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ORIGINAL ARTICLE *Infertility*

Individualised luteal phase support in artificially prepared frozen embryo transfer cycles based on serum progesterone levels: a prospective cohort study

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STUDY QUESTION: Does an individualised luteal phase support (iLPS), according to serum progesterone (P4) level the day prior to euploid frozen embryo transfer (FET), improve pregnancy outcomes when started on the day previous to embryo transfer?

SUMMARY ANSWER: Patients with low serum P4 the day prior to euploid FET can benefit from the addition of daily subcutaneous P4 injections (Psc), when started the day prior to FET, and achieve similar reproductive outcomes compared to those with initial adequate P4 levels.

WHAT IS KNOWN ALREADY: The ratio between FET/IVF has spectacularly increased in the last years mainly thanks to the pursuit of an ovarian hyperstimulation syndrome free clinic and the development of preimplantation genetic testing (PGT). There is currently a big concern regarding the endometrial preparation for FET, especially in relation to serum P4 levels around the time of embryo transfer. Several studies have described impaired pregnancy outcomes in those patients with low P4 levels around the time of FET, considering 10 ng/ml as one of the most accepted reference values. To date, no prospective study has been designed to compare the reproductive outcomes between patients with adequate P4 the day previous to euploid FET and those with low, but restored P4 levels on the transfer day after iLPS through daily Psc started on the day previous to FET.

STUDY DESIGN, SIZE, DURATION: A prospective observational study was conducted at a university-affiliated fertility centre between November 2018 and January 2020 in patients undergoing PGT for aneuploidies (PGT-A) IVF cycles and a subsequent FET under hormone replacement treatment (HRT). A total of 574 cycles (453 patients) were analysed: 348 cycles (leading to 342 euploid FET) with adequate P4 on the day previous to FET, and 226 cycles (leading to 220 euploid FET) under iLPS after low P4 on the previous day to FET, but restored P4 levels on the transfer day.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Overall we included 574 HRT FET cycles (453 patients). Standard HRT was used for endometrial preparation. P4 levels were measured the day previous to euploid FET. P4 > 10.6 ng/ml was considered as adequate and euploid FET was performed on the following day (FET Group 1). P4 < 10.6 ng/ml was considered as low, iLPS was added in the form of daily Psc injections, and a new P4 analysis was performed on the following day. FET was only performed on the same day when a restored P4 > 10.6 ng/ml was achieved (98.2% of cases) (FET Group 2).

MAIN RESULTS AND THE ROLE OF CHANCE: Patient's demographics and cycle parameters were comparable between both euploid FET groups (FET Group 1 and FET Group 2) in terms of age, weight, oestradiol and P4 levels and number of embryos transferred. No statistically significant differences were found in terms of clinical pregnancy rate (56.4% vs 59.1%: rate difference (RD) −2.7%, 95% CI [−11.4; 6.0]), ongoing pregnancy rate (49.4% vs 53.6%: RD −4.2%, 95% CI [−13.1; 4.7]) or live birth rate (49.1% vs

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52.3%: RD −3.2%, 95% CI [−12; 5.7]). No significant differences were also found according to miscarriage rate (12.4% vs 9.2%: RD 3.2%, 95% CI [−4.3; 10.7]).

LIMITATIONS, REASONS FOR CAUTION: Only iLPS through daily Psc was evaluated. The time for Psc injection was not stated and no serum P4 determinations were performed once the pregnancy was achieved.

WIDER IMPLICATIONS OF THE FINDINGS: Our study provides information regarding an 'opportunity window' for improved ongoing pregnancy rates and miscarriage rates through a daily Psc injection in cases of inadequate P4 levels the day previous to FET (P4 < 10.6 ng/ml) and restored values the day of FET (P4 > 10.6 ng/ml). Only euploid FET under HRT were considered, avoiding one of the main reasons of miscarriage and implantation failure and overcoming confounding factors such as female age, embryo quality or ovarian stimulation protocols.

