


Investigation of the effect of protocol changes in IVF treatment on pregnancy outcomes: A retrospective analysis

 ¹Ramazan MURDAN

 ²Enis ÖZKAYA

 ³Yağmur MİNARECİ

¹Department of Gynecological Oncology, Hatay Training and Research Hospital, Hatay, Turkey

²Department of Gynecology and Obstetrics, Kırklareli University, Kırklareli, Turkey

³Department of Gynecology and Obstetric Oncology, Istanbul University Faculty of Medicine, Istanbul, Turkey

ORCID ID

RM : 0000-0002-9049-6913

EÖ : 0000-0001-6580-1237

YM : 0000-0003-1420-9318



ABSTRACT

Objective: The effect of changing the ovarian stimulation protocol on treatment outcomes after an unsuccessful IVF treatment is still a controversial issue. In this study, we investigated the effect of protocol changes in the second attempt on treatment outcomes in patients who failed in the first attempt.

Material and Methods: Our study included 60 patients who applied to Zeynep Kamil Training and Research Hospital due to infertility between 01.01.2013 and 01.01.2017, who had previously failed IVF treatment and were trying for the second time. The patients were divided into two groups: those with and without protocol changes in the second trial. The success of these two groups in pregnancy outcomes was compared. Of the 60 patients, 30 were selected from those who underwent protocol changes, while 30 were selected from those who did not undergo protocol changes.

Results: A total of 60 patients between the ages of 22 and 41 were included in our study. Fifty of the 60 patients were primarily infertile, while 10 were secondarily infertile. There was no significant difference in β -hCG positivity in the first and second IVF attempts between the groups with and without protocol changes ($p>0.05$). There was no clinical pregnancy in either group in the first IVF attempt. In our study, no statistically significant difference was found in clinical pregnancy outcomes in the second attempt between the two groups ($p>0.05$).

Conclusion: In couples whose first IVF attempt was unsuccessful, making a protocol change did not create a statistically significant difference in terms of β -hCG positivity and clinical pregnancy success in the second attempt compared to not making a protocol change.

Keywords: Failed IVF attempt, GnRH agonist, GnRH antagonist, IVF pregnancy, pregnancy outcomes, protocol change.

Cite this article as: Murdan R, Özkaya E, Minareci Y. Investigation of the effect of protocol changes in IVF treatment on pregnancy outcomes: A retrospective analysis. Zeynep Kamil Med J 2025;56(3):127–132.

Received: November 10, 2023

Revised: June 25, 2025

Accepted: June 28, 2025

Online: August 15, 2025

Correspondence: Ramazan MURDAN, MD. Hatay Eğitim ve Araştırma Hastanesi, Jinekolojik Onkoloji Kliniği, Hatay, Türkiye.

Tel: +90 555 612 76 48 **e-mail:** ramazanmurdan@hotmail.com

Zeynep Kamil Medical Journal published by Kare Publishing. Zeynep Kamil Tıp Dergisi, Kare Yayıncılık tarafından basılmıştır.

OPEN ACCESS This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).



INTRODUCTION

Infertility can be defined as the inability to achieve pregnancy despite unprotected intercourse for one year. Approximately 85–90% of healthy couples achieve pregnancy after one year of unprotected intercourse. Delaying marriage to a later age, postponing the desire to have children, increasing use of contraceptive methods, changing roles of women in society, and environmental and socioeconomic factors are effective in decreasing fertility.^[1] Anamnesis is very important in the evaluation of infertile couples. Age, duration of infertility, previous pregnancy, menstrual cycle, last menstrual period, systemic diseases, habits, previous surgeries, and whether infertility treatment has been received before should be questioned.^[2,3] Male patients also need to be evaluated by urologists in infertility clinics.

