td>
<td>25</td>
<td>9</td>
<td>100</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>2,227</td>
<td>20</td>
<td>12</td>
<td>67</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>2,648</td>
<td>22</td>
<td>10</td>
<td>60</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>2,133</td>
<td>23</td>
<td>19</td>
<td>68</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>2,453</td>
<td>18</td>
<td>16</td>
<td>75</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Conversion factor to SI unit, 3.671.
† Twin pregnancy.
‡ Spontaneous abortion at 10 weeks.
adopting a policy of cryopreservation of all embryos. Furthermore, our data suggest that embryos resulting from such cycles are of good quality and are associated with superior PRs in subsequent thaw replacement cycles. In centers experiencing success with embryo cryopreservation, this approach would seem to increase the safety of COH without compromising the outcome. We advocate a stepwise approach to the management of the stimulation cycle in patients considered to be at risk of OHSS. Based on these criteria, the hCG trigger to ovulation may be withheld, or ET may be delayed by allocating all suitable embryos to cryopreservation. Other adjunctive measures such as the use of IV albumin or the continuation of GnRH-a in the luteal phase of at-risk cycles may prove to be of benefit in future randomized trials. We advocate that a randomized study be undertaken to confirm the findings of this retrospective study.

REFERENCES