ASSOCIATION BETWEEN SERUM PROGESTERONE LEVELS AND ENDOMETRIAL STATUS ON HCG INJECTION DAY IN IVF CYCLE

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ABSTRACT

BACKGROUND: Endometrial thickness is a determining factor in successful implantation and pregnancy. So, this study is aimed at assessing the association between the serum levels of progesterone and endometrial status on the day of hCG injection in women treated with in-vitro fertilization.

METHODS: Totally, 71 women who underwent in-vitro fertilization cycle were prospectively recruited in this study. On the day of hCG injection, their serum progesterone levels were evaluated with the ELISA kit. The endometrial thickness was examined using transvaginal ultrasound. Patients were divided into two groups, including patients with progesterone level ≤ 1 ng/ml and those with progesterone level > 1 ng/ml. Eventually, patients' demographic and clinical features were statistically compared between two groups.

RESULTS: Among all the evaluated factors, the rate of gravidity in patients with progesterone level ≤ 1 ng/ml was significantly lower than those with progesterone level > 1 ng/ml (P=0.02). But the rate of abortion in patients with progesterone level ≤ 1 ng/ml was significantly higher than another group (P=0.02). Moreover, the mean endometrial thickness was not significantly different between the two groups (P= 0.83).

CONCLUSION: The progesterone levels on the day of hCG injection had no significant effect on the endometrial thickness. Nevertheless, a strong negative correlation between the progesterone levels and the abortion rate implies that the progesterone may probably affect the IVF clinical outcomes in a way other than changing the endometrial thickness.

KEYWORDS: Progesterone, Endometrial thickness, Human chorionic gonadotropin, In-vitro fertilization

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INTRODUCTION

The human endometrium is a unique, elastic, dynamic, steroid-sensitive tissue that undergoes periodic changes during the menstrual cycle, including proliferation, differentiation, destruction, and repair[1]. These changes are regulated by estrogen and progesterone hormones to allow implantation during the relatively short "implantation window" period of the menstrual cycle. In fact, the physiological functions of the endometrium is to allow blastocyst replacement and support the pregnancy after the implantation[1,2]. Therefore, endometrial receptivity is essential for successful implantation, both in normal pregnancy cycles and in vitro fertilization (IVF) cycles[3].

Discovering the relationship between biological and hormonal markers with the clinical outcomes in various diseases increases our understanding of pathophysiology and the exact mechanism behind those clinical outcomes[4]. Several factors can affect endometrial receptivity, and endometrial thickness is one of the parameters that can be used as an indirect indicator of its acceptability[5,6]. During the normal ovulation cycle, a female sex hormone called progesterone is released from the corpus luteum, which its interruption causes menstruation through expelling the extra layer of the uterus. In addition, this hormone is released during pregnancy. Progesterone is used in medical strategies such as contraception, abortion, amenorrhea treatment, hormone therapy in postmenopausal women[7]. Also, immediately after oocyte implantation, human chorionic gonadotropin (hCG) hormone is produced by the placenta, which is essential for maintaining pregnancy. During the first weeks of pregnancy, hCG causes more natural progesterone to be produced, which ultimately leads to menopause[8].

Injections of hCG for pregnancy play a major role in the treatment of infertility in both men and women. This polypeptide hormone plays a vital role in the normal growth of oocytes in a woman's ovaries[9]. Moreover, only 30 to 35% of assisted reproductive techniques (ART) cycles are successful to treat infertility while infertile couples expect a high probability of success with high financial and time costs. Therefore, in ART cycles such as IVF, intracytoplasmic sperm injection (ICSI), ovarian stimulating hormones are used[10]. In this regard, progesterone plays an important role in preparing the endometrium for embryo implantation and successful pregnancy in ART-related cycles. Therefore, this study was performed to determine the relationship between the serum levels of progesterone and endometrial status on the day of hCG injection in the IVF cycle.

MATERIAL AND METHODS

Study Design and Population

This prospective study was performed on infertile women treated with IVF in the infertility ward of Imam Khomeini Hospital. This research was approved by Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran with Ethical Code: IR.AJUMS.HGOLESTAN.REC.1399.132, and all participants signed the informed consent prior to enrollment. All proceedings and documentation were overseen by local institutional ethics committee of hospital center.

All volunteer patients treated with IVF were recruited in the study. But those with adenomyosis, myoma, and patients undergoing GnRH agonist triggering or hCG triggering were excluded from the study.

Procedure

All couples referred to in the infertility ward of Imam Khomeini Hospital between 2020-2021 were invited to this study, and received the consent form after informing about the study procedure. Women, who voluntary agreed to participate and signed their consent form, received a baseline questionnaire form. A clinic nurse along with an investigator visited and followed up the patients during the treatment period until the end of the study, and collected their data. A physician added the medical details related to ART procedure and results of laboratory tests.

