Transfer of frozen–thawed embryos in artificially prepared cycles with and without prior gonadotrophin-releasing hormone agonist suppression: a prospective randomized study

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Transfer of frozen–thawed embryos is usually carried out in a natural cycle or in a programmed cycle in which the endometrium is exogenously stimulated following down-regulation of the hypophysis. To analyse the possibility that the programmed cycle for embryo transfer can still be hormonally manipulated without the use of gonadotrophin-releasing hormone agonist (GnRHa) we have conducted a prospective randomized study that compared the outcome of frozen–thawed embryo transfer cycles using micronized 17β-oestradiol and micronized progesterone preparations with and without the concomitant use of GnRHa. One hundred and six patients were randomly divided into two groups. In group A (53 patients) 4 mg/day of micronized 17β-oestradiol was initiated following down-regulation of hypophysis. In group B (53 patients) oestrogen stimulation started on day 1 of the cycle without prior pituitary down-regulation using a dose of 6 mg/day for 7 days. In both groups, micronized progesterone in a dose of 900 mg/day was administered vaginally after at least 12 days of oestrogen stimulation. Embryo transfer embryo transfer took place 48–72 h thereafter according to the cryopreserved embryonic stage. Overall, none of the patients had any follicular development and only one cycle in group B served embryonic stage. Overall, none of the patients had any follicular development and only one cycle in group B had to be cancelled because of premature progesterone secretion. The two groups did not differ in age (31 ± 5.6 and 31 ± 5.0 years), number of embryos transferred per patient (3.4 ± 1.2 and 3.3 ± 1.0), and day of progesterone initiation (15 ± 2.2 and 15 ± 1.9 for groups A and B respectively). The endometrial thickness on the day of progesterone initiation was comparable in both groups (11 ± 1.6 and 10 ± 1.6 mm for groups A and B respectively). Similarly, the pregnancy rate per embryo transfer and implantation rate in group A (26.4% and 9.5%) were comparable to those of group B (21.1% and 9%). These results indicate that programmed cycles can be successfully applied by administering a high dose of micronized 17β-oestradiol starting on day 1 of the cycle. Compared to GnRHa programmed cycles, this approach is simpler, more convenient for both the patient and medical staff, and results in a similar success rate at a lower cost.

Key words: artificial cycle/cryopreservation/embryo/endometrial preparation/GnRH agonist/thawing

Introduction

The use of gonadotrophin-releasing hormone agonists (GnRHa) in in-vitro fertilization (IVF) has eliminated the concern of premature ovulation and extended the length of ovarian stimulation. As a result, a higher number of follicles may be recruited, yielding an increased number of oocytes during a single retrieval. Concomitantly, improvements in embryo culture conditions have resulted in an increased number of embryos available for transfer. In order to decrease the multiple pregnancy rate, many IVF programmes limit the transfer to only two or three embryos (Roest et al., 1997). These developments lead to an increased number of extra embryos that are not used for immediate transfer. The ability to cryopreserve these supernumerary embryos and obtain viable pregnancies after thawing and embryo transfer served as an excellent solution to the problem of surplus embryos and increased the overall pregnancy rate from one oocyte retrieval attempt (Trounson and Mohr, 1983; Trounson, 1990). Cryopreservation of embryos has additional advantages as it may be employed in cases in which ovarian hyperstimulation syndrome is anticipated to occur and fresh embryo transfer is not recommended (Navot et al., 1992). In addition, although the pregnancy rate for frozen–thawed embryos is somewhat lower than that for fresh embryo transfer, the expenses for such embryo transfer cycles are far below the total cost incurred by a repeat fresh cycle. Taken together, these considerations emphasize the role of cryopreservation as an indispensable tool in any assisted fertilization programme.

