Medical Science

pISSN 2321–7359; eISSN 2321–7367

Evaluation of the association between progesterone levels on the day of HCG injection and the clinical outcomes of IVF treatment cycles in infertile women

Masoumeh Rezaei[1], **Fariba Seyedoshohadaei**[1], **Nasrin Soufizadeh**[2], **Mohamad Jafar Rezaei**[3], **Tayyebe Ghazavi**[4*]

To Cite:
Rezaei M, Seyedoshohadaei F, Soufizadeh N, Rezaei MJ, Ghazavi T. Evaluation of the association between progesterone levels on the day of HCG injection and the clinical outcomes of IVF treatment cycles in infertile women. Medical Science, 2022, 26, ms168e2120.
doi: https://doi.org/10.54905/disssi/v26i123/ms168e2120

Authors' Affiliation:
[1]Associated professor, Obstetrics and gynecology Department infertility ward, Besat Hospital, Kurdistan University of Medical Sciences, Sanandaj, Iran
[2]Associated professor, Department of Obstetrics and Gynecology, Besat Hospital, Kurdistan University of Medical Sciences, Sanandaj, Iran
[3]Associated Professor of Anatomy, Kurdistan University of Medical Science, Department of Anatomy, Infertility Center of Besat Hospital. Sanandaj, Iran
[4]Resident of Obstetrics and Gynecology, Besat hospital, Medical Faculty, Kurdistan University of Medical Sciences, Sanandaj, Iran

[*]**Corresponding author**
Resident of Obstetrics and Gynecology, Besat hospital, Medical Faculty, Kurdistan University of Medical Sciences, Sanandaj, Iran
Email: t.ghazavi2015@gmail.com

Peer-Review History
Received: 16 February 2022
Reviewed & Revised: 18/February/2022 to 29/April/2022
Accepted: 30 April 2022
Published: 09 May 2022

Peer-review Method
External peer-review was done through double-blind method.

URL: https://www.discoveryjournals.org/medicalscience

ABSTRACT

Introduction: Progesterone has a major role in menstrual cycles as well as implantation of fertilized oocytes and induction of pregnancy, but its effects on controlled ovarian stimulation cycles are still debated. The aim of this research was to investigate the association between progesterone levels on the day of HCG injection and the clinical outcomes of IVF treatment cycles in infertile women. *Materials and Methods:* The present research is a cross-sectional descriptive-analytical study. The study population included 300 infertile women, and then had IVF by Gnarl agonist and antagonist cycles in the infertility clinic of Besat Hospital in Kurdistan. Patient records and researcher-made checklists were used for collecting the data, and SPSS 24 software was implemented for analyzing the data. *Results:* Of the total patients studied, 224 people (74.7%) had progesterone levels less than 1.5, and 76 (25.3%) had progesterone levels greater than 1.5. The average age of patients was 33 years old and the mean duration of infertility was and 5.73 years. 79.3% of women had primary infertility and 20.7% had secondary infertility. There was no considerable association among progesterone levels and type of infertility, agonist and antagonist cycles, age and oocyte count ($P > 0.05$), implantation rate, clinical pregnancy, and spontaneous abortion. However, the progesterone levels had a considerable association with the grading and quality of oocytes. *Conclusion:* The findings indicated that cases with progesterone level less than 1.5 have higher fertility rates compared to cases with progesterone level greater than 1.5 (P-value = 0.6).

Keywords: Progesterone, HCG injection, Clinical outcome, IVF, Infertile women

1. INTRODUCTION

In a normal menstrual cycle, one of the 10-20 growing follicles will dominate. The objective of controlled ovarian stimulation (COS) in assisted reproductive technique (ART) protocols is to maturate further follicles (Fertilisation et al., 2018). This strategy can increase the oocytes and embryos count that can be transferred in one cycle. But this will cause hormonal changes that would, unfortunately, affect the acceptance rate of the endometrium and therefore the implantation rate. These hormonal changes will reduce the success of fertility (Ecochard et al., 2017). Although progesterone has major effects on menstrual cycles as well as implantation of fertilized oocytes and induction of pregnancy, its role in controlled ovarian stimulation cycles is still debated among gynecologists (Al-Inany et al., 2016; Vikas et al., 2017). An increase in premature progesterone is referred to as an incremented level of progesterone on the day of injection of HCG. Accordingly, the researchers found that a slight increase in progesterone before ovulation was inversely related to gestation rate, which causes an increased pregnancy failure rate. The pathogenesis and effects of premature progesterone elevation are still the subjects of scientific debate among infertility specialists (Kolibianakis et al., 2012; Vaegter et al., 2017).

