Analysis of Factors Influencing Pregnancy Rate in Frozen-thawed Embryo Transfer

Lu Li, Xiao-xi Sun, Jun-ling Chen, Xiao-hong Gao, Yong-wei Wang, Jie-wei Tao, Li-nan Cheng
Assisted Reproductive Center, International Peace Maternity and Child Health Hospital, China Welfare Institute, Shanghai 200030, China

Objective To analyse factors influencing the outcome of frozen-thawed embryo transfer (FET)

Method A retrospective analysis was performed in our center on 129 thawing cycles from March 2001 to April 2003. The related parameters were compared between conceived and non-conceived cycles.

Results There were totally 129 clinical pregnancies in these transfers (pregnancy rate: 27.1%). Frozen-thawed embryos were transferred to natural cycles and CC cycling and hormone replacement treatment had equal success. Groups of IVF and ICSI did not differ significantly in pregnancy rates (P>0.05). The pregnancy rates for one, two, three and four pre-embryos transfer were 0, 20.0%, 44.1% and 75.0%, respectively (P<0.05). There were statistical differences between pregnancy group or non-pregnancy group in the endometrial thickness, CES, CES/No. of embryo. A higher pregnancy rate was observed in embryo transfers which had at least one 4-cell grade I embryo (d 2)(P<0.01).

Conclusions The most important factors influencing the implantation rate and pregnancy rate of frozen-thawed embryo transfer are age, endometrium thickness, and the number, morphology and growth rate of transferred frozen embryos of women participants.

Key words: frozen thaw; embryo transfer; pregnancy rate

Since the first report on successful frozen embryo transfer (Trounson et al., 1983)[1], the cryopreservation of embryos has been an important supplementary procedure in the treatment of infertility. Embryo cryopreservation has also provided additional clinical safety
in the presence of ovarian hyperstimulation and contributed to lowering the risk of multiple births by reducing the need for multiple fresh embryos transfer. A retrospective analysis was performed in our center on 129 thawing cycles to analyse the factors influencing the outcome of a frozen-thawed embryo transfer (FET), including age, as well as the number, morphology and growth rate of transferred frozen embryos of women participants.

Subjects & Methods

Subjects

Among a total of 520 couples undergoing in vitro fertilization-embryo transfer (IVF-ET) or intracytoplasmic sperm injection (ICSI) in our center between March 2001 to April 2003, 129 couples who had supernumerary cryopreserved embryos were selected with their informed consent and the outcome of transferred frozen-thawed embryos were reviewed. Cycles were excluded if the embryos were derived from donated gametes.

Embryo cryopreservation and evaluation criteria

In our IVF programme ovarian stimulation was achieved mainly using the gonadotropin-releasing hormone agonist (GnRHa) triptorelin (decapeptyl 3.75 mg, Ferring, Germany) for pituitary suppression and recombinant FSH (rFSH; Gonal-F, Serono) in a long protocol. Oocyte pick-up was performed 34–36 h after the injection of 10 000 IU of hCG (Profasi, Ares-Serono). The general procedures associated with the programmes of assisted reproduction have been previously described. After fertilization and fresh embryos/oocytes transfer on d 2 or 3 after oocyte retrieval, the remaining embryos were assessed by standard classification system and embryos of grade 1 or 2 were cryopreserved. Cryopreservation of most embryos was done at early cleavage stages following instructions of the standard freezing-thawing protocol. Embryo quality was evaluated 40–42 h (d 2 of transfer) or 65–67 h (d 3 of transfer) after fertilization and embryo was selected solely by its quality. Cryopreservation was carried out according to a slow-freeze and fast incubation protocol\(^2\) by a programmable biological freezer (Kryo 10 series II, Planer Products Ltd., UK). Embryos were scored according to the commonly used morphological criteria\(^3\): grade I (4 points): no fragments are found and blastomeres are symmetrical; grade II (3 points): blastomeres are not symmetrical and fragmentation is <10%; grade III (2 points): blastomeres are not symmetrical and/or fragmentation is between 20%–25%; grade IV (1 point): fragmentation is >50%.

Cumulative embryo score (CES) = No. of cells in embryo × score of that embryo.

