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Short Communication

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Serum Progesterone Concentration and Ongoing Pregnancy Rate in Frozen-Thawed Embryo Transfers with Intramuscular Plus Vaginal Progesterone Administration for Endometrial Preparation

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Abstract

Background: Insufficient serum progesterone level in the implantation phase may reduce the rate of pregnancy during freeze embryo transfer (FET) cycles. The present study aimed to evaluate the impact of FET day serum progesterone level on pregnancy outcomes in patients receiving intramuscular plus vaginal progesterone administration for endometrial preparation.

Methods: Based on serum progesterone level on FET day, patients were divided into four quartiles: first (<25%), second (26–50%), third (51%–75%), and fourth (>75%). There was no significant difference among groups in basal characteristics.

Results: No statistically significant difference was seen among groups concerning the mean number of retrieved and mature oocytes, embryos transferred, and endometrial thickness (EnT). The rate of implantation ($P=0.5$), biochemical ($P=0.75$), clinical ($P=0.54$), and ongoing pregnancy ($P=0.5$) were not associated with serum progesterone level on embryo transfer day.

Conclusion: We found that there is no association between serum progesterone level on ET day and pregnancy outcome during FET cycles. It seems that combination therapy using intramuscular and vaginal progesterone, keeps the serum progesterone on ET day high enough that eliminates the need for serum progesterone measurement.

Keywords: Progesterone, Intramuscular, Endometrial, Pregnancy, Embryo transfer

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Introduction

Progesterone plays an important role in secretory endometrium and it allows implantation and maintenance of pregnancy in the early stages. Insufficient progesterone in the implantation phase may reduce the rate of pregnancy (1).

In frozen-thawed artificial endometrial preparation, estrogen is administrated from cycle day 2 or 3 to induce endometrial development and when endometrium thickness reaches approximately 7 mm, exogenous progesterone takes part in a process similar to what occurs in the natural cycle of the body (2,3). Exogenous progesterone supports the synchronization of embryo and endometrium development (4). The best route of administration, the optimal duration and dosage, and the period of exposure to progesterone before embryo transfer have not been identified well.

Identification of the adequate serum progesterone level for optimizing favorable pregnancy outcomes may allow gynecologists to individualize the hormone replacement therapy for freeze embryo transfer (FET). The data are currently sparse on this topic; most data are on the vaginal route of progesterone administration (5-7) and other data on the intramuscular route (8,9).

The present study aimed to evaluate the impact of serum progesterone level on the day of FET on pregnancy outcomes in patients receiving intramuscular plus vaginal progesterone administration for endometrial preparation.

Methods

The present cross-sectional analytical study was conducted at Mehr Medical Institute, Rasht, Iran from March to October 2020. One hundred and twenty intracytoplasmic sperm injection (ICSI) patients undergoing hormone replacement therapy (HRT) for FET were recommended

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for serum progesterone measurement on the day of embryo transfer. The study protocol was approved by the institutional review board (MEHR.1398.10.965).

The basal characteristics of patients including age, body mass index (BMI), infertility duration, and hormonal profile of luteinizing hormone (LH), follicle stimulating hormone (FSH), and anti-Mullerian hormone (AMH) were collected from electronic files of patients. Following controlled ovarian hyperstimulation protocols, oocytes were retrieved under an ultrasound guide. ICSI was performed 4-6 hours after oocyte retrieval. Three to four days after fertilization, embryos were cryopreserved. Ongoing pregnancy as the primary endpoint was defined being the continuation of pregnancy over 12 weeks of gestation. The secondary endpoint was biochemical (positive β-hCG) and clinical pregnancy (the presence of gestational sac and fetal heartbeat on ultrasound) rate.

Endometrial preparation

Endometrial preparation for vitrified-thawed embryos was started on cycle day 3 with estradiol valerate 2 mg, twice a day, and continued at 6 mg/day from cycle day 7 until endometrial thickness (EnT) reaches more than 7 mm. EnT was measured on cycle day 15 or 16, and if the growth of EnT was not adequate the duration and dose of estrogen therapy were increased. Endometrial pattern A was considered a triple line pattern under ultrasound. Once EnT has reached more than 7 mm, 400 mg vaginal micronized progesterone accompanied by 100 mg intramuscular progesterone was administrated for three days for 8-cells embryos and five days for blastocysts before embryo transfer. Only top and good-quality embryos were transferred. On the day of embryo transfer, blood samples were taken for progesterone measurement.

