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Serum luteal phase progesterone in women undergoing frozen embryo transfer (FET) in assisted conception: a systematic review and meta-analysis

Running title: Serum progesterone in FET cycles

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Capsule: For thresholds below 10 ng/ml, women with lower progesterone levels experience fewer live births and increased risk of miscarriage compared to women with higher serum progesterone.

Abstract

Objective

To investigate the association between luteal serum progesterone levels and frozen embryo transfer (FET) outcomes.

Design

Systematic review and meta-analysis.

Setting

Not applicable.

Patients

Women undergoing FET.

Interventions

We conducted electronic searches of MEDLINE, PubMed, CINAHL, EMBASE, the Cochrane Database of Systematic Reviews, CENTRAL, Web of Science, ClinicalTrials.gov and grey literature (not widely available) from inception to March 2021 to identify cohort studies where serum luteal progesterone was measured around the time of FET.

Main Outcome Measures

Ongoing pregnancy or live birth rate, clinical pregnancy rate and miscarriage rate.

Results

Among studies analyzing serum progesterone thresholds below 10 ng/ml, higher serum progesterone was associated with increased rates of ongoing pregnancy or live birth (relative risk [RR] 1.47, 95% confidence interval [CI] 1.28 to 1.70), higher chance of clinical pregnancy (RR 1.31, 95% CI 1.16 to 1.49) and lower risk of miscarriage (RR 0.62, 95% CI 0.50 to 0.77) in cycles using exclusively vaginal progesterone and blastocyst embryos. There was uncertainty about whether progesterone thresholds higher than 10 ng/ml were associated with FET outcomes in sensitivity analyses including all studies, owing to high inter-study heterogeneity and wide confidence intervals.

Conclusion

Our findings indicate that there may be a minimum clinically important luteal serum concentration of progesterone required to ensure an optimal endocrine milieu during embryo implantation and early pregnancy following FET treatment. Future clinical trials are required to assess whether administering higher-dose luteal phase support improves outcomes in women with low serum progesterone at the time of FET.

PROSPERO Number

CRD42019157071

Key Words

Progesterone, endometrial receptivity, frozen embryo transfer, luteal phase support, live birth, miscarriage

Introduction

The use of freeze-thaw embryo transfer (FET) treatment is an important option in assisted conception practice. FET allows clinicians to comply with single embryo transfer (SET) policies while significantly reducing the chance of ovarian hyperstimulation syndrome (OHSS) associated with controlled ovarian stimulation (COS) (1). Furthermore, it is thought that FET mitigates the risk of endometrial asynchrony by avoiding the need for treatment with gonadotropins and by allowing greater flexibility on the timing of embryo transfer (2). For these reasons, some clinics have started to offer a universal freeze-all policy (3). According to the Human Fertilisation and Embryology Authority (HFEA), the number of FET undertaken in the UK nearly doubled between 2013 and 2018, now accounting for 38% of all in vitro fertilization (IVF) cycles. Conversely, the number of fresh embryo transfers decreased by 11% in the same time period (4). This mirrors similar trends in continental Europe (5), the USA (6) and across the world (7). The success rate of FET cycles remains relatively low nonetheless, with fewer than one in three women achieving a live birth following FET treatment worldwide (7).

There has been a growing body of research aimed at identifying ways to predict and improve FET outcomes in the past decade. It is widely accepted that treatment success depends on a plethora of factors including embryo ploidy status and endometrial receptivity (8). Endometrial thickness as measured by ultrasound has been historically used as a marker of receptivity, while recent studies have focussed on identifying receptive molecular patterns through histological and transcriptomic analyses of endometrial tissue (9, 10). Despite these advances, there remains a significant level of uncertainty about strategies to optimize day-to-day clinical practice and increase success rates in FET. For example, there is no consensus on how to best prepare the endometrium for embryo implantation in freeze-thaw treatment (2). Some authors advocate that in ovulatory women it is optimal to transfer the embryo in the mid-luteal phase of a natural cycle without administering exogenous hormones (NC-FET), whereas others support the artificial preparation of the endometrium with hormone replacement therapy (HRT-FET). To date, however, both approaches have been shown to be largely equivalent in effectiveness and safety (11-14).

There is some evidence that circulating progesterone may be associated with treatment success in FET (15-17). Progesterone is a steroid hormone produced by the corpus luteum, shortly after ovulation in a natural menstrual cycle. It is through the regulatory effect of progesterone that the endometrium becomes secretory and receptive to the implanting embryo, during a four-day interval in the luteal phase of the menstrual cycle termed “window of implantation” (WOI) (18). Before or after this strictly timed interval, implanting embryos face an asynchronous endometrium where the immune-endocrine milieu may impede rather than facilitate implantation (19). Serum progesterone levels peak during the WOI, and have been used as a marker of endometrial receptivity in natural conception and assisted reproductive technology (ART) treatment (20). During the follicular phase, high circulating progesterone identifies premature luteinization and endometrial asynchrony (21-23). By contrast, in the luteal phase, it has been suggested that single progesterone measurements lower than 10 ng/ml indicate luteal phase deficiency (LPD), which in turn has been associated with infertility and recurrent pregnancy loss (24). Further, fresh IVF cycles have long been known to lack endogenous progesterone support in the luteal

phase, mainly due to the inhibition of luteinizing hormone (LH) secretion. The administration of exogenous hormones such as progesterone remains therefore critical to achieve treatment success in IVF (25).

