Reducing multiple pregnancies by restricting the number of embryos transferred to two at the first embryo transfer attempt

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Though the multiple pregnancy rate in in-vitro fertilization–embryo transfer must be reduced, strict and uniform regulation of the number of embryos transferred may make it impossible for women with little natural fecundity to carry children. We therefore restricted the number of embryos per transfer. In the first half of the observation period (n = 100), we limited the number of embryos transferred to three regardless of the number of previous transfers. In the second half (n = 137), we strictly regulated the number of embryos transferred to two at the first attempt and three in the second and later attempts. The multiple pregnancy rate per pregnancy was significantly lower (P < 0.005) in the second period (20.4%) than in the first period (52.9%), while the clinical pregnancy rate and the implantation rate per transfer were similar in both observation periods. 60.7% (17/28) of the multiple pregnancies arose from the first embryo transfer attempt, and 17.9% (5/28) of them arose from a second attempt. The 18 multiple pregnancies in the first period involved six sets of triplets, while the 10 multiple pregnancies that arose in the second period all involved twins. The implantation rate per transfer at the first attempt was significantly higher than that at the second or later attempts. The criterion for determining the number of embryos transferred should be simple to minimize errors of judgement. We believe that our method of restricting the numbers of embryos transferred may be one method of reducing multiple pregnancies without eliminating the possibility of having children for women with low fecundity.

Key words: embryo transfer/IVF/multiple pregnancy/triplets

Introduction

The rate of multiple pregnancy is unacceptably high in the field of assisted reproduction, and is considered a major complication of assisted reproduction technology. Whereas only 31% of couples who underwent in-vitro fertilization (IVF) wished for one child as their ideal outcome (Murdoch, 1997), any multiple pregnancy over triplets is an unwelcome event for couples receiving assisted reproduction treatment. One way to reduce the multiple pregnancy rate is to enforce laws that provide for a predetermined number of embryos to be transferred. This approach has been adopted in Germany and the United Kingdom, where the maximum number of embryos allowed to be transferred is three in any single treatment cycle. However, strict and uniform regulation of the number of embryos transferred may make it impossible for women with little natural fecundity to carry children. Although great variation in the number of embryos transferred is reported in practice, it is still difficult to predict a patient’s fecundity and determine for each individual the number of embryos to be transferred. In this study, we restricted the number of embryos transferred simply by limiting the number of embryos per embryo transfer attempt, and repeatedly attempting embryo transfer until a pregnancy was achieved.

Materials and methods

Patients

From April 1995 to February 1998, a total of 237 IVF cycles of 165 patients who produced more than three cleaved embryos were included in this study. Cycles with intracytoplasmic sperm injection (ICSI) were excluded, as the success of ICSI increased year-by-year because of technical improvement over the years in which we conducted the study. Patients who were diagnosed as having primary habitual abortions and were undergoing their secondary infertility treatment were also excluded. Data were analysed in two separate periods. In the first half of the observation period (n = 100), from April 1995 to September 1996, we limited the number of embryos transferred to three regardless of the number of attempted transfers, except for a few patients who were aged ≥42 years. In the second study (n = 137), from November 1996 to February 1998, we strictly regulated the number of embryos transferred, limiting it to two in the first embryo transfer attempt and three in the second and later attempts, except for a few patients who were aged ≥42 years.

During both periods, the number of embryos being transferred was determined by doctors after obtaining informed consent from all patients.

