

Article

Artificial versus stimulated cycles for endometrial preparation prior to frozen–thawed embryo transfer



Kristen Page Wright graduated from the Pennsylvania State University with a Bachelor's degree in Biology and a minor in Women's Studies. She then attended the University of Vermont College of Medicine. She is currently completing her final year of residency in Obstetrics and Gynecology at Women & Infants' Hospital of Rhode Island and Brown Medical School. She plans to return to the University of Vermont in July to complete a fellowship in Reproductive Endocrinology and Infertility.

Dr Kristen Page Wright

Kristen Page Wright^{1,3}, Juliette Guibert², Sherry Weitzen¹, Celine Davy², Patricia Fauque², Francois Olivennes²

¹Department of Obstetrics and Gynecology, Women and Infants' Hospital, Brown Medical School, Providence, RI;

²Unit of Reproductive Medicine, Department of Obstetric and Gynecology II, Cochin Hospital, Paris, France

³Correspondence: e-mail: kristen.wright@vtmednet.org

Abstract

The objective of this study was to compare the implantation rate, pregnancy rate and endometrial thickness of frozen–thawed embryo transfers using endometrial preparation with either an artificial cycle or stimulated cycle. This was a prospective randomized trial at a single academic IVF centre. Seventy-seven patients undergoing artificial cycles received oral oestradiol; patients with endometrium < 7 mm on day 9–10 were switched to vaginal oestradiol. Eighty-six patients undergoing stimulated cycles received recombinant FSH followed by human gonadotrophin hormone injection. Vaginal progesterone was begun 2 or 3 days prior to embryo transfer. There was no difference in implantation rate (8.5% versus 7.3%), pregnancy rate (16% versus 13%), cancellation rate (both 23%) or endometrium thickness (8.7 ± 1.1 mm versus 8.7 ± 1.0 mm) between artificial and stimulated cycles. Stimulated cycles had a higher incidence of thin endometrium (27% versus 5%, $P < 0.01$). In artificial cycles, patients switched to vaginal oestradiol had improved pregnancy rate (31%) versus patients who received oral oestradiol alone (13%) ($P = 0.05$). It is concluded that artificial and stimulated cycles produce comparable pregnancy rates, implantation rates, cancellation rates and endometrial thickness, although stimulated cycles have a higher incidence of thin endometrium. Vaginal oestradiol supplementation improved implantation rates.

Keywords: cryopreservation, endometrial receptivity, endometrial thickness, implantation, vaginal oestradiol

Introduction

Improvements in IVF have led to an increased number of embryos not available for immediate transfer due to concern about multiple pregnancies. Cryopreservation of supernumerary embryos with subsequent frozen–thawed embryo transfer (FET) is an excellent solution to surplus embryos and has become a common practice in infertility centres. FET increases the pregnancy rate per oocyte retrieval and is a cost-effective practice (Ubaldi *et al.*, 2004).

Timing transfers of frozen–thawed embryos can be accomplished using the natural menstrual cycle in women with regular

cycles. However, timing transfers in women with irregular or long cycles becomes problematic. To overcome this problem, the endometrium can be prepared using either mild ovarian stimulation or artificial priming with oestrogen. Manipulated cycles allow increased flexibility in timing of transfers, as they afford better control over the cycle. Many IVF centres today use either artificial or stimulated cycles for all frozen–thawed embryo transfers because of their convenience. Embryo quality, implantation rate and overall pregnancy rates have been shown to be similar in natural versus stimulated cycles (Imthurn *et al.*, 1996; Ziebe *et al.*, 2004).

