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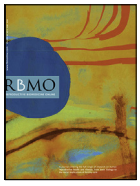

ARTICLE

Can combining vaginal and rectal progesterone achieve the optimum progesterone range required for implantation in the HRT-FET model?

BIOGRAPHY

Dr Alsbjerg obtained her MD in 2001, her specialty degree in obstetrics and gynaecology in 2011, and has undertaken expert education in reproductive medicine (EXPU). She currently works at Fertility Clinic, Skive and is an Associated Professor at Aarhus University, Denmark. Research interests include reproductive endocrinology and protocols for frozen–thawed embryo transfer.

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KEY MESSAGE

Combined rectal and vaginal progesterone in hormone replacement frozen embryo transfer cycles secure high median progesterone levels. Our results show that an upper threshold for serum progesterone may exist and high serum progesterone levels may be associated with decreased ongoing pregnancy rate. Rectally administered progesterone is well accepted by patients.

ABSTRACT

Research question: What is the ongoing pregnancy rate (OPR) in frozen embryo transfer (FET) cycles, using combined rectal and vaginal progesterone in hormonal replacement therapy (HRT)?

Design: A prospective cohort study (n = 277) including 239 HRT-FET cycles with serum progesterone measurements studying combined vaginal (90 mg/12 h) and rectal (90 mg/12 h) progesterone administration and single blastocyst transfer on the sixth day of progesterone administration. A total of 134 responses to questionnaires covering convenience and side-effects were collected.

Results: The median serum progesterone level was 45 nmol/l (range 2–150 nmol/l). Overall positive HCG rate, OPR at week 12 and pregnancy loss rates were 62%, 44% and 29%, respectively. A non-linear relationship between serum progesterone levels and OPR was found. Crude odds ratio for OPR in the high progesterone group (>45 nmol/l) was 0.56 (95% CI 0.32 to 0.98; P = 0.04) compared with the intermediate progesterone group (28–45 nmol/l).

Discomfort after rectal progesterone administration was reported on the embryo transfer day and on the day of pregnancy scan 5 weeks later by a total of 18% (16/87) and 17% (8/47) of patients, respectively. Discomfort related to vaginal administration increased significantly over time and was reported by 18% (16/87) on the day of embryo transfer compared with 45% (21/47) on the day of pregnancy scan (P < 0.002).

Conclusions: Combined rectal and vaginal progesterone in HRT-FET cycles resulted in higher median progesterone levels compared with vaginal administration alone. This study suggests that an upper threshold for serum progesterone exists and that above this concentration serum progesterone levels decrease the OPR. Rectally administered progesterone was well tolerated by patients.

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Declaration: BA received unrestricted research grant and honoraria for lectures from Gedeon Richter and Merck. TH received honoraria for lectures from Merck and Ferring. PH received unrestricted research grants from MSD, Merck, and Ferring as well as honoraria for lectures from MSD, Merck, Gedeon Richter, Theramex, and IBSA. P.H is a co-founder in the Poseidon group.

INTRODUCTION

Progesterone is necessary to ensure normal implantation and pregnancy. In hormone replacement therapy frozen embryo transfer (HRT-FET) cycles, the implanting embryo depends entirely on the exogenous progesterone supplied. Growing evidence supports the notion that reproductive outcomes correlate with serum progesterone levels during the 'luteal phase' in HRT-FET (*Brady et al., 2014*; *Kofinas et al., 2015*; *Yovich et al., 2015*; *Basnayake et al., 2017*; *Labarta et al., 2017*; *Alsbjerg et al., 2018*; *Gaggiotti-Marre et al., 2018*; *Cédrin-Durnerin et al., 2019*).

