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Impact of administration route on serum progesterone levels in women undergoing artificial endometrial preparation

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KEYWORDS
Assisted reproduction technology;
Endometrial preparation;
Administration route;
Serum progesterone

Abstract
Objective: Are there differences in serum progesterone levels between different routes of exogenous progesterone administration for artificial endometrial preparation?
Material and methods: This prospective, observational, single-centre study included 9 infertile female patients who underwent cycles of artificial endometrial preparation between January and June 2019 with different progesterone formulations (3 cycles in 2 patients; 2 cycles in 2 patients; and 1 cycle in 5 patients). Oestrogen stimulation was followed by vaginal progesterone 400 mg every 12 h (first cycle), subcutaneous progesterone 25 mg every 12 h (second cycle), and intramuscular progesterone 50 mg every 24 h (third cycle). Progesterone therapy was continued for 5 days and daily serum progesterone was recorded. The primary outcome was day 5 serum progesterone.
Results: Day 5 mean ± standard deviation serum progesterone levels after vaginal, subcutaneous, and intramuscular administration were 14.6 ± 5.5, 47.9 ± 22.3, and 60.3 ± 65.5 ng/mL, respectively (p = 0.032 across routes). From day 1 to day 5, the coefficients of variation for serum progesterone were 66% and 75% with the vaginal and subcutaneous routes, respectively, indicating low variability, and 146% with the intramuscular route, indicating high variability. Two linear regression models were conducted: a normal linear regression model, which found no

Abbreviations: ART, assisted reproduction technology; BMI, body mass index; CV, coefficient of variation D, day; E2, oestradiol; FET, frozen–thawed embryo transfer; SD, standard deviation.

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significant effect of administration route on serum progesterone, and a mixed-effects linear regression model, which also showed no statistically significant differences between routes.
Conclusion: All routes of progesterone administration showed satisfactory day 5 mean serum progesterone levels, regardless of administration route.

PALABRAS CLAVES
Ciclo sustituido;
Progesterona sérica;
Fase lútea

Impacto de la vía de administración en los niveles de progesterona sérica en mujeres sometidas a ciclos artificiales

Resumen
Objetivo: ¿Existen diferencias en los niveles de progesterona sérica entre las diferentes vías de administración de progesterona exógena para los ciclos sustituidos?
Material y métodos: Este estudio prospectivo observacional incluyó a nueve pacientes infértiles que se sometieron a ciclos de preparación endometrial artificial entre enero y junio de 2019 con diferentes formulaciones de progesterona, 5 pacientes con cada tipo de progesterona finales (3 ciclos en 2 pacientes; 2 ciclos en 2 pacientes y 1 ciclo en 5 pacientes) . La estimulación con estrógenos fue seguida por progesterona vaginal 400 mg cada 12 horas (primer ciclo), progesterona subcutánea 25 mg cada 12 horas (segundo ciclo) y progesterona intramuscular 50 mg cada 24 horas (tercer ciclo). La terapia con progesterona se continuó durante 5 días y se registró la progesterona sérica diaria. El resultado primario fue la progesterona sérica del día 5, auqnue se midio diariamente durante los 5 dias de fase lutea, siendo un objetivo secundario la variabilidad durante la misma.
Resultados: Los niveles de progesterona sérica en Día 5 fueron expresados como la media ± desviación estándar, siendo respectivamente después de la administración vaginal, subcutánea e intramuscular: 14,6 ± 5,5, 47,9 ± 22,3 y 60,3 ± 65,5 ng/mL, respectivamente (p = 0,032 en todas las vías). Desde el día 1 hasta el día 5, los coeficientes de variación de la progesterona sérica fueron del 66% y el 75% con las vías vaginal y subcutánea, respectivamente, lo que indica una baja variabilidad, y del 146% con la vía intramuscular, lo que indica una alta variabilidad (secundario a una paciente que obtuvo niveles mayores de los standar). Se realizaron dos modelos de regresión lineal: un modelo de regresión lineal normal, que no encontró ningún efecto significativo de la vía de administración sobre la progesterona sérica, y un modelo de regresión lineal de efectos mixtos, que tampoco mostró diferencias estadísticamente significativas entre las vías.
Conclusión: Todas las vías de administración de progesterona mostraron niveles medios de progesterona sérica óptimos en el día 5, independientemente de la vía de administración, y en la vía vaginal baja variabilidad durante los diferentes días de la fase lútea, lo cual nos permite concluir que una medición nos permite saber si dicha paciente tiene niveles óptimos de progesterona previos a la transferencia.

