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RESEARCH

Effects of vaginal vs oral progesterone supplementation before embryo transfer on live birth rates and levels: a randomized trial

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Abstract

The increase in frozen embryo transfers (FETs) is a consequence of advances in embryo vitrification and the implementation of genetic screening of embryos. There is debate over the best progesterone administration route in substituted cycles and the relationship between progesterone levels on embryo transfer (ET) day and reproductive outcomes. This trial aimed to compare the clinical results of different progesterone supplementation schedules before ET and assess the relationship between plasmatic progesterone levels on transfer day and clinical outcomes. In a prospective, randomized, controlled study, 500 patients were randomly divided into two groups based on the progesterone administration route (oral or vaginal) before ET. Progesterone levels were measured on ET day (PP1) and B-HCG determination (PP2). The primary endpoint was the live birth rate according to different progesterone schedules and levels on transfer day. Despite higher plasmatic progesterone levels with oral administration compared to the vaginal route, there were no significant differences in clinical pregnancy (50.20 vs 47.37%), live birth (43.67 vs 40.89%) or miscarriage rates (13.01 vs 13.68%). Progesterone levels on transfer day were significantly higher among ongoing pregnancies (24.96 ± 1.00 ng/mL) compared to non-ongoing pregnancies (19.39 ± 1.47 ng/mL) and non-pregnancies (17.56 ± 0.99 ng/mL) (P = 0.0001).

Lay summary

The use of FET for the treatment of infertility is increasing. There are two key ongoing debates. First, consensus still needs to be reached regarding the most effective method for preparing the endometrial lining regarding maternal and neonatal outcomes. Second, the optimal progesterone level on the day of ET remains unclear. While how progesterone is administered affects its concentration, no significant differences in clinical outcomes have been observed. However, successful pregnancies tend to have higher progesterone levels than others. Given that the way progesterone is administered does not affect pregnancy rates, patient preferences should guide protocol choices, and further research is needed to explore the association between low progesterone levels on the day of ET and poorer reproductive outcomes.

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Trial registration number: EUDRACT: 2022-000382-41. The clinical trial was approved by the AEMPS (Agencia Española de Productos Sanitarios y Medicamentos; Spanish Agency for Medicines and Health Products) and the local Drugs Research Ethics Committee (CEIm-E). The Institutional Review Board approved the trial.

Trial registration date: 24 August 2022. Date of first patient's enrolment: 10 September 2022.

Keywords: RCT; IVF; PGT-A; progesterone supplementation; progesterone levels; endometrial receptivity; frozen-thawed embryo transfer; sET; clinical pregnancy; live birth rates; miscarriage

Introduction

Successful embryo implantation is a process that requires synchronous development and relies upon crosstalk between the implanting euploid embryo at the blastocyst stage and a receptive endometrium (Patel *et al.* 2019, Casper 2020). Implantation failure of even euploid embryos is one of the most critical limiting factors in ART success and remains a 'black hole' in our knowledge.

Endometrial receptivity can be compromised in fresh cycles by controlled ovarian hyperstimulation protocols and inadequate secretory endometrial transformation. However, endometrial development in frozen-thawed cycles (FET) mitigates the risk of endometrial asynchrony, allowing greater flexibility in the timing of embryo transfer (Eftekhar *et al.* 2013, Mackens *et al.* 2017).

The utilization of FET treatment, particularly employing single-embryo transfer policies, significantly diminishes the likelihood of ovarian hyperstimulation syndrome and complications arising from multiple pregnancies linked with assisted reproductive procedures (Wong *et al.* 2017, Barrenetxea *et al.* 2020, Melo *et al.* 2021, Zhu *et al.* 2023). Since the first live birth by FET in 1983, these procedures have progressively advanced and become essential tools in the treatment of infertility (Wei *et al.* 2019).

Despite the worldwide increase in FET for various indications, the search for the best protocol to prepare the endometrium continues (Mumusoglu *et al.* 2021), and there is still no consensus on which regimen is most optimal for endometrial preparation in FET cycles (Ghobara & Vandekerckhove 2008, Wangren *et al.* 2022). Hormonally substituted AC-FET requires less monitoring than other FET protocols, and thus offers flexibility in the timing of the thaw and transfer of the embryo (Ortega & García Velasco 2015), although recent studies have shown that a correctly conducted natural cycle is superior to hormone replacement therapy (HRT) cycle regarding obstetrical outcome (Melado Vidales *et al.* 2023). It has been reported that circulating progesterone during hormone replacement therapy in AC cycles may affect ongoing pregnancy in FET cycles (Bulletti *et al.* 2022).

Given the conflicting data on the optimal route of progesterone replacement for vitrified-warmed blastocyst transfer, highlighted by studies such as Akaeda *et al.* (2019), Labarta *et al.* (2017) and Yarali *et al.* (2016), which demonstrate existing conflicts on the best administration method, the increasing use of this treatment method and evidence of different preferences of patients, we conducted a trial to determine whether different progesterone replacement schedules before embryo transfer are better than others in terms of live birth rate (LBR) after transfer of vitrified euploid blastocysts. We also designed the trial to evaluate the potential influence of progesterone plasmatic levels around FET on a favorable clinical outcome.

Materials and methods

Study design and randomization

This was a prospective, randomized, controlled study performed between September 2022 and June 2023 at the University-associated Assisted Reproduction Center to properly investigate the potential influence of different progesterone administration protocols before embryo transfer and plasmatic progesterone levels on implantation and pregnancy rates in frozen euploid embryo transfers.

After ovarian stimulation and egg retrieval, a blastocyst trophoectoderm biopsy and cryopreservation of all embryos were performed. Since only single embryo transfers of frozen euploid blastocysts are performed at our center, no double embryo transfer of non-screened embryos was included.

