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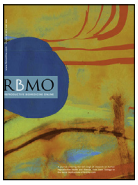

ARTICLE

Addition of intramuscular progesterone to vaginal progesterone in hormone replacement therapy in vitrified–warmed blastocyst transfer cycles

BIOGRAPHY

Mehtap Polat, MD, has been working as Associate Professor at Anatolia IVF and Women's Health Center, Ankara, Turkey, since 2005. She is interested in all aspects of clinical IVF, and has been a member of the Executive Committee of Turkish Society of Reproductive Medicine (TSRM) since 2016.

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KEY MESSAGE

Intramuscular progesterone supplementation did not enhance ongoing pregnancy rates compared with vaginal progesterone only hormone replacement therapy in vitrified–warmed embryo transfer cycles.

ABSTRACT

Research question: Does intramuscular progesterone supplementation ensure ongoing pregnancy rates (OPR) comparable with vaginal progesterone only in hormone replacement therapy cycles for vitrified–warmed embryo transfer; and is there a window of serum progesterone concentration out of which reproductive outcomes may be negatively affected?

Design: Retrospective longitudinal cohort study carried out at a single IVF clinic. In total, 475 consecutive, day-5 to day-6 vitrified–warmed embryo transfer cycles using hormone replacement therapy regimen were included. Vaginal progesterone only was given to 143 patients; supplementation of vaginal progesterone only with intramuscular progesterone supplementation every third day was given to 332 patients. On the sixth day of progesterone administration, immediately before frozen–thawed embryo transfer, circulating progesterone levels were measured. Main outcome measure was OPR.

Results: The baseline demographic features and embryological data of the vaginal progesterone only and intramuscular progesterone supplementation groups were comparable. The OPR were 48.3% and 51.8%, respectively ($P = 0.477$). Neither the circulating progesterone level nor the type of progesterone administration were independent predictors of OPR. The effect of serum progesterone levels on OPR was evaluated by percentiles (<10%, 10–49%, 50–90% and >90%), taking 50–90% as the reference sub-group. All percentiles in the intramuscular progesterone supplementation group and in the vaginal progesterone only group had similar OPR.

Conclusions: Intramuscular progesterone supplementation every third day, overall, does not enhance OPR compared with vaginal progesterone only.

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KEYWORDS

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Im progesterone supplementation
IVF
Serum progesterone
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INTRODUCTION

During recent years, the availability of surplus embryos, as well as freeze-all being suggested for various indications, has led to an increasing trend towards 'freeze all' followed by frozen–thawed embryo transfer (FET) in IVF (*Roque et al., 2019*). Despite this increasing trend, the best priming protocol of the endometrium before FET is yet to be defined, mainly because of the paucity of well-designed randomized controlled trials (RCT) (*Yarali et al., 2016*; *Ghobara et al., 2017*).

The hormone replacement therapy (HRT) cycle is one of the most common protocols for priming the endometrium before FET. Despite the worldwide common use of the HRT protocol, the most optimal route and dosing of progesterone is still unknown. Moreover, common practice in HRT cycles has been to not monitor serum progesterone levels during the treatment cycle, assuming that 'one size fits all' and, therefore, that the same dosage secures the optimal circulating and intra-endometrial progesterone levels in all women. This assumption, however, may not be valid as reported in recent studies (*Brady et al., 2014*; *Kofinas et al., 2015*; *Yovich et al., 2015*; *Labarta et al., 2017*; *Alsbjerg et al., 2018*; *Basnayake et al., 2018*; *Gaggiotti-Marre et al., 2019*; *Cedrin-Durnerin et al., 2019*). Despite the use of the same route and dose of progesterone administration, however, significant inter-personal variations in circulating progesterone levels exist, resulting in differences in reproductive outcomes after transfer of high-quality embryos (*Brady et al., 2014*; *Kofinas et al., 2015*; *Yovich et al., 2015*; *Labarta et al., 2017*; *Alsbjerg et al., 2018*; *Basnayake et al., 2018*; *Cedrin-Durnerin et al., 2019*; *Gaggiotti-Marre et al., 2019*). A threshold level, ranging from 10–20 ng/ml, has been suggested in these studies, below which significantly poorer reproductive outcomes were seen (*Brady et al., 2014*; *Labarta et al., 2017*; *Alsbjerg et al., 2018*; *Basnayake et al., 2018*; *Cedrin-Durnerin et al., 2019*; *Gaggiotti-Marre et al., 2019*).