The transfer of frozen–thawed embryos can be performed in a natural ovulatory cycle or in a hormonally manipulated cycle with a comparable pregnancy rate of 15–20% per embryo transfer (Pattinson et al., 1992; Society for Assisted Reproductive Technology, 1995, 1996). Although embryo transfer in a natural cycle is less expensive, a transfer in an artificial cycle is more applicable in patients who are not menstruating regularly such as those with polycystic ovary disease (PCOD) and in cases where a better control or a flexible transfer is indicated. When a hormonally modulated embryo transfer cycle is scheduled for patients with functioning ovaries, an artificial endometrial preparation is carried out after down-regulation of the pituitary with GnRHa. After pituitary desensitization, the patient is given an oestrogen preparation to mimic the proliferative phase followed by concomitant administration of progesterone to imitate the luteal phase (Younis et al., 1996). An adequate proliferative phase before progesterone initiation is considered to be that of 12–20 days (Younis et al., 1992) and embryo transfer usually takes place 48–72 h after progesterone initiation, depending
on the stage in which the embryos were cryopreserved after fertilization. Various replacement protocols using different routes of oestrogen and progesterone delivery have been successfully used without a consensus as to the most effective regimen (Younis et al., 1996). Oestradiol may be administered as an oral preparation, skin patches, vaginal preparation and recently as s.c. micronized implants (Younis et al., 1996; Ben-Nun and Shulman, 1997). Progesterone supplementation may be administered either as i.m. progesterone in oil injections or as micronized progesterone tablets given by either mouth or vaginally.

Omitting GnRHα from programmed embryo transfer cycles while using oestrogen and progesterone alone has the obvious advantages of simplicity and decreased expenses. However, a prospective randomized study to evaluate the effect of omitting GnRHα and simplifying the frozen–thawed embryo transfer cycles was not previously reported.

This work was undertaken in order to prospectively compare the results of frozen–thawed embryo transfer cycles with prior GnRHα administration to those in which the agonist was omitted.

Materials and methods

Subjects

The study population consisted of 106 patients with functioning ovaries who had previously undergone IVF with embryo cryopreservation and were candidates for frozen–thawed embryo transfer.

Embryos were cryopreserved 48 or 72 h after ovum retrieval at the 2–8-cell stage according to a protocol previously described using 1,2-propanediol and sucrose solution in phosphate-buffered saline (PBS) as cryoprotectants (Testart et al., 1986). Embryos of all patients were scored before freezing according to the blastomere symmetry, and the relative proportion of anucleate fragments presence in the zona pellucida. Four types of embryos were defined: type A embryos had regular blastomeres with no fragmentation, type B embryos had irregular and asymmetrical blastomeres but no fragmentation, type C embryos had either regular or irregular blastomeres with ≤20% of their content filled with anucleated fragments, and type D embryos had 20–50% of their volume filled with anucleated fragments. Embryos with >50% of their surface filled with anucleated fragments were not eligible for freezing or for transfer and were discarded.

Thawing was performed by the transfer of cryotubes into a warm bath at a temperature of 35°C. After complete thawing, embryos were taken through a series of decreasing concentrations of propanediol and sucrose (Testart et al., 1986), washed three times in PBS and employed a solid-phase, chemiluminescent enzyme immunoassay (Immulite; Diagnostic Products Corp., Los Angeles, CA, USA). The interassay and intra-assay coefficients of variation were 9.3 and 8.6%

Statistical analysis

The two groups were compared as to their clinical profile, laboratory results and outcome of embryo transfer cycles. Data are presented as mean ± SD. The results were analysed using Student’s t-test and χ²-test. P < 0.05 was considered statistically significant. Statistical analyses were performed using a standard computer program of Microsoft Excel 5 for Windows. Based upon initial estimates, it was calculated that for the sample size of 106 subjects, only a difference >20% in pregnancy rate per embryo transfer would be considered to be of statistical significance with a power of >80%.
Table I. Comparison between the group pretreated with gonadotrophin-releasing hormone agonist (group A) prior to artificial endometrial preparation and the group having the endometrium prepared for embryo transfer without the use of the agonist (group B)

<table>
<thead>
<tr>
<th>Endometrial preparation for embryo transfer</th>
<th>Pretreated by Decapeptyl Group A (53 cycles)</th>
<th>Without Decapeptyl Group B (52 cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31 ± 5.6</td>
<td>31 ± 5.0</td>
</tr>
<tr>
<td>Embryos frozen</td>
<td>211</td>
<td>193</td>
</tr>
<tr>
<td>Grade A (%)</td>
<td>25 (12)</td>
<td>21 (11)</td>
</tr>
<tr>
<td>Grade B (%)</td>
<td>111 (52)</td>
<td>99 (51)</td>
</tr>
<tr>
<td>Grade C (%)</td>
<td>52 (25)</td>
<td>53 (28)</td>
</tr>
<tr>
<td>Grade D (%)</td>
<td>23 (11)</td>
<td>20 (10)</td>
</tr>
<tr>
<td>Embryos survived (%)</td>
<td>179 (84.8)</td>
<td>167 (86.5)</td>
</tr>
<tr>
<td>No. of embryos transferred per patient</td>
<td>3.4 ± 1.2</td>
<td>3.3 ± 1.0</td>
</tr>
<tr>
<td>Day of progesterone initiation</td>
<td>15 ± 2.2</td>
<td>15 ± 1.9</td>
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<tr>
<td>Endometrial thickness (mm)</td>
<td></td>
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</tr>
<tr>
<td>Day 1</td>
<td>5.0 ± 2.0</td>
<td>5.0 ± 2.0</td>
</tr>
<tr>
<td>Day 7</td>
<td>9.2 ± 2.1</td>
<td>8.9 ± 1.9</td>
</tr>
<tr>
<td>Day of progesterone initiation</td>
<td>11.0 ± 1.6</td>
<td>10.0 ± 1.6</td>
</tr>
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</table>

