Rectal progesterone administration secures a high ongoing pregnancy rate in a personalized Hormone Replacement Therapy Frozen Embryo Transfer (HRT-FET) protocol - a prospective interventional study

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Running title (50 characters): Rectal administration of progesterone in HRT-FET.

Abstract

Study question

Can supplementation with rectal administration of progesterone secure high ongoing pregnancy rates (OPR) in patients with low serum progesterone (P4) on the day of blastocyst transfer (ET)?

Summary answer

Rectally administered progesterone commencing on the blastocyst transfer day secures high OPRs in patients with serum P4 levels below 35nmol/l (11ng/ml).

What is known already

Low serum P4 levels at peri-implantation in HRT-FET cycles impact reproductive outcomes negatively. However, studies have shown that patients with low P4 after a standard vaginal progesterone treatment can obtain live birth rates (LBR) comparable to patients with optimal P4 levels if they receive additional subcutaneous progesterone, starting around the day of blastocyst transfer. In contrast, increasing vaginal progesterone supplementation in low serum P4 patients does not increase LBR. Another route of administration rarely used in ART is the rectal route, despite the fact that progesterone is well absorbed and serum P4 levels reach a maximum level after approximately two hours.

Study design, size, duration

This prospective interventional study included a cohort of 488 HRT-FET cycles, in which a total of 374 patients had serum P4 levels ≥35nmol/l (11ng/ml) at ET, and 114 patients had serum P4 levels <35nmol/l (11ng/ml). The study was conducted from January 2020 to November 2022.

Participants/materials, setting, methods

Patients underwent HRT-FET in a public Fertility Clinic, and endometrial preparation included oral oestradiol (6mg/24hours), followed by vaginal micronized progesterone, 400mg/12hours. Blastocyst transfer and P4 measurements were performed on the 6th day of progesterone administration. In patients with serum P4 <35nmol/l (11ng/ml) “rescue” was performed by rectal

administration of progesterone (400mg/12hour) starting that same day. In pregnant patients, rectal administration continued until week 8 of gestation, and oestradiol and vaginal progesterone treatment continued until week 10 of gestation.

Main results and the role of chance

Among 488 HRT-FET single blastocyst transfers, the mean age of the patients at oocyte retrieval (OR) was 30.9 ±4.6 years and the mean BMI at ET 25.1 ±3.5 kg/m^2.

The mean serum P4 level after vaginal progesterone administration on the day of ET was 48.9 ±21.0nmol/l (15.4 ±6.6ng/ml), and a total of 23% (114/488) of the patients had a serum P4 level lower than 35nmol/l (11ng/ml). The overall, positive hCG rate, clinical pregnancy rate, OPR week 12, and total pregnancy loss rate were 66% (320/488), 54% (265/488), 45% (221/488), and 31% (99/320), respectively. There was no significant difference in neither OPR week 12 nor total pregnancy loss rate between patients with P4 ≥35nmol/l (11ng/ml) and patients with P4 <35nmol/l, who received rescue in terms of rectally administered progesterone, 45% vs. 46%, p=0.77 and 30% vs. 34%, p=0.53, respectively.

OPR did not depend on whether patients had initially low P4 and rectal rescue or were above the P4 cut-off. Logistic regression analysis showed that only age at OR and blastocyst scoring correlated with OPR week 12, independently of other factors like BMI and vitrification day of blastocysts (day 5 or 6).

Limitations, reasons for caution

In this study vaginal micronized progesterone (Cyclogest®), a solid pessary with progesterone suspended in vegetable hard fat, was used vaginally as well as rectally. It is unknown whether other progesterone pessaries could be used rectally with the same rescue effect.

Wider implications of the findings

A substantial part of HRT-FET patients receiving vaginal progesterone treatment has low serum P4. Adding rectally administered progesterone in these patients increases the reproductive outcome. Importantly, rectal progesterone administration is considered convenient, and progesterone pessaries are easy to administer rectally and of low cost.

Study funding/competing interest(s)

Gedeon Richter Nordic supported the study with an unrestricted grant as well as study medication.

