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The window for embryo transfer in oocyte donation cycles depends on the duration of progesterone therapy

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In 192 oocyte donation cycles performed between January 1993 and July 1996, we examined the width of 'the window for embryo transfer' using standard hormonal replacement methods. All transfers were performed within 48 h of insemination. We varied the day of embryo transfer with regard to the initiation of progesterone therapy and, thus, the duration of endometrial exposure to progesterone and analysed the resulting pregnancy rates. Patients were divided into five groups (I–V) and embryo transfers were performed 2, 3, 4, 5 or 6 days following initiation of progesterone therapy. The number of pregnancies per transfer cycle achieved in groups I–V were 0 (0%), 3 (12%), 16 (40%), 29 (48.3%), and 10 (20.4%) respectively. The increased pregnancy rate in group III in comparison to group II is statistically significant ($P < 0.03$). Furthermore, the pregnancy rate in group IV (5 days of progesterone administration before embryo transfer) was significantly higher than in group V (6 days of progesterone administration before embryo transfer; $P < 0.005$). We also noted that, when embryos were transferred 4 or 5 days after initiation of progesterone therapy, the pregnancy rates were not significantly different between menopausal and cycling recipients (50% vs 43.7%). Our results indicate that the window for embryo transfer is dependent on duration of treatment with progesterone; it begins approximately 48 h after starting progesterone administration and lasts for approximately 4 days. The optimum period for transferring embryos at the 4- to 8-cell stage corresponds to cycle days 18 and 19. Transfers performed on the 17th and 20th days of the cycle can result in successful implantation, although the rates of implantation are highest when transfers are done on days 18 and 19.

Key words: embryo transfer window/implantation/menopausal recipients/oocyte donation/replacement therapy

Introduction

Many protocols of hormonal replacement therapy for endometrial preparation have been proposed for recipients in oocyte donation programmes (Medical Research International, 1991). One of the greatest challenges of oocyte donation programmes is to improve implantation rates. The endometrium appears to have a temporally restricted capacity to sustain embryo implantation. Thus, it is crucial to synchronize accurately the developmental stage of donated embryos with the developmental maturation of recipient's endometrium (Navot *et al.*, 1986; Rosenwaks, 1987).

The term 'uterine receptivity' was introduced to define the short period during which the uterus allows nidation to occur (Psychoyos, 1976; Yoshinaga, 1988). This short 'phase of receptivity' (implantation window) is preceded by a pre-receptive (neutral) state followed by a refractory, non-receptive phase (Psychoyos and Martel, 1985). The pre-receptive phase and the receptive phase combined constitute 'the window for embryo transfer' (Navot *et al.*, 1991). Embryos transferred outside of this chronological window generally do not result in successful pregnancies (Rosenwaks, 1987; Mandelbaum *et al.*, 1994). The implantation window depends on the developmental stage of the conceptus at the time of transfer, and the maturity of the endometrium and, thus, the specific hormone replacement regimen used.

The elapsed time between the initiation of progesterone administration and embryo transfer appears to be important. Thus, the aim of this study was to assess the width of the window for embryo transfer using standard hormonal replacement regimens, while varying the day of embryo transfer and, thus, the duration of exposure of the endometrium to progesterone.

Materials and methods

A total of 192 oocyte donation cycles were retrospectively studied in 98 cycling and 45 non-cycling women between January 1993 and July 1996. All cases had anonymous donors. All donors were healthy and had normal ovulatory function. The mean ages of the cycling and the non-cycling recipients were 39.8 ± 1.6 and 42 ± 2.2 years, respectively. Endometrial preparation was achieved using a sequential regimen of oestrogen and progesterone designed to mimic a natural 28-day cycle. Oestradiol valerate (Cyclacur®; Schering, Berlin, Germany) was used to induce endometrial maturation in recipients at a dose of 2 mg per day for the first 4 days, 4 mg daily on days 5–8, and 6 mg per day starting from the 9th day of the cycle until progesterone administration was initiated. Progesterone was administered in 100 mg vaginal suppositories t.i.d. or 100 mg i.m. until a β-human chorionic gonadotrophin (HCG) test was performed to detect pregnancy 14 days following embryo transfer. When progesterone therapy was initiated, the dose of oestradiol was decreased to 4 mg daily for the remainder of the cycle. A gonadotrophin releasing hormone analogue (GnRH-a)(Daronda®; Abbottlab-Hellas, Athens, Greece, 1 mg s.c.)

was administered to cycling recipients beginning on the 21st day of the previous cycle to achieve pituitary suppression. Artificial cycles were initiated when serum oestradiol was 40 pg/ml or less.

