Comparison between Soft and Conventional Flexible Antagonist Protocols Regarding ICSI Outcome and Pregnancy Rate in Women with Poor Ovarian Response

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Abstract

Background: Poor ovarian responders (POR) are defined as the group of infertile women who are characterized by decreased the response of the ovarian follicles to gonadotropins (Gn) and/or diminished ovarian reserve making them not respond well to standard protocols leading to recruit less number of oocytes. Until now no ideal ovarian stimulation protocol has be proved for such group of women. The conventional or classical ovarian stimulation protocols using highest doses of Gn have been used for many years proposing to harvest more oocytes but recently the concept of using soft protocol using low doses of Gn with/without oral compounds such as letrozole. Soft ovarian stimulation protocol was suggested and accepted as a good option for this group of women. Gonadotropin-releasing hormone (GnRH) - antagonist protocols is the best choice for poor responders especially with low-dose Gn protocols. Objective: To evaluate the effect of soft versus conventional flexible GnRH-antagonist stimulation protocol in POR concerning IVF/ICSI outcome and pregnancy rate. Patients, Materials and Methods: This was a prospective randomized controlled trial study. It included 80 infertile poor responder's women according to Bologna criteria divided to two equal groups and undergoing IVF/ICSI antagonist protocols. One group underwent soft protocol by using letrozole 2.5mg 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. Both groups continued stimulation throughout the ovarian stimulation cycle until the day of trigger using the flexible GnRH-antagonist protocol in which all women were received 0.25 mg/day Cetrorelix when at least two or more follicles reached a size of 12-14 mm. Basal physical examination and basal hormonal assessment was performed including FSH, LH, and S. prolactin, TSH, E2, P4 and AMH associated with AFC by transvaginal ultrasound. At day of trigger, serum levels of some hormones (LH, Progesterone and Estradiol) with no. of growing follicles and endometrial thickness measurement was performed. Both groups were undergone oocyte pickup, ICSI, and embryo transfer (ET) if embryos were available or case closed if no ET and cycle outcomes with pregnancy rate were compared. The data analyzed using Statistical Package for Social Sciences (SPSS) version 25 using Pearson Chi-square test (α2-test). Independent t-test and Analysis of Variance (ANOVA) (two tailed) was used to compare the continuous variables accordingly. Result(s): The demographic characteristics between the two study groups and the comparison of baseline LH and Progesterone showed no significant difference. At day of trigger, there was a statistically significant difference in the mean levels of LH and progesterone between the studied groups. The mean levels of LH and progesterone in soft protocol group were significantly higher than in conventional protocol (P= 0.001). There is no statistically significant difference (P> 0.05) in the mean of endometrial thickness. Concerning the ICSI outcome parameters including no. of retrieved oocyte, no of MII, no. of fertilized oocyte, total no. of embryos, and no. of embryo transferred to uterus, there was no significant difference (P > 0.05). Pregnancy rate and case closed rate also showed no significant difference Conclusion: The current study showed evidence in favor of soft protocol for poor responders since it is resulted in no difference in ICSI outcome and pregnancy rates associated with much less cost effectiveness.

Keywords: poor ovarian responders, soft stimulation, conventional stimulation, letrozole, gonadotropins, gonadotropins releasing hormone- antagonist.

1. 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 the clinical management of patients who have a poor ovarian response (POR) which is still a challenge in everyday practice and disappointing for both the patient and the fertility expert (Drakopoulos, 2020). The percentage of women who have a poor ovarian response range