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Key words: frozen embryo transfer / progesterone / euploid embryo / hormone replacement treatment / preimplantation genetic testing

Introduction

Frozen embryo transfer (FET) is increasingly adopted in modern IVF. The ratio between FET and fresh embryo transfer in ART cycles has increased both in Europe and USA: from 28% to 40.3% (2010–2015) and from 22.9% to 69.4% (2010–2017), respectively (De Geyter *et al.*, 2018; ART Success Rates | CDC, 2020). Among the many factors that have contributed to such change, the pursuit of an ovarian hyperstimulation syndrome free clinic has been determinant. Improvements in the vitrification and warming processes and the excellent cryosurvival rates have turned FET in our main tool for preventing this complication (Devroey *et al.*, 2011). Moreover, a freeze all strategy has proven to provide excellent or even better pregnancy rates (PRs), not only in high (Chen *et al.*, 2016) but also in normal responders (Shi *et al.*, 2018; Vuong *et al.*, 2018; Wei *et al.*, 2019; Stormlund *et al.*, 2020). Furthermore, techniques such as preimplantation genetic testing (PGT) have also highly benefited from FET, in which the preimplantation embryo is ideally biopsied at the blastocyst stage and subsequently vitrified to allow for chromosomal analysis (Rodriguez-Purata *et al.*, 2016; Sermon *et al.*, 2016).

While ART have rapidly evolved in the areas of embryo culture, vitrification and understanding of the embryo development, little progress has been achieved regarding endometrial preparation for FET. Undoubtedly, correct implantation requires a good quality embryo and a suitable decidualised endometrium. In order to achieve an adequate environment for implantation, endometrial transformation for FET can be achieved through a natural cycle (NC-FET) or an artificial preparation (AC-FET). Artificial cycles require hormone replacement treatment (HRT) with oestradiol and progesterone (P4). However, there is not a single standardised treatment described for optimal endometrial preparation and no protocol has proven superiority in terms of reproductive outcomes (Ghobara *et al.*, 2017; Groenewoud *et al.*, 2018).

Although artificial preparation is the most convenient method to schedule FET cycles, recent reports have highlighted a potentially detrimental effect of low P4 levels prior to FET on miscarriage and live birth rates (LBRs). These results have been observed both in homologous and oocyte recipient FET cycles (Labarta *et al.*, 2017; Cédrin-Durnerin *et al.*, 2019; Volovsky *et al.*, 2020), but also in FET cycles of embryos that had undergone PGT for aneuploidies (PGT-A) (Gaggiotti-Marre *et al.*, 2019).

Nonetheless, despite the accumulating reports on the value of pre-transfer P4 levels on pregnancy outcomes, to our knowledge, no prospective study has been published up to date aiming at overcoming this risk factor. Additional P4 supplementation may be a way to improve reproductive outcomes in these patients. The current prospective study aims to investigate whether patients with low serum P4 levels the day before euploid FET under standard HRT can benefit in terms of ongoing pregnancy and miscarriage rates (MRs) from an individualised luteal phase support (iLPS) consisting in the addition of a daily subcutaneous P4 injection (Psc).

Materials and methods

Study setting

A prospective observational study was performed at a university-affiliated fertility centre between November 2018 and January 2020 in patients undergoing PGT-A IVF cycles and a subsequent FET under HRT.

The www.clinicaltrials.gov registration number is NCT03740568.

Sample size calculation

Sample size calculation was based on previous studies (Alsbjerg *et al.*, 2018; Cédrin-Durnerin *et al.*, 2019; Gaggiotti-Marre *et al.*, 2019), according to which the estimated percentage of patients with low progesterone levels that needed Psc supplementation was 46%. The study hypothesis was that the ongoing pregnancy rate (OPR) in the group with normal P4 levels would be 54%, equivalent to the group with low P4 levels receiving Psc. Based on this assumption we calculated that, by using a two-sided 95% confidence interval in an equivalence study design, at least 592 patients (46% in the supplementation group and 54% in the standard group) are needed in order to exclude a difference between the standard and supplemental groups, with an

equivalence limit set at the level of 10%, which we considered clinically relevant.

Endpoints

The primary endpoint in this study is to compare OPR, defined as the ultrasound confirmation of a foetus with heart activity beyond 12 weeks of pregnancy per transfer, between patients with adequate P4 before FET under standard HRT to those with initial low P4 before FET and restored value after additional P4 supplementation through a daily Psc injection (iLPS).

Pregnancy rate (PR) (defined as a rise in serum beta hCG concentration >25 UI/L per transfer), clinical pregnancy rate (CPR) (defined as the presence of at least one gestational sac in ultrasound per transfer) and MR (defined as the spontaneous loss of an intra-uterine pregnancy prior to 12 completed weeks of gestational age) between both groups were considered as secondary endpoints. Biochemical pregnancy rate (BP), defined as a pregnancy diagnosed only by the detection of beta hCG in serum per transfer, LBR, defined as the number of deliveries that resulted in a live born neonate per transfer, were also included in the analysis. We also considered as secondary endpoints the % of rescued cycles (defined as cycles were a normal P4 level was achieved after iLPS) and percentage of cancelled FET due to lack of response to iLPS (defined as cycles were a normal P4 level was not achieved after iLPS).

