Intramuscular Versus Vaginal Progesterone in Luteal Phase Support: Measuring Serum Progesterone in Frozen Embryo Cycles – A Randomized Controlled Trial

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Abstract:

Background and Aim: Although, progesterone supplementation during the luteal support phase can improve the outcomes, there is not a general consensus regarding the best delivery method for progesterone administration. Our study aimed at comparing the pregnancy success rate and ongoing pregnancy between vaginal versus intramuscular administration of progesterone supplementation among infertile patients receiving assisted reproductive technology and who were in the luteal support phase.

Methods and Materials: We included 120 candidates for hormone replacement therapy (HRT) for embryo transfer in this prospective cohort study. Luteal phase support was done in one group with 100 mg of intramuscular progesterone daily and in the other group with 800 mg of vaginal progesterone daily. Sixteen days after embryo transfer, beta human chorionic gonadotropin (β-hCG) was measured to confirm pregnancy. If pregnancy was confirmed, luteal phase support continued until the 12th week of pregnancy, in each patient based on the last dose and route received. The pregnancy success rate and ongoing pregnancy were compared between the two groups.

Results: Overall, 120 women were included in our study, 64 in the intramuscular group and 56 in the vaginal support group. The most prevalent cause of infertility was male factor (62, 52.99%) and ovarian tube dysfunction (17, 14.53%). Overall, 27 patients had positive hCG serum test, among which 17 (62.96%) and 10 (37.04%) received intramuscular and vaginal progesterone support, respectively. Also, among those who had ongoing pregnancy, 12

(57.14%) received intramuscular progesterone and 9 (42.86%) received vaginal progesterone. We found no significant difference between the two groups regarding serum progesterone level (*p-value = 0.58*).

Conclusion: Our results show that there was no significant difference between the success rate of pregnancy and ongoing pregnancy among those treated with vaginal supplementation of progesterone compared to those treated with intramuscular progesterone supplementation, hence, intramuscular vaginal supplementation had no superior clinical efficacy.

Keywords: Assisted reproductive technology, embryo transfer, luteal phase support, progesterone supplementation.

1. Introduction

After the first live birth following frozen embryo transfer in 1983, this method has been met with increasing acceptance for the treatment of infertility globally to the extent that it includes up to 25% of all assisted reproductive technology (ART) procedures performed [1, 2]. In this method, during the luteal support phase, external supplementation of progesterone is indicated. Several studies have shown that the serum level of progesterone is an effective intervention for improvement of the rate of pregnancy success. The first clinical trials evaluated the effect of progesterone supplementation versus placebo. The findings of those studies indicated improved rate of live birth and ongoing pregnancies among those who received progesterone supplementation. Further studies assessed the effect of human Gonadotropin-releasing hormone versus progesterone administration, which suggested lower ovarian hyperstimulation syndrome, a common complication of ART, among those who were treated with progesterone [3-7].

The third wave of publications focused on the comparison of progesterone supplementation alone versus in combination with esterogen or combined with GnRH agonist. The results reported no significant difference in outcome when progesterone was combined with esterogen, however, advantageous outcomes were reported when progesterone was combined with GnRH agonist. Finally, recent body of research is interested to evaluate the effect of different progesterone delivery routes (oral, vaginal, intramuscular, subcutaneous, and rectal)

on pregnancy outcomes [7-11]. There is inconsistency in the literature regarding the optimal delivery route for progesterone supplementation. Hence, this study attempted at comparing the pregnancy success rate and ongoing pregnancy between vaginal versus intramuscular administration of progesterone supplementation among infertile patients receiving assisted reproductive technology and who were in the luteal support phase.

2. Methods and Materials

This study was a prospective cohort study from February, 2019 to January, 2020 among candidates for hormone replacement therapy (HRT) for embryo transfer. These were referred patients to Mahdieh Hospital, Tehran University of Medical Sciences, Tehran, Iran. The sample size was calculated to be 120 cases based on the "clinical pregnancy rate" in similar studies. The first type of error and power were considered 5% and 90%, respectively.

HRT candidate patients for embryo transfer who are referred to the infertility department were first consulted, and after providing sufficient explanation about the present study, were examined only after obtaining a written consent based on the eligibility and exclusion criteria. The eligibility criteria included women aged 20 to 40 who underwent HRT for frozen embryo transfer and had an endometrium with a thickness of at least 7 mm after receiving progesterone from any of the intramuscular or vaginal routes on the day of transfer to the secretory phase. Based on the exclusion criteria, patients with uterine diseases, subcutaneous myoma, a history of abortion or embryo transfer failure more than three times, and receiving drugs affecting reproductive function or metabolism were excluded from the study. Patients were then randomly divided into two groups of intramuscular and vaginal progesterone.