Early protocols for priming the endometrium for implantation of frozen–thawed embryos used mild ovarian stimulation with low-dose gonadotrophins or suppression of ovarian function using a gonadotrophin-releasing hormone agonist (GnRHa) followed by replacement of oestradiol and progesterone (Lornage *et al.*, 1990; Schmidt *et al.*, 1989). Cycles in which oestradiol and progesterone are administered following a GnRHa offered low cancellation rates and implantation rates comparable to natural or stimulated cycles (Sathanandan *et al.*, 1991). However, this approach is complicated, as it requires administration of three different hormones and intensive patient monitoring. Moreover, patients are exposed to the adverse effect of the GnRHa desensitization, with menopause-type symptoms. A successful method for preparing the endometrium using oestradiol and progesterone without prior GnRHa desensitization was later described (Lelaidier *et al.*, 1992). A prospective randomized study by Simon *et al.* found that endometrial preparation with an artificial cycle using oestradiol and progesterone alone is simpler, less expensive and more convenient for patients and providers, with similar success rates, when compared with GnRHa-primed cycles (Simon *et al.*, 1998). Other studies have confirmed no improvement in pregnancy rates with GnRHa-primed cycles versus artificial cycles or stimulated cycles. (Sathanandan *et al.*, 1991; Benshushan *et al.*, 1993; Yee *et al.*, 1995).

Artificial cycles without GnRHa desensitization have widely replaced stimulated cycles; however, as far as is known, there are no prior studies that prospectively compare pregnancy and implantation rates between these two protocols. Bals-Pratsch *et al.* (1999) compared a prospective case series of artificial cycles with a retrospective cases series of stimulated cycles and noted a significant improvement in convenience of artificial cycles due to decreased ultrasound and endocrine monitoring (Bals-Pratsch *et al.*, 1999). Interestingly, these authors also noted a trend towards increased pregnancy rates with artificial cycles; however, this study was not randomized. This study presents a prospective randomized trial that compares these two treatments with respect to implantation rate, pregnancy rate, cycle cancellation rate and endometrial thickness at a single academic IVF centre.

Materials and methods

Study population

After internal review board approval and informed consent, 165 women undergoing 199 cycles at a single academic IVF centre were randomized to receive either the artificial cycle or the stimulated cycle prior to frozen–thawed embryo transfer. There were 88 patients undergoing 99 cycles in the artificial cycle group and 87 patients undergoing 100 cycles in the stimulated cycle group. All women had functioning ovaries and both normo-ovulatory and oligo-ovulatory women were included. All patients had previously undergone IVF with embryo cryopreservation and were candidates for FET. This included women who had failed a fresh cycle, those who had cryopreservation of all embryos in a fresh cycle, and those who conceived previously and were undergoing frozen embryo transfer to achieve a second pregnancy. **Table 1** depicts the patient characteristics of the two groups, which were similar with respect to age, day 3 FSH concentration and percentage undergoing intracytoplasmic sperm injection (ICSI). Recent data for FSH concentration was unavailable for 26 patients in the artificial cycles and for 19

patients in the stimulated cycles because their oocyte retrievals occurred prior to 2002.

Embryos were cryopreserved 48–72 h after ovum retrieval at the 2–8 cell stage according to a previously published protocol using 1,2-propanediol and sucrose solution in phosphate-buffered saline (PBS) (Frydman and Testart, 1986). Prior to freezing, the embryos of all patients were scored with respect to symmetry and proportion of anucleated fragmentation. Embryos received one of four scores based on their percentage of fragmentation: A: 0%; B: <20%; C: 20–50%; D: >50%. C and D embryos and embryos with no cell division between days 2 and 3 were not eligible for freezing or transfer and were discarded. Embryos were frozen in liquid nitrogen at –196°C; thawing was accomplished rapidly by removing the embryos from the freezing apparatus. Once thawed, the embryos were washed with decreasing concentrations of propanediol and sucrose, then washed three times with PBS and finally placed in fresh, equilibrated warmed culture medium (Zenke and Chetkowski, 2004). Embryos with < 50% cell necrosis after the thawing process were considered suitable for transfer. Embryos that survived the thaw were placed in culture medium until transfer was performed.

Thawed embryo transfer was performed 48–72 h after beginning vaginal progesterone in both the artificial and stimulated cycles. Transfers were performed by one of three physicians who have similar pregnancy rates per embryo transfer as evaluated by the institution every 6 months. Transfers were not performed using ultrasound guidance.