Ethical approval

The clinical trial was approved by the Institutional Ethics Committee of Clinical Research of Euskadi (CEIC-E) and the Institutional Review Board of the University of the Basque Country (UPV/EHU (Clinical Trial Number EUDRACT: 2022-000382-41) on August 20, 2022, and the AEMPS (Agencia Española de Productos Sanitarios y Medicamentos; Spanish Agency for Medicines and medical devices). The study was conducted in compliance with Good Clinical Practice guidelines.

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CONSORT reporting guidelines have been used (Schulz *et al.* 2010).

This study was approved in accordance with the ethical principles that have their origin in the Declaration of Helsinki and Ethical Guidelines for Biomedical Research on Human Participants. All patients received and signed a written informed consent form.

Patient allocation and blinding

Patients with available euploid blastocysts derived from own oocytes after PGT-A were included in the trial. All women reported regular length of the menstrual cycle.

Exclusion criteria were age ≥42 years, body mass index (BMI) above 32 or less than 18, endocrine or autoimmune disease (e.g., diabetes, thyroid disease or presence of anti-thyroid antibodies or PCOS), chromosomal abnormality, uterine malformations, history of intra-abdominal infectious pathology or surgery, diagnosis of endometriosis, adenomyosis, uterine fibroids, absolute or relative contraindication for a follicular puncture and the use of testicular sperm for ICSI. Couples involved in other clinical or embryological trials were also excluded. Embryo transfers from donated oocytes were excluded.

A total of 500 with planned embryo transfers of vitrified-warmed euploid blastocysts were invited to participate in this randomized controlled trial (RCT) and were randomized 1:1 into two groups: group A, in which progesterone before embryo transfer (pre-ET) was administered orally, and group B, with patients who received pre-ET progesterone vaginally. Of these, 492 patients ultimately reached study completion (Fig. 1).

A block randomization process was performed before initial treatment. Patients were allocated to the mentioned treatment groups using the computer-generated randomization codes, which were then placed in the sealed, opaque, sequentially numbered envelopes by a third party (nurse practitioner), who was not directly involved in the patient management or the randomization process. The envelopes were handed out to the participants upon completing the informed consent. Both the patients and the clinicians were aware of the allocated arm.

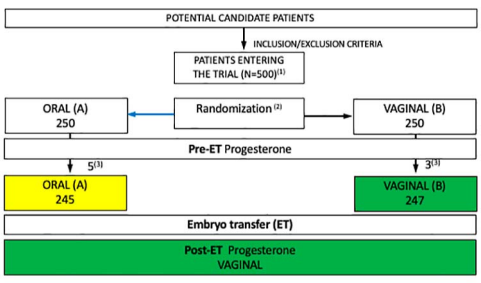

Figure 1

Randomization chart of the trial and trial design.

The participants were followed through one completed ART cycle until all frozen embryos generated from the index cycle were transferred or until delivery in those who achieved pregnancy.

Ovarian stimulation, oocyte retrieval, ICSI, and PGT-A

All patients underwent ovarian stimulation, egg retrieval and ICSI procedures described elsewhere (Barrenetxea *et al.* 2024). A GnRH antagonist protocol was scheduled for ovarian stimulations. A single injection of corifollitropin alpha (Elonva®, Organon NV, The Netherlands) on day 2 of the menstrual cycle was followed by the administration of recombinant follicle-stimulating hormone (rFSH) (Bemfola 225–300 IU/day, depending on ovarian reserve, Gedeon Richter Plc. Budapest Hungary). GnRH antagonist (Ganirelix 250 μg/day, Organon NV, The Netherlands) was started when a leading follicle of 14 mm was achieved. A GnRH agonist (Decapeptyl 0.2 mg, Ferring Pharmaceuticals, Switzerland) trigger was administered when at least one follicle was above 18 mm.

Ultrasound-guided transvaginal oocyte retrieval was performed 36–38 h after the trigger injection using an 18-gauge double-lumen needle and a vacuum pump (Cook Medical Incorporated, USA) under pressure at 150 mmHg. Follicle flushing was not performed. The cumulus–oocyte complexes were removed from the collection fluid using a sterile glass pipette and washed in G-IVF Plus media (Vitrolife, Sweden).

All embryos were cultured until the blastocyst stage. A trophectoderm biopsy of developed blastocysts was performed. Embryo vitrification was performed according to the Cryotop method (Kitazato, Japan) on day 5 or 6 of embryo development after a laser-induced collapse. Blastocysts were transferred from the culture medium (G2, Vitrolife) into an equilibration solution using 130 μm strippers. Blastomeres spontaneously began shrinking but gradually returned to their original size. This process took approximately 13 min. After that, blastocysts were transferred to a vitrification solution. Embryos were placed in VS1 for 30 s, VS2 for 30 s and then placed in the Cryotop sheet with minimal volume (<0.1 μL) of VS2. Excess volume was removed and the Cryotop was quickly plunged into fresh liquid nitrogen. Cryotops were stored in Espace 151 (Air Liquid, France) in the gas phase.

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Endometrial preparation and ultrasound assessment

Once a euploid embryo was available for transfer, endometrial preparation was induced with the sequential provision of oral estradiol valerate (Progyluton Bayer Hispania, Spain) in a fixed dose of 4 mg (per os) per day from cycle day 2 onward. After 8–10 days of estrogen therapy, a blood sample was collected, and a vaginal ultrasound was performed for measurements of progesterone levels (PP0) and endometrial features, respectively. Endometrial features included endometrial thickness (measured in the midsagittal plane of the uterus as the maximum distance between the two interfaces of endometrial–myometrial junction) and pattern (Fig. 2).

Pre-ET progesterone administration was started before the 14th day of the cycle and only if plasmatic progesterone determination (PP0) was lower than 1.0 ng/mL (Coughlan *et al.* 2023). Based on the progesterone administration approach before embryo transfer (pre-ET progesterone), patients were divided into group A with natural micronized progesterone (Utrogestan, SEID, Spain) administered orally at a dose of 200 mg/8 h (600 mg/day) starting 6 days (in the morning) before scheduled embryo-transfer and group B with natural micronized progesterone vaginal administration (400 mg/12 h; 800 mg/day) starting (in the evening) 6 days before embryo transfer (Fig. 2).