The chemical and clinical pregnancy was confirmed by a positive β-hCG test and the presence of the intrauterine gestational sac with fetal heartbeat on vaginal ultrasound. The luteal phase support was continued until 12 weeks of pregnancy.

Hormonal measurement

Serum progesterone level was measured using the VIDAS® Progesterone (PGR) assay as an automated quantitative enzyme-linked fluorescent immunoassay. The sensitivity of the assay was 0.25 ng/mL.

Statistical analysis

Patients were divided into quartiles: 1st < 25%, 2nd 26–50%, 3rd 51–75%, and 4th > 75%. The chi-square test and student *t* test were used for categorical and continuous variables, respectively. Multinomial logistic regression analysis was used for investigating the impact of confounding variables (*P* value < 0.2). The first quartile was considered as the reference.

Results

A total of 120 patients in frozen-thawed cycles were evaluated in the present study. The mean age and BMI of patients were 36 ± 7.9 years and 27 ± 4.1 kg/m^2. They had a mean EnT of 8.7 ± 1 mm at the end of estradiol therapy. The mean serum progesterone level on the day of embryo transfer was 32.9 ± 18.7 ng/mL. The serum progesterone range for each quartile was as follows: Q1: 10.3-25ng/mL, Q2: 25-29.3 ng/mL, Q3: 29.3-40.75 ng/mL, Q4: 40.75-79.

The baseline characteristics of participants were presented in Table 1. There was no significant difference among groups in terms of age, BMI, LH, FSH, AMH, and duration of infertility. No statistically significant difference was seen among groups concerning the mean number of retrieved and mature oocytes, the number of embryos transferred, and EnT (Table 1). The rate of implantation ($P = 0.50$), biochemical ($P = 0.75$), clinical ($P = 0.54$), and ongoing pregnancy ($P = 0.50$) were not associated with serum progesterone level on embryo transfer day.

The univariate logistic regression indicated that age, BMI, EnT, number of retrieved and mature oocytes, the total number of embryos, and embryos transferred had *P* value < 0.2. The results of univariate and multivariate logistic regression analysis are summarized in Table 2.

Discussion

As observed in the present study, there is no association between serum progesterone level on ET day and pregnancy outcome during FET cycles. The present results do not match with the previous findings which described low serum progesterone levels on FET day impair pregnancy outcomes in patients undergoing endometrial preparation with vaginal or intramuscular progesterone (9-11).

Optimal timing and concentration of administrated progesterone during HRT for FET to establish and continue a pregnancy are unclear. It seems that the intrauterine progesterone measurement could be the best marker for analyzing endometrial receptivity, but it is not practical, and serum progesterone level as an alternate marker would be applicable.

In order to maintain the optimal level of serum progesterone with the same level as natural cycles, progesterone can be administrated through different routes. The similar efficacy of intramuscular (50 mg), subcutaneous (25 mg), and micronized vaginal progesterone (600 mg) have been reported by the European Society of Human Reproduction and Embryology (ESHRE) guidelines (12).

To the best of our knowledge, the route of progesterone administration in all studies on serum progesterone level on FET day was intramuscular or intravaginal. In a study by Brady et al (8), it was indicated that there is a positive correlation between ET day serum progesterone