Moreover, a recent RCT in HRT cycles reported lower ongoing pregnancy rates (OPR) using vaginal progesterone only (VP4), compared with daily intramuscular progesterone (imP4) and intramuscular progesterone every third day supplemented to vaginal progesterone (imP4-suppl) (*Devine et al., 2018*). No data on circulating progesterone levels, however, were reported in that study.

The aim of the present study was twofold; first, to evaluate whether imP4-supplementation ensures sufficient serum progesterone levels and good OPR compared with VP4 in HRT cycles for FET; second, to identify a possible window of serum progesterone beyond which reproductive outcomes are negatively affected.

MATERIALS AND METHODS

Patients

This study was an observational cohort study conducted between October 2017 and October 2019, including a total of 475 consecutive HRT cycles, using day-5 or day-6 vitrified blastocysts for FET. The inclusion criteria were as follows: female age between 20 and 44 years, body mass index (BMI) 35 kg/m^2 or lower and having available day-5 or day-6 vitrified blastocyst(s) after warming. Only the first FET cycle was included for each patient, although some patients might have had previous FET cycles elsewhere. Frozen embryo transfer from freeze-all cycles were also included. The exclusion criteria were as follows: pre-implantation genetic testing for aneuploidy, monogenic disorders or structural re-arrangements.

Monitoring of serum progesterone levels began on the day of FET in HRT cycles in October 2017. Between October 2017 and February 2018, VP4 was used for luteal support (n = 110). After the publication of the study by *Devine et al. (2018)*, our HRT-FET policy changed from March 2018 and onwards (n = 332) to include imP4 every third day to the VP4 supplementation. Thirty-three patients in the later period, however, refused to be supplemented with imP4 every third day, but chose to be treated with VP4; these 33 cases were added to the VP4 arm to make a total of 143 patients.

Protocols

Vitrification at the blastocyst stage (day 5 and day 6) was used in all patients. Blastocyst grading was in accordance with Gardner's staging (*Gardner and Schoolcraft, 1999*) and categorized as excellent (3AA, 4AA, 5AA), good (3, 4, 5, 6 AB or BA), average (3, 4, 5, 6 BB or AC or CA) or poor (3, 4, 5, 6 BC or CC) (*Capalbo et al., 2014*). When more than one blastocyst was transferred, only the one with the best morphological grading was included in the analysis. Collapsed embryos after warming were also transferred, although limited in number (n = 11); however, morphology assessment could not be made in such embryos.

October 2017 to February 2018

Hormone replacement therapy without gonadotrophin releasing hormone agonist suppression was used for preparation of the endometrium in all patients as follows: oral oestrogen treatment (Estrofem) (Novo Nordisk, Istanbul, Turkey) started on the second or third day of the menstrual cycle at a dose of 6 mg per day. After 12–14 days, transvaginal ultrasonographic examination was carried out to measure endometrial thickness. Once the bi-layer endometrial thickness was 7 mm or greater, vaginal progesterone gel (Crinone 8%) (Merck Sereno, Bedfordshire, UK) twice daily was administered. Embryo transfer was scheduled on the sixth day of progesterone supplementation. In conception cycles, luteal support continued up to the tenth week of gestation.

March 2018 to October 2019

Between March 2018 and October 2019, our policy on progesterone supplementation changed but the oestrogen regimen remained the same. Therefore, 50 mg imP4 (Progynex) (Farmako, Istanbul, Turkey) was administered from the first day of vaginal progesterone administration, and from then onwards in the mornings before 9.00 am every third day (day 1, 4, 7, 10, 13) of vaginal progesterone administration.

Blood sampling and luteal phase support

On day 6 of vaginal progesterone administration, 4–5 h after the morning administration of vaginal gel and immediately before the scheduled embryo transfer (12.00–13.00), a blood sample was drawn to measure circulating progesterone levels. Importantly, the embryo transfer was carried out without knowledge of the progesterone level; hence, the level did not affect the decision to transfer. Furthermore, after embryo transfer, the dose of progesterone remained unchanged for those patients with sub-optimal serum

TABLE 1 COMPARISON OF THE DEMOGRAPHIC FEATURES OF PATIENTS TREATED WITH VAGINAL PROGESTERONE GEL ONLY OR VAGINAL PROGESTERONE GEL SUPPLEMENTED WITH INTRAMUSCULAR PROGESTERONE EVERY THIRD DAY