Vitrified euploid blastocysts were warmed on the day of embryo transfer in those embryos vitrified on day 6 and the day before transfer in those vitrified on day 5, using thawing, equilibration and washing media and the protocol from the package insert (Irvine Scientific, USA). Single embryos were loaded into a transfer catheter (Sureview, Wallace, Smith's Medical, UK) and transferred into the patient's uterus. During the transfer procedure, the placement of the catheter within the uterus was confirmed by abdominal ultrasound.

After embryo transfer, a vaginal protocol was administered (micronized progesterone, 800 mg/day, Utrogestan SEID, Spain).

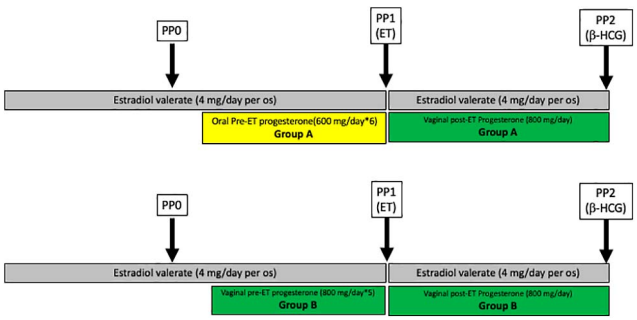

Figure 2

Treatment schedule.

After an initial plasmatic progesterone determination to rule out patients before exogenous progesterone administration (PP0) (see above), two additional progesterone concentration determinations were included in the trial protocol. An analysis was performed on the day of embryo transfer (PP1). Finally, a new analysis was performed on day of B-HCG determination (10 days after ET) (PP2). Blood samples for progesterone concentration measurements were performed at 8:00 and 10:00 h. Levels of progesterone were analyzed by time-resolved fluoroimmunoassay (Roche® G3 Elecsys cobas e411, Switzerland), with a coefficient of variation of 0.10%. The detection limit was 0.05 ng/mL.

If B-HCG determination was positive, hormonal treatment (including estrogen and progesterone) was maintained, and an ultrasound assessment was performed 2 weeks later. Once clinical pregnancy was diagnosed, patients continued with the prescribed hormonal supplementation until the 10th week of gestation. In the present study, the effects on birth outcomes also were investigated. All pregnant patients were followed until delivery.

Outcome measures

The primary outcome measure was to assess LBRs depending on the protocol of progesterone supplementation before embryo transfer (ET) and progesterone levels on the day of ET. LBR was defined as a viable intrauterine pregnancy resulting in the delivery of a viable infant after 24 weeks of gestation per cycle.

The secondary outcomes included the clinical pregnancy, miscarriage rates and LBR depending on various factors, including the plasmatic progesterone levels on the day of B-HCG determination and endometrial thickness. Clinical pregnancy was defined as a visible gestational sac determined by vaginal ultrasonography performed 2 weeks after a positive pregnancy test (7 weeks of gestation). Miscarriage was defined as the loss of pregnancy within 14 weeks of confirmation of a clinical pregnancy.

Statistical analysis

Sample size calculation

The sample size calculation for this study was based on the rate of newborns as the primary outcome. The required sample size to detect a difference of 15% in the primary outcome measure (40%) (Ferrando *et al.* 2020) with a two-tailed alpha of 0.05 and power (1-β) of 0.80 was estimated at 240 women in each arm.

Data were expressed as the means and standard error of means (SEMs) for continuous variables or as frequency

rates for categorical variables. The Student's *t*-test and one-way ANOVA for within-group variance analysis were used to compare continuous variables between the groups depending on the data distribution. Pearson's chi-squared was used to compare categorical variables. Receiver operator characteristic (ROC) curves and the analysis of the area under the curve (AUC) were performed to evaluate the discriminatory ability of the assessed parameters to predict a successful clinical outcome. Serum progesterone levels on the day of embryo transfer were analyzed as both a continuous variable and a dichotomous variable (according to percentiles 10th, 25th and 50th).

Statistical significance was set at a probability (*P*) value <0.05. Analyses were performed using the SPSS (IBM SPSS Statistics for Windows, version 29.0.2.0, released in 2023, IBM Corp., USA).

Results

The baseline features of patients were similar between the randomized groups. The flowchart of the patients entering the trial is shown in Fig. 1. Both groups were comparable in age, years of infertility, ovarian reserve and number of previous ART cycles. There were no differences in clinical pregnancy (50.20 vs 47.37%), live birth (43.67 vs 40.89%) or miscarriage rates (13.33 vs 6.48%) depending on the route of progesterone administration before embryo transfer (Pre-ET) (Table 1). However, the plasmatic levels of progesterone on the day of ET (PP1) were significantly higher when pre-ET progesterone administration was oral (group A) compared to the vaginal route (group B) (35.10 ± 5.61 vs 15.13 ± 2.73 ng/mL; t = 1.559; P = 0.002).

All 208 newborns were available for analysis, including sex, weight and type of delivery. There were no differences in the percentage of preterm deliveries and newborns' mean weight between the study's two arms (Table 1). There was a higher percentage of cesarean deliveries among the patients who received vaginal progesterone before embryo transfer (group B) (45.55 vs 28.97%; chi-square = 6.12; P = 0.013). The proportion of females/males was higher among group A patients (oral pre-ET progesterone) (Table 1).

Progesterone levels on the day of embryo transfer (PP1) were significantly higher among patients with evolutive pregnancies than among those without evolutive pregnancies and no pregnancies (Table 2). The P1 levels were similar between patients with non-ongoing pregnancies (including miscarriages and non-ongoing positive pregnancy tests) and non-pregnant patients (Table 2).

To go further, progesterone values on the ET day (PP1) were examined dichotomously, stratified by the 10th, 25th and 50th percentiles. It was noted that while the 10th percentile lacked discriminatory ability, significance was observed at the 25th and 50th percentiles (Table 3 and Fig. 3).