The first step in infertility treatment is to determine the cause of infertility. Ovulatory dysfunction, tubal and peritoneal pathologies, male factor, uterine pathologies, and unexplained factors should be taken into consideration. In normal couples, the fertility rate per cycle is 20–25%. The aim of treatment in infertile couples is to increase the fertility rate per cycle. When untreated, the average cycle fertility rate in infertile couples is around 1.3–4.1%. This rate is 3.8% with IUI, 5.6% with clomiphene citrate, 8.3% with clomiphene citrate+IUI, 7.7% with gonadotropin use, 8–15% with gonadotropin+IUI, and 20.7% with IVF.^[3]

Controlled ovarian hyperstimulation (COH) is the most important step in in vitro fertilization (IVF) treatment. Pituitary suppression with gonadotropin-releasing hormone (GnRH) agonists or GnRH antagonists is important in preventing premature luteinization. Oocyte number and quality play a major role in embryo development. The optimum number of oocytes retrieved will result in more embryos available for transfer and higher pregnancy rates in fresh IVF cycles.^[4,5] For optimum oocyte number and quality, an appropriate stimulation protocol should be selected. The stimulation protocol and gonadotropin dose are determined by the patient's characteristics, including age, body mass index (BMI), baseline serum follicle-stimulating hormone (FSH) level, serum estradiol (E2) level, and antral follicle count (AFC).

In the GnRH antagonist protocol, there is increased patient compliance due to the shorter duration of ovarian stimulation and the lower total dose of gonadotropins used for stimulation, and there is a chance of preventing ovarian hyperstimulation syndrome (OHSS) by inducing final oocyte maturation with GnRH agonists.^[6] In the GnRH agonist protocol, suppression of endogenous gonadotropin secretion in the early follicular phase results in synchronization of antral follicles and thus enables a greater number of oocytes to be retrieved.^[7] However, the longer duration of treatment, greater risk of OHSS, and agonist-related side effects are disadvantages associated with the GnRH agonist protocol.

There are several studies comparing the GnRH agonist protocol with the antagonist protocol. In normoresponder patients, clinical pregnancy and ongoing pregnancy rates were found to be higher with the long agonist protocol than with the antagonist protocol. In patients with polycystic ovary syndrome (PCOS) and low ovarian reserve, ongoing pregnancy rates were similar across the two stimulation protocols.^[8] In this study, we investigated the effect of protocol changes in the second attempt on treatment outcomes in patients who failed in the first attempt.

MATERIAL AND METHODS

In our study, patients who had previously failed IVF treatment and were undergoing a second IVF attempt were evaluated. For this purpose, the files of 60 patients who received IVF treatment at Zeynep Kamil Training and Research Hospital between 01.01.2013 and 01.01.2017 were scanned and evaluated retrospectively. The patients were divided into two groups: those who had a protocol change in their second IVF attempt and those who had not. There were 30 patients in both groups. Patients in the group without protocol change received IVF treatment with either the antagonist or agonist protocol, provided that the same treatment protocol was used in both trials. The patients in the protocol change group were those who had their first attempt with the antagonist protocol and their second attempt with the agonist protocol, or those who had their first attempt with the agonist protocol and their second attempt with the antagonist protocol. These two groups were compared in terms of success in pregnancy rates.

The indications for IVF treatment in our study were male factor (oligoasthenospermia, azoospermia), low ovarian reserve, tubal factor, endometriosis, hormonal-ovulation disorders, and unexplained infertility. Patients with normal TSH and prolactin levels were included, while those with systemic or endocrine diseases were excluded. Patients with multiple failed IVF treatments were not included, as this would hinder randomization. The cycle was cancelled in case of failure in fertilization, failure to obtain follicles >18 mm, a decrease of more than 50% in estrogen levels between two control days, or development of OHSS. Our study was approved by the ethics committee of our hospital.