Introduction

Advances in assisted reproduction technology (ART) include improvements to controlled ovarian stimulation protocols and in vitro fertilisation technologies. There has been increased use of embryo cryopreservation and frozen–thawed embryo transfer (FET) worldwide (Pabuccu et al., 2020), partly because embryo cryopreservation is a reasonable approach to avoiding ovarian hyperstimulation syndrome in high-risk patients (Corbett et al., 2014). The success of FET depends on preparation of the endometrium and luteal phase support, which can be done artificially using hormone replacement therapy with both oestrogen and progesterone. However, the optimal approach to endometrial preparation is not yet well established (Pabuccu et al., 2020; Poletto et al., 2019). The mid-luteal phase is a critical point in natural and artificial cycles because this is the point at which implantation is likely to occur (Pabuccu et al., 2020).

Assessing serum progesterone levels prior to FET has gained increasing attention because thresholds associated with improved pregnancy outcomes have been recently identified. Labarta et al. (2017) demonstrated that, after artificial endometrial preparation in women undergoing oocyte donation cycles, progesterone levels <9.2 ng/mL on the day of FET were associated with significantly lower ongoing pregnancy rates than those with levels ≥9.2 ng/mL (odds ratio: 0.297; 95% confidence interval: 0.113, 0.779; p = 0.013). Similarly, Gaggiotti-Marre et al. have reported higher rates of

miscarriage and inferior live birth rates when progesterone levels were < 10.64 ng/mL compared with when they were above this cut-off (Gaggiotti-Marre et al., 2019). Therefore, previous relevant studies suggest that the minimum serum progesterone level in the mid-luteal phase is >9–10 ng/mL in artificial FET cycles (Pabuccu et al., 2020).

Several different progesterone regimens, varying in route, formulation, and dosage, have been investigated for endometrial preparation and luteal phase support during FET cycles. Although a 2015 Cochrane review of routes of administration (vaginal, oral, intramuscular, subcutaneous, and rectal) found that no route was superior over any other for key outcomes, such as live births or ongoing pregnancy rates (van der Linden et al., 2015), the standard protocol in most ART clinics worldwide involves the use of vaginal progesterone. This is mainly because oral and intramuscular formulations are not available in all countries, while the subcutaneous formulation is reserved for cases when vaginal progesterone is ineffective. Given the importance of achieving a minimum serum progesterone level prior to FET (Labarta et al., 2021), many ART clinics have adopted a 'rescue protocol' of administering progesterone subcutaneously or intramuscularly when serum progesterone levels are below threshold levels. Labarta et al. (2021) recently reported a serum progesterone threshold of 8.8 ng/mL in artificial endometrial preparation cycles, below which the likelihood of successful pregnancy is reduced.

It has not yet been established whether there is any variability in serum progesterone levels between different routes of administration of exogenous progesterone. We performed a study to evaluate whether day 5 serum progesterone levels are influenced by different routes of administration, using progesterone formulations that are commercially available in Spain, in women undergoing artificial endometrial preparation for FET.

Materials and methods

Study design and population

This was a prospective, observational, single-centre study conducted at IVIRMA Madrid (Spain) between January and June 2019. Women scheduled to receive hormone replacement therapy for artificial endometrial preparation were included if they were aged 30–45 years, had a body mass index (BMI) of 18–25 kg/m^2, were in good physical and mental health, had negative serological tests for hepatitis B and C, HIV, and syphilis, and were not scheduled to undergo frozen embryo transfer in that cycle. Patients using hormonal contraception or with an intrauterine device, and those with known endometrial diseases, such as endometritis and uterine endometriosis, were excluded.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki Ethical Principles. The ethics committee of the University Hospital Puerta de Hierro Majadahonda, Spain, approved the study protocol (Identification Code 1809-MAD-063-MC; 26 February 2018), which also complied with Spanish law on ART. All patients gave written informed consent to participate in the study.

