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ARTICLE

Endometriosis patients benefit from high serum progesterone in hormone replacement therapy–frozen embryo transfer cycles: a cohort study

BIOGRAPHY

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KEY MESSAGE

This cohort study of endometriosis patients explores the effect of progesterone concentrations during luteal phase in hormone replacement therapy frozen embryo transfer. Concentrations above 118 nmol/l (37.1ng/ml) resulted in significantly higher live birth rate compared with lower serum progesterone levels, suggesting that a threshold for optimal serum progesterone exists.

ABSTRACT

Research question: What is the optimal serum progesterone cut-off level in patients with endometriosis undergoing hormone replacement therapy frozen embryo transfer (HRT-FET) with intensive progesterone luteal phase support?

Design: A cohort study, including 262 HRT-FET cycles in 179 patients all diagnosed with endometriosis either by laparoscopy or by ultrasound in patients with visible endometriomas. Pre-treatment consisted of 42 days of oral contraceptive pills and 5 days' wash-out, followed by 6 mg oral oestrogen daily. Exogenous progesterone supplementation with vaginal progesterone gel 90 mg/12h commenced when the endometrium was 7 mm or thicker. From the fourth day of vaginal progesterone supplementation, patients also received intramuscular progesterone 50 mg daily. Blastocyst transfer was scheduled for the sixth day of progesterone supplementation.

Results: The overall positive HCG, live birth (LBR) and total pregnancy loss rates were 60%, 39% and 34%, respectively. The optimal progesterone cut-off level was 118 nmol/l (37.1 ng/ml) defined as the maximum of the Youden index. The unadjusted LBR was significantly higher in patients with progesterone measuring 118 nmol/l or above compared with patients with progesterone measuring less than 118 nmol/l (51% [44/86] versus 34% [59/176], $P = 0.01$), whereas the adjusted odds ratio for a live birth was 2.1 (95% CI 1.2 to 3.7) after adjusting for age, body mass index, blastocyst score, blastocyst age, quality and number of blastocysts transferred.

Conclusions: Serum progesterone levels above 118 nmol/l (37.1ng/ml) resulted in significantly higher LBR compared with lower serum progesterone levels, suggesting that a threshold for optimal serum progesterone exists.

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KEYWORDS

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Serum progesterone

INTRODUCTION

The association between endometriosis and infertility is well known, and numerous patietns with endometriosis undergo assisted reproductive technology (ART), mainly IVF, to obtain a pregnancy and live birth. Contradictory results, however, have been reported in cohort studies when comparing the reproductive outcomes of endometriosis patients with patients with other diagnoses; therefore, some studies have suggested a negative effect of endometriosis whereas others reported no difference in reproductive outcome (*Burney et al., 2007*; *de Ziegler et al., 2010*; *Ata and Telek, 2021*). The reasons for the decreased pregnancy and live birth rates (LBR) in endometriosis patients have been discussed, and factors such as lower ovarian sensitivity, lower oocyte yield, impaired oocyte quality, effect of endometriosis on the endometrial environment and an increased risk of miscarriage have been debated (*Hamdan et al., 2015*; *Miravet-Valenciano et al., 2017*; *Horton et al., 2019*).

During a natural cycle, the endometrium undergoes physiological changes to facilitate implantation and support the early pregnancy. These processes are regulated by circulating oestrogen and progesterone. Oestrogen, apart from inducing proliferation of the endometrium through oestrogen receptors (ESR1 and ERS2), also up-regulates progesterone receptor expression (PR-A and PR-B), and progesterone inhibits the proliferation of stromal cells and mediates decidualization through its receptors. Furthermore, the ratio of the two isoforms of the progesterone receptor is important for endometrial receptivity. Endometriosis is dominated by a disruption of progesterone and oestrogen signalling pathways, resulting in oestrogen dominance and progesterone resistance at the receptor level (*Wu et al., 2006*; *Lessey and Kim, 2017*; *Marquardt et al., 2019*). To understand the effect of endometriosis on implantation, *Prapas et al. (2012)* previously conducted a study of 240 menopausal oocyte recipients with and without endometriosis, who shared sibling oocytes from the same donor. The investigators reported significantly lower implantation and LBR in the group of endometriosis patients compared with the reference group (24% versus 31%, $P = 0.019$ and 35% versus 51%, $P = 0.013$), respectively, in support of the hypothesis of an impaired endometrial function in endometriosis patients (*Prapas et al., 2012*).

