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Vaginal Progesterone (VP) versus VP plus Intermittent Intramuscular Progesterone (IMP) Use in Frozen/Thawed Blastocyst Transfer Cycles: An Observational Cohort Study

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Abstract

Objective: Comparison of vaginal progesterone (VP) versus VP and intermittent intramuscular progesterone (IMP) use in frozen/thawed blastocyst transfer cycles. **Study Design:** A single center retrospective analyses of 470 elective FET cycles which were performed between January 2015 and September 2019 were evaluated. Patients were divided into two groups. Control group was consisted of VP (n = 272), the study group was consisted of VP plus IMP (n = 198) users. **Results:** The number of transfer attempts in control and study groups was 272 and 198, respectively. Age (29.8 ± 4 vs 30.6 ± 4; p = 0.09), BMI (22 ± 2 vs 21.9 ± 3; p = 0.79) and the number of transferred embryos (1.4 ± 0.5 vs 1.4 ± 0.5; p = 0.48) were comparable between groups. Altough, implantation rates (43.7% vs 43.6%; p = 0.9), ectopic pregnancy (0.8% vs 0.3%; p = 0.46) and abortion rates (8.2% vs 4.8%; p = 0.07) were similar. Biochemical pregnancy rate (8.4% vs 3.4% p = 0.01) in control group and ongoing pregnancy rate (OPR) (27.9% vs 38.1%; p = 0.005) in study group were significantly higher. **Conclusion:** Within the FET cycles in which good quality blastocyst are being transferred additional IMP supplementation to VP may increase OPR while reducing the biochemical pregnancy rate.

Keywords

Vaginal Progesterone Supplementation, Progesterone Supplementation, IVF, Cryopreserved Embryo Transfer, Hormone Replacement Therapy

1. Introduction

In *in vitro* fertilization (IVF) cycles, frozen-thawed embryo transfer (FET) cycles

have been increasing due to many reasons, such as transfer of surlups embryo and freeze all strategies preferred to avoid the risk of ovarian hyperstimulation syndrome (OHSS) or to use the embryos underwent preimplantation genetic diagnosis (PGD) [1].

In addition to a good quality embryo, a well-prepared endometrium is also needed for an optimal pregnancy outcome in FET cycles. However, it has not fully been demonstrated which endometrial preparation protocol is the best in FET cycles [2]. Considering the fact that the corpus luteum is not formed in programmed FET cycles, it is very important to use progesterone until the luteal-placental shift occurs. However, the current literature still lacks strong evidence regarding the ideal route of administration and optimal dose of progesterone [3]. In order to provide the luteal phase support, progesterone can be used orally, intramuscularly, vaginally, or rectally [4]. A recent web-based survey revealed that 74% of the clinicians preferred to use vaginal progesterone for luteal-phase support [3]. The vaginal route is generally preferred over intramuscular as it does not cause injection site pain and abscess formation associated with intramuscular administration [5] [6] [7]. A Cochrane review analyzing the clinical pregnancy rate showed a higher trend in vaginal progesterone compared to intramuscular progesterone (IMP) [8]. On the other hand, another study showed findings in favor of IMP, and also studies with similar results were also published [9] [10] [11].

In the recent studies comparing IMP with IMP added to vaginal progesterone for Luteal phase support (LPS) in FET cycles, conflicting results were published [12] [13]. In their randomized controlled trial (RCT), Devine *et al.* reported that live birth rates (LBR), comparing micronized vaginal progesterone (MVP) (200 mg twice daily), IMP (50 mg daily), and MVP plus IMP every third day as LPSin FET cycles, significantly decreased in MVP alone group alone [12]. On the other hand, in their retrospective analysis, Polat *et al.*, found similar ongoing pregnancy rates in IMP added to vaginal progesterone (VP) gel group and VP only group [13]. Due to these conflicting results, protocol for LPS in FET cycles and the best progesterone formulation still remains unclear.

