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ASSISTED REPRODUCTION TECHNOLOGIES

Women utilizing oocyte donation have a decreased live birth rate if they displayed a low progesterone level in a previous hormonal replacement mock cycle

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Abstract

Purpose Is serum progesterone(P) level on day 2 of vaginal P administration in a hormonally substituted mock cycle predictive of live birth in oocyte donation(OD)?

Methods Retrospective analysis of 110 mock cycles from 2008 to 2016 of OD recipients having at least one subsequent embryo transfer (ET). Endometrial preparation consisted of sequential administration of vaginal estradiol, followed by transdermal estradiol and 600 mg/day vaginal micronized P. In mock cycles, serum P was measured 2 days after vaginal P introduction. OD was performed 1 to 3 years later, without P measurement.

Results In mock cycles, mean serum P level on day 2 was 12.8 ± 4.5 ng/mL (range: 4–28 ng/mL). A total of 32% patients had P < 10 ng/mL. At the time of first OD, age of recipients and donors, number of retrieved and attributed oocytes, and number of transferred embryos were comparable between patients with P < 10 ng/mL in their mock cycles compared with P ≥ 10 ng/mL. Pregnancy and live birth rate after first ET were significantly lower for patients with P < 10ng/mL (9% vs. 35 %; $P = 0.002$ and 9% vs. 32%; $P = 0.008$, respectively). Considering both fresh and subsequent frozen-thawed ET, cumulative live birth rate per-patient and per-transfer were significantly lower in patients with P < 10 ng/mL in their mock cycle (14% vs. 35%; $P = 0.02$ and 11% vs. 27%; $P = 0.03$).

Conclusion A low P level in hormonally substituted cycles several years before ET performed with the same endometrial preparation is associated with a significantly lower chance of live birth. This suggests that altered vaginal P absorption is a permanent phenomenon. Monitoring serum P in hormonally substituted cycles appears mandatory to adjust luteal P substitution.

Keywords Serum progesterone level · Vaginal progesterone administration · Mock cycle · Hormone replacement therapy · Oocyte donation · Live birth

Abbreviations

0D	oocyte donation
LBR	live birth rate
HRT	hormonal replacement therapy
E2	estogen
P	progesterone

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OPR ongoing pregnancy rate
FET frozen embryo transfer
ICSI intracytoplasmic sperm injection
SD standard deviation

Introduction

Since the first successful pregnancy obtained from egg donation reported in 1984, the practice of oocyte donation (OD) has widely increased [1–3]. Initially indicated in case of premature ovarian insufficiency or genetic disease, its use has been extended to women with pathologically or age-linked impaired ovarian status. Despite encouraging results reporting higher implantation rates, clinical pregnancy rates, and live birth rates (LBR) in OD compared with autologous cycles, further improvements are expected to maximize OD success rates [4].

Notably, successful implantation of OD embryos relies on an optimal synchronization between the embryo and the endometrium of the recipient. This point is particularly critical in synchronous OD when fresh oocytes are attributed to recipients. Endometrial preparation is usually achieved by hormonal replacement therapy (HRT), consisting of sequential supplementation by estrogen (E2) and progesterone (P) that mimicks the physiological conditions of a natural cycle. Yet, the ideal HRT protocol remains to be established [5–9]. As described previously [10], in patients with persistent ovarian function, E2 is started at the beginning of the cycle, which enables endometrial growth while preventing FSH rise and follicular growth. Then, P is administered once appropriate endometrial thickness is achieved. In case of pregnancy, hormonal supplementation is continued up to the luteo-placental shift or the 12th week of gestation. Vaginal administration seems to be the preferred route, as it not only prevents the first pass in the liver (source of thrombo-embolic adverse events), but also provides higher serum levels than the oral route [10] and higher uterine levels than the intramuscular or subcutaneous routes [11]. Studies reported that serum P levels did not increase proportionally to the increase of vaginal P doses [12] and that uterine concentrations were 10-fold higher than serum levels after vaginal administration of P [13].