Patients who respond strongly to ovarian stimulation cycles have a higher rate of premature progesterone rise that may cause impaired receptivity of endometrium (Ecochard et al., 2017). The prevalence of increase in premature progesterone varies in ovarian stimulation cycles. Despite the fact that gonadotropin-releasing hormone agonists and antagonists reduce or prevent premature progesterone elevation, this process still occurs in some patients (van et al., 2010). It was found that premature progesterone elevation occurs in approximately 35% of cases undergoing treatment with GnRH agonists and up to 38% of patients undergoing therapy with GnRH antagonists (Sunkara et al., 2014). In addition, some studies have demonstrated that elevated premature progesterone may have inverse effects in cases that received therapy with GnRH antagonists but had no effect on the clinical outcomes of ovarian cycles in patients treated with GnRH agonists (Vaegter et al., 2017). It was previously demonstrated that an increase in improper progesterone secretion on the day of administration of HCG can have adverse efficacies on fetal quality and cumulative live birth rate (Robati et al., 2020).

The objective of our research was to assess the efficacy of progesterone levels on the day of hCG injection on clinical outcomes (implantation and fertility rate, oocyte quality, and number of oocytes removed) of IVF treatment cycles in infertile women referring to Besat Hospital.

2. MATERIALS AND METHODS

Study design

The study population of this cross-sectional descriptive-analytical study included all female patients with infertility referring to the infertility clinic of Besat Hospital in Kurdistan in November 2020-2021 and underwent IVF. The study was registered in the Iranian Registry of Clinical Trials (IR.MUK.REC 1398.137) after approval of the research and ethics committees at the university.

Sampling method and sample size

According to previous experiences reference books, between 25 and 30% of cases will lead to successful clinical pregnancy; based on this point and considering the 95% confidence level (1.96), the p-value equal to 0.25 and error value equal to 20 percent above the prevalence, i.e. (0.05), the sample size was calculated using the estimation formula that is mentioned in the following. Lastly, the size of the statistical population was estimated at 600 people, but due to the outbreak of the new coronavirus, the closure of the infertility clinic, and the absence of women, and after coordination with the medical school, 300 infertile women were studied in this study.

$$n = \frac{Z_{1-\frac{\alpha}{2}} P(1-P)}{d^2}$$

Inclusion and exclusion criteria

In this study, inclusion criteria included infertile women undergoing therapy with IVF in the GnRH agonist and antagonist cycles and infertile women who used fresh embryos. Exclusion criteria included women who planned for oocyte cryopreservation before HCG injections, those who were unwilling to participate, those who intended to conceive with a donated egg, and those with OHSS.

Data Collection tools

The data collection tool was a checklist that included information about infertile women participating in the study. This information included maternal age, type of drugs used, progesterone levels, number of oocytes removed, oocyte quality, implantation rate, and clinical fertility rate.

Methods

This descriptive-analytical research was performed in 2019. In this study, after coordination with the medical school and the research assistant for obtaining a license, information was collected. This research was done in a two-year period and the target group included infertile women who had the inclusion criteria, and written consent was obtained from all individuals. The study population included 300 infertile women referring to the clinic of infertility. To collect information about the women, a checklist was used, and the collected information included maternal age, type of drugs used, progesterone level, number of oocytes removed, oocyte quality, rate of implantation, and rate of clinical fertility. In these subjects, progesterone levels were assessed on the day of administration of HCG using the Infinitum Biotech laboratory kit and according to the standard protocol in the kit. The cut-off point was contemplated to be 1.5 and then the gestation outcomes were evaluated after embryo transfer.

