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Comparing the Implications of IVF-Induced Pregnancy by Two Regimens with a Flexible Dose of GnRH Antagonist and a Half Fixed Dose of GnRH Antagonist

Fateme Sarvi[1], Melika Assefi[2], Maryam Nurzadeh[3*]

[1]Department of Obstetrics and Gynecology, School of Medicine, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
[2]Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Fetomaternal, Faculty of Medicine, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Introduction: In an IVF cycle after ovulation stimulation, the goal is to prevent LH surge and wasting follicles. The use of GnRH antagonists to achieve this goal has been considered by infertility specialists in recent years. Two fixed or flexible drug regimens have been tested. The aim of this study was to compare the implications of induced pregnancy by two regimens with a flexible dose of GnRH antagonist and a half fixed dose of GnRH antagonist that was evaluated for the first time.

Materials and Methods: This randomized double-blinded controlled trial was conducted on 140 patients referring to the infertility department of Shariati Hospital between June 2017 and June 2018 who were candidates for IVF. Patients who were candidates for cyclic therapy with GnRH antagonists were randomly assigned (with a randomized table of numbers) to either the fixed dose group with a half dose of the GnRH antagonist (Group A, n = 70) or the flexible GnRH antagonist dose (Group B, n = 70).

Results: There was also difference in the numbers of IVF or IUI cycles across the two groups. The mean degree of the sperm motility and sperm morphology percentages were similar in the spouses of women in the two groups. With regard to the outcome of IVF, no difference was revealed in the mean number of mature follicles, number of retrieved oocytes, and number of transferred embryos (Table 3). The successful clinical pregnancy rate in the groups A and B was 20.0% and 18.6% respectively with no difference (p = 0.830). The successful clinical pregnancy rate in the groups A and B was 20.0% and 18.6% respectively with no difference (p = 0.830). Based on the multivariable logistic regression analysis, the fixed dose regimen with half dose of GnRH antagonist or GnRH flexible dose antagonist dose did not affect the IVF success. The final determinants of the success of IVF were the percentage of sperm morphology (OR = 1.237, P = 0.015) and BMI of the patients (OR = 0.836, P = 0.019).

Conclusion: Both GnRH antagonist regimens with flexible doses and the GnRH antagonist with a half fixed dose have similar effects on the outcome of IVF. Among the factors predicting the success rate of IVF, the role of reducing the morphology of sperm and obesity is very prominent.

ARTICLE HISTORY

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KEYWORDS

IVF, GnRh antagonist, flexible dose, half dose, pregnancy.

INTRODUCTION

Gonadotropins were first introduced in the early 1960s and were used to stimulate ovarian cycles to induce the development of ovarian follicles. This issue has been exploited in the last three decades, especially in women, to treat fertility induction by in-vitro fertilization (IVF). The analogues of the GnRH hormone together with gonadotropins are used to prevent LH hormone surge that occurs before achieving the follicular diameter greater than 17 mm for induction of ovulation via hCG injection. Without using GnRH analogues, we will

* **Contact** Maryam Nurzadeh Department of Fetomaternal, Faculty of Medicine, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran Drnurzadeh@gmail.com Tel: +98 2184902415

see an increase in LH in approximately 20% of cases (1, 2). Recently, high-power GnRH antagonists with minimized side effects have been introduced and marketed to the IVF as the alternatives for GnRH agonists. Unlike the GnRH agonists, these GnRH antagonists immediately inhibit gonadotropins through the block of GnRH receptors in the anterior pituitary and consequently prevent the secretion of endogenous gonadotropin by inducing secretion of LH and FSH in the anterior pituitary gland (3-5). Various pharmacological mechanisms of these antagonists have led to the introduction of these drugs selectively in IVF to prevent the premature LH secretion (6). The benefits of using GnRH antagonists have been studied extensively in comparison with GnRH agonists, which can reduce the duration of injecting, the lack of vasomotor symptoms, the lower risk of misuse the drug during pregnancy, the prevention of ovarian cysts and needing lower doses of gonadotropins during each cycle, which ultimately lead to a greater compliance of the patient (7). However, some disadvantages and limitations have been reported for the GnRH antagonist protocol compared to the agonists, which includes a reduced programing of the GnRH antagonists in terms of cycle planning, as well as a slight reduction in pregnancy rate per cycle (8, 9).