In HRT cycles using vaginal P, low serum P levels have been significantly associated to lower ongoing pregnancy rates (OPR) and LBR in autologous frozen-thawed embryo transfers (FET) [14–18]. Consistently, using intramuscular P, the association between low serum P levels and lower clinical pregnancy rates and LBR in autologous FET cycles was also significant [19–21]. Similar results were observed for OD cycles, in which low serum P levels using either vaginal P [22] or intramuscular P [23] led to significantly decreased OPR and LBR. Despite these results, P levels are not routinely measured in HRT cycles, and their interpretation is made difficult by important interindividual variability of P absorption [10, 24]. Furthermore, the deleterious impact of serum P levels on outcome has only been reported in cycles with ET in which serum P levels were measured. Indeed, it remains unknown whether altered P absorption is a permanent phenomenon reproducible from one cycle to another in a single patient. In our OD practice prior to 2016, oocyte recipients underwent a mock HRT cycle in which serum P level was measured, before achieving several years later their first OD cycle without P measurement in view of embryo transfer.

The aim of the present retrospective study was to determine, in oocyte recipients, whether serum P level measured on day 2 of vaginal P administration in a hormonally substituted mock cycle was associated to implantation and pregnancy outcomes in the first subsequent OD cycle.

Materials and methods

Patients

Oocyte recipients undergoing an OD cycle in view of embryo transfer from January 1st, 2008 to December 31st, 2016 at University Hospital Jean Verdier (France) and having previously performed a mock cycle were included in the analysis. For each patient, baseline characteristics were retrieved from electronic patient files registered in our database. Data relating to the mock cycle were collected from electronic patient files when performed after 2011 and from paper patient files if required. A retrospective analysis of the data was performed. The primary endpoint was the relationship between serum P level measured 2 days after vaginal P administration in the mock cycle and LBR in the first subsequent OD cycle. Secondary endpoints included (i) biochemical pregnancy rate (positive β-hCG test), (ii) clinical pregnancy rate (ultrasound visualization of fetal heartbeat), (iii) first trimester pregnancy loss rate (the difference between the number of positive pregnancy tests and the number of ongoing pregnancies at 12 weeks of amenorrhea), (iv) cumulative LBR per patient, and (v) cumulative LBR per transfer. This retrospective study was approved by an ethical review board committee (Centre Hospitalier Intercommunal de Créteil) on July 2nd, 2020.

Protocol of endometrial preparation for mock cycles

Vaginal micronized estradiol (Provames® 1 mg; Merus Labs Luxco, Luxembourg) was started twice a day from the first day of a natural menstrual cycle, without previous down-regulation with gonadotrophin-releasing hormone (GnRH) agonist. A first monitoring by blood sample and vaginal ultrasound was performed on Monday, 10 to 12 days after the introduction of E2 to assess serum E2 and P levels and endometrial thickness. In case of triple-line endometrium ≥ 7 mm

and low P level below 1.5 ng/mL: (i) vaginal micronized P (Progestan®; Besins International, Montrouge, France) was initiated in the evening at the dose of 200 mg, three times a day (referred to as day 0 of P administration); (ii) and E2 administration was switched from the vaginal to transdermal route (Vivelledot®; Novartis Pharma, Rueil-Malmaison, France) at 100 μg patch × 2 every 3 days. Serum P4 measurements were performed on Wednesday in the morning, between 7:30 AM and 9:00 AM, after 5 pessaries of vaginal micronized P4: 1 on Monday evening, 3 on Tuesday, and 1 on Wednesday morning. A transfer test was performed on Friday.

Protocol of endometrial preparation for subsequent OD cycles

Endometrial preparation for subsequent fresh OD cycles was performed either by HRT or HRT with previous down-regulation by GnRH agonist in patients with persistent ovulatory function to allow synchronization with the matched donor. Adequate E2 preparation according to the mock cycle was administered in a timely manner with respect to the donor's ovarian stimulation. P administration was started in the evening of donor oocyte retrieval (D0) at the dose of 200 mg, three times a day, and P level was not measured. Embryo transfer was performed on day 2 of P administration for day 2 embryos and on day 3 for day 3 embryos. The treatment was continued until the pregnancy test 15 days later, and until 10–12 weeks of gestation in case of pregnancy. Supplementation was stopped in case of negative pregnancy test. A vaginal ultrasound scan was performed at 6 weeks of amenorrhea to assess the number of gestational sacs containing an embryo with positive cardiac activity.