Procedures for IVF

Ovarian stimulation and other IVF procedures were performed as previously described (Fujii et al., 1997a). In the long-based protocol, gonadotrophin releasing hormone (GnRH) agonist (Suprecur; Hoechst, Tokyo, Japan) 900 µg/day was administered transnasally from the midluteal phase of the previous cycle, and pure follicle stimulating hormone (FSH, Fertinorm-P; Serono, Tokyo, Japan) 225 IU was administered i.m. for 2 days, followed by 150 IU for 5 days. In the short-based protocol, GnRH agonist was administered from the second or third day of menstruation, and pure FSH was administered beginning on the day of or the day following the start of the GnRH agonist regimen. After 7 days of pure FSH administration, the
follicular development was evaluated using transvaginal ultrasound. If the mean diameter of the dominant follicles was >14 mm, human menopausal gonadotrophin (HMG, Humegon; Organon, Tokyo, Japan) 150 IU/day was administered additionally until the mean diameter of the dominant follicles reached 18 mm. Twenty-four to 48 h after the last HMG administration, 5000 or 10 000 IU of human chorionic gonadotrophin (HCG, HCG-Mochida; Mochida, Tokyo, Japan) was administered i.m. Oocyte retrieval was performed 34–36 h later by ultrasound-guided transvaginal aspiration. Insemination with 100 000 washed motile spermatozoa/ml was performed 4 h after oocyte retrieval. Embryos were transferred into the uterine cavity 48–72 h after oocyte retrieval. In the first study period, the luteal phase was supported by progesterone depot (Oophorin Luteum Depot; Teikoku Zoki, Tokyo, Japan), 125 mg on the day of embryo transfer, 7 days after oocyte retrieval, and 11 days after oocyte retrieval. In the second study period, the luteal phase was supported by daily oral administration of dydrogesterone (Duphaston; Daiichi-seiyaku, Tokyo, Japan), 10 mg for 14 days, and by administration of HCG 2500 IU i.m. on the day of embryo transfer and 7 days after oocyte retrieval. A clinical pregnancy was defined as ultrasound visualization of a gestational sac or histological evidence of trophoblast.

Statistics
Results were analysed by either Fisher’s exact probability test of the frequency distribution or Student’s unpaired t-test. Differences were considered significant when \( P < 0.05 \).

Results
The mean age, age range, period of infertility, cause of infertility, and the percentages of GnRH agonist protocols were similar in patients from both observation periods. The number of oocytes retrieved and of oocytes fertilized, as well as the fertilization rate were similar in both observation periods. The number of embryos transferred per cycle was significantly different \( (P < 0.001) \) between the two observation periods as a matter of course. The multiple pregnancy rate per pregnancy was significantly lower \( (P < 0.005) \) in the second observation period than in the first period, although the clinical pregnancy rate per transfer, the implantation rate per transfer, and the abortion rate per pregnancy were similar in both observation periods (Table I).

The relationship between the number of times transfers were performed and the number of embryos transferred is shown in Table II for each observation period. A total of 60.7% (17/28) of the multiple pregnancies arose from the first embryo transfer attempt, and 17.9% (5/28) of them arose from the second attempt. That is, 78.6% (22/28) of multiple pregnancies were produced by the first two embryo transfer attempts. The 10 multiple pregnancies that arose in the second observation period all involved twins, while the 18 multiple pregnancies in the first observation period involved six sets of triplets. The implantation rate per transfer for the number of transfer attempts was also analysed (Table III). Though the implantation rate of each observation period analysed over five separate attempts was similar, a significantly \( (P < 0.05) \) higher implantation rate was obtained in the first period at the second attempt. As a whole, the implantation rate at the first embryo transfer attempt was significantly higher than that at the second embryo transfer attempt \( (P < 0.05) \), that at the third or fourth embryo transfer attempt \( (P < 0.005) \), and that at the fifth or more attempt \( (P < 0.0005) \). The difference between the implantation rate at the second embryo transfer attempt and that at the fifth or more embryo transfer attempt was also significantly different \( (P < 0.05) \).

Discussion
The maternal complications associated with multiple pregnancies were abortion, threatened preterm delivery, premature rupture of membrane, gestational diabetes mellitus, pregnancy-induced hypertension, and so on. A recent investigation revealed that all women who delivered triplets experienced emotional distress at 4 years after giving birth due to difficulties in their relationships with the children (Garel et al., 1997). The percentages of newborns who required ventilation (Seoud et al., 1992) and the percentages of at least one handicapped child (Yokoyama et al., 1995a,b) increase in proportion to the number of fetuses. Some of these maternal complications and an unfavourable perinatal outcome are more common in multiple pregnancies following infertility treatment than in spontaneous multiple pregnancies (Nyirati et al., 1997).