The aim of our study is to investigate the effect of intermittent IMP added to VP gel for luteal support in FET cycles undergoing vitrified blastocyst transfer on clinical and ongoing pregnancy outcomes.

2. Material and Method

We conducted an observational cohort study at Baku Medical Plaza IVF Centers between January 2015 and September 2019. In our study, we retrospectively retrieved patients' data from our electronic health record system. In this study, we included the data obtained from the cases diagnosed and managed by a single physician. This study was approved by the Azerbaijan National Academy of Sciences Genetic Resources lnstitute ethics committee with an approval number 58-13/242. Also all participants signed the written consent forms, and clinical

research commission approval was obtained.

We recruited the patients, who were 20 - 43 years old, had body mass index (BMI) < 35 kg/m^2, and underwent *in vitro* fertilization (IVF) treatment with an indication, such as oligoasthenoteratozoospermia (OAT), and tubal factor, and then underwent elective frozen thawed embryo transfer (ET).

Our inclusion criteria were 1) the transferred embryos were elective frozen-thawed embryos at the blastocyst stage (either 5th or 6th day), 2) the ET was the first FET cycle, and 3) intracytoplasmic sperm injection (ICSI) procedure was performed.

Furthermore, pathological conditions, such as patients with endometrial polyp or type 1 - 2 myoma who underwent hysteroscopic polypectomy or myomectomy, tubal factor (cases with hydrosalpinx) treated with laparoscopic surgery were also included in our study.

In our study, we excluded surlups embryos after the first frozen-thawed embryo transfers. Moreover, natural or modified natural cycles for endometrial preparation in ET were excluded from our study. We also excluded the cases using transdermal estradiol, oral, subcutaneous and only intramuscular progesterone for endometrial preparation and luteal phase support. Embryo transfers with preimplantation genetic diagnosis (PGD) due to aneuploidy or other genetic evaluation were also excluded. Furthermore, we excluded the uterine malformations, such as unicornuate uterus and uterus didelphys, that may adversely affect implantation and cannot be corrected from our study. The cases with missing data, uncertain outcome or outliers were excluded from the study.

We compared the following baseline characteristics of the patients included in our study: age, BMI, duration of infertility, causes of infertility, number of previous IVF attempts, the number of transferred embryos in the present cycle, the levels of estradiol and progesterone and also endometrial thickness on the day of the initiation of progesterone supplementation, blastocyst quality based on the morphology (excellent, good, average, or poor), and pregnancy outcomes (pregnancy test positivity, implantation rate, biochemical pregnancy, clinical pregnancy, ongoing pregnancy, pregnancy loss, ectopic pregnancy, and multiple pregnancy).

During the artificial endometrial preparation in FET, if we detect the serum progesterone level as >1.5 ng/mL on the 12th - 13th day of estradiol treatment, ET is canceled based on the ET protocol in our clinic [14].

The patients who applied VP gel (Crinone 8%, 90 mg, BID, Watson Pharmaceuticals, Morristown, NJ) (Group 1) and VP gel (BID) in addition to IMP oil in oil alone (50 mg/day) (Group 2) every other day were analyzed and included in our study for the LPS for artificial endometrial preparation in FET.

Artificial Endometrial Preparation Protocol

Folliculometry and endometrial thickness were evaluated by transvaginal ultrasound (TV-USG) on the second or third day of the menstrual cycle of all cases and also measured serum estradiol and progesterone levels. Next, estradiol

hemihydrate tablet (Estrofem, Novo Nordisk, Istanbul, Turkey) at a dose of 4 mg/day on the same day were administered. After four days of use, the drug dose was increased to 6 mg/day and 8 mg/day, respectively, at the same intervals. At the end of 12 days of estradiol treatment, endometrial thickness by TV-USG and checked serum estradiol and progesterone levels were evaluated. LPS were initiated if serum progesterone value was <1.5 ng/dl and endometrial thickness was ≥7 mm and also trilaminar [14] [15].