Subsequent FET was performed in HRT cycles without GnRH agonist suppression or occasionally in natural or mildly stimulated cycles in patients with ovulatory cycles.

Embryo transfer

Fertilization of donor oocytes was systematically performed using intracytoplasmic sperm injection (ICSI), and embryos were replaced fresh or vitrified and warmed as previously described on day 2 or day 3 [25]. All embryo transfers were guided by ultrasound. Number, stage, and quality of transferred embryos were recorded. Embryo quality was qualified as Q+ if at least one embryo of good quality was transferred. Good quality embryos (Q+) were defined as the presence of 3 to 5 cells without fragmentation for day 2 embryos, and as the presence of 6 to 10 cells with less than 20% fragmentation according to the Holte classification for day 3 embryos [26].

Serum hormonal measurement

Hormonal measurements were performed using commercially available chemo-luminescence immunoassays with an automated Elecsys immunoanalyser (ECLIA, Roche Diagnostics, Meylan, France). The sensitivity of the assay was 5 pg/mL for E2, 0.03 ng/mL for P, and 0.07 IU/L for LH. Intra- and inter-assay coefficients of variation were, respectively, 5 and 10% for E2, 3% and 5% for P, and 2.3% and 2.6% for LH.

Statistical analysis

Outcomes were compared between patients below or above the threshold of serum P level currently used to define adequate corpus luteum, i.e., 10 ng/mL [27, 28]. Data were expressed in terms of frequencies and percentages or by mean values ± standard deviations (SD). Depending on their distribution, Student or Mann-Whitney tests were used to analyze continuous variables. Discrete variables were compared with Chi^2 tests. Explanatory factors, significantly associated with the outcome on univariate analysis for the first subsequent embryo transfer, were included in a multivariate model by logistic regression with a stepwise enter. $P < 0.05$ was considered as statistically significant. Analyses were performed with Statistical Analysis System Version 9.3 for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Our analysis included 110 OD recipients having performed a mock cycle between 2008 and 2016 and having undergone at least one subsequent embryo transfer. In mock cycles, mean serum P level on day 2 after vaginal P administration was 12.8 ± 4.5ng/mL (range: 4–28 ng/mL). A total of 31.8% (n = 35/110) of patients had serum P < 10ng/mL on day 2 of P administration.

Baseline and mock cycle characteristics according to P threshold of 10 ng/mL on day 2 of P administration in the mock cycle are detailed in Table 1. Every first embryo transfer was performed in a HRT cycle. Overall, 96% (n = 106) corresponded to fresh embryo transfers. Four percent (n = 4) were differed due to bleeding or inadequate endometrial thickness during the fresh cycle and were performed further in HRT FET (n = 1/35 in the group serum P < 10 ng/mL; n = 3/75 in the group serum P ≥ 10 ng/mL). Age of recipients and donors, number of oocytes retrieved from donors, number of oocytes attributed to recipients, and number of embryos transferred were comparable between patients with serum P < 10 ng/mL in their mock cycle compared with patients with serum P ≥ 10 ng/mL. However, patients with P < 10 ng/mL in their mock cycle had lower E2 levels, both on the last ultrasound

Table 1 Baseline characteristics of cycles (mock and oocyte donation) according to serum Progesterone level threshold of 10 ng/mL on day 2 after P administration in mock cycle with HRT