Medical specialists and expensive supplies and equipment are required for the management of multiple pregnancies. Given that such complications are unavoidable, and that managing multiple pregnancies demands a great deal of resources, it is obvious that in the field of infertility treatment medical staff must have a policy of reducing high multiple pregnancy rates. It has been postulated that transfers of one embryo might be more cost-efficient than transfers of two

<table>
<thead>
<tr>
<th>No. of cycles (n)</th>
<th>First period</th>
<th>Second period</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. oocytes retrieved/ aspiration (n)</td>
<td>6.9 ± 3.4</td>
<td>6.7 ± 3.3</td>
<td>NS</td>
</tr>
<tr>
<td>No. oocytes fertilized (n)</td>
<td>5.4 ± 2.5</td>
<td>5.4 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>81.2 ± 16.4</td>
<td>84.9 ± 18.2</td>
<td>NS</td>
</tr>
<tr>
<td>No. embryos transferred/ cycle (n)</td>
<td>3.1 ± 0.5</td>
<td>2.7 ± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clinical pregnancies/ transfer</td>
<td>34/100 (34.0)</td>
<td>49/137 (35.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Multiple pregnancies/ pregnancy</td>
<td>18/34 (52.9)</td>
<td>10/49 (20.4)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Implantations/ transferred embryos</td>
<td>58/331 (18.5)</td>
<td>59/370</td>
<td>NS</td>
</tr>
<tr>
<td>Abortions/ pregnancy</td>
<td>4/34 (11.8)</td>
<td>8/49 (16.3)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD. Values in parentheses are percentages. NS = not significant.
Table II. Relationship between the order of transfer attempts and the number of embryos transferred for each observation period (first, April 1995 to September 1996; second, November 1996 to February 1998)

<table>
<thead>
<tr>
<th>Transfer attempt</th>
<th>No. of embryos transferred</th>
<th>First period</th>
<th></th>
<th></th>
<th>Second period</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cycles (n)</td>
<td>Pregnancies (%)</td>
<td>Multiple pregnancies (%)</td>
<td>Cycles (n)</td>
<td>Pregnancies (%)</td>
<td>Multiple pregnancies (%)</td>
<td></td>
</tr>
<tr>
<td>First</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>60</td>
<td>25 (41.7)</td>
<td>7 (28.0)</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td></td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>18 (38.3)</td>
<td>10 (55.6)%</td>
<td>29</td>
<td>9 (31.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Second</td>
<td>3</td>
<td>10 (43.5)</td>
<td>4 (40.0)%</td>
<td>4</td>
<td>0 (0.0)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>2 (66.7)</td>
<td>1 (50.0)</td>
<td>1</td>
<td>0 (0.0)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>3</td>
<td>1 (16.7)</td>
<td>1 (100.0)</td>
<td>19</td>
<td>7 (36.8)</td>
<td>2 (28.6)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>1 (20.0)</td>
<td>1 (100.0)%</td>
<td>2</td>
<td>0 (0.0)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Fourth</td>
<td>3</td>
<td>1 (16.7)</td>
<td>1 (100.0)</td>
<td>7</td>
<td>2 (28.6)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1 (50.0)</td>
<td>0 (0.0)</td>
<td>4</td>
<td>1 (25.0)</td>
<td>1 (100.0)</td>
<td></td>
</tr>
<tr>
<td>Fifth</td>
<td>3</td>
<td>0 (0.0)</td>
<td>–</td>
<td>1</td>
<td>0 (0.0)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0 (0.0)</td>
<td>–</td>
<td>7</td>
<td>2 (28.6)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0 (0.0)</td>
<td>–</td>
<td>4</td>
<td>2 (50.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>34 (34.0)</td>
<td>18 (52.9)</td>
<td>137</td>
<td>49 (35.8)</td>
<td>10 (20.4)%</td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

- *a* Included three sets of triplets;
- *b* included two sets of triplets;
- *c* included one set of triplets;
- *d* all involved twins.