Endometrial preparation

Patients randomized to the artificial cycle began taking oral 17- β oestradiol (Provames®, Laboratoires Aventis, France) 2 mg twice per day beginning on day 1 of their menstrual cycle. Ultrasound and hormone assays were performed on day 9–10 and if the endometrial thickness was >7 mm, the patient began vaginal micronized progesterone (Utrogestan®, Besins-Iscovesco Pharmaceuticals, Paris, France). Progesterone was dosed 100 mg in the morning and 200 mg in the evening and oral oestradiol was continued. If the endometrial thickness was <7 mm on day 9–10, patients were switched to vaginal oestradiol (Provames, Laboratoires Aventis) 2 mg once per day. In those patients with thin endometria, ultrasound and hormone surveillance was continued until the endometrium was >7 mm, at which point they began vaginal progesterone. If the endometrial thickness was not >7 mm by day 20 then the cycle was cancelled due to inadequate endometrial response despite the switch to the vaginal route.

Table 1. Patient characteristics.

	Artificial cycle	Stimulated cycle
Age in years (mean \pm SD)	34 \pm 4.00	34 \pm 4.25
FSH (mean \pm SD)	7 \pm 1.5	6 \pm 1.3
ICSI (%)	62	60

ICSI = intracytoplasmic sperm injection.

Oestradiol and progesterone were continued following embryo transfer and serum β -human chorionic gonadotrophin (β HCG) was measured 12 days post-transfer. Hormone replacement was continued if the pregnancy test was positive, due to absence of the corpus luteum, and pregnant patients were followed with serial ultrasounds to determine viability. In viable pregnancies, hormone replacement was discontinued at 8 weeks' gestation, when placental autonomy was assured. Hormone replacement was discontinued immediately in patients whose pregnancy test was negative.

Patients randomized to the stimulated protocol received FSH injections 150 IU (FSH; Gonaf F[®], Serono, Geneva, Switzerland; or Puregon[®], Organon, Serfontaine, France) on days 6, 8 and 10 of their menstrual cycle. Ultrasound and hormone assays were performed on day 9–10 and FSH was continued in patients with thin endometrium if follicle size was <15 mm and no LH surge or premature progesterone rise was present. Ultrasound and hormonal surveillance was continued until the endometrial thickness was >7 mm and a follicle reached 16–20 mm, at which time HCG (Ovitrelle[®], Serono, Boulogne, France) was administered. Cycles with endometrial thickness significantly <7 mm by day 20 were cancelled due to poor endometrial response. Vaginal progesterone (Utrogestan[®]) 100 mg in the morning and 200 mg in the evening was begun on the day following HCG administration. Thawed embryo transfer was performed after beginning progesterone supplementation 48 h in advance for embryos frozen on day 2 and 72 h in advance for embryos frozen on day 3. Vaginal progesterone was continued until 12 days post-transfer when a pregnancy test was performed. Progesterone was continued until 8 weeks' gestation in viable pregnancies and was discontinued immediately in patients with negative pregnancy tests.

The outcome values in this study were implantation rate, pregnancy rate and endometrial thickness. Implantation rate was calculated as the number of viable first trimester pregnancies divided by the number of embryos transferred. Successful pregnancy was defined as an appropriately rising β HCG; the only patients with a positive β HCG who were excluded were those patients with biochemical pregnancies. Endometrial thickness was measured on day 9–10 of the cycle for all patients by two trained operators. Patients with a thin endometrium in the artificial cycle group began vaginal oestradiol on day 9–10; for these patients the endometrial thickness was re-measured 3–5 days later. The maximal endometrial thickness that the patients attained prior to FET is reported.

Statistical analysis

Data for age, FSH concentration, number of embryos replaced and endometrial thickness is presented as mean \pm SD. Outcome measures of pregnancy rate and cycle cancellation rate between artificial and stimulated cycles were compared using the chi-squared test with $P \leq 0.05$ considered statistically significant. Endometrial thickness between groups was evaluated using comparison of means (t -test for two groups, analysis of variance for three groups) or non-parametric test (Wilcoxon rank sum for two groups, Kruskal–Wallis for three groups) with $P \leq 0.05$ considered statistically significant.