	Progesterone < 10 ng/mL n = 35	Progesterone ≥ 10 ng/mL n = 75	P value
Recipient age (years) on OD cycle	37.6 ± 4.4	36.7 ± 4.3	0.29
Recipient BMI (kg/m^2) on OD cycle	26.6 ± 4.5	25.3 ± 4.5	0.16
Age of the donor (years)	32.0 ± 3.8	31.3 ± 4.2	0.43
Number of oocytes retrieved from the donor	13.5 ± 7.5	13.7 ± 7.6	0.89
Number of oocytes attributed to recipients	3.6 ± 1.1	3.6 ± 1.3	0.88
Number of fertilized oocytes	2.7 ± 1.2	2.9 ± 1.2	0.53
Number of embryos obtained from OD	2.4 ± 1.3	2.6 ± 1.1	0.37
Number of transfers (fresh and frozen) for the recipient	1.3 ± 0.6	1.3 ± 0.5	0.85
Mock cycle endometrial thickness on ultrasound day (mm)	8.8 ± 2.4	8.7 ± 2.4	0.78
Mock cycle E2 level on ultrasound day (pg/mL)	954 ± 505	1469 ± 796	0.0009
Mock cycle E2 level on D2 of P administration (pg/mL)	231 ± 95	391 ± 411	0.03
Mock cycle P level on D2 of P administration (ng/mL)	7.9 ± 1.3	15.2 ± 4.4	< 0.0001

Results are expressed as mean ± SD

SD standard deviation, *P* progesterone, *HRT* hormone replacement therapy, *OD* oocyte donation, *BMI* body mass index, *E2* estrogen, *ultrasound day*: at least after 10 days of E2 treatment, *D2* day 2

day (prior to the introduction of P with vaginally administrated E2, $P = 0.0009$) and two days after the switch to transdermal administration of E2 (the day of P measurement, $P = 0.03$).

Concerning outcomes after the first subsequent embryo transfer, LBR (9% vs. 32%, respectively; $P = 0.008$), biochemical pregnancy rate (20% vs. 44%, respectively; $P = 0.015$), and clinical pregnancy rate (9% vs. 35%; $P = 0.002$, respectively) were significantly lower for patients with serum P < 10 ng/mL in their mock cycle. Considering both fresh and subsequent frozen thawed embryo transfers up to the first live birth, cumulative LBR per patient and per transfer was significantly lower in patients with P < 10 ng/mL in their mock cycle (14% vs. 35%, respectively; $P = 0.02$ and 11% vs. 27%, respectively; $P = 0.03$) (Table 2). One patient achieved a second live birth among patients with P < 10 ng/mL in her mock cycle, versus 2 in patients with P ≥ 10 ng/mL. No patient with P < 10 ng/mL in her mock cycle had remaining frozen embryos, while 5 patients with P ≥ 10 ng/mL had remaining frozen embryos.

In univariate analysis of the first embryo transfer, factors significantly associated with live birth were higher P levels on day 2 of P administration in mock cycle, higher E2 levels on ultrasound day in mock cycle and first embryo transfer cycle, the use of HRT protocol instead of GnRH-agonist HRT protocol, and higher LH levels on ultrasound day in first embryo transfer cycle (Table 3).

After adjustment for age of the recipient, number of transferred embryos and all significant variables of univariate analysis, and serum P < 10 ng/mL on day 2 of P administration in mock cycle remained significantly associated to live birth in multivariate analysis ($P = 0.018$), as well as the type of protocol ($P = 0.010$) (Table 4).

Discussion

Our results demonstrate that serum P level < 10ng/mL on day 2 after vaginal P administration in mock cycles of OD recipients is significantly associated to decreased LBR in the first subsequent OD cycle, even after adjustment on confounding variables. Low serum P level on the mock cycle was also significantly correlated to lower pregnancy rates and clinical pregnancy rates on the first subsequent OD cycle, as well as to lower cumulative LBR per patient when all transfers were considered (fresh and FET), and to lower LBR per transfer.