Based on the serum levels of progesterone on the day of hCG injection, patients were categorized into two groups, including patients with the progesterone level ≤ 1 ng/ml and those with the progesterone level > 1 ng/ml. All demographic and clinical features [e.g., gravity, parity, the number of cesarean sections, the number of natural deliveries, the history and number of abortions, cause and type of infertility, duration of infertility, the number of IVF failures] were statistically assessed and compared between the two groups.

Measurements

To separate serum, 2cc of intravenous blood was taken from all patients on the day of HCG injection and all serum samples were kept at minus 20°C until the end of sampling time. At the end of the sampling time, the progesterone level of the samples was measured with a sensitivity of 0.1ng/ml with the ELISA kit in the Dr. Mostofi's laboratory. Also, the condition of the endometrium, including the appearance and the thickness of the endometrium were evaluated by transvaginal ultrasound.

Statistical Analysis

To describe the data, the mean and standard deviation for quantitative variables and frequency and percentage for qualitative variables were used. Data were analyzed using a t-test (Mann-Whitney test if necessary), and Chi-square test when appropriate. $P < 0.05$ is considered as statistically significant. All analyzes were done using statistical software SPSS version 26.0 (IBM Corporation, Armonk, NY).

RESULTS

In this study, 71 patients in age ranging from 25 to 44 years (35.05 ± 5.10) with a history of infertility and IVF were evaluated. As shown in Table 1, all patients were evaluated for demographic and clinical information. Most patients have been treated with IVF at least once but this treatment process has failed for them. Overall, the mean serum progesterone level and endometrial thickness on the day of hCG injection were 1.44 ± 1.97 ng/ml and 8.99 ± 1.89 mm, respectively (Table 1). Also, the most prevalent causes of infertility in patients were male factors (53.5%), female factors (32.4%), male and female factors (9.9 %), and unexplained causes (4.2%), respectively.

Table 1. Description of demographic information, clinical manifestations in the studied patients.

Variables	Mean ±SD	Frequency (%)
Age	35.05 ± 5.10	-
weight	71.62 ± 9.69	-
height	164.72 ± 7.40	-
BMI	27.03 ± 3.96	-
Gravidity		
Nulligravid	_	29 (40.8)
		42 (59.2)
Parity		
Nulliparous	_	9 (12.7)
		62 (87.3)
Number of natural deliveries	_	1 (1.4)
Number of cesareans	_	8 (11.3)
Abortion	_	24 (33.8)
Infertility period (years)	5.63 ± 3.90	-
Number of IVF failures	_	62 (87.3)
Serum progesterone levels	1.44 ± 1.97	-
Endometrial thickness	8.99 ± 1.89	-
Type of abortion		
Spontaneous	-	18 (25.4%)
Curettage	-	5 (7%)
Right salpingectomy	-	1 (1.4%)
Type of infertility		
Primitive	-	39 (54.9%)
Secondary	-	30 (42.3%)
Primitive/secondary	-	2 (2.8%)
Cause of infertility		
Male & Female factors	-	7 (9.9 %)
Unexplained factor	-	3 (4.2%)
Male factor	-	38 (53.5%)
Female factor		23 (32.4%)

Comparison of demographic and clinical features between two groups

As illustrated in Table 2, there was no significant difference between the two groups in terms of age, body mass index (BMI), parity, live child birth,

number of natural deliveries, number of cesarean sections, type of abortion, infertility period (years), and number of IVF failures (P >0.05). Among all the evaluated factors, the rate of gravidity in patients with the progesterone level ≤ 1 ng/ml was significantly lower than those with progesterone level > 1 ng/ml (P=0.02). But the rate of abortion in patients with the progesterone level ≤ 1 ng/ml was significantly higher than those with progesterone level > 1 ng/ml (P=0.02). Also, the rate of parity in cases with the progesterone level > 1 ng/ml was higher than those with progesterone level ≤ 1 ng/ml, but not significantly so (P= 0.13).

The number of pregnancies is defined as gravidity. Parity refers to the number of times a woman with a gestational age of 24 weeks or more gives birth to a fetus, regardless of whether the baby is alive or dead.

Comparison of endometrial thickness between two groups

As shown in Table 2, the mean (SD) endometrial thickness was not significantly different between the two groups (P= 0.83).