Trial registration number (25)

EudraCT no.: 2019-001539-29

Key words

HRT-FET; low serum progesterone; vaginal progesterone; rectal progesterone, ongoing pregnancy

Introduction

A growing scientific evidence supports that the reproductive outcome depends on serum progesterone (P4) levels during the mid-luteal phase and early pregnancy in Hormone Replacements Therapy Frozen Embryo Transfer (HRT-FET). Recently, in a meta-analysis Melo et al. including HRT-FET cohort studies using vaginal progesterone only for luteal phase support (LPS) reported that cycles with P4 levels less than 32nmol/l (10ng/ml) had a significantly lower ongoing pregnancy rate (OPR) and live birth rate (LBR) compared to cycles with higher P4 levels. Furthermore, a significantly higher risk of early pregnancy loss was seen in patients with low luteal P4 levels (Melo *et al.*, 2021). The meta-analysis was based on studies where different cut-off levels of P4 were used. In a previous study, we based the P4 cut-off level on a sensitivity analysis of different P4 levels in relation to OPR. In that study 35nmol/l (11ng/ml) was defined as the most optimal cut-off level and OPR was significantly decreased if P4 was less than 35nmol/l (11ng/ml), OR=0.54, 95 % Confidence Interval (CI) [0.32;0.91], p=0.022, corresponding to a reduction in chance of an ongoing pregnancy of 14%, CI [-26;-2 %], *p*=0.024 (Alsbjerg *et al.*, 2018).
To increase the reproductive outcome different LPS rescue regimens have been applied. Thus, Cédrin-Durnerin et al. doubled the vaginal progesterone dose if P4 was less than 32nmol/l (10ng/ml) measured on the 2nd to 5th progesterone administration day. In their study, a total of 69% of patients reached P4 levels over 32nmol/l (10ng/ml) after doubling the vaginal progesterone dose; however, the LBR was significantly reduced compared to the LBR of patients with initially high P4 (17% vs. 31%, p=0.02) (Cédrin-Durnerin *et al.*, 2019). Subsequently, three studies added subcutaneous progesterone, 25 mg daily from the day of embryo transfer (ET) as a rescue regimen (Álvarez *et al.*, 2021; Yarali *et al.*, 2021; Labarta *et al.*, 2022). Álvarez et al., Yarali et al. and Labarta et al. used all different cut-off levels 34nmol/l (10.6ng/ml), 27.8nmol/l (8.75ng/ml) and 29nmol/l (9.2ng/ml), respectively. Interestingly, no significant difference in reproductive outcomes were reported in either of these studies. The LBR´s between the high progesterone group compared to the low progesterone rescue group in two of the studies were, RD=-3.2% (95% CI [-12; 5.7]) and adjusted OR=0.99 (95% CI [0.79-1.25]) (Álvarez *et al.*, 2021; Labarta *et al.*, 2022). Meaning that additional progesterone administration from the time of blastocyst transfer increased the LBR in patients with an initial low P4 after a standard vaginal

progesterone LPS. Interestingly, some studies have suggested that too high mid-luteal P4 levels might decrease reproductive outcomes, in support of a ceiling effect of P4 (Yovich *et al.*, 2015; Alyasin *et al.*, 2021; Alsbjerg *et al.*, 2020).

Progesterone is usually administered vaginally, subcutaneously, orally or intramuscularly, but can also be administered rectally. A few studies used rectally administered progesterone, but until now never as a rescue regimen in HRT-FET cycles (Nillius and Johansson, 1971; Chakmakjian and Zachariah, 1987; Aghsa *et al.*, 2012; Alsbjerg *et al.*, 2020).

The aim of the present study was to investigate the effect on the OPR by administering additional progesterone rectally in HRT-FET cycles in patients with mid-luteal P4 levels lower than 35nmol/l (11ng/ml) after a standard vaginal progesterone regimen.

Materials and methods

Study design

A prospective interventional study based on serum P4 levels on the day of blastocyst transfer.

Participants

The study population was recruited at The Fertility Clinic, Skive Regional Hospital, a public fertility clinic in Denmark from January 2020 to November 2022.

The inclusion criteria were: age between 18 and 45 years with at least one autologous vitrified blastocyst for transfer and body mass index (BMI) >18.5 <34 kg/m². Exclusion criteria were an endometrial thickness <7 mm after 12-20 days of 6 mg estradiol treatment, no blastocyst for transfer after thawing, uterine abnormalities, oocyte donation and dysregulated severe chronical medical diseases.

In total 488 patients participated in the study, of whom 374 patients had a serum P4 level ≥35nmol/l (11ng/ml) at the blastocyst transfer day, and 114 patients had a serum P4 level <35nmol/l (11ng/ml). See study flow chart figure 1.

Treatment protocols

Endometrial preparation was performed using 6 mg oestradiol valerate daily starting from the second day of the cycle. An ultrasound examination was performed after 12-20 days of treatment and in patients with an endometrial thickness ≥7 mm and quiescent ovaries, treatment with vaginal micronized progesterone 400 mg Cyclogest® (7 am and 7 pm) was initiated. Blastocyst transfer was scheduled for the 6th day of vaginal progesterone treatment, and on this day serum P4 was measured in a standardized manner two hours after vaginal progesterone administration. Patients were allocated to one of two groups depending on the serum progesterone levels; less than 35nmol/l (11ng/ml) or ≥35nmol/l (11ng/ml).