Results

Of the 199 cycles randomized, eight were excluded due to missing data on the endometrium, 23 were excluded due to failure of the embryos to survive thawing and five patients receiving donated oocytes were excluded because of medical conditions that could affect implantation (such as chemotherapy or radiotherapy). The breakdown of patient exclusion from analysis of implantation rate is depicted in **Table 2**. Overall, 77 artificial cycles and 86 stimulated cycles were included in the final analysis of implantation and pregnancy rate. Data on endometrial thickness were missing in eight patients, resulting in analysis of 93 artificial cycles and 98 stimulated cycles. Patients whose embryos did not survive the thaw and patients lost to follow-up in the analysis of endometrial thickness were included. The number of embryos replaced per transfer was 1.77 ± 0.57 in the artificial cycles and 1.66 ± 0.56 in the stimulated cycles. The overall cycle cancellation rate for any reason was 23% for both artificial and stimulated cycles. This included cycles that were cancelled due to poor endometrial response, patients with premature ovulation in the stimulated group and patient-initiated cancellations.

Endometrial thickness for artificial cycles was 8.7 ± 1.1 mm (range 7.3–13.0 mm) and for stimulated cycles was 8.7 ± 1.0 mm (range 5.5–12.4 mm). In the artificial cycles, endometrial thickness was 8.8 ± 1.1 mm for patients receiving oral oestradiol alone and 8.5 ± 0.3 mm for patients receiving oral and vaginal oestradiol. The difference in endometrial thickness between each of these groups was not significant. However, the proportion of patients with endometrial thickness ≤ 8 mm was significantly different: 5/93 (5%) in artificial cycles compared with 26/98 (27%) in stimulated cycles ($P < 0.01$). Within the artificial cycles, the oral oestradiol group had 5/75 (7%), and the vaginal oestradiol group had 0/17 (0%) of patients with endometrial thickness ≤ 8 mm.

Table 3 depicts the pregnancy rates and number of patients in each of the described protocols. The overall pregnancy rate for artificial cycles was 16% versus 13% for stimulated cycles; this difference was not statistically significant. The overall implantation rate for artificial cycles was 8.5% versus 7.3% for stimulated cycles. The post-hoc power to detect a difference between the artificial cycle and the stimulated cycle is 33%. A future study assuming an 18% increase in pregnancy rate by using artificial compared with stimulated cycle would require 138 patients in each group, assuming 80% power and $\alpha = 0.05$.

In the artificial cycles, 26% of patients required vaginal oestradiol supplementation due to endometrium <7 mm. The overall implantation and pregnancy rates in patients receiving oral oestradiol alone and in patients switched to vaginal oestradiol were 6% versus 18% and 11% versus 31% respectively. Patients receiving vaginal oestradiol had significantly improved pregnancy rates compared with patients receiving oral oestradiol alone ($P = 0.05$). The post-hoc power for this comparison is 38%. A three-way comparison of pregnancy rate between artificial cycle–oral oestradiol alone versus artificial cycle–oral oestradiol plus vaginal oestradiol supplementation versus stimulated cycles was not significantly different.

Table 2. Patients excluded from analysis of implantation rate.

	Artificial cycle	Stimulated cycle
Enrolled in study	99	100
Excluded – lost to follow-up	3	5
Excluded – egg donation recipients ^a	5	0
Excluded – embryos did not survive thawing	14	9
Included in final analysis	77	86

^aExcluded due to medical conditions that could affect implantation such as chemotherapy or radiotherapy.

Table 3. Pregnancy outcome for each protocol.

Protocol	No. pregnant patients	No. non-pregnant patients	Total	Pregnancy	P-value
Artificial cycle	12	65	77	16	–
Oral oestrogen	7	54	61	11	–
Oral and vaginal oestrogen	5	11	16	31	0.05
Stimulated cycle	11	77	86	13	NS

NS = not significant.

Pregnancy rates were then re-calculated restricting the entire sample to only those with endometrial thickness ≥ 8 mm because thin endometria are associated with decreased implantation rates (Noyes *et al.*, 2001; Kovacs *et al.*, 2003; Zenke and Chetkowski, 2004). When comparing artificial and stimulated cycles, there was no statistically significant difference in pregnancy rate and a three-way comparison of oral oestradiol alone, oral oestradiol switched to vaginal oestradiol and stimulated cycles also showed no statistically significant difference. Within the artificial cycles, the trend persisted towards an improvement in implantation rate in patients receiving vaginal oestradiol (31%) versus those receiving oral oestradiol alone (13%); however, these results were not statistically significant when considering only patients with endometrial thickness ≥ 8 mm.