However, whenever the endometrial thickness was less than 7 mm, estradiol treatment was continued for 7 more days. LPS was started to the women who had the aforementioned criteria at their second control. In cases where adequate endometrial thickness could not be detected by TV-USG, we did not extend estradiol treatment beyond 20 days and cancelled those cycles.

Study Groups and Luteal Phase Support

As the LPS protocol in FET cycles at our clinic, while we initially administered VP as a gel, after March 2018 according to well-accepted randomized controlled trial by Devine *et al.*, we updated it as an intermittent 50 mg IMP added to the VP gel BID [12].

Since the first day of VP gel application and every two days following it (day 0, 2, 4, 6, 8, 10), 50 mg of IMP was administered. While progesterone treatment was given in both study groups, 8 mg/day estradiol treatment was continued at the same time. ET was performed on the 6th day of progesterone supplementation treatment.

Serum beta human chorionic gonadotrophin (β-hCG) level was measured 11 days after the ET procedure. β-hCG >5 mIU/mL was considered as pregnancy [14]. In both study groups, LPS were continued in the same way until the 6th - 8th week of pregnancy.

The Protocol for Vitrification and Thawing Blastocysts

Freeze-thawing procedures were performed by using the commercial vitrification kits (Vit Kit®-Freeze, 90,133-SO, and Vit Kit®-Thaw, 90137-SO, Irvine Scientific, CA, USA).

Blastocyst vitrification and thawing processes were performed as per described by Boynukalın *et al.* [16].

Embryo and Blastocyst Morphology

The morphologic assessment of the blastocysts was performed by means of a staging algorithm, as described by Gardner *et al.* [17]. Embryos were categorized as very good (≥3 AA), good (3, 4, 5, 6, AB, and BA), medium (3, 4, 5, 6 BB, AC, and CA), and weak (<3 BB) [18]. A total of 17 embryos collapsed during the transfer after thawing, we performed ET procedures but we did not include those embryos in morphological grading.

Pregnancy Definitions

Ongoing pregnancy was accepted as the primary outcome measure. Clinical pregnancy and biochemical pregnancy were evaluated as secondary outcomes.

Biochemical pregnancy rate (BPR) was defined as the number of pregnancies diagnosed only by β-hCG detection where a gestational sac was not visualized by

TV-USG at the 6th week of pregnancy per number of pregnancies. The implantation rate was calculated by dividing the number of gestational sacs observed by the number of embryos transferred.

Clinical pregnancy was described as the intrauterine gestational sac detected via TV-USG at the 6th week of gestation. The spontaneous expulsion of an embryo or fetus before 20 weeks of gestational age was accepted as miscarriage. The abortion rate was obtained via dividing the number of miscarriages by the total number of clinical pregnancies.

Ongoing pregnancy was defined as a positive heartbeat at or beyond 20th week of gestation.

The ongoing pregnancy rate was calculated by dividing the number of pregnancies beyond 20th week by the number of patients.

Statistical Analysis

We assessed the distribution characteristics of variables via histograms, Shapiro-wilk, and Kolmogorov-Smirnov tests. The numerical data was presented as median, minimum, and maximum, whereas the categorical data was presented as frequency and their percentages.

The quantitative variables were analyzed via the Mann-Whitney U test because the continuous variables were not found to be normally distributed.

The categorical variables were compared by using Chi-Square test. We considered p-value < 0.05 as statistically significant. Logistic regression analysis to investigate factors that affected ongoing pregnancy. Outcomes were run. The statistical analysis by using the Statistical Package for the Social was performed. Sciences (SPSS), version 21.0 (Chicago, IL, USA).

3. Results

In our study, we reviewed IVF-ET treatment records of a total of 470 patients. VP gel (Group 1) and VP gel + intermittent IMP (Group 2) had 272 cases and 198 cases, respectively. We performed 391 and 291 ETs in the VP gel group and the VP + intermittent IMP group, respectively.

