Prevalence of ovarian hyperstimulation in normal responders receiving letrozole in assisted reproductive technology: A randomized controlled trial

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Introduction
The use of letrozole, a selective aromatase inhibitor, induces follicular maturation. Randomized controlled trials addressing the utility of letrozole in normal responders are few. We evaluated whether incorporation of letrozole could be effective in decreasing ovarian hyperstimulation syndrome (OHSS) in normal responders undergoing intracytoplasmic sperm injection cycles.

Objective
The objective of this study was to compare the aromatase inhibitor (letrozole) with low-dose gonadotropins versus the standard long protocol for controlled OHSS in normal responder women undergoing intracytoplasmic sperm injection in terms of OHSS.

Patients and methods
This was a randomized noninferiority clinical trial registered in clinicaltrials.gov (NCT02429999). This study was conducted in Assiut University Fertility Center, from January 2015 to April 2016, and included 61 normal responder women randomized in a 1: 2 ratio. Twenty-one women (study group) received letrozole at a 10 mg daily dose from days 3 to 7 together with follicle-stimulating hormone (FSH) 75 IU/day from day 5. Gonadotropin-releasing hormone antagonist (orgalutran 0.25) is given when the follicle size is equal to 14 mm until human chorionic gonadotropin injection. Forty women (control group) received 0.1 mg decapeptyl from day 21 in the preceding cycle and continuously stimulated by FSH (150–225 IU/day) from day 2. The total dose of FSH received, number of mature oocytes, good-quality embryos, and OHSS were evaluated in both groups. Statistical analysis was done using Student's *t*-test and χ^2-test when appropriate.

Results
Comparable rates for maturation index, fertilization, and good-quality embryos were yielded by both groups. The incidence of OHSS was significantly lower in the letrozole group compared with the long protocol group ($P = 0.03$).

Conclusion
Minimal stimulation protocol using letrozole with gonadotropins may form an effective tool to decrease the incidence of OHSS in in-vitro fertilization cycles in normal responding women.

Keywords:
letrozole, normal responder, ovarian hyperstimulation syndrome

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Introduction

It is well known that ovarian hyperstimulation syndrome (OHSS) is often induced by ovarian stimulation protocols in in-vitro fertilization (IVF). Prevention of OHSS is the main consideration when performing this procedure.

OHSS remains a lethal iatrogenic complication of the early luteal phase and/or early pregnancy after ovarian stimulation [1]. OHSS has been classified based on severity (mild, moderate, severe, and critical). In addition, based on the time of occurrence, OHSS has been classified into early (<9 days) and late. Moderate or severe OHSS affects 2–3% of patients, whereas milder forms may develop in 20–30% of all IVF cycles. The significant risks are associated with moderate/severe OHSS, which could lead to acute renal insufficiency, acute respiratory distress syndrome, and venous thromboembolism [2].

Pathophysiological changes of OHSS that underlie these events include arteriolar vasodilatation and increased capillary permeability. This facilitates a shift in fluid from the intravascular to the extravascular compartment and the induction of a state of hypovolemic hyponatremia with hemoconcentration. Although various systemic and local vasoactive mediators contribute to the

pathogenesis of OHSS, vascular endothelial growth factor appears to be critical to the development of the condition [3].

The current work is aimed at evaluating the efficacy of letrozole (LTZ) as a modality for minimal ovarian stimulation aiming to decrease OHSS.

Patients and methods

Study design

The present study is a randomized clinical noninferiority trial. The study was conducted at Assisted Reproductive Technology (ART) Unit of Women's Health Hospital, Assiut University, Assiut, Egypt, from January 2015 to April 2016. The protocol of the study has been approved by the Ethical Review Board of Faculty of Medicine, Assiut University. This study was registered in the website clinicaltrials.gov (NCT02429999, *http://www.clinicaltrials.gov*).