Our results are in line with previous studies showing an association between serum P levels in HRT cycles and outcomes. Using the same dose of micronized vaginal P (600 mg daily), our previous analysis of 227 autologous HRT-FET showed that patients with P < 10ng/mL on transfer day had significantly lower pregnancy rates (34% vs. 48%, respectively, $P = 0.04$) and LBR (17% vs. 31%, respectively, $P = 0.01$) [16], and Gaggiotti et al. analysis of 244 autologous HRT-FET also reported lower pregnancy rates and LBR in case of low serum P levels (≤ 10.64 ng/mL) measured 1 day before ET [17]. Consistently, other studies have described the association between serum P levels and outcomes in autologous HRT-FET, whether P was administered using the vaginal route [14, 15, 18] or the intramuscular route [19–21]. Moreover, a recent prospective study combining vaginal P (90 mg/12 h) and rectal P (90 mg/12 h) administration in

Table 2 Results for first transfer, frozen-thawed embryo transfer, and total number of transfers according to serum progesterone level threshold of 10 ng/mL on day 2 after P administration in mock cycle with HRT

	Progesterone < 10 ng/mL	Progesterone ≥ 10 ng/mL	*P* value
First transfer, *n* = 110	*n* = 35	*n* = 75	
Protocol			
HRT	54.3%	45.3%	0.38
GnRH-agonist HRT	45.7%	54.7%	
Endometrial thickness (mm)	9.1 ± 2.5	8.8 ± 2.1	0.55
E2 level (pg/mL) on ultrasound day	*1016 ± 562*	*1513 ± 795*	*0.0012*
LH level (IU/L) on ultrasound day	11.4 ± 13.1	12.1 ± 18.1	0.83
P level (ng/mL) on ultrasound day	0.3 ± 0.15	0.3 ± 0.2	0.87
Number of transferred embryos	1.6 ± 0.5	1.7 ± 0.5	0.12
Fresh/frozen embryo transfers	34/1	72/3	0.76
Good-quality transferred embryos (Q+)	54.3%	56%	0.86
Biochemical pregnancy rate (*n*)	*20% (7)*	*44% (33)*	*0.015*
Clinical pregnancy rate (*n*)	*9% (3)*	*35% (28)*	*0.002*
Live birth rate (*n*)	*9% (3)*	*32% (24)*	*0.008*
First trimester pregnancy losses (*n*)	57% (4)	24% (8)	0.20
Additional FET up to first live birth, *n* = 32	*n* = 10	*n* = 22	
Protocol			
HRT	70%	59%	0.55
Modified natural cycle	30%	41%	
Number of transfers	10	21	
Number of transferred embryos	1.4 ± 0.5	1.4 ± 0.5	0.88
Good-quality transferred embryos (Q+)	50%	66%	0.37
Biochemical pregnancy rate (*n*)	30% (3)	10% (2)	0.14
Clinical pregnancy rate (*n*)	20% (2)	10% (2)	0.41
Live birth rate (*n*)	20 % (2)	10% (2)	0.41
Total transfers up to first live birth, *n* = 142	*n* = 45	*n* = 97	
Cumulative live birth rate/patient	14%	35%	0.02
Live birth rate/transfer	11%	27%	0.03

Results are expressed as mean ± **SD**

SD standard deviation, *HRT* hormone replacement therapy, *GnRH* gonadotropin-releasing hormone, *E2* estrogen, *LH luteinizing hormone, P* progesterone, *ultrasound day:* at least after 10 days of E2 treatment

autologous HRT-FET cycles reported a non-linear relationship between serum P levels and OPR [29]. Concerning the specific context of OD, a previous prospective cohort study of 211 OD cycles using 800 mg of vaginal P in HRT observed significantly lower OPR ((OR: 0.297; 95%CI: 0.113–0.779); $P = 0.013$) in OD recipients with serum P < 9.2 ng/mL the day of ET, after adjustment on all potential confounders[22]. Similar results were found in fresh OD cycles when using intramuscular P [23]. Altogether, these previous findings highlight the impact of serum P levels on outcome, all are based on cycles with ET in which serum P levels were measured at the same time. Despite its retrospective design and small sample size, our study is the first to our knowledge to report that low serum P levels measured in a mock cycle have a negative impact on the outcomes of subsequent ET cycles. Our results suggest that altered P absorption is a permanent phenomenon, reproducible from one cycle to another in a single patient, although it could not be proven due to the lack of P measurement on the cycle in which the OD embryo transfer occurred.