Treatment Protocols

All patients started ovarian stimulation on the third day of their menstrual cycle. When determining the starting dose, the estimated ovarian response for each case was taken into account. Accordingly, subcutaneous injections were administered to the abdominal area by the patient at doses starting from 300 IU. On the 6th and 7th days of stimulation, the number and size of follicles were checked with ultrasound. A new dose adjustment was made according to the ovarian response by looking at serum estradiol levels. Stimulation was continued until the day of hCG application.

hCG application criteria for oocyte maturation were the same in both groups. Ovulation triggering was performed when the leading follicle was 18 mm or when two of the follicles were 17 mm. Oocyte retrieval was performed 35–36 hours after hCG administration. During the oocyte retrieval procedure, all follicles measuring 14 mm and above were aspirated. Fertilization and embryo quality were evaluated with a microscope at the 20th, 28th, 44th, and 68th hours by a specialist embryologist in our clinic. By applying the IVF standard procedure, 1–3 of the graded embryos, preferably of good quality type A, were transferred to the uterine cavity 3 days after oocyte retrieval. Luteal support was provided to all patients. If pregnancy occurred, vaginal progesterone support continued until the third month of pregnancy was completed.

Table 1: Distribution of demographic characteristics

	n	%
Duration (year); Min–Max, Mean±SD	1–16	5.41±3.22
Infertility		
Primary	50	83.3
Secondary	10	16.7
Etiology		
Unexplained	21	35
Low ovarian reserve	14	23.3
Low ovarian reserve + male factor	2	3.3
Low ovarian reserve + tubal factor	1	1.7
Hypogonadotropic hypogonadism	2	3.3
Male factor	5	8.3
PCOS	1	1.7
Tubal factor	13	21.7
Uterus septus	1	1.7
Parity		
1	1	1.7
0	59	98.3
Abortus		
1	7	11.7
2	1	1.7
4	1	1.7

Min: Minimum; Max: Maximum; SD Standard deviation; PCOS: Polycystic ovary syndrome.

Statistical Analysis

While evaluating the findings obtained in the study, IBM SPSS Statistics 22.0 program was used for statistical analysis. To compare the 1st and 2nd trial measurements of quantitative data, the Wilcoxon signed-rank test was used, along with descriptive statistical methods (mean, standard deviation). Continuity Correction (Yates) test was used to compare qualitative data. Significance was evaluated at the $p < 0.05$ level.

The Zeynep Kamil Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 25.11.2016, number: 164). Our study was conducted in accordance with the Declaration of Helsinki.

RESULTS

The study was conducted with a total of 60 cases, aged between 22 and 41 years, who applied to Zeynep Kamil Training and Research Hospital between 01.01.2013 and 01.01.2017. The mean age of the cases was 34.53±4.49 years.

Infertility duration varied between 1 and 16 years, with a mean duration of 5.41±3.22 years. When the distribution of infertility was

examined, it was observed that it was primary in 50 (83.3%) cases and secondary in 10 (16.7%) cases. The etiology distribution is shown in Table 1.

As seen in Table 2, the decrease in the number of antral follicles in the second IVF trial compared to the first IVF trial was statistically significant in both groups ($p < 0.01$). The increase in the second IVF trial compared to the first IVF trial of the gonadotropin initial and total dose was statistically significant in both groups ($p < 0.05$).

According to the results in Table 3, no significant difference was detected in terms of β -hCG positivity in the first and second IVF attempts in the groups with and without protocol changes ($p > 0.05$). There was no clinical pregnancy in any patient during the first IVF attempt. No statistically significant difference was detected between the two groups in terms of clinical pregnancy success in the second IVF attempt, depending on whether a protocol change was made or not ($p > 0.05$).

DISCUSSION

This study was conducted to investigate the effect of protocol changes on pregnancy success in the second IVF treatment attempt in patients whose first attempt was unsuccessful. In our study, making a protocol change in the second attempt did not have a positive effect on either β -hCG positivity or clinical pregnancy success. No significant difference was found in terms of pregnancy outcomes between the groups with and without protocol changes.

In our study, there was no statistically significant difference between the two groups in terms of β -hCG positivity in the first IVF attempt. While 6 patients were β -hCG positive in the group without protocol change, 4 patients were β -hCG positive in the group with protocol change. As a result, clinical pregnancy could not be achieved in any patients in the first IVF attempt. In the second IVF trial, no difference was found in terms of β -hCG positivity and clinical pregnancy numbers in the groups with and without protocol changes. While the number of β -hCG positive patients in both groups was 12, the number of patients who developed clinical pregnancy was 8.