Data presented as mean ± SD. There were no significant differences between groups A and B.

Results

Group A had 53 frozen–thawed embryo transfer cycles with prior pituitary desensitization. None of the embryo transfer cycles had to be cancelled for premature luteinization and progesterone secretion. In group B, one cycle was cancelled for premature progesterone secretion detected only on day 14 of the cycle, a day in which exogenous progesterone was scheduled to be initiated. None of the remaining 52 cycles showed follicular development or premature progesterone secretion. This was true even when the artificial follicular phase was extended as long as 18 days.

Patients’ profiles were similar in the two groups (Table I). The proportion of embryos surviving the freezing–thawing procedure was similar (84.8 and 86.5% for groups A and B, respectively). In both groups, almost half of the frozen embryos were of grade B; thus, 64 and 62% of the cryopreserved embryos were of excellent or good quality for groups A and B respectively. Similarly, the two groups did not differ in the number of embryos transferred (3.4 ± 1.2 and 3.3 ± 1.0 for groups A and B respectively).

As a rule, we attempted to initiate exogenous progesterone administration following 14 days of oestrogen exposure. However, for patients’ preferences, because of weekends and, most importantly, because the endometrium did not reach the desired thickness we had either to shorten the follicular phase down to 12 days or to extend it up to 18 days in both groups. The day of progesterone initiation was similar in both groups and averaged 15 days. Endometrial thickness increased gradually, in both groups, from a mean of 5 mm to reach a maximal width on day of progesterone initiation, 11 ± 1.6 mm and 10 ± 1.6 mm in groups A and B, respectively (Table I). This increase in thickness was paralleled by an increase in serum oestradiol concentrations (Figure 1A).

Figure 1 demonstrates oestradiol and progesterone serum concentrations throughout the treatment cycles. For any day analysed, oestradiol concentrations were significantly lower in group A than those of group B ($P < 0.003$, Figure 1A). On day 1, this difference represents the efficient down-regulation achieved by the agonist in group A, while for other days it reflects the higher dose of Estrofem used in group B. In both groups, oestradiol concentrations increased after day 7. This represents an increase in Estrofem dose given to some of the patients in order to reach an appropriate oestradiol concentration and endometrial thickness as had been previously determined in the study design. Serum progesterone concentrations (Figure 1B) were similar throughout the cycle in both groups and low until progesterone supplementation was added.

Table II represents the clinical outcome of the treatment cycles in both groups. A similar pregnancy rate (26.4% and 21.1%) and implantation rate (9.5% and 9%) was achieved in groups A and B respectively. Although not statistically significant, a higher early abortion rate (28%, 4/14) occurred in group A as compared to group B. In group A there were additionally three late abortions resulting in an ongoing pregnancy rate of 13.2%. This rate, although somewhat lower, did not differ significantly from that of group B (21.1%).

Discussion

This is the first prospective randomized study that compares the results of frozen–thawed embryo transfer cycles in an artificially prepared endometrium with and without prior use of GnRHa for pituitary suppression in women with functioning ovaries.

The results of this study indicate that transfer of frozen–thawed embryos can be successfully performed in a programmed cycle without the use of GnRHa prior to endometrial preparation. A similar pregnancy rate was achieved for artificially prepared cycles in which the agonist was applied (26.4%) and in simplified cycles without its use (21.1%).