Thereby, our results raise the question of the underlying mechanisms of P absorption. The efficacy of the oral route of micronized P for luteal support is limited due to the first pass metabolism in the liver. Vaginal administration of P was reported to achieve higher serum levels compared with the oral route [10], and higher uterine levels than the intramuscular or subcutaneous routes despite lower serum P levels [11, 12]. Compared with vaginal P supplementation only, the addition of intramuscular P was not reported to enhance OPR in autologous HRT-FET cycles when no P measurement was

Table 3 Baseline and cycle characteristics between patients with live birth and no live birth after the first embryo transfer

	Live birth $n = 27$	No live birth $n = 83$	P value
Recipient age (years)	35.7 ± 3.9	37.4 ± 4.4	0.07
Recipient BMI (kg/m^2)	26.8 ± 5.4	25.5 ± 4.2	0.26
Age of the donor (years)	31.4 ± 4.4	31.7 ± 4.0	0.76
Number of oocytes retrieved from the donor	13.3 ± 9.1	14.0 ± 7.3	0.66
Number of oocytes attributed to the recipient	3.7 ± 1.5	3.6 ± 1.2	0.92
Number of fertilized oocytes	2.9 ± 1.0	2.8 ± 1.2	0.75
Number of embryos obtained from OD	2.7 ± 1.0	2.5 ± 1.2	0.26
Mock cycle endometrial thickness (mm)	8.4 ± 2.2	8.8 ± 2.5	0.41
Mock cycle E2 level on ultrasound day (pg/mL)	1557 ± 766	1219.1 ± 734	0.046
Mock cycle P level on D2 of P administration (ng/mL)	14.5 ± 4.9	12.3 ± 5.0	0.044
Mock cycle E2 level on D2 of P administration (pg/mL)	389 ± 466	324 ± 307	0.41
Protocol for first embryo transfer			
HRT	67%	33%	0.027
GnRH-agonist HRT	42%	58%	
Endometrial thickness (mm)	8.9 ± 2.1	8.9 ± 2.3	0.95
E2 level on ultrasound day (pg/mL)	1620± 847	1268 ± 718	0.037
LH level on ultrasound day (IU/L)	17.6 ± 20.4	10.0 ± 14.8	0.038
P level on ultrasound day (ng/mL)	0.3 ± 0.2	0.3 ± 0.2	0.71
Number of embryos transferred	1.8 ± 0.4	1.6 ± 0.5	0.12
Good-quality embryos (Q+) transferred	48%	58%	0.38

Results are expressed as mean ± SD

SD standard deviation, *BMI* body mass index, *OD* oocyte donation, *E2* estrogen, *P* progesterone, *D2 day 2*, *P* progesterone, *HRT* hormone replacement therapy, *GnRH* gonadotropin-releasing hormone, *LH* luteinizing hormone, *ultrasound day: at least after 10 days of E2 treatment*

considered [30]. Conversely, in OD cycles, the combination of intramuscular and vaginal P was associated to higher LBR and decreased miscarriage rates compared with vaginal P only [5]. When administered vaginally, P is preferentially absorbed by uterine endometrial tissue, referred to as the first uterine pass effect [11, 13, 31]. Nonetheless, important inter-individual variabilities of serum P levels have been described after either route of P administration, thus making it particularly difficult to predict serum concentrations after a given dosage [10]. Apart from P absorption, differences in terms of metabolism or protein binding phenomena could have influenced serum P levels. Notably, large variations of serum P levels were reported despite all women receiving the same HRT protocol [18]. After vaginal administration, the pattern of mean P levels is known to rapidly rise (with detectable levels measured in as little as 30 min), achieve steady state within 24 h, and then gradually decrease in all individuals [12]. In HRT, no endogenous P secretion from the corpus luteum is present, thus avoiding the rapid fluctuating levels in the mid-late luteal phase of ovalutory cycles of normal subjects. Therefore, variations of P levels in HRT cannot be explained by the pulsatility of P secretion present in the classical luteal phase of a normally ovulatory cycle. Age, weight, history of previous cryopreserved embryo transfers with serum P levels < 10 ng/mL and time of blood sampling were suggested as potential determinants of P levels in autologous HRT-FET cycles [32]. Some factors such as sexual intercourse, poor patient compliance, and inter-individual differences in vaginal absorption, distribution, and metabolism were identified as possibly affecting P levels after vaginal