The patients included in the study - all of whom have undergone HRT - received intramuscular triptorelin 3.75 mg one week before the onset of menstruation to ensure suppression of ovarian function. Afterwards, patients received oral estradiol valerate 4 mg daily for four days and then 6 mg daily for three days from the second day of menstruation. Patients underwent ultrasound on the 8th day of menstruation; The dose of estradiol valerate in patients with endometrial thickness less than 7 mm was increased to 8 mg daily and these patients underwent ultrasound again. If the endometrial thickness was still less than 7 mm after increasing estradiol valerate to 8 mg, the patient was excluded from the study. Eligible patients' serum progesterone levels were measured one hour prior to embryo transfer, and these patients then received 3 to 5-day frozen embryos based on the age and quality of the available embryos. In case of 3-day embryo transfer, the patient received 100 mg of

intramuscular progesterone daily for the first two days, and then 800 mg of vaginal progesterone daily for the next two days. If the patient received a 5-day-old embryo, the patient received 100 mg of intramuscular progesterone daily for two days and 800 mg of vaginal progesterone daily for the next four days.

Luteal phase support after this stage was done in one group with 100 mg of intramuscular progesterone daily and in the other group with 800 mg of vaginal progesterone daily. Sixteen days after embryo transfer, beta human chorionic gonadotropin (β-hCG) was measured to confirm pregnancy. If pregnancy was confirmed, luteal phase support continued until the 12th week of pregnancy, in each patient based on the last dose and route received. Serum progesterone levels, pregnancy rates, miscarriages, and live births were measured to determine the preferred method of progesterone administration.

SPSS software (version 26) was used for statistical analysis of data. The Kolmogorov-Smirnov test was used to measure the normal distribution of the data, and the variables were analysed using Student's t-test, Chi-square, Fisher's exact test, and Mann-Whitney.

3. Results

Overall, 120 women were included in our study, among which 64 were included in the intramuscular support group and 56 were included in the vaginal support group. The mean age (±SE) was 32.24 (±0.65) in the intramuscular support group and 32.62 (±0.68) in the vaginal support group. There was no significant difference of age between the two groups. The mean (±SE) interval from initiation of estradiol treatment to embryo transfer was 15.22 (±0.20) in the intramuscular support group and 14.38 (±0.32) among the vaginal support group, respectively (*p-value = 0.02*). This interval was significantly higher among the intramuscular support group compared to the vaginal support group. The most prevalent cause of infertility was male factor (n = 62, 52.99%) and ovarian tube dysfunction (n = 17, 14.53%) among the included patients in our study. Overall, among who had ongoing pregnancy, 12 (57.14%) received intramuscular progesterone and 9 (42.86%) received vaginal progesterone. There was no significant difference regarding the ongoing pregnancy and route of delivery.

Table 1. The association of intramuscular and vaginal delivery of progesterone with continuous variables

Variable	Categories	Mean	SE	*p-value*

Age	Intramuscular	32.24	0.65	*0.68*
	Vaginal	32.62	0.68	
	HCG -	32.53	0.55	*0.66*
	HCG +	32.03	0.91	
BMI	Intramuscular	25.91	0.59	*0.20*
	Vaginal	24.80	0.62	
	HCG -	25.41	0.50	*0.98*
	HCG +	25.43	0.83	
Interval to Embryo Transfer	Intramuscular	15.22	0.20	***0.02****
	Vaginal	14.38	0.32	
	HCG -	14.76	0.22	*0.43*
	HCG +	15.13	0.34	
Progesterone Level	Intramuscular	31.41	2.01	*0.58*
	Vaginal	29.72	2.29	
	HCG +	29.46	1.87	*0.26*
	HCG -	33.28	2.72	
Endometrial Thickness	Intramuscular	7.43	0.11	*0.23*
	Vaginal	7.68	0.17	
	HCG +	7.64	0.12	*0.11*
	HCG -	7.25	0.16	

The mean (±SE) serum progesterone level was 31.41 (±2.01) among the intramuscular support group and 29.72 (±2.29) among the vaginal support group, respectively. We found no significant difference between the two groups regarding serum progesterone level (*p-value = 0.58*). Those with positive serum hCG test had lower serum progesterone level (33.28 ± 2.72) compared to those with negative test results (29.46 ± 1.87), however, this difference was not significant (*p-value = 0.26*). Further details, regarding the BMI and endometrial thickness among the two luteal support groups and hCG negative or positive groups are available in Table 1.