If **serum P4 was <35nmol/l** (11ng/ml) (study group) the vaginal progesterone regimen was supplemented with additional 400 mg Cyclogest® bid (7 am and 7 pm) administered rectally starting on the evening of the day of transfer. In case of a positive pregnancy test patients continued their rectal rescue LPS regimen (2 vaginal +2 rectal suppositories) until gestational week 8+0. If the ultrasound examination visualised an intrauterine viable pregnancy, the vaginal progesterone administration continued alongside 6 mg oestradiol valerate until gestational week 10+0.

If **serum P4 was ≥35nmol/l** (11ng/ml) on the day of blastocyst transfer, the standard LPS regime continued and in pregnant patients all LPS was discontinued at gestational week 10+0. Pregnancy scan was performed in gestational week 8 and week 12.

An ongoing pregnancy was defined as a viable pregnancy at the ultrasound scan performed at gestational week 12.

Study flow diagram are shown in figure 2.

Embryos and embryo transfer

Double embryo transfer (DET) was permitted according to the protocol; however, only two DET were performed. Four hundred and eighty six were Single Embryo Transfer (SET) and all blastocyst transfers were autologous blastocysts vitrified on day 5 or 6, using the 'Cryotec method' by Masashige Kuwayama (Gandhi *et al.*, 2017). Blastocysts were scored according to the Gardner and Schoolcraft grading system (Gardner and Schoolcraft, 1999) and blastocyst transfer was scheduled 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 and no poor-quality blastocysts (score 3) were transferred.

Blood sampling and hormone analyses

Blood sampling was carried out in a standardized way on the blastocyst transfer day (9 am to 11 am) two to four hours after vaginal progesterone administration. Pregnancy testing was performed 9-11 days after ET at a random time for patient convenience and serum P4 was included in the analysis. Serum P4 levels were analysed using direct chemiluminecent tecnology (Atellica, Siemens), routinely used for analysis at the local department of biochemistry. All measurements were performed according to the manufacturer's instructions. The assay provided results from 1.0-1908nmol/l and was designed to have a within-laboratory precision of ±12% (2 Coefficient of Variation) for samples level 6.3nmol/l and ±8% (2 CV) at samples level 39nmol/l. All blood samples were analysed for progesterone immediately.

Sample size calculation

The power calculation was based on previous studies reporting that about half of patients had serum P4 levels less than 35nmol/l following two different standard vaginal LPS regimens (Labarta *et al.*, 2017; Alsbjerg *et al.*, 2018). Furthermore, we previously reported an ongoing pregnancy rate of 51% in the ≥35nmol/l group and 38% in the <35nmol/l group if no additional progesterone was administered in HRT-FET (Alsbjerg *et al.*, 2018).

Based on the assumption that progesterone supplementation in the low P4-group would increase from 38% to 51%, a Power (1-β) of 80%, α = 5%, one-sided test, a sample of 112 participants would be required in each of the groups.

The one-side test was chosen given that increasing the progesterone dose by rectal administration would increase the serum level, and hence the pregnancy rate would either remain the same or increase. Furthermore, increasing the serum progesterone from ET day has shown to be beneficial (Álvarez *et al.*, 2021; Yarali *et al.*, 2021; Labarta *et al.*, 2022).

In total, 224 patients were needed with 112 in the low P4 group (<35nmol/l). Two patients with P4 <35nmol/l (11ng/ml) violated the protocol; one did not administer rectally progesterone at all and the other did not administer rectally progesterone for one week (gestational week 5).

Consequently, the total number of patients with P4 less than 35nmol/l was increased to 114. All 488 patients enrolled were included in the final analysis.
The following assumptions were made: very limited loss to follow-up, near full compliance to study medication and homogeneity in the treatment effect.

Statistical methods

Normality was evaluated using quantile-quantile plots, and the assumption of equal variance was tested using the F-test. Fisher´s exact, chi-squared and t-test were used as appropriate.
Furthermore, a logistic regression model was used to adjust for potential confounders.
All statistical analyses were performed using STATA version 13, StataCorp LLC, USA.

Ethics

Approval by the Regional Ethical Committee, the Danish Data Protection and the Danish Medicines Agency was given on the 10th of October 2019. The study was registered in EudraCT no.: 2019-001539-29. Furthermore, the trial was monitored by the Good Clinical Practice (GCP) unit at Aarhus University with GCP protocol number: 787/2019.
All participating patients signed a letter of consent before enrolment in the study and no patients withdrew consent during the study.