Study protocol

Both ovarian stimulation protocols and PGT-A technique have been previously described elsewhere. Briefly, ovarian stimulation was performed under gonadotrophins and pituitary suppression with gonadotrophin-releasing hormone analogues (agonists or antagonists) according to established standard protocols (Alvarez *et al.*, 2019). Mature oocytes were microinjected 40 h after hCG or GnRH agonist trigger, upon indication. Embryos were cultured in a time-lapse incubator (Geri®, Merck, Germany) using single-step culture media (G-TL™, Vitrolife, Sweden). All developing embryos on Day 3 had their zona pellucida opened. Hatching blastocysts were biopsied using laser thermolysis (Veiga *et al.*, 1997) and vitrified immediately afterwards using Kitazato methodology (Kitazato Medical Group, Japan). Preimplantation genetic testing aneuploidies analysis was performed by next generation sequencing using the VeriSeq™PGS—MiSeq® platform from Illumina® (USA) following the manufacturer's protocols and guidelines. Embryo quality and grading is determined by morphologic and development criteria (ASEBIR, 2015). Euploid embryos were transferred in a subsequent cycle (Parriego *et al.*, 2007).

Endometrial preparation

Hormonal replacement under standard protocol (Martínez *et al.*, 2011) was used for endometrial preparation and FET. In brief, patients underwent treatment with either 2 mg/8 h oral oestradiol (E2) valerate (Progynova®, Schering, Spain) or 150 µg every 3 days transdermal patches (Evopad®, Janssen-Cilag, Spain) for 12–14 days. Vaginal micronized P4 treatment at 200 mg/8 h was started from the night of Day 15 (D0) until the day of plasma β-hCG determination (D14). The day prior to FET (D4) a vaginal ultrasound to assess endometrial thickness and a blood analysis for E2 and P4 were performed.

Serum analysis

Blood samples were obtained and processed in our laboratory for E2 and P4 measurements, using an electrochemiluminescence immunoassay (Cobas® e-411 analyser, Roche diagnostics, Germany). For E2, the lower limit of detection was 5 pg/ml with intra and interassay variation of 2.4–4.6% and 4.3–9.9%, respectively. For P4, the lower limit of detection was 0.03 ng/ml, with intra and interassay variation of 1.5–2.7% and 3.7–5.5%, respectively.

Patient selection

Only patients undergoing FET of an euploid blastocyst between the established time period were included. Patients who underwent mosaic FET and those who did not follow our standard supplementation protocol were excluded.

All patients undergoing FET of an euploid embryo were prospectively followed up and categorized into two groups according to their serum P4 values one day before FET: low P4 (<10.6 ng/ml) and adequate P4 (>10.6 ng/ml) (Fig. 1). The cut-off value to define low and adequate progesterone was stated at 10.6 ng/ml, in relation to a previous retrospective study (Gaggiotti-Marre *et al.*, 2019) in which 244 euploid FET were included under HRT, and patients with serum P4 < 10.6 ng/ml the day before FET had significantly higher MR (26.6% vs 9.5%, $P = 0.007$) and lower LBR (47.5% vs 62.3%, $P = 0.029$) than those with serum P4 > 10.6 ng/ml.

Treatment plan

Patients were treated as shown in Fig. 1. Patients with adequate serum P4 level (P4 > 10.6 ng/ml) on D4 (Group 1) continued standard P4 supplementation treatment (vaginal micronized P4 200 mg every 8 h) until serum β-hCG determination. Embryo warming and transfer were performed on the following day (D5) (FET Group 1) under ultrasound guidance as previously described (Coroleu *et al.*, 2002).

Group 2 was defined in patients with low serum P4 level (P4 < 10.6 ng/ml) on D4. In this group a daily subcutaneous P4 injection of 25 mg (Prolutex® 25 mg, IBSA, Spain) was added to HRT on the same day. Patients underwent a second serum P4 analysis on D5. Embryo warming and FET were performed only in case P4 level on D5 was >10.6 ng/ml (FET Group 2). Embryo transfer was cancelled in those patients in which P4 level on D5 was <10.6 ng/ml.

The treatment was continued in the same regimen until around gestational week 10 if pregnancy was confirmed.

Ethical approval

Patients signed an informed consent form. The study was approved by our Institutional Review Board: number 172018101003.