Another important point is the comparison of the different dosing protocols of GnRH antagonists especially the fixed or flexible doses. The flexible doses are used to reduce the number of antagonistic injections and to reduce the duration of the stimulation period. It is recommended that a fixed dose regimen should be initiated from day 5 to 6 of stimulation (10, 11) while flexible doses should be scheduled when the follicle diameters reach over 14 mm (12-14). It has been shown that the use of flexible therapeutic doses has led to better pregnancy induction in patients treated with GnRH antagonists (15). The results of several studies have shown the high efficacy and safety of the flexible regimen with ganirelix (16, 17). Evidence suggests that flexible dosing regimens can improve the outcome of ovarian stimulation cycles. In some studies, although there is no difference between the two fixed and flexible dose regimens, the use of rFSH in a flexible dose regimen has decreased (18). However, there is still ambiguity about the efficacy and efficacy of two regimens with fixed and flexible doses and further evaluation is needed. The present study aimed to compare the efficacy and safety of GnRH antagonist with flexible dose and fixed dose of GnRH antagonist with half the usual dose.

Table 1: Baseline characteristics of study population

Item	Fixed dose	Flexible dose	P value
Mean age (women)	32.81 ± 5.09	32.23 ± 4.58	0.614
Mean age (men)	37.49 ± 7.07	36.16 ± 4.45	0.195
Mean weight	68.89 ± 9.31	66.43 ± 11.57	0.171
Mean BMI	23.64 ± 3.85	25.08 ± 5.00	0.195
Mean duration of infertility	5.16 ± 5.13	4.99 ± 4.25	0.838
Abortion			0.065
Non	55 (78.6)	65 (92.9)	
One time	7 (10.0)	4 (5.7)	
Two times	6 (8.6)	1 (1.4)	
Three times	2 (2.9)	0 (0.0)	
Ectopic pregnancy	0 (0.0)	1 (1.4)	0.998
Curettage	4 (5.7)	0 (0.0)	0.120
Cesarean section	2 (2.9)	1 (1.4)	0.622
Salpingectomy	2 (2.9)	2 (2.9)	1.000
Hypothyroidism	22 (31.4)	23 (32.9)	0.856
PCOS	13 (18.6)	47 (67.1)	0.081
Endometriosis	0 (0.0)	3 (4.3)	0.245
Hyperprolactinemia	6 (8.6)	5 (7.1)	0.753
Azospermia	4 (5.7)	8 (11.4)	0.227
Mulerian anomaly	1 (1.4)	5 (7.1)	0.209
Septoplasty	10 (14.3)	6 (8.6)	0.288
Previous laparoscopy	10 (14.3)	15 (21.4)	0.123
Previous hysteroscopy	21 (30.0)	30 (42.9)	0.114
Uterine fibroma	2 (2.9)	4 (5.7)	0.681
Uterine polyp	0 (0.0)	2 (2.9)	0.496

MATERIALS AND METHODS

This randomized double-blinded controlled trial was conducted on 140 patients referring to the infertility department of Shariati Hospital between June 2017 and June 2018 who were candidates for IVF. The inclusion criteria were age under 39 years,

less than three times of repeated IVF, BMI lower than 29 kg/m^2, regular menstrual cycles, lack of history of ovarian surgery, and lack of history of PCOS. Patients who were candidates for cyclic therapy with GnRH antagonists were randomly assigned (with a randomized table of numbers) to either the fixed dose group with a half dose of the GnRH antagonist (Group A, n = 70) or the flexible GnRH antagonist dose (Group B, n = 70). Ethics code was prepared for this process from Tehran University of Medical Sciences. At first, hormonal evaluation was performed in both groups including measurement of serum levels of LH and FSH and estradiol on the third day of menstruation and repeat these tests plus progesterone levels on the day of HCG injection. In group A, gonadotropin started with the Gonal F marker on the second day of menarche. The required dose was determined based on AMH and antral follicles. From day 5, of cyclic stimulation, it was administered a half fixed dose of Cetrotide. This fixed dose continued until at least two follicles of more than 17 mm were obtained. If these conditions were reached, HCG was injected to stimulate oocytes and 36 hours later, the puncture was performed. In method B, the process of ovulation stimulation with gonadotropins started on day 2 of the menstrual period, and when the size of the follicles reached 14 mm, one Cetrotide was injected daily to prevent LH surge and, with the follicles reaching 17 mm, 36 hours later from the injection of HCG, the puncture was done. The study endpoint was to compare the outcomes of the procedure in terms of oocyte quality, number of retrieved oocytes, number of transferred embryos, fertility rate, and oocyte implantation rate, rate of chemical and clinical pregnancy and live birth rate.