The baseline characteristics of the included patients were presented in Table 1. Women's age, age ranges BMI, duration of infertility, number of embryos transferred in the present cycle, number of previous IVF attempts, the transferred embryo morphology, and the levels of estradiol and progesterone and also endometrial thickness on the day of the initiation of progesterone supplementation were similar in both study groups (p-value > 0.05).

Unexplained infertility as the most common cause of infertility in both groups (Group 1: 45.2% vs. Group 2: 40.9%, respectively, p-value = 0.78). Other causes of infertility were detected at similar rates in both groups (p-value = 0.78) (Table 1).

We presented pregnancy results in Table 2. When the pregnancy outcomes in both groups were compared, VP + intermittent IMP group (Group 2) had statistically significantly higher implantation rate [49.1% and 41.2%, respectively, p =

0.03, OR = 0.72 (0.53 - 0.98)] and ongoing pregnancy rate [52.0% and 37.1%, respectively, p = 0.01, OR 1.83 (1.26 - 2.66]] than the VP group (Group 1). Moreover, VP + intermittent IMP group (Group 1) had statistically significantly lower biochemical pregnancy rate [5.1% and 12.1%, respectively, p = 0. 009] and

Table 1. Data are given as number (percentage). IMP, VP, denote intramuscular progesterone, vaginal progesterone.

	Daily VP (n = 272)	Daily VP + IMP (n = 198)	P value
Age	30 (20 - 43)	30 (21 - 43)	0.09
Age brackets			
<35	227 (60.1)	151 (39.9)	0.05
≥35	45 (48.9)	47 (51.1)	
Body mass index (kg/m^2)	21.9 (17.3 - 34.8)	22.6 (15.4 - 31.6)	0.17
Body mass index (kg/m^2) brackets			
<30	265 (57.6)	195 (42.4)	0.43
≥30	7 (70)	3 (30)	
Duration of infertility (years)	3 (1 - 14)	3 (1 - 22)	0.58
Number of transferred embryos	1 (1 - 2)	1 (1 - 2)	0.48
Number of previous attempts	0 (0 - 9)	0 (0 - 7)	0.2
Day of starting progesterone			
Progesterone level (ng/mL)	0.22 (0.03 - 1)	0.2 (0.05 - 1.4)	0.11
Estrogen level (pg/mL)	255 (109 - 984)	283 (110 - 817)	0.12
Endometrial thickness (mm)	9.2 (7.1 - 13)	9.1 (7.1 - 14.6)	0.12
Causes of infertility			
Diminished ovarian reserve	9 (3.3)	9 (4.5)	0.78
Endometrioma	8 (2.9)	4 (2)	
Male factor	58 (21.3)	56 (28.3)	
Ovulatory dysfunction	28 (10.3)	18 (9.1)	
Recurrent implantation failure	20 (7.4)	11 (5.6)	
Recurrent miscarriage	3 (1.1)	2 (1)	
Tubal factor	23 (8.5)	17 (8.6)	
Unexplained	123 (45.2)	81 (40.9)	
Blastocyst morphology			
Excellent	62 (23.3)	61 (31.4)	0.08
Good	60 (22.6)	46 (23.7)	
Average	71 (26.7)	51 (26.3)	
Poor	73 (27.4)	36 (18.6)	

Table 2. Data are given as number (percentage). IMP, VP, denote intramuscular progesterone, vaginal progesterone. OR and CI denote odds ratio and confidential interval.

	Daily VP (n = 272)	Daily VP + IMP (n = 198)	OR (95%CI)	P value
Positive pregnancy test	171/272 (62.9)	125/198 (63.1)	0.98 (0.67 - 1.44)	0.95
Implantation rate	161/391 (41.2)	143/291 (49.1)	0.72 (0.53 - 0.98)	0.03
Biochemical pregnancy	33/272 (12.1)	10/198 (5.1)	0.38 (0.18 - 0.8)	0.009
Clinical pregnancy	135/272 (49.6)	114/198 (57.6)	1.37 (0.95 - 1.99)	0.08
Miscarriage rate	34/272 (12.5)	11/198 (5.6)	0.41 (0.2 - 0.83)	0.01
Ongoing pregnancy	101/272 (37.1)	103/198 (52)	1.83 (1.26 - 2.66)	0.01
Ectopic pregnancy	3/272 (1.1)	1/198 (0.5)	0.45 (0.47 - 4.4)	0.64
Multiple pregnancy	26/272 (9.6)	28/198 (14.1)	1.55 (0.88 - 2.75)	0.12

