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ARTICLE

# Increasing vaginal progesterone gel supplementation after frozen–thawed embryo transfer significantly increases the delivery rate

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**Abstract** The aim of this study was to evaluate the reproductive outcome in patients receiving frozen–thawed embryo transfer before and after doubling of the vaginal progesterone gel supplementation. The study was a retrospective study performed in The Fertility Clinic, Skive Regional Hospital, Denmark. A total of 346 infertility patients with oligoamenorrhoea undergoing frozen–thawed embryo transfer after priming with oestradiol and vaginal progesterone gel were included. The vaginal progesterone dose was changed from 90 mg (Crinone) once a day to twice a day and the reproductive outcome during the two periods was compared. The pregnancy rate increased significantly after doubling of the progesterone dose (26.7% (90 mg) versus 38.4% (180 mg); $P = 0.021$). Moreover, the early pregnancy loss rate decreased significantly (67.4% versus 43.7%, respectively; $P = 0.014$), which significantly increased the delivery rate (8.7% versus 20.5%, respectively; $P = 0.002$). Doubling of the vaginal progesterone gel supplementation during frozen–thawed embryo transfer cycles decreased the early pregnancy loss rate, resulting in a significantly higher delivery rate.



**KEYWORDS:** early pregnancy loss, frozen–thawed embryo transfer, luteal-phase support, progesterone, substituted cycle

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## Introduction

Frozen–thawed embryo transfer (FET) has become increasingly important in modern assisted reproduction treatment, which partly reflects the increased use of single-embryo transfer. During the last decade, cryopreservation techniques have received considerable interest, whereas priming and preparation of the endometrium prior to and after embryo transfer has received limited attention.

A recent Cochrane review, analysing a number of different protocols used prior to FET, concluded that there is currently no evidence to support the superiority of any one protocol over the other (Ghobara and Vandekerckhove, 2008).

Preparation of the endometrium with oestradiol and progesterone before FET is commonly used due to convenience of scheduling and it is also the preferred protocol for women with irregular cycles and for oocyte donation cycles. Priming with oestradiol and progesterone prepares the endometrium for implantation, transforming the uterine glands to the secretory state, which is necessary for implantation to take place (Bourgain et al., 1990; Fatemi et al., 2007).

Previously, Hull et al. (1982) reported a mean mid-luteal progesterone serum concentration in spontaneous conception cycles of 12.8 ng/ml (41 nmol/l) with a range of 8.5–16.7 ng/ml (28–53 nmol/l). In contrast, in non-conception cycles there was a wider range of the progesterone serum concentration. This could indicate that the optimal condition for a successful implantation is within a relatively narrow serum progesterone window. Progesterone is not only important at the time of implantation, but also during early pregnancy; thus, it has previously been reported that from 5–8 weeks of gestation the chance of an ongoing pregnancy is significantly higher with progesterone concentrations higher than 10 ng/ml (31 nmol/l) (Lin and Liu, 1995). Earlier studies confirm this, as removal of the corpus luteum before 7 weeks of gestation resulted in miscarriage, whereas no negative impact of a removal was seen after 7 weeks (Csapo et al., 1972).

While oestradiol is usually administered orally, different routes of progesterone administration are used. It was previously shown that although intramuscular progesterone administration leads to a higher serum concentration, the vaginal route results in a higher endometrial concentration (Miles et al., 1994). Vaginal application is commonly administered either in a tablet form or as a gel and a previous meta-analysis did not find any difference in the clinical outcome between the two modes of administration (Polyzos et al., 2010). However, clinical evidence supports the choice of vaginal progesterone administration compared with intramuscular in terms of effectiveness and side effects (Fatemi, 2009).

There is no evidence from fresh or frozen embryo transfer cycles to suggest an 'optimal luteal' serum progesterone concentration to secure a successful implantation and early pregnancy; moreover, mid-luteal phase concentrations do not necessarily reflect endometrial maturation concentrations (Batista et al., 1993). This study retrospectively analysed the impact of an increase in the vaginal progesterone dose among patients receiving FET in this study unit.

From 2006, vaginal progesterone gel, once daily (90 mg) was used in the unit for luteal-phase support during FET, according to the recommendations by the manufacturer. However, from 2007 the daily progesterone gel dose administration was doubled (180 mg) due to a high number of early pregnancy losses. This retrospective analysis evaluated the change in practice by analysing the cumulative ongoing pregnancy and delivery rates among patients receiving FET with either one or two daily vaginal progesterone doses during a 5-year period (2006–2011).