	VP4 (n = 143)	*ImP4-suppl (n = 332)*	P-*value*
Female age, years	31.3 ± 4.5	31.4 ± 4.5	0.879
Male age, years	34.2 ± 4.8	34.5 ± 6.0	0.676
Female body mass index (kg/m^2)	24.9 ± 4.1	25.2 ± 4.2	0.536
Duration of infertility (months)	53.1 ± 41.3	51.4 ± 34.8	0.685
Number of previous cycles	2.0 (1; 3)	2.0 (1; 3)	0.452
Previous childbirth, *n* (%)	33 (23.1)	77 (23.2)	0.978
Number of patients with freeze-all strategy, *n* (%)	58 (40.6)	124 (37.3)	0.509

Values are given as mean ± SD or number (%).

ImP4-suppl, vaginal progesterone gel supplemented with intramuscular progesterone every third day; VP4, vaginal profesterone gel only.

progesterone levels (<10 ng/ml) (*Labarta et al., 2017*; *Cedrin-Durnerin et al., 2019*; *Gaggiotti-Marre et al., 2019*).

Progesterone analysis

Progesterone was measured using commercially available ImmunoDiagnostic Assay System as an automated quantitative enzyme-linked fluorescent immunoassay (VIDAS Progesterone) (Bio-Merieux, Marcy l'Etoile, France). The assay sensitivity was 0.25 ng/ml. The intra-assay coefficient of variation was 3.97–14.30%; the inter-assay coefficient of variation was 3.10–24.30%.

Pregnancy

Ongoing pregnancy was the primary outcome measure. A positive pregnancy test was defined as a serum beta-HCG level exceeding 30 IU/l (9.5 ng/ml) on day 9 after embryo transfer. Implantation rate per patient is calculated as the number of gestational sacs observed at transvaginal ultrasonography divided by the number of embryos transferred. A clinical pregnancy was defined as the visualization of a gestational sac at transvaginal ultrasonographic examination, documentation of trophoblastic tissue in a miscarriage specimen, or both. Miscarriage was defined as spontaneous loss of intrauterine pregnancy before 12 weeks' gestation, excluding those cases with biochemical pregnancy loss. Ongoing pregnancy was defined as pregnancy beyond 12 weeks of gestational age.

The Institutional Review Board of Hacettepe University approved the study protocol on October 10, 2019 (KA-19115/2019).

Statistical analysis

Distribution characteristics of variables were visually assessed with the use of histograms, box plots, and Q-Q plots, and analysed with the use of Kolmogorov–Smirnov and Shapiro–Wilk tests. Continuous variables were expressed as mean ± SD or median and interquartile range, as appropriate. Comparisons were made with the use of independent-samples t-test or Mann–Whitney-U test according to distribution characteristics. Chi-squared and Fisher's exact tests were used to compare the categorical variables. Logistic regression analysis was conducted to delineate the covariates affecting OPR. The statistical package SPSS 22.0 (IBM Corp., USA) was used for all statistical analyses.

RESULTS

The baseline demographic features of the VP4 and imP4-suppl groups were comparable (TABLE 1).

The mean circulating progesterone levels on the day of FET were 13.9 ± 4.9 ng/ml and 21.0 ± 8.2 ng/ml in the VP4 and imP4-suppl groups, respectively (P < 0.001). The endometrial thickness was significantly higher in the imP4-suppl group compared with the VP4 group (9.7 ± 1.7 versus 9.3 ± 1.6 mm; P = 0.03). The number of embryos transferred, blastocyst quality and day of vitrification (day 5 to day 6) were comparable between the two groups (TABLE 2). The reproductive outcomes plus positive HCG per embryo transfer, implantation, clinical pregnancy, biochemical loss, miscarriage, ongoing pregnancy and multiple pregnancy rates were comparable between the two groups (TABLE 2).

Logistic regression analyses

When ongoing pregnancy was chosen as the dependent factor, and female age, BMI, previous childbirth, freeze-all strategy, serum progesterone levels, number of embryos transferred, day of vitrification (day 5 or 6), blastocyst morphology and type of progesterone administration (VP4 and imP4-suppl) were chosen as independent factors, only blastocyst morphology was found to be a significant independent prognosticator (P = 0.047) (TABLE 3). Importantly, neither the circulating progesterone nor the type of progesterone administration were found to be significant predictors of ongoing pregnancy.