Table III. Order of transfer attempt in relation to the implantation rate per transfer

<table>
<thead>
<tr>
<th>Transfer attempt</th>
<th>Implantation rate per transfer</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First period</td>
<td>Second period</td>
<td>P-value between periods</td>
<td>Entire period</td>
</tr>
<tr>
<td>First</td>
<td>31/142 (21.8)</td>
<td>32/120 (26.7)</td>
<td>NS</td>
<td>63/262 (24.0)</td>
</tr>
<tr>
<td>Second</td>
<td>19/81 (23.5)</td>
<td>9/91 (9.9)</td>
<td>&lt;0.05</td>
<td>28/172 (16.3)</td>
</tr>
<tr>
<td>Third</td>
<td>5/58 (13.2)</td>
<td>9/65 (13.5)</td>
<td>NS</td>
<td>14/103 (13.6)</td>
</tr>
<tr>
<td>Fourth</td>
<td>3/31 (9.7)</td>
<td>4/42 (9.5)</td>
<td>NS</td>
<td>7/73 (9.6)</td>
</tr>
<tr>
<td>Fifth or more</td>
<td>0/26 (0)</td>
<td>5/52 (9.6)</td>
<td>NS</td>
<td>5/78 (6.4)</td>
</tr>
<tr>
<td>Total</td>
<td>58/313 (18.5)</td>
<td>59/370 (15.9)</td>
<td>NS</td>
<td>117/683 (17.1)</td>
</tr>
</tbody>
</table>

*See Table I.*

Significant differences: *a* first versus second, second versus fifth or more, *P* < 0.05; *b* first versus third, first versus fourth, *P* < 0.005; *c* first versus fifth, *P* < 0.0005. NS = not significant.

embryos because of the lower incidence of twin pregnancies (Wølner-Hansen and Rydhstroem, 1998). Multiple pregnancies, which involve greater risks to maternal and neonatal health, should be prevented.

It is indicated that a uniform restriction to transfers of three embryos has a small effect on reducing the rate of multiple pregnancy (Bollen et al., 1991), and the difference in pregnancy rates between transfers of two and three embryos has been reported as statistically non-significant, if good quality embryos are transferred (Tasdemir et al., 1995). Yaron et al. (1997) concluded that, in oocyte donation programmes, the only way to eliminate the risk of triplet pregnancy is by limiting the number of embryos transferred to two. However, if a uniform restriction to transfers of two embryos were enacted, the multiple pregnancy rate would decrease (Staessen et al., 1993; Roest et al., 1997) but the possibility of achieving pregnancy in some patients would be lost. The transfer of two embryos may be practical by the strict selection of patients (Vauthier-Brouzes et al. 1994).

It seems certain that some female patients are predisposed to becoming pregnant by IVF-embryo transfer. However, it is quite difficult to determine case-by-case the number of embryos to be transferred, as routine evaluations of infertility provide us with useful but incomplete information about patients’ fecundity. In particular, measuring the factors which influence implantation, such as the uterine receptivity and the ability of embryos to develop, is apparently beyond our reach at present.

As mentioned above, we do not agree with a uniform restriction on the number of embryos transferred. We therefore established a clear and simple method of monitoring the number of embryos transferred. In the first observation period in which the number of embryos transferred was limited to three, the rate of multiple pregnancy was not reduced, and six sets of triplets were produced. For the second observation period, we restricted the number of embryos transferred in the first embryo transfer attempt to two. As shown in Tables I and II, the results were satisfactory. Though it might be fortunate that no triplets arose in the second observation period, the implantation rate per transfer shown in Table III indicated that the excessive implantations of embryos might be prevented by restricting the number of embryos transferred at least at the first IVF attempt.

Though this study was conducted on non-contemporaneous groups, medical staff involved had been fixed and there had been no major changes in IVF procedures during the observa-
tion period. We therefore think that a potential for bias could be avoided. The only exception was the change of the drugs used for luteal phase support. This change was performed mainly aiming at reducing the frequency in attending a hospital and in receiving painful muscular injections for patients. Though the effect due to the difference is uncertain, we think that the difference had little effect on IVF results, as the implantation rate per unit of transfer was similar in both observation periods.