Hormone replacement therapy for artificial endometrial preparation

Patients underwent 3 consecutive artificial cycles with a different route of administration being used in each cycle (i.e., vaginal, subcutaneous, or intramuscular). According to the protocol used in our clinic, all cycles began with oral oestradiol valerate 2 mg every 8 h (Meriestra®), given within the first 3 days of the menstrual period and after ovarian quiescence was confirmed by ultrasound examination. On the 10th day of the cycle, ultrasound was performed, and serum oestradiol (E2) and progesterone levels were measured. Exogenous progesterone was started if the serum progesterone level was <1 ng/mL and endometrial thickness was ≥6.5 mm.

Progesterone regimens were as follows: vaginal micronised progesterone 400 mg every 12 h (first cycle), subcutaneous progesterone 25 mg every 12 h (second cycle), and intramuscular progesterone 50 mg every 24 h (third cycle). Progesterone was continued for 5 days per cycle. FET was not performed in any of these cycles.

Serum hormone determination

Daily serum E2 and progesterone levels were recorded from the day the patients started receiving exogenous progesterone. Blood samples were taken at the same time in all patients (2–4 h after administration) and coded, guaranteeing confidentiality in accordance with the provisions of the European Union General Data Protection Regulation 2016/679 established by the European Parliament and Council of the European Union. After clotting for 30 min, the samples were centrifuged at 4000 rpm for 10 min in order to obtain the blood serum for hormone level analysis. Serum E2 and progesterone were measured using automated immunoassay with the Cobas e411 analyser (Roche Diagnostics, Switzerland), which has lower detection limits of 10 pg/mL for E2 and 0.05 ng/mL for progesterone.

Study endpoints

The 3 different routes of administration were compared in terms of day 5 serum progesterone levels (the primary study objective) and endometrial thickness, and the proportions of patients achieving serum progesterone levels of >8.8 ng/mL, based on the cut-off described by Labarta et al. (2021).

Statistical analysis

The coefficient of variation of serum progesterone levels was calculated by dividing the standard deviation by the mean for each route of progesterone administration. Two linear regression models were conducted with day 5 serum progesterone levels as the response variable and exogenous progesterone administration route as the explanatory variable. The first model used a normal linear regression and the second used a mixed-effects multiple linear regression, adjusting for patient age and BMI. The mixed-effects model

took into account the availability of multiple data per patient. A normal linear regression model assumes that data are independent of each other, but as the data in this study come from the same patient, they are not independent. In the mixed-effects model, the patient was included as a random effect and the serum progesterone level variable was considered a fixed effect; therefore, the model had both fixed and random effects (i.e., mixed effects). All analyses were performed using the R statistical programming language (version 3.6.0) from The R Project for Statistical Computing (R Core Team, 2019).

Results

The study included 9 women who were scheduled to undergo artificial endometrial preparation with exogenous progesterone. Two patients underwent 3 consecutive cycles with 3 different progesterone formulations, 2 had 2 cycles using vaginal progesterone in one and subcutaneous progesterone in the other, and 5 patients had just 1 cycle with only one type of progesterone (subcutaneous in 1 patient, vaginal in 1 patient, and intramuscular in 3 patients). None of the women underwent embryo transfer in these cycles.

The basal characteristics of age and BMI are shown in Table 1. The mean ± standard deviation (SD) age was 37.4 ± 4.4 years in patients who received vaginal progesterone, 36.6 ± 3.9 years in those who received subcutaneous progesterone, and 39.2 ± 3.1 years in those who received intramuscular progesterone. The mean ± SD BMI by administration route was 24.3 ± 3.0 kg/m^2 in the vaginal group, 24.2 ± 3.1 kg/m^2 in the subcutaneous group, and 24.2 ± 3.2 kg/m^2 in the intramuscular group (Table 1), both age and BMI are similar in three groups.

Individual patient serum progesterone levels during each endometrial preparation cycle are shown in Fig. 1. Mean serum progesterone levels at day 5 showed statistically significant differences across the administration routes (p = 0.032). At day 5, mean ± SD serum progesterone levels were 14.6 ± 5.5 ng/mL for vaginal progesterone, 47.9 ± 22.3 ng/mL for subcutaneous progesterone (p = 0.021 vs vaginal), and 60.3 ± 65.5 ng/mL for intramuscular progesterone (p = 0.059 vs vaginal).