Results

Patients characteristics

Among the 488 HRT-FET cycles, the mean age of the patients at oocyte retrieval (OR) was 30.9 ±4.6 years and the mean BMI at ET 25.1 ±3.5 kg/m^2. Patients in the rescue group (2+2) had higher BMI (24.8 ±3.5kg/m^2 vs 26.0 ±3.4, p=0.65); however, this difference was not significant.
The mean serum P4 level on the day of blastocyst transfer was 48.9 ±21.0nmol/l (15.4 ±6.6ng/ml). A total of 23% (114/488) of the patients had a serum P4 level lower than 35nmol/l (11ng/ml). The 90% centile on the ET day was 73nmol/l (23ng/ml) and only 12 patients (2%) had P4 >100nmol/l (31.4ng/ml).

Single embryo transfer (SET) was performed in 486 cycles (99.6%). Significantly more anovulation/oligoovulation patients were seen in the 2+2 group; 21% (24/114) compared to 9% (35/374) in the standard group (p=0.001). For further characteristics see table 1.
No adverse events were reported during the study.

Reproductive outcomes

The overall, positive hCG rate, clinical pregnancy rate, OPR week 12 and total pregnancy loss rate were 66% (320/488), 54% (265/488), 45% (221/488), and 31% (99/320), respectively.
There was no significant difference in OPR week 12 between the standard group and the 2+2 group 45% (168/374) vs. 46% (53/114) p=0.77, respectively. Neither were differences seen as regards total pregnancy loss 30% vs. 34% (p=0.53) nor biochemical pregnancy loss 18% vs. 16% (p=0.73)
A logistic regression analysis adjusting for Body Mass Index, age at OR, day of vitrification and blastocyst score showed that OPR did not depend on whether patients had initially low P4 and were rescued with rectal progesterone or were above the P4 cut-off, OR 1.06 (95% CI [0.68 - 1.64] p=0.80). Age at OR and blastocyst score correlated with OPR week 12, OR 0.95 (95% CI [0.91 – 0.99] p=0.03) and OR 0.51 (95% CI [0.33 – 0.77] p<0.01) See Table 2.

Discussion

This prospective interventional study in HRT-FET shows that additional rectal administration of progesterone in patients with P4 <35nmol/l after a standard vaginal administration rescued the luteal phase and resulted in a non-significant difference in OPR week 12 in patients below and above the cut-off level of ≥35nmol/l (11ng/ml).
It has become evident that a substantial part of all HRT-FET cycles treated with a standard vaginal progesterone end up with an insufficient luteal phase when evaluated by serum P4 measurement (Melo *et al.*, 2021); even though, serum P4 must be regarded as a proxy marker of the endometrial micro-environment (Labarta *et al.*, 2021). In the present study 35nmol/l (11ng/ml) was chosen as the optimal P4 cut-off level as this was the result of a sensitivity analysis, comparing different cut-off levels and their relation to ongoing pregnancy in a previous study (Alsbjerg *et al.*,

2018). Furthermore, the cut-off level is in accordance with a recent meta-analysis showing a significantly higher LBR/OPR using cut-off levels between 32 and 64nmol/l (10-20ng/ml) (Melo *et al.*, 2021).

The 2+2 rescue regimen used in the present study showed a non-significant difference in OPR after rescue between patients with P4 levels above and below above 35nmol/l and our results are consistent with the results reported by Álvarez et al. (2021) and Labarta et al. (2022), using 25 mg progesterone SC daily as a rescue regimen in patients with P4 levels lower than 33.7nmol/l (10.6ng/ml) and 29.3 (9.2ng/ml); respectively. The percentage of patients needing recue was 39% and 25% in those studies. In the studies the dosing of micronized vaginal progesterone was different (200mg/8h vs 400mg/12h) and, furthermore, the cut-off levels were defined differently as one study used the median level (Álvarez *et al.*, 2021) and the other used the lower quartile (Labarta *et al.*, 2022). This might explain the difference in patients needing rescue. Compared to the standard vaginal progesterone dosing regimen and a cut-off level of 35nmol/l (11ng/ml) used in our study, only 23% of patients needed rescue. The advantages of the 2+2 rescue regimen are that patients already have the medication at home, the cost is lower compared to SC progesterone, some patients find the rectal route with less side-effects compared to the vaginal route and they avoid taken injections.