In non-endometriosis IVF patients undergoing hormone replacement therapy frozen embryo transfer (HRT-FET), several studies reported the importance of serum progesterone levels, and cut-off levels for reproductive success have been suggested in patients receiving vaginal progesterone for luteal phase support (LPS) (*Yovich et al., 2015*; *Labarta et al., 2017*; *Alsbjerg et al., 2018*; *Cédrin-Durnerin et al., 2019*). Therefore, a recent meta-analysis suggested an optimal cut-off level for successful implantation and live birth of 32 nmol/l (10 ng/ml) in HRT-FET (*Melo et al., 2021*). Importantly, to date, no study has explored luteal phase serum progesterone cut-off levels in patients with endometriosis.

The aim of the present study was to investigate the optimal luteal phase serum progesterone level in endometriosis patients undergoing HRT-FET, who received intensive LPS, including a combination of vaginal and intramuscular progesterone.

MATERIALS AND METHODS

Study design and eligibility criteria

Cohort study, conducted in a public fertility centre between January 2016 and August 2019.

Participants

All patients included in this cohort were diagnosed with endometriosis. Endometriosis, however, is a heterogeneous disease and the present cohort reflects the broad spectrum of different categories of endometriosis patients seen in daily clinical practice, including patients who underwent surgery for endometriosis for ovarian endometriomas as well as deep infiltrating endometriosis. Moreover, some patients were diagnosed during their first consultation in the clinic, but before fertility treatment, with endometriomas or adenomyosis visible on ultrasound and a typical history of endometriosis-related pain. This clinical cohort of 262 cycles in 179 patients was included in the study.

Endometrial priming

All patients were pre-treated with 6 weeks of oral contraceptive pills (levonorgestrel, ethinylestradiol 150 + 30 µg) followed by 5 days wash-out before treatment with 6 mg oral oestradiol valerate (Estrofem) (Novo Nordisk, Bagsvaerd, Denmark) was administered once daily (*de Ziegler et al., 2010*). An ultrasound examination was carried out after 12–19 days, and if the endometrial thickness was 7 mm or thicker, progesterone supplementation commenced. All patients received the same micronized progesterone dose, 90 mg/12 h (Crinone©) (Merck, Søborg, Denmark) administered vaginally. In the evening of the fourth day of vaginal progesterone treatment, daily intramuscular 50 mg progesterone was added to the LPS protocol. In patients with a positive pregnancy test, treatment with vaginal progesterone (90 mg) twice daily and 6 mg oral oestradiol continued until the 10th week of gestation, whereas intramuscular progesterone treatment stopped after the first ultrasound scan carried out between gestational weeks 7 + 0 and 8 + 0. See Supplementary Figure 2 for treatment protocol.

Embryos and embryo transfer

All transfers were autologous blastocyst transfers with blastocysts previously vitrified on day 5 or 6, using the 'Cryotec method' by Masashige Kuwayama (*Gandhi et al., 2017*). Embryo transfer was scheduled in all patients for the sixth day of vaginal progesterone administration. A top-quality blastocyst (score 1) was defined as a 3AA, 3AB, 3BA, 4AA, 4AB, 4BA, 5AA, 5AB and 5BA. An intermediate blastocyst (score 2) was defined as a 3BB, 4BB and 5BB. No poor-quality blastocysts (score 3) were transferred according to the Gardner and Schoolcraft grading system (*Gardner and Schoolcraft, 1999*).