## Materials and methods

Data derive from The Fertility Clinic, Skive Regional Hospital during the period from 2006 to 2011. All women included had either amenorrhoea or a cycle length exceeding 34 days and received FET after endometrial preparation with oestradiol and progesterone. From the second day of bleeding, oestradiol 6 mg was administered daily (Estrofem; Novo Nordisk Scandinavia, Copenhagen, Denmark). After 12 days, an ultrasound was preformed evaluating the endometrium and the ovaries. When the endometrial thickness was $>$7 mm and had a triple line pattern and no follicular development was seen, treatment with vaginal progesterone 90 mg (Crinone; Merck Serono, Hellerup, Denmark) commenced. If the endometrium was $<$7 mm, the oral oestradiol treatment continued or the patient started co-treatment with transdermal oestradiol (Evorel; Janssen, Birkeroed, Denmark). From January 2006 until November 2007, 90 mg vaginal progesterone (Crinone) was applied once daily and after November 2007 vaginal progesterone (Crinone) was applied twice daily. The progesterone gel was administered in the morning and in the late afternoon. The endometrium and embryos were synchronized; thus, day-3 embryos were thawed and transferred on day 4 of progesterone treatment. Embryos were frozen according to the slow-freezing protocol and thawed using a standard protocol as described elsewhere (Elder and Dale, 2010; Lassalle et al., 1985). The embryos were frozen on the second or third day. Embryos suitable for freezing required a minimum of 4 or 6 blastomeres, respectively, and $<$20% fragmentation (Ziebe et al., 1997). To be eligible for transfer after thawing, at least 50% of the blastomeres should have survived. A maximum of two embryos was transferred.

In the event of a pregnancy the oestradiol and progesterone treatment continued until the end of 10 weeks of gestation in both groups.

During the study period, assisted hatching was performed with acidified Tyrode's solution for all women older than 38 years of age, for patients who had more than two previous unsuccessful fresh embryo transfers and for embryos with a thick zona pellucida. Oocyte donation cycles were not included in the analysis. A pregnancy test was preformed 12 days after embryo transfer and a serum β-human chorionic gonadotrophin $>$10 IU/l was considered positive. A biochemical pregnancy was defined as a positive human chorionic gonadotrophin but no visible gestational sac. The overall early pregnancy loss rate was calculated as

**Table 1** Embryos and reproductive outcome during the two periods analysed.

| *Characteristics* | *January 2006–October 2007 (Crinone once daily)* | *November 2007–December 2010 (Crinone twice daily)* | *P-value* |
|---|---|---|---|
| No. of patients | 161 | 185 | |
| Patient age (years) | 31.8 ± 4.4 | 31.6 ± 4.4 | NS |
| Body mass index (kg/m$^2$) | 24.2 ± 3.7 | 24.2 ± 3.2 | NS |
| Secondary diagnosis | | | |
| Male infertility (%) | 39.2 | 30.9 | |
| Tubal factor (%) | 18.4 | 14.9 | |
| Others (%) | 3.2 | 6.2 | |
| Embryos thawed | 2.48 ± 1.25 | 2.68 ± 1.47 | NS |
| Embryos transferred per cycle | 1.64 ± 0.48 | 1.63 ± 0.48 | NS |
| Transfer of ≥1 top-quality embryo (7.0)/FET[a] | 123/161 | 131/185 | NS |
| Transfer of ≥1 top-quality embryo (7.0 + 7.1)/FET[b] | 153/161 | 168/185 | NS |
| ICSI embryos/embryos transferred | 163/264 (61.7) | 168/302 (55.6) | NS |
| AH embryos/embryos transferred | 43/264 (16.3) | 59/302 (19.5) | NS |
| Implantation rate | 20/264 (7.6) | 61/302 (20.2) | 0.0001 |
| Pregnancy rate | 43/161 (26.7) | 71/185 (38.4)[c] | 0.021 |
| Delivery rate | 14/161 (8.7) | 38/185 (20.5) | 0.002 |
| Early pregnancy loss rate | 29/43 (67.4) | 31/71 (43.7) | 0.014 |
| Biochemical pregnancy rate | 25/43 (58.1) | 16/71 (22.5) | 0.0001 |

AH = assisted hatching; FET = frozen–thawed embryo transfer; ICSI = intracytoplasmic sperm injection.
[a]7.0; all blastomeres survived the thawing process.
[b]7.1; >75% of the blastomeres survived the thawing process.
[c]Pregnancies included one extrauterine pregnancy and one abortion after 12 weeks of gestation.

the sum of biochemical pregnancies and abortions before 12 weeks of gestation.

Each woman contributed to the analysis only once and in the case of more than one cycle the first cycle was included.

