THE ROLE OF LETROZOLE (LE) IN CONTROLLED OVARIAN STIMULATION (COS) DURING IVF IN PCOS PATIENTS: RANDOMIZED CONTROLLED STUDY

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Abstract

Background: Polycystic ovarian syndrome (PCOS) is a common endocrine disorder, characterized by hyperandrogenism, anovulation, and polycystic ovaries, which significantly increase risk of infertility. The mainstay treatment for infertility in PCOS patients is ovulation induction which may cause one of the most serious complication which is Ovarian hyper stimulation syndrome.

Objective: The aim of the current study was to compare conventional GnRH-antagonist protocol with and without LE in PCOS patients at high risk of ovarian hyperstimulation syndrome (OHSS).

Patients and methods: This was double blinded: A prospective randomized controlled study which was conducted in In Vitro Fertilization (IVF) unit, Ain Shams Maternity Hospitals on 44 women, during period from February 2020 to December 2020. The patients were randomly divided into 2 equal groups: Group (A): Control group: consisted of PCOS women who received COS with conventional multiple-dose GnRH- antagonist protocol and placebo, and Group (B): Study group: consisted of women with PCOS who received COS with a conventional antagonist protocol and LE.

Results: The results of the current study revealed that no statistical significant difference between studied groups as regard OHSS & low count before trigger. There was 7 patient (31.8%) developed OHSS in group A. While, there was 2 patients (9.1%) developed OHSS in group B before trigger.In group A, there were 5 patients (71.4%) of mild OHSS and 2 patients (28.8%) of moderate OHSS.In group B, there were 2 patients (100%) of mild OHSS. There was 3 patient (13.6%) of low count in group A and no patients of low count in group B. Also it was found that no statistical significant difference between studied groups as regard OHSS after trigger. There was 1 patient (4.5%)developed OHSS of moderate grade in group A while there were no patients developed OHSS in group B after trigger.

Conclusion: The current study concluded that the Addition of LH to conventional GnRH- antagonist protocol in PCOS patients which are at high risk of OHSS may be the same as using conventional GnRH-antagonist protocol only in prevention of OHSS in controlled ovarian stimulation (COS) during IVF.

Keywords: Letrozole, Controlled Ovarian Stimulation, IVF, PCOS.

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Introduction:

Polycystic ovarian syndrome (PCOS) one of the most common endocrine/metabolic disorders of women. This syndrome was first described by **Stein and Leventhal [(1]),** although the presence of sclerocystic ovaries had been recognized for at least 90 years prior to their report **[(2]).**

PCOS is a leading cause of female infertility and the most common endocrine disorder in women of reproductive age **[(3]).** IVF in PCOS patients remains a challenge **[(4]).** Due to the high sensitivity of the polycystic ovaries to gonadotropin stimulation, treatment with gonadotropins is difficult to control and may result in OHSS **[(5]).** Thus, while in COS cycles in the general population,the incidence of moderate and severe OHSS is 3% to 8%, in PCOS patients, it increases from 10%to 20% **[(3]).**

There are a number of risk factors for OHSS, including, previous episode of OHSS, Polycystic ovary syndrome (PCOS), basal serum anti-müllerian hormone (AMH) concentration >3.3 ng/mL, and an antral follicle count (AFC) >8 **[(6]).**

Among several suggestions that have been made to prevent OHSS **[(7]),** the use of a gonadotropin releasing factor antagonist protocol (GnRH-ant), agonist triggering, low-dose gonadotropins and a „freeze all" concept with elective frozen embryo transfer (FET) are the most commonly accepted **[(8]).** Gonadotropin-releasing hormone (GnRH) antagonist protocol**[(9, 10)]**, GnRH agonist for oocyte

maturation triggering are suggested to be effective in reducing OHSS risk. Nonetheless, the luteal lysis effect following GnRH agonist triggering may reduce the chance of pregnancy in fresh embryo transfer cycle [(11)].

There was evidence of a lower live birth rate, reduced ongoing pregnancy rate and higher miscarriage rate in women who received a GnRH agonist for final oocyte maturation triggercompared to women given HCG, in fresh autologous cycles (woman's own eggs) [(12)]. Letrozole is now considered to be the drug of choice for ovulation induction in women with PCOS. Clomiphene citrate has been the first-line drug for this population for many years, with metformin used as an alternative. However, both clomiphene and metformin appear to be less effective for live birth rates than letrozole [(13)].