Cycles were divided into five groups (I–V) according to the number of days of progesterone administration in relation to embryo transfer. Groups I–V had embryos transferred 2, 3, 4, 5, and 6 days, respectively, following the initiation of progesterone administration. Protocols for ovarian stimulation, oocyte retrieval, oocyte and embryo culture, and embryo transfer were the same for all groups.

The stimulation protocol for all donors consisted of GnRH-a analogues (Daronda®, 1 mg s.c.) and human menopausal gonadotrophin (HMG) (Pergonal® 75 IU; Ares-Serono, Rome, Italy; or Humegon® 75 IU; Organon Hellas, Athens, Greece). GnRH-a was started on day 1 of the cycle. HMG was initiated (3 ampoules/day) when serum oestradiol concentration was 40 pg/ml or less. Seven days after starting HMG, serum oestradiol was determined and follicular growth was evaluated ultrasonographically. HCG was administered when three or more follicles measured 19–21 mm in diameter and oestradiol concentration was at least 750 pg/ml. Ultrasound directed transvaginal follicle aspiration was performed 36 h later under intravenous sedation and local anaesthesia. Oocytes were inseminated 4–6 h after retrieval. To assess embryo quality, all embryos were observed at ×400 magnification under an inverted stage microscope equipped with a temperature controlled stage and graded at the time of embryo transfer using the grading system described by Veeck (1988). All embryo transfers were performed 44–48 h after oocyte retrieval; each transferred embryo consisted of at least four blastomeres.

Pregnancies were confirmed by radioimmunoassay for serum β-HCG levels and transvaginal ultrasound. Clinical pregnancy was defined as a distinct intrauterine gestational sac seen on transvaginal ultrasound. Statistical analysis of the data was performed using the χ^2 test and analysis of variance.

Results

The data for the five groups of recipients are partitioned according to number of days of progesterone treatment prior to embryo transfer. Table I shows the number of oocytes retrieved from the donors, as well as the number and the quality of embryos transferred to the recipients. The numbers of clinical pregnancies and abortions are presented according to the number of days of progesterone administration prior to embryo transfer. Pregnancy rates were significantly related to the number of days of progesterone administration prior to transfer ($P < 0.0001$). No pregnancy occurred when embryo transfer was performed on the second day of progesterone administration (group I). In the remaining groups, pregnancies were obtained. The highest pregnancy rates occurred in groups III and IV, although there was a statistically significant difference between these groups. The pregnancy rate in group II was significantly lower than in group III ($P < 0.03$), and the pregnancy rate in group V was significantly lower than in group IV ($P < 0.005$). No significant differences in first trimester spontaneous abortion rates were found among any of the five groups ($P = 0.18$) (Table I). All five groups had comparable mean number of oocytes retrieved ($P > 0.05$), mean number of embryos transferred ($P > 0.05$), and mean embryo quality score ($P > 0.05$; Table I).

The effect of the day of embryo transfer in relation to the initiation of progesterone replacement on embryo implantation rates is presented in Table II. A total of 610 embryos were transferred in 192 cycles representing a mean of 3.48 embryos per cycle. Sixty-six intrauterine sacs resulted yielding an overall implantation rate of 7.8% per embryo. The implantation rate per embryo transferred varied significantly in relation to the number of days of progesterone administration ($P < 0.0001$). The highest implantation rates were observed in groups III and IV. The implantation rate in group II was significantly lower than in group III ($P < 0.01$), and the implantation rate in group V was lower than that in group IV ($P < 0.005$; Table II). The ages of donors (mean ± SD) were 27.7 ± 4.6 (group I), 28.0 ± 4.6 (group II), 27.4 ± 4.1 (group III), 26.9 ± 3.2 (group IV), and 27.1 ± 3.5 (group V). The ages of donors were comparable across all groups ($P > 0.05$). The ages of recipients (mean ± SD) were 41.3 ± 3.5 (group I), 41.0 ± 1.8 (group II), 42.3 ± 2.2 (group III), 41.0 ± 2.5 (group IV), and 41.3 ± 2.3 (group V). There was no significant difference in ages of recipients across the groups ($P > 0.05$). The average duration of oestrogen administration before embryo transfer was comparable in all five groups.