Data analysis method

After collecting information and completing the questionnaires, the data were entered into SPSS software version 25. Qualitative descriptive variables (frequency and frequency percentage) and quantitative descriptive variables (mean and standard deviation) were calculated. For analyzing the data and evaluating the associations among variables, two-way tables, Chi-square test, Fisher's exact test, and logistic regression were implemented.

Ethical considerations

Before starting the research, the code of ethics was obtained from the ethics committee of Kurdistan University of Medical Sciences. The information was analyzed at each stage without the patient's name, and the outcomes of the research were kept completely confidential by the researcher.

3. RESULTS

Of the total patients studied, 224 people (74.7%) had progesterone levels less than 1.5, and 76 (25.3%) had progesterone levels greater than 1.5. The average age of individuals was 33 years and the average duration of infertility in the cases was and 5.73 years. It was observed that 242 patients (80.7%) had an agonist cycle and 58 patients (19.3%) had an antagonist cycle. Also, the evaluation of the rates of clinical pregnancy in infertile women based on progesterone levels demonstrated that 62 people in the group of cases with progesterone levels less than 1.5 ng/ml and 18 of the patients with progesterone levels higher than 1.5 ng/ml had clinical pregnancies (p=0.6). The investigation of oocyte quality in infertile women demonstrated that 6% of cases were in metaphase 1, 88.7% in metaphase 2, and 5.3% were in GV (Table 1).

Table 1 Frequency distribution of infertility, clinical pregnancy rate, progesterone level, and oocyte quality in cases

Variable		Frequency	Percentage
Infertility	Primary	238	79.3
	Secondary	62	20.7
Type of drug used	Agonist cycle	242	80.7
	Antagonist cycle	58	19.3
Clinical pregnancy rate	BHCG positive	80	26.7
	BHCG negative	220	73.3
Progesterone level	<1.5	224	74.7
	≥1.5	76	25.3
Oocyte quality	Metaphase 1	18	6
	Metaphase 2	266	88.7
	GV	16	5.3

Based on the obtained results, no considerable relationship was found among progesterone levels and age, duration of infertility, and quantity of oocytes and embryos in infertile women (p >0.05) (table 3).

Table 2 Association between progesterone levels and age, duration of infertility, and quantity of oocytes and embryos in infertile women studied

Variable	Progesterone level	Frequency	Percentage	Standard deviation	p-value*
Duration of infertility	<1.5	224	5.5	3.54	0.24
	≥1.5	76	6.38	4.98	
Age	<1.5	224	33.13	5.72	0.62
	≥1.5	76	32.61	5.86	
Number of oocytes	<1.5	224	5.81	4.35	0.07
	≥1.5	76	7.26	3.89	
Number of embryos	<1.5	224	3.3	2.73	0.11
	≥1.5	76	4.21	3.71	
* T-test was used for analysis.					

Also, the evaluation of the effective factors on progesterone levels in infertile women using the Chi-square test indicated that there were no considerable associations between progesterone levels with infertility, agonist and antagonist cycles, oocytes count, implantation rate, clinical pregnancy, and spontaneous abortion. However, there was a significant association among progesterone levels and oocyte quality and grading (p = 0.03, p = 0.007) (Table 3 and Fig 1).

Table 3 Association between progesterone levels and effective factors in infertile women studied

Variable		Progesterone level		Total	p-value*
		Frequency percentage less than 1.5	Frequency percentage equal to or greater than 1.5		
Infertility	Primary	74.8	25.2	238	0.94
	Secondary	74.2	25.8	62	
Type of drug used	Agonist	73.6	26.4	242	0.52
	Antagonist	79.3	20.7	58	
Implantation	Singleton	77.5	22.5	72	0.57
	Twins	100	0	6	
Clinical pregnancy	BHCG positive	77.5	22.5	80	0.63
	BHCG negative	73.6	26.4	220	
Oocyte quality	Metaphase 1	100	0	18	0.03
	Metaphase 2	71.4	28.6	266	
	GV	100	0	16	
Spontaneous abortion	Yes	100	0	4	0.4
	No	76.3	23.7	76	
Grading	A	81.3	18.8	96	0.007
	B	48	52	50	
	A ,B	77.3	22.7	88	
Total		74.7	25.3	300	-
* Chi-square test was used					