Very recently, the co-administration of letrozole (LE) for ovarian stimulation has been suggested to lower the risk of OHSS in high-risk women and PCOS patients [(14)]. However, the data remain controversial [(3)].

The aim of the current study was to compare conventional GnRH-antagonist protocol with and without LE in PCOS patients at high risk of OHSS.

Patients and Methods:

This was double blinded: A prospective randomized controlled study which was conducted in IVF unit, Ain Shams Maternity Hospitals on 44 women, during period from February 2020 to December 2020.

An approval of the study was obtained from Ain Shams University academic and ethical committee. Every patient signed an informed written consent for acceptance of the operation.

Inclusion criteria: Patients whom ages between 21 and 38, no hormonal treatment during last 3 months, body mass index (BMI) 25-30 kg/m^2, and AMH > 7 ng/mL.

Exclusion criteria: Patients with systemic diseases, patients with endocrinological disorders, body mass index (BMI) >30kg/m^2 or < 25kg/m^2, unfit for surgery.

Randomization:

Were done using computer generated randomization sheet using MedCale© Version 13. The patients were randomized to either Group A or Group B based on the below tables with random numbers assigned to each group. The random numbers were generated by online Random number Generator computer program (stattrek.com/statistics/random-number-generator).

Sample size calculation:

Using PASS (11) Program, for sample size calculation an according to **Tshzmachyan and Hambartsoumian** [(3)], the expected frequency of OHSS in study group 8% and control group = 42%, sample size of 20 patients per group can detect this difference with power 80% and a-error 0.05. Sample size were increased by 10% to accommodate for dropouts (22 per each group).

Randomization and allocation:

We randomly assigned all women who accepted to participate in the study were after their randomly assigned into one of the following two groups:

- **Group (A):** Control group: consisted of PCOS women who received COS with conventional multiple-dose GnRH- antagonist protocol and placebo.
- **Group (B):** Study group: consisted of women with PCOS who received COS with a conventional antagonist protocol and LE.

Study intervention: The outcome: Occurrence of at least moderate OHSS.

Transvaginal sonography (TVS):

We examined all participants with TVS. The women were scanned using TVS with 5-9-MHz transducer. Endometrial thickness and lining, as well as the day of ovulation were documented according to the protocol of our unit.

Controlled ovarian stimulation (COS):

We supplied A COS with multiple-dose GnRH antagonist protocol according to the protocol of our unit for patients at high risk for OHSS. We administrated Follicle-stimulating hormone (FSH) (Fostimon, IBSA, Switzerland) were from day 2 of the cycle with a starting dose 150 IU until the day of triggering final follicular maturation. Then we added Cetrotide "the antagonist" at a dose of 0.1 mg /day s/c (Cetrotide, Serono Merck, Italy) from day 6. Following that we monitor Follicular maturation were every 1 to 2 days. Once the dominant follicle reached 19-20 mm in size, oocyte maturation were gave 0.2 mg of triptorelin (Decapeptyl, Ferring Pharmaceuticals, Switzerland) for trigger. We added Placebo in the form of two tablets for each patients in thecontrol group from day 3 to day 7 of the cycle.

Criteria of abruptly cancelled cycle patients before giving the trigger:

Relevant symptoms from a woman suspected to be suffering from OHSS: Abdominal bloating, abdominal discomfort/pain, need for analgesia, nausea and vomiting, breathlessness, inability to lie flat or talk in full sentences Reduced urine output, leg swelling, vulval swelling, and associated comorbidities such as thrombosis.

Examination and investigation of women with suspected OHSS:

Examination:

- General: assess for dehydration, oedema (pedal, vulvar and sacral); record heart rate,respiratory rate, blood pressure, body weight.
- Abdominal: assess for ascites, palpable mass, peritonism; measure girth.
- Respiratory: assess for pleural effusion, pneumonia, pulmonary oedema.

Investigations :

- Full blood count.
- Haematocrit (haemoconcentration).
- C-reactive protein (severity).
- Urea and electrolytes (hyponatraemia and hyperkalaemia).
- Serum osmolality (hypo-osmolality).
- Liver function tests (elevated enzymes and reduced albumin).
- Coagulation profile (elevated fibrinogen and reduced antithrombin).
- Ultrasound scan: ovarian size, pelvic and abdominal free fluid. Consider ovarian Doppler if torsion suspected [15].