In an oocyte donation programme in HRT cycles, using vaginally administered micronized progesterone (Utrogestan 400 mg/12 h), *Labarta et al. (2017)* reported significantly lower ongoing pregnancy rates (OPR) in the patients with progesterone lower than 35 nmol/l (<11 ng/ml) on day 5 of vaginal progesterone administration, compared with patients whose progesterone was over 35 nmol/l (<11 ng/ml) (35.3% versus 55.6%, RR 0.64; $P = 0.005$). The same cut-off level was observed by *Alsbjerg et al. (2018)* in a cohort study including 244 HRT-FET cycles treated with 90 mg vaginal progesterone gel (Crinone) three times daily. The unadjusted OPR was significantly lower in the lower than 35 nmol/l progesterone group (38 %) compared with the 35 nmol/l and higher progesterone group (51 %); $P = 0.043$. Furthermore, in a logistic regression analysis adjusted for maternal age, body mass index (BMI), smoking, number of embryos transferred and blastocyst age at cryopreservation (day 5 or 6), a significant decrease in OPR in the low progesterone group was found (OR 0.54, 95% CI 0.32 to 0.91; $P = 0.022$), corresponding to a reduction in the chance of an ongoing pregnancy of 14% (95% CI –26 to –2%; $P = 0.024$) if serum progesterone was less than 35 nmol/l.

In a study by *Yovich et al. (2015)*, 529 artificial frozen-thawed cycles with single blastocyst transfer were evaluated. The investigators reported a non-linear pattern in the association between serum progesterone levels and reproductive outcome: serum progesterone levels below, but also above an optimal progesterone range significantly reduced the chance of a clinical pregnancy with 20% points (64% versus 44%).

The largest and most recently published prospective study included 1155 HRT cycles using vaginal micronized progesterone and reported the optimal threshold for OPR to be 28 nmol/l (8.8 ng/ml) (*Labarta et al. 2019*). Taken together, the association between reproductive outcomes and serum progesterone levels seem reproducible despite different vaginal progesterone preparations and dosing in HRT cycles.

In the studies discussed, 50% of patients using standard vaginal progesterone treatment had low progesterone levels, underlining the fact that insufficient progesterone levels in HRT-FET cycles seem to be an important clinical problem. In a recent work in which vaginal micronized progesterone (Uterogestan) 200 mg three times daily was used for luteal phase support, 50% of the patients had serum progesterone level less than 10.6 ng/ml (33.8 nmol/l) (*Gaggiotti-Marre et al., 2018*).

Exogenous progesterone can be administered in different formulations, e.g. for oral, vaginal, subcutaneous, intramuscular or rectal use. Vaginal progesterone supplementation has clinical advantages, such as a rapid absorption, sustained plasma concentration and local endometrial effects. One standard vaginal luteal phase support regimen (Crinone 90 mg x 2) induces an average steady-state concentration of 37 nmol/ml (11.6 ng/ml) with steady-state concentration being achieved within the first 24 h after treatment initiation (Crinone 8% product monograph). Rectal progesterone administration is not widely used as luteal phase support; however, one study used rectal progesterone pessaries as single agents for luteal phase support during fresh IVF cycles, reporting comparable clinical pregnancy rates between vaginal and rectal administration (*Aghsa et al., 2012*). Likewise, similar serum progesterone levels can be obtained with the same dose of progesterone administered either vaginally or rectally (*Nillius and Johansson, 1971*). The intra-endometrial progesterone level may be substantially different, however, owing to the uterine first pass effect after vaginal administration (*Miles et al., 1994*; *Cicinelli and de Ziegler, 1999*).

To date, no study has explored the efficacy of combined vaginal and rectal administration of progesterone in HRT-FET cycles. Moreover, focus on patient compliance and convenience in HRT-FET cycles using different types of progesterone administrations has been limited. In the present study, the OPR in HRT-FET cycles using a combination of rectal and vaginal progesterone was investigated, along with patient compliance and possible side-effects of the treatment.

MATERIALS AND METHODS

Study design, eligibility criteria and blastocyst scoring

This prospective observational cohort study was conducted in a public fertility centre between March 2018 and April 2019. Inclusion criteria were as follows: autologous embryos frozen on either day 5 or 6, single embryo transfer, BMI less than 34 kg/m^2, and age older than 18 years and younger than 46 years. Exclusion criteria were as follows: oocyte donation cycles, cycles supplemented with intramuscular progesterone, uterine abnormalities and severe comorbidities.