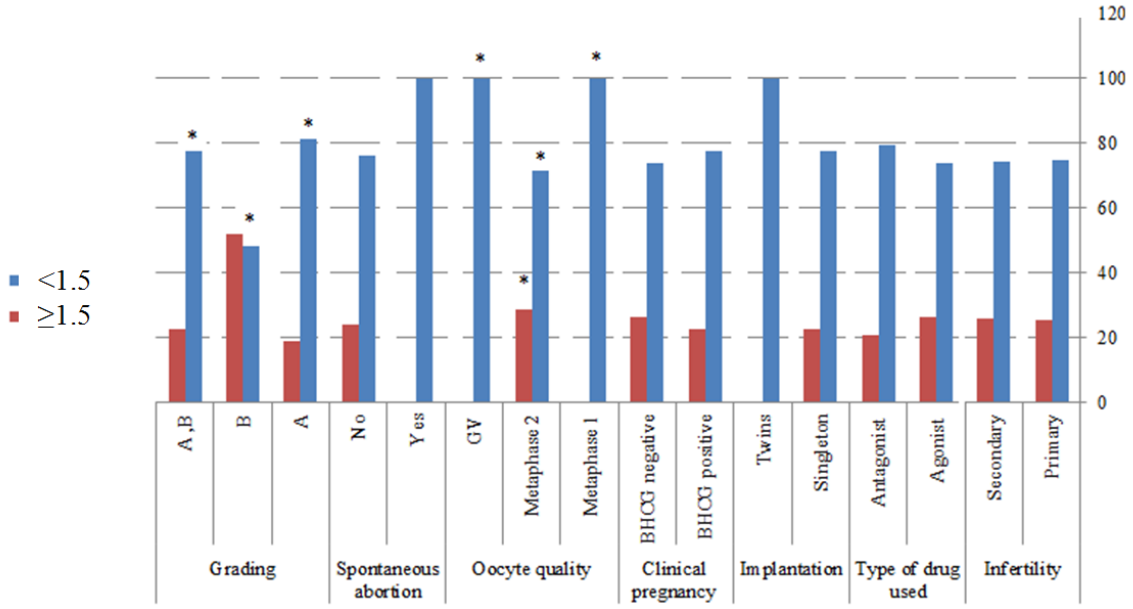

Figure 1 Association between progesterone levels and effective factors

4. DISCUSSION

An average increment in serum progesterone levels is observed in the majority of ovulatory cycles on the day of injection of human chorionic gonadotropin (HCG). Studies show that an increase in luteinizing hormone (LH) in the late follicular phase increases progesterone, and attempts are made to use gonadotropin-releasing hormone agonists and antagonists to prevent LH increase and premature luteinization (Lahoud et al., 2012). In general, progesterone has a major role in menstrual cycles as well as implantation of fertilized oocytes and induction of pregnancy; however, its effects on ovarian stimulation cycles are still debated among gynecologists (Huang et al., 2015). According to this research, no considerable association was found between progesterone levels and clinical pregnancy in infertile women. However, progesterone levels greater than 1.5ng/ml had a lower correlation with pregnancy rates than progesterone levels lower than 1.5ng/ml.

In a study by Huang et al., (2016) the efficacy of increased progesterone levels on high-quality fetal IVF cycles was examined. In this research, the progesterone level of 1.5ng/ml on the day of injection of HCG was considered as the best threshold for determining the destructive level of progesterone for the outcomes of the IVF / ICSI-ET cycle. In our study, 1.5 ng / ml was also considered as the cut-off point, which was consistent with the study by Huang et al., (2016). The findings of a study by Bosch et al., (2010) showed that the level of progesterone following hCG injection was inversely related to fertility rates, and accordingly, the fertility rate of pregnant women with progesterone levels lower than 1.5 ng/ml was considerably higher than the other group, which was consistent with our study.