Statistical analysis

Continuous outcomes were presented as mean and standard deviation whereas categorical outcomes were presented as frequencies and percentages.

Univariate analysis was carried out to describe and compare the cycle characteristics and reproductive outcomes between the two groups of progesterone. *T*-test or Mann Whitney *U* test were applied for continuous variables and Chi-square test or Fisher's test for categorical variables. Normality distribution was analysed by the

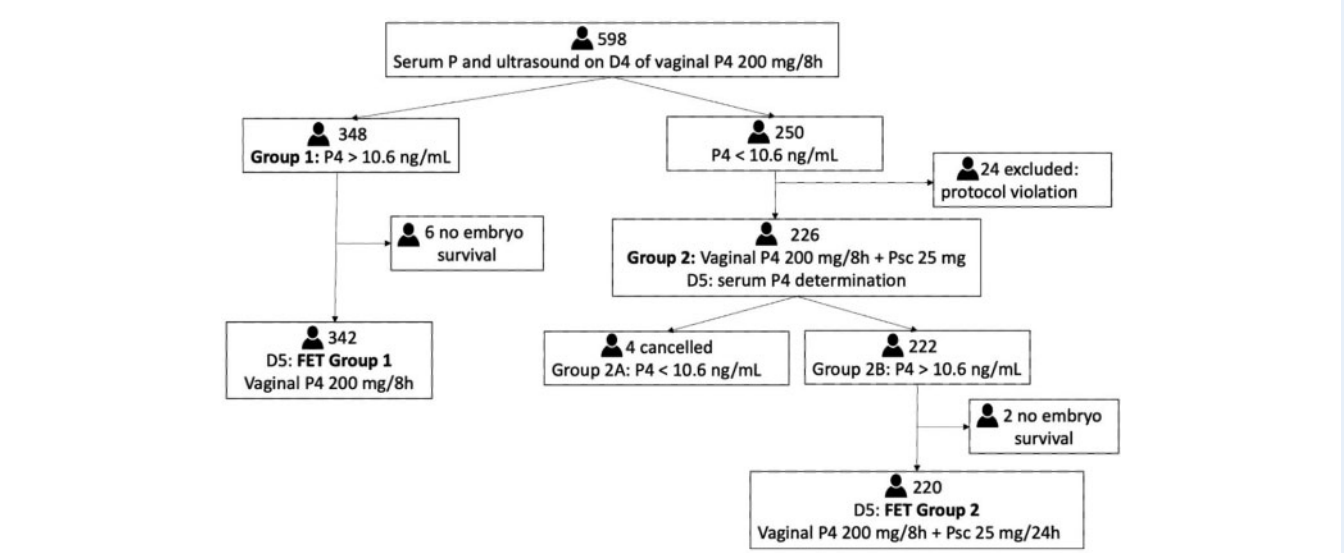

Figure 1. Flowchart showing patient distribution into groups according to serum progesterone levels on the day previous to frozen embryo transfer. P4: progesterone, FET: frozen embryo transfer, D4: day previous to frozen embryo transfer, D5: day of embryo transfer.

Kolmogorov–Smirnov test and Boxplot. The 95% confidence intervals for differences between proportions were calculated for main outcomes (PR and OPR). All tests were two tailed, and $P < 0.005$ was considered statistically significant. Statistical analyses were performed with IBM© SPSS© Statistics v 22 software.

Results

Patients' demographics and cycle characteristics

A total of 598 FET cycles were included in the study. Although most of women with low serum P4 levels received Psc as per protocol, 24 patients undergoing FET cycles did not proceed with Psc and were excluded from the analysis. These patients did not receive treatment either because they were remotely located with no access to medication before the embryo transfer or because they were not willing to initiate Psc (despite being advised so) either for convenience or cost reasons. A total of 574 FET cycles (453 patients) were finally considered for analysis.

Patient's demographics and cycle parameters for the 574 FET cycles meeting inclusion criteria and for the two groups are described in Table I. In summary, the mean age of all intended mothers was 39.7 ± 3.8 years and mean weight was 63.4 ± 11.4 kg. The mean serum P4 level the day before FET was 12.9 ± 6.9 ng/ml. Patients and cycle characteristics were comparable between the group with initial adequate P4 level (Group 1) and the group with low initial P4 who received additional P4 supplementation (Group 2). Group 1 included 58.2% (348) patients. Group 2 included 37.7% (226) women, who received an additional Psc injection. On the following day, 98.2% (222/226) had reached serum P4 levels >10.6 ng/ml and FET was performed. Overall, only four FET cycles were cancelled (1.8%) due to inadequate serum P4 levels despite additional P4 treatment.