Institutional Review Board approval was not required for the present study owing to its retrospective nature and the fact that the study data completely excluded the identification of subjects. All patients had given written authorization at the time of treatment for the future use of their clinical data.

## Statistical methods

For all dichotomous parameters, chi-squared or Fisher's Exact tests were was adopted as appropriate. Continuous outcomes were analysed, using either independent T-test or Mann–Whitney *U*-test based on the normality of the distribution. The level of significance for all outcomes was set at 0.05. All analyses were performed using the Statistical Package for Social Sciences version 20.0 (SPSS, USA).

## Results

This analysis included 346 FET cycles in which 161 patients were treated with vaginal progesterone gel once daily and 185 patients twice daily. No differences were seen between the two groups as regards body mass index, the use of IVF or intracytoplasmic sperm injection, age of the patient, the frequency of assisted hatching, numbers of embryos transferred and the embryo quality. All women had oligo- or amenorrhoea and a diagnosis of polycystic ovary syndrome, anovulation or ovulation defect. Some patients had a secondary diagnosis of tubal factor, male infertility and other factors including endometriosis (Table 1). A significant difference was found in pregnancy rates between the two groups (26.7% (once daily) versus 38.4% (twice daily); $P = 0.021$). The delivery rate was 8.7% versus 20.5% ($P = 0.002$) and the early pregnancy loss rate was 67.4% versus 43.7% ($P = 0.014$), respectively. The majority of early pregnancy losses were biochemical pregnancies (58.1% versus 22.5%; $P = 0.0001$).

## Discussion

This study shows a significantly higher ongoing pregnancy rate after doubling of the vaginal progesterone gel supplementation in patients undergoing stimulated FET cycles, concomitantly with a significantly lower early pregnancy loss rate. Collectively, this resulted in a significantly higher delivery rate, highlighting the importance of the vaginal progesterone dose for the establishment of a successful pregnancy after FET.

The results of the present study confirm and expand previous studies. Thus, in a study by Orvieto et al. (2007) doubling of the progesterone dose in patients receiving endometrial priming with oestradiol and progesterone prior

to FET increased the clinical pregnancy rate per transfer from 7.9% to 41.4% and significantly reduced the biochemical pregnancy rate. The study included a total of 114 patients and progesterone was administered either intramuscularly (50 mg versus 100 mg) or vaginally (100 mg versus 200 mg). Moreover, Check et al. (2010) increased the progesterone dose during FET when the vaginal ultrasound examination on the 7th day of progesterone treatment showed either triple-line or an isoechogenic pattern. One hundred and thirty-six patients were quasi randomized to a standard progesterone dose or an increased dose, and a significantly higher delivery rate was reported in the group of patients who had an increase in the daily progesterone dose.

In the current study, no changes were made in laboratory handling, freezing, thawing or embryo transfer procedures during the period analysed. Moreover, there was no significant difference in the pregnancy rates (42.0% versus 39.1%) or ongoing pregnancy rate (39.3% versus 36.5%) after fresh IVF/intracytoplasmic sperm injection before and during the observational period and there was no change in the freezing frequency during the period, indicating that the results were not caused by a change in the population of patients treated.

Furthermore, the two groups were comparable regarding body mass index, infertility diagnosis, numbers of top-quality embryos transferred, the age of the patient and the frequency of assisted hatching. Thus, although being retrospective, the data seem to be valid. Moreover, the data suggest that the viability of an early pregnancy is dependent on a certain threshold of progesterone. The unanswered question regards how high the progesterone concentration should be. Interestingly, the concentration does not seem to be constant in all women as visualized in earlier studies, which showed significant histological endometrial differences in patients treated according to the same protocol (Sudoma et al., 2011).

There is still a lack of evidence regarding the best regimen of FET. This study only included irregularly cycling women, but the substituted regimen is also used in regularly cycling women. A large retrospective study reported a higher pregnancy loss after FET in substituted cycles compared with natural cycles; however, the delivery rate was similar independent of whether the women had a regular or irregular cycles. More randomized controlled trails are needed to clarify the best regimen for FET (Tomas et al., 2012).

Taken together, several studies now collectively suggest that a significantly better reproductive outcome is obtained after FET by administering a higher progesterone dose than previously suggested — in the current study by increasing the vaginal progesterone gel dose to 180 mg daily, thus exceeding the recommended dose of the manufacturer. This simple intervention significantly reduced the early pregnancy loss rate, leading to a significantly higher delivery rate. Future studies should focus on exploring the luteal phases of both stimulated and natural cycle FET in order to optimize the design of currently used protocols. These studies might also have important implications for the outcome of fresh IVF transfer cycles.

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