Blood sampling and hormone analyses

Blood sampling was carried out 9 or 11 days after embryo transfer, corresponding to the day of the pregnancy test. The LPS was standardized for all patients, and blood sampling was carried out 2–4 h after vaginal progesterone administration in the morning and 9–15 h after the last administration of intramuscular progesterone.

All blood samples were analysed for HCG and progesterone immediately at the Department of Biochemistry, Viborg Regional Hospital, Denmark, using automated electro-chemiluminescent

immunoassays (Cobas® Modular analytics E170) (Roche Diagnostics, Rotkreuz ZG, Switzerland) according to the manufacturer's instructions. 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%.

A positive pregnancy test was defined as a serum HCG concentration above10 IU. Therefore, clinical pregnancy was defined as a sonographically verified gestational sac, and a live birth was defined as a live birth after gestational week 22. Total pregnancy loss was defined as loss of a pregnancy before gestational week 12, including both biochemical pregnancy losses and clinical losses. Biochemical pregnancy loss was defined as a pregnancy, diagnosed only by the detection of HCG in serum or urine and not verified sonographically.

Statistics

The data were prospectively registered and retrospectively analysed and presented as percentages and means with SD, if normal distribution was found after testing, using histograms and Q-Q plots. Fisher's exact test, Pearson's chi-squared test or t-test were used as appropriate; all *P*-values are two-sided and a level less than 0.05 was considered as significant.

The associations between serum progesterone concentrations and LBR are presented in 10 equally sized groups using percentiles (Supplementary Figure 1), and the best estimated cut-off value was found by use of the Youden Index. The Youden Index is defined as J= max (Sensitivity[c]+Specificity [c]−1) where c is the cut-off point and the value range from 1 to –1. If the Youden Index has the value 1 it represents the perfect test, and value of 0 or lower than 0 indicate that the test is not fit for use (*Fluss et al., 2005*; *Shan, 2015*).

A logistic regression model was used to evaluate the effect of progesterone concentrations higher or equal to 118 nmol/l on the LBR. The model was adjusted for body mass index (BMI) (continuous), age (continuous), number of blastocysts transferred, blastocyst vitrification day (day 5 or 6) and blastocyst quality (high or intermediate). As some patients participated more than once, a robust standard error was calculated to account for non-independency of data. STATA 16.0 (StataCorp LP, College Station, TX,USA) was used for all statistical analyses.

Ethics

This cohort study was registered by the local Ethics Review Board on the 8 May 2017, registration number 1-10-72-4-17. No further approval was need owing to the design.

RESULTS

Basic characteristics

This study included a total 262 HRT-FET cycles carried out in 179 patients; 118 patients had only one treatment, and 61 patients had more than one treatment. The mean serum progesterone concentration of all cycles was 103.1 ± 44.4 nmol/l (32.4 ± 14.0 ng/ml), and in 33% (86/262) of the cycles serum progesterone concentrations were higher or equal to 118 nmol/l (37.1 ng/ml). Single embryos were transferred in 76% of cycles. More day-5 blastocysts than day-6 blastocysts (71% versus 51 %) were transferred in the low progesterone group (P = 0.001). All other basic characteristics were equally distributed in the two serum progesterone groups (TABLE 1).

Reproductive outcomes

The overall pregnancy, implantation and LBR were 60% (157/262), 44% (144/325) and 39% (103/267), respectively. A significantly higher LBR was seen in the group of patients with serum progesterone concentrations 118 nmol/l or above compared with the group with serum progesterone concentrations less than 118 nmol/l, 51% versus 34% (P = 0.01), respectively. Furthermore, a trend for a lower total pregnancy loss rate was seen in the serum progesterone group 118 nmol/l or above (25% versus 41%, P = 0.07). The total twinning rate was 9% (9/103).