Replacement of frozen-thawed embryo

Natural cycles

Patients with regular menstrual cycles and biphasic BBT were monitored for serum LH surge in natural cycles. FET was performed 3 d after LH surge (or 2 d after ovulation) and progesterone (P) or hCG (bid) was used in luteal phase. Serum $\beta$ hCG was tested 14
days after transfer. Those patients with elevated hCG level underwent B ultrasonic examination for gestational sac and they stopped taking P or hCG 12 weeks after confirmation of pregnancy.

**Clomiphene citrate cycles**

Nonovulatory patients with regular/long cycles or an absence of estradiol rise/luteinizing hormone (LH) surge in regular menstrual cycle were given clomiphene citrate (50–150 mg) daily for 5 d and their serum LH surge was monitored to decide the best time for transfer.

**Hormone replacement cycles**

Hormone replacement treatment was offered to nonovulatory patients after taking clomiphene citrate. No information was recorded on cycles pattern or length for patients in the different groups. As for down-regulation, decapeptyl (1.88 mg, Ferring, France) was sc injected once in the mid-luteal phase, then progynova injection (E2V, 6 mg/d) started on the second day of the next menstrual cycle. If ultrasound scanning detected an endometrial thickness >8 mm, E2V was then reduced to 4 mg/d and P injection (100 mg/d) started. Embryo transfer was carried out on d 4 of P injection.

A positive serum hCG test (>50 U/L) was conducted 14 d after pregnancy confirmation. The clinical pregnancy was documented by the presence of gestational sac(s) with or without fetal heart beat on transvaginal sonography 2 weeks later.

**Statistical analysis**

Data were analyzed by Student t-test and SPSS statistics software. Comparisons between proportions were performed by a chi square test. Data were provided as $\bar{x} \pm s$ and compared by a two-tailed unpaired Student’s test. Differences were considered significant if $P<0.05$.

**Results**

The mean age of women patients at transfer was 29.9 ± 9.6 years (ranging between 21-43 years). In these cycles, 437 frozen embryos were thawed and 306 embryos survived the thawing process (survival rate: 70.0%), with an average number of embryos transferred per cycle of 2.4 ± 0.7, an overall implantation rate of 12.8%, an overall pregnancy rate of 27.1%, a miscarriage rate of 28.6% and a multiple births rate of 8.6% (Table 1, 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Cycles</th>
<th>Pregnancies (n)</th>
<th>Pregnancy rate(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural cycle</td>
<td>99</td>
<td>25</td>
<td>25.2</td>
</tr>
<tr>
<td>Clomiphene cycle</td>
<td>15</td>
<td>7</td>
<td>46.7</td>
</tr>
<tr>
<td>HRT cycle</td>
<td>15</td>
<td>3</td>
<td>20.0</td>
</tr>
<tr>
<td>Total</td>
<td>129</td>
<td>35</td>
<td>27.1</td>
</tr>
</tbody>
</table>
Table 2  Outcome of frozen-thawed embryo transfer of IVF and ICSI

<table>
<thead>
<tr>
<th>Group</th>
<th>Pregnancy rate(%)</th>
<th>Implantation rate(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF</td>
<td>23/90(25.6)</td>
<td>27/205(13.2)</td>
</tr>
<tr>
<td>ICSI</td>
<td>12/39(30.8)</td>
<td>13/101(12.8)</td>
</tr>
</tbody>
</table>

In our study, there was no difference in pregnancy rates between the three groups, and no difference was found in the pregnancy rates and implantation rates between the IVF and ICSI group. Table 3 shows the clinical data of frozen embryo transfer cycles in 3 groups of women stratified by age. The implantation rates in the three groups were significantly different from each other. The overall pregnancy rate and implantation rate of women aged 30-34 were significantly higher than those of the other two groups (Table 3, \(P<0.05\)).

Table 3  Relationship between age and outcome of frozen-thawed embryo transfer

<table>
<thead>
<tr>
<th>Age(yr)</th>
<th>Cycles</th>
<th>Transfers (n)</th>
<th>Implantation rate(%)</th>
<th>Pregnancy rate(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>63</td>
<td>148</td>
<td>16/148(10.8)*</td>
<td>15/63(23.8)*</td>
</tr>
<tr>
<td>30-34</td>
<td>44</td>
<td>103</td>
<td>20/103(19.4)</td>
<td>17/44(38.6)</td>
</tr>
<tr>
<td>35-44</td>
<td>22</td>
<td>55</td>
<td>3/55(5.5)*</td>
<td>3/22(13.6)*</td>
</tr>
<tr>
<td>Total</td>
<td>129</td>
<td>306</td>
<td>39/306(12.8)</td>
<td>35/129(27.1)</td>
</tr>
</tbody>
</table>

*: \(P<0.05\), compared with patients aged 30-34

Table 4 shows the chance of pregnancy after 1-, 2-, 3- or 4-embryo transfer. The risk of multiple implantation and the chance of pregnancy were documented. There were statistical differences between pregnancy group or nonpregnancy group in the endometrial thickness, CES, CES/No. of embryo and at least one 4-cell grade I embryo transferred (Table 4).