The hypothesis that serum progesterone levels may be used as a predictor of FET success stems from data linking higher circulating progesterone with increased live birth rates and reduced risk of miscarriage in freeze-thaw cycles (26-28). Some researchers also postulate that women with low serum progesterone may benefit from additional progesterone supplementation to achieve optimal luteal phase support (LPS), although good-quality interventional evidence remains scarce (29, 30). Existing data are conflicting, however, with other studies suggesting that excessive progesterone may be equally detrimental to treatment outcomes as low progesterone (15, 31, 32). It remains therefore unclear whether serum progesterone levels may constitute a reliable predictor of FET outcomes, and whether additional progesterone supplementation in women with low circulating levels may lead to luteal rescue and improved treatment success.

The aim of this systematic review and meta-analysis was to summarize and appraise published data on the association between circulating progesterone in the luteal phase and treatment outcomes in FET cycles.

Methods

Registration

This review was registered with PROSPERO (CRD42019157071) before commencement.

Search strategy

We performed extensive bibliographic searches according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (33). The following computerized databases were searched from their inception until March 15, 2021: MEDLINE, PubMed, CINAHL, EMBASE, the Cochrane Database of Systematic Reviews, the Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science, ClinicalTrials.gov and grey literature (not widely available). The key MeSH/Emtree expressions and search terms (and respective word variants) used to identify studies investigating serum progesterone levels in frozen embryo transfer cycles were the following: progesterone AND (in vitro fertilization OR intracytoplasmic sperm injection, frozen embryo, embryo transfer, assisted reproduction techniques). The search strategy was reviewed by two medical sciences librarians. Additional studies were identified from the reference lists of selected abstracts and manuscripts to avoid missing relevant data. We applied no language restrictions to any of our searches.

Study selection and quality assessment

We selected studies in a two-step process using Covidence (34). Two independent reviewers (P.M. and Y.C., or P.M. and O.P.) carried out the study selection, first by assessing titles and then by screening the abstracts of selected citations. The full manuscripts of studies that were deemed likely to meet our eligibility criteria were obtained. Any disagreements about inclusion were resolved by consensus or arbitration by a third author (A.C.).

We included observational studies investigating the relationship between serum progesterone levels around the time of FET and treatment outcomes. In natural FET cycles, we included publications where serum progesterone was measured at any time in the luteal phase of participants' menstrual cycles. In HRT-FET cycles, due to variation in existing HRT regimens for endometrial preparation and differences in study protocols, we included manuscripts where serum progesterone levels were measured at any time from the onset of exogenous progesterone supplementation (by any route available) to at least the first pregnancy test following embryo transfer.

We excluded studies performed solely in fresh cycles, or where serum progesterone measurement was undertaken as part of a trial (for example, comparing different routes of progesterone administration) but not directly correlated with treatment outcomes. We also excluded interventional studies where women with low serum progesterone around the time of FET received additional LPS, either by increasing the progesterone dosage or by adding a progestogen with a different route of administration.

All articles meeting the selection criteria underwent quality assessment using the Newcastle-Ottawa scale for quality assessment of observational studies by two independent reviewers (P.M. and Y.C.), as recommended by the Cochrane Collaboration (35, 36).

Study outcomes

The primary outcome of interest was the composite rate of ongoing pregnancy or live birth (OPR or LBR) per cycle. Ongoing pregnancy was defined as a viable intrauterine pregnancy of at least 12 weeks' gestation confirmed on ultrasound, while live birth was defined as the delivery of a live fetus after 22 completed weeks of gestational age (37). Secondary outcomes included the clinical pregnancy rate (CPR) per cycle, defined as the presence of one or more gestational sacs on ultrasound; the miscarriage rate (MR) per pregnancy, defined as the spontaneous loss of an intrauterine pregnancy before 22 completed weeks of gestation (37); and the rate of adverse events attributable to exogenous progesterone supplementation (e.g. abdominal pain, bloating, nausea, depression and headache).

Data extraction process

Two authors (P.M. and Y.C.) independently extracted data from the eligible studies, including publication date, country of origin, study objective, participants (number and demographic characteristics), methods and statistical analysis, study findings and study conclusions. Disagreements were resolved by consensus or arbitration by a third reviewer (A.C.).

Data synthesis and analysis

All data analyses were performed using Stata Statistical Software (Release 16, TX, USA). We described effect sizes using relative risk (RR) and 95% confidence intervals (CI). We assessed statistical heterogeneity by measure of the I^2 statistic and set the acceptability of heterogeneity at I^2<50%, in line with the recommendations from the Cochrane handbook (36). We performed meta-analyses of dichotomous outcomes using a random effects model.

Where studies reported progesterone concentrations in nmol/l, we converted them to ng/ml (conversion factor 3.18), as this is how serum progesterone was expressed in most of the included papers. For the purposes of meta-analysis, we grouped studies according to the serum progesterone thresholds they reported (<10 ng/ml, 10-20 ng/ml, >20-30 ng/ml and >30 ng/ml). We dichotomized outcome data according to whether they pertained to "high" or "low" serum progesterone as defined by each study (i.e., respectively above or below the threshold specified for serum progesterone in each individual study).