The findings of a research by Venetis et al., (2013) demonstrated a negative relation among increased progesterone and fertility-related variables in progesterone levels above 0.8ng/ml. In our study, the level of 1.5 ng/ml was considered as a threshold and the findings were similar to the outcomes of a study of Venetis et al., (2013) also, a study by Vikas et al., (2017) demonstrated that the rate of clinical gestation in cases with progesterone levels <1.5 ng/ml was considerably higher than patients with progesterone levels, P ≥ 1.5 ng / ml, which was consistent with our study. But the findings of a research by Roque et al., (2014) concluded that progesterone levels had no effect on fertility in pregnant women. Another study by Griesinger et al., (2013) showed that progesterone levels above 1.5 ng/ml had no efficacy on pregnancy, which was inconsistent with the results of our study; these discrepancies may be caused by the differences in the size of the statistical population, the discrepancy between the analyzes performed, and the threshold value of progesterone levels.

In a study by Vikas et al., (2017) there was no considerable difference between the average age and duration of infertility in the evaluated patients. However, a higher number of oocytes were seen in the patients with increased levels of progesterone and a significant relationship was shown between the oocyte count and progesterone levels. However, no considerable difference was seen in the other studied parameters. In another study by Xu et al., (2012) no considerable relationship was found among age,

duration of infertility, and progesterone levels, but a significant association was reported between oocyte count and progesterone levels.

In another study by Griesinger et al., (2013), a significant association was reported between oocyte count and progesterone levels. However, in our study, no significant relationship was found among progesterone levels with age, duration of infertility, and the quantity of oocytes and embryos in infertile women. According to our research, progesterone level had no considerable relation with the type of drug used (agonist or antagonist) in infertile women, which was similar to a study by Vikas et al., (2017).

5. CONCLUSION

According to research, serum progesterone level of 1.5 ng/ml on the day of injection of hCG can be contemplated as the best threshold for determining progesterone destructive levels for the outcomes of the IVF / ICSI-ET cycle. The findings of our research demonstrated that in cases with progesterone levels lower than 1.5ng/ml the fertility rate was higher compared to women with progesterone levels higher than 1.5ng/ml.

Research Limitations

Due to the outbreak of the new coronavirus and also patients' dissatisfaction with participating in this project, the calculated sample size was not studied, which could affect the outcomes of this research.

Acknowledgements

The authors thank all study participants for their cooperation.

Funding

This study has not received any external funding.

Conflicts of interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Al-Inany HG, Youssef MA, Ayeleke RO, Brown J, Lam WS, Broekmans FJ. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. Cochrane Database Syst Rev 2016; 4(4):Cd001750. doi:10.1002/14651858.CD001750.pub4.
2. Bosch E, Labarta E, Crespo J, Simón C, Remohí J, Jenkins J, Pellicer A. Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for in vitro fertilization: analysis of over 4000 cycles. Hum Reprod 2010; 25(8):2092-2100. doi:10.1093/humrep/deq125.
3. Ecochard R, Bouchard T, Leiva R, Abdulla S, Dupuis O, Duterque O, Garmier Billard M, Boehringer H, Genolini C. Characterization of hormonal profiles during the luteal phase in regularly menstruating women. Fertil Steril 2017; 108(1):175-182.e171. doi:10.1016/j.fertnstert.2017.05.012.
4. Fertilisation H, Authority E. Fertility treatment 2014–2016: Trends and figures. London: HFEA. 2018.
5. Griesinger G, Mannaerts B, Andersen CY, Witjes H, Kolibianakis EM, Gordon K. Progesterone elevation does not compromise pregnancy rates in high responders: a pooled analysis of in vitro fertilization patients treated with recombinant follicle-stimulating hormone/gonadotropin-releasing hormone antagonist in six trials. Fertil Steril 2013; 100(6):1622-1628.e1621-1623. doi:10.1016/j.fertnstert.2013.08.045.
6. Huang B, Ren X, Wu L, Zhu L, Xu B, Li Y, Ai J, Jin L. Elevated Progesterone Levels on the Day of Oocyte Maturation May Affect Top Quality Embryo IVF Cycles. PLoS One 2016; 11(1):e0145895. doi:10.1371/journal.pone.0145895.
7. Huang Y, Wang EY, Du QY, Xiong YJ, Guo XY, Yu YP, Sun YP. Progesterone elevation on the day of human chorionic gonadotropin administration adversely affects the outcome of IVF with transferred embryos at different developmental stages. Reprod Biol Endocrinol 2015; 13:82. doi:10.1186/s12958-015-0075-3.
8. Kolibianakis EM, Venetis CA, Bontis J, Tarlatzis BC. Significantly lower pregnancy rates in the presence of progesterone elevation in patients treated with GnRH antagonists and gonadotrophins: a systematic review and

