

EVALUATION OF CORRELATION OF SERUM PROGESTERONE LEVEL WITH PREGNANCY OCCURRENCE IN FROZEN EMBRYO TRANSFER

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ABSTRACT

BACKGROUND

Progesterone is an endogenous hormone secreted by ovaries that prepares uterine endometrium for implantation. The aim of this study was to evaluate the relation of serum progesterone level on the day of transferring frozen embryo with pregnancy occurrence.

MATERIALS AND METHODS

161 women were chosen for frozen embryo transfer. From the second day of their cycle, they were treated with 2 mg per 8 hours estradiol. From the 13th day, 5 mg intramuscular (IM) progesterone was injected every 12 hours to increase endometrium thickness above 8 mm. 4 days after progesterone administration, embryo transfer was performed, and progesterone level was measured. Pregnancy occurrence was also evaluated.

RESULTS

The average progesterone level in 25 women with positive beta HCG was 26.26 ± 8.58 on 1st day, 26.63 ± 8.22 on 17th day and 24.3 ± 11.48 on the 18th day of transfer. In 136 non-pregnant women it was 25.61 ± 16.01 on 1st day, 29.03 ± 14.45 on 17th day and 15.42 ± 2.77 on 18th day.

CONCLUSION

There was no relationship between serum level of progesterone on the day of transfer and pregnancy occurrence.

KEY WORDS

Frozen Embryo Transfer, Progesterone, Pregnancy.

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BACKGROUND

Infertility is defined as the failure to engender after one year of unprotected proximity in women less than 35 years of age and after 6 months in women over 35 years of age.⁽¹⁾ Epidemiological data indicated that about 10 - 15% of couples are suffering from infertility.⁽²⁾ Management includes fertility counselling, lifestyle modifications, medical/ surgical treatment of underlying conditions, fertility medications, Assisted Reproductive Technologies (ARTs), serving as Intrauterine Insemination (IUI) and In Vitro Fertilisation (IVF)/ Intracytoplasmic Sperm Injection (ICSI).⁽³⁾ The harmonic differentiation of embryo to blastocyst and uterine preparation for admission of blastocyst are essential factors defining women's fertility.⁽⁴⁾ Distraction in transferred embryo implantation is one of the most important issues in the failure of ART method.⁽⁵⁾

There are two methods of embryo transfer in IVF: frozen embryo transfer and fresh embryo transfer. Frozen embryo transfer increases cumulative pregnancy ratio and decreases costs, is easy to perform and could be administered successfully in a relatively shorter time span compared with repeated fresh cycles.⁽⁶⁾ Frozen embryo transfer is done in two manners: slow and very fast. Compared with the traditional slow freezing method, the very fast method (embryo vitrification) that is introduced recently includes an ultrafast cryopreservation that inhibits ice formation within the suspension which is transformed to a glass-like solid, avoiding damage to the cells or tissues.⁽⁷⁾ In the ovaries, progesterone is synthesized in the mitochondria of granulosa cells from cholesterol.⁽⁸⁾ Progesterone is an endogenous hormone secreted by ovaries that prepares the endometrium of uterine by delivering nutrients to the foetus. It also suppresses uterine contractility by attaching to its receptors.^(9,10) Although, some studies rejected the relationship between progesterone and pregnancy ratio,^(11,12) others reported an inverse relationship.^(13,14)

In this study, we evaluated the rate of implantation and abortion in patients whose embryos were frozen due to complications such as inappropriate thickness of the endometrium, uterine bleeding etc. Frozen embryos were subsequently transferred to prepared endometrium and the effect of progesterone in foetal implantation in the uterine

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cavity was investigated. According to the serum progesterone level in the examined cycles and to prevent unsuccessful occurrence of pregnancy, we transferred immediately to postpone the transfer of frozen embryo.

MATERIALS AND METHODS

Study Design

Patients referring to IVF center of Urmia Kosar Hospital between May 2013 and December 2015, being candidates for IVF were evaluated in this prospective observational study. We got an approval from the Ethical Committee of Urmia University of Medical Sciences and informed consent from all participants. Totally, 161 patients received ICSI-ET treatment. The study was designed to determine the pregnancy outcomes and abortion according to progesterone serum level on the day of transfer.