Pregnancy loss after HRT

The risk of pregnancy loss is decreased in HRT-FET cycles if serum P4 is optimal. Based on a meta-analysis Melo et al. reported a risk ratio of 0.62 (95% CI [0.50 – 0.77]) for miscarriage if serum P4 was higher than 32nmol/l (10ng/ml). Interestingly, a previous study highlighted that the risk of miscarriage was higher in HRT-FET cycles compared to the true Natural cycle (t-NC) with LPS and the modified natural cycle (m-NC) triggered with HCG; the analysis included a total of 4474 FET cycles and the total pregnancy loss rates were 41.5%, 22.4% and 33.6% ($p<0.0001$), for HRT-FET, true natural cycle and modified natural cycle, respectively (Tomas *et al.*, 2012). Ten years ago luteal phase serum P4 levels where not routinely measured which might explain the higher pregnancy loss as the standard LPS used in the Tomas et al. cohort was vaginal micronized progesterone 90 mg twice daily which we subsequently learned results in a mean serum P4 level of 24.2 ±10.1nmol/l (7.6 ± 3.2 ng/ml) (Alsbjerg *et al.*, 2021). In the present study no significant

difference was seen in total pregnancy loss rate between the patient group with P4 ≥35nmol/l (11ng/ml) and the low P4 rescue group 30% vs 34% (p=0.53), respectively.
Whether a further decrease in pregnancy loss rate is achievable in a cohort of un-screened blastocysts is unknown. As In comparison Gaggiotti-Marre et al. found an overall miscarriage rate of 13.5% in a cohort of tNC FET with no LPS; however, in that study 29% of the blastocyst were euploid (PTG-A screened) (Gaggiotti-Marre *et al.*, 2018).

Is there a risk of too high luteal phase P4 levels in HRT-FET?

A few studies previously suggested that too high mid-luteal P4 levels in HRT-FET negatively impacted reproductive outcomes. Thus, Yovich et al. reported that, although not significant, patients with P4 levels higher than 100nmol/l (31.4ng/ml) treated with progesterone 400mg/8h had a lower LBR compared to patients with P4 between 70 and 100 nmol/l (22-31ng/ml) (Yovich *et al.*, 2015). Similarly, Alsbjerg et al. reported a significant decrease in OPR in patients with P4 levels higher than 45nmol/l (14ng/ml) (Alsbjerg *et al.*, 2020). The same negative influence of high P4 was reported by Alyasin et al. using a combined LPS, including vaginal as well as and intramuscular (IM) progesterone. In that study, the LBR was significantly lower in the highest quartile group (Q4) compared with the lowest quartile group (Q1).
Common for these three studies is a high starting and continued progesterone dose. Based on this it can be hypothesized that the starting progesterone dose is critical in order to avoid advancement of the window of implantation and; consequently, a suboptimal implantation. In contrast, once the embryo implanted, the P4 level should be sufficiently high to secure growth and development as the typical pattern of low luteal P4 is a high biochemical pregnancy loss rate as seen in both fresh transfer IVF and HRT-FET (Humaidan *et al.*, 2005; Alsbjerg *et al.*, 2013).
The standard vaginal progesterone regimen used in the present HRT-FET protocol ensured that 77% of patients had a P4 level >35nmol/l (11ng/ml) and only 12 patients (2%) had P4 levels >100nmol/l on the day of ET (31.4ng/ml), previously shown to be “too high” (Yovich *et al.*, 2015). Interestingly, nine of these patients did not achieve an OP week 12; five with negative pregnancy test, two with biochemical pregnancy loss and two with clinical pregnancy loss; though, this was not significant.

Parameters influencing P4 levels

Cédrin-Durnerin et al. in a retrospective analysis showed that doubling the vaginal progesterone dose in patients with P4 <10 ng/ml (31.8nmol/l) on the ET day did not increase live birth rates. Furthermore, the mean serum progesterone in the low P4 group was the same before and after increasing the vaginal progesterone dose (Cédrin-Durnerin *et al.*, 2019). Thus, it might be hypothesized that after a certain progesterone dose, the maximal vaginal absorption capacity is reached and that additional administration will not increase P4 levels further. However, this vaginal absorption capacity might be different from patient to patient and importantly, will differ between vaginal products.

Serum P4 levels fluctuate in relation to time of administration and consequently blood sampling time in the present cohort was strictly standardized from two to four hours after the administration of vaginal progesterone. The P4 level during this time frame for most patients reflects the highest P4 level (Duijkers *et al.*, 2018). However, it has never been studied whether this is the best prognostic serum P4 value as regards to reproductive outcomes, compared to the P4 level just before administration which mirrors the lowest P4 level.

It has been shown that BMI, age, a history of low P4 and the time of blood sampling in relation to the administration influence the P4 levels (González-Foruria *et al.*, 2020). In another cohort reported by Maignien et al. parity and a non-European geographic origin were also associated with low P4 levels. Furthermore, active smoking appears to be associated with higher P4 levels which may be explained by decreased metabolic clearance of steroid hormones (Maignien *et al.*, 2022). In the present cohort, significantly, more patients were anovulatory in the 2+2 group compared to the standard group (21% vs 9%). One explanation for the uneven distribution between groups may be that patients with anovulation tend to have higher weight and BMI resulting in lower P4. We found that patients with anovulation in the 2+2 group weighed 3.6 ±3.0 kg more and had a 1.2 ±0.9 kg/m^2 higher BMI than patients with anovulation in the non-rescue group, though, these differences were not significant.