A final cohort of 277 patients was included and serum progesterone measurements were obtained from 239 patients 9 or 11 days after embryo transfer (the study flow is presented in Supplementary Figure 1). Only blastocysts with at least an expansion score grade 3 and score A or B for inner cell mass and trophectoderm, according to the Gardner and Schoolcraft grading system, were vitrified, using the 'Cryotec method' by Masashige Kuwayama (*Gandhi et al., 2017*). A European Society of Human Reproduction and Embryology certified senior embryologist allocated a final score from 1 to 3 to each embryo; 1 being a top-quality embryo, 2 being an intermediate embryo, 3 being a low-quality embryo (*Gardner and Schoolcraft, 1999*).

Hormone replacement therapy protocol

A total of 6 mg oral oestradiol valerate daily from the second day of the menstrual cycle was used for endometrial preparation. An ultrasound examination was carried out after 12–19 days of treatment and when the endometrial thickness was 7 mm or thicker, progesterone supplementation was initiated. All patients received the same micronized progesterone dose, administered vaginally 90 mg/12 h

(Crinone©) (Merck, Søborg, Denmark) and rectally 90 mg/12 h, i.e. four doses daily (referred to in our daily clinic as the '2 + 2' regimen). No additional progesterone supplementation was given to any patient. Single embryo transfer was scheduled for all patients on the sixth day of progesterone administration.

Patients had blood samples drawn 1–8 h after progesterone administration in the morning 9 or 11 days after embryo transfer, corresponding to the day of their pregnancy test. If the pregnancy test was positive the '2 + 2' regimen continued. Once a clinical pregnancy was visualized by ultrasound examination in the seventh week of gestation, rectal administration was discontinued and only vaginal progesterone (90 mg) twice daily and 6 mg oral estradiol was administered until the tenth week of gestation.

Progesterone measurements

Serum progesterone (nmol/l) levels were measured, using automated electrochemiluminescent immunoassays (Cobas® Modular analytics E170) (Roche Diagnostics, Rotkreuz ZG, Switzerland), routinely used for analysis at the Department of Biochemistry, Viborg Regional Hospital, Denmark. All measurements were carried out according to the manufacturer's instructions, using a commercially available radioimmunoassay kit intended for measurements in serum. The limit of detection for progesterone was 0.2 nmol/l. The intra-assay and inter-assay coefficients of variation for progesterone were both below 5%.

Reproductive outcomes

A HCG test was carried out 9 or 11 days after embryo transfer, and the test was considered positive if the serum HCG level was over 10 IU/l. Levels lower than 45 IU/l were repeated after 48 h. 'Ongoing pregnancy' was defined as a viable pregnancy detected by ultrasound examination at the twelfth week of gestation. Biochemical pregnancy was defined as a pregnancy detected by HCG in blood or urine, only, but never confirmed clinically. The pregnancy loss rate was defined as both biochemical as well as clinical pregnancy loss up to week 12 per 100 cycles with a positive HCG test.

Questionnaire

An electronic questionnaire was presented to patients on a tablet on the day of embryo transfer. Patients were asked whether they had experienced administration problems, administration pain, discharge from the rectum or vagina, perianal irritation, discomfort from the vagina or rectum, abdominal discomfort and change in flatulence. The answer options were graded as no, mild, moderate or severe. Where pregnancy was confirmed, the questionnaire was repeated at the time of ultrasound examination at gestational week 7. The survey was conducted between October 2018 and April 2019.

Statistical analysis

Data are presented as percentages for categorical variables, mean and SD for continuous variables and median and range for continuous variables, depending on the criteria for equal variances and normal distribution.