Table 2. Comparing certain pre-pregnancy characteristics with the success rate of pregnancy

Variable	**Categories**	**hCG (-)**	**hCG (+)**	***p-value***
Parity	0	74 (83.15%)	21 (84%)	*0.74*
	1	13 (14.61%)	4 (16%)	
	2	2 (2.25%)	0 (0%)	
Luteal Support	Intramuscular	47 (50.54%)	17 (62.96)	*0.25*
	Vaginal	46 (49.46%)	10 (37.04)	
Type of Infertility	Primary	71 (77.17%)	20 (76.92%)	*0.97*

	Secondary	21 (22.83%)	6 (23.08%)	
Type of Fetus	3-day	58 (63.74%)	8 (32%)	***0.005****
	5-day	33 (36.26%)	17 (68%)	

Overall, 27 patients had positive hCG serum test, among which 17 (62.96%) and 10 (37.04%) received intramuscular luteal support and vaginal luteal support, respectively. Those who received 5-day embryos had significantly higher rate of successful pregnancy (68%) compared to those who received 3-day (32%), respectively (*p-value = 0.005*). Table 2 presents association of successful pregnancy with parity and type of infertility.

4. Discussion

Our study was designed to compare the pregnancy success rate outcome and ongoing pregnancy between vaginal versus intramuscular administration of progesterone supplementation among infertile patients receiving assisted reproductive technology and were in the luteal support phase. Our results showed no significant difference between the two methods of delivery on success rate and ongoing pregnancy.

Progesterone supplementation during the luteal support phase can be administered via different entry routes including oral, vaginal, subcutaneous, intramuscular, or rectal [7, 12]. It is also the most prevalent preferred method for luteal phase support for in vitro fertilisations and intrauterine sperm injection. Possible complication to this procedure is the ovarian hyperstimulation syndrome. Among different delivery routes, vaginal support during LPS is more common compared to the other modalities of delivery [7, 13, 14]. Several studies have shown beneficial effect of progesterone supplementation on both serum and intrauterine progesterone levels [7, 15, 16]. However, based on a comprehensive review by Cochrane, there is no significant difference between the success rate of induced pregnancy, ongoing pregnancy, and live births, similar to the results of our study.

The available literature regarding the comparison of different progesterone supplementation delivery regiment, dosage, and entry route are scarce and have high risk of methodological and explanatory bias. Beside delivery route, based on a recent systematic review and meta-analysis study, different dosage of progesterone supplementation had no significant effect on the pregnancy outcome, similarly [5, 7, 17-19]. However, these results should be interpreted with caution as the number of randomized clinical trials designed to evaluate this difference

are scarce. More importantly, there is controversy in the literature regarding the effect of increasing progesterone supplementation level and improvement in the pregnancy outcomes. One recent study found no significant difference between two groups treated with 200 mg and 300 mg daily progesterone supplementation, whereas another recent study reported promising results on endometrial thickness and endometrial secretory potency. However, this study did not report any pregnancy related outcomes [7, 20, 21]. Vaginal progesterone supplementation can cause several adverse side effects such as pain, increased vaginal discharge, emesis, nauseous sensation, and itching [7, 17, 22-26].

Intramuscular administration of progesterone during the luteal phase was hypothesized to have a more efficacious effect compared to vaginal progesterone supplementation. As estradiol can improve uterus contraction, progesterone, on the other hand, can diminish this effect by lowering the frequency and strength of endometrial contraction waves [7, 25-28]. This lowering effect on the uterine contractility can help improving the chance of pregnancy success and ongoing pregnancy, due the fact that high rate of contraction can lower ongoing pregnancy rate [7, 26, 29, 30]. It has since been thought that as the intramuscular supplementation of progesterone can deliver a more constant delivery of progesterone, it may improve the pregnancy outcomes. However, based on the results of this study and similar previous studies, no significant difference was found between these two methods of delivery. Interestingly, patients prefer the vaginal delivery, as intramuscular delivery is more irritable and discomforting than the vaginal delivery of progesterone [7]. Hence, intramuscular administration of progesterone supplementation has no clinical superiority to vaginal administration.

Conclusion

Based on the results of our study there was no significant difference between the success rate of pregnancy and ongoing pregnancy among those treated with vaginal supplementation of progesterone compared to those treated with intramuscular progesterone supplementation. Also, as patients prefer the vaginal supplementation it can be the clinically preferred method of delivery for progesterone supplementation.

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