

Comparison between Soft and Conventional Antagonist protocols for Estradiol Level and Number of Growing Follicles in Women of Poor Ovarian Response

Nidhal Salim Alwan*, Manal Taha Al-Obaid , Lubna Al-Anbari

High Institute for Infertility Diagnosis and Assisted Reproductive Technologies,
Al-Nahrain University, Baghdad, Iraq

Corresponding author: Nidhal Salim Alwan, Mobile: 009647901749879, Email: Nalooly@yahoo.com

ABSTRACT

Introduction: Poor ovarian responders (PORs) are defined as the group of infertile women who are characterized by decreased response of the ovaries to stimulation. Recently using soft protocol with low doses of Gn with/without oral compounds like letrozole seems superior to the conventional high doses since it is less cost-effective.

Objective: Comparing of soft versus conventional GnRH-antagonist protocol in POR for estradiol level and number of growing follicles.

Patients and Methods: This was a prospective randomized controlled trial including eighty infertile POR according to Bologna criteria that were divided into two equal groups and undergoing IVF (In vitro fertilization)/ICSI (Intracytoplasmic sperm injection) antagonist protocols. One group underwent soft protocol by using letrozole 2.5 mg twice daily for 5 days starting from cycle day 2-3 overlapped low-dose Gn 225 IU from cycle day 4-5. Other group received high dose Gn only 450 IU from cycle day 2-3.

Results: Basal demographic characteristics and hormones, comparison showed no significant difference. The mean AFC was not significantly different ($P > 0.05$) between the two groups with the number of the growing follicles at day of starting antagonist and at day of trigger. There is a significant difference in the total units of Gn used.

Conclusion: Soft protocol is a good option for POR since obtaining the same ovarian response with less cost.

Keywords: Poor ovarian responders, Soft stimulation, Conventional stimulation, Letrozole, Gonadotropins.

INTRODUCTION

The live birth of a single and healthy infant is the aim of the recent assisted reproduction technology (ART). This aim is intended to be accomplished with less time and cost to increase patient satisfaction and safety. In spite of the rapid progress that has been made in ARTs over the period of the past 40 years, there are still several issues that have not been resolved like the problem of managing clinically the women who have POR, which is still a matter of debate and disappointing for the women and the clinician ⁽¹⁾. The percentage of women who have a poor ovarian response ranges from 5.6% to 35.1%, depending on how the term "poor response" is defined ⁽²⁾.

The etiology and pathogenesis of POR is complex and only parts of it have been understood and recognized like the effect of age and its relation to decrease the number and quality of ovarian follicles, chromosomal and genetic abnormalities and advanced endometriosis ⁽²⁾. The technologies of assisted reproduction have shown progressive development in the field of clinical knowledge and technology in order to increase the success rate of pregnancy, which is still in relation to the number of eggs obtained after ovulation induction by Gn ⁽³⁾.

There have been numerous trials done for management of POR using different protocols, but these efforts have not been successful in identifying the exact protocol, which is most effective ⁽⁴⁾. The heterogeneity of this group of women may be the reason behind the difficulty in identifying the most effective strategies in management of POR ⁽⁵⁾. In 2011, the European Society of Human Reproduction and Embryology (ESHRE) developed the Bologna criteria with the intention of

standardizing the description of POR. These criteria consist of the following:

- (1) The age of women (more than 40 years old) or any other factor that increases the risk of POR.
- (2) The women had prior experience with IVF or ICSI and were able to collect at least three (or less than 3) oocytes using a conventional stimulation procedure.
- (3) An abnormal ovarian reserve test, which is defined as an antral follicle count that is lower than 5–7 follicles or an AMH level that is lower than 0.5–1.1 ng/ml ⁽⁶⁾.

After the age of 35, the chance of getting pregnant and having live birth begins to reduce dramatically and providing successful treatment for these patients continues to be an important issue for ART programs ⁽⁷⁾.