An area under the curve (ROC) was analyzed to determine the potential predictive value of progesterone levels on the day of embryo transfer in a full-term pregnancy. The PP1 levels did not predict an evolutive gestation (AUC 0.491 with a 95% confidence interval between 0.380 and 0.601) (Table 5).

Table 1 Clinical, analytic and ultrasound parameters. Comparative data according to pre-ETprogesterone administration schedule.

		Pre-ET progesterone administration					
	All patients	**Orally administered**	**Vaginally administered**	***t***	***P***	**Pearson's χ^2**	***P***
Age (years)	36.49 ± 0.18	36.52 ± 0.21	36.33 ± 0.29	0.528	0.599		
BMI (kg/m^2)	23.44 ± 0.25	23.28 ± 0.26	24.29 ± 0.79	1.216	0.230		
Years of infertility	2.20 ± 0.05	2.21 ± 0.07	2.19 ± 0.07	0.102	0.919		
Previous ART cycles (yes/no)	162/495 (33.01%)	83/245 (33.72%)	80/247 (32.32%)			0.061	0.805
Number of previous cycles	1.56 ± 0.06	1.55 ± 0.08	1.57 ± 0.08	0.108	0.914		
Antral follicle count	9.24 ± 0.21	9.46 ± 1.30	9.56 ± 1.09	0.677	0.654		
Endometrial thickness (mm)	8.03 ± 0.84	8.01 ± 0.09	8.16 ± 0.25	0.613	0.540		
PP1*	31.85 ± 4.74	35.10 ± 5.61	15.13 ± 2.73	1.559	0.002		
PP2†	17.51 ± 1.86	16.88 ± 1.10	19.53 ± 1.59	0.942	0.348		
Positive pregnancy test (rate)	264/492 (53.66%)	137/245 (55.92%)	127/247 (51.42%)			0.458	0.506
Clinical pregnancy (rate)	240/492 (48.78%)	123/245 (50.20%)	117/247 (47.37%)			0.166	0.920
Live birth (rate)	208/492 (42.28%)	107/245 (43.67%)	101/247 (40.89%)			0.031	0.860
Miscarriage (rate)	32/240‡ (13.33%)	16/123‡ (13.01%)	16/117 (13.68%)			0.020	0.879
Preterm deliveries (<37 weeks)	18/208 (8.65%)	10/107 (9.35%)	8/101 (7.92%)			0.041	0.831
Newborns: female/male	98/110	59/48	39/62			5.702	0.017
Vaginal/cesarean delivery	131/77	76/31	55/46			6.12	0.013
Weight of newborn (g)	3,305.95 ± 81.00	3,323.25 ± 91.83	3,238.08 ± 176.79	0.420	0.676		

BMI, body mass index.

*Plasma progesterone levels on the day of embryo transfer (ET) (ng/mL). †Plasma progesterone levels on the day of B-HCG determination (ET) (ng/mL). ‡Includes one ectopic (tubal pregnancy).

Table 2 Comparative progesterone determinations and endometrial thickness data.

	EP[‡] (*n* = 208)	Miscarriage[§] (*n* = 56)	Not pregnant (*n* = 228)	F	*P*
PP1*[(1)]	24.96 ± 1.00	19.39 ± 1.47	17.56 ± 0.99	28.8561	0.0001
PP2[†(2)]	16.93 ± 1.17	15.72 ± 1.07	19.44 ± 1.02	2.9874	0.0500
Endometrial thickness, mm	8.11 ± 0.10	7.85 ± 0.38	8.10 ± 0.12	6.63.70	0.0010

EP, evolutive pregnancy.
*Plasmatic progesterone levels on the day of embryo transfer (ET) (ng/mL). †Plasmatic progesterone levels on the day of B-HCG determination (ng/mL). ‡Evolutive pregnancy included full-term and preterm deliveries (LBR). §Included miscarriages and non evolutive positive pregnancy test.

Regarding secondary outcomes, progesterone levels on the day of B-HCG determination (PP2) were not associated with pregnancy success or miscarriage rates (Tables 2 and 4 and Fig. 4).

Endometrial thickness showed significant differences when comparing ongoing pregnancies to non-ongoing pregnancies. However, no significant differences were observed between ongoing pregnancies and non-pregnancies (Table 2). By assessing ROC curves, the predictive value of endometrial thickness for determining progressive pregnancy was not statistically significant (Table 5).

Discussion

This randomized clinical trial comprehensively evaluates different progesterone administration schedules and plasma levels on the day of embryo transfer (ET) on full-term pregnancy rates in patients undergoing frozen-thawed single embryo transfer (FET) of euploid blastocysts. With the global increase in FET, unresolved debates persist. First, no consensus has been reached on the optimal regimen for endometrial preparation in these cycles despite advancements that leave significant uncertainty in strategies to enhance day-to-day clinical practice and success rates in FET (Mackens *et al.* 2017). Moreover, focusing on (LBRs) and maternal, obstetrical and neonatal outcomes is crucial (Hubayter & Muasher 2008, Mumusoglu *et al.* 2021). Recent studies suggest that a well-conducted natural cycle is superior to an HRT cycle regarding obstetrical outcomes (Melado Vidales *et al.* 2023).

The optimal plasmatic progesterone levels around the day of ET for achieving satisfactory clinical outcomes after FET are debated, where the timing and concentration of progesterone exposure are crucial for establishing and maintaining a pregnancy (Kofinas *et al.* 2015, Devine *et al.* 2021). In addition, studies highlight conflicts over the best route for progesterone administration, reflecting differing opinions on oral versus vaginal routes (Yarali *et al.* 2016, Labarta *et al.* 2017, Akaeda *et al.* 2019).