In analyzing the data, the chi-square test, student t-test and its non-parametric equivalents (Mann Whitney U test) were used if necessary. To determine the main determinants of successful fertility induced by IVF, the multivariable logistic regression modeling was planned. For the statistical analysis, the statistical software SPSS version 16.0 for windows (SPSS Inc., Chicago, IL) was used. P values of 0.05 or less were considered statistically significant.

Table 2: Baseline sexual and hormonal conditions in study population

Item	Fixed dose	Flexible dose	P value
Number of IUI			0.303
One time	5 (7.1)	9 (12.9)	
Two times	4 (5.7)	7 (10.0)	
Number of IVF			0.261
One time	65 (92.9)	59 (84.3)	
Two times	5 (7.1)	6 (8.6)	
Three times	0 (0.0)	2 (2.9)	
Four times	0 (0.0)	2 (2.9)	
Five times	0 (0.0)	1 (1.4)	
Tube condition			0.208
Normal	65 (92.9)	63 (90.0)	
Closed	5 (7.1)	4 (5.7)	
Adhesion	0 (0.0)	3 (4.3)	
Mean sperm motility degree	2.30 ± 2.02	2.37 ± 2.16	0.860
Mean sperm morphology percentage	6.61 ± 6.22	6.84 ± 6.32	0.829
Mean serum AMH	3.78 ± 4.27	4.94 ± 4.71	0.143
Mean serum FSH	6.77 ± 3.22	7.53 ± 4.91	0.287
Mean serum prolactin	95.54 ± 53.62	122.80 ± 82.69	0.351
Mean serum FBS	90.93 ± 10.16	90.44 ± 14.16	0.820
Mean serum LH	5.71 ± 3.32	6.22 ± 3.38	0.385
Mean serum TSH	2.96 ± 2.32	2.71 ± 2.55	0.546

Table 3: Outcomes of IVF in the two regimens

Item	Fixed dose	Flexible dose	P value
Mean number of follicles	5.56 ± 2.14	6.75 ± 5.32	0.380
Mean number of retrieved oocytes	3.97 ± 2.76	3.77 ± 2.92	0.678
Mean number of embryos	2.73 ± 2.08	3.53 ± 2.74	0.054
Mean number of transferred embryos	1.33 ± 1.28	1.61 ± 1.36	0.204
Chemical pregnancy	14 (20.0)	13 (18.6)	0.830
Clinical pregnancy	14 (20.0)	13 (18.6)	0.830
OHSS leading cycle cancelation	8 (11.4)	13 (18.6)	0.237

Table 4: Main determinants of successful IVF

Factor	Beta	SE	Univariate p-value	Multivariate p-value	OR
Drug regimen	0.239	0.543	0.830	0.660	1.270
BMI	-0.179	0.076	0.041	0.019	0.836
Sperm motility	-0.366	0.252	0.039	0.146	0.639
TSH level	-0.196	0.224	0.003	0.384	0.822
Previous abortion	0.125	0.419	0.001	0.766	1.133
hypothyroidism	-0.813	0.671	0.032	0.226	0.444
Sperm morphology	0.212	0.087	0.001	0.015	0.237
Constant	2.268	0.872	0.032	0.226	9.659