A logistic regression analysis showed that patients with high serum progesterone concentrations (≥118 nmol/l) were more likely to achieve a live birth compared with patients with low progesterone concentrations less than 118 nmol/l (OR 2.10, 95% CI 1.20 to 3.68) after adjusting for age, BMI, number of blastocysts transferred, blastocyst quality and blastocyst age. Furthermore, as expected, older patients had a lower LBR compared with younger patients (OR 0.93, 95% CI 0.88 to 0.98) (TABLE 2).

DISCUSSION

To the best of our knowledge, this is the first study to evaluate the optimal luteal serum progesterone concentration in HRT-FET cycles in endometriosis patients. We found that endometriosis patients with serum progesterone concentrations higher than 118 nmol/l (37.1 ng/ml) had double the LBR compared with patients with lower serum progesterone concentrations in HRT-FET cycles.

As previously explored and described by *Lessey and Young (2014)*, a homeostasis imbalance exists in the endometrium of the endometriosis patient, associated with chronic inflammation, resulting in progesterone resistance (*Young and Lessey, 2010*). Oestrogen is a well-known proinflammatory factor (*Straub, 2007*) and the main driver of inflammation in the endometriosis patient seems to be oestrogen via oestrogen receptor 2, which is increased in endometriosis (*Hudelist et al., 2005*; *Bulun et al., 2012*); moreover, the endogenous endometrial mechanisms usually involved in inflammation resolution seem to be defective in endometriosis. In contrast, progesterone has specific anti-inflammatory characteristics, also promoting immunotolerance (*Straub, 2007*); however, the progesterone resistance seen in endometriosis leads to a reduction in progesterone actions, and thus increased inflammation, further imbalance of homeostasis and resistance to progesterone at the receptor level (*Lessey and Young, 2014*).

Inspired by basic research conducted by Lessey et al., we hypothesized that the cut-off for serum progesterone in the endometriosis patient undergoing HRT-FET would be higher compared with the 'non-endometriosis' patient, and the way to overcome the endometrial progesterone resistance would be by increasing the progesterone supplementation used for HRT-FET, in line with what has previously been reported for FSH receptor resistance (*Greb et al., 2005*). On the basis of these physiological facts, the LPS of the present study was designed.

To mimic the natural cycle progesterone peak at peri-implantation, patients initially received vaginal progesterone gel, 90 mg twice daily for 4 days. This has previously been shown to result in a 'modest'

TABLE 1 BASIC CHARACTERISTICS AND REPRODUCTIVE OUTCOME

Characteristics	All	Progesterone <118 nmol/l	Progesterone ≥118 nmol/l	*P*-value
Cycles, *n*	262	176 (67)	86 (33)	
Serum progesterone	103.1 ± 44.4	79.8 ± 25.7	150.8 ± 35.7	<0.001
Age, years	33.4 ±5.0	33.4 ± 5.1	33.2 ±5.1	0.96
Body mass index, kg/m^2	24.6 ±4.1	25.1 ± 4.1	23.8 ±3.8	0.35
Smoking, *n* (%)	4 (2)	2 (1)	2 (2)	0.46
Blastocysts transferred, *n*	325	215	110	0.53[a]
Single embryo transfer, *n* (%)	199 (76)	137 (69)	62 (31)	0.31[a]
Double embryo transfer, *n* (%)	63 (24)	39 (62)	24 (38)	
Day of vitrification, *n* (%)				0.001[a]
Day-5 blastocyst	245	174 (71)	71 (29)	
Day-6 blastocyst	80	41 (51)	39 (49)	
Fertilization method, *n* (%)				0.95[a]
IVF	215	142 (66)	73 (34)	
ICSI	110	73 (66)	37 (34)	
Cycles with at least one high-quality blastocyst,[b] *n* (%)	186	122 (66)	64 (34)	0.39[a]
Pregnancy per embryo transfer, *n* (%)	157 (60)	98 (56)	59 (69)	0.05[a]
Implantation rate, *n* (%)	144/325 (44)	87/215 (40)	57/110 (52)	0.05[a]
Live birth[c] per embryo transfer, *n* (%)	103/262 (39)	59/176 (34)	44/86 (51)	0.01
Twinning, *n* (%)	9/103 (9)	5/58 (9)	4/45 (9)	0.98[a]
Total pregnancy loss, *n* (%)	54/157 (34)	39/96 (41)	15/61 (25)	0.07[a]
Biochemical pregnancy loss, *n* (%)	27/157 (17)	19/98 (19)	8/59 (14)	0.35