The optimal route for progesterone replacement in FET remains unknown, with administration types including oral, vaginal, oil-based intramuscular (IM) and aqueous subcutaneous (SC) progesterone. Introducing non-intramuscular formulations provides physicians and patients with additional supplement options (Aflatoonian & Mohammadi, 2021). Traditionally, vaginal and intramuscular routes have been the most effective and widely used (Bulletti *et al.* 2022, Zhu *et al.* 2023). Micronized progesterone shows enhanced absorption through both vaginal and intestinal mucosa; it is widely used for luteal phase supplementation in ART via the vaginal route, whereas oral progesterone is less commonly utilized (Enatsu *et al.* 2018, Melo *et al.* 2021).

Table 3 Ongoing (live birth) pregnancies according to plasmatic progesterone levels on the day of embryo transfer (PP1). Comparative results between values below or above are 10th, 25th and 50th percentiles.

PP1-ET	OPR	Pearson's χ^2	*P*
Percentile 10 (8.35 ng/mL)		1.438	0.230
<10	31.11% (14/45)		
≥10	43.40% (194/447)		
Percentile 25 (10.24 ng/mL)		4.560	0.033
<25	28.13% (27/96)		
≥25	45.71% (181/396)		
Percentile 50 (16.14 ng/mL)		12.241	0.000
<50	28.98% (71/245)		
≥50	52.26% (130/247)		

PPI-ET, plasmatic progesterone levels on day of embryo transfer (ET) (ng/mL); OPR, ongoing pregnancy rate.

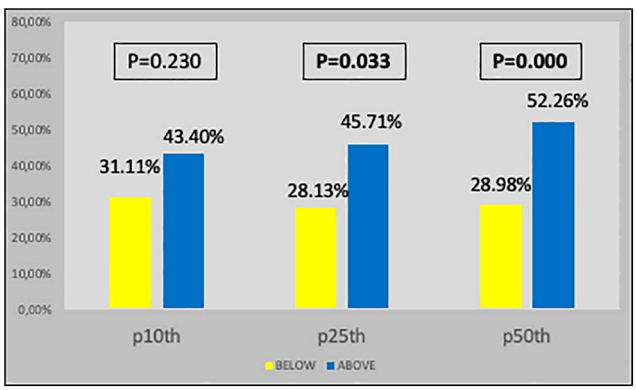

Figure 3

LBR according to progesterone levels stratified by percentiles on the day of embryo transfer (PP1).

Table 4 Ongoing (live birth) pregnancies according to plasmatic progesterone levels on day of pregnancy test (PP2). Comparative results between values below or above are 10th, 25th and 50th percentiles.

PP2_B-HCG	OPR	Pearson's χ^2	*P*
Percentile 10 (7.85 ng/mL)		0.222	0.638
<10	45.83% (22/48)		
≥10	41.89% (186/444)		
Percentile 25 (9.49 ng/mL)		0.080	0.777
<25	40.34% (48/119)		
≥25	42.90% (160/373)		
Percentile 50 (13.40 ng/mL)		0.141	0.707
<50	44.31% (109/246)		
≥50	40.24% (99/246)		

PP2_B-HCG, plasmatic progesterone levels on the day of B-HCG determination (pregnancy test) (ng/mL); OPR, ongoing pregnancy rate.

The vaginal route has become more popular due to reduced discomfort and fewer adverse effects, yielding high progesterone levels in the uterine endometrium, potentially favoring pregnancy outcomes (Jiang *et al.* 2019). In our study, vaginal and oral progesterone administration before ET were evaluated, as these are commonly used alternatives to intramuscular and subcutaneous routes, assuming that oral administration post-ET is ineffective (Saucedo *et al.* 2000). Our findings align with previous studies indicating no significant differences in live birth or clinical pregnancy rates between different protocols (Ghobara & Vandekerckhove 2008, Groenewoud *et al.* 2013). Despite lower progesterone concentrations with vaginal administration on the day of ET, clinical outcomes did not differ between treatment schedules. Our findings showed no difference in clinical outcomes, including live birth and miscarriage rates, between the two administration routes despite variations in plasma levels. Plasma progesterone levels are lower with the vaginal route compared to intramuscular

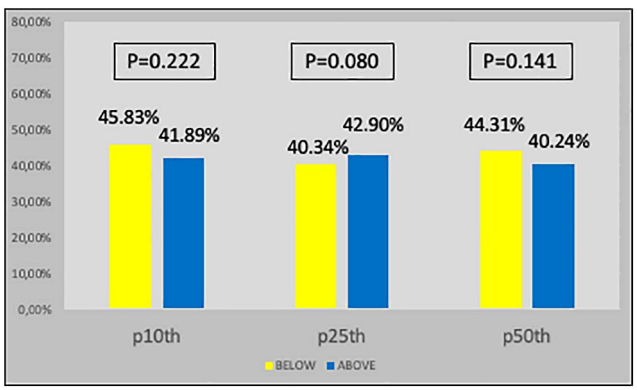

Figure 4

Live birth rate according to progesterone levels stratified by percentiles on the day of B-HCG determination (PP2).

Table 5 Area under the curve (AUC) (ROC) (parameters related to live births).

			95% A-CI	
	Area	Asymptotic significance*	Lower limit	Higher limit
Age (years)	0.439	0.257	0.333	0.544
Endometrial thickness (mm)	0.509	0.580	0.417	0.648
PP1-ET (ng/mL)	0.491	0.869	0.380	0.601
PP2_B-HCG (ng/mL)	0.491	0.852	0.392	0.589

*Null hypothesis: area = 0.5.

A-CI, asymptomatic confidence interval; PP1-ET, plasma progesterone levels on the day of embryo transfer; PP2_B-HCG, plasma progesterone levels on the day of B-HCG determination.

(Cicinelli *et al.* 2000, Enatsu *et al.* 2018). However, all administration routes have been shown to achieve satisfactory mean serum progesterone levels by day 5 (Labarta *et al.* 2021, Melo *et al.* 2021).

This trial identified a significant association between plasma progesterone levels on ET day and ongoing pregnancies. However, progesterone levels measured 10 days post-ET showed no correlation with pregnancy outcomes, suggesting that pre-implantation progesterone exposure plays a more critical role.