meta-analysis. Curr Pharm Biotechnol 2012; 13(3):464-470. doi:10.2174/138920112799361927.

9. Lahoud R, Kwik M, Ryan J, Al-Jefout M, Foley J, Illingworth P. Elevated progesterone in GnRH agonist down regulated in vitro fertilisation (IVFICSI) cycles reduces live birth rates but not embryo quality. Arch Gynecol Obstet 2012; 285(2):535-540. doi:10.1007/s00404-011-2045-0.
10. Robati S, Saab W, Durán-Retamal M, Saab W, Theodorou E, Cawood S, Serhal P, Seshadri S. The Association Between Elevated Progesterone Level on Day of hCG Trigger and Live Birth Rates in ART Cycles: A Single Centre Observational Study. J Reprod Infertil 2020; 21(4):283-290. doi:10.18502/jri.v21i4.4333.
11. Roque M, Valle M, Sampaio M, Geber S, Checa MA. Ratio of progesterone-to-number of follicles as a prognostic tool for in vitro fertilization cycles. J Assist Reprod Genet 2015; 32(6):951-957. doi:10.1007/s10815-015-0487-1.
12. Sunkara SK, Coomarasamy A, Faris R, Braude P, Khalaf Y. Long gonadotropin-releasing hormone agonist versus short agonist versus antagonist regimens in poor responders undergoing in vitro fertilization: a randomized controlled trial. Fertil Steril 2014; 101(1):147-153. doi:10.1016/j.fertnstert.2013.09.035.
13. Vaegter KK, Lakic TG, Olovsson M, Berglund L, Brodin T, Holte J. Which factors are most predictive for live birth after in vitro fertilization and intracytoplasmic sperm injection (IVF/ICSI) treatments? Analysis of 100 prospectively recorded variables in 8,400 IVF/ICSI single-embryo transfers. Fertil Steril 2017; 107(3):641-648.e642. doi:10.1016/j.fertnstert.2016.12.005.
14. van Loendersloot LL, van Wely M, Limpens J, Bossuyt PM, Repping S, van der Veen F. Predictive factors in in vitro fertilization (IVF): a systematic review and meta-analysis. Hum Reprod Update 2010; 16(6):577-589. doi:10.1093/humupd/dmq015.
15. Venetis CA, Kolibianakis EM, Bosdou JK, Tarlatzis BC. Progesterone elevation and probability of pregnancy after IVF: a systematic review and meta-analysis of over 60 000 cycles. Hum Reprod Update 2013; 19(5):433-457. doi:10.1093/humupd/dmt014.
16. Vikas S, Swati G. The impact of progesterone level on day of hCG injection in IVF cycles on clinical pregnancy rate. J human reprod sci 2017; 10(4):265.
17. Xu B, Li Z, Zhang H, Jin L, Li Y, Ai J, Zhu G. Serum progesterone level effects on the outcome of in vitro fertilization in patients with different ovarian response: an analysis of more than 10,000 cycles. Fertil Steril 2012; 97(6):1321-1327.e1321-1324. doi:10.1016/j.fertnstert.2012.03.014.