The cancelled cycle patients were analyzed as failure for extreme hyper response. Transvaginal ultrasound-guided oocyte retrieval were conducted 35–36 h after the trigger, which was done by the most expert doctor. In the study group (group B), the only difference were the addition of Femapent (letrozole, Penta Pharma Egypt, Egypt) to the COS protocol, at a dosage of 5 mg per day starting from day 3 to day 7 of the cycle. Aspirated oocytes were fertilized by IVF and embryos were transferred on day 3 or days 5 from the day of ovum pickup, which was done by the most expert doctor.

Statistical analysis:

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage. Independent-samples t-test of significance was used when comparing between two means. Chi-square (x^2) test of significance wasused in order to compare proportions between two qualitative parameters. The confidence interval was set to 95% and the margin of error accepted was set to 5%. P value < 0.05 was considered significant.

RESULTS:

Table (1): Comparison between studied groups as regard age and BMI.

		Group A(N = 22)	Group B(N = 22)	P-value
Age (years)	**Mean**	27.8	28.1	0.812 NS
	±SD	5.5	4.6	
BMI (kg/m^2)	**Mean**	28.06	27.9	0.822 NS
	±SD	1.43	1.36	

NS: p-value > 0.05 is considered non-significant.

There were no statistical significant difference **(p-value > 0.05)** between studied groups as regard age and BMI (Table 1).

Table (2): Comparison between studied groups as regard AMH.

		Group A(N = 22)	Group B(N = 22)	P-value
AMH (ng/ml)	**Mean**	8.4	8.3	0.368 NS
	±SD	0.57	0.54	

NS: p-value > 0.05 is considered non-significant.

There was no statistical significant difference **(p-value > 0.05)** between studied groups as regard AMH (Table 2).

Table (3): Comparison between studied groups as regard endometrial thickness.

		Group A(N = 22)	Group B(N = 22)	P-value
Endometrial thickness (mm)	**Mean**	8.9	8.1	0.151 NS
	±SD	1.8	1.6	

NS: p-value > 0.05 is considered non-significant.

There was no statistical significant difference **(p-value > 0.05)** between studied groups as regard endometrial thickness (Table 3).

Table (4): Comparison between studied groups as regard OHSS before trigger.

Before trigger		Group A(N = 22)		Group B(N = 22)		P-value
OHSS	**No**	15	68.2%	20	90.9%	0.061 NS
	Yes	7	31.8%	2	9.1%	
OHSS grade	**Mild**	5	71.4%	2	100%	0.391 NS
	Moderate	2	28.8%	0	0%	
Low count	**No**	19	86.4%	22	100%	0.072 NS
	Yes	3	13.6%	0	0%	

NS: p-value > 0.05 is considered non-significant.

There was no statistical significant difference **(p-value > 0.05)** between studied groups as regard OHSS & low count before trigger. There was 7 patient (31.8%) developed OHSS in group A while there was 2 patients (9.1%) developed OHSS in group B before trigger. In group A, there were 5 patients (71.4%) of mild OHSS and 2 patients (28.8%) of moderate OHSS. In group B, there were 2 patients (100%) of mild OHSS. There were 3 patients (13.6%) of low count in group A (Table 4).

Table (5): Comparison between studied groups as regard OHSS after trigger.

After trigger		Group A(N = 22)		Group B(N = 22)		P-value
OHSS	**No**	21	95.5%	22	100%	0.311 NS
	Yes	1	4.5%	0	0%	

NS: p-value > 0.05 is considered non-significant.

There was no statistical significant difference **(p-value > 0.05)** between studied groups as regard OHSS after trigger. There was 1 patient (4.5%) developed OHSS of moderate grade in group A while there were no patients developed OHSS in group B after trigger (Table 5).

DISCUSSION:

The age group used in our study was between 21 and 38 years. The mean age of the patients included in Group A was 27.8 and 28.1 in group B there was no statistical significant difference between both groups as regard age.