Study Population

Inclusion criteria involved patients who were candidates for IVF, but after oocyte retrieval their embryos were frozen due to complications such as ovarian hyperstimulation syndrome, uterine bleeding, inappropriate thickness of endometrium, polyp, any space occupying mass in uterus and cervical stenosis. Totally, 200 patients met inclusion criteria. Exclusion criteria included smokers, patients with systemic diseases and those who had received hormone therapy. So 39 people were excluded from exclusion list. A total of 161 women with ICSI-ET (Intracytoplasmic Sperm Injection followed by Embryo Transfer) treatment were evaluated.

Treatment Protocol

Patients were applied for endometrial preparation from the first or second day of their period cycle after oocyte retrieval via ultrasound-guided transvaginal aspiration. They were treated with estradiol at a dose of 2 mg per 8 hours. On the 13th or 14th day of the cycle, endometrium thickness was examined. In the cases where endometrium thickness was more than 8 mm, 50 mg of intramuscular (IM) progesterone were injected each 12 hours and estradiol treatment was continued. On the 4th day of progesterone administration, blood was drawn for each patient prior to the embryo transfer. Embryo transfer was performed, but progesterone and estradiol injections were continued. After 14 - 15 days, BHCG level was checked. In the cases where the results were positive, they were referred to ultrasound center. When gestational sac in uterus along with foetal cardiac activity were observed, the case was considered as positive pregnancy. For surveillance of miscarriage, all patients were followed up for about 14 weeks of gestation. Embryos were graded according to their morphologies and cleavage rate. 122 (75.8%) embryos were transferred on 17th day and 39 (24.2%) of them on 18th day.

Sampling

Blood samples were obtained for progesterone measurement from the day of embryo transfer. 2.5 mL of venous blood samples were collected from the patients and sent to clinical laboratory of the hospital to measure the serum progesterone level. All isolated serum samples were kept frozen at -18°C. The serum samples were melt at room temperature and progesterone levels on the day of transferring frozen embryo

were measured by luminescence assay using traditional kits (LIAISON, DiaSorin, USA).

Statistical Analysis

Data analysis was performed by student's t-test using Statistical Packages of Social Sciences (SPSS) version 20 (IBM, Chicago, IL). Results were reported as mean $\pm$ SD, and percentages for outcomes of the patients were calculated. We considered a P value of < 0.05 as statistically significant.

RESULTS

Basic characteristics and Assisted Reproductive Technique (ART) parameters are shown in Table 1. The overall ongoing pregnancy and miscarriage ratio are shown in Table 2. An average progesterone level in 25 women with positive BHCG was 26.26 ± 8.58 and in 136 non-pregnant women was 25.61 ± 16.01 (P value= 0.84). There was no significant relationship between serum progesterone levels with pregnancy rates. In this study, we compared the average level of progesterone according to embryo transfer day (Table 3).

There was no significant difference in serum progesterone level of women with positive BHCG in comparison with non-pregnant women according to embryo transfer day. As seen in Table 4, A-B grade in 26 cases (16.1%) and grade A in 23 cases (14.3%) were the most prevalent grading of transferred embryos. There was no significant difference observed in serum progesterone level and pregnancy occurrence ($p > 0.05$, Table 5).

Variable	Mean $\pm$ SD
Age (years)	30.82 $\pm$ 5.41
Number of embryos transferred	2.63 $\pm$ 0.93
Progesterone on transfer day (ng/mL)	15.08 $\pm$ 25.71
Basal FSH (mIU/mL)	2.13 $\pm$ 6.57
FSH: Follicle-Stimulating Hormone	

Table 1. Demographic Characteristics of the Patients

Variable	Positive	Negative
Clinical pregnancy rate	25 (15.5%)	136 (84.5%)
Early abortion	5 (3.1%)	156 (96.9%)

Table 2. Pregnancy and Miscarriage Rate

Variable	BHCG		P value
	Positive	Negative	
Serum P level of mothers with transferred embryo on the 17 th day	26.63 $\pm$ 8.22	29.03 $\pm$ 14.45	0.46
Serum P level of mothers with transferred embryo on the 18 th day	24.30 $\pm$ 11.48	15.42 $\pm$ 2.77	0.31
P= Progesterone (ng/mL)			