The coefficient of variation of serum progesterone levels from the start of administration (day 1) to day 5 was 66% with the vaginal route and 76% with the subcutaneous route (Fig. 1). These values were both less than 80%, indicating stable serum progesterone levels were achieved with these administration routes. The coefficient of variation with the intramuscular route was 146% (i.e., over 80%), because one patient showed much higher serum progesterone levels than the others.

In the normal linear regression model, mean serum progesterone levels on day 5 showed no significant difference between the vaginal and subcutaneous routes or between the vaginal and intramuscular routes (Fig. 2a). Serum progesterone levels increased by a mean of 33 ± 25.3 ng/mL between the vaginal and the subcutaneous routes (p = 0.213) and by a mean of 45 ± 25 ng/mL between the vaginal and intramuscular routes (p = 0.096).

We consider interesting, although there were only 2 patients who performed 3 cycles with 3 types of progesterone, to graphically show the progesterone levels evolution, to exclude interpatient variability, and is showed in Fig. 3.

In the mixed-effects linear regression model, which was adjusted for patient age and BMI, the estimated effect of changing from the vaginal to the intramuscular route was a mean increase in serum progesterone of 33 ng/mL (Fig. 2b), which was notably lower than the 45 ng/mL increase that was estimated with the normal linear regression model. This is despite inclusion of the patient whose serum progesterone levels rapidly increased after intramuscular progesterone administration (Fig. 1). In the graph of the mixed-effects model, the confidence intervals of the estimated serum progesterone levels overlapped, indicating that the different

Table 1 Patient age, body mass index, and day 5 endometrial thickness and oestradiol levels according to exogenous progesterone administration route.

Parameter	Administration route		
	Vaginal	Subcutaneous	Intramuscular
Age, years			
Mean ± SD	37.4 ± 4.4	36.6 ± 3.9	39.2 ± 3.1
p-value vs vaginal	–	0.910	0.460
Global p-value	0.480		
BMI, kg/m^2			
Mean ± SD	24.3 ± 3.0	24.2 ± 3.1	24.2 ± 3.2
p-value vs vaginal	–	1.000	1.000
Global p-value	0.990		
D5 Endometrial thickness, mm			
Mean ± SD	11.1 ± 1.0	10.3 ± 1.2	11.5 ± 2.0
p-value vs vaginal	–	0.280	0.830
Global p-value	0.46		
D5 E2, ng/mL			
Mean ± SD	199.6 ± 70.1	117.9 ± 22.0	153.0 ± 39.8
p-value vs vaginal	-	0.350	0.830

BMI, body mass index; D5, day 5; E2, oestradiol; SD, standard deviation.