Where authors reported adjusted effect estimates to evaluate the impact of confounders on their crude analyses (e.g. adjusted odds ratios [aOR]), we conducted meta-analyses to investigate the pooled adjusted effect size of "low" serum progesterone versus "high" serum progesterone according to the specified cut-off.

Subgroup analyses and sensitivity analyses

The primary analysis included studies where only blastocysts were used in FET treatment. In addition, we planned to analyze data according to the route of progesterone administration where a sufficient number of studies were available (intramuscular [IM], oral [PO], rectal [PR], subcutaneous [SC], or vaginal [PV]). We performed sensitivity analyses for all outcomes of interest (OPR or LBR, CPR and MR) to determine whether our

conclusions were robust to arbitrary decisions regarding eligibility and analysis. The sensitivity analyses aimed to assess whether our conclusions would have differed if (i) eligibility for the meta-analyses had been restricted to studies at low risk of bias and (ii) all studies had been included in the meta-analysis, regardless of cycle type (NC-FET and HRT-FET), progesterone route (IM, PO, PV, PR and PV), stage of embryo development (cleavage and blastocyst) or risk of bias.

Results

Search results

The PRISMA Flow Diagram (Supplemental Fig. 1) shows details of the study selection process. The systematic search on March 15, 2021 identified 4974 articles which were imported into Covidence. Following the removal of duplicates, 3639 titles and abstracts were screened, of which 3586 were excluded as they were clearly not relevant. One study was ongoing (38). The remaining fifty-two articles underwent full-text screening, and thirty-one were excluded for the following reasons: seven studies were interventional; seven did not correlate progesterone levels with treatment outcomes; four were review or commentary papers; two measured serum progesterone before the luteal phase (Supplemental Table 1); and eleven studies are awaiting classification due to unanswered or unsuccessful correspondence with the authors (Supplemental Table 2). We included twenty-one studies in the narrative synthesis, sixteen of which were suitable for meta-analysis.

We contacted the authors of thirty-two manuscripts to obtain additional study details and data (Supplemental Table 2). To date, we have received responses for ten publications (15, 26-28, 39-44).

Included studies

Study characteristics

Table 1 contains a detailed description of the twenty-one included studies. All included manuscripts were cohort studies, of which eight were prospective (26, 28, 43, 45-49) and thirteen were retrospective (15, 17, 27, 31, 32, 40-42, 44, 50-53). Twenty studies have been published as full articles (15, 17, 26-28, 31, 32, 40-45, 47-53) and one as a conference abstract (46). Five of the included studies were conducted in Spain (26-28, 50, 52); three in Japan (32, 44, 47); two each in Australia (15, 51), Denmark (17, 45), France (40, 41) and Turkey (42, 43); and one each in China (53), India (46), Iran (49), Iraq (48) and the USA (31). Eighteen studies focused solely on HRT-FET cycles; two included HRT-FET and fresh donor recipient cycles (26, 28); and one study included only NC-FET cycles (52). Finally, although the largest publication featured results from both HRT-FET and NC-FET cycles, only the HRT-FET data were suitable for meta-analysis (51).

In aggregate, the quantitative synthesis included 6175 FET cycles, of which 5881 were HRT-FET and 294 were NC-FET. Participants were aged between 18 and 50 years, and the body mass index (BMI) of included women ranged from 18 to 37 kg/m^2. Furthermore, eleven studies analyzed autologous cycles only (15, 17, 27, 31, 41, 42, 45, 49, 50, 52, 53), while one publication focused exclusively on donor cycles (26) and two included both autologous and donor cycles (28, 51). Oocyte source was not specified in the remainder of included manuscripts.

Most of the included studies (n = 15) analyzed cycles including only day 5/6 blastocysts; four studies included both cleavage-stage and blastocyst embryos (32, 40, 47, 51); and two publications analyzed exclusively cleavage-stage embryos (48, 53). Three of the included studies analyzed FET cycles using euploid embryos only (31, 43, 50), while in the remaining studies the ploidy status of the embryos was not specified.

Serum progesterone measurement

The timing of serum progesterone measurement varied across all included studies. Ten studies measured progesterone on the day of FET (26, 28, 31, 32, 42, 43, 46-49), three measured serum progesterone on the day before FET (27, 50, 52), and one study one or two days before transfer (41). The remaining studies tested progesterone levels later on in the luteal phase: one study two to three days after FET (15); one sixteen days after the onset of progesterone supplementation (51); three studies measured progesterone on the day of pregnancy test (17, 45, 53); and in one study progesterone was tested on the day of pregnancy test, and then again 46h and 96h later (40). Finally, one study measured serum progesterone every five days from the day after FET until 9w 1d gestation (44).

Twelve studies reported on the time interval between the last dose of progesterone and venepuncture in HRT-FET cycles (15, 17, 27, 28, 31, 42, 45, 47-50, 54). Of these, ten studies were included in the quantitative synthesis, with dose-to-measurement intervals ranging between one and twelve hours (15, 17, 26, 28, 31, 42, 45, 47, 49, 50).