Two FET cycles from 222 in Group 2 and six from 348 in Group 1 were not performed as embryos did not survive the warming process.

Reproductive outcomes

Reproductive outcomes were similar between FET Group 1, with initial adequate P4 level, and FET Group 2, with a restored adequate P4 level after additional treatment with Psc (Fig. 2).

The PR and CPR in FET Group 1 was 62.3% (213/342) and 56.4% (193/342) compared to 64.5% (142/220) and 59.1% (130/220) in FET Group 2 (rate difference (RD) −2.2%, 95% CI [−10.8; 6.3]; RD −2.7%, 95% CI [−11.4; 6.0]). Similarly, the OPR was comparable between FET Group 1 (49.4% [169/342]) and FET Group 2 (53.6% [118/220]) respectively (RD −4.2%, 95% CI [−13.1; 4.7]).

Miscarriage rate was 12.4% (24/193) in FET Group 1, compared to 10.8% (14/130) in FET Group 2 (RD 1.6%, 95% CI [−6.1; 9.4]), with no statistically significant differences. There were also no significant differences according to biochemical pregnancy rate that were 5.85% (20/342) and 5.45% (12/220) in FET Group 1 and FET Group 2 respectively.

Finally, we also did not find significant differences according to LBR between FET Group 1 (49.1% [168/342]) and FET Group 2 (52.3% [115/220]) (RD −3.2%, 95% CI [−12; 5.7]).

The 24 FET with P4 < 10.6 ng/ml excluded from the study for protocol violation as no Psc was added, albeit small in sample, had poor reproductive success, with an OPR of 20.8% (5/24) and MR of 37.5% (3/8).

All four cancelled cycles due to unrestored P4 despite additional Psc underwent FET in a subsequent cycle under HRT with both vaginal and Psc treatment. All women achieved serum P4 level >10.6 ng/ml the day before FET and FET was performed.

Table I. Patients' demographics and cycle characteristics.

	Overall (n = 574)	Group 1 (n = 348)	Group 2 (n = 226)	P value
Age (years)	39.7 ± 3.8	40.0 ± 3.9	39.2 ± 3.6	0.021
Weight (kg)	63.4 ± 11.4	63.0 ± 11.4	64.0 ± 11.3	0.387
Endometrial thickness (mm)	10.5 ± 1.9	10.5 ± 1.9	10.5 ± 2.0	0.980
Oestradiol (pg/ml)	221.4 ± 99.0	220.9 ± 101.2	222.1 ± 95.6	0.894
Number of embryos transferred	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.2	0.125
Good quality embryos (A + B)*	0.6 ± 0.5	0.6 ± 0.5	0.5 ± 0.5	0.073

Group 1: Patients with adequate serum P4 level (P4 > 10.6 ng/ml) on the day before frozen embryo transfer (D4).
Group 2: Patients with low serum P4 level (P4 < 10.6 ng/ml) on the day before frozen embryo transfer (D4) who received additional daily subcutaneous P4 injection.
*According to ASEBIR's morphological scoring system (ASEBIR, 2015).