RESULTS

In the present study, a total of 70 women under IVF were enrolled in the study, of which 70 were administered a fixed dose with a half dose of the GnRH antagonist (group A) and 70 subjects were administered the GnRH antagonistic dose (Group B). As shown in Table 1, there was no difference between the two groups in terms of baseline parameters including mean age, mean BMI, duration of infertility, history of abortion, history of premature labor or ectopic pregnancy, history of cesarean section, salpingectomy, number of children, history of hypothyroidism, endometriosis, uterine anomalies, hperprolactinemia, previous laparoscopy or hysteroscopy. There was also difference in the numbers of IVF or IUI cycles across the two groups. The mean degree of the sperm motility and sperm morphology percentages were similar in the spouses of women in the two groups (Table 2). There was also no difference in baseline level of hormones including AMH, FSH, LH, prolactin, and TSH between the two groups (Table 2). With regard to the outcome of IVF, no difference was revealed in the mean number of mature follicles, number of retrieved oocytes, and number of transferred embryos (Table 3). The successful clinical pregnancy rate in the groups A and B was 20.0% and 18.6% respectively with no difference (p = 0.830). There were no cases of miscarriage, abortion following pregnancy induction, curettage or hysterocele in any of the examined cases following procedure. Reproduction cancellation following OHSS was reported in 11.4% and 18.6% respectively (p = 0.237). In assessing the success of fertility between the two groups and in the Univariate analysis, among the indices of the study, the high BMI of women (P value = 0.041), reduction of sperm motility (P value of 0.039), reduction of morphology percentage of sperm (P value = 0.001), high TSH level (P value 0.003), abortion history (P value> 0.001) and previous history of hypothyroidism (P value = 0322) were the factors related to IVF failure. Based on the multivariable logistic regression analysis (Table 4), the fixed dose regimen with half dose of GnRH antagonist or GnRH flexible dose antagonist dose did not affect the IVF success. In that analysis, the final determinants of the success of IVF were the percentage of sperm morphology (odds ratio equal to 1.237, P value = 0.015) and BMI of the patients (odds ratio equal to 0.836, P value = 0.019).

DISCUSSION

For microinjection cycles, there are various treatment protocols. Since various protocols can affect the outcome of IVF, since the introduction of IVF, various protocols have been devised. In an IVF cycle after ovulation stimulation, the goal is to prevent LH surge and release and wasting follicles. In the first IVF cycle, in the past, the patient underwent sonography repeatedly to make a puncture before the oocyte is released. Due to the difficulty of this process, later on the GnRH agonist was used to prevent early release of oocytes. However, the use of GnRH antagonists to achieve this goal has been considered by infertility specialists in recent years. These antagonists are used with various protocols. In this regard, two fixed or flexible drug regimens have been tested. The aim of this study was to compare the implications of induced pregnancy in two regimens with a flexible dose of GnRH antagonist and a constant half fixed dose of GnRH antagonist that was evaluated for the first time. What we found in this study was that both types of regimens led to similar outcomes in terms of successful pregnancy. In fact, either in terms of follicle production, oocyte retrieval and embryo transfer, or in terms of the final outcome as successful pregnancy, two regimens achieve similar results. It also seems that the only use of a half dose of a fixed dose of GnRH antagonist can be as useful as the GnRH antagonist with a flexible dose. A review of the literatures also suggests a similarity between our study and previous results, although some also contradicted our study. In the study by Al-Inany et al (18), contrary to our survey, pregnancy rate was significantly higher in the flexible dose than in the fixed dose, which may have been due to differences in the drug dosage used in the two regimens, but similar to our study, the difference in LH level was not different in the two regimens. In a study by Rashidi et al (19), the days for stimulation and the number of prescriptive antagonists did not differ

between the two methods. However, the number of administered gonadotropin was significantly lower in the flexible group. The number of oocytes retrieved and the number of embryos transferred in the flexible dose was significantly higher, which was openly in contradiction with our study. In a study by Mochtar et al(14) and quite similar to our study, the average number of retrieved oocytes was similar between the two regimens. The rate of pregnancy in the flexible group was 22.2% and in the fixed group 31.1%, which did not differ significantly between the two groups.