There was also variation in the progesterone cut-off levels reported, ranging between 5 ng/ml (32) and 53.2 ng/ml (43). The most common threshold was 8.8 ng/ml, used in two studies (28, 45). Eight studies reported on treatment outcomes according to serum progesterone percentile analyses (42, 43, 45, 47, 49-51, 53); three studies reported on progesterone thresholds using a “high” versus “low” dichotomy (26, 28, 51); three publications reported thresholds according to previously published literature or what the authors perceived to be accepted standard practice (17, 31, 52); one study reported outcomes according to a receiver operating characteristic curve (ROC) analysis of serum progesterone levels (46); and in two manuscripts no rationale was given for the reported progesterone thresholds (15, 32). Finally, two publications identified a linear positive correlation between serum progesterone and live birth, without a significant cut-off point associated with treatment success (27, 44).

Ten of the included studies reported on intra- and inter-assay coefficients of variation associated with serum progesterone measurements, ranging between 1.2% and 23.1% (15, 17, 26, 28, 43, 45, 49, 55-57). In the remaining studies this was not stated.

Outcomes

Twelve publications reported on live birth (15, 27, 28, 31, 44, 46, 47, 49-53). However, the gestational age threshold to define this outcome varied between >20 weeks in two studies (15, 51), >22 weeks in another two manuscripts (44, 52), and >28 weeks in the study by Liu and Wu (53), while the remaining six publications did not specify a gestational age for live birth (27, 28, 31, 46, 47, 49, 50).

Eleven studies reported on ongoing pregnancy, defined by most as the presence of fetal heart activity at ≥12 weeks' gestation (17, 26, 28, 40-42, 45, 46, 48), with one study using the threshold of 16 weeks (43) and another leaving the threshold unspecified (31).

Seventeen studies reported on clinical pregnancy, defined as the presence of a gestational sac on ultrasound in ten studies (15, 26, 28, 32, 42, 43, 47, 51-53), while the remaining seven publications defined clinical pregnancy as the presence of an intra-uterine gestational sac with visible fetal heart activity (17, 31, 40, 41, 45, 48, 49).

Finally, seventeen studies reported on miscarriage, of which eleven used thresholds between 6-12 weeks' gestation (17, 26, 28, 31, 40, 42-45, 48, 51); one used a cut-off of 20 weeks (49); another study used a threshold of 22 weeks (52); and four studies did not specify gestational ages for miscarriage (41, 46, 47, 50).

No studies reported on adverse events attributable to progesterone.

Quantitative synthesis of serum progesterone effects

We performed meta-analyses of studies reporting on OPR or LBR and CPR per cycle, and MR per pregnancy. We grouped study results according to different threshold categories used by authors to present outcome data. Within each threshold category (<10 ng/ml, 10-20 ng/ml, >20-30 ng/ml and >30 ng/ml), outcome data were presented for women with "high" (i.e., above that particular cut-off) and "low" (i.e., below the cut-off) progesterone. While it was possible to conduct meta-analyses of studies using PV-only progesterone and blastocyst embryos, there were an insufficient number of studies to perform meta-analyses for cleavage-stage embryos and for the IM, PO, PR and SC routes of progesterone administration.

Progesterone threshold category <10 ng/ml

Among studies using solely PV progesterone and blastocyst embryos, meta-analysis of outcome data for cut-off values lower than 10 ng/ml showed that serum progesterone higher than the specified threshold was associated with increased OPR or LBR (RR 1.47, 95% CI 1.28 to 1.70; five studies; n = 1990; I^2 = 0%) (Fig. 1), increased CPR (RR 1.31, 95% CI 1.16 to 1.49; three studies; n = 1603; I^2 = 0%) (Fig. 2) and reduced MR (RR 0.62, 95% CI 0.50 to 0.77; five studies; n = 1990; I^2 = 0%) (Fig. 3). The aforementioned sensitivity analyses did not change the conclusions (Supplemental Fig. 2, Supplemental Fig. 3 and Supplemental Fig. 4).

Pooled aOR for studies using PV-only progesterone showed that lower serum progesterone was associated with a reduction in OPR or LBR (aOR 0.48, 95% CI 0.35 to 0.62; two studies; n = 1074; I^2 = 30.9%) (Supplemental Fig. 5). Meta-analysis of adjusted effect estimates was not possible for CPR and MR within this threshold category due to insufficient data.

Progesterone threshold category 10-20 ng/ml

For studies using PV-only progesterone and blastocyst embryos where serum progesterone cut-off values ranged between 10 and 20 ng/ml, the primary analysis showed that higher progesterone was associated with increased OPR or LBR (RR 1.27, 95% CI 1.09 to 1.48; three studies; n = 916; I^2 = 0%) (Fig. 1) and improved CPR (RR 1.28, 95% CI 1.10 to 1.50; two studies; n = 773; I^2 = 0%). However, sensitivity analyses including all studies, regardless of progesterone route, type of FET or risk of bias, showed uncertainty about whether higher progesterone was associated with increased OPR or LBR (RR 1.19, 95% CI 0.98 to 1.45; eleven studies; n = 4436;

I^2 = 77.6%) (Supplemental Fig. 2) and higher CPR (RR 1.21, 95% CI 0.99 to 1.47; eleven studies; n = 4252; I^2 = 77.5%) (Supplemental Fig. 3).