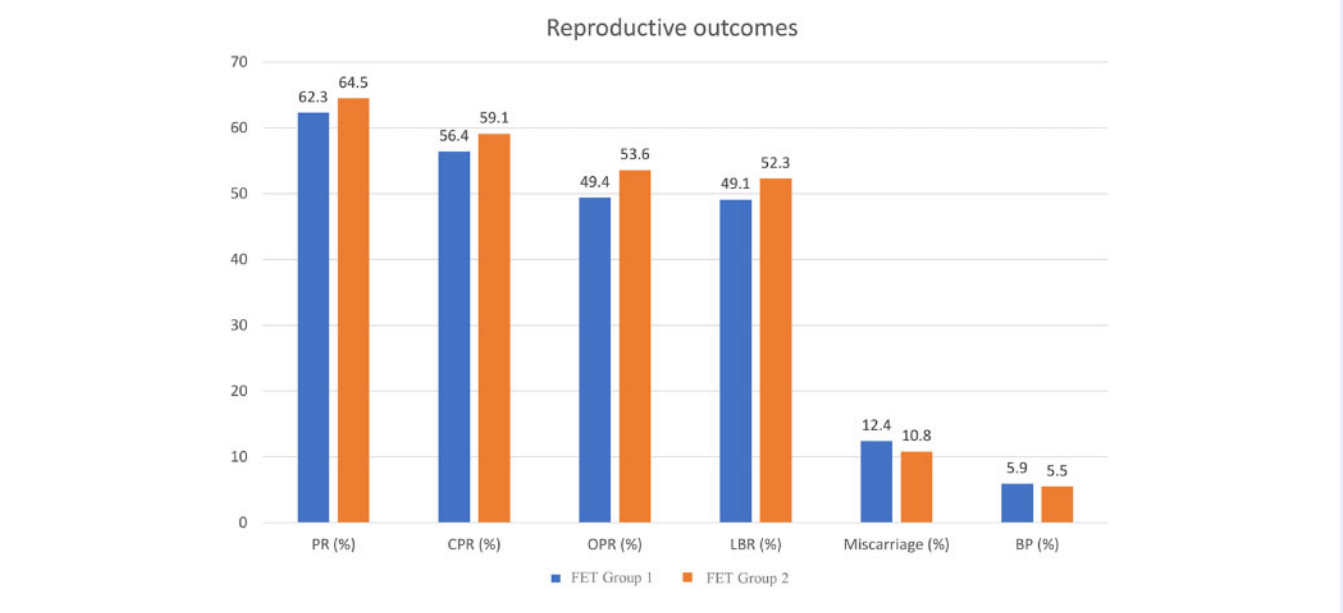

Figure 2. Reproductive outcomes. CPR: clinical pregnancy rate, PR: pregnancy rate, OPR: ongoing pregnancy rate, LBR: live birth rate, BP: biochemical pregnancy rate. Blue: FET Group 1; Orange: FET Group 2.

Discussion

To our knowledge this is the first study providing evidence that an individualised LPS can result in a very high OPR and LBR in patients undergoing euploid FET cycle under HRT in cases of low serum P4 levels prior to embryo transfer. In this context, addition of daily Psc injection to our standard HRT in patients with low P4 levels (<10.6 ng/ml) the day prior to euploid FET (D4) results in excellent OPR and LBR, similar to those in women with adequate initial P4 levels (>10.6 ng/ml).

Serum P4 levels and FET has become a main topic in ART. Recent retrospective studies have described P4 levels as an independent prognostic factor associated not only with OPR (Boynukalin *et al.*, 2019), but also with LBR (Cédrin-Durnerin *et al.*, 2019; González-Foruria *et al.*, 2020) in patients undergoing FET. In fact, previous studies have demonstrated a detrimental effect of low P4 levels around the time of embryo transfer on reproductive outcomes in women undergoing FET under HRT. Altogether, the mixed data and the retrospective basis of these studies called for a prospective design comparing the reproductive outcomes between FET under standard HRT and FET under iLPS when low P4 serum level is registered prior to FET.

Even though there is no clear consensus concerning the optimal P4 threshold in FET, one of the most accepted reference values is around 10 ng/ml (Labarta *et al.*, 2017; Cédrin-Durnerin *et al.*, 2019; Gaggiotti-Marre *et al.*, 2019), which correlates to an adequate P4 production by the corpus luteum in a natural cycle (Hull *et al.*, 1982; Jordan *et al.*, 1994). In most of the recent publications on this topic, serum P4 is measured on the day of embryo transfer (Brady *et al.*, 2014; Labarta

et al., 2017; Cédrin-Durnerin et al., 2019) or the day of pregnancy test (Alsbjerg et al., 2018), both timepoints at which little or no intervention is possible before transferring the embryo. However, in a previous study by our group we determined the optimal cut-off value for serum progesterone not on the day of embryo transfer or the day of pregnancy test, but on the day prior to blastocyst transfer (Gaggiotti-Marre et al., 2019), a timepoint at which an individualised LPS can be initiated. Based on our results, this cut-off has been set at 10.6 ng/ml and patients with levels beyond this value were supplemented with a daily 25 mg. Psc injection. The percentage of patients with low serum P4 values appears to be relatively constant among studies published up to date. In one study, 37% of the patients under HRT for FET had a serum P4 value the day of FET below 10 ng/ml (Cédrin-Durnerin et al., 2019) whereas in another 25% had levels below 9.2 ng/ml on the day of the embryo transfer (Labarta et al., 2017), following vaginal administration of 200 mg micronized progesterone every 8 h and 400 mg every 12 h, respectively. While both studies have shown that low P4 levels are associated with compromised PRs, Cédrin-Durnerin et al. (2019) also found that doubling the vaginal P4 dosage from the day of FET did not improve the reproductive outcome. Similarly, other reports (Archer et al., 1995; Paulson et al., 2014) also described a limited beneficial effect of increasing the vaginal dosage of P4, probably due to a rate-limited absorption by the vaginal epithelium. Likewise, Brady et al. (2014) described detrimental effects of P4 < 20 ng/ml the day of embryo transfer in oocyte recipients under HRT with intramuscular (IM) P4 replacement. They also did not report improved outcomes when additional IM dosages were prescribed to these patients with low P4 levels. Similarly, a recent retrospective study (Alur-Gupta et al., 2020) conclude that increasing doses of IM P when P4 levels are lower than 15 ng/ml give similar outcomes to patients with P4 levels >15 ng/ml. On the other hand, Alsbjerg et al. (2013) did report improved reproductive outcomes when vaginal P4 was doubled in patients undergoing FET under HRT, or when additional rectally administered P4 was provided (Alsbjerg et al., 2020).