In both the VP4 and imP4-suppl groups, the effect of different circulating progesterone levels on the reproductive outcome was evaluated by percentiles (<10%, 10–49%, 50–90% and >90%), taking 50–90% as the reference sub-group. In the VP4 group, the threshold for the 10% was 8.75 ng/ml (n = 14) (TABLE 4). Of interest, a borderline significant lower positive HCG rate was reported (42.9% versus 70.7%; crude OR = 0.3, 95% CI 0.09 to 1.0; P = 0.06) in this sub-group compared with the reference 50–90% sub-group with a threshold of 12.95–20.42 ng/ml. The remaining percentiles, including the 10–49% and over 90% subgroups, had comparable positive HCG rates. In the VP4 group, no significant differences were found in OPR in all subgroups (TABLE 4).

In the imP4-suppl group, the threshold for the 10% was 11.75 ng/ml (n = 33) (TABLE 5). The less than 10% sub-group had similar OPR compared with the 50–90% subgroup; this was also the case for the 10–49% and the over 90% sub-groups (TABLE 5).

The adjusted odds ratios of serum progesterone percentiles on outcome measures (positive HCG, overall pregnancy loss, ongoing pregnancy) were also calculated by taking female age, BMI, number of embryos transferred and blastocyst morphology as independent variables (Supplementary Table 1 and Supplementary Table 2). In the VP4 group, as concordant with the crude odds ratio, adjusted odds ratio was lower for positive HCG in the less than 10% group compared with the 50–90%

TABLE 2 COMPARISON OF THE FROZEN–THAW EMBRYO TRANSFER CYCLE CHARACTERISTICS AND OUTCOME BETWEEN OR VAGINAL PROGESTERONE GEL SUPPLEMENTED WITH INTRAMUSCULAR PROGESTERONE EVERY THIRD DAY

	VP4 (n=143)	*ImP4-suppl (n=332)*	P-*value*
Serum progesterone level on the day of embryo transfer, ng/ml	13.9 ± 4.9	21.0 ± 8.2	<0.001
Endometrial thickness, mm	9.3 ± 1.6	9.7 ± 1.7	0.030
Number of embryos transferred	1.20 ± 0.40	1.30 ± 0.40	0.580
Blastocyst morphology[a]			0.544
Excellent	15 (10.6)	41 (12.7)	
Good	66 (46.8)	153 (47.4)	
Average	56 (39.7)	119 (36.8)	
Poor	4 (2.8)	10 (3.1)	
Day of vitrification 5/6, *n* (%)	121/22 (84.6/15.4)	284/48 (85.5/14.5)	0.794
Positive HCG (>29 mIU/ml), *n* (%)	94 (65.7)	231 (69.6)	0.408
Implantation rate, %	55.9 ± 47.7	58.1 ± 46.7	0.645
Biochemical pregnancy loss, *n* (%)	8/94 (8.5)	19/231 (8.2)	0.932
Clinical pregnancy rate, *n* (%)	86 (60.1)	212 (63.9)	0.442
Miscarriage rate, *n* (%)	17/86 (19.8)	38/212 (17.9)	0.710
Ongoing pregnancy, *n* (%)	69 (48.3)	172 (51.8)	0.477
Multiple pregnancy rate, *n* (%)	6/86 (7.0)	12/212 (5.7)	0.666

Values are given as mean ± SD or number (%).

[a] Elevan blastocysts were collapsed after warming and hence embryo quality could not be assessed.

ImP4-suppl, vaginal progesterone gel supplemented with intramuscular progesterone every third day; VP4, vaginal progesterone gel only.

sub-group (adjusted OR 0.4, 95% CI 0.2 to 0.9; P = 0.022); however, no significant differences were found in OPR in all subgroups (Supplementary Table 1). In the imP4-suppl group, taking 50–90% as the reference sub-group, all percentiles had comparable adjusted odds ratios for positive HCG and OPR (Supplementary Table 2). The OPR of all serum progesterone percentiles (<10%, 10–49%, 50–90% and >90%) of the VP4 and imP4-suppl groups were comparable; however, in the less than 10% sub-group, OPR was 28.6% in the VP4 group compared with 54.5% in the imP4-suppl group; however, the difference did not reach statistical significance (P = 0.103).