Poor ovarian response (POR) is characterized by a decline in ovarian function that is substantially greater than what is considered to be within the normal range for the woman's age ⁽⁸⁾. The oocyte donation is now regarded the most effective and reliable option for POR but the vast majority of patients are insisting to use their own oocytes even though doing many trails of ART cycles in order to be pregnant ⁽⁹⁾.

Many of the procedures that are employed for patients who are POR are concentrating on reducing the amount of gonadotropins they received ⁽¹⁰⁾.

They are prone to longer and more expensive cycles because of the higher cancellation rates that are experienced. In addition to the financial burden that results from limited working days and expensive treatment, they also face with the emotional load that result from repeated failed cycles ⁽¹¹⁾.

PATIENTS AND METHODS

This prospective randomized-controlled trial was conducted in the IVF center of the High Institute for Infertility Diagnosis and Assisted Reproductive Technology/AL-Nahrain University from November 2020 to May 2022. It included 80 infertile poor responder's women according to Bologna criteria undergoing ICSI protocols.

All infertile couple was asked detailed questions about their medical history including the length of time they had been infertile, type of infertility whether primary or secondary associated with complete general physical examination including body weight with height and complete gynecological examination and then all were sent for full infertility investigations including husband's seminal fluid analysis, basal hormonal assay at day 2-3 of the cycle including FSH, LH, prolactin, TSH, estradiol (E2), progesterone (P4) and anti-mullerian hormone (AMH) associated with transvaginal ultrasound to exclude any ovarian cyst and to check the antral follicles count (AFC) in both ovaries and endometrial thickness, which should be less than 4 mm to start IVF/ICSI cycle.

Hystrosalpingography was done previously to all women for assessment of uterus and the patency of the tubes. All participants were poor responders according to Bologna criteria and undergone intracytoplasmic sperm injection (ICSI) programs using the flexible GnRH-antagonist protocol. They were divided into two equal groups. First group received letrozole tablets 2.5 mg twice daily for 5 days starting from cycle day 2-3 and then overlapped with Gn (225 IU) from cycle day 4-5. The second group received high doses Gn (450 IU) only from cycle day 2-3. Both continued throughout the antagonist stimulation protocol until the day of trigger for ovarian stimulation. Then, patients received 0.25 mg/day Cetrotorelix until at least two or more follicles reached a size of 12-14 mm.

Inclusion criteria: Patient with poor ovarian response according to Bologna Criteria (At least two of the aforementioned three criteria had to be present to establish the definition of POR).

Exclusion criteria:

Day-3 FSH > 30 IU/L and AMH below 0.5, age above 45 years. Uncontrolled endocrine or metabolic disorders like diabetes mellitus, Cushing's diseases, thyroid diseases, chronic liver and renal diseases, moderate & severe endometriosis and any significant abnormalities of the uterus or endometrium such as uterine congenital anomalies, fibroids or tubal diseases such as hydrosalpinges.

Both groups underwent oocyte pickup (OCP) and oocytes were classified according to maturity after denudation and then injected with the sperm. Embryo

transfer (ET) were performed if embryos were available. But when there was no oocytes retrieved and no fertilization or embryos formed (they did not complete division), in such cases there was no embryo transfer and such cases called case closed. Endometrial preparation with progesterone supplementation started on day of OCP that may be given vaginally, rectally, intramuscularly or orally and continued by the same regimen until 2 weeks after ET. Low molecular weight heparin (2000 IU) initiating daily on the evening of ET to enhance blood flow to the endometrium.

The compared parameter includes number of growing follicles, number of retrieved oocytes, number of total embryos formed, number of embryo transferred, duration of stimulation in days, total unites of gonadotropins used and biochemical pregnancy and clinical pregnancy rates.

Ethical Consideration:

The study was approved by the local medical Ethical Committee of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Iraq. Ethical approval was taken from each participant in the study. This work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The calculation was carried out with Statistical Package for the Social Sciences (SPSS) version 25, using Pearson Chi-square test (X^2 -test). Independent t-test and analysis of variance (ANOVA) (two tailed) were used.