Fischer et al. [16] agreed with our study as regard age group of patients. This study aimed at comparing the occurrence of OHSS, under a lower, individualized gonadotrophin dosing mainly in the long agonist protocol between PCOS patients (group A) and patients without PCOS (group B). The mean age of this study was 34.1 in group A and 32.7 in group B, also there was no significant difference between the two groups as regard age.

This agreement is due to that the women between 21 and 38 years are in the child bearing period and also give us a wide range of patients with PCOS.

As regard body mass index (BMI) patients included in our study were 25-30 kg/m^2.therewas no statistical significant difference between studied groups, as mean of BMI in group A was
28.06 and was 27.9in group B in the present study.

Guang et al. [17] study supports our study in the chosen range of BMI. This retrospective study investigated the efficacy and safety of letrozole for patients with polycystic ovary syndrome (PCOS) as it included 136 cases allocated into two groups: 68 cases underwent letrozole (Letrozole group). The other 68 cases received clomiphene (clomiphene group). All cases in both groups
received the treatment up to 5 cycles. The mean BMI in the letrozele group was 28.4 and in the clomiphene group was 27.8. There was no significant difference between two groups.

In the current study, anti-Mullerian hormone (AMH) was > 7ng/ml. Mean of AMH in group A was 8.4 and was 8.3 in group B, there was no statistical significant difference between both groups as regard AMH.

In the study done by **Fischer et al. [16]**, agreed with our study as they concluded that mean AMH in group A (PCOS patients) was 7.5, and the mean AMH in group b (patients without PCOS) stated that AMH were higher in the PCOS-group and thus there was a significant difference between the two studied groups.

Also, **Tshzmachyan and Hambartsoumian [3]** agreed with our study as regard AMH. They conducted a prospective randomized controlled pilot study presenting the role of Letrozole in controlled ovarian stimulation in patients at high risk to develop OHSS.

In their control and study groups, they included patients with extremely high level of serum AMH > 50 pmol/L or AMH > 7 ng/mL, and stated that, (AMH) is a sensitive marker of ovarian reserve that is highly correlated with the ovarian response to

stimulation during IVF. Thus, women with PCOS and high levels of AMH undergoing IVF constitute a specific group at high risk for OHSS. The optimal threshold of AMH to predict OHSS was suggested to be 49.63 pmol/L (6.9 ng/mL). These patients experienced a nine times higher frequency of OHSS.

Our study concluded that, as regard low count of oocytes, there was low count of oocytes in 3 patients in our control group (non letrozole group) which their cycles were canceled and there wasno patients with low count in the study group (letrozole group), But there was no statistical significant difference between the studied groups as low count before trigger.

Ben-Haroush et al. [18] studied the effect of co-administration of letrozole and gonadotropins during ovarian stimulation on oocyte yield and maturation in breast cancer patients prior to chemotherapy and their results come in agreement with our study which is that the addition of letrozole to gonadotropins does not increase the number of oocytes retrieved or the oocyte maturation rate.

In contrast to our study, **Chen et al. [19]** was a retrospective clinical review included 181 cases of women with PCOS who underwent IVF cycles with intracytoplasmic sperm injection (ICSI) and embryo transfer (IVF/ICSI-ET). The day before the use of human chorionic gonadotropin (hCG), cases were divided into a letrozole-treated group (N=78) and a non-letrozole group (N=103). An oral dose of 2.5 mg qd of letrozole was given when the peak level of estradiol (E_2) was ≥ 4000 pg/ml during ovarian stimulation and ceased before the day of egg retrieval and concluded that the letrozole-treated group had a significant increase in the number of retrieved oocytes.

Also, **Garcia-Velasco et al. [20]** study comes in contrast with our study as regard the effect of letrozole on the number of the oocytes. This study aimed to evaluate the impact of aromatase inhibitors as adjuvant treatment in IVF cycles on intraovarian androgens and cycle outcome. It included 147 patients low responder patients with a previous canceled IVF cycle; 71 patients were treated with letrozole 2.5 mg plus a high-dose FSH/hMG-antagonist regimen, and 76 patients were similarly treated but letrozole was not employed. This study concluded that Letrozole-treated patients had a higher number of oocytes retrieved and a higher implantation rate despite similar doses of FSH/hMG.