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from 5.6% to 35.1%, depending on how the term "poor response" is defined (Mehtap Polat, 2021). 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, advanced endometriosis, previous ovarian surgery and pelvic adhesions, metabolic and enzymatic diseases, as well as toxic, autoimmune, and infectious diseases (Blumenfeld, 2020).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 related to the number of eggs obtained after hormonal stimulation by gonadotropins (Ubaldi, 2014). 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 (Drakopoulos, 2017). The heterogeneity of this group of women may be the reason behind the difficulty in identifying the most effective strategies in management of POR (ErrÃ¡zuriz, 2019). The European Society of Human Reproduction and Embryology (ESHRE) published the Bologna criteria in 2011 in order to standardize the definition of poor ovarian response (POR). At least two of the following three criteria had to be present to establish the definition of Bologna criteria: (1) Advanced maternal age (>40 years) or any other risk factor for POR like ovarian surgery, endometriosis or chemotherapy. (2) A previous POR (≤3 oocytes with a conventional stimulation protocol). (3) An abnormal ovarian reserve test [i.e, antral follicle count (AFC) less than 5–7 follicles or anti-Müllerian hormone (AMH) below 0.5–1.1 ng/ml] (Younis, 2015) 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 (Weissman, 2018). 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 (Bunpei Ishizuka 2021). The oocyte donation is now regarded the most effective and reliable option for POR, but the vast majority of patients are insisting about using their own oocytes in many attempts in order to obtain the desired pregnancy (Boudry, 2021). Many of the procedures that are employed for patients who are POR are concentrating on reducing the amount of gonadotropins they received (Adrija Kumar Datta, 2021). 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 (Wonga, 2019).

2. Materials and Methods

This is a prospective randomized controlled trial conducted in the IVF center of the High Institute for Infertility Diagnosis and Assisted Reproductive Technology / AL-Nahrian University from November 2020 to May 2022. Ethical approval of the present study was issued by the Local Medical Ethical Committee of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University. It includes 80 infertile poor responder's women according to Bologna criteria undergoing ICSI protocols. Eighty poor responder's women were enrolled in this study. All infertile couples were subjected to a full history taking including duration of infertility, 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 trans-vaginal ultrasound to exclude any ovarian cyst, 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 evaluation of uterine cavity and tubal patency. All participants were poor responders according to Bologna criteria and undergone intracytoplasmic sperm injection (ICSI) programs using the flexible GnRH-antagonist protocol and divided to 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 gonadotropins (225 IU) from cycle day (4-5) continued throughout the ovarian stimulation cycle till the day of trigger. Second group received high doses gonadotropins (450 IU) only from cycle day (2-3) for ovarian stimulation and continued throughout the ovarian stimulation cycle until the day of trigger. Patients were then received 0.25 mg/day Cetrorelix when at least two or more follicles reached a size of 12-14 mm. Inclusion criteria included patient with poor ovarian response according to Bologna Criteria, which includes the following (ferritte, 2011): (1) Advanced maternal age (≥40 years) or any other risk factors for POR like ovarian surgery, chemotherapy or radiotherapy. (2) A previous POR (cycles cancelled or gave ≤3 oocytes with a conventional stimulation protocol). (3) An abnormal ovarian reserve test (AFC < 5–7 follicles or AMH < 0.5–1.1 ng/mL). At least two of the above three criteria had to be present to establish the definition of POR. All cycles included for each group represented their first IVF attempt. Exclusion Criteria included: Day-3 FSH > 30 IU/L and AMH below 0.5, age above 45 years. uncontrolled endocrine or metabolic disorders like diabetes mellitus, and cushings' 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 were undergone oocyte pickup and oocytes classified according to maturity after denudation and then injected with the sperm. Embryo transfer were performed if embryos available but when there was no oocytes retrieved, no fertilization or embryos formed but they did not complete division (arrest) and 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 rate. The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. The significance of difference of different percentages (qualitative data) were tested using Pearson Chi-square test (α2-test) with application of Yate's correction or Fisher Exact test whenever applicable. Independent t-test and Analysis of Variance (ANOVA) (two tailed) was used to compare the continuous variables accordingly.

3. Results

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 regard 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 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

Basal LH and Progesterone hormones Analysis

The comparison between the two groups according to baseline hormones showed no statistically significant difference (P>0.05) between the soft protocol group and conventional protocol group in consideration to mean levels of LH, progesterone since all women were poor ovarian responders according to Bologna criteria (table 2). No comparison for basal endometrial thickness (EM) since all women had basal EM>4mm.

Table 2: Baseline LH and Progesterone hormones Analysis

Basal Hormonal Profile	Study Groups		P – Value
	Soft protocol Mean ± SD	Conventional protocol Mean ± SD	
LH (mIU/mL)	3.78 ± 1.40	3.76 ± 0.89	0.945
Progesterone (ng/mL)	0.45 ± 0.27	0.46 ± 0.18	0.918

SD: standard deviation, LH: luteinizing hormone

Comparison between study groups for Progesterone and LH levels and

Endometrial Thickness at day of trigger

On day of trigger, there was a statistically significant difference in the mean levels of LH and progesterone between the studied groups. The mean levels of LH and progesterone in the soft protocol group were significantly higher than in the conventional protocol

(3.88 mIU/mL vs. 3.04 mIU/mL, P=0.001) and (1.06 ng/mL vs. 0.90 ng/mL), respectively. Concerning endometrial thickness there was no significant difference between the two study groups (P=0.724). (Table 3) (Figure 1).