Table 2. Comparison of studied factors between the two groups

Variables	Progesterone levels ≤ 1 N=48	Progesterone levels > 1 N=23	p. value
Age	34.65 ± 5.34	35.67 ± 4.74	0.41
weight	73.71 ± 10.55	68.47 ± 7.34	0.09
height	164.90 ± 8.01	164.46 ± 6.54	0.81
BMI	27.93 ± 4.37	25.65 ± 2.76	0.09
Endometrial thickness (mm)	9.03 ± 1.2	8.93 ± 2.6	0.83
Nulligravid	33 (68.7)	9 (39)	0.02*
Gravidity	15 (31.3)	14 (61)	
Parity	4 (8.4)	5 (21.7)	0.13
Nulliparous	44 (91.6)	18 (78.3)	
Live child birth	3 (6.2)	5 (21.7)	0.1
Stillborn	1 (2.1)	0	1
Number of natural deliveries	0	1 (4.3)	0.32
Number of cesarean sections	3 (6.2)	5 (21.7)	0.1
Abortion	21 (43.7)	3 (13)	0.02*
Length of infertility (years)	5.10 ± 3.72	6.44 ± 4.09	0.15
Number of IVF failures	45 (93.8)	18 (78.3)	0.1
Type of abortion			
Spontaneous	14 (29.1%)	3 (13%)	0.5
Curettage	5 (10.4%)	0 (0%)	
Right salpingectomy	2 (4.1%)	0 (0%)	
Type of infertility			
Primitive	25 (52.1%)	17 (73.9%)	0.2
Secondary	21 (43.7%)	5 (21.7%)	
Primitive/secondary	2 (4.1%)	1 (4.3)	
Cause of infertility			
Male & Female	6 (12.5%)	1 (4.3%)	0.5
Unexplained	2 (4.1%)	1 (4.3%)	
Male Factor	23 (47.9%)	15 (65.2%)	
Female Factor	17 (35.5%)	6 (26.2%)	

DISCUSSION

In the present study, the most common causes of infertility in both groups were respectively male and or female factors. No significant association was found between the serum progesterone levels on the day of hCG injection and demographics (age and BMI) and most clinical features, including the rates of live child birth, stillborn, natural deliveries, cesarean sections, length of infertility, history of IVF failures, and types of abortion and infertility, and causes of infertility. By contrast, Whynott et al. reported a significant negative correlation between the serum progesterone level after a cryopreserved embryo transfer cycle and both BMI and weight in patients undergoing IVF; approximately 30% of patients with body weight ≥ 90.7 had serum progesterone levels <15 ng/mL. However, no significant effect of the progesterone levels on live birth rate was found[11], which was in agreement with the corresponding result in the present study.

The serum progesterone level was significantly associated with gravidity and abortion. But, no significant effect of the progesterone level on the day of hCG injection on the endometrial thickness was detected in women treated with IVF. These findings confirm the results of the previous studies[12,13] that reported a positive correlation between the endometrial thickness and the successful process of implantation and pregnancy, but a negative relationship with abortion.

Previous studies demonstrated that low luteal progesterone levels had a significant adverse effect on ongoing pregnancy and live birth rates in patients undergoing ART procedures[14]. In the present study, although the progesterone levels were significantly associated with gravidity and abortion, the rates of stillborn and live child birth seem not to be significantly affected by the progesterone levels. This contradictory result may be due to the insufficient sample size, which demands further investigations in larger population.

The findings of Davar et al.'s study showed no relation between the endometrial thickness and the serum levels of estradiol and progesterone at 5 to 7 days after ovulation. However, a significant correlation was observed between the endometrial thickness and estradiol serum level only in the age group of <20 years[15]. On the other hand, several studies have reported conflicting results about endometrial thickness changes during IVF cycle[16]. Bu et al. assessed the endometrial thickness change in response to progesterone injection and found that the endometrial thickness increased or remained constant after progesterone injection. Also, Bu et al. reported that an increase in endometrial thickness after progesterone injection was associated with better pregnancy outcomes[16]. Contrary to the results of Bu et al.'s study, Haas et al. reported that endometrial thickness may become thinner after progesterone administration[17]. In this regard, Kim et al. reported that progesterone levels at 14 days after oocyte retrieval could be a good indicator of pregnancy during infertility treatment[18]. Such conflicting results in different studies may be affected by differences in the sample size and/or type or methodology of the study, which requires more precise investigations.

CONCLUSION

Results of the present study proposed that the serum level of progesterone on the day of hCG injection had no significant effect on the endometrial thickness. However, a strong negative correlation was detected between the serum progesterone levels and the abortion rate, implies that the progesterone may probably affect the IVF clinical outcomes in a way other than changing the endometrial thickness. Considering the challenging role of progesterone in changing the endometrial thickness and IVF outcomes, clinical trials on more diverse populations are required to obtain definite results.

ACRONYMS:

IVF: in vitro fertilization
hCG: human chorionic gonadotropin
ART: assisted reproductive techniques
ICSI: Intracytoplasmic sperm injection
BMI: body mass index

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