The three progesterone groups were defined using cut-offs at the 10th and 50th percentiles corresponding to 28 and 45 nmol/l, respectively. The cut-off was defined according to a sensitivity analysis of different progesterone levels as a factor variable and their association with ongoing pregnancy. The chi-squared, Kruskal–Wallis test or analysis of variance test was used depending on the outcome and the criteria for equal variances and normal distribution.

A multiple logistic regression model was used to assess the association between the three progesterone level groups and the HCG test result (positive/negative), clinical pregnancy (yes/no) and ongoing pregnancy (yes/no). The model included the following independent variables: maternal age, maternal BMI, blastocyst age and blastocyst quality for estimates of positive HCG, clinical pregnancy and ongoing pregnancy.

Where covariate data (n = 1) or progesterone levels (n = 38) were missing, patients were excluded from the final regression analysis. STATA version 13 was used for all statistical analyses.

Ethics

This study was approved on 28 August 2018 by the local ethical review board committee, carrying the registration number 1-10-72-233-18. ClinicalTrials.gov number: NCT03470610

RESULTS

The present study included 277 patients. Of these, 239 had progesterone measured on the day of pregnancy testing. The cohort had a mean age of 31.9 (±4.8) years and mean BMI of 24.5 (±3.8) kg/m^2. The distribution across primary infertility diagnoses was 31% idiopathic, 29% male infertility, 13% ovulation factor, 6% tubal factor, 2% endometriosis, 13% single and 5% others. A total of 25 out of 239 of patients (10%) had serum progesterone levels lower than 28 nmol/l (8.8 ng/ml) and a total of 118/239 (49%) had progesterone levels higher than 45 nmol/l (14.2 ng/ml). Serum progesterone levels ranged from 2 nmol/l to 150 nmol/l; however, only five patients had serum progesterone levels higher than 99 nmol/l. Baseline characteristics of the study population, broken down into serum progesterone range sub-groups, are presented in TABLE 1.

Reproductive outcomes

Overall, the positive HCG rate was 62% (173/277), the clinical pregnancy rate 47% (131/277), the ongoing pregnancy rate (OPR) 44% (123/277) and the pregnancy loss rate 29% (50/173). Two twin pregnancies occurred: one pregnancy spontaneously reduced to a singleton ongoing pregnancy and the other one ended as a clinical pregnancy loss.

The unadjusted OPR for the three different progesterone groups are presented in FIGURE 1. A non-linear relationship between serum progesterone levels and OPR was found with decreasing chances of OPR with progesterone levels lower than 28 nmol/l (36%) and higher than 45 nmol/l (39%) compared with the optimal progesterone group (28–45 nmol/l) which had an ongoing pregnancy rate of 53%; however, this did not reach statistical significance (P = 0.08). The crude OR for ongoing pregnancy in the high progesterone group (P > 45 nmol/l) was 0.56 (95% CI 0.32 to 0.98; P = 0.04) compared with the optimal progesterone group (28–45 nmol/l) (TABLE 2).

A logistic regression analysis, adjusting for maternal age, maternal BMI, blastocyst quality and blastocyst age, showed an odds ratio for ongoing pregnancy of 0.48 (0.18 to 1.24; P = 0.13) in the group of patients with progesterone level less than 28 nmol/l and a significant OR of 0.53 (0.30 to 0.97; P = 0.04) in the progesterone higher than 45 nmol/l group, both compared with the optimal progesterone group (28–45 nmol/l) (TABLE 2). The risk difference for OPR