What was found in our study results compared to other studies was the lower success rate of IVF-induced pregnancy in both groups compared to other studies. In some studies, even the success rate in IVF-induced pregnancy has reached more than 50%. In some studies, it has been shown that over the past three decades, the success rate of IVF leading live birth has reached 80% even though the number of transferred embryos has decreased (20). However, it seems that we are facing a wide range of success rates in IVF from about 10% to over 80%, and therefore, various factors contributing to the success or failure of IVF therapy will be effective. In this regard, maternal age, duration of infertility, causes of infertility (male or female), the number of previous failures in IVF, baseline hormone deficiency, and the number of cycles are all predictors of IVF success. What we found out in our study was that the most important predictors of success in IVF were ultimately the two factors of BMI and sperm morphology. Several studies have been carried out on the effect of sperm morphology on the success rate of IVF. In this regard, the techniques used to improve sperm morphology have significantly improved the success of fertility induction. In some studies, this improvement has been reported from 21% to 34% (21). In various studies, sperm morphology more than 5% was considered as a predictor of successful IVF (22). It has also been reported that in cases with sperm morphology less than 4%, considering ICSI as the first one is preferred (23). With regard to the effect of BMI on the results of IVF, the evidence is also consistent with our finding. In a study by Sarais et al., a BMI over 25 kg/m^2 was related to a significant reduction in the success rate of IVF (24). In Pandey et al. study, obesity has been reported as a strong prognostic factor for failure of IVF, and in this context, planned weight loss has led to a gradual increase in the success of this procedure (25). In terms of the mechanism of the effect of obesity on fertility, obesity is associated with a decrease in the production of liver hormones, and in particular the disruption of the androgen-estrogen-induced aromatase pathway, and hence the process of folliculogenesis is impaired. Also, adiposity accumulation in obesity will be accompanied by an increased incidence of inflammation, coagulation and fibrinolysis as well as metabolic syndrome, which will be effective in the hormonal production and balance pathways (24). In general, considering the effect of the two indicators, the reduction of sperm morphology and BMI increase on the results of IVF and due to the low significance of the success rate of IVF in our country, considering measures to improve sperm morphology and recommend treatment for obesity should be considered in candidates for IVF.

CONCLUSION

In the present study, both GnRH antagonist regimen with flexible doses and the GnRH antagonist with a half fixed dose had similar effects on the outcome of IVF and therefore, the selection of either of these two prognostic agents has failed to predict induced fertility by IVF. However, in both methods, the success rate in achieving successful pregnancy has been low. Among the factors predicting the success rate of IVF, the role of reducing the morphology of sperm and obesity has been very prominent in our study.

REFERENCES

1. Edwards RG, Lobo R, Bouchard P. Time to revolutionize ovarian stimulation. Oxford University Press; 1996.
2. Janssens R, Lambalk C, Vermeiden J, Schats R, Bernards J, Rekers-Mombarg L, et al. Dose-finding study of triptorelin acetate for prevention of a premature LH surge in IVF: a prospective, randomized, double-blind, placebo-controlled study. Human reproduction. 2000;15(11):2333-40.
3. Engel JB, Griesinger G, Schultze-Mosgau A, Felberbaum R, Diedrich K. GnRH agonists and antagonists in assisted reproduction: pregnancy rate. Reproductive biomedicine online. 2006;13(1):84-7.
4. Group EOS, Borm G, Mannaerts B. Treatment with the gonadotrophin-releasing hormone antagonist ganirelix in women undergoing ovarian stimulation with recombinant follicle stimulating hormone is effective, safe and convenient: results of a controlled, randomized, multicentre trial. Human Reproduction. 2000;15(7):1490-8.
5. Al-Inany H, Aboulghar M. GnRH antagonist in assisted reproduction: a Cochrane review. Human Reproduction. 2002;17(4):874-85.
6. Huirne J, Homburg R, Lambalk C. Are GnRH antagonists comparable to agonists for use in IVF? Human Reproduction. 2007;22(11):2805-13.
7. Tarlatzis B, Kolibianakis E. GnRH agonists vs antagonists. Best Practice & Research Clinical Obstetrics & Gynaecology. 2007;21(1):57-65.