Power calculation

The total number enrolled in this study was 488 patients, although, it was originally powered to 224 patients of which 112 would have P4 <35nmol/l (11ng/ml). The explanation is that the power

calculation was based on another standard vaginal progesterone regimen in which 50% of patients had P4 levels <35nmol/l (11ng/ml) (Alsbjerg *et al.*, 2018). The vaginal progesterone product used in the present study (Cyclogest® 400 mg/12hours) resulted in a mean serum P4 of 48.9 ±21.0nmol/l (15.4 ±6.6ng/ml) and only 23% of patients had P4 levels <35nmol/l (11ng/ml). Consequently, more patients were enrolled to include enough patients to the rescue group.

Limitations

Formulations of vaginal micronized progesterone products differ, as progesterone can be suspended in vegetable hard fat, in a vegetable lipophile liquor, are prepared in an oil-in-water emulsion carrier, or mixed in a tablet. The product used in this study was suspended in vegetable hard fat, and it is unknown whether other products would have the same effect when administered rectally.

Due to national clinical guidelines recommending weight restriction for IVF patients no patients with BMI more than 34 kg/m^2 were included; consequently, it is unknown whether patients with higher BMI will benefit from the rectal rescue regimen.

Future research

There are still a number of unanswered questions regarding the most optimal HRT-FET protocol, and individualization of the luteal phase support has only just begun. Sub-groups of patients may need higher luteal P4 levels for successful implantation as recently shown for the endometriosis/adenomyosis patient (Alsbjerg *et al.*, 2023). Furthermore, it needs to be clarified at which time point P4 should be measured to obtain the best predictive value. Vaginal progesterone products may also need to be re-evaluated to ensure the best dosing regimen for each product.

Conclusion

A substantial part of HRT-FET patients receiving vaginal progesterone treatment has low luteal serum P4. Rectal rescue in these patients secures the reproductive outcome.

Author's roles

BA and PH have planned and designed the study. MBJ, BBP, RJ, HOE, BA and PH conducted the

study. BA and USK prepared the statistics and all authors participated in drafting the manuscript and accepted the final version.

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Conflict of interest

BA have received unrestricted grant from Gedeon Richter Nordic and Merck and honoraria for lectures from Gedeon Richter, Merck, IBSA and Marckyrl Pharma. PH have received honoraria for lectures from Gedeon Richter, Merck, IBSA and USK has received grant from Gedeon Richter Nordic, IBSA and Merck for studies outside this work and honoraria for teaching from Merck and Thillotts Pharma AB. The other co-authors have none conflict of interest to declare.

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Figure 1. Study flow diagram.