TABLE 1 BASELINE CHARACTERISTICS AND REPRODUCTIVE OUTCOME IN THREE PROGESTERONE GROUPS

	Serum progesterone <28 nmol/l	*Serum progesterone ≤28 ≥45 nmol/l*	*Serum progesterone >45 nmol/l*	*P-value*
Cycles, *n* (%)	25 (10)	96 (40)	118 (49)	–
Median serum progesterone level, nmol/l (range)	24 (2.2–27)	36 (28–45)	59 (46–150)	–
Mean BMI, kg/m^2 (SD)	25.8 (3.4)	25.3 (4.2)	23.4 (3.4)	0.0006
Mean age, years (SD)	31.9 (5.4)	32.5 (4.7)	31.5 (4.8)	0.35
Mean blastocyst quality (SD)	1.3 (0.6)	1.3 (0.5)	1.3 (0.5)	0.98
Day-5 blastocyst, *n* (%)	16 (64)	67 (70)	84 (71)	0.78
Day-6 blastocyst, *n* (%)	9 (36)	29 (30)	34 (29)	0.78
Positive HCG, *n* (%)	14 (56)	68 (71)	67 (57)	0.08
Implantation, *n* (%)	9 (36)	57[1] (59)	54[1] (46)	0.05
Clinical pregnancy with heartbeat, *n* (%)	9 (36)	53[2] (55)	50 (42)	0.09
Ongoing pregnancy week 12, *n* (%)	9 (36)	51 (53)	46 (39)	0.08
Biochemical pregnancy,[a]*n* (%)	5 (20)	10 (10)	13 (11)	0.39
Total loss, *n* (%)	5 (36)	17 (25)	21 (31)	0.57

One double implantation counted as one implantation and one double clinical pregnancy counted as one clinical pregnancy.
[a] Pregnancy only detected by HCG in blood or urine and never confirmed clinically.BMI, body mass index.

in the low (<28 nmol/l) and high (>45 nmol/l) progesterone group was –0.16 (CI –0.37 to 0.04; P = 0.12) and –0.14 (CI –0.27 to –0.002; P = 0.046), respectively.

The early pregnancy loss rate (biochemical pregnancy rate) was highest in the low progesterone group (20%) compared with both the middle (10%) and high (11%) progesterone group, although this difference was not found to be statistically significant (P = 0.39) (TABLE 1).

A retrospective comparison using a cohort of single embryo transfer HRT-FET cycles (*Alsbjerg et al., 2018*) treated with vaginal progesterone, only (90 mg Crinone three time a day) and the combined 2 + 2 regime is shown in Supplementary Table 1. Median serum progesterone values are significantly higher (45 nmol/l: range 2–150 nmol/l) in patients administered progesterone via the combined 2 + 2 regime than in those who received progesterone only through the vaginal route (34 nmol/l: range 0.3–110; P < 0.001). No significant difference was observed in reproductive outcomes (Supplementary Table 1).

Questionnaire

A tablet with an electronic questionnaire was handed out to 91 patients after embryo transfer, of whom 87 responded. Forty-seven of the 48 patients completed the questionnaire again after ultrasound confirmation of pregnancy in gestational week 7. The overall response rate was 96% (134/139).

Rectal discomfort was reported by 18% (16/87) of patients at embryo transfer and by 17% (8/47) at the time of the pregnancy scan in gestational week 7. In comparison, vaginal discomfort related to vaginal progesterone administration was reported by 18% and 45% during the same time period (Supplementary Figure 2 and Supplementary Figure 3).

Abdominal discomfort was reported by 31% (27/87) at embryo transfer. Only eight patients (9%) rated this discomfort as moderate or severe, and no patient discontinued rectal progesterone administration because of abdominal side-effects. A total of 33% (29/87) reported an increase in flatulence following the progesterone administration at embryo transfer and 51% (24/47) in gestational week 7. Further side-effects are described in Supplementary Figure 2 and Supplementary Figure 3.