In the present study, the percentage of cycles with low serum P4 progesterone levels (<10.6 ng/ml) was 37.8% (226 cycles). Among them in 140 cycles (61.95%) P4 levels were between 8 and 10.6 ng/ml and 86 cycles (38.05%) with P4 levels <8 ng/ml. All these cycles fulfilled the criteria for iLPS through the addition of daily 25 mg Psc injection. Most of them (98.2%) reached adequate serum P4 levels with the administration of only one dosage of Psc. This can be explained by the pharmacokinetics of the two different routes for P4 administration, given that while the vaginal route has been shown to provide a rapid endometrial absorption and local effect via the uterine first-pass effect (Miles et al., 1994), it also yields lower circulating levels due to its shorter half-life (Miles et al., 1994; Levy et al., 1999; Cicinelli et al., 2000). Thus, addition of P4 through a parental route could be an option to rapidly and effectively increase the serum P4 levels in case of low values after only vaginal progesterone exposure.

Up to date, literature regarding the best route for P4 replacement is mixed. In terms of reproductive outcomes, while some authors describe better results in women receiving IM P4 supplementation compared to only vaginal (Haddad et al., 2007; Kaser et al., 2012; Devine et al., 2018), others do not confirm these results (Williams et al., 2000; Shapiro et al., 2014; Wang et al., 2015). Still, a combined treatment with different routes seems a plausible option to ensure adequate P4 exposure for patients that fail to achieve sufficient serum P4 levels under one selected treatment. In fact, there is published evidence on improved reproductive outcomes when the combined route is used compared to only vaginal (Feinberg et al., 2013; Devine et al., 2018). In this regard, Psc has proven its efficacy for both endometrial preparation and luteal phase support in ART and FET (Baker et al., 2014; Lockwood et al., 2014; Turkgeldi et al., 2020), providing higher serum P4 levels than the vaginal route (Sator et al., 2013; Paulson et al., 2014) and a good acceptance, comfort and ease of use among patients (Venturella et al., 2018). We could also hypothesize about a possible lower subendometrial wave activity under Psc that has been described when P4 was switched to the IM route during the three days before FET compared to those who continued on the vaginal route (Casper, 2014), although a recent randomized clinical trial did not confirm this data (Klement et al., 2018).

Another possible explanation behind the biological rationale of our study could be related to what we could define as an 'opportunity window' in which additional parenteral P4 administration may offer an advantage when is provided before FET but no later than hCG test. In this regard, Delcour et al. (2019) describe no improved outcomes when IM P4 is administered after hCG test. On the contrary, we have to note the low ongoing pregnancy (20.8%) rate and high MR (37.5%) observed in the 24 patients that did not strictly follow the iLPS protocol. Altogether, the present study provides the advantages of both administration routes (vaginal and subcutaneous) with reduced discomfort compared to the IM administration, which requires training and can cause pain in the site of injection, skin inflammation or even sterile abscesses (Penzias, 2002; Phy et al., 2003).

One of the main strengths of present study is its prospective design in a single centre, under the same standardised clinical setting, treatment and laboratory conditions. Also, the inclusion of only chromosomally normal embryos avoids one of the main reasons of miscarriage and implantation failure (Marconi et al., 2003) and overcomes confounding factors such as female age (Harton et al., 2013; Rubio et al., 2017), embryo quality or ovarian stimulation protocols. The determination of P4 the day before FET allowed an iLPS through an alternative route for P4 supplementation according to our own data in a previous study (Gaggiotti-Marre et al., 2019). In this regard, other authors have recognized that serum P4 analysis on the transfer day may be too late, as doubling vaginal dosage did not influence in ongoing or LBRs, and advise on the possibility of cancelling FET with such low levels (Cédrin-Durnerin et al., 2019). In this sense, our study does not only provide an alternative route for additional P4 supplementation, but also introduces for the first time the possibility of rescuing cases of P4 deficiency along the 'opportunity window' (before the FET). This approach could provide an individualised strategy based on each patient's need.