We also compared 64 cycling and 34 menopausal recipients from groups III and IV to assess the effect of the status of ovarian function on the outcome of oocyte donation. Pregnancy rates in menopausal women and in cycling women were 17/34 (50%) and 28/64 (43.8%), respectively. This difference did not reach statistical significance ($P > 0.05$). There was also no significant difference in the abortion rates between these groups with the pregnancy loss of 5/17 (29.4%) among menopausal recipients and 9/28 (32.1%) among cycling recipients. Furthermore, the outcome was not influenced by the presence or absence of endometriosis in the cycling women ($P > 0.05$).

Discussion

Normal endometrial development is crucial for successful implantation. Lutjen *et al.* (1984) were the first to report a successful pregnancy in an agonadal woman whose endometrium had been primed with exogenous steroids. Many different regimens mimicking physiological replacement of oestradiol and progesterone are capable of supporting implantation (Navot *et al.*, 1986; Rosenwaks, 1987; Sauer *et al.*, 1990; de Ziegler *et al.*, 1992). Pregnancy rates in donor in-vitro fertilization (IVF) programmes, though generally based on small series, have been higher than those reported for IVF or gamete intra-Fallopian transfer (GIFT) cycles (Rosenwaks, 1987; de Ziegler and Frydman, 1990).

One of the intriguing issues raised by oocyte donation programmes pertains to the 'temporal window' of endometrial receptivity which is conducive to embryo implantation. The endometrial receptive period seems to last 24–48 h in humans (Martel *et al.*, 1981, 1989). According to Psychoyos and Prapas (1987), the human endometrium has a neutral phase which precedes the receptive phase. Embryo transfer during the neutral phase may result in implantation. The neutral and receptive phases combined comprise the window of embryo transfer. Although the window of endometrial receptivity lasts 24–36 h in rats (Psychoyos and Prapas, 1987), it may last up to 3 days in monkeys (Hodgen, 1983).

Table I. Effect of the day of embryo transfer in relation to initiation of progesterone administration on clinical outcomes

	Groups				
	I (n = 18)	II (n = 25)	III (n = 40)	IV (n = 60)	V (n = 49)
Oocytes retrieved (no.)[c]	11 ± 6.4[a]	10.5 ± 4.7[a]	10 ± 4.8[a]	11.1 ± 3[a]	10.7 ± 3.6[a]
Embryos transferred (no.)[c]	3.2 ± 1.1[a]	3.3 ± 1.1[a]	3.4. ± 1[a]	3.5 ± 1[a]	3.7 ± 0.9[a]
Quality of embryos transferred[c]	3.1 ± 1.5[a]	3.2 ± 1.2[a]	3.0 ± 1.5[a]	3.3 ± 1.5[a]	3.1 ± 1.2[a]
Clinical pregnancies	0	3 (12%)[a]	16 (40%)[b]	29 (48.3%)[b]	10 (20.4%)[a]
Clinical abortions	0	1 (33.3%)[a]	4 (25%)[a]	10 (34.4%)[a]	4 (40%)[a]

[a,b]Means and proportions with no superscripts in common are significantly different ($P < 0.05$).
[c]Results are presented as means ± SD.

Table II. Effect of the day of embryo transfer in relation to initiation of progesterone administration on implantation rates

	Groups				
	I	II	III	IV	V
Number of embryos	58	86	135	214	177
Number of intrauterine sacs	0	3	19	34	10
Implantation rate per embryo	0	3.5%[a]	14.1%[b]	15.8%[b]	5.6%[a]

[a,b]Values with no superscripts in common are significantly different ($P < 0.01$).

Synchrony between the developmental stage of the embryo and endometrial maturation is a crucial factor for implantation in IVF cycles. Normally, a 4- to 8-cell stage embryo coincides with endometrial development 3–4 days after the LH surge *in vivo* (Navot *et al.*, 1986). Navot *et al.* (1986) presented a series of eight women with ovarian failure who participated in a donor IVF programme. Embryo transfer was performed on days 16–21 after starting progesterone administration on day 15. They observed that days 18 and 19 (i.e. after 4 or 5 days of progesterone administration) were the optimum period for embryo transfer in their programme. Rosenwaks (1987) found that the best period for embryo transfer was from day 17–19 of the recipient's cycle, after starting progesterone administration on recipient cycle day 15. In contrast, a prospective study by Navot *et al.* (1991) involving 60 recipients participating in their oocyte donation programme indicated that embryo transfers performed on days 15, 16, 17, 18, 19 or 20, or 1, 2, 3, 4, 5, and 6 days after starting progesterone administration respectively, had no significant effect on pregnancy and abortion rates. Since the above reports evaluated a relatively small number of patients and yielded conflicting results, this study re-evaluated the role of the timing of embryo transfer on a greater number of cycles.