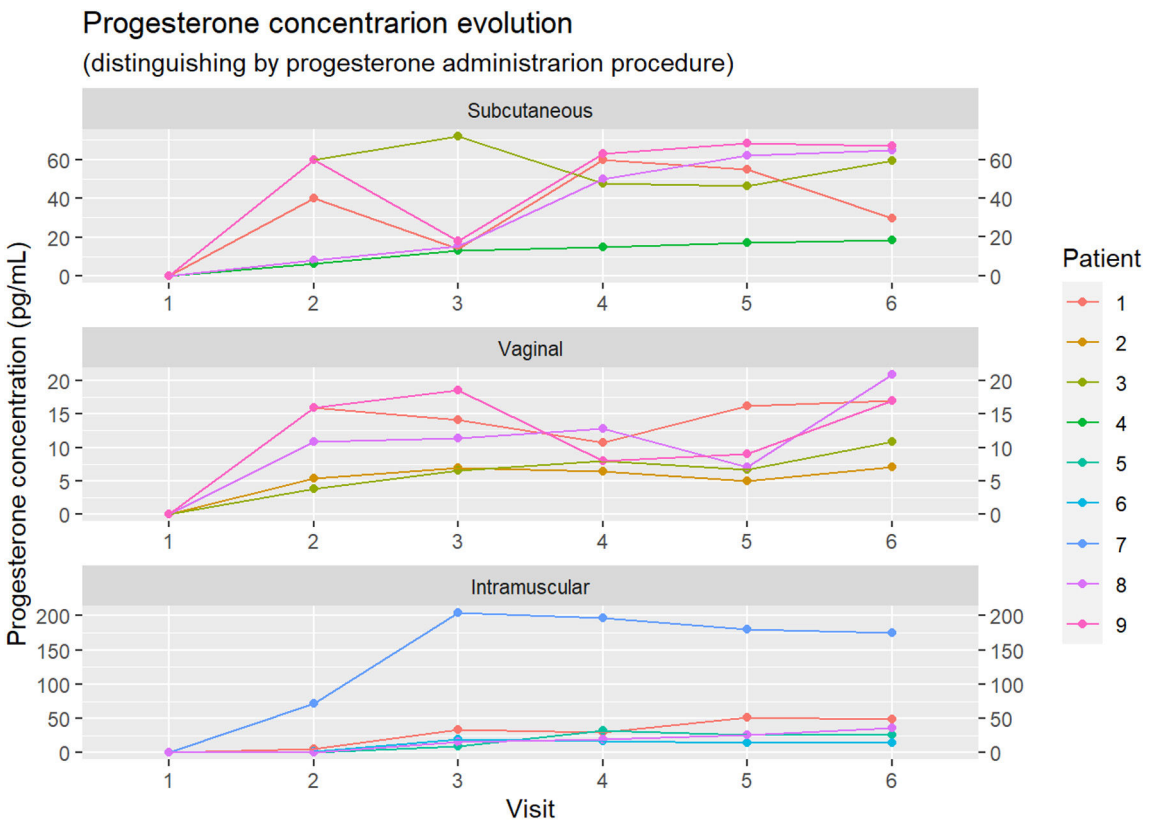

Fig. 1 Individual patient serum progesterone levels over time from day 1 to day 5 after exogenous progesterone administration via the vaginal route (cycle 1), the subcutaneous route (cycle 2) and the intramuscular route (cycle 3). CV, coefficient of variation; D, day. Each colour represents an individual patient.

routes of progesterone administration do not have a statistically significant effect on day-5 serum progesterone levels (Fig. 2b).

After adjustment for patient age and BMI, the mixed-effects linear regression analysis showed that the route of progesterone administration did not significantly affect day 5 endometrial thickness (Fig. 4).

Discussion

In our single-centre prospective study, exogenous progesterone administered to women undergoing artificial endometrium preparation for FET provided similar day 5 serum progesterone levels in our 2 statistical analyses and resulted

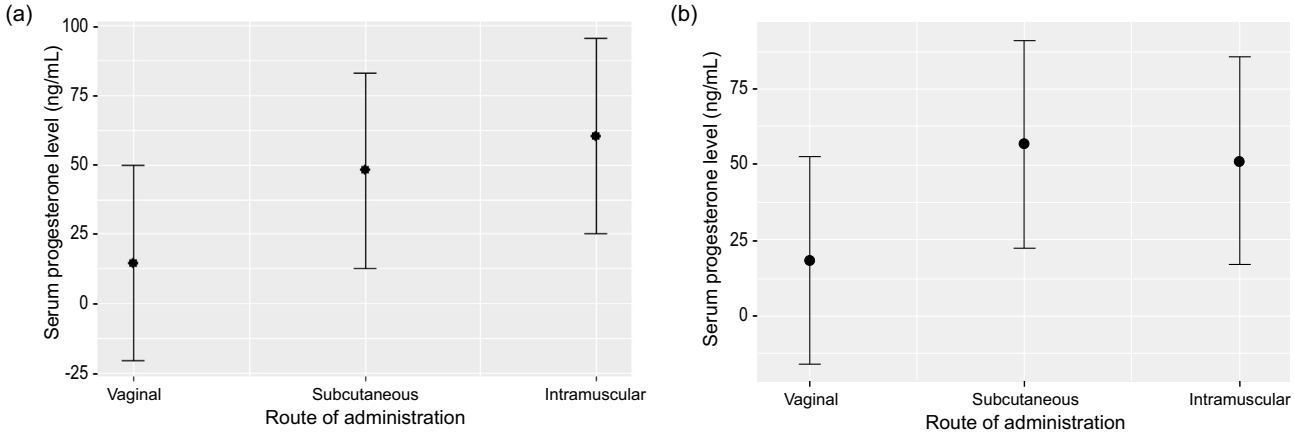

Fig. 2 Linear regression model prediction of serum progesterone levels according to exogenous progesterone route of administration by (a) normal linear regression and (b) mixed-effects linear regression (adjusted for age and body mass index). Data points show mean serum progesterone levels and error bars indicate 95% confidence intervals.