The main limitation of our study is that a single serum P4 determination was performed without a specific time interval since the last vaginal dose administration or the first subcutaneous injection. Our group has recently published that lower P4 levels on the day prior to FET are in relationship with the further apart the time of blood collection from the latest dose of vaginal progesterone administration ($R = -0.090$; $P = 0.018$) (González-Foruria et al., 2020). However, the exact time of injection was not stated in the present study. Another limitation is the lack of serum P4 determinations on the day of β-hCG testing or once the pregnancy is achieved. Patients continued on either only vaginal or both regimens from the day of FET until

β-hCG testing, but no additional determinations were performed in order to ensure adequate P4 exposure during the first weeks of pregnancy. Treatment discontinuation was individualised but not strictly defined, usually at around 10th week of pregnancy.

Another important limitation of the current prospective study is that, per protocol, Psc supplementation was adopted for patients with serum progesterone levels <10.6 ng/mL, given that several previous reports in our setting demonstrated that such values are likely to be associated with lower PRs (Gaggiotti-Marre *et al.*, 2019). However, caution is needed because we haven't proven that low progesterone levels were also associated with inferior PRs in the current study. Consequently, our finding that iLPs through Psc supplementation results in excellent PRs in the patients with P4 levels below 10.6 ng/mL, may only indirectly support that iLPS improves pregnancy outcomes, given the absence of evidence that the patients without the adjustment would have had lower pregnancy or higher MRs.

In summary, this is the first prospective study to provide an individualised strategy for P4 replacement treatment in patients undergoing euploid FET with low P4 serum level the day prior to transfer. Our results suggest a minimum P4 threshold to improve reproductive outcomes in FET under HRT with vaginal progesterone, which, if detected, can be overcome in most cases by the addition of a daily subcutaneous shot. Such a benefit could be provided not only by the different routes of P4 administration to ensure adequate P4 exposure for patients, but also taking into account the 'opportunity window' related to adding P4 before the embryo transfer. Furthermore we cannot neglect the high patient's satisfaction in regard to Psc, especially as compared with the side effects associated with IM administration. Based on our findings we demonstrate that the approach described in the present study could provide clinicians a standardised and individualised protocol for luteal phase replacement in women undergoing FET HRT, securing excellent PRs even in cases of low serum P4 levels. Undoubtedly more studies are needed to confirm whether iLPS through the addition of daily Psc is the optimal treatment for cases of low P4 levels around the time of FET.

In conclusion, according to our results, iLPS through Psc co-administration with vaginal P4 in cases of low serum P4 values before FET under HRT can result in excellent OPRs and LBRs. Although our study design is not a randomized trial and thus cannot prove superiority of co-treatment with Psc with vaginal progesterone vs only vaginal progesterone in women with low levels, it is unclear whether such a study should be considered ethically appropriate today, especially taking into account the consistent and accumulating evidence demonstrating very low PRs in women with low serum P4 levels who continue treatment only with vaginal P4.

Data availability

Derived data supporting the findings of this study are available from the corresponding autor (M.A) on reasonable request. Data cannot be shared for ethical/privacy reasons. This research was performed under the auspices of the Càtedra d'Investigació en Obstericia i Ginecologia of the Department of Obstetrics, Gynaecology and Reproductive Medicine, Dexeus University Hospital, Universitat Autònoma de Barcelona.

Authors' roles

BC, MA and SGM conceived and designed the study. All the authors analysed and interpreted the data. SG and IR contributed to data collection and performed the statistical analysis. IGF, FM, MP, LC, NPP and BC revised the article for important intellectual content. SGM, MA and NPP wrote the article. All the authors approved the final version of the manuscript.

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Conflict of interest

B.C. reports personal fees from MSD, Merck Serono, Ferring Pharmaceuticals, IBSA and Gedeon Richter outside the submitted work. N.P. reports grants and personal fees from MSD, Merck Serono, Ferring Pharmaceuticals, Theramex and Besins International and personal fees from IBSA and Gedeon Richter outside the submitted work. The remaining authors have no conflicts of interest to declare.

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