In our study, the decrease in the number of antral follicles in the second IVF trial compared to the first IVF trial was found to be statistically significant both in the group with and without a protocol change. This was attributed to the decrease in ovarian reserve over time. Similarly, due to the decrease in ovarian reserve, the initial gonadotropin dose and total gonadotropin dose were found to be higher in both groups in the second IVF trial compared to the first IVF trial. There was no significant difference in the patients' basal FSH and estradiol values between the two trials in either group. Although estradiol levels on the day of hCG were slightly higher in the second trial than in the first trial, no significant difference was found between the two trials in either group. The number of oocytes collected and the number of embryos transferred were similar in both groups for both trials.

There are many studies comparing GnRH agonist protocols with antagonist protocols. One meta-analysis concluded that in patients with normal ovarian reserve, clinical pregnancy and live birth rates were similar between the two protocols, but the incidence of OHSS

Table 2: Clinical parameters evaluated in the first and Second IVF trial

	1.IVF trial	2.IVF trial	p
	Mean±SD	Mean±SD	
Group with protocol changes			
FSH	7.33±3.40	6.78±4.34	0.359
E2	71.90±127.41	58.41±63.49	0.838
Number of antral follicles	11.32±4.31	8.98±4.09	0.001**
Induction time	8.78±2.19	9.58±1.89	0.356
Hcg day E2 level	1274.65±819.83	1351.62±869.79	0.577
Collected oocyte	6.26±4.49	6.21±3.79	0.601
M1	3.89±3.7	3.93±3.97	0.891
M2	1.05±2.02	1.26±2.59	0.567
GV	0.65±1.24	0.51±0.92	0.702
Number of embryos transferred	1.03±0.7	1.14±0.61	0.192
Transfer day	2.41±1.32	2.36±1.22	0.886
Gonadotropin starting dose	294.42±93.83	318.25±95.75	0.014*
Gonodotropin total dose	2587.34±1226.33	2885.08±1234.07	0.015*
Group without protocol change			
FSH	7.25±3.80	6.71±4.08	0.351
E2	76.70±128.32	56.47±57.48	0.829
Number of antral follicles	11.07±4.37	8.75±4.02	0.001**
Induction time	8.77±2.13	9.03±1.82	0.355
Hcg day E2 level	1277.65±813.83	1354.62±868.79	0.572
Collected oocyte	6.16±4.44	6.25±3.81	0.607
M1	3.85±3.91	3.94±3.98	0.898
M2	1.08±2.05	1.23±2.53	0.565
GV	0.65±1.26	0.55±0.98	0.704
Number of embryos transferred	1.06±0.8	1.15±0.68	0.199
Transfer day	2.47±1.33	2.34±1.24	0.886
Gonadotropin starting dose	298.42±97.83	317.25±97.77	0.014*
Gonodotropin total dose	2582.37±1221.35	2889.08±1235.06	0.015*

Mann-Whitney U test used. *: P<0.05; **: P<0.01; IVF: *In vitro* fertilization; FSH: Follicle stimulating hormone; E2: Estradiol; M1: Metafaz 1; M2: Metafaz 2; GV: Germinal vesicle, Hcg: Human chorionic gonadotropin.

was lower with the antagonist protocol.^[9] Another meta-analysis concluded that the antagonist protocol was associated with lower ongoing pregnancy rates compared with the agonist protocol, but that the antagonist protocol should be the first choice of treatment in patients with PCOS and poor responders, although ongoing pregnancy rates were similar with both protocols.^[6] Another study comparing the two protocols concluded that the success rates of achieving a live birth in the first IVF attempt were similar.^[10]