The relationship between serum progesterone levels and pregnancy outcomes has been extensively studied, with mixed results (Stavridis *et al.* 2023). Low serum progesterone levels have been linked to poor pregnancy outcomes in several studies (Brady *et al.* 2014, Yovich *et al.* 2015, Labarta *et al.* 2017, Cedrin-Durnerin *et al.* 2019, Gaggiotti-Marre *et al.* 2019, Alsbjerg *et al.* 2020, Gonzalez-Foruria *et al.* 2020, Labarta *et al.* 2021, Shiba *et al.* 2021). However, the predictive value remains debated (Melo *et al.* 2021, Bulletti *et al.* 2022, Lawrenz *et al.* 2023, 2024).

Although low progesterone levels on the ET day are associated with poor reproductive outcomes, the exact thresholds for optimal outcomes remain unclear (Labarta *et al.* 2021, González-Foruria *et al.* 2023). Previous studies assessing progesterone levels and pregnancy outcomes have methodological differences. Timing of serum progesterone measurement varied, with some measuring on ET day and others before or after ET. Some researchers propose additional progesterone supplementation for low serum levels to optimize luteal phase support (Álvarez *et al.* 2021, Labarta *et al.* 2022), but good-quality interventional evidence is lacking (Alsbjerg *et al.* 2020).

Although thin endometrium (<7 mm) is generally considered unfavorable, emerging evidence suggests that endometrial receptivity may depend more on functional characteristics than on thickness alone (Liu *et al.* 2018, Zhang *et al.* 2018, Kai-Lun Hu *et al.* 2021, Shakerian *et al.* 2021, Ata *et al.* 2023). Our study showed that endometrial thickness was greater in

patients with ongoing pregnancies than non-ongoing pregnancies. Paradoxically, endometrial thickness was also greater in non-pregnant patients than in those who experienced a miscarriage. This apparent contradiction highlights the need for a more nuanced understanding of endometrial receptivity markers.

This trial exhibited a high cesarean section rate. HRT cycles are associated with increased risks of postpartum hemorrhage and cesarean section compared to natural cycles. FETs after hormonal replacement have a higher likelihood of hypertensive disorders and overall maternal complications (Schattman 2023).

Clinical implications and future directions

This study contributes to the ongoing effort to optimize FET protocols by demonstrating that different progesterone administration regimens result in comparable clinical outcomes, despite differences in plasma levels. These findings support the use of patient-preferred routes of administration, particularly vaginal progesterone, which is associated with fewer side effects and greater convenience.

Future research should focus on standardizing the timing and methods of progesterone measurement, as variations in study designs have led to inconsistent conclusions. In addition, well-designed interventional studies are needed to determine whether targeted progesterone supplementation for patients with low levels could improve outcomes.

Conclusion

Our findings reinforce the equivalence of oral and vaginal progesterone administration in FET cycles regarding clinical pregnancy and LBRs. While plasma progesterone levels on ET day were associated with pregnancy outcomes, other parameters, such as post-ET progesterone levels, showed no predictive value. These results emphasize the importance of personalized approaches to FET preparation and highlight areas for future investigation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the work reported.

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Author contribution statement

GB conceived the study idea. GB, OP, RC and JB participated in the design of the trial to recruit participants and assessed clinical outcomes. EM, MDLH, OG and OA were involved in the critical revision of the manuscript. GB and OP coordinated the data collection, performed the analysis and wrote the first draft of the manuscript. All authors approved the final manuscript.

Data availability

The data underlying this article will be shared by the corresponding author upon a reasonable request.

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References

Aflatoonian A & Mohammadi B 2021 Subcutaneous progesterone versus vaginal progesterone for luteal-phase support in frozen-thawed embryo-transfer: a cross-sectional study. *Int J Reprod BioMed* **19** 115–120. (https://doi.org/10.18502/ijrmv19i2.8469)

Akaeda S, Kobayashi D, Shioda K, *et al.* 2019 Relationship between serum progesterone concentrations and pregnancy rates in hormone replacement treatment-frozen embryo transfer using progesterone vaginal tablets. *Clin Exp Obstet Gynecol* **46** 695–698. (https://doi.org/10.12891/ceog4360.2019)

Alsbjerg B, Labarta E & Humaidan P 2020 Serum progesterone levels on day of embryo transfer in frozen embryo transfer cycles-the truth lies in the detail. *J Assist Reprod Genet* **37** 2045–2046. (https://doi.org/10.1007/s10815-020-01851-1)

Álvarez M, Gaggiotti-Marre S, Martínez F, *et al.* 2021 Individualised luteal phase support in artificially prepared frozen embryo transfer cycles based on serum progesterone levels: a prospective cohort study. *Hum Reprod* **36** 1552–1560. (https://doi.org/10.1093/humrep/deab031)

Ata B, Liñán A, Kalafat E, *et al.* 2023 Effect of the endometrial thickness on the live birth rate: insights from 959 single euploid frozen embryo transfers without a cutoff for thickness. *Fertil Steril* **120** 91–98. (https://doi.org/10.1016/j.fertnstert.2023.02.035)

Barrenetxea G, Romero I, Celis R, *et al.* 2020 Obstetrical and perinatal complications after assisted reproduction; what matters is age, not the procedure. *Res Int J Obst Gynecol* **1** 001–008. (https://doi.org/10.37179/rijog.000001)

Barrenetxea G, Celis R, Barrenetxea J, *et al.* 2024 Intraovarian platelet-rich plasma injection and IVF outcomes in patients with poor ovarian response: a double-blind randomized controlled trial. *Hum Reprod* **39** 760–769. (https://doi.org/10.1093/humrep/deae038)

Brady PC, Kaser DJ, Ginsburg ES, *et al.* 2014 Serum progesterone concentration on day of embryo transfer in donor oocyte cycles. *J Assist Reprod Genet.* **31** 569–575. (https://doi.org/10.1007/s10815-014-0199-y)