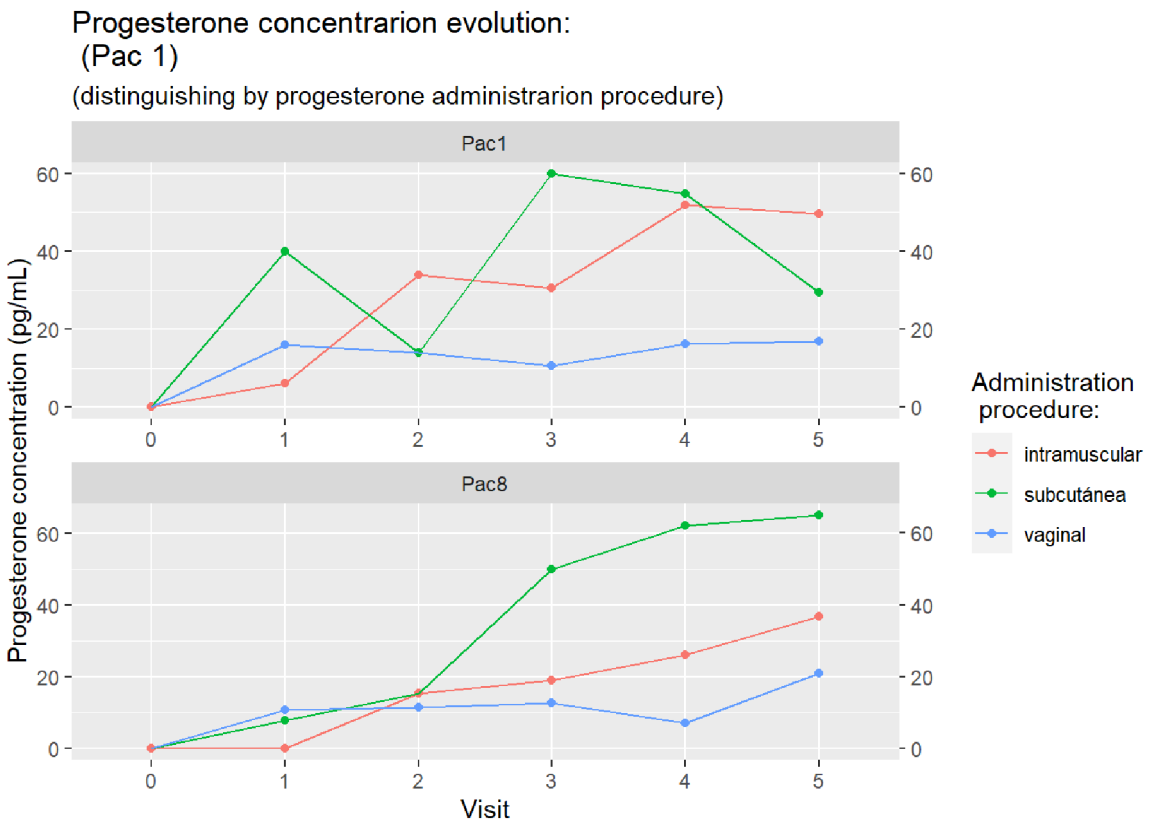

Fig. 3 Progesterone levels evolution by three ways in the same patients.

in similar endometrial thickness at the same time-point, regardless of whether it was administered via the vaginal, subcutaneous, or intramuscular routes. Neither patient age nor BMI significantly influenced serum progesterone levels. Other factors that may influence serum progesterone levels following an artificial cycle, including the timing of blood sampling or a previous history of low serum progesterone levels (< 10 ng/mL) (González-Foruria et al., 2020), were not assessed in this study.