TABLE 3 LOGISTIC REGRESSION ANALYSIS TO IDENTIFY INDEPENDENT PREDICTORS OF ONGOING PREGNANCY (*N* = 475)

	Odds ratio (95% CI)	P-*value*
Female age, years	0.958 (0.902 to 1.016)	0.155
Body mass index, kg/m^2	0.970 (0.920 to 1.022)	0.254
Previous childbirth	0.900 (0.523 to 1.550)	0.704
Progesterone concentration, ng/ml	1.001 (0.972 to 1.032)	0.922
Freeze all strategy	1.121 (0.684 to 1.835)	0.651
Number of embryos transferred, *n*	1.731 (0.977 to 3.064)	0.060
Day of embryo vitrified (day 5/6)	0.877 (0.455 to 1.691)	0.695
Blastocyst morphology[a]		0.047
Excellent	6.423 (1.185 to 34.825)	0.031
Good	4.486 (0.895 to 22.498)	0.068
Average	3.061 (0.612 to 15.317)	0.173
Poor	1	
The type of progesterone administration (VP4-only; imP4-suppl)	1.070 (0.633 to 1.811)	0.800

[a] Blastocyst grading was categorized as excellent (3AA, 4AA, 5AA), good (3,4,5,6 AB or BA), average (3,4,5,6 BB or AC or CA) and poor (3,4,5,6 BC or CC). When more than one embryo was transferred, the one with the best morphological grading was included in analysis.

ImP4-suppl, vaginal progesterone gel supplemented with intramuscular progesterone every third day; VP4, vaginal progesterone gel only.

DISCUSSION

In this HRT study, 50 mg imP4 administered every third day, starting from the first day of the VP4 administration did not enhance the OPR compared with VP4, in the overall population. Serum progesterone levels on the day of FET, as a continuous variable, were not an independent prognostic factor for ongoing pregnancy in the whole cohort. Despite the same dosing in both the VP4 and imP4-suppl sub-group, a marked inter-personal difference in serum progesterone levels was found. In the VP4 group, when serum progesterone levels were stratified as percentiles, patients with less than 10% (8.75 ng/ml) had a numerically lower, but not statistically significant, OPR compared with the 10–50%, 50–90% or over 90% sub-groups. In the imP4-

TABLE 4 PREGNANCY OUTCOMES IN VAGINAL PROGESTERONE GEL-ONLY ARM ACCORDING TO PERCENTILE OF SERUM PROGESTERONE CONCENTRATIONS ON THE DAY OF VITRIFIED BLASTOCYST TRANSFER (*N* = 143)

Serum progesterone percentiles (n) range	*Positive HCG, % (n)*	*Crude OR (95% CI)*	*Overall pregnancy losses % (n)*	*Crude OR (95% CI)*	*Ongoing pregnancy % (n)*	*Crude OR (95% CI)*
<10 (*n* = 14) 0–8.75 ng/ml	42.9 (6)	0.3 (0.09 to 1.0); *P* = 0.06	33.3 (2)	0.5 (0.4 to 9.5); *P* = 0.7	28.6 (4)	0.5 (0.6 to 7.7); *P* = 0.2
10–49 (*n* = 57) 8.76–12.94 ng/ml	61.4 (35)	1.5 (0.7 to 3.3); *P* = 0.3	20.0 (7)	2.2 (0.8 to 6.1); *P* = 0.1	49.1 (28)	0.9 (0.4 to 1.9); *P* = 0.8
50–90 (*n* = 58) 12.95–20.42 ng/ml	70.7 (41)	1	34.1 (14)	1	46.6 (27)	1
>90 (*n* = 14) >20.42 ng/ml	85.7 (12)	2.4 (0.5 to 12.3); *P* = 0.3	16.7 (2)	0.5 (0.1 to 2.6); *P* = 0.7	71.4 (10)	2.8 (0.8 to 10.2); *P* = 0.1

OR, odds ratio.

suppl group, however, all percentiles had comparable adjusted odds ratios for OPR. No ceiling effect of the serum progesterone level on OPR was noted in the over 90% arm for neither the VP4 nor the imP4-suppl sub-groups.