Table 1. The baseline and stimulation characteristics and pregnancy outcomes

Variable	Q_1 (10.3-25) n = 30	Q_2 (25-29.3) n = 30	Q_3 (29.3-40.75) n = 30	Q_4 (40.75-79) n = 30	*P* value
Progesterone	19.43 ± 5.6	26.73 ± 1.41	33.14 ± 4.3	50.7 ± 10.7	-
Age	34.9 ± 7.5	39.13 ± 7.51	37.2 ± 9.3	36.5 ± 7.6	0.24
BMI	27.5 ± 4.1	28.2 ± 5.1	25.4 ± 3.3	26.8 ± 3.3	0.1
LH	5.9 ± 3.3	4.6 ± 2.7	5.6 ± 4.6	4.62 ± 2.1	0.5
FSH	7.6 ± 2.3	7.05 ± 2.3	7.2 ± 1.8	7.1 ± 2.2	0.8
AMH	4.2 ± 2.6	3.9 ± 2.3	5.2 ± 2.98	3.9 ± 2.99	0.3
Duration of infertility					
Primary	81.57 ± 38.7(28-180)	67.1 ± 70.97(15-264)	66.3 ± 46.6(5-216)	79.2 ± 68.81(12-246)	0.21
Secondary	40.8 ± 23.4(4-94)	48.8 ± 39.01(7-132)	59.33 ± 61.7(6-172)	72.1 ± 82.5(12-348)	0.7
Previous embryo transfer failure	0.7 ± 0.8	0.83 ± 1.23	1.03 ± 1.03	0.63 ± 1.23	0.5
Embryo quality (Top-quality): 1 (Good-quality): 2 (Low-quality): 3	1.3 ± 0.5	1.2 ± 0.5	1.3 ± 0.4	1.2 ± 0.4	0.81
EnT (mm)	9.2 ± 1.15	8.6 ± 1.1	8.5 ± 0.94	8.7 ± 0.9	0.1
Endometrial type A (%)	22 (73.3)	20 (66.7)	21 (70)	27 (90)	0.4
Fertilization rate	0.7 ± 0.2	0.8 ± 0.23	0.8 ± 0.24	0.74 ± 0.3	0.2
No. of oocytes retrieved	12.4 ± 6.9	16.3 ± 10.7	14.98 ± 7.1	13.9 ± 7	0.2
No. of metaphase II oocytes	9.97 ± 5.66	13.31 ± 7.5	12.4 ± 6.2	11.4 ± 6.8	0.25
No. of embryos transferred	2.63 ± 0.9	2.8 ± 0.7	2.63 ± 0.81	2.6 ± 0.81	0.8
Embryo transfer day	3.1 ± 0.6	3.1 ± 0.3	3.4 ± 0.8	3 ± 0.4	0.09
Implantation rate	0.21 ± 0.31	0.2 ± 0.3	0.13 ± 0.3	0.22 ± 0.3	0.5
Chemical pregnancy (%)	11 (36.7)	10 (33.3)	12 (40)	14 (46.7)	0.75
Clinical pregnancy (%)	11(36.7)	9 (30)	8 (26.7)	13 (43.3)	0.54
Ongoing pregnancy (%)	10 (33.3)	8 (26.7)	7 (23.3)	12 (40)	0.5

Abbreviations: Q, quartile; BMI, body mass index; LH, luteinizing hormone; FSH, follicle stimulating hormone; AMH, anti-Mullerian hormone; EnT, endometrial thickness.

Table 2. The results of univariate and multivariate logistic regression analysis

	Univariate logistic regression			Multiple logistic regression		
	P value	OR	95% CI	*P* value	OR	95% CI
Group	0.52			0.638		
Group (1)	0.6	0.73	(0.24-2.21)	0.826	0.867	(0.243-3.097)
Group (2)	0.4	0.61	(0.2-1.9)	0.658	0.747	(0.205-2.722)
Group (3)	0.6	1.3	(0.5-0.8)	0.430	1.644	(0.478-5.658)
Age	0.34	0.98	(0.93-1.03)	0.644	0.985	(0.926-1.049)
BMI	0.9	0.991	(0.9-1.1)	0.999	1.000	(0.895-1.117)
EnT	0.04	1.5	(1.02-2.21)	0.041	1.598	(1.020-2.505)
No. of oocytes retrieved	0.5	1.02	(0.97-1.1)	0.998	1.000	(0.851-1.176)
No. of metaphase II oocytes	0.7	1.014	(0.95-1.08)	0.257	0.889	(0.725-1.09)
No. of embryos	0.3	1.044	(0.97-1.13)	0.069	1.199	(0.986-1.458)
No. of embryos transferred	0.74	1.1	(0.66-1.8)	0.550	1.206	(0.653-2.228)
Constant				0.085	0.006	