There are few studies comparing IVF outcomes in patients who tried the same or different protocols after a failed IVF attempt. Şükür et al.^[11] concluded that changing the protocol in the next attempt did not change pregnancy rates after a failed IVF attempt

with GnRH agonist or antagonist protocols. In another study, after multiple unsuccessful IVF attempts with a GnRH agonist protocol, a trial of a GnRH antagonist protocol resulted in improved embryo quality and higher pregnancy rates.^[12] Li et al.^[13] compared GnRH agonist and antagonist protocols and concluded that after an unsuccessful IVF trial with the antagonist protocol, implantation, clinical pregnancy, and live birth rates were similar in subsequent cycles with either the agonist or the antagonist protocol. In addition, a trial of the GnRH antagonist protocol after a failed trial with the agonist protocol resulted in higher live birth rates compared to stimulation using the agonist protocol, although implantation and clinical pregnancy rates were similar.

Table 3: β -hCG and clinical pregnancy evaluation by protocol change

	Protocol change		p
	Negative n (%)	Positive n (%)	
First trial β -hCG measure			0.729
Negatif	24 (80)	26 (86.7)	
Pozitif	6 (20)	4 (13.3)	
Second trial β -hCG measure			1.000
Negatif	18 (60)	18 (60)	
Pozitif	12 (40)	12 (40)	
First trial clinic pregnancy			–
Negative	30 (100)	30 (100)	
Positive	–	–	
Second trial clinic pregnancy			1.000
Negative	22 (73.3)	22 (73.3)	
Positive	8 (26.7)	8 (26.7)	

Yates Continuity Correction Test.

Depalo et al.^[14] reported that patients who underwent IVF with an agonist protocol had significantly higher mean oocyte and mature oocyte counts compared to those who underwent an antagonist protocol, but the number of good-quality embryos, implantation, clinical pregnancy, and ongoing pregnancy rates were similar in both groups. In this study, the groups with and without protocol changes after unsuccessful IVF attempts were evaluated, and it was concluded that there was no significant difference between the groups in terms of the number of oocytes retrieved, mature oocytes, fertilized oocytes, and good-quality embryos. Aldemir et al.^[15] concluded in their study that a protocol change after an unsuccessful IVF trial with a GnRH agonist protocol increased live birth rates. However, after an unsuccessful trial with a GnRH antagonist protocol, they concluded that the protocol change had no effect on clinical pregnancy and live birth rates.

In a study conducted in 2024, Kahn et al.^[16] investigated the effect of protocol change on the next IVF treatment in patients whose previous IVF cycle was canceled. In the study, 13,135 patients were evaluated retrospectively. While the protocols of 6434 patients were not changed, the protocols of 6701 patients were changed in the next attempt. After IVF cycle cancellation, compared to those who repeated the same stimulation protocol, those who changed their protocol had higher odds of live birth and lower odds of recurrent cycle cancellation.

The retrospective design and small number of patients were the main limitations of our study. Prospective studies with larger patient numbers are needed to reach definitive conclusions on this issue.

CONCLUSION

As a conclusion, in our study, in patients whose first IVF attempt was unsuccessful, making a protocol change in the second IVF attempt did not have a positive effect on either β -hCG positivity or clinical pregnancy success. No significant difference was found in terms of pregnancy outcomes between the groups with and without protocol changes.

Statement

Ethics Committee Approval: The Zeynep Kamil Maternity and Children's Diseases Health Training and Research Center Clinical Research Ethics Committee granted approval for this study (date: 25.11.2016, number: 164).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Conflict of Interest: The authors declare that there is no conflict of interest.

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: Artificial intelligence was not used in writing the article.

Author Contributions: Concept – Concept – EÖ; Design – EÖ; Supervision – RM; Resource – RM; Materials – RM; Data Collection and/or Processing – RM; Analysis and/or Interpretation – RM; Literature Search – RM; Writing – RM; Critical Reviews –YM.

Peer-review: Externally peer-reviewed.