miscarriage rate [5.6% and 12.5%, respectively, p = 0.01] than the VP group (Group 1). Both Group 1 and 2 had similar rates for positive pregnancy result [62.9% vs 63.1%, respectively, p = 0.95], clinical pregnancy [49.6% vs 57.6%, respectively, p = 0.08], ectopic pregnancy [1.1% vs 0.5%, respectively, p = 0.64], and multiple pregnancy rates [9.6% vs 14.1%, respectively, p = 0.12] (Table 2).

The effects of female age at the time of embryo transfer, BMI, type of LPS treatment, cause of infertility, the number of embryos transferred, and transferred embryo morphologies on the ongoing pregnancy outcomes were evaluated separately. Female age, type of LPS treatment, and the number of embryos transferred were detected as the factors determining the ongoing pregnancy (p-values = 0.02, 0.01, and <0.01, respectively). BMI, the levels of estradiol and endometrial thickness on the day of the initiation of progesterone supplementation, the transferred embryo morphology, and the cause of infertility were found to be non-significant variables (p-values = 0.38, 0.12, 0.85, 0.78, and 0.06, respectively) (Table 3).

The effects of age, BMI, the number of embryos transferred, the level of estradiol and endometrial thickness on the day of the initiation of progesterone supplementation on the ongoing pregnancy outcomes were investigated by using binary logistic regression analysis.

The Nagelkerke R^2 0.09 and Hosmer-Lemeshow goodness of fit test (p-value = 0.16) where the estimation percentage of model was 56.6%. We detected age, type of luteal phase support, and the number of embryos transferred as the risk factors for ongoing pregnancy (p-values = 0.005, 0.001, and <0.001, respectively). BMI, serum estradiol value and the endometrial thickness measured on the day of the initiation of progesterone supplementation were not found to be risk factors (p-value = 0.2, 0.18, and 0.89, respectively) (Table 4). Age, type of luteal phase support, and the number of embryos transferred were identified as the significant variables in both univariate and multivariate analysis.

Table 3. Factors effecting ongoing pregnancy outcomes are displayed. Women age, type of luteal support and number of transferred embryos has increased ongoing pregnancy.

	Negative (n = 266)	Ongoing pregnancy (n = 204)	P value
Age	29 (20 - 43)	29 (21 - 43)	0.02
Age brackets			
<35	203 (53.7)	175 (46.3)	0.01
≥35	63 (68.5)	29 (31.5)	
Body mass index (kg/m^2)	22.3 (15.4 - 34.8)	21.8 (16.7 - 30.8)	0.38
Body mass index (kg/m^2) brackets			
<30	259 (56.3)	201 (43.7)	0.52
≥30	7 (70)	3 (30)	
Type of treatment Luteal phase support			
VP + IMP	95 (48)	103 (52)	0.01
VP	171 (62.9)	101 (37.1)	
Day of starting progesterone			
Estrogen level (pg/mL)	260.1 (109 - 984)	275.9 (119 - 848)	0.12
Endometrial thickness (mm)	9.2 (7.1 - 13.1)	9.2 (7.2 - 14.6)	0.85
Causes of infertility			
Unexplained	122 (58.1)	88 (41.9)	0.06
Tubal factor	20 (51.3)	19 (48.7)	
Diminished ovarian reserve	15 (83.3)	3 (16.7)	
Endometrioma	3 (25)	9 (75)	
Male factor	61 (53.5)	53 (46.5)	
Recurrent implantation failure	20 (64.5)	11 (35.5)	
Ovulatory dysfunction	25 (54.3)	21 (45.7)	
Number of transferred embryos	1 (1 - 2)	2 (1 - 2)	<0.01
Blastocyst morphology			
Excellent	71 (55)	58 (45)	0.78
Good	46 (51.7)	43 (48.3)	
Average	76 (55.5)	61 (45.5)	
Poor	58 (59.2)	40 (40.8)	