Data presented as *n*, *n* (%) or mean ± SD.

[a] Pearson's chi-squared test.

[b] Defined as 3AA, 3AB, 3BA, 4AA, 4AB, 4BA, 5AA, 5AB and 5BA (Gardner classification).

[c] Defined as birth of one or two children.

ICSI, intracytoplasmic sperm injection.

TABLE 2 LOGISTIC REGRESSION ANALYSIS OF THE ASSOCIATION BETWEEN PROGESTERONE CONCENTRATION 9–11 DAYS AFTER BLASTOCYST TRANSFER AND CHANCE OF A LIVE BIRTH, ADJUSTED FOR BODY MASS INDEX, AGE, BLASTOCYST AGE, BLASTOCYST QUALITY AND NUMBER OF BLASTOCYSTS TRANSFERRED

Characteristics	Odds ratio	95% CI	*P*-value
Serum progesterone concentration			
<118 nmol/l	1		
≥118 nmol/l	2.10	1.20 to 3.68	0.01
Body mass index	1.00	0.94 to 1.07	0.98
Age, years	0.93	0.88 to 0.98	0.01
Day of vitrification			
5	1		
6	0.85	0.43 to 1.69	0.65
Blastocyst score, quality			
High	1		
Medium	0.70	0.38 to 1.30	0.26
Blastocyst transfer, *n*			
Single embryo	1		
Double embryo	1.22	0.66 to 2.23	0.53

increase in serum progesterone with a mean of 24.2 ± 10.1 nmol/l (7.6 ± 3.2 ng/ml) (*Alsbjerg et al., 2021*). On the fourth day of vaginal progesterone, a daily dose of 50 mg intramuscular progesterone was added to the protocol, which has been reported to result in an increase in serum progesterone of 96 ± 24 nmol/l (30.2 ±7.5 ng/ml) (*Paulson et al., 2014*).

It is important to remember that serum progesterone is a pseudo-parameter, and that serum concentrations do not directly reflect the progesterone load to the uterus. Especially, when vaginal and intramuscular progesterone regimens have been compared, large differences between serum progesterone concentrations and endometrial progesterone concentrations have been found, and this paradox has been explained by the 'first pass effect' after vaginal progesterone administration (*Miles et al., 1994*; *Paulson et al., 2014*; *Cicinelli et al., 2000*). Moreover, recently, *Labarta et al. (2021)* in a cohort of 79 patients undergoing IVF

and HRT-FET who only received vaginal progesterone for LPS, reported that serum progesterone concentrations were not correlated to endometrial progesterone concentrations nor to endometrial receptivity as determined by the ERA® test. Following this report, others questioned the ability of the ERA test to predict receptivity at all (*Lawrenz and Fatemi, 2022*).

A growing body of scientific evidence supports the notion that luteal serum progesterone used as a pseudo-marker of endometrial receptivity plays an important role for the reproductive outcomes of HRT-FET cycles. Most reported studies have been conducted in HRT-FET regimens, using vaginal progesterone LPS, only, and a great variation in the reported cut-off concentrations for progesterone have been seen, ranging from concentrations higher than 26 nmol/l (8.2 ng/ml) (*Gaggiotti-Marre et al., 2018*) to concentrations between 70 (22.0 ng/ml) and 99 nmol/l (31.1 ng/ml) (*Yovich et al., 2015*); however, most recent evidence suggests that the optimal cut-off concentration is around 32 nmol/l (10 ng/ml) (*Melo et al., 2021*). Interestingly, the cut-off concentration seems to depend on the vaginal progesterone formulation, the administration regimen and the statistical method used for calculation; importantly, differences in previously suggested cut-off concentrations could be related to differences in pharmacokinetics of the progesterone formulation.