8. Tarlatzis B, Fauser B, Kolibianakis E, Diedrich K, Devroey P, Group BGACW. GnRH antagonists in ovarian stimulation for IVF. Human Reproduction Update. 2006;12(4):333-40.
9. Huirne J, Hugues J, Pirard C, Fischl F, Sage J, Pouly J, et al. Cetrorelix in an oral contraceptive-pretreated stimulation cycle compared with buserelin in IVF/ICSI patients treated with r-hFSH: a randomized, multicentre, phase IIIb study. Human Reproduction. 2006;21(6):1408-15.
10. Devroey P, Boostanfar R, Koper N, Mannaerts B, Ijzerman-Boon P, Fauser B. A double-blind, non-inferiority RCT comparing corifollitropin alfa and recombinant FSH during the first seven days of ovarian stimulation using a GnRH antagonist protocol. Human Reproduction. 2009;24(12):3063-72.
11. Andersen AN, Witjes H, Gordon K, Mannaerts B. Predictive factors of ovarian response and clinical outcome after IVF/ICSI following a rFSH/GnRH antagonist protocol with or without oral contraceptive pre-treatment. Human reproduction. 2011;26(12):3413-23.
12. 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). Reproductive biology and endocrinology. 2012;10(1):26.
13. Ludwig M, Katalinic A, Banz C, Schröder A, Löning M, Weiss J, et al. Tailoring the GnRH antagonist cetrorelix acetate to individual patients' needs in ovarian stimulation for IVF: results of a prospective, randomized study. Human Reproduction. 2002;17(11):2842-5.
14. Mochtar M. The effect of an individualized GnRH antagonist protocol on folliculogenesis in IVF/ICSI. Human Reproduction. 2004;19(8):1713-8.
15. Oberyé J. Study Group on Weight Adjusted Dosing of Ganirelix: No need for dose adjustment of GnRH antagonist based on patient's body weight in controlled ovarian hyperstimulation with recombinant follicle stimulating hormone. Fertil Steril. 2003;80(Suppl 3):S9.
16. Escudero E, Bosch E, Crespo J, Simón C, Remohí J, Pellicer A. Comparison of two different starting multiple dose gonadotropin-releasing hormone antagonist protocols in a selected group of in vitro fertilization–embryo transfer patients. Fertility and sterility. 2004;81(3):562-6.
17. Kolibianakis EM, Venetis CA, Kalogeropoulou L, Papanikolaou E, Tarlatzis BC. Fixed versus flexible gonadotropin-releasing hormone antagonist administration in in vitro fertilization: a randomized controlled trial. Fertility and sterility. 2011;95(2):558-62.
18. Al-Inany H, Aboulghar MA, Mansour RT, Serour GI. Optimizing GnRH antagonist administration: meta-analysis of fixed versus flexible protocol. Reproductive biomedicine online. 2005;10(5):567-70.
19. Rashidi BH, Lak TB, ShahrokhTehrani E, Tanha FD. Fixed versus Flexible Gonadotropin Releasing Hormone Antagonist Protocol in Controlled Ovarian Stimulation for Invitro Fertilization in Women with Polycystic Ovary Syndrome. Journal of family & reproductive health. 2015;9(3):141.
20. Kovacs G. The success rate of IVF has significantly improved over the last decade. 2014.
21. Van der Zwalmen P, Bertin-Segal G, Geerts L, Debauche C, Schoysman R. Sperm morphology and IVF pregnancy rate: comparison between Percoll gradient centrifugation and swim-up procedures. Human Reproduction. 1991;6(4):581-8.
22. Nikbakht R, Saharkhiz N. The influence of sperm morphology, total motile sperm count of semen and the number of motile sperm inseminated in sperm samples on the success of intrauterine insemination. International journal of fertility & sterility. 2011;5(3):168.
23. Li B, Ma Y, Huang J, Xiao X, Li L, Liu C, et al. Probing the effect of human normal sperm morphology rate on cycle outcomes and assisted reproductive methods selection. Plos one. 2014;9(11):e113392.
24. Sarais V, Pagliardini L, Rebonato G, Papaleo E, Candiani M, Viganò P. A comprehensive analysis of body mass index effect on in vitro fertilization outcomes. Nutrients. 2016;8(3):109.
25. Best D, Avenell A, Bhattacharya S, Stadler G. New debate: is it time for infertility weight-loss programmes to be couple-based? Human Reproduction. 2017;32(12):2359-65.