4. Discussion

In this FET study, 50 mg IMP administered every other day starting from the first day of VP administration increased OPR compared with VP in the general population. LPS as a continuous variable, were an independent prognostic factor for ongoing pregnancy in the whole cohort.

Table 4. Logistic regression analysis to identify independent predictors of ongoing pregnancy.

	P value	Odds ratio (95% CI)
Luteal phase support	<0.001	1.959 (1.331 to 2.884)
Age	0.005	0.944 (0.907 to 0.983)
Body mass index (kg/m^2)	0.275	0.964 (0.904 to 1.029)
Day of starting progesterone		
Estrogen level (pg/mL)	0.183	1.001 (0.999 to 1.003)
Endometrial thickness (mm)	0.896	1.010 (0.868 to 1.176)
Number of transferred embryos	<0.001	2.012 (1.375 to 2.945)

In normal pregnancies, progesterone secreted from the corpus luteum is essential for implantation and continuation of pregnancy. Insufficient progesterone induction during the follicular phase or insufficient progesterone levels reaching the endometrium from the ovary frequently causes abnormal implantation or early pregnancy loss [19]. During the normal menstrual cycle, the increased follicle stimulating hormone (FSH) levels begin to decrease in the mid-follicular period for single follicle development. In IVF cycles, there are high gonadotropin levels that are administered daily for multimolecular development. Supraphysiological estradiol and progesterone levels, that were increased with multifollicular development, inhibit luteinizing hormone (LH) secretion from the pituitary. Additionally, during the oocyte pick up procedure, the follicle fluid is aspirated, and damage may occur in the granulosa and theca cells the procedure. Because of these reasons, luteal phase defect occurs, and implantation failures frequently occurs in IVF cycles [20] [21].

After ovarian stimulation treatments, progesterone or medications that provide progesterone secretion are applied together with different treatment protocols in order to compensate the luteal phase defect. Moreover, in embryo donation or FET cycles, endometrial development is achieved by mimicking the normal menstrual cycle, and LPS is successfully provided [22] [23].

In the present study, we found that for LPS after FET, women using IMP (50 mg) every 2 days added to VP had higher ongoing pregnancy rates than women using only VP. Furthermore, we detected that intermittent IMP added to VP for luteal support in FET cycles also reduced the rates of biochemical pregnancy and miscarriage.

Hormone replacement therapy (HRT) is frequently used for the endometrial preparation in FET cycles, however, the optimal route and dose of progesterone for luteal support has not been clearly determined. In the literature, we identified 3 studies comparing combined (intermittent IMP + VP) and VP in the FET cycles [12] [13] [24]. In their retrospective study, Feinberg *et al.*, compared the results of Endometrin (100 mg, 3 times a day) together with IMP (50 mg, at least every 3 days) and Endometrin (100 mg, 3 times a day) for luteal support [24].