The evidence was also uncertain about whether women with progesterone levels above the specified threshold within this category experienced fewer miscarriages than those with lower progesterone (RR 0.76, 95% CI 0.47 to 1.22; four studies; n = 1160; I^2 = 60.2%) (Fig. 3). Sensitivity analyses for MR did not change the conclusions (Supplemental Fig. 4).

Pooled aOR for studies using PV-only progesterone showed that lower serum progesterone was associated with a reduction in OPR or LBR (aOR 0.37, 95% CI 0.27 to 0.47; three studies; n = 2068; I^2 = 43.6%) (Supplemental Fig. 5) and CPR (aOR 0.45, 95% CI 0.32 to 0.57; two studies; n = 1824; I^2 = 0%) (Supplemental Fig. 6), while there was uncertainty about whether lower progesterone was associated with increased risk of miscarriage (aOR 1.64, 95% CI 0.73 to 2.56; three studies; n = 2068; I^2 = 36.9%) (Supplemental Fig. 7).

Progesterone threshold category >20-30 ng/ml

When pooling data from studies using exclusively PV progesterone and blastocysts where results were presented for progesterone thresholds between >20 and 30 ng/ml, serum progesterone measurements did not confidently correlate with OPR or LBR (RR 1.27, 95% CI 0.92 to 1.76; two studies; n = 672; I^2 = 55.9%) (Fig. 1) and MR (RR 1.05, 95% CI 0.70 to 1.57; two studies; n = 672; I^2 = 0%) (Fig. 3). Only one study in the PV progesterone group reported within this threshold for CPR, and its findings were uncertain (RR 1.15, 95% CI 0.97 to 1.36; n = 529). Sensitivity analyses did not materially change the conclusions (Supplemental Fig. 2, Supplemental Fig. 3 and Supplemental Fig. 4).

Meta-analysis of adjusted effect estimates was not possible for OPR or LBR, CPR and MR within this threshold category due to insufficient data.

Progesterone threshold category >30 ng/ml

For progesterone thresholds above 30 ng/ml, only one study using blastocysts and the PV route of administration reported on OPR or LBR, CPR and MR, with uncertain findings for all three outcomes (Fig. 1, Fig. 2 and Fig. 3). The aforementioned sensitivity analyses did not change the effect size of progesterone levels within this category (Supplemental Fig. 2, Supplemental Fig. 3 and Supplemental Fig. 4).

Meta-analysis of adjusted effect estimates was not possible for OPR or LBR, CPR and MR within this threshold category due to insufficient data.

Additional analyses

There were an insufficient number of studies administering IM, PO, PR or SC progesterone for additional analyses according to administration route. Furthermore, only three studies analyzed cycles using exclusively euploid embryos, thus precluding sensitivity analyses according to embryo ploidy status.

Risk of bias across studies

Most of the included studies were deemed to be at a low risk of bias (15, 17, 26-28, 31, 40, 42, 43, 45-49, 51-53), while four publications were judged to be at a moderate risk of bias (32, 41, 44, 50) due to a lack of data on

whether women within different serum progesterone ranges were comparable in baseline demographic characteristics such as age and BMI (Supplemental Table 3).

Discussion

Summary of evidence

In this systematic review and meta-analysis of twenty-one cohort studies, we aimed to investigate how serum progesterone around the time of FET correlates with treatment outcomes. The primary analysis showed that for thresholds below 10 ng/ml, women with higher progesterone levels experienced more ongoing pregnancies or live births, more clinical pregnancies, and fewer miscarriages than those with serum progesterone lower than the specified cut-off. This was corroborated by our sensitivity analyses. For progesterone thresholds equal or above 10 ng/ml, however, there was uncertainty in whether higher progesterone was associated with better treatment outcomes, owing to significant inter-study heterogeneity, a paucity of prospective data and wide confidence intervals around pooled effect estimates.

Among the included studies, progesterone was administered via different routes and in varying doses. To date, there has been no consensus on the best form of LPS in frozen embryo cycles, due to a lack of robustly designed randomized trials. In a seminal study by Miles et al. (58) including twenty ovulatory women, the authors compared the pharmacokinetic profile of micronized progesterone administered vaginally versus intramuscularly. Although serum concentrations of progesterone were more than twice as high following IM administration, women assigned to the vaginal route had a mean endometrial progesterone concentration nearly eight times higher than the IM group. Importantly, however, the study participants did not have a diagnosis of infertility, and reproductive outcomes were not assessed. In a subsequent randomized trial, Lightman et al. (59) compared micronized progesterone PV 200 mg three times daily with IM progesterone 100 mg once daily in 354 women undergoing HRT-FET cycles. Serum progesterone on the day of pregnancy test was more than double in the IM progesterone group, yet crucially did not result in higher clinical pregnancy rates. These findings support the hypothesis that endometrial rather than serum progesterone is more important in determining treatment success in FET cycles. This may additionally reflect the phenomenon of first uterine pass, whereby a targeted delivery of vaginal progesterone to the uterus enhances the local endocrine milieu and hence facilitates implantation and pregnancy success (60). Overall, however, studies comparing different routes and regimens of LPS in FET have failed to categorically identify a formulation with superior results (42, 47, 61-65).