The vaginal route has been traditionally used in luteal phase supplementation (Griesinger and Meldrum, 2018). This route is preferred over micronised oral progesterone because the oral route is associated with more side effects than the vaginal route and first-pass liver metabolism can

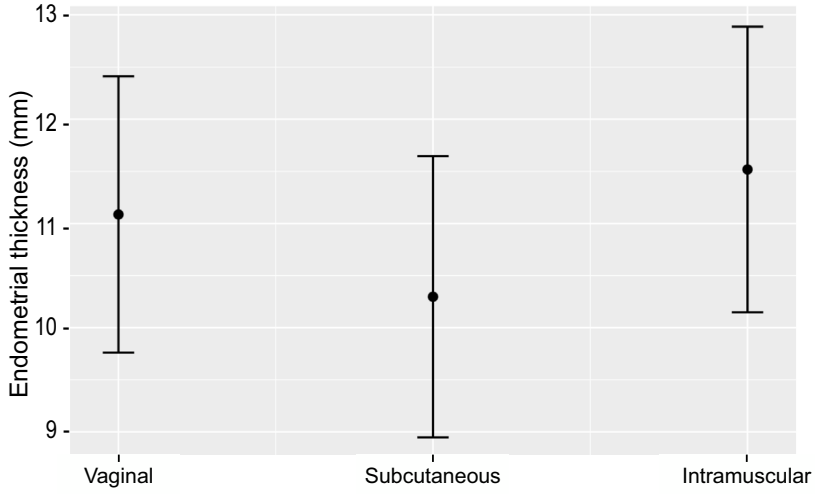

Fig. 4 Mixed-effects linear regression model prediction of endometrial thickness on day 5 according to exogenous progesterone route of administration, adjusted for patient age and body mass index. Data points show mean endometrium thickness and error bars indicate 95% confidence intervals.

reduce bioavailability. The subcutaneous route has become more widely used during the past decade, partly to avoid some of the adverse effects associated with vaginal or intramuscular administration (Cometti, 2015; de Ziegler et al., 2013; Doblinger et al., 2016). The intramuscular route of administration is often associated with significant pain and discomfort, and is infrequently used in Europe; however, it has been widely used in North America (Ciampaglia and Cognigni, 2015).

Measuring serum progesterone levels before FET has become increasingly important. In the study by Labarta et al., which reported a progesterone level cut-off of 9.2 ng/mL, serum progesterone levels were assessed on the day of embryo transfer, which equated to day 5 of vaginal progesterone 400 mg every 12 h (Labarta et al., 2017). Subsequently, these authors reported a serum progesterone threshold of 8.8 ng/ml, but in a broader range of patients undergoing artificial endometrial preparation cycles prior to embryo transfer (i.e., regardless of oocyte origin [own or donated]) (Labarta et al., 2021). In this study, serum progesterone levels were also measured on the day of embryo transfer, at a mean of 5.7 h before the procedure (Labarta et al., 2021). In our study, we measured serum progesterone levels from day 1 to day 5 of progesterone administration and found that all patients exceeded the pre-designated study threshold of 9.2 ng/mL; we selected this level prior to publication of the new threshold of 8.8 ng/mL. Our results are reassuring, given that 1 in 3 patients show inadequate levels of serum progesterone when undergoing artificial cycles; these patients have significantly lower pregnancy rates than those with adequate levels (Labarta et al., 2017). Of note, the patients in our study did not undergo embryo transfer following the artificial cycle, so our findings are limited by not being able to compare pregnancy rates in relation to the evolution of serum progesterone levels with different routes of administration.

Some authors report that it is standard practice at their ART clinics to measure serum progesterone levels the day before FET (Gaggiotti-Marre et al., 2020). In the 2 recent studies by the Dexeus group, serum progesterone levels were measured the day before FET (Gaggiotti-Marre et al., 2020; Gaggiotti-Marre et al., 2019); in another study by Ramos et al., levels were measured either 1 or 2 days before FET (Ramos et al., 2020). In the study by Gaggiotti-Marre et al., in which patients received endometrial preparation with estradiol and vaginal micronised progesterone, serum progesterone was measured on day 4 of progesterone administration (Gaggiotti-Marre et al., 2019). A cut-off threshold of 10.5 ng/mL was obtained by Ramos et al. when serum progesterone levels were assessed on either day 4 or 5 of progesterone administration. In this study, patients received combined administration of vaginal progesterone (800 mg/day) and subcutaneous progesterone (25 mg/day) (Ramos et al., 2020).