Upon evaluation of a total of 194 FET cycles, they reported that both clinical pregnancy rate and live birth rate (LBR) were be higher in cases treated with Endometrin + IMP (clinical pregnancy rate 47.9% vs. 23.5%, p-value = 0.0004; LBR 37.5% vs. 17.3%, p-value = 0.0015) [24]. Moreover, the implantation rates were reported to be higher in the combination therapy group (35.8% vs 15.3%, p-value = 0.0001) [24]. Noteworthy, the retrospective nature of the study and the low number of FET cycles might have affected the meaningful interpretation of the data [24]. In a recently published randomized controlled trial (RCT), VP was administered as 400 mg/day for LPS which was different from Feinberg *et al.* [12] [24]. They divided the study patients into three groups as VP 400 mg/day, IMP 50 mg/day, and IMP 50 mg every 3 days and VP 400 mg/day, and the ongoing pregnancy rates in the VP only arm were significantly lower than in the other groups (31%, 50%, and 47%, respectively) [12]. The investigators showed that this was due to higher biochemical pregnancies (33%, 20%, and 13%, respectively, p-value = 0.0008) in the VP arm of the study, thus, they terminated the VP arm of the study earlier than anticipated [12]. In another similar and recently published prospective observational study, the patients were divided into two groups as VP only (Crinone 8%) (Merck Sereno, Bedfordshire, UK) twice daily, and VP and IMP 50 mg every 3 days. Ongoing pregnancy (48.3% and 51.8%, respectively p-value = 0.477), implantation (55.9 ± 47.7 and 58.1 ± 46.7, respectively, p-value = 0.645), and biochemical pregnancy (8.5% and 8.2%, respectively, p-value = 0.932) rates were detected similarly, and also the type of progesterone administration for LPS was not found to be an independent prognostic factor for ongoing pregnancy rates [13]. In this study, serum progesterone level was also measured on the 6th day of VP application, 4 - 5 hours after vaginal gel application in the morning, and just before the planned ET [13]. Serum progesterone percentiles (<10%, 10% - 49%, 50% - 90%, >90%) were separately calculated in both groups [13]. Ongoing pregnancy rates in both VP and combination groups were similar among percentiles [VP group: p-values = 0.8, 0.06, 1, 0.3, respectively; and combination group: p-values = 0.2, 0.8, 1, 0.1, respectively] [13]. However, the type of VP used in this study was differed from the other two studies [13].

While Polat *et al.* used VP 180 mg/day vaginal gel, Feinberg *et al.* and Devine *et al.* used 300 and 400 mg/day vaginal inserts, respectively [12] [13] [24]. We could not find any study in the current literature comparing the pharmacokinetics and pharmacodynamics of vaginal gel twice a day and VP insert twice a day. However, in the pharmacokinetic study of 100 mg and 200 mg endometrin and 90 mg vaginal gel, progesterone concentrations over 10 ng/mL for 24 hours were detected on the 5th day of the application in the use of both inserts, but not in the use of vaginal gel [25]. In our study, while the ongoing pregnancy rate was higher in combined progesterone application for luteal phase support, we found lower rates of biochemical pregnancy and miscarriage (p-value = 0.01, 0.009, 0.01, respectively). Our results were similar to the studies of Feinberg *et al.* and

Devine *et al.* [12] [24]. In the combine group, we applied vaginal gel 90 mg BID and intermittent IMP 50 mg every other day for luteal support. Not measuring the patients' serum progesterone values before ET was a limitation in our study.

In the HRT cycles, external administration of estradiol and progesterone is essential to obtain a receptive endometrium for embryo implantation. However, the ideal progesterone administration route and dose for ongoing pregnancy is still unclear. VP and IMP are two commonly used forms [21] [26].

In comparison to vaginal progesterone, serum progesterone level is higher after IMP administration. However, the progesterone level in the endometrial tissue was found to be higher in vaginal applications [27]. Despite the fact that endometrial progesterone measurement is more sensitive, endometrial progesterone measurement is not possible in the clinical practice. Therefore, serum progesterone level is implemented in daily practice.

In the prospective cohort and retrospective studies, as the dose of VP used for LPS increases, the level of serum progesterone measured in the midluteal phase also increases, but the pregnancy outcomes were contradictory [28] [29] [30] [31] [32]. In order to optimize pregnancy outcomes, the recommended serum progesterone level in the midluteal phase for FET cycles was determined as >9 - 10 ng/ml [33], but serum progesterone levels in the midluteal phase were found to be below 10 ng/ml in 25% - 33% of the women using VP [30] [31]. Although doubling the dose of VP in the patients with a serum p level of <10 ng/ml on the day of ET increased the serum progesterone level, it was insufficient to change the pregnancy outcomes [30]. Similar findings were observed in IMP applications [34] [35]. In their study examining the fresh donor cycles, Brady *et al.* found that serum P (<20 ng/ml vs ≥20 ng/ml) value measured on the day of ET was found to be associated with clinical pregnancy [42 (56.0%) and 113 (73.4%), respectively, p-value = 0.01] [34]. However, in this study, the IMP dose was not standardized (50 or 100 mg/day), and also increasing the IMP dose by 50% - 100% when the serum progesterone value measured on the ET day as <20 ng/ml, was insufficient to save pregnancy rates [34].