Research on intramuscular progesterone regimens and HRT-FET, particularly the optimal serum progesterone concentration, is limited, and contradictory results have been reported. *Brady et al. (2014)* reported a significantly higher LBR (65% versus 51%, $P = 0.04$) if serum progesterone concentrations were higher than 64 nmol/l (20 ng/ml) in a cohort of 229 postmenopausal oocyte recipients. In contrast, *Kofinas et al. (2015)* found a significantly higher LBR (65% versus 49%, $P = 0.02$) if serum progesterone concentrations were lower than 64 nmol/l (20 ng/ml) in a cohort of 213 HRT-FET cycles in which autologous euploid blastocysts were used for transfer. In both cohorts, patients were treated with intramuscular progesterone 50–75 mg daily for LPS without any vaginal support (*Brady et al., 2014*; *Kofinas et al., 2015*). A negative effect of high serum progesterone concentrations was also reported by *Alysin et al. (2021)* in an HRT-FET study, combining both vaginal and intramuscular progesterone for LPS. In that study, the LBR was higher in patients with serum progesterone concentrations lower than 32.5 ng/ml (103 nmol/l) (*Alyasin et al., 2021*). Concurrently, *Alsbjerg et al. (2020)* reported a negative effect on reproductive outcomes if serum progesterone concentrations were higher than 14 ng/ml (45 nmol/l) in 277 HRT-FET cycles, using both vaginal and rectal progesterone for LPS (*Alsbjerg et al., 2020*). The studies included patients with varying fertility diagnoses and did not exclusively focus on endometriosis patients as in the present study. The endometrial physiology of the endometriosis patient, especially the progesterone resistance, may explain why the present cohort benefitted from higher serum progesterone concentrations compared with groups of non-endometriosis patients.

Endometriosis is a heterogeneous disease with variation in symptoms and anatomical findings; furthermore, disease burden and symptoms do not always correspond (*Birmingham, 1997*; *Riiskjær et al., 2017*). The cohort of endometriosis patients in the present study represent different categories of endometriosis as found in most fertility clinics. Patients, however, were treated equally whether they had been operated for deep infiltrating endometriosis, endometriomas or were diagnosed in the fertility clinic in case of endometriomas visible on ultrasound and a history of pain. It is unknown whether patients with different endometriosis severity or different endometriosis subgroups have the same progesterone requirement to achieve an ongoing pregnancy, and this may influence the results of the present study as the cohort was heterogeneous regarding these parameters. This is, however, the first clinical study showing that patients diagnosed with endometriosis overall seem to benefit from higher progesterone concentrations in HRT-FET cycles. Consequently, larger studies are needed to investigate subgroups in the future.

This novel clinical approach for the endometriosis patient was implemented in our unit in 2016 and only patients fulfilling the mentioned criteria were treated, using the intensive LPS protocol. Consequently, we do not have any local data that include patients without endometriosis being treated with an intensive LPS regimen. The findings of a very recent paper using an intensive LPS regimen in patients without endometriosis, however, suggest that it would be un-ethical to treat this group with an intensive LPS regimen. Therefore, *Alyasin et al. (2021)* showed a significantly reduced LBR if the mid-luteal serum progesterone was higher than 103 nmol/l (32.5 ng/ml) in a cohort of patients without endometriosis. This contrasts with the present study, which only includes endometriosis patients. Higher LBR was achieved if progesterone concentrations were above 118 nmol/l. The *Alyasin et al. (2021)* cohort is comparable to the present cohort despite each being conducted in a different fertility centre, as both cohorts included non-screened vitrified blastocyst transfers and intensive LPS, including vaginal and intramuscular progesterone. Importantly, no significant differences in age, BMI and mean serum progesterone concentrations between the two cohorts were found. Differences were found, however, in the timing of progesterone measurement and embryo transfer day versus 12 days later. Theoretically, however, no differences were found in progesterone concentrations as no corpus luteum is present and placenta is not secreting progesterone this early in case of pregnancy. The comparison is presented in Supplementary Table 1.