Abbreviations: OR, odds ratio; CI, confidence interval; EnT, endometrial thickness.

level and clinical pregnancy and live birth rate in fresh donor IVF/ICSI cycles that underwent intramuscular progesterone as needed in HRT. In another study on donor oocyte cycles, it was concluded that maintenance of progesterone levels in the range of 10-20 ng/mL before implantation will maximize ongoing pregnancy rate using single euploid frozen embryo transfer (9). In a study on patients undergoing endometrial preparation with estradiol valerate and vaginal micronized progesterone, the correlation of serum progesterone level less than 10.64 ng/mL one day before frozen embryo transfer with lower pregnancy rate was proved (10). In another retrospective

study, it was indicated that serum progesterone level just before embryo transfer is related to the rate of live birth in patients who underwent HRT with estradiol and 600 mg vaginal micronized progesterone (6). In a prospective cohort study, it was indicated that serum progesterone level less than 9.2 ng/mL on ET day may lead to a significantly lower pregnancy rate in patients undergoing HRT with estradiol valerate and vaginal micronized progesterone (400 mg/12 h) (11). In a study by Boynukalin et al (13), it was shown that serum progesterone concentration one hour before embryo transfer has a predictive value in ongoing pregnancy rate. The sensitivity and specificity were calculated respectively to be:71.7% and 56.5% for the 20.6 ng/mL cutoff value.

There is an obvious intrapersonal variation in serum progesterone levels regardless of similar progesterone supplementation. It has been stated that the fast uptake of intramuscular administration will result in a more rapid increment in the serum progesterone level (14). The intramuscular route of progesterone administration has a longer half-life and better absorption than the vaginal route which leads to opening the window of implantation for an extended period and reducing the endometrial contraction on the day of embryo transfer (15). Some studies have demonstrated that the intramuscular route of progesterone administration is more effective than the vaginal route (16,17). There is some evidence that shows higher pregnancy outcomes as a result of combination therapy. In a retrospective study on blastocyst FET cycles, vaginal progesterone 300 mg/day was compared with vaginal progesterone 300 mg/day plus intramuscular progesterone 50 mg for three or more days. They reported significantly higher pregnancy outcomes using the combination therapy and a comparable miscarriage rate has been observed between the two groups (18). In another randomized clinical trial on frozen blastocyst transfer, ongoing pregnancy was compared among three groups: vaginal progesterone 400 mg/day, intramuscular progesterone 50 mg/day, vaginal progesterone 400 mg plus intramuscular progesterone 50 mg/3 days. Results indicated a significantly less ongoing pregnancy rate in the vaginal progesterone group compared to the other two groups (19).

Depending on the route of progesterone administration, the optimal progesterone level on ET day convenient for maintaining pregnancy is unclear. In the present study, the mean progesterone levels on ET day were higher than in other studies (with a mean level of 32.5 ng/mL ranging from 10.3 to 79 ng/mL), perhaps because we used combination therapy. In a retrospective cohort study on the natural cycle FET, patients did not receive any hormone therapy for endometrial preparation or luteal support. The results indicated that regular ovulatory women undergoing natural cycle FET with serum progesterone levels less than 10 ng/mL result in a significantly lower pregnancy rate compared to those with more than 10 ng/mL (7). It seems that a threshold around 10 ng/mL, which is close to the concentration reported as an optimal progesterone level released by corpus luteum in natural cycles (20), should be retained.

Contrary to the aforementioned studies, no significant difference was detected among quartiles regarding pregnancy outcomes. Also, the ongoing pregnancy rate has adjusted for variables with $P<0.2$ as univariate logistic regression has indicated. When confounding variables were included in the logistic regression model, EnT significantly increased the pregnancy rate.

Conclusion

It seems that combination therapy using intramuscular and vaginal progesterone keeps the serum progesterone on ET day high enough that eliminates the need for serum progesterone measurement. More randomized clinical trial studies should be designed to evaluate the effect of combination therapy on pregnancy outcomes.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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