Conflicting results were also found in studies comparing the use of VP with IMP. Some authors reported similar pregnancy rates with IMP [36] [37] while other authors reported higher pregnancy rates with it [12] [26] [38]. IMP doses are usually between 50 and 100 mg/day, while VP doses varies as 90 mg/day once or twice a day, 100 mg vaginal progesterone tablets twice or three times a day, and micronized progesterone capsule 200 mg/day 3 - 4 times a day [12] [26] [36] [38].

In 2013, Alsbjerg *et al.*, in their retrospective study, reported that increasing the dose of 90 mg/day VP gel applied in FET cycles to 180 mg/day increased live birth rates [14/161 (8.7%) vs 38/185 (20.5%), respectively, p-value = 0.002), and it was also shown to reduce biochemical pregnancy rates [25/43 (58.1%) vs 16/71 (22.5%), respectively, p-value = 0.0001) [39].

Despite the same progesterone dose administration in all the patients, the mechanisms, that might explain the wide range in serum progesterone levels,

remain unclear [16]. Thus, it can be argued that due to daily variations in serum progesterone level, one measurement alone might not reflect the objective progesterone level and might not indicate the appropriate state of luteal support [33]. When administered vaginally, decrease in progesterone levels has been shown to after sexual intercourse [40]. Also, there is a negative correlation between serum progesterone and BMI [16]. In our study, the patient's BMI in both groups were similar (p-value = 0.43).

In our clinic, VP administration for the LPS in the FET cycles is preferred by the patients owing to its advantages, including easy administration, application by the patient, less pain, and higher patient satisfaction. However, the concern of decrease in clinical pregnancy rates after vaginal progesterone administration changed our practical approach [12]. Moreover, we administer VP gel BID in our clinic for the luteal support in the FET cycles, since increasing the dose of VP gel applied in FET cycles from 90 mg/day to 180 mg/day reduces biochemical pregnancy and increases the live birth rate [39]. Due to higher pregnancy rates after IMP administration for luteal support compared to VP administration [12] [26] [38], less endometrial wave-like movements and uterine contractions [9], lower patient satisfaction compared to VP administration, and side effects such as cold abscess, we add 50 mg of IMP to the vaginal progesterone gel every 2 days in our clinic.

Our study included several limitations. First of all, our study is retrospective, and its unmeasured confounding factors are a limitation of our study. Secondly, we did not measure patients' serum progesterone levels on the ET day. However, VP administration has a direct effect on the uterus independent from serum progesterone levels. Hence, the serum progesterone level may not reflect the reality. Third, we had two embryo transfers in some women in both arms of our study (n = 119/272 (43%), n = 93/198 (46%)). Fourthly, using "the excellent blastocyst quality" to detect embryo quality is a limitation. However, we believe that this should not be a major concern, as the female age, BMI, and the number of embryos transferred, as well as the distribution between study groups, which may affect pregnancy outcomes, was similar.

We concluded that in HRT autologous vitrified blastocyst transfer cycles, supplementing with 90 mg/twice daily VP every other day with IMP increases ongoing pregnancy rates compared to 90 mg/twice daily VP administration alone. The combination treatment group achieves this by reducing the biochemical pregnancy and miscarriage rates. Further studies and RCTs comparing combined progesterone and VP gel administrations are required to determine the ideal LPS protocol in adequate number of single euploid blastocyst transfer cycles.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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