Table 3: Comparison Between Study Groups for Progesterone and LH Levels and Endometrial Thickness at Day of Trigger			
Hormones and endometrial thickness at day of trigger	Study Groups		P – Value
	Soft protocol Mean ± SD	Conventional protocol Mean ± SD	
LH (mIU/mL)	3.88 ± 0.42	3.04 ± 0.63	0.001
Progesterone (ng/mL)	1.06 ± 0.16	0.90 ± 0.12	0.001
mm: millimeter			

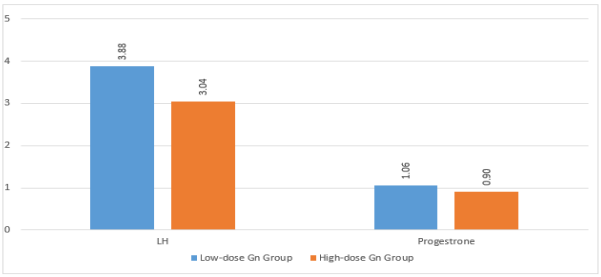

Figure 1: Mean levels of LH, progesterone between study groups at day of trigger

Comparison of ICSI Outcome

In the present study, although there was no significant difference (P>0.05) among the two study groups with regard to the ICSI outcome parameters including number of retrieved oocyte, MI, MII, germinal vesical, number of fertilized oocyte, total number of embryo, and embryo transferred to uterus but the no. of retrieved oocytes and no. of MII was higher in soft protocol group than the conventional protocol group (Table 2).

Table 4: Comparison between study groups by ICSI outcome parameters			
ICSI outcome parameters	Study Groups		P value
	Soft protocol Group Mean ± SD	Conventional protocol Group Mean ± SD	
Number of Oocytes Retrieved	5.89 ± 2.52	4.56 ± 2.67	0.052
MII	4.22 ± 2.15	3.18 ± 2.18	0.066
MI	0.81 ± 0.79	0.88 ± 0.98	0.772
Germinal Vesical	0.30 ± 0.47	0.21 ± 0.48	0.461
Abnormal	0.52 ± 0.75	0.29 ± 0.52	0.176
Number Of Fertilized Oocytes	3.11 ± 2.15	2.38 ± 2.03	0.180
Total Number of Embryo	2.48 ± 1.74	2.09 ± 1.76	0.388
Embryo Transferred to Uterus	**1.89 ± 1.28**	**1.65 ± 1.35**	**0.479**

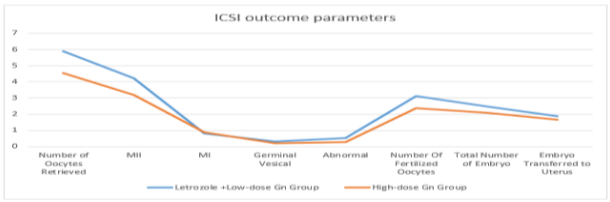

***Figure (1):** Comparison between study groups by ICSI outcome parameters*

Comparison of Pregnancy Outcome

It was obvious that the pregnancy rate in the soft protocol group was lower than in the control group, but the difference in the pregnancy rate between the two study groups was not significant. Furthermore, there was no statistically significant difference (P>0.05) in the case closed between the two studied groups (Table 3).