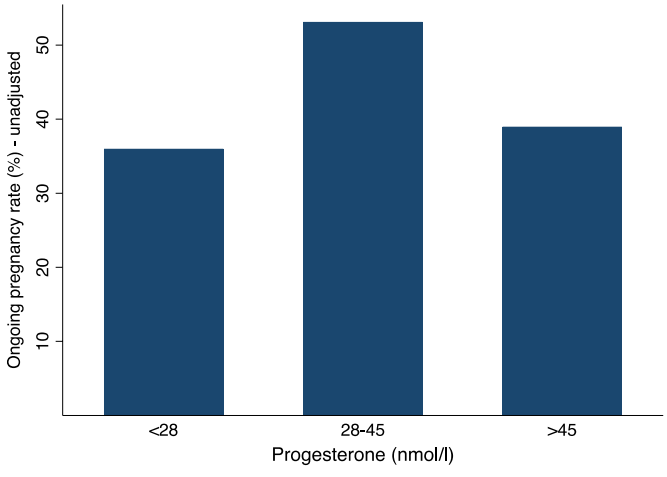

FIGURE 1 Unadjusted ongoing pregnancy rate for the three progesterone groups. The differences between the three progesterone groups did not reach statistical significance (P = 0.08).

DISCUSSION

In the present study, we explored for the first time the reproductive outcomes of HRT-FET cycles using a combination of rectal and vaginal progesterone for luteal phase support (the '2 + 2' regimen). The percentage of patients with low serum progesterone (<28 nmol/l) was 10% in the 2 + 2 regimen compared with around 25% in a previous HRT-FET study by *Labarta et al. (2017)*,

TABLE 2 LOGISTIC REGRESSION ANALYSIS OF REPRODUCTIVE OUTCOMES

	Crude odds ratio (95% CI)					*Adjusted odds ratio (95% CI)*					*Adjusted risk difference (95% CI)*				
Proges-teronen-mol/l	**P4<28**	***P*-value**	**P428–45**	**P4>45**	***P*-value**	**P4<28**	***P*-value**	**P428–45**	**P4>45**	***P*-value**	**P4<28**	***P*-value**	**P428–45**	**P4>45**	**P-*value***
Positive HCG	0.52 (0.21 to 1.31)	0.16	1	0.54 (0.30 to 0.96)	0.03	0.50 (0.19 to 1.30)	0.16	1	0.54 (0.29 to 0.99)	<0.05[a]	−0.14 (−0.34 to 0.7)	0.19	1	−0.13 (−0.26 to −0.007]	0.04
Clinical pregnancy	0.46 (0.18 to 1.15)	0.09	1	0.60 (0.34 to 1.03)	0.06	0.44 (0.17 to 1.14)	0.09	1	0.56 (0.32 to 1.01)	0.06	−0.19 (0.39 to 0.02)	0.08	1	−0.13 (−0.27 to 0.003)	0.06
Ongoing pregnancy	0.50 (0.20 to 1.25)	0.13	1	0.56 (0.32 to 0.98)	0.04	0.48 (0.18 to 1.24)	0.13	1	0.53 (0.30 to 0.97)	0.04	−0.16 [−0.37 to 0.04]	0.12	1	−0.14 (-0.27 to −0.002)	<0.05[b]
Early pregnancy loss	2.15 (0.65 to 7.08)	0.20	1	1.06 (0.44 to 2.55)	0.89	2.25 (0.68 to 7.41)	0.18	1	1.27 (0.51 to 3.17)	0.60	0.11 [−0.06 to 0.28]	0.21	1	0.03 (−0.05 to 0.11)	0.50

P-values describe the comparison between the optimal progesterone group (P4 28–45 nmol/l) and the low progesterone group (P4 <28 nmol/l) and the high progesterone group (P4 >45 nmol/l), respectively.

SI conversion factor for progesterone: nmol/l = 3.18* ng/ml.

[a] $P = 0.046$.

[b] $P = 0.046$.P4, progesterone.

using a standard vaginal luteal phase support. Furthermore, the median serum progesterone level was 45 nmol/l (range 0.2–150) compared with 34 nmol/l (0.3–110) in a previous study by our group in which progesterone was administered vaginally only (*Alsbjerg et al., 2018*). The combined vaginal and rectal progesterone regimen revealed a non-linear relationship between the serum progesterone level and ongoing pregnancy with lower ongoing pregnancy rates in the low progesterone group (<28 nmol/l) as well as in the high (>45 nmol/l) group, compared with the intermediate progesterone group. The combination of vaginal and rectal progesterone supplementation was well tolerated by patients.