Women attending the ART Unit of Women's Health Hospital, Assiut University, were considered for enrollment. In the study period, 120 eligible women were selected from the clinic of the ART Unit. They were randomized by sealed envelope into two groups. Group 1 included 80 women who received long midluteal protocol, whereas group 2 included 40 women who received minimal stimulation protocol (LTZ/gonadotropin). Eligible women were counseled and offered to join the study after obtaining written informed consent. The consent forms were signed after thorough discussion with the couples and explanation of the study purpose, interventions, outcomes, and adverse events.

Enrollment criteria

Women were included if they 20-35 years old, with a BMI of 18–29 kg/m^2, infertile with an indication for IVF [including unexplained infertility, tubal factor, and male factor (including concentrations down to 5–10 million sperms/ml)], with anticipated adequate ovarian response, antral follicle count more than five follicles in one ovary, and/or anti-Müllerian hormone (AMH) more than 1 ng/ml [4].

We excluded patients with endometriosis and poor ovarian response (POR). In addition, we excluded poor responders based on Bologna criteria. At least two of the following three criteria had to be present to establish the definition of poor responder: (i) advanced maternal age (>40 years) or any other risk factor for POR; (ii) a previous POR (≤3 oocytes with a conventional stimulation protocol); (iii) an abnormal ovarian reserve test (i.e. antral follicle count less than 5–7 follicles or AMH < 0.5–1.1 ng/ml) [5].

Randomization

Allocation of participants in the two groups was intentionally made unequal with a lesser number of women treated with LTZ, as this was a relatively new drug in the field of controlled OHSS in normal responders. Among most of the studies of controlled ovarian stimulation, only two studies used LTZ in normal responders. The randomization ratio of LTZ/gonadotropins protocol to the gonadotropin-releasing hormone (GnRH) antagonist protocol was taken to be 1: 2. Sequentially numbered sealed envelopes were prepared and provided by the study coordinator, according to random-number tables.

Interventions

Group 1 (control group) received 0.1 mg decapeptyl (triptorelin; Ipsen Pharma, Barcelona, Spain) from day 21 in the previous cycle and continuously stimulated by follicle-stimulating hormone (FSH) (150–225 IU/day) from day 2, whereas group 2 received (study group) LTZ (Jiangsu Hengrui Medicine, Lianyungang, China) 10 mg daily from days 3 to 7 and FSH 75 IU/day from day 5 continuously. GnRH antagonist (orgalutran 0.25 mg ganirelix; N.V. Organon, Oss, The Netherlands) is given when the follicle size is equal to 14 mm until human chorionic gonadotropin injection.

Outcome measures

The incidence of OHSS was the primary outcome measure of this study. Mild OHSS was diagnosed by mild abdominal pain, abdominal bloating, and ovarian size usually less than 8 cm. Moderate OHSS was concluded after moderate abdominal pain, nausea and vomiting, ultrasound evidence of ascites, and ovarian size usually 8–12 cm, whereas severe OHSS was suspected if there is clinical ascites (occasionally hydrothorax), oliguria, hemoconcentration, hematocrit more than 45%, hypoproteinemia, and ovarian size usually more than 12 cm. Critical OHSS is a condition with tense ascites or large hydrothorax, hematocrit more than 55%, white cell count more than 25 000/ml, oligo/anuria, thromboembolism, and acute respiratory distress syndrome [6].

The total dose of FSH injection, maturation index, good-quality embryos, as well as endometrial thickness at the time of human chorionic gonadotropin trigger, has been evaluated in both groups as secondary outcomes.

Sample size

According to Mukherjee *et al.* [7], the pregnancy rate was increased in the LTZ–FSH group (36%) compared with that in the control group (33%). We calculated the sample size at α equal to 0.05, 95% confidence interval of 1.96 for normal distribution, and statistical power of 80% Statistical analysis was performed using SPSS version 24 STATA software, version 13. Estimated sample size was 120 patients distributed in a 1: 2 ratio [8].

Statistical analysis

Statistical analysis was performed using SPSS, version 24. First, Shapiro–Wilk's test was used in the tested population as a test of normality for continuous variables. Parametric variables were expressed as mean ± SD. χ^2 and Fisher's exact tests were used to compare proportions as appropriate. Interim analysis will be done once half of the sample size is achieved as part of the periodic assessment of the ART Unit results.