Table 5: Comparison between study groups according to pregnancy outcome			
Clinical Pregnancy	Study Groups		P- Value
	Soft protocol Group no (%)	Conventional protocol Group no (%)	
Positive	4 (10.0)	7 (17.5)	0.560
Negative	23 (57.5)	27 (67.5)	
Case Closed			
Yes	13 (32.5)	6 (15.0)	0.065
No	27 (67.5)	24 (85.0)	
no: number			

4. Discussion

Overview

Infertility is considered as a medical condition, which can cause psychological, physical, mental, spiritual, and medical detriments to the patient (Walker 2021). Women with POR represent about 20% of all women undergoing IVF/ICSI cycles, they usually faced a big problem due to reduced number of oocyte retrieved, increased cancellation rates, decreased pregnancy rates and live birth rate. The most suitable protocol used in ovarian stimulation for POR is still a matter of debate and represent a big challenge in ART field (Di Guardo, Blockeel et al. 2022). There are many protocols for COH for POR but there was no superiority of the effectiveness of one stimulation protocol over another. Mild ovarian stimulation

protocols using low doses of gonadotropins with or without oral compounds have significant advantages over the other protocols including mainly cost effectiveness. Letrozole is one of the main oral drugs used in mild protocols for ovarian stimulation of poor responders (Ali, et al. 2022). The idea of the present study indicated that the adding of letrozole to Gn in the ovarian stimulation protocol in POR patients could enhance pregnancy outcomes and decrease gonadotropin use. Although the results showed no difference between the two groups in the clinical and chemical pregnancy rates. Usually, POR present with a shortened follicular phase, which decreases the time available for follicular recruitment and lower FSH receptor expression levels in granulosa cells. Scientists suggested letrozole as an aromatase inhibitor that can prolong the follicular phase and give more time and chance for follicular recruitment through raised intra-ovarian androgens and suppression to estrogens formation (Tal Lazer, 2014). Letrozole prolongs the action of FSH by affecting the pituitary gland as a feedback action to decrease estrogen and more FSH and LH secreted. Raised intra-ovarian androgens may improve ovarian response to exogenous Gn by increasing the sensitivity of FSH receptors and play a role in preantral and antral follicular development and so the gonadotropin doses that required for stimulation are decreased (Kaçar, Biler et al. 2022).

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 (Table 1). This means that our result will depend on our interference since all women involved in this study are poor responders selected according to bologna criteria to decrease the rate of bias. Age is one of the most significant factors that predict the success of IVF cycles because it is associated with reduced number and the quality of oocytes (McLernon et al. 2016) The female fecundity declines significantly starting approximately at age 35 and decreases more rapidly after age 40 (Steiner et al., 2016). 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 (Ilse Delbaere, 2020). Most women become PORs at their late thirties or early forties, therefore, this study show no significant difference between the two study groups.

Obesity may affect the fecundability of the women causing ovulatory infertility. (Gaskins AJ, et al. 2018). There is an inverse association of obesity with AMH concentration (Ashley M. Eskew, 2022). Mohamad Irani, (2017) studied the correlation BMI and IVF outcomes and found that women with BMI > 25 kg/m2 exhibited lower implantation and pregnancy rates and higher spontaneous abortion rate following IVF compared to women with BMI ≤ 25 kg/m2. Therefore, in current study we tried to exclude obese women with high BMI>30.

This study revealed no statistically significant difference with regard to the duration of infertility. The duration of infertility for more than 2 years reduced the chances of natural pregnancy and may need ART interventions (So Hyun Ahn, et al. 2021). There is adverse effect on pregnancy outcome with increased infertility duration following IVF especially if associated with advancing age (Bhattacharya et al., 2013). The rate of primary or secondary infertility revealed no significant difference in both study groups since all women were poor responders.

Basal LH and Progesterone Hormones Analysis

It is very essential to assess the basal hormonal levels of women as part of IVF setting to exclude any underlying endocrine abnormalities and to predict the ovarian response. Comparison of the basal hormones between the two study groups showed no significant difference because all patients enrolled in this study were POR according to bologna criteria. Basal hormonal levels can affect directly the outcomes of IVF/ICSI cycles, starting with LH, follicular growth and development are also related LH, which enhances ovarian steroidogenesis in synergy with FSH. In granulosa cells, FSH stimulates the development of ovarian follicles, while in theca cells, LH action is involved in follicle development and maturation and this can be achieved with less than 1% of LH receptors has been occupied (J Smitz, 2020). Aging is one of the factors causes deficiency or defect in LH and FSH action affecting and ovarian steroid production and reduced ovarian response (Bosch, 2021). Therefore, LH supplementation in IVF/ICSI cycles improves poor ovarian response and may increase the sensitivity of the receptors of granulosa cells of the small antral follicles to Gn promoting follicular growth and survival by suppressing granulosa cell apoptosis and follicular atresia, resulting in multiple follicular development. All women enrolled in the study since they were POR had basal LH levels at lowest normal and for this reason; we used urinary menotropin (Human menopausal gonadotropins (HMG) to get more no. of growing follicles. There was no significant difference in the baseline serum level of LH between the two groups (P= 0.377) (Table 2). Concerning the basal progesterone, level means in this study, there was no statistically significant difference among the two study groups (P=0.918). It is uncommon the elevation of basal progesterone during ovarian stimulation cycles and may suggest the incomplete luteolysis of the corpus luteum, endogenous production by the adrenals or ovarian aging (Carlos Hernandez-Nieto, 2020).