Table 4 Parameters associated to live birth after the first embryo transfer, in multivariate analysis

	Chi-squared test	P value
Mock cycle P level on D2 of P administration	5.60	0.018
Protocol for first embryo transfer	6.72	0.010
Interaction P level * protocol	4.90	0.027

P progesterone, *D2* day 2

administration. We observed that patients of the low P group (P < 10 ng/mL) also had significantly lower E2 levels, which suggests a variability of absorption through the vaginal and cutaneous epithelium. Sexual intercourse was shown to reduce P levels after vaginal administration [24], and interindividual patient variations in metabolism were also observed following the parenteral route of P administration [20, 23].

Ultimately, no consensus exists on the optimal threshold for P levels. Neither the length of exposure to P before ET nor what serum P levels are required to optimize cycle outcome have been firmly established. In our study, outcomes were compared between patients below or above the threshold of serum P level currently used to define adequate corpus luteum, i.e., 10 ng/mL [27, 28]. It is important to emphasize that the serum P threshold in this study was suggested for HRT using vaginal P, but that serum P thresholds probably differ according to the administration regimen. The day on which P levels were measured also varies between studies. For instance, Basnayake et al. [15] identified serum P < 15ng/mL 16 days after ET as being significantly associated to decreased LBR (11.3% vs. 26.4%; adjusted odds ratio (OR) 3.14 (95% CI 2.21–4.48)). Furthermore, Yovich et al. [18] measured P levels at day 2 or day 3 after ET and suggested the existence of an upper limit, identifying an optimal P range between 15 and 31 ng/mL ($P < 0.005$). Some discrepancy was also reported concerning the optimal threshold when using intramuscular P. While most studies observed suboptimal pregnancy outcomes in case of serum P < 20 ng/mL after intramuscular administration of P [19, 21, 23], one reported that serum P level greater than 20 ng/dL on ET day was associated with lower LBR and higher pregnancy loss rates [20]. It is possible that the parenteral routes of administration may require a higher P threshold than the vaginal route due to the absence of first pass in the uterus. Altogether, 31.8% of patients in our study had P levels below the 10 ng/mL threshold after 600 mg of vaginal P, which is similar to the 37% previously reported by our team [16]. Labarta et al. [22] showed, in 211 OD cycles, that P < 9.2 ng/mL on the day of ET was associated to lower OPR ((OR: 0.297; 95%CI: 0.113–0.779); $P = 0.013$), 24.6% patients had serum P levels under the threshold. For Alsbjerg et al. [14], up to 51% of patients had serum P < 11 ng/mL, level under which significantly decreased OPR were reported (38% vs. 51%; $P = 0.04$). Hence, given that an important percentage of patients have inadequate P levels in HRT, these results highlight the necessity to monitor serum P in HRT protocols. A recent study reported that patients undergoing HRT-FET with a surveillance protocol consisting of E2 and P measurement the day before ET were significantly more likely to achieve live birth (aOR 1.6; 95%CI [1.2, 2.2]) compared with cycles with no surveillance [33].

Our results suggest that altered P absorption is a permanent phenomenon, reproducible from one cycle to another in a single patient. Given the impact of serum P levels on outcomes in HRT, and knowing that a large proportion of patients have inadequate P levels, it appears mandatory to monitor serum P in HRT protocols in order to adjust luteal P substitution. Finally, since the success of ET is multifactorial and does not solely depend on P levels, further studies are warranted to identify other impacting factors to maximize the chance of favorable pregnancy outcomes in HRT.

Data availability All data are available on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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