1. Demographic characteristics of studied groups:

The mean age of soft protocol group was 37.05 ± 5.64 years, and 19 patients (47.5%) aged ≥ 40 years. In the conventional group, the mean age was 38.20 ± 4.89 years, and 23 patients (57.5%) aged ≥ 40 years. Regarding infertility, the highest proportion of the studied patients had primary infertility, 26 patients (65%) in the soft protocol group and 25 patients (62.5%) in the conventional group.

The most common duration of infertility was 1-4 years in 16 patients (40%) of soft protocol group and 22 (55%) of the conventional group. Both groups had comparable baseline characteristics and there were no significant differences ($P > 0.05$) between the two studied groups in regards to age, type of infertility, and duration of infertility. Also, the mean of BMI of soft protocol group was 26.34 ± 3.31 Kg/m² while for the conventional group it was 25.05 ± 3.55 Kg/m² and P value was 0.096 (no significant difference, $P > 0.05$) as shown in (Table 1).

Table (1): Demographic characteristics of the study groups

Demographic characteristics	Study groups		P- Value
	Soft protocol group no. (%) n=40	Conventional protocol group no. (%) n=40	
Age (Years)			
Mean ± SD	37.05 ± 5.64	38.20 ± 4.89	
< 30	3 (7.5)	3 (7.5)	0.736
30 - 34	7 (17.5)	7 (17.5)	
35 - 39	11 (27.5)	7 (17.5)	
≥ 40	19 (47.5)	23 (57.5)	
Type of infertility			
Primary	26 (65.0)	25 (62.5)	0.816
Secondary	14 (35.0)	15 (37.5)	
Duration of infertility (Years)			
1 - 4	16 (40.0)	22 (55.0)	0.275
5 - 9	14 (35.0)	8 (20.0)	
≥ 10	10 (25.0)	10 (25.0)	
	Mean ± SD	Mean ± SD	
BMI (kg/m²)	26.34 ± 3.31	25.05 ± 3.55	0.096

no.: number, SD: standard deviation, BMI: body mass index

2. Comparison of estradiol levels

The comparison between the two groups according to the mean levels of estradiol (baseline, at day of starting GnRH antagonist and day of Trigger) levels showed no statistically significant difference ($P > 0.05$) between the soft protocol group and conventional protocol group (Table 2) (Figure 1).

Table (2): Comparison by Estradiol levels (Baseline, Day of Starting and Day of Trigger) of the study groups

Estradiol levels	Study groups		P – Value
	Soft protocol Gn group Mean ± SD	Conventional protocol group Mean ± SD	
Baseline level	33.01 ± 11.98	32.78 ± 11.33	0.930
Day of starting GnRH antagonist	227.4 ± 67.11	264.9 ± 112.07	0.073
Day of trigger	831.1 ± 466.2	1032 ± 563.2	0.086

SD: standard deviation

3. Comparison of antral follicular count (AFC)

The comparison between the two groups according to the baseline AFC count and numbers of growing follicles at day of starting GnRh antagonist and day of trigger showed no significant difference between the soft protocol group and conventional protocol group (Table 3).

Table (3): Comparison by numbers of AFC and growing follicles on day of starting GnRH antagonist, day of trigger and in ovarian response parameters

No. of AFC and growing follicles	Study groups		p-value
	Soft protocol group Mean ± SD	Conventional Protocol group Mean ± SD	
Baseline AFC	6.12 ± 3.76	5.89 ± 3.52	0.816
No. of growing follicles on day of starting GnRH antagonist	6.04 ± 3.65	5.35 ± 3.20	0.612
No. of growing follicles on day of trigger	5.89 ± 3.14	5.13 ± 3.	0.612

4. Comparison of Ovarian Stimulation Response

The comparison between the two study groups according to ovarian response characteristics revealed that patients in the soft protocol group required a significantly lower total dose of Gn than that required for the conventional protocol group (2298.1 vs. 4517.4, P=0.001). The mean number of growing follicles and mean duration of stimulation were not significantly different (P > 0.05) between the two groups (Table 4) (Figure 1).