In HRT-FET cycles using vaginal progesterone, several studies have shown that low serum progesterone levels have a negative effect on reproductive outcomes (*Labarta et al., 2017*; *Alsbjerg sset al., 2018*; *Gaggiotti-Marre et al., 2018*). Furthermore, in these studies, up to 50% of all patients treated with vaginally administered progesterone at standard doses had suboptimal progesterone levels. This underlines the question asked in the present study: will the proportion of patients having low serum progesterone in HRT-FET be reduced with the '2 + 2' regimen and will this result in a reduced early pregnancy loss and increased OPR? Furthermore, is the correlation between luteal progesterone levels and reproductive outcome linear or is there a ceiling effect, whereby lower ongoing pregnancy rates are observed in patients with high serum progesterone levels?

In the present study as well as in a previous study by *Yovich et al. (2015)*, a non-linear relationship between serum progesterone levels and reproductive outcomes was found, indicating that also a high as well as low serum progesterone levels may negatively affect reproductive outcome. In this study, however, only five patients had serum progesterone levels higher than 99 nmol/l, a level previously described as a ceiling level resulting in suboptimal reproductive outcomes (*Yovich et al., 2015*). It can be hypothesized that different modes of administration may result in different optimal cut-off levels. *Yovich et al. (2015)* used vaginal administration only; in contrast, the present study used a combination of vaginal and rectal progesterone administration.

In a small pilot study carried out by our group, we found that adding rectal progesterone to the standard vaginal progesterone dosage increased the mean serum progesterone from 26 nmol/l to 41 nmol/l in a group of patients with low progesterone levels (unpublished data). This demonstrates that the '2 + 2' regimen can be used in patients with insufficient progesterone absorption in a standard HRT-FET regimen to increase serum progesterone levels but limits the risk of excessive serum progesterone concentrations that might negatively affect pregnancy rates. The use of standard combined vaginal and rectal regimen may lead to excessive progesterone levels in some patients. As seen in the retrospective comparison (Supplementary Table 1), if a standard regimen is compared with an extended progesterone regimen, the overall reproductive outcome is the same. This paradox may be explained by the fact that some patients who would be sufficiently supported using a standard vaginal regimen may end up with progesterone levels high enough to reduce reproductive outcomes when treated with the '2 + 2' regimen. The results presented in this paper indicate that using the combined vaginal and rectal progesterone regimen, the optimal serum progesterone level range is between 28 and 45 nmol/l.

During the natural cycle, progesterone secretion from the corpus luteum increases from the early luteal phase towards the mid-luteal phase with peak progesterone levels of 30–60 nmol/l 7 days after ovulation (*Landgren et al., 1980*; *Stricker et al., 2006*). The lower threshold for live birth in the natural cycle was evaluated by *Hull et al. (1982)* who examined 212 cycles and reported that no pregnancies were obtained with serum progesterone levels below 27 nmol/l or above 50 nmol/l. These cut-off levels are consistent with the optimal range identified in this study. In contrast, others have reported

pregnancies in natural cycles with mid-luteal progesterone levels as low as 7 nmol/l, demonstrating a huge biological variation in conception cycles (*Takaya et al., 2018*).

The HRT cycle aims to mimic the hormonal luteal pattern of the natural cycle. With supplementation of exogenous progesterone in the HRT cycle, the progesterone level is more constant during the luteal phase with maximum progesterone levels achieved from day 1. These levels are then maintained for the duration of the luteal phase and do not peak at the time of implantation as they would during the natural cycle.

Previously, we showed that increasing vaginally administered progesterone support using 90 mg Crinone once a day in HRT-FET cycles to 180 mg Crinone per day increased the live birth rate from 8.7% to 20.5% ($P = 0.002$), following cleavage-stage embryo transfer in HRT-FET cycles (*Alsbjerg et al., 2013*). The increase in live birth rate was mainly mediated by a reduction in the early pregnancy loss rate. Previously, *Cédrin-Durnerin et al. (2019)* showed that doubling of the vaginal progesterone dose from 200 mg three times a day to 400 mg three times a day did not significantly increase either mean serum progesterone levels or live birth rates. This indicates that the vaginal capacity to absorb progesterone has a maximum and that, thereafter, additional vaginal administration will not increase the serum progesterone levels any further.