Table 4  Outcome of different number of embryos transfer

<table>
<thead>
<tr>
<th>Embryos</th>
<th>Cycles</th>
<th>Pregnancies (n)</th>
<th>Pregnancy rate (%)</th>
<th>Multi-pregnancy rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>12</td>
<td>20.0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>26</td>
<td>44.1</td>
<td>7.7</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>3</td>
<td>75.0</td>
<td>33.3</td>
</tr>
</tbody>
</table>

\(P \text{ value} <0.01\) >0.05

Discussion

In spite of a lower pregnancy rate compared with fresh embryo protocol, cryopreservation is nowadays an integrated part of assisted reproductive technology\(^{(4)}\). In our study, the pregnancy rate was 27.1%, conforming to the findings of other studies.
The overall implantation rate of embryos following frozen embryo transfer was 12.8% in this study, which is comparable with the results obtained from other large study groups (8%-11%).

There have been debates on the pregnancy outcomes of ICSI cryopreservation and subsequent embryo transfer after thawing. Several studies supported the view that ICSI does not impair pregnancy rates or survival rates of frozen-thawed embryos. In our study, there were no significant differences in pregnancy rate or survival rate between frozen IVF cycles and frozen ICSI cycles, which demonstrated that fertilization by ICSI does not adversely affect the implanting capacity of frozen-thawed embryos.

We found significant differences in endometrial thickness between pregnancy groups and nonpregnancy groups (9.6 ± 1.6 and 8.9 ± 1.8, P<0.05) which suggested that endometrial thickness may have effects on pregnancy rates after frozen embryo transfer.

The classification of zygote morphology and the growth rate of embryos are observed by using noninvasive method to assess the developmental potential of human preimplantation embryos. Poor classification of zygote morphology is often associated with chromosomal abnormality. Slow embryo division suggests worse prognosis. Cumulative embryo score (CES) could help to evaluate classification of zygote morphology and the growth rate of embryos. Zhang et al. calculated and analysed the scores of 559 IVF/embryo transfer cycles and found the clinical pregnancy rate was 28.3% when CES>40, and the pregnancy rate was 13.9% when CES≤40, which demonstrated statistical significance. We also found statistical significance between the clinical pregnancy rates below or above the value of 11 of CES/No. of embryos (16.8% and 26.2%, respectively).

Age is one of the important factors influencing pregnancy rate after frozen-thawed embryo transfer. Many recent studies showed some common characteristics in the women above 30, especially above 35, include diminishing ovarian reserve, poor quality of remaining oocytes, decreasing endometrial receptivity and abnormal genetic factors. Studies by Kaltrom and Wang found that with ageing, the pregnancy rate reduced after frozen embryo transfer, and the major reduction occurred in women above 40 years. The implantation rates in the three age groups were significantly different from each other. The overall pregnancy rate of the younger women (aged 30–34) was significantly higher than that of those women aged 35-44 and under 30. One possible reason for this might be that for infertile couples under 30, male factor is more common cause while for women aged 30–34, tubal factor consists the main cause. Younger women also had a significantly higher implantation rate than the old women.

Wang found that the pregnancy rate for single frozen-thawed embryo was 10%–14% in younger patients (under 40). The expectation of pregnancy for two embryos could be >20% in younger women and the risk of multiple births was 15%–29%. However, three-embryo transfer would not further enhance the pregnancy in most subjects; it did have
higher risk of multiple births. We performed single frozen-thawed embryo transfer in 15 patients but none of them were pregnant. The reasons could be for poor quality of the thawed embryos since the ratio of CES/No. of embryo in these patients was only 8.8 (132/15). By increasing the number of embryos, clinical pregnancy rates and multiple births rates improved significantly. In the light of increasing pregnancy rate and decreasing multiple births rate, it is recommended that before deciding how many embryos should be transferred, patients' general condition and the quality of embryos should be taken into consideration.

References


(Received on September 27, 2004)