Table (4): Comparison between study groups by ovarian response parameters

Ovarian stimulation response parameters	Study Groups		P – Value
	Soft protocol group Mean ± SD	Conventional protocol group Mean ± SD	
No. of growing follicles	6.0 ± 3.25	5.67 ± 2.89	0.756
Duration of stimulation (Days)	10.38 ± 1.51	10.65 ± 1.75	0.892
Total units of Gn administered	2298.1 ± 578.9	4517.4 ± 1428.1	0.001

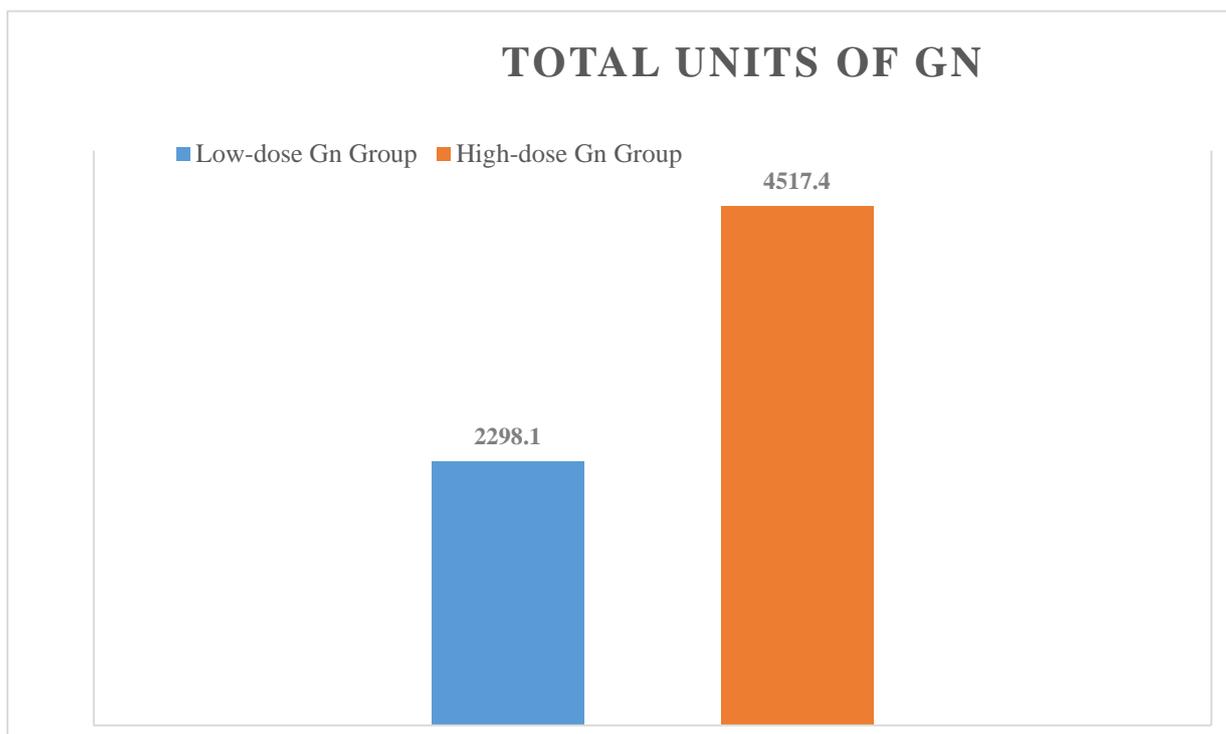


Figure 1: Mean total units of gonadotropins among the studied groups

DISCUSSION

1. Demographic Characteristics of Study groups

Regarding the demographic features of women involved in this study, which include age, duration of infertility, types of infertility and BMI there was no significant difference between them. This means that our result will depend on our interference since all women involved in this study were poor responders selected according to Bologna criteria to decrease the rate of bias. Because older age is linked to a reduction in both the quantity and the quality of oocytes, it is one of the most important factors that determines whether or not an IVF cycle will be successful⁽¹³⁾. The female fecundity declines significantly starting approximately at age 35 and decreases more rapidly after age of 40⁽¹⁴⁾. Young woman with age less than 30 has a chance of 85% to conceive within 1 year and at the age of 30 becomes 75% and declines to 66% at the age of 35 and 44% at the age of 40⁽¹⁵⁾. Most women become PORs at their late thirties or early forties, therefore, this study showed no significant difference between the two study groups.