Discussion

The results demonstrate similar implantation and pregnancy rates between artificial and stimulated cycles for endometrial preparation prior to transfer of frozen-thawed embryos. As far as is known, this is the first prospective randomized study confirming the equivalent pregnancy rate of artificial cycles. A significant improvement in implantation rates using artificial cycles, as observed in the case series by Bals-Pratsch *et al.* (Bals-Pratsch *et al.*, 1999), was not noted. The findings are consistent with prior studies demonstrating equivalent pregnancy rates

between GnRHa primed artificial cycles and stimulated cycles (Benshushan *et al.*, 1993).

Stimulated and artificial cycles are both widely used prior to FET. Stimulated cycles require precise timing of HCG administration, and subsequent FET, to avoid natural ovulation and endometrium outside the implantation window at the time of transfer. Conversely, in artificial cycles, oestradiol may be added at any time that is convenient once oestradiol stimulation has resulted in an endometrial thickness >7 mm. Timing transfers of frozen-thawed embryos is much more convenient for patients and providers using artificial cycles because natural ovulation is avoided and the day of transfer can be chosen by selecting the start date of progesterone. Many infertility centres are already taking advantage of the flexibility in the artificial cycle and the results of this study validate this practice. The only drawback is the prolonged dual hormonal treatment required in case of pregnancy in patients undergoing artificial cycles.

An increased number of patients with thin endometrium were noted in the stimulated cycles; however, this discrepancy did not translate into a difference in implantation rate. Given that thin endometria are associated with decreased implantation rates, it is possible that a difference would become apparent with a larger sample size (Noyes *et al.*, 2001; Kovacs *et al.*, 2003; Zenke and Chetkowski, 2004). Larger studies would be needed to address this issue.

In artificial cycles, a statistically significant improvement in implantation rates was noted in patients who required vaginal oestradiol supplementation due to a thin endometrium, compared with patients who developed adequate endometrial thickness on oral oestradiol alone. Previous studies have demonstrated increased serum oestradiol concentrations and an improvement in endometrial thickness and uterine perfusion in patients receiving vaginal oestradiol compared with oral oestradiol (Fanchin *et al.*, 2001; Tourgeman *et al.*, 2001a). The vaginal route of administration also results in higher endometrial concentrations of oestradiol, compared with oral or endogenous oestradiol (Tourgeman *et al.*, 2001b). However, prior data have suggested no effect of vaginal oestradiol supplementation on pregnancy rates in patients with a thin endometrium and in-vitro studies have indicated that high oestrogen concentrations in the endometrium are deleterious to implantation (Valbuena *et al.*, 2001; Check *et al.*, 2004). The improved implantation rate in the subset of poor-responding patients was thus unexpected. It is important to note that endometrial unresponsiveness to oestrogen can be indicative of a pathological state, as there is a case report of atypical endometrial hyperplasia presenting in this manner (Marikinti, 2006).

The improved implantation rates in patients receiving vaginal oestradiol is not explained by an obvious discrepancy in endometrial preparation, given that the measured endometrial thickness was similar in each of the protocols. Furthermore, this trend persisted when the data was re-analysed to include only patients with endometrium ≥ 8 mm, suggesting that the difference in implantation rate is not due to a discrepancy in the number of patients with a thin endometrium between the samples. Serum oestradiol concentrations and infertility diagnoses were not recorded during this study and this may have biased the pregnancy rate. The number of patients requiring vaginal oestradiol supplementation is small and it is possible that the results are due to sampling error. However, it is also possible that the prolonged exposure to oestradiol stimulation, or the vaginal route of administration, is advantageous in priming the endometrium for implantation. Further studies are needed to investigate this issue.

In summary, similar implantation rates, pregnancy rates and cycle cancellation rates in artificial and stimulated cycles for endometrial preparation prior to implantation of frozen-thawed embryos have been demonstrated in this initial study. Mean endometrial thickness was also equivalent between the two protocols; however, stimulated cycles had a significantly higher incidence of thin endometrium. The most interesting observation is the significant improvement in pregnancy rate in patients requiring vaginal oestradiol supplementation compared with the patients who responded well to oral oestradiol. Prospective studies comparing these two routes of administration are indicated.

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