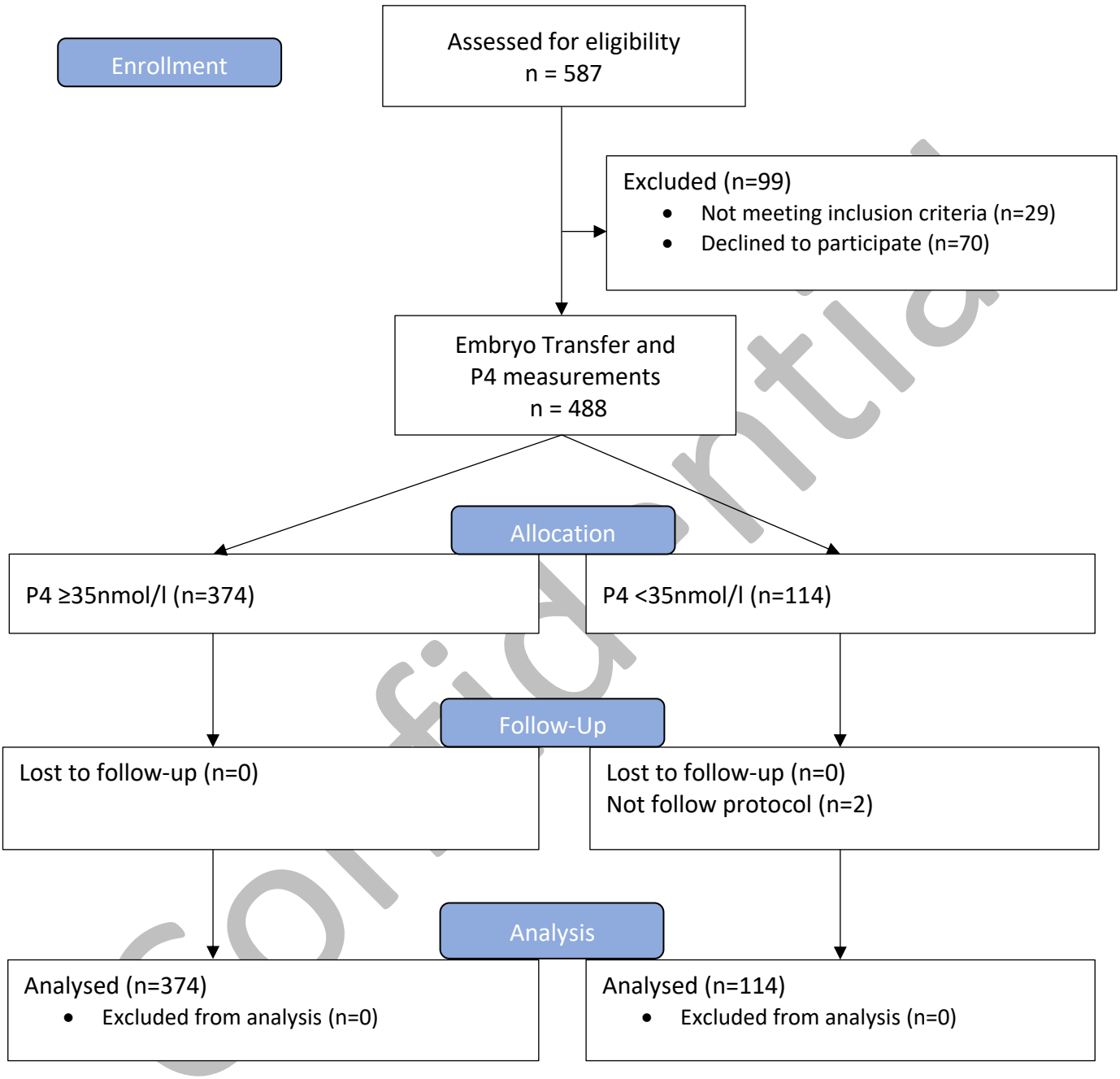

Figure 2. Study flow diagram

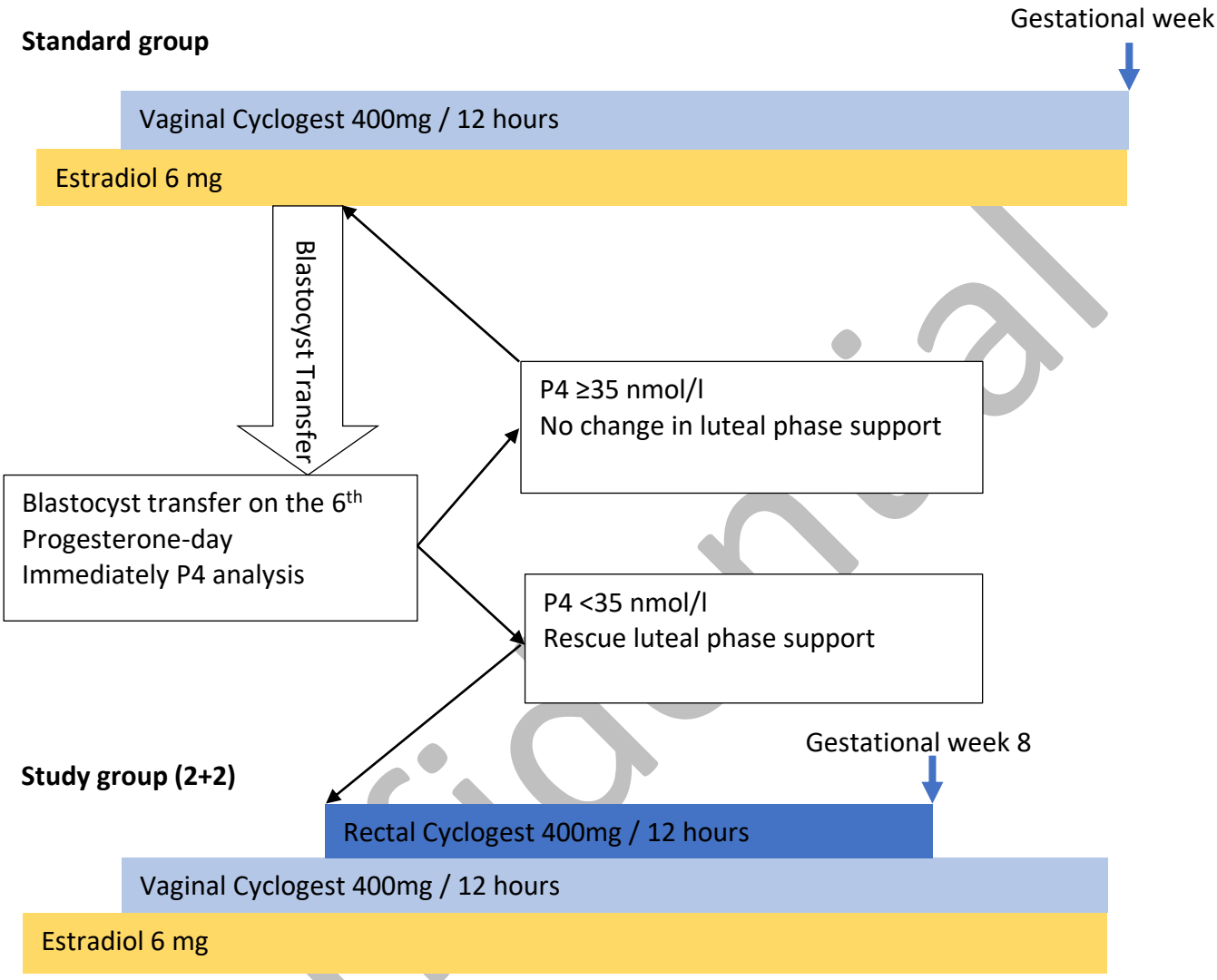

Table 1: Basic characteristics of patients.

	all	Standard P4 ≥35nmol/l	2 + 2 P4 <35nmol/l	p-value
Cycles, n	488	374	114	
Serum P4 ET-day, mean (±SD)	48.9 (21.0)	55.1 (20.0)	28.3 (4.4)	
Serum P4 hCG-test day, mean (±SD)	51.6 (26.3)	46.4 (19.5)	69.2 (36.7)	
Age OR, years (±SD)	30.9 (4.6)	30.9 (4.3)	30.9 (4.6)	0.46
Age ET, years (±SD)	31.5 (4.4)	31.4 (4.4)	31.6 (4.7)	0.39
Body mass index, kg/m^2 (±SD)	25.1 (3.5)	24.8 (3.5)	26.0 (3.4)	0.65
Smoking, n (%)	6 (1.2)	4 (1.0)	2 (1.8)	0.56
Single embryo transfer, n (%)	486 (99.6)	372 (99.5)	114 (100)	0.43
Developmental stage				0.82
Day 5 blastocyst transfer, n (%)	425 (86.9)	325 (86.6)	100 (87.7)	
Day 6 blastocyst transfer, n (%)	63 (12.9)	49 (13.1)	14 (12.3)	
Insemination method				0.81
IVF, n (%)	222 (45.5)	169 (45.2)	53 (46.5)	
ICSI, n (%)	266 (54.5)	205 (54.8)	61 (53.5)	
No. of cycles with at least one high quality blastocyst[1], n (%)	345 (70.7)	263 (70.3)	82 (71.9)	0.71
Primary diagnosis				<0.01
Anovulation, n (%) (1)	59 (12)	35 (9)	24 (21)	
Idiopathic, n (%) (11)	133 (27)	103 (28)	30 (26)	