Several factors influencing the IVF outcomes have been mentioned. First, the quality of the embryo has been identified as a major indicator of the number of embryos to be transferred (Schulman et al., 1993; Austin et al., 1996; Fuji et al., 1996; Hu et al., 1998). The relationship between the quality of the embryo and its ability to give rise to a pregnancy is described in detail (Cummins et al., 1986; Bolton et al., 1989). The absence of developmental potential in embryos, however, may be caused by low genetic competence, which is not measurable by the traditional method of estimating embryo quality by appearance. It was also reported that pregnancy rate was decreased in aged women even though good quality embryos were transferred (Giorgetti et al., 1995). Possible way currently to ensure the high quality of the embryos transferred by a non-invasive method is to perform embryo transfer at the blastocyst stage. Some debates (Alikani and Wiener, 1997; Bavister and Boatman, 1997; Bronson, 1997; Jonge and Wolf, 1997) indicated that this may be the most realistic solution for the reduction of iatrogenic multiple pregnancies. The selection of blastocyst stage embryos, which should have high implantation efficiency, would make the transfer of an excess number of embryos unnecessary to ensure embryo quality.

Second, the patient's age must be considered. It is obvious that increased maternal age causes a decrease in pregnancy and implantation rates (Templeton et al., 1996; Elsner et al., 1997). Navot et al. (1991) described a possible relationship between oocyte quality and the age-related decline in female fertility. It was also reported that, with advancing maternal age, genetic abnormality in oocytes increases and the developmental potential of embryos decreases (Fujino et al., 1996; Lim and Tsakok, 1997). This factor has little relevance for the majority of couples, in which the woman is <40 years old (Senöz et al., 1997). Though Adonakis et al. (1997) reported that pregnancy rate was increased in women aged ≥40 years when at least four embryos were transferred, they often do not have the option to transfer more than two embryos, as they will grow few follicles.

Third, the condition of tubes is also a factor that influences success rates. It has been generally accepted that implantation is remarkably impaired by the presence of hydroosalpex, especially when visible by transvaginal ultrasound (Andersen et al., 1994; Fuji et al., 1997b; Wit et al., 1998).

Fourth, the outcome of any previous IVF treatments is also a relevant factor in the determining how many embryos to implant (Templeton et al., 1996). The best chance of achieving a live birth is on the first attempt; the live birth rates of subsequent attempts gradually decrease.

Templeton et al. (1996) identified by logistic regression the factors that affect the outcome of IVF treatment. Their regression model by maternal age, duration of infertility, previous pregnancy, and previous unsuccessful IVF attempts, can clearly give us a predicted probability of a live birth, and is very useful to inform patients of their fecundity. However, it is still difficult to judge how many embryos should be transferred, because their model took no account of the number of embryos transferred. Coetsier and Dhont (1998) made a trial calculation on the basis of their IVF results, and referred to the selection criteria for the population in which one embryo was to be transferred. Their following criteria might possibly reduce multiple pregnancy rate with a minimal effect on overall pregnancy rate: age <36 years; first to third embryo transfer attempts; ≥3 embryos available; good quality embryos. Consequently, since many factors have to be considered in evaluating patients' fecundity, restricting the maximum number of embryos to be transferred in each patient is complex and may easily be misjudged by the person who makes the decision. We think that the method for making this decision should be simple and clear to minimize errors of judgement by health professionals.

One problem with our simplified method might be that it is not easy to evaluate the results of IVF–embryo transfers previously performed in other hospitals, as there are great differences among hospitals in IVF results. If the patient previously received embryo transfer at hospitals which have reliable clinical IVF–embryo transfer results, we did not consider the first embryo transfer in our hospital as the first treatment, and we determined the maximum number of embryos to be transferred should be three.

Iatrogenic multiple pregnancies, especially pregnancies involving multiples above triplets, have to be reduced in any way possible. We believe that our method of determining the number of embryos transferred by regulating the number per embryo transfer may be one method of reducing the rate of multiple pregnancies without eliminating the possibility of having children for women with low fecundity.

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Received on April 6, 1998; accepted on October 1, 1998.