Obesity may affect the fecundability of the women causing ovulatory infertility⁽¹⁶⁾. There is an inverse association of obesity with AMH concentration⁽¹⁷⁾. Mohamad⁽¹⁸⁾ conducted research on the relationship between BMI and the results of in vitro fertilization (IVF), and they discovered that women with a BMI of more than 25 kg/m² had lower implantation and pregnancy rates, as well as a higher rate of spontaneous abortion following IVF, when compared to women with a BMI of less than 25 kg/m². Therefore, in current study we tried to exclude obese women with high BMI > 30.

This study revealed no statistically significant difference regarding the duration of infertility. The duration of infertility for more than 2 years reduced the chances of natural pregnancy and may need ART interventions⁽¹⁹⁾. There is adverse effect on pregnancy outcome with increased infertility duration following IVF especially if associated with advancing age⁽²⁰⁾. The rate of primary or secondary infertility revealed no significant difference in both study groups since all women were poor responders.

2. Estradiol levels (Baseline, day of starting GnRH antagonist and day of trigger):

It is extremely important to determine the women's basal hormonal levels in order to rule out any underlying endocrine problems and to estimate the ovarian response. Because all of the patients who participated in this study met the criteria for POR outlined in the Bologna statement. Comparison of the basal hormone levels between the two study groups did not reveal any significant differences. Starting with the basal FSH level, which plays a significant part in follicular formation, basal hormone levels have the potential to directly influence the results of IVF as well as in cystic oophorectomy (ICSI) cycles. Measurement of serum

estradiol (basal and serial) during IVF/ICSI cycles is a significant indicator of response of ovaries to ovulation induction⁽²¹⁾.

The mean basal serum estradiol (E2) levels were compared between the two study groups and there was no significant difference (P=0.930). Moreover, as the serum estradiol level suppress FSH secretion, these two hormones should be measured together as high serum E2 level can falsely normalize FSH. The results of the present work agree with that published in **Ebrahimi et al.**⁽²²⁾ study in 2017, in which he enrolled 70 women with POR complain of infertility based on Bologna criteria in two equal groups: (n=35/each). Group (1) received only Gn 225 IU (SC) at cycle day 3. Group (2) received oral letrozole from cycle day 3 (2.5 mg twice daily) for 5 days incorporated with 225 IU of Gn. Both groups underwent flexible antagonist protocol. They found no differences in mean peak E2 level and serum progesterone level at the day of GnRH antagonist administration. This explained that letrozole showed no significant effect at day of starting antagonist and it is mainly related to the number of growing follicles. Some studies demonstrated that GnRH-antagonists showed a significant decrease in dosage and days of Gn administration because it administered in the beginning of late follicular phase after recruitment of the follicles. This protocol found to be the most suitable one for POR because there is no suppression to early endogenous gonadotropins secretion and can obtain more natural recruitment of follicles⁽²²⁾. No significant difference was found in current study between the study groups (P=0.612). This result may related to insignificant difference in estradiol levels, which is directly proportional to AFC. On the day of the trigger in the present research, there was no significant difference found in the mean levels of estradiol (P=0.086). **Ebrahimi et al.**⁽²²⁾ agree with our study by showing no significant difference in peak E2 level at trigger (P=0.36). According to **Liu et al.**⁽²³⁾, letrozole has been shown to suppress the production of ovarian estradiol, which results in an accumulation of oestrogen synthesis precursors as well as androgens (e.g. testosterone and androstenedione) In current study, although showed no significant difference in estradiol (E2) levels but we noticed that the level of E2 is much lower in soft protocol group than conventional protocol group but not reach to the level of significance. **Bulow et al.**⁽²⁴⁾ found that letrozole significantly decreased E2 levels on the ovulation trigger day, which also does not come in harmony with the current study that may be due to those normal responders women who were enrolled and fixed dose of Gn used in their study. **Tal et al.**⁽²⁵⁾ compared two groups of POR according to Bologna criteria. First group (MS) received minimal dose of letrozole 2.5 mg daily for five days, beginning from day 2 of the cycle overlapped low dose Gn (150 IU) on day 4 of the cycle and the dose of Gn was either remained constant or