Table 3. Comparison of Serum Progesterone Level due to Embryo Transfer Time

Grading	Frequency	Percent
a-b	26	16.1
a	23	14.3
b	20	12.4
a2-b	15	9.3
b-c	13	8.1
a-b-c	10	6.2
a-b-c2	1	0.6
a-b2	1	0.6
a-c	4	2.5
a1-b2	6	3.7
a1-b2-c1	4	2.5
a2	2	1.2
a2-b2	6	3.7
a2-b2-c	1	0.6
a3	2	1.2
a4-b	1	0.6
B-c2	7	4.3
B1-c4	1	0.6
B2-c	8	5
B2-c2	8	5
B2-c3	1	0.6
C2-b3	1	0.6
Total	161	100

Table 4. Distribution of Pregnancy and Percentage of Embryo's Grading

Variable	BHCG		P value
	Negative	Positive	
Serum progesterone level at the day of transfer	25.61 ± 16.01	26.26 ± 8.58	0.84

Table 5. Comparison of Serum Progesterone Level due to occurrence of Pregnancy

DISCUSSION

The number of women accomplishing frozen embryo transfer has been increased in this decade, that has many reasons including elective and medically indicated oocyte and embryo conservation. There are three different routes of exogenous progesterone treatment: vaginal, intramuscular and oral.^(13,14) Comparing to human chorionic gonadotropin (BHCG), the use of progesterone level has better FET cycles performance support and is superior to the use of BHCG.⁽¹⁵⁾ Progesterone results in a lower chance of ovarian hyperstimulation syndrome.⁽¹³⁾ So it is necessary to understand the impact of progesterone support and optimal progesterone values during FET. Progesterone prepares uterine endometrium for implantation and delivers nutrient substances to the foetus by stimulation of endometrial glands. It also suppresses uterine contractions.^(9,10) The impact of progesterone rise on IVF outcomes has been the subject of many studies.^(11,16,17) Some studies rejected the relationship between progesterone and pregnancy rates,^(11,12) while others reported an inverse association.^(13,14) The early studies reported no relationship between pre-ovulatory progesterone rise and pregnancy rates.^(11,16,17) In most related studies, high serum progesterone level prevents the increase in pregnancy rate and likelihood of live birth. Although, progesterone levels on the day of transfer have not yet been investigated extensively in humans, planning the exact time of frozen embryo transfer based on progesterone values alone concludes with higher pregnancy rates.⁽¹⁸⁾

The aim of this cross-sectional study was to evaluate the rate of implantation and abortion in patients whose embryo has been frozen due to reasons such as inappropriate endometrial thickness, uterine bleeding etc. and their embryos have been subsequently transferred followed by preparing the endometrium. In this study, we evaluated the effect of progesterone on embryo implantation in the uterus. The results of this study was contradicted with the study of Nawroth F (2015), which deduced that pregnancy rates have decreased with the increase of serum progesterone level in pregnant.⁽¹⁹⁾ Although, there was no significant difference in our study, serum progesterone levels were in pregnant women.

In a study done by Kofinas JD et al in 2015, the increase in serum progesterone level resulted in a reduction in the incidence of pregnancy to live birth. They concluded that serum progesterone levels above 20 ng/mL on the day of transfer decreases the rate of pregnancy of live births.⁽²⁰⁾ These results are completely different with our findings. Because in our study we have just investigated the occurrence of pregnancy and live births were not among our goals of study and so have not been studied. However, they need to be studied in future studies.

Considering the fact that in most of the studies, the embryo transfer day was effective in pregnancy, we evaluated the occurrence of pregnancy according to embryo transfer day with serum progesterone levels. 75.8% of embryos were transmitted on 17th day and 24.2% on 18th day and the results showed that mean serum progesterone level on the day of transfer in 25 women with positive BHCG was 26.26±8.22 on 17th day and 24.3 ±11.48 on 18th day of period cycle and in 136 non-pregnant women it was 29.03 ± 14.45 on 17th day and 15.42 ± 2.77 on 18th day of the period cycle. There was no statistically significant difference in progesterone levels in women regarding pregnancy incidence. These findings also showed that there is a controversial effect of serum progesterone on pregnancy occurrence.

CONCLUSION

Our findings showed that there was no association between serum progesterone level on the day of transfer and pregnancy occurrence.

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