The success of embryo transfer in agonal patients undergoing egg donation led to the use of the same principles of endometrial preparation in patients with functioning ovaries, by employing GnRHa prior to endometrial hormonal stimulation (Schmidt et al., 1989; Muasher et al., 1991). A similar success rate was achieved after the transfer of cryopreserved–thawed embryos by using either natural cycles or hormonally controlled endometrial preparation with prior GnRHa suppression (Muasher et al., 1991; Sathanandan et al., 1991; Al-Shawaf et al., 1992; Queenan et al., 1994). The use of GnRHa has several advantages: it enables an easier synchronization between embryo and endometrial development, especially in patients who are anovulatory or have irregular cycles (Schmidt et al., 1989; Muasher et al., 1991); it decreases the need for repetitive sonographic and endocrine monitoring required for embryo transfer in a natural cycle; it decreases the cancellation rate and enables a better control of embryo transfer timing because the transfer can be modified according to the patients’ or staff preferences. However, the use of GnRHa has some disadvantages: the treatment cycle is prolonged; the patients may suffer from menopausal symptoms resulting from the
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Figure 1. Hormonal profile of serum oestradiol (A) and progesterone (B) concentrations throughout the cycles in patients undergoing programmed cycles for frozen–thawed embryo transfer with (group A) and without (group B) the concomitant use of gonadotrophin-releasing hormone agonist. P = progesterone; ET = embryo transfer.

Table II. Clinical outcome of frozen–thawed embryo transfer cycles in group A and group B

<table>
<thead>
<tr>
<th>Endometrial preparation for embryo transfer</th>
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<tr>
<td>Pretreated by Decapeptyl</td>
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<tr>
<td>Group A (53 cycles)</td>
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<td></td>
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<tr>
<td>No. of pregnancies</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Ongoing</td>
<td>7  (3 twins)</td>
<td>11  (2 twins, 1 triplet)</td>
</tr>
<tr>
<td>Early abortion</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Late abortion</td>
<td>3  (Rh incompatibility, Cx incompetence, malformed fetus)</td>
<td>0</td>
</tr>
<tr>
<td>Pregnancy/embryo transfer (%)</td>
<td>26.4</td>
<td>21.1</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>9.5</td>
<td>9.0</td>
</tr>
<tr>
<td>Ongoing pregnancy rate (%)</td>
<td>13.2</td>
<td>21.1</td>
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P value

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<th>P value</th>
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<tr>
<td>NS</td>
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NS, not significant.

hypo-oestrogenic state induced by the agonist; and GnRHa administration is sometimes associated with a paradoxical stimulation of the ovaries resulting in cyst formation (Smitz et al., 1992; Keltz et al., 1995).

Applying a programmed transfer cycle without the use of GnRHa seems attractive as it has the obvious advantage of further simplifying and lowering the cost of a treatment cycle. Previous studies have reported on the successful use of exogenous oestrogen and progesterone without prior ovarian suppression by GnRHα for controlled preparation of the endometrium in women who have functioning ovaries and were candidates for frozen–thawed embryo transfer (de Ziegler et al., 1991; Pattinson et al., 1992; Queenan et al., 1997) or were included in an oocyte donation programme (Remohı ´ et al., 1993; Michalas et al., 1996; Ben-Nun and Shulman, 1997). However, none of these studies prospectively compared the results of a programmed cycle with and without prior use of GnRHα for frozen–thawed embryo transfer. Our study has addressed this question and indicated that programmed cycles for frozen–thawed embryo transfer without prior use of GnRHα can be successfully applied. Our results are in agreement with those of Pattinson et al. (1992) who in a prospective non-randomized study, reported on a comparable pregnancy rate of 14.4% and 20% for frozen–thawed embryo transfer cycles performed in natural or exogenously prepared cycles without the use of GnRHα, respectively. Our observations also corroborate those of Queenan et al. (1997) who have recently reported a 29.9% pregnancy rate and a 16.4% ongoing pregnancy rate for the transfer of cryopreserved-thawed pre-embryos in a programmed cycle without prior use of GnRHα.

Several methods for oestrogen preparation administration were used and included skin patches, oral tablets and s.c. implants given in a fixed (Pattinson et al., 1992; Michalas et al., 1996; Ben-Nun and Shulman, 1997) or gradually increased dose (de Ziegler et al., 1991; Remohı ´ et al., 1993; Queenan et al., 1997). We chose to employ a fixed oestrogen dose for endometrial stimulation as it is simple and much more convenient for the patients, and had previously been applied successfully in programmed cycles with pituitary suppression (Younis et al., 1996).