All endometriosis patients in the present study were pre-treated with oral contraceptive pills for 6 weeks, followed by a 5-day wash-out period before stimulation with exogenous oestradiol. The physiological rationale for this regimen was to decrease intrauterine prostaglandin concentrations and pro-inflammatory factors as previously described by *De Ziegler et al. (2010)*, who used the same concept before IVF treatment in a pilot study in endometriosis patients who underwent fresh embryo transfer. In that study, a significant increase in clinical pregnancy rate was seen in endometriosis patients pre-treated with the oral contraceptive pill compared with the reference group without oral contraceptive pill pre-treatment (41.4% versus 12.9, $P = 0.1$) (*de Ziegler et al., 2010*).

In the present study, progesterone concentrations were measured 9–11

days after embryo transfer; however, at this time point, the progesterone concentration will be similar to the progesterone concentration seen during the mid-luteal phase, as no corpus luteum is present in the HRT-FET cycle. Furthermore, in case of implantation, endogenous progesterone production from the placenta will be negligible 9–11 days after embryo transfer; therefore, *Neumann et al. (2020)* reported the mean progesterone concentration to be 0.24 ± 0.17 ng/ml (0.76 ± 0.54 nmol/l) in the fifth gestational week, and *Kawachiya et al. (2019)* reported a median serum progesterone of 0.6 ng/l (1.9 nmol/l) at 5 + 4 weeks of gestation in HRT-FET cycles after administration of dydrogesterone.

No consensus has been reached on estimated serum progesterone cut-off concentrations. Consequently, different calculation methods have been used, e.g. lowest quartile or median of serum progesterone concentrations and the correlation to reproductive outcomes, sensitivity analysis in relation to reproductive outcomes and progesterone concentrations correlated to the highest sensitivity and specificity of a specific reproductive outcome (*Yovich et al., 2015*; *Alsbjerg et al., 2018*; *2020*; *Gaggiotti-Marre et al., 2018*; *Álvarez et al., 2021*; *Yarali et al., 2021*; *Labarta et al., 2022*). In the present study, the Youden Index was introduced, which is an objective statistical method to detect a cut-off level of a test in relation to the highest possible specificity and sensitivity, in which specificity and sensitivity are equally weighted. Although the Youden Index, as with all other performance measures, does not include or take into account other criteria, such as efficiency and potential misclassification of the test, it is indeed objective.

A strength of the present study is that it was a single centre study conducted in a population of endometriosis patients only, diagnosed by either laparoscopy or ultrasound (endometriomas). As in any cohort study, data were prospectively registered. A major limitation relates to the fact that the estimated luteal serum cut-off concentration for progesterone of 118 nmol/l (37.1 ng/ml) applies only to endometriosis patients treated with a combination of vaginal progesterone for 4 days and intramuscular progesterone 50 mg from the fourth progesterone day. Whether the present cut-off applies to other progesterone regimens needs to be explored.

In conclusion, we herein reported a cut-off concentration of 118 nmol/l or above (37.1 ng/ml) for luteal progesterone in endometriosis patients undergoing HRT-FET with intensive LPS. This concentration is significantly higher compared with the non-endometriosis IVF patient. From a physiological point of view, this might be explained by progesterone resistance at the receptor concentration in the endometriosis patient. Future studies are needed to confirm the present findings before clinical recommendations can be made, and to explore differences in pharmacokinetics of the existing progesterone preparations used for LPS in HRT-FET.

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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.2022.09.005.

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