Bulletti C, Bulletti FM, Sciorio R, *et al.* 2022 Progesterone: the key factor of the beginning of life. *Int J Mol Sci* **23** 14138. (https://doi.org/10.3390/ijms232214138)

Casper RF 2020 Frozen embryo transfer: evidence-based markers for successful endometrial preparation. *Fertil Steril* **113** 248–251. (https://doi.org/10.1016/j.fertnstert.2019.12.008)

Cedrin-Durnerin I, Isnard T, Mahdjoub S, *et al.* 2019 Serum progesterone concentration and live birth rate in frozen- thawed embryo transfers with hormonally prepared endometrium. *Reprod Biomed Online* **38** 472–480. (https://doi.org/10.1016/j.rbmo.2018.11.026)

Cicinelli E, Schonauer LM, Galantino P, *et al.* 2000 Mechanisms of uterine specificity of vaginal progesterone. *Hum Reprod* **15** (Supplement 1) 159–165. (https://doi.org/10.1093/humrep/15.suppl_1.159)

Coughlan C, Ata B, Gallego RD, *et al.* 2023 Interindividual variation of progesterone elevation post LH rise: implications for natural cycle frozen embryo transfers in the individualized medicine era. *Rep Biol Endocrinol* **21** 47. (https://doi.org/10.1186/s12958-023-01096-4)

Eftekhar M, Rahsepar M & Rahmani E 2013 Effect of progesterone supplementation on natural frozen-thawed embryo transfer cycles: a randomized controlled trial. *Int J Fertil Steril* **7** 13–20.

Enatsu Y, Enatsu N, Kishi K, *et al.* 2018 Effectiveness of high-dose transvaginal progesterone supplementation for women who are undergoing a frozen-thawed embryo transfer. *Reprod Med Biol* **17** 242–248. (https://doi.org/10.1002/rmb2.12096)

Ferrando M, Coroleu B, Rodríguez-Tabernero L, *et al.* 2020 The continuum of ovarian response leading to BIRTH, a real world study of ART in Spain. *Fertil Res Pract* **6** 13. (https://doi.org/10.1186/s40738-020-00081-4)

Gaggiotti-Marre S, Martinez F, Coll L, *et al.* 2019 Low serum progesterone the day prior to frozen embryo transfer of euploid embryos is associated with significant reduction in live birth rates. *Gynecol Endocrinol* **35** 439–442. (https://doi.org/10.1080/09513590.2018.1534952)

Ghobara T & Vandekerckhove P 2008 Cycle regimens for frozen-thawed embryo transfer. *Cochrane Database Syst Rev* **7** (Supplement 1) CD003414. (https://doi.org/10.1002/14651858.CD003414.pub3)

Gonzalez-Foruria I, Gaggiotti-Marre S, Alvarez M, *et al.* 2020 Factors associated with serum progesterone concentra- tions the day before cryopreserved embryo transfer in artificial cycles. *Reprod Biomed Online* **40** 797–804. (https://doi.org/10.1016/j.rbmo.2020.03.001)

González-Foruria I, García S, Álvarez M, *et al.* 2023 Elevated serum progesterone levels before frozen embryo transfer do not negatively impact reproductive outcomes: a large retrospective cohort study. *Fertil Steril* **120** 597–604. (https://doi.org/10.1016/j.fertnstert.2023.04.038)

Groenewoud ER, Cantineau AE, Kollen BJ, *et al.* 2013 What is the optimal means of preparing the endometrium in frozen-thawed embryo transfer cycles? A systematic review and meta-analysis. *Hum Reprod Update* **19** 458–470. (https://doi.org/10.1093/humupd/dmt030)

Hubayter ZR & Muasher SJ 2008 Luteal supplementation in in vitro fertilization: more questions than answers. *Fertil Steril* **89** 749–758. (https://doi.org/10.1016/j.fertnstert.2008.02.095)

Jiang L, Luo ZY, Hao GM, *et al.* 2019 Effects of intramuscular and vaginal progesterone supplementation on frozen-thawed embryo transfer. *Sci Rep* **9** 15264. (https://doi.org/10.1038/s41598-019-51717-5)

Kai-Lun Hu AK, Sarah H, Wentao L, *et al.* 2021 Endometrial thickness (EMT) in the prediction of neonatal adverse outcomes in frozen cycles for singleton pregnancies. *Reprod Biomed Online* **43** 553–560. (https://doi.org/10.1016/j.rbmo.2021.04.014)

Kofinas JD, Blakemore J, McCulloh DH, *et al.* 2015 Serum progesterone levels greater than 20 ng/dl on day of embryo transfer are associated with lower live birth and higher pregnancy loss rates. *J Assist Reprod Genet* **32** 1395–1399. (https://doi.org/10.1007/s10815-015-0546-7)

Labarta E, Mariani G, Holtmann N, *et al.* 2017 Low serum progesterone on the day of embryo transfer is associated with a diminished ongoing pregnancy rate in oocyte donation cycles after artificial endometrial preparation: a prospective study. *Hum Reprod* **32** 2437–2442. (https://doi.org/10.1093/humrep/dex316)

Labarta E, Mariani G, Paolelli S, *et al.* 2021 Impact of low serum progesterone levels on the day of embryo transfer on pregnancy outcome: a prospective cohort study in artificial cycles with vaginal progesterone. *Hum Reprod* **36** 683–692. (https://doi.org/10.1093/humrep/deaa322)

Labarta E, Mariani G, Rodríguez-Varela C, *et al.* 2022 Individualized luteal phase support normalizes live birth rate in women with low progesterone levels on the day of embryo transfer in artificial endometrial preparation cycles. *Fertil Steril* **117** 96–103. (https://doi.org/10.1016/j.fertnstert.2021.08.040)

Lawrenz B, Ata B, Kalafat E, *et al.* 2023 Are systemic progesterone levels in true natural cycle euploid frozen embryo transfers with luteal phase support predictive for ongoing pregnancy rates? *Hum Reprod* **38** 1318–1324. (https://doi.org/10.1093/humrep/dead104)