Our results show that of the 18 cycles in which embryo transfer was performed on day 16 or after 2 days of progesterone administration none resulted in pregnancy. According to our findings, a minimum of 48 h of progesterone administration is required for successful implantation. The highest pregnancy rates were obtained when embryo transfer was performed on the 4th or the 5th day after initiation of progesterone administration. By the 6th day of progesterone administration (cycle day 20) pregnancy rates declined significantly. Corresponding differences were observed in the implantation rate per embryo transferred. The present findings cannot be attributed to differences in the number or the quality of the embryos, or to the variability in the age or ovulatory status of the donors. Furthermore, it should be stressed that in all groups, embryo transfers were performed at a constant time interval, namely, within 44–48 h after retrieval.

There is evidence suggesting that the appearance of pinopods represents a marker for identification of the endometrial receptivity window in normal cycling and menopausal women undergoing hormone replacement for embryo transfer. The pinopods begin to appear on the 6th day or later following the initiation of progesterone administration (Psychoyos and Martel, 1990; Nikas *et al.*, 1995). The formation of uterine pinopods is strictly dependent on progesterone, whereas oestrogen induces their regression. It has been suggested that the appearance of endometrial pinopods signifies the end of the window for embryo transfer (Psychoyos and Martel, 1990) unless embryos are transferred at the blastocyst stage (Bolton, 1994); in donor cycles the embryos are usually transferred at earlier stages of development. Recently, Nikas *et al.* tested for pinopods during a mock cycle to identify the optimal timing for embryo transfer (Nikas *et al.*, 1997). At present, the significance of detection of pinopods still remains to be confirmed. A search for reliable markers of endometrial receptivity continues and involves various proteins participating in the process of implantation such as interleukin-1, proteases digesting the basement membrane and integrins anchoring the embryo (Simon *et al.*, 1995; Bischof and Campana, 1996).

Bergh and Navot (1992) suggested that within the assumed

window of endometrial receptivity the human embryo could be the principal determinant in the timing of nidation and that the endometrium has no apparent impact on the timing of implantation. They based this suggestion on the detection of HCG (the first embryonic signal) which occurs between days 19 and 23 of the cycle at a mean embryonic age of 7 days. In contrast, other studies have suggested that implantation may occur much later. Naaktgeboren *et al.* (1986) used close monitoring of serum hormones to detect implantations delayed by 2–3 weeks after ovulation induction. Edwards (1994) also reported IVF patients with delayed implantation where the rising concentrations of HCG typical of a normal pregnancy occurred 4–5 days later than expected. Use of the antiprogestin RU 486 in rats has shown that the window of implantation can be postponed or advanced according to the progesterone treatment (Sarantis *et al.*, 1988). Comparable studies with other antiprogestins lilopristone (ZK 98.734) or onapristone (ZK 98.299) were performed on rabbits (Beier *et al.*, 1994). However, once the endometrium has entered the receptive period, it is impossible to prevent progression of maturation to the refractory period (Psychoyos and Prapas, 1987; Sarantis *et al.*, 1988). Our results support the concept of embryo-endometrial cross-talk since different lengths of endometrial exposure to progesterone, before same stage embryos were transferred, resulted in significantly different implantation and pregnancy rates.

Borini *et al.* (1995) found that pregnancy and implantation rates in cycling women undergoing oocyte donation were improved after long-term down-regulation with GnRH analogues. Psychoyos (1993) supported this idea, suggesting that long-term down-regulation in the recipients eliminates endogenous factors that may interfere with implantation and that the endometrium treated with oestrogen–progestin replacement therapy is better prepared than in routine IVF cycles. Our findings are not in agreement with the above observations since we noted that the pregnancy and abortion rates were comparable in menopausal and cycling recipients. Although this discrepancy could be related to endometriosis in cycling women, we found no correlation between endometriosis in the recipient and the pregnancy and abortion rate.

This study has demonstrated that the window for embryo transfer is dependent on the duration of progesterone exposure. The window for embryo transfer begins 48 h after starting progesterone administration, and lasts for at least 4 days. The best time for transferring embryos at the 4- to 8-cell stages coincides with cycle days 18 and 19. Transfer on the 17th or 20th day of the cycle can result in successful implantation, but the success rate is significantly lower than that which occurs as a result of transfer on cycle day 18 or 19.

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