elevated to 225 IU according to the response of the ovary but not higher. The second group (control group) received high levels of Gn (≥ 300 IU/day) starting from the cycle day 2. They followed antagonist protocol. The level of estrogen at the day of triggering with hCG showed significant difference. This result may be due to half dose of letrozole was used resulting in higher level of estradiol and related to the number of follicles grown since there was significant difference in the number of AFC basically⁽²⁵⁾. **Ebrahimi et al.**⁽²²⁾ also showed no significant difference in peak E2 level at trigger day.

3. Number of AFC and growing follicles

Antral follicle count is one of the most commonly parameter used to evaluate the ovarian reserve and predict the ovarian response to Gn stimulation in ART cycles after accounting the age⁽²⁶⁾. There was no significant difference regarding AFC in both study groups ($P = 0.816$). The ovarian reserve is predicted from the AFC seen within the ovary that visualized by transvaginal ultrasound and it has close correlation with the primordial follicular pool⁽²⁷⁾. While, at the day of initiating GnRH antagonist provided at the beginning of the late follicular phase following the recruitment of the follicles, many studies have shown that using GnRH-antagonists leads to a large reduction in the amount of Gn that must be given over the course of several days. This protocol found to be the most suitable one for POR because there is no suppression to early endogenous gonadotropins secretion and can obtain more natural recruitment of follicles⁽²²⁾. **Ebrahimi et al.**⁽²²⁾ agree with our study by showing no significant difference in number of growing follicles ($p=0.08$).

4. Comparison of ovarian stimulation response in study groups

The comparison between current study groups according to ovarian response characteristics revealed that soft protocol group surely needed a significantly lower total dose of Gn than for conventional protocol group ($P=0.001$). The mean number of growing follicles and mean duration of stimulation were not significantly different between the two groups ($P > 0.05$). The results published in **Ali et al.**⁽²⁸⁾ study goes in accordance to the current results, as they reported that patients with poor ovarian response managed with soft protocol required a lower dose of GnRH-antagonist in comparison with those treated with the conventional GnRH-antagonist with significant relation between both groups ($P<0.001$). Mean duration of stimulation was not significantly different between study groups ($P = 0.137$). **Tal et al.**⁽²⁵⁾ mentioned no significant difference in number of oocytes harvested, number of oocytes fertilized and number of transferred embryos but in significant clinical pregnancy rate. Moreover, results of **Ecemis et al.**⁽²⁹⁾ study yielded that ICSI cycles with letrozole, significantly had lower gonadotropin consumption. In contrary, **Olgan et al.**⁽³⁰⁾ study enrolled 45 cycles using conventional GnRH antagonist protocol

were compared to 76 cycles using a soft protocol. They found that total gonadotropin dose, and duration of stimulation, were non-significantly different between the study groups ($p > 0.05$). The study of **Ebrahimi et al.**⁽²²⁾ mentioned that GnRH antagonist used for poor responders to get benefit from the initial release of endogenous Gn that may augment the action of exogenous Gn to recruit more oocytes and to prevent mature LH surge. They found no differences in dose and duration of Gn administrated because they compared the same dose of Gn used in both groups, one with letrozole and another without letrozole.

CONCLUSION

Soft protocol is a good option for POR since obtaining the same ovarian response with less cost.

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