Lawrenz B, Kalafat E, Ata B, *et al.* 2024 The combination of dydrogesterone and micronized vaginal progesterone can render serum progesterone level measurements on the day of embryo transfer and rescue attempts unnecessary in an HRT FET cycle. *J Assist Reprod Genet* **41** 885–892. (https://doi.org/10.1007/s10815-024-03049-1)

Liu KE, Hartman M, Hartman A, *et al.* 2018 The impact of a thin endometrial lining on fresh and frozen-thaw IVF outcomes: an analysis of over 40 000 embryo transfers. *Hum Reprod* **33** 1883–1888. (https://doi.org/10.1093/humrep/dey281)

Mackens S, Santos-Ribeiro S, van de Vijver A, *et al.* 2017 Frozen embryo transfer: a review on the optimal endometrial preparation and timing. *Hum Reprod* **32** 2234–2242. (https://doi.org/10.1093/humrep/dex285)

Melado Vidales L, Lawrenz B, Vitorino RL, *et al.* 2023 Clinical and laboratory parameters associated with cycle outcomes in patients undergoing euploid frozen blastocyst transfer. *Reprod Biomed Online* **46** 917–925. (https://doi.org/10.1016/j.rbmo.2023.02.014)

Melo P, Chung Y, Pickering O, *et al.* 2021 Serum luteal phase progesterone in women undergoing frozen embryo transfer in assisted conception: a systematic review and meta-analysis. *Fertil Steril* **116** 1534–1556. (https://doi.org/10.1016/j.fertnstert.2021.07.002)

Mumusoglu S, Polat M, Ozbek IY, *et al.* 2021 Preparation of the endometrium for frozen embryo transfer: a systematic review. *Front Endocrinol* **12** 688237. (https://doi.org/10.3389/fendo.2021.688237)

Ortega I & García Velasco JA 2015 Progesterone supplementation in the frozen embryo transfer cycle. *Curr Opin Obst Gynecol* **27** 253–257. (https://doi.org/10.1097/gco.0000000000000184)

Patel JA, Patel AJ, Banker JM, *et al.* 2019 Personalized embryo transfer helps in improving in vitro fertilization/ICSI outcomes in patients with recurrent implantation failure. *J Hum Reprod Sci* **12** 59–66. (https://doi.org/10.4103/jhrs.JHRS_74_18)

Saucedo LLE, Galache VP, Hernandez AS, *et al.* 2000 Randomized trial of three different forms of progesterone supplementation in ART: preliminary results. *Fertil Steril* **74** (Supplement 1) S150. (https://doi.org/10.1016/s0015-0282(00)01151-1)

Schattman G 2023 Subcutaneous progesterone: a more patient-friendly approach for programmed frozen embryo transfer cycles. *Fertil Steril* **4** 159–160. (https://doi.org/10.1016/j.xfre.2023.03.003)

Schulz KF, Altman DG, Moher D, *et al.* 2010 CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMJ* **340** c332. (https://doi.org/10.1136/bmj.c332)

Shakerian B, Turkgeldi E, Yildiz S, *et al.* 2021 Endometrial thickness is not predictive for live birth after embryo transfer, even without a cutoff. *Fertil Steril* **116** 130–137. (https://doi.org/10.1016/j.fertnstert.2021.02.041)

Shiba R, Kinutani M, Okano S, *et al.* 2021 Serum progesterone levels with the use of four different types of vaginal progesterone in frozen-thawed embryo transfer cycles and related pregnancy outcomes. *Int J Fertil Steril* **15** 34–39. (https://doi.org/10.22074/IJFS.2021.6235)

Stavridis K, Kastora SL, Triantafyllidou O, *et al.* 2023 Effectiveness of progesterone rescue in women presenting low circulating progesterone levels around the day of embryo transfer: a systematic review and meta-analysis. *Fertil Steril* **119** 954–963. (https://doi.org/10.1016/j.fertnstert.2023.02.007)

Wånggren K, Dahlgren Granbom M, Iliadis SI, *et al.* 2022 Progesterone supplementation in natural cycles improves live birth rates after embryo transfer of frozen-thawed embryos—a randomized controlled trial. *Hum Rep* **37** 2366–2374. (https://doi.org/10.1093/humrep/deac181)

Wei D, Liu JY, Sun Y, *et al.* 2019 Frozen versus fresh single blastocyst transfer in ovulatory women: a multicentre, randomised controlled trial. *Lancet* **393** 1310–1318. (https://doi.org/10.1016/s0140-6736(18)32843-5)

Wong KM, van Wely M, Mol F, *et al.* 2017 Fresh versus frozen embryo transfers in assisted reproduction. *Cochrane Database Syst Rev* **3** CD011184. (https://doi.org/10.1002/14651858.cd011184.pub2)

Yarali H, Polat M, Mumusoglu S, *et al.* 2016 Preparation of endometrium for frozen embryo replacement cycles: a systematic review and meta-analysis. *J Assist Reprod Genet* **33** 1287–1304. (https://doi.org/10.1007/s10815-016-0787-0)

Yovich JL, Conceicao JL, Stanger JD, *et al.* 2015 Mid-luteal serum progesterone concentrations govern implantation rates for cryopreserved embryo transfers conducted under hormone replacement. *Reprod Biomed Online* **31** 180–191. (https://doi.org/10.1016/j.rbmo.2015.05.005)

Zhang T, Li Z, Ren X, *et al.* 2018 Endometrial thickness as a predictor of the reproductive outcomes in fresh and frozen embryo transfer cycles. A retrospective cohort study of 1512 IVF cycles with morphologically good-quality blastocyst. *Medicine* **97** e9689. (https://doi.org/10.1097/MD.0000000000009689)

Zhu Q, Huang J, Lin Y, *et al.* 2023 Association between serum progesterone levels on the day of frozen-thawed embryo transfer and pregnancy outcomes after artificial endometrial preparation. *BMC Pregnancy Childbirth* **23** 401. (https://doi.org/10.1186/s12884-023-05596-4)