Nillius and Johansson (1971) investigated plasma progesterone levels after vaginal, rectal and intramuscular progesterone administration. They found a rapid absorption after rectal administration with high serum progesterone levels after 2 h of peak serum levels within 8 h, followed by a gradual decline to near baseline levels within 24 h. They concluded that, to maintain stable progesterone serum levels, rectal progesterone should optimally be administered every 12 h. Considerable individual variations in serum progesterone levels were seen between patients receiving the same rectal progesterone dose, most likely due to large inter-individual variations in venous drainage of the rectum and the presence of anastomoses between the inferior, middle and superior rectal vein.

Furthermore, *Chakmakjian and Zachariah (1987)* compared the absorption of progesterone after different routes of administration, reporting higher peak serum concentrations after rectal administration (14.0 ± 4.2 ng/ml compared with 8.2 ±1.0 ng/ml after vaginal administration). Moreover, the area under the curve was higher after rectal administration compared with vaginal administration (169.9 8 h/cm^2 versus 96.0 8 h/cm^2, respectively). The study included small patient numbers (12 and 13 in the treatment groups, respectively) and it was concluded that it was impossible to predict the most effective route of administration, owing to both variable absorption between patients, but also to differences in absorption within patients after different modes of administration.

The present study shows that the group of patients with low serum progesterone levels can be reduced using a combination of vaginal and rectal progesterone, although progesterone absorption seems to be highly variable between patients. Standard vaginal luteal phase support, e.g. Crinone 90 mg per 12 h, induces an average steady-state progesterone concentration of 37 nmol/ml (11.6 ng/ml) within 24 h, assuming optimal absorption, which will cover the progesterone requirement of the HRT-FET cycle. The present study also shows that a large individual variation in progesterone absorption exists between patients: one patient had a progesterone level of 23 nmol/l whereas another patient, receiving the same treatment, had a serum progesterone level of 150 nmol/l. Importantly, we must bear in mind that serum progesterone is a surrogate marker and the endometrial progesterone concentration may be substantially different (*Fanchin et al., 1997*; *Cicinelli and de Ziegler, 1999*).

The main limitation of this observational cohort study describing a novel '2 + 2' regimen is that it did not include a control group. The investigators previously published a retrospective cohort study of HRT-FET cycles using a standard vaginal progesterone regimen. The results from this study showed that 50% of patients had suboptimal serum progesterone levels which negatively affected reproductive outcomes. To increase the progesterone levels, all patients in the present study period were treated with the combined vaginal and rectal regimen as it would have been unethical to continue the suboptimal treatment in what would have been the control group. Furthermore, micronized progesterone (Crinone 90 mg) was used for all patients in the study, and whether these results can be applied to other progesterone preparations is currently unknown. Finally, only one blood sample was collected on the day of pregnancy testing and, owing to the broad time frame after administration (1–8 h), it is possible that results could have been affected by circadian variation and that the combination of two different routes of administration could have influenced this variation further.

In conclusion, to the best of our knowledge, this is the first study to investigate the use of combined rectal and vaginal progesterone in HRT-FET cycles. It showed a higher mean progesterone serum level, compared with a vaginally administered progesterone regimen, only. The study indicates that high and low serum progesterone may be associated with decreased ongoing pregnancy rates. Rectally administered micronized progesterone was well accepted, and we suggest that this route of administration can be used as a supplementary route for patients with low progesterone after standard vaginal progesterone treatment in HRT-FET cycles. Randomized controlled studies are needed to identify the optimal HRT regimen and how to monitor and individualize treatment.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2020.02.007.

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