Comparison between Study Groups for Progesterone and LH levels and Endometrial Thickness at Day of Trigger

At day of trigger in the current study, a significant

difference in the mean levels of LH and progesterone observed. The mean levels of LH and progesterone in the soft protocol group were significantly higher than in the conventional dose (P=0.001), Concerning the assessment of endometrial thickness, current study showed no significant difference P= 0.724. Ebrahemi 2017 agreed our study by showing no significant difference in endometrial thickness p=0.1 and no significant difference regarding level of progesterone P= 0.12. In comparison to other studies, Liu and other co-workers in a study conducted in 2022 worked on letrozole supplementation to reduce costs and increase ovarian sensitivity and the increased risk of elevated progesterone levels on trigger day. Liu, et al. observed a similar finding and found that patients managed with letrozole, and gonadotropin significantly presented with higher progesterone levels (P<0.001) and a significant higher LH levels on trigger day (P <0.001).

The study of liu, and et al (2022) hypothesized that late increase in follicular blood progesterone might be related to the following:

I. An increase in progesterone secretion from the recruited follicles; the greater the number of follicles, the higher the progesterone level.
II. Pathological progestogen, which is related to premature luteinisation of follicles and decreased follicle quality, which is always accompanied with LH surge.
III. Use of letrozole in controlled ovarian stimulation protocol (liu, et al. 2022).

The study of (Liu, 2022) concluded that increased progesterone levels strongly related to letrozole use. He explained this:

1. Elevated progesterone level manifested at day of trigger with women in the soft protocol group even when the number of growing follicles was similar in comparison with women not receiving letrozole.
2. With increasing numbers of growing follicles, women in the soft protocol group had a greater increase in progesterone levels than women in the GnRH-antagonist group without letrozole.

These evidences abolish the possibility that high progesterone levels in the soft protocol group are referred to the increase in the number of follicles. Regarding the mechanism through which letrozole elevates progesterone.

Once the accumulation of androgens reaches a certain threshold, progesterone accumulates, increasing its levels in the blood. This hypothesis also has been proven in previous studies like (Bülow, 2022). In Bülow et al study compared two groups: one received oral letrozole either 5 mg/day for 5 days (letrozole group) or placebo (placebo group), along with the same dose (150 IU) FSH and normal responders' women. They reported that premature progesterone >1.5 ng/ml was similar and no significant difference in both groups (Bülow, 2021). The result of Bülow, et al study not agreed with result of current study concerning relation of increased level of progesterone to administration of letrozole as adjuvant treatment. This result may be related that women involved in Bülow study were normal responders and both groups received the same Gn dose. Concerning LH levels comparison between the study groups in current study showed significant difference P=0.001. Higher levels of LH in soft protocol group may be due to letrozole administration, which enhance the pituitary gland to secrete more FSH and LH as an effect of feedback inhibition to hypoesterogenima produced by inhibition of aromatization (Eftekhar, 2020).