Despite the lack of evidence attesting to superior efficacy, the use of micronized vaginal progesterone for LPS in ART has become widespread in recent decades. There were originally concerns with reduced bioavailability of natural progesterone due to its large molecular size, but in its micronized form progesterone has since been shown to be easily absorbed by the vaginal and intestinal mucosa (66, 67). Furthermore, gel-based formulations containing micronized progesterone exhibit a sustained-release mechanism which leads to steady levels of absorption over time and prevents excessive variation in serum progesterone levels (63, 64). The vaginal formulation is most commonly used in ART treatment across the world, and the preferred route of administration for up to one in four ART practitioners (68). In this review, most studies used PV progesterone, IM progesterone, or a combination of both routes for LPS. While it is difficult to link the route of administration

with progesterone measurements across different studies, the analysis by Polat et al. (42) identified higher progesterone levels in the group receiving PV plus IM progesterone in comparison to those assigned to PV progesterone only. However, there was no evidence that treatment outcomes differed between the two cohorts. In addition to PV-only progesterone, we aimed to conduct meta-analyses evaluating other routes of administration. Although the number of studies using IM, PO, PR and SC progesterone were insufficient to perform grouped analyses, we carried out sensitivity analyses including all studies, regardless of route of administration and stage of embryo development. The primary analysis of studies using PV-only progesterone showed that higher circulating levels were associated with increased OPR or LBR for the threshold categories of <10 ng/ml and 10-20 ng/ml, yet our sensitivity analyses including all studies showed that this was no longer the case for progesterone thresholds ranging between 10 and 20 ng/ml. These data strengthen the argument that although systemic routes of administration such as SC or IM may result in higher circulating progesterone, this does not necessarily reflect the amount that actually reaches the endometrium, which may be lower, thus explaining the dilution in effect size observed in the sensitivity analysis for the outcome of OPR or LBR.

There was variation across the studies in the timing of progesterone measurement, although those assessing blastocyst transfers undertook venepuncture at least 4 days after the onset of LPS. The pharmacokinetics of different progesterone formulations have been the subject of much research. For example, there have been concerns regarding low bioavailability of progesterone when administered orally due to the effect of food on intestinal absorption and the phenomenon of first hepatic pass leading to the rapid metabolization and excretion of progesterone metabolites (69). In the past decade, however, dydrogesterone, a synthetic progestogen, has been increasingly utilized in ART due to its improved bioavailability, safety and convenience of use (70). When progesterone is administered vaginally or intramuscularly, a steady state in serum concentration is usually achieved within 24-48 hours (66, 71, 72). In the case of subcutaneous progesterone, time to steady state concentrations ranges between 48 to 72 hours (73). Therefore, in this review, it is reasonable to postulate that serum progesterone measurements in all studies evaluating HRT-FET provided a reliable estimate of steady state concentrations, and were thus reflective of circulating progesterone at the time of embryo transfer. In addition, existing evidence also suggests that serum progesterone levels remain above 10 ng/ml for over eighteen hours following vaginal administration (27). Our primary analysis included only studies whose dose-to-measurement interval ranged between one and six hours, and are therefore likely to accurately reflect the correlation between serum progesterone and FET treatment outcomes in this cohort of patients.

Our results confidently identified that for cut-offs lower than 10 ng/ml, higher progesterone levels were on average associated with better treatment outcomes. However, as the threshold values increased, there was an overall shift towards the line of no effect. For the highest cut-off levels (>30 ng/ml), it was in fact unclear whether progesterone might even be detrimental to ongoing pregnancy and live birth rates (Fig. 1, Supplemental Fig. 2). It is therefore possible that a minimally important level of serum progesterone is required to ensure sufficient binding to endometrial progesterone receptors and facilitate their secretory activity. Above this minimum threshold, however, the data suggest that treatment outcomes may not be optimized when higher circulating levels of progesterone are identified. While mechanistic studies investigating the pharmacodynamics of

progesterone receptors in the uterus of women undergoing ART are scarce, existing evidence from humans and other mammals indicates that endometrial progesterone receptors undergo saturation at or below physiological concentrations of endogenous progesterone (74, 75). Furthermore, adding progesterone to endometrial samples has shown that specific binding was saturable after a threshold of approximately 3.14 ng/ml (74, 76), suggesting that beyond that level further progesterone may not enhance the secretory activity of the endometrium.

While it may be difficult to extrapolate in vitro data to clinical practice, a recent study by Deng et al. (77) has added strength to the hypothesis of progesterone receptor saturation. The authors demonstrated that in 726 women with threatened miscarriage in the first trimester of naturally conceived pregnancies, the risk of pregnancy loss decreased incrementally as serum progesterone levels rose, but only up to a concentration of 28.5 ng/ml. Above this value, the risk of miscarriage was no longer associated with circulating progesterone levels (77). Although progesterone in its natural and micronized forms has a proven safety record in pregnancy (78, 79), studies in mammals have indicated that excessive progesterone may impair implantation and decidualization (80). There is hence a need for additional data investigating the effect of higher levels of circulating progesterone upon FET outcomes.