REFERENCES

- Mosher WD, Pratt WF. Fecundity and infertility in the United States: Incidence and trends. *Fertil Steril* 1991;56:192–3.
- Forti G, Krausz C. Clinical review 100: Evaluation and treatment of the infertile couple. *J Clin Endocrinol Metab* 1998;83:4177–88.
- Kahraman S, Yakın K. Ovulasyon indüksiyonu. İstanbul: Memorial Hastanesi Yardımcı Üreme Teknikleri ve Reprodüktif Endokrinoloji Merkezi; 2000. [In Turkish]
- Cai Q, Wan F, Huang K, Zhang H. Does the number of oocytes retrieved influence pregnancy after fresh embryo transfer? *PLoS One* 2013;8:e56189.
- Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: An analysis of 400 135 treatment cycles. *Hum Reprod* 2011;26:1768–74.
- Depalo R, Jayakrishan K, Garruti G, Totaro I, Panzarino M, Giorgino F, et al. GnRH agonist versus GnRH antagonist in in vitro fertilization and embryo transfer (IVF/ET). *Reprod Biol Endocrinol* 2012;10:26.
- Daya S. Gonadotropin releasing hormone agonist protocols for pituitary desensitization in in vitro fertilization and gamete intrafallopian transfer cycles. *Cochrane Database Syst Rev* 2000;2000:CD001299. Update in: *Cochrane Database Syst Rev* 2007;:CD001299.
- Lambalk CB, Banga FR, Huirne JA, Toftager M, Pinborg A, Homburg R, et al. GnRH antagonist versus long agonist protocols in IVF: A systematic review and meta-analysis accounting for patient type. *Hum Reprod Update* 2017;23:560–79.
- Wang R, Lin S, Wang Y, Qian W, Zhou L. Comparisons of GnRH antagonist protocol versus GnRH agonist long protocol in patients with normal ovarian reserve: A systematic review and meta-analysis. *PLoS One* 2017;12:e0175985.

10. Toftager M, Bogstad J, Løssl K, Prætorius L, Zedeler A, Bryndorf T, et al. Cumulative live birth rates after one ART cycle including all subsequent frozen-thaw cycles in 1050 women: Secondary outcome of an RCT comparing GnRH-antagonist and GnRH-agonist protocols. *Hum Reprod* 2017;32:556–67.
11. Şükür YE, Ulusoy CO, Özmen B, Sönmezer M, Berker B, Aytaç R, et al. Protocol shift from agonist to antagonist or vice versa after an unsuccessful intracytoplasmic sperm injection cycle on the same patient does not improve outcome. *Taiwan J Obstet Gynecol* 2018;57:417–20.
12. Takahashi K, Mukaida T, Tomiyama T, Goto T, Oka C. GnRH antagonist improved blastocyst quality and pregnancy outcome after multiple failures of IVF/ICSI-ET with a GnRH agonist protocol. *J Assist Reprod Genet* 2004;21:317–22.
13. Li Y, Li Y, Lai Q, Zhang H, Zhu G, Jin L, et al. Comparison between a GnRH agonist and a GnRH antagonist protocol for the same patient undergoing IVF. *J Huazhong Univ Sci Technolog Med Sci* 2008;28:618–20.
14. Depalo R, Lorusso F, Palmisano M, Bassi E, Totaro I, Vacca M, et al. Follicular growth and oocyte maturation in GnRH agonist and antagonist protocols for in vitro fertilisation and embryo transfer. *Gynecol Endocrinol* 2009;25:328–34.
15. Aldemir O, Ozelci R, Dilbaz S, Dilbaz B, Baser E, Ustun Y. Impact of changing the ovarian hyperstimulation protocol in the sequential cycle on ICSI outcomes in the same patient population. *J Gynecol Obstet Hum Reprod* 2021;50:101999.
16. Kahn JL, Wu H, Gerber R, Shah T, Lieman H, Pollack SE, et al. Protocol change improves live birth and recurrent cycle cancellation rates after a previous IVF cycle cancellation: An analysis of 13 000 autologous cycles reported to SART CORS. *Hum Reprod* 2024:deae102.