Comparison of ICSI Outcome of Study Groups

In the present study, despite no significant difference between study groups concerning to the ICSI outcome parameters as number of retrieved oocyte (p= 0.052) MI (P= 0.772) , MII (P= 0.066) , abnormal (P=0.176), number of fertilized oocyte (P=0.180), total number of embryo(P=0.388), and embryo transferred to uterus(P= 0.479) but the no. of retrieved oocytes and no. of MII was higher in soft protocol group than the high dose group as shown in Table (5). This result mostly due to adding letrozole that prevent androgen conversion to estrogen. Letrozole is a preferable drug due to its oral administration and low cost. Mahbod Ebrahimi et al. (2017) agreed with our study and showed no significant difference in the number of oocytes retrieved and fertilization rate with adding letrozole to GnRH antagonist cycles in women with POR which means adding letrozole in COH protocol in POR needs well-designed prospective randomize trials with large sample size to precise the role of letrozole in COH protocols of POR. In a different manner, Ali and other co-authors in their study in 2022, which include 120 poor responder women divided into Group 1: 60 patients received letrozole+ minimum dose (150 IU) GnRH-antagonist, and Group 2: included 60 patients received conventional dose (300-450IU) GnRH-antagonist protocol. They observed no significant difference between study groups regarding fertilization rate and transferred embryos. Also, there is not statistically difference between groups in consideration to number of days of stimulation. There was significant difference regarding all dosage of gonadotropin, mature follicles number, endometrial thickness and number of M2oocytes were comparable with statistically significance difference between soft group and conventional group may leads to such results since it blocks aromatization and enhance pituitary Gn secretion. (Ali, Abdellah et al. 2022). In ICSI cycles managed with letrozole in Ecemis et al study, mean number of metaphase II and fertilized oocytes retrieved were significantly higher compared to cycles without letrozole (P<0.05), concluded that number of oocytes retrieved is higher in letrozole might indicate that letrozole contribute to successful ovarian stimulation with a lower dosage of

gonadotropins (Ecemis, Tasci et al. 2016). Lazer and co-workers compared pregnancy and IVF outcomes of poor responder patients to the treatment with high doses of Gn and those treated with Gn and letrozole with minimal doses (150IU). Even though no significant differences were observed in the number of oocytes retrieved and the eggs fertilized between the two protocols, a significantly higher rates of clinical pregnancy and live birth were evident in the group received minimal dose of gonadotropin + letrozole (Lazer Tal, 2014). Poor responders generally have markedly low number of follicles available for recruitment and the ovarian response is mostly affected by the number of follicles available for recruitment (AFC) rather than the levels of circulating gonadotropins which at some point saturation kinetics are attained. Usually, the eggs of POR women are of bad quality and can produce bad quality embryos and high doses of FSH recruit these "resistant" follicles to save them from death leading to bad quality embryos formation or even no fertilization (Eftekhar, 2014).

Comparison of Pregnancy Outcome of study groups

In current study, pregnancy rate considered a secondary outcome since there was many factors that can affect the pregnancy especially laboratory factors or male factors. Therefore, we cannot rely on pregnancy rate in this study. PR in the soft protocol group (10.0%) was lower than in the conventional protocol group (17.5%), but the difference in the pregnancy rate was not significant. This may be attributed to adding letrozole to Gn in GnRH antagonists' protocol that lead to significant increase in progesterone level which has a determinant effect on the endometrium. High level of progesterone significantly altered DNA methylation and gene expression of adhesion molecules on endometrium on the day of hCG administration in IVF cycle (Yujing Xiong, 2020). Since our study involved comparing soft protocol group with high dose, we cannot be certain that our results were not related to the use of letrozole rather than high versus low dose gonadotropins. Tal Lazer disagree with our result and concluded that mild stimulation protocol improved both clinical pregnancy and live birth rates, compared to the conventional protocol (Tal Lazer, 2016). Furthermore, no significant difference in the case closed between the two studied groups (P>0.05). This result goes with Tal Lazer, 2016 who found also no significance in cancellation rate. The current results agreed to that published in Bülow et al study who reported that ongoing pregnancy rate was similar between both groups (31% vs 39% (p = 0.55)) (Bülow, Skouby et al. 2021). Finally, Lazer and co-workers in their study a significantly higher rates of clinical pregnancy and live birth were evident in the group of soft protocol because too much Gn has a negative effect on the endometrium rather than on oocyte quality as well as the significant modulation of genes and altered gene expression profiles that participate in processes of implantation and not due to lertrozole (Lazer and Dar 2014).

5. Conclusion

This study gives evidence in direction of using soft protocols in antagonist ICSI cycles as first line treatment protocol for POR women since it consumed less doses of gonadotropins that means less cost and gives no significant difference in ICSI outcome or pregnancy rate.

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Author Contribution

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Conflict of Interest

The author declares no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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