Crucially, it remains uncertain whether providing additional LPS to women with low serum progesterone improves outcomes in FET. Cédrin-Durnerin et al. (81) conducted a retrospective analysis of 227 FET cycles where women received a total daily dose of 600 mg of PV micronized progesterone for endometrial preparation. Although the dose of progesterone was increased to 800 mg daily in participants whose serum progesterone was lower than 10 ng/ml on the day of FET, these women still experienced fewer live births than those with progesterone ≥10 ng/ml. In a subsequent retrospective study, Volovsky et al. (82) analyzed 2010 FET cycles where women whose serum progesterone on the day of transfer was <8.0 ng/ml received additional supplementation. In contrast to Cédrin-Durnerin et al. (81), Volovsky et al. (82) observed no differences in CPR and LBR between women with serum progesterone <10 ng/ml and those where progesterone was ≥10 ng/ml, suggesting that additional LPS may have led to luteal rescue. More recently, Álvarez et al. (30) published a non-randomized prospective study where women with serum progesterone lower than 10.6 ng/ml on the day before FET received additional SC progesterone supplementation and experienced similar outcomes to participants whose serum progesterone was >10.6 ng/ml. Importantly, this study was restricted to euploid-only cycles, thus eliminating the contribution of aneuploidy to miscarriage rates in this cohort. High-quality randomized interventional data are needed to further inform the above findings by ascertaining whether women with low progesterone (<10 ng/ml) who receive additional LPS (for example, by adding a second route of administration) experience non-inferior outcomes to those with higher progesterone levels.

Most studies included in this review stated that LPS was continued at least until the day of pregnancy test, with the exception of four publications where the duration of LPS was not stated (17, 43, 46, 50), and one study where women underwent NC-FET without LPS (27). In natural conception, the corpus luteum is the main source of progesterone from ovulation until approximately 7-8 weeks of gestation, when the luteoplacental shift occurs and endogenous steroid synthesis continues predominantly in the placenta (83). Due to the LPD phenomenon

in fresh IVF cycles, it has been recommended that exogenous LPS should be undertaken until at least the day of pregnancy test (84), although an international survey of over a thousand clinicians has shown that worldwide most practitioners continue LPS until twelve weeks (68). However, a recent systematic review identified no difference in live birth, ongoing pregnancy or miscarriage rates between women who ceased progesterone early (weeks four to seven) and those who discontinued it later on in the first trimester (up to twelve weeks) (85). Fresh cycle data are nonetheless difficult to extrapolate to HRT-FET treatment, because the lack of a corpus luteum in the latter results in diminished levels of endogenous progesterone. It is possible that this may affect implantation and impact upon LPS requirements in FET (86). Furthermore, it has recently been suggested that vasoactive molecules produced by the corpus luteum, such as relaxin and vascular endothelial growth factor (VEGF), play a key role in facilitating initial placentation, and that their absence may contribute to higher rates of hypertensive disorders observed in pregnancies resulting from HRT-FET treatment (87, 88).

Strengths and limitations

The findings of this systematic review are strengthened by the large number of studies analyzing progesterone levels within different threshold categories, which allowed for meta-analyses for all outcomes of interest. We conducted our primary analyses according to progesterone administration route and embryo development stage, therefore significantly reducing clinical heterogeneity. There were an insufficient number of studies evaluating euploid-only FET cycles, however, and we were therefore unable to conduct sensitivity analyses adjusting for ploidy status, a crucial determinant of pregnancy success. There is a need for additional prospective data evaluating how serum progesterone correlates with FET outcomes in the absence of embryonic aneuploidy.

In addition to the retrospective nature of most of the included studies, there was a significant degree of variation in vaginal progesterone formulations, doses, timing of administration and serum levels found to be associated with treatment outcomes. Due to the wide range of serum progesterone measurements reported in relation to pregnancy outcomes, we opted to group the various cut-offs by regular 10 ng/ml intervals. This classification, while arbitrary, allowed for meta-analyses of studies reporting on similar ranges of serum progesterone. For the primary analyses, most of the cut-offs in each threshold category were in fact within 5 ng/ml intervals, hence reducing heterogeneity.

There were multiple coefficients of variation reported for different study assays in this review, ranging between 1.2% and 23.1%. This may have affected the precision and reproducibility of the included manuscripts, and crucially it may have skewed the cut-off levels reported as significant by different studies, thus potentially impacting on the real-life applicability of our findings. Further, it was not possible to conduct separate meta-analyses evaluating non-PV administration routes (IM, PO, PR and SC), or for natural cycle FET, although these were included in the sensitivity analyses which corroborated our primary findings for serum progesterone levels <10 ng/ml. For serum levels ≥10 ng/ml, however, the sensitivity analyses featured high statistical and clinical heterogeneity, thus precluding generalizable recommendations.

Finally, some of the included studies did not find a linear dichotomy whereby “high” and “low” progesterone levels inversely correlated with treatment outcomes, and reported instead on serum measurement ranges that were associated with improved treatment success. This was further compounded by the use of mid-range

progesterone levels as the reference group where studies calculated adjusted effect estimates, therefore preventing their inclusion in the supplemental adjusted analyses evaluating “high” versus “low” progesterone. We conducted exploratory analyses by comparing “high-” versus “mid-” versus “low-level” progesterone for publications that analyzed interval data, although the high degree of variation in mid-level progesterone measurements between studies represented a significant challenge and precluded informative conclusions. Future cohort studies should focus on a percentile evaluation that goes beyond the dichotomous approach. Furthermore, a collaborative effort between study authors to undertake an individual participant data meta-analysis may help clarify the trends of effect of serum progesterone upon FET outcomes.