This disagreement with our study is due the large number of patients, 181 patients in **Chen et al. [19]** study and 147 patients in **Garcia-Velasco et al. [20]** study thus there were statistical difference in the results of both studies. The low number of patients in our study may be cause that there was no statistical difference between the two groups despite the difference in number of patients between them.

In our study we concluded that before giving the trigger, there was 7 patients developed OHSS in group A (5 mild and 2 moderate OHSS) while there was 2 patients developed OHSS in group B and both were of mild OHSS. There was no statistical difference between the two groups.

As regard occurrence of OHSS after trigger: There was 1 patient developed OHSS of moderate grade in group A while there were no patients developed OHSS in group B. There was no statistical significant difference between studied groups as regard OHSS after trigger.

These findings are reinforced with the results of a study conducted by **Wang et al. [21]** to explore the efficiency of using aromatase inhibitors during luteal phase in IVF stimulated cycles for patients at high risk for OHSS, and concluded that the Treatment with letrzole in luteal phase decreases serum estrogen levels of patients after oocyte retrieval, but it couldn't reduce the risk of severe OHSS as there was no statistical difference between both groups.

The advantage of our study over the **Wang et al. [21]** study is that we reported the different grades of OHSS not only the severe cases.

Also, in our study we did not encounter a single case of severe OHSS, neither in the control nor the study groups. Also, we did not observe cycle cancellations related to the risk of OHSS in both groups. This is mainly because of the GnRH-agonist trigger, which results in the so-called "safe IVF-clinic protocol".

Chen et al. [19] agreed with our study as regard the incidence OHSS, and reported that it was lower in the letrozole-treated group 7.77 (8/103) than in the letrozole-non treated group 2.56 (2/78) but this difference did not reach statistical significance (P>0.05).

Although **Chen et al. [19]** study is same as our study in the timing of giving LE (during ovarian stimulation and ceased before egg retrieval), it didn't report which cases of OHSS were before the trigger and which were after.

On the other hand, **Tshzmachyan and Hambartsoumian [3]** study comes in contrast with our study as it aimed to explore the role of Letrozole in controlled ovarian stimulation in PCOS patients at high risk to develop OHSS and they concluded that in the LE group, there was a significantly lower rate of OHSS (2 cases of mild OHSS) compared to 10 cases (9 mild and 1 moderate case) of OHSS in the control group. All patients responded to COS and therefore no cyclecancellations were registered.

This difference between our study and the **Tshzmachyan and Hambartsoumian [3]** study is that the last has more number of patients (51 patients) than our study (44 patients). Also they followthe protocol of "freeze all" but in our study fresh emberyo transfer was done.

Also, one of the most important differences is the chosen range of BMI as **Tshzmachyan and**

Hambartsoumian [3] chose PCOS patients with low BMI (<25 kg/m2) which was shown to be an additional marker of a high risk of OHSS so the incidence of OHSS was higher in their study.

The **Tshzmachyan and Hambartsoumian** [3] study supports our study regarding role of LE combined with GnRH-agonist trigger which completely eliminate severe and critical stages of OHSS.

Our data also suggest a comparable effectiveness of the GnRH-ant plus LE and conventional GnRH-ant regimens in terms of cost effectiveness as we found that the use of LE in our study not only resulted in a significant reduction in the duration of COS but also in the amount of gonadotropins used. This data was reinforced by the results of **Tshzmachyan and Hambartsoumian** [3] study.

The results of **Mai et al.** [14] study differs from the present study for many reasons. First of all was the large number of patients (238 patients) in the **Mai et al.** [14] study. Secondly, they used HCG as a trigger which may be a factor in the occurrence of the severe cases of OHSS. Also, LE was given after the trigger not during ovarian stimulation.

Also, unlike our study, **Fatemi et al.** [22] used letrozole in the early luteal phase for prevention of OHSS in a 21-yearold patient which was at high risk for the development of OHSS during COH.

In a case report, **Fatemi et al.** [22] tested the co-administration of letrozole and cabergoline inthe early luteal phase for prevention of OHSS in a high risk patient undergoing ovarian stimulation for IVF. Neither early nor late OHSS was developed.

CONCLUSION:

The current study concluded that the Addition of LH to conventional GnRH-antagonist protocol in PCOS patients which are at high risk of OHSS may be the same as using conventional GnRH-antagonist protocol only in prevention of OHSS in controlled ovarian stimulation (COS) during IVF.

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