Assessment of serum progesterone levels the day before embryo transfer creates the opportunity to increase levels if required, for example, by increasing the exogenous progesterone dosage or administering a dose of subcutaneous or intramuscular progesterone, to help improve pregnancy outcomes. a recent prospective study found no significant differences with regard to the rates of clinical pregnancy, ongoing pregnancy, live birth, or miscarriage with this individualised treatment strategy compared with standard hormone replacement therapy when they measurent the levels of progesterone the day before the embryo transfer and the level is lower than 10.6 mg (Álvarez et al., 2021). Further investigation of this strategy is required (Gaggiotti-Marre et al., 2019). Based on the previous study by Labarta et al. (Labarta et al., 2017; Labarta et al., 2021; Labarta et al., 2022), our group established a protocol of supplementation with subcutaneous progesterone in patients receiving vaginal progesterone for endometrial preparation.

Although it was not the main objective of this study, daily measurement of serum progesterone illustrates how levels vary over time in individual patients. Some patients had higher levels on one day and lower levels the next, although the overall trend was for serum progesterone levels to gradually increase. Our results for each route of administration were broadly consistent with the known pharmacokinetic profiles of vaginal (Blake et al., 2010; Paulson et al., 2014), intramuscular (Miles et al., 1994; Paulson et al., 2014), and subcutaneous (Sator et al., 2013) progesterone. These observations suggest that serum progesterone levels obtained likely depend on the exact timing of the blood sample in relation to initiation of exogenous progesterone administration. A single serum progesterone measurement is likely to be insufficient, especially in cases where serum progesterone levels are expected to be relatively low because of the route of administration (e.g., the vaginal route, because of the uterine 'first-pass' effect (Paulson et al., 2014)).

In our patients, measurement of serum progesterone was conducted 2–4 h after progesterone administration. Based on published evidence, the timing of blood collection following vaginal progesterone administration could be influenced by the slow increase in serum progesterone levels with this route; however, given that a single daytime progesterone measurement can be sufficient to establish corpus luteum function in patients with low serum levels, as described by Thomsen et al. (2018), we used a single measurement in our study and the variability between the days in vaginal route is low, when we compared day 4 with day 5 the results were similar and achieve the optimal cut-off; then if possible to measurement in day 4 or day 5 for checking the optimal point before the embryo transfer.

In our study, the route of progesterone administration did not significantly influence day 5 endometrial thickness. Although endometrial progesterone levels are influenced by route of administration, with higher levels after vaginal administration compared with intramuscular administration (Cicinelli et al., 2000; Miles et al., 1994), it is not surprising no evidence of differences in endometrial thickness was found in our study, given that all three routes have been shown to be efficacious in studies of endometrial preparation (Ciampaglia and Cognigni, 2015; Cometti, 2015).

The main limitation of our study is its small sample size, which reduces its statistical power; our results should be confirmed in a study with a much larger patient population. Another limitation could be that our patients didn't undergo embryo transfer. In addition, the results of this observational study may have been influenced by other confounding factors that were not adjusted for, as well as possible selection bias. Omission of a cycle with oral dydrogesterone is not strictly a study limitation because it is not possible to measure dydrogesterone serum levels because of its rapid metabolism to dihydrogesterone. Our results are limited to the exact hormone replacement protocol used, and the

specific progesterone formulations for each route of administration, and thus may not be generalisable to other protocols, formulations, or dosages, or to other types of embryo transfer (fresh vs frozen).

In conclusion, there is increasing interest in measuring and adjusting pre-transfer serum progesterone levels as a means of improving pregnancy outcomes. In our study, the route of exogenous progesterone administration for endometrial preparation during ART did not influence serum progesterone levels in our two linear regression analyses, and most women achieved adequate serum progesterone levels after 5 days of continuous treatment, regardless of route. This suggest that the progesterone administration route can be selected based on tolerability and individual patient preference.

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Merck Serono, Spain, contributed to manuscript writing and the decision to submit the manuscript for publication.

Authorship contributions

MC designed the study. MC and MM recruited patients and conducted the artificial endometrial preparation. AP performed serum hormonal determinations. MC was responsible for the statistical analysis. MC and GNC wrote the first manuscript draft. JAGV critically revised the manuscript. All the authors approved the final version of the manuscript, and agree to be accountable for all aspects of the work in ensuring questions relating to the accuracy or integrity of the work or any part the work are investigated and resolved.

Declarations of interest

None.

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