It seems that administration of oestrogen in a non-GnRHα-suppressed cycle should take place at the very beginning of the period. Initiating oestrogen treatment from day three of the cycle was associated with a higher spontaneous LH secretion and postovulatory changes observed on day 15 (Remohı ´ et al., 1993) with cycles in which oestrogen was given from day 1 (de Ziegler et al., 1991). In addition, giving a fixed dose of 2 mg/day of micronized 17β-oestradiol from...
day 2 to day 5, Pattinson et al. (1992) reported a cancellation rate of 7.4% for programmed cycles in which GnRHa was not used. This relatively high cancellation rate may be associated with either postponing oestrogen initiation within the cycle (Remohi et al., 1993) or with the low oestradiol dose used throughout the cycle which may not be sufficient for pituitary suppression in all cases. Considering these observations and to avoid the undesirable event of premature LH and possible progesterone secretion whenever GnRHa was not used (group B), we administered an initially high dose of micronized 17β-oestradiol (6 mg/day) beginning on day 1 of the cycle. Applying this protocol we had only a 2% (1/53) cancellation rate which was similar to that reported by Queenan et al. (1997) who also started oestrogen stimulation on day 1.

Initiating treatment from day 1 with persistent exposure to oestradiol enabled adequate endometrial proliferation and is associated with sustained pituitary suppression and prevention of LH surge (Nezhat et al., 1980; Goodman et al., 1981; Yaron et al., 1995). Accordingly, when a high dose of 17β-oestradiol was used in group B, pituitary function was suppressed and we were able to extend the proliferative phase up to 18 days without observing breakthrough bleeding, follicular development, or cyst formation. In only one case was premature progesterone secretion detected which led to cancellation of the cycle. One can argue that high oestradiol concentrations >740 pmol/l (200 pg/ml) for >50 h may be associated with an abrupt LH secretion (Speroff et al., 1994). Although this event might have occurred, we did not observe any significant increase in progesterone concentrations during the artificial follicular phase of group B. These findings are in agreement with those of de Ziegler et al. (1991) who reported that no follicular growth was observed in six women with functioning oocytes treated by exogenous oestrogen for controlled preparation of the endometrium. Moreover, despite increased plasma LH to preovulatory surge concentrations, plasma progesterone concentrations remained at low follicular concentrations resulting in adequate histological appearance of the endometrium on day 20 (de Ziegler et al., 1991).

Manipulating the proliferative phase up to 18 days should be appropriate if one aims to apply our protocol for frozen–thawed embryo transfer programmed cycles. However, for oocyte donation programmes in which a longer proliferative phase is sometimes indicated, our protocol for programmed cycles without prior GnRHa suppression has still to be tested. Encouraging results were reported by several authors who, in an oocyte donation programme, were able to manipulate the proliferative phase for 27 days and up to 60 days by using either oestradiol valerate per os (Michalas et al., 1996) or s.c. oestradiol implants (Ben-Nun and Shulman, 1997).

Several studies of endometrial thickness have shown a correlation between thickness and cycle outcome and that there is a minimum threshold of 5–8 mm for successful implantation (Al-Shawaf et al., 1993; Dickey et al., 1993; Abdalla et al., 1994). Our study demonstrated that this threshold can be reached by using 4 and 6 mg/day of micronized 17β-oestradiol in groups A and B, respectively. Increasing the oestrogen dose in group B over 6 mg/day after day 7 resulted in an increased oestradiol plasma concentration (Figure 1A), but gained no further advantage in terms of endometrial thickness on the day of progesterone initiation (Table I). This observation led to the conclusion that endometrial thickness is determined mainly by the interval length during which the endometrium is exposed to oestrogen rather than oestradiol plasma concentrations.

Taken together, it may be suggested that using 6 mg/day of micronized 17β-oestradiol would be sufficient to induce pituitary suppression, preventing follicular development on the one hand and inducing an optimal endometrial thickness for implantation on the other.

The two study groups were similar in their profile including age, embryo grading before cryopreservation and number of embryos transferred per patient (Table I). Similarly, both groups demonstrated a similar pregnancy rate per embryo transfer of 26.4 and 21.1%, and similar implantation rate of 9.5 and 9.0% (groups A and B, respectively, Table II). The ongoing pregnancy rate for group B (21.1%) although somewhat higher than in group A (13.2%) did not differ significantly. The comparable outcome for the treatment cycles associated with a similar patients profile in both groups, indicates that programmed cycles for frozen–thawed embryo transfer without prior use of GnRHa can be successfully applied by administering a high dose of 17β-oestradiol starting on day one of the cycle. As compared to GnRHa programmed cycles, it is much simpler, more convenient for both the patient and the medical staff, and results in a similar success rate but with lower cost.

References
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