Although, HRT is commonly used to prepare the endometrium for FET, the optimal route of progesterone administration has yet to be established. To our knowledge, only two studies have compared the administration of vaginal progesterone to vaginal progesterone supplemented with intramuscular progesterone in HRT cycles (*Feinberg et al., 2013*; *Devine et al., 2018*). In a retrospective study of 194 vitrified and warmed blastocyst FET cycles, *Feinberg et al. (2013)* compared 300 mg vaginal progesterone daily with a combination of vaginal progesterone 300 mg daily and imP4 50 mg 'at least once every 3 days'. The investigators reported significantly higher positive pregnancy test rates and live births using the combination therapy (60.4% versus 33.7%, *P* = 0.002; 37.5% versus 17.4%, *P* = 0.0015, respectively); however, the biochemical pregnancy loss and miscarriage rates were comparable between the two groups. An important limitation of that study was the limited sample size as well as the somewhat unclear definition of imP4 administration, stated as 'at least once every 3 days', which indicates that some patients might have had more frequent administrations.

More recently, a RCT using vitrified blastocyst transfer in HRT cycles compared the OPR rates in three arms consisting of 200 mg vaginal progesterone twice daily, 50 mg daily imP4, only, and 200 mg vaginal progesterone twice daily supplemented with 50 mg imP4 every third day (*Devine et al., 2018*). A significantly lower OPR was reported in the vaginal progesterone group: 31% compared with the other two groups (50% and 47%); this was caused mainly by a significantly higher biochemical loss and miscarriage rate rather than a lower positive HCG rate in the vaginal progesterone group, only. After the interim analysis, randomization to vaginal progesterone only, was stopped. Unfortunately, no data on serum progesterone levels in the three groups are available from that study.

In contrast to the studies by *Devine et al. (2018)* and *Feinberg et al. (2013)*, we did not find any significant differences between the VP4 and VP4 and imP4-suppl groups in positive pregnancy test rates, implantation, biochemical loss, miscarriage and OPR rates. Importantly, the type of progesterone administration was not found to be an independent prognosticator for OPR in this study when tested by logistic regression analysis. These discordant findings might be a result of the use of different types and, importantly, doses of micronized vaginal progesterone administered. To our knowledge, to date, no direct comparison of the pharmacokinetics and pharmacodynamics of vaginal gel 90 mg twice daily versus twice daily 200 mg vaginal inserts has been made.

Optimal endometrial progesterone exposure (timing and concentration) is essential to establish and maintain an ongoing pregnancy in HRT cycles. Although the use of endometrial progesterone measurements would be ideal, this is not feasible in clinical practice and, therefore, serum measurement so far is the most straightforward monitoring method; however, it serves only as a surrogate marker of the intrauterine progesterone

TABLE 5 PREGNANCY OUTCOMES IN VAGINAL PROGESTERONE GEL SUPPLEMENTED WITH INTRAMUSCULAR PROGESTERONE EVERY THIRD DAY ARM ACCORDING TO PERCENTILES OF SERUM PROGESTERONE LEVEL ON THE DAY OF VITRIFIED BLASTOCYST TRANSFER (*N* = 332)

Serum progesterone percentiles (n) range	*Positive HCG % (n)*	*Crude OR (95% CI)*	*Overall pregnancy losses % (n)*	*Crude OR (95% CI)*	*Ongoing pregnancy % (n)*	*Crude OR (95% CI)*
<10 (*n* = 33) 0–11.75 ng/ml	69.7 (23)	0.8 (0.5 to 2.8), *P* = 0.7	21.7 (5)	1.0 (0.3 to 3.1), *P* = 0.9	54.5 (18)	0.7 (0.5 to 2.5), *P* = 0.8
10–49 (*n* = 133) 15.76–19.86 ng/ml	66.2 (88)	1.4 (0.8 to 2.4), *P* = 0.2	28.4 (25)	1.6 (0.8 to 2.5), *P* = 0.3	45.9 (61)	1.6 (1.0 to 2.6), *P* = 0.06
50–90 (*n* = 133) 19.87–31.79 ng/ml	73.7 (98)	1	21.4 (21)	1	57.9 (77)	1
>90 (*n* = 33) >31.79 ng/ml	66.7 (22)	0.7 (0.3 to 1.6), *P* = 0.5	27.3 (6)	1.2 (0.4 to 3.2), *P* = 0.8	48.5 (16)	0.7 (0.3 to 1.5), *P* = 0.3

OR, odds ratio.