Male factor, n (%) (5)	186 (38)	146 (39)	40 (35)	
Tubal factor, n (%) (3)	34 (7)	32 (9)	2 (2)	
Single, n (%) (8)	54 (11)	42 (11)	12 (11)	
Others[2], n (%) (12)	22 (5)	16 (4)	6 (5)	

Table 2: Reproductive outcome

	all	Standard P4 >35nmol/l	2 + 2 P4 <35nmol/l	p-value
Pregnancy per ET, n (%)	320 (66)	240 (64)	80 (70)	0.29
Clinical pregnancy per ET, n (%)	265 (54)	198 (53)	67 (59)	0.27
Ongoing week 12 per ET, n (%)	221 (45)	168 (45)	53 (46)	0.77
Ongoing gemelli week 12, n (%)	1	1	0	
Total pregnancy loss, n (%)	99 (31)	72 (30)	27 (34)	0.53
Biochemical pregnancy loss, n (%)	56 (18)	43 (18)	13 (16)	0.73

Table 3: Logistic regression analysis of the association between group and chance of an ongoing pregnancy week 12, adjusted for Body Mass Index, age at oocyte retrieval (OR), day of vitrification and blastocyst score.

Characteristics	Odds ratio	95% CI	p-value
Group			
Standard group	1		
2+2 group	1.06	0.68 - 1.64	0.80
Body mass index	0.98	0.93 - 1.04	0.58
Age at OR, years	0.95	0.91 – 0.99	0.03
Day of vitrification			
5	1		
6	0.60	0.33 – 1.11	0.10
Blastocyst score, quality			
High	1		
Medium	0.51	0.33 – 0.77	<0.01

OR= oocyte retrieval