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Authors’ roles

P.M. and A.C. designed the study and wrote the protocol. P.M., Y.C. and O.P. performed the searches, abstract screening and full-text selection. P.M. and Y.C. undertook the data extraction, synthesis and quality assessment. P.M. and M.J.P. carried out the data analyses. P.M., A.C., I.G. and M.J.P. wrote the final draft of the manuscript. Y.C., O.P., S.F., M.K., C.K., P.L., G.P., M.R., V.S., A.T., S.W., E.L., M.W. and A.D. provided critical input into the final manuscript. All authors approved the final version and accept responsibility for the paper as published.

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Conflicts of Interest

S.F. is a minor shareholder of CARE Fertility, but has no financial or other interest with progesterone testing or manufacturing companies.

P.L. reports personal fees from Pharmasure, outside the submitted work.

G.P. reports personal fees from Besins Healthcare, outside the submitted work.

E.L. was the lead author in two of the manuscripts in this review (Labarta et al., 2017; and Labarta et al., 2021). She has additionally received a grant from Ferring in 2020, has provided consultancy services for MSD and Ferring Pharmaceuticals and is part of the Ferring Pharmaceuticals LIFE program and Merck Global program for Fertility Innovation Leaders. During the past 12 months, E.L. has received honoraria from Angelini/IBSA, Gedeon Richter and Ferring Pharmaceuticals for lecturing.

M.W. reports personal fees from Ferring Pharmaceuticals, outside the submitted work.

A.C. has conducted two RCTs evaluating the role of progesterone in miscarriage prevention (the PROMISE trial, published in 2015 (89); and the PRISM trial, published in 2019 (79)). However, he has no financial or other interest with progesterone testing or manufacturing companies.

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Figure Legends

Figure 1. Forest plot describing the association between different threshold categories of serum progesterone and ongoing pregnancy or live birth rates for women undergoing frozen embryo transfer cycles using blastocyst embryos and PV progesterone only. CI, confidence interval; PV, vaginal route.

Figure 2. Forest plot describing the association between different threshold categories of serum progesterone and clinical pregnancy rates for women undergoing frozen embryo transfer cycles using blastocyst embryos and PV progesterone only. CI, confidence interval; PV, vaginal route.

Figure 3. Forest plot describing the association between different threshold categories of serum progesterone and miscarriage rates for women undergoing frozen embryo transfer cycles using blastocyst embryos and PV progesterone only. CI, confidence interval; PV, vaginal route.

Supplemental Figure 1. Flow diagram of study selection process.

Supplemental Figure 2. Forest plot describing the association between different threshold categories of serum progesterone and ongoing pregnancy or live birth rates for women undergoing frozen embryo transfer cycles. Sensitivity analysis including all studies suitable for meta-analysis. CI, confidence interval; IM, intramuscular route; NA, not applicable (natural cycle); PR, rectal route; PV, vaginal route; SC, subcutaneous route.

Supplemental Figure 3. Sensitivity analysis describing the association between different threshold categories of serum progesterone and clinical pregnancy rates for women undergoing frozen embryo transfer cycles. Sensitivity analysis including all studies suitable for meta-analysis. CI, confidence interval; IM, intramuscular route; NA, not applicable (natural cycle); PR, rectal route; PV, vaginal route; SC, subcutaneous route.

Supplemental Figure 4. Sensitivity analysis describing the association between different threshold categories of serum progesterone and miscarriage rates for women undergoing frozen embryo transfer cycles. Sensitivity analysis including all studies suitable for meta-analysis. CI, confidence interval; IM, intramuscular route; NA, not applicable (natural cycle); PR, rectal route; PV, vaginal route; SC, subcutaneous route.

Supplemental Figure 5. Meta-analysis describing the pooled adjusted effect of low serum progesterone (below specified cut-off) versus high serum progesterone (above specified cut-off) on ongoing pregnancy or live birth rates in women undergoing FET. aOR, adjusted odds ratios; CI, confidence interval; FET, frozen embryo transfer; IV, inverse variance; PV, vaginal route.

Supplemental Figure 6. Meta-analysis describing the pooled adjusted effect of low serum progesterone (below specified cut-off) versus high serum progesterone (above specified cut-off) on clinical pregnancy rates in women undergoing FET. aOR, adjusted odds ratios; CI, confidence interval; FET, frozen embryo transfer; IV, inverse variance; PV, vaginal route.

Supplemental Figure 7. Meta-analysis describing the pooled adjusted effect of low serum progesterone (below specified cut-off) versus high serum progesterone (above specified cut-off) on miscarriage rates in women undergoing FET. aOR, adjusted odds ratios; CI, confidence interval; FET, frozen embryo transfer; IV, inverse variance; PV, vaginal route.

Table Legends

Table 1. Characteristics of included studies.

Supplemental Table 1. Characteristics of excluded studies.

Supplemental Table 2. Correspondence with study authors.

Supplemental Table 3. Quality of included studies according to the Newcastle-Ottawa Scale for the assessment of cohort studies.