level and, thus, the endometrial receptivity. Until recently, serum progesterone was not monitored in HRT cycles, assuming that 'one size fits all'. In this study, however, we noted a marked interpersonal variation in serum progesterone levels despite using the same luteal phase support. To our knowledge, to date, the effect of circulating serum progesterone levels in HRT cycles has been reported in a total of eight studies; imP4 was only used in two studies (*Brady et al., 2014*; *Kofinas et al., 2015*), whereas vaginal progesterone was used in the remaining six studies (*Yovich et al., 2015*; *Labarta et al., 2017*; *Alsbjerg et al., 2018*; *Basnayake et al., 2018*; *Cedrin-Durnerin et al., 2019*; *Gaggiotti-Marre et al., 2019*). As expected, these eight studies are heterogeneous, making comparisons difficult. The differences include population; autologous cycles (*Kofinas et al., 2015*; *Alsbjerg et al., 2018*; *Cedrin-Durnerin et al., 2019*; *Gaggiotti-Marre et al., 2019*); oocyte recipient cycles (*Brady et al., 2014*; *Yovich et al., 2015*; *Labarta et al., 2017*; *Basnayake et al., 2018*); type and dose of vaginal progesterone; stage of embryo transfer (day 2–3 blastocyst); and the day of measuring serum progesterone levels. Despite these differences, a threshold level, ranging from 10–20 ng/ml above which significantly higher reproductive outcomes was seen, was reported in six studies (*Brady et al., 2014*; *Labarta et al., 2017*; C*alsbjerg et al., 2018*, *Basnayake et al., 2018*; *Edrin-Durnerin et al., 2019*; *Gaggiotti-Marre et al., 2019*). In one study, using a homemade pessary, an optimal window for serum progesterone levels on the sixth day of progesterone of 22.01–31.1 ng/ml and a ceiling effect above 31.1 ng/ml was reported (*Yovich et al., 2015*). In another study using imP4, patients underwent preimplantation genetic testing for aneuploidy followed by euploid embryo transfer, a ceiling effect was reported in patients with serum progesterone levels over 20 ng/ml on the sixth day of progesterone (*Kofinas et al., 2015*).

In the VP4 group, data are inconclusive whether a rescue bolus of either intramuscular or subcutaneous progesterone might change the outcome positively for this sub-group. Although two studies did not support this idea (*Brady et al., 2014*; *Cedrin-Durnerin et al., 2019*), a recent study, in 1462 autologous or egg donation cycles reported rectified OPR when subcutaneous progesterone was administered on the day of embryo transfer (*Labarta, 2019a*; *2019b*). Moreover, whether these patients may benefit from higher starting doses of progesterone or a combination of the vaginal and intramuscular routes needs to be delineated in future RCTs. As steady state serum levels are attained after 3 days (*Wu et al., 2017*), it might be more practical to measure the serum progesterone level on the fifth day of progesterone, i.e. one day before scheduled embryo transfer (*Gaggiotti-Marre et al., 2019*). As for the imP4-suppl group, there seems to be no benefit from monitoring serum progesterone levels as adjusted odds ratios for OPR were comparable for all percentiles. Therefore, in terms of OPR, imP4 supplementation seems to be an advantage. Importantly, no ceiling effect of serum progesterone levels on the OPR was found in the over 90% arm in either the VP4 or the imP4-suppl groups; however, the reported disadvantages, including pain, risk of sterile abscess formation or allergic reactions of imP4 supplementation, also need to be taken into account (*Phipps et al., 1988*).

Despite comparable baseline demographic features, the inherent selection bias caused by immeasurable confounding factors of a retrospective study design is a limitation of the present study. As some patients in both groups had a double embryo transfer, using 'the best blastocyst quality' to represent embryo quality is a limitation, considering that blastocyst morphology is a poor predictor of viability (*Capalbo et al., 2014*). We believe, however, that this should not be a concern as blastocyst morphology and number of embryos transferred were considered along with female age and BMI as potential confounders while calculating the adjusted odds ratios, (Supplementary Table 1 and Supplementary Table 2).

In conclusion, in HRT, autologous vitrified blastocyst transfer cycles, supplementing with imP4 every third day, does not enhance overall OPR compared with VP4 at a dose of 90 mg twice daily. As supplementing vaginal progesterone with imP4 every third day results in similar OPR in all serum progesterone percentiles, monitoring of serum progesterone may not be justified.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2020.01.031.

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