Results

Forty women received the standard long protocol, whereas 21 underwent stimulation by LTZ. The present work was

Table 1 Demographic data in study groups

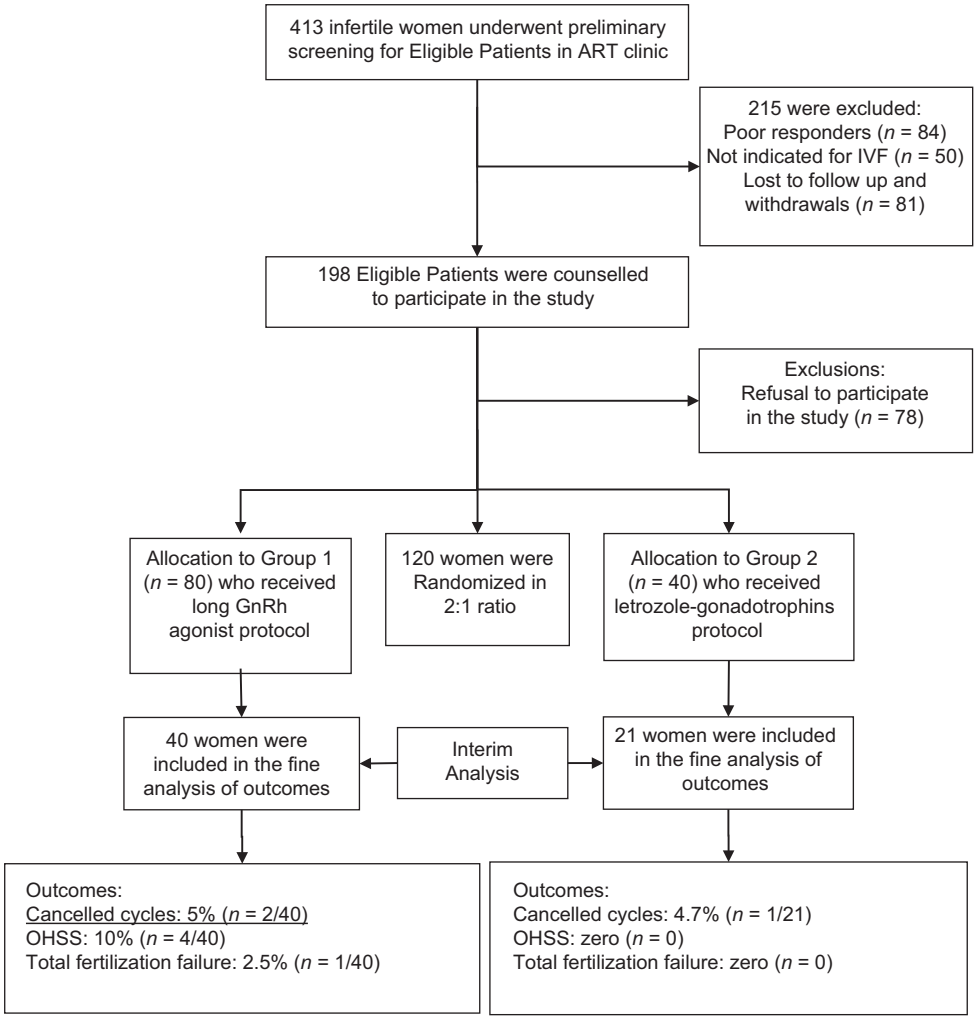
Item	Long protocol (*n*=40)	Letrozole protocol (*n*=21)	*P*
Age (years)			
Mean±SD	26.95±3.97	28.91±3.76	0.073
BMI (kg/m^2)			
Mean±SD	24.78±3.29	26.12±1.82	0.079

Table 2 Baseline characteristics of study groups

Item	Long protocol (*n*=40)	Letrozole protocol (*n*=21)	*P*
Duration of infertility	6.21±1.20	5.25±1.9	0.593
AFC	10.62±4.26	10.08±1.95	0.69
E2	31.15±5.21	29.12±5.52	0.228
AMH	3.21±1.18	2.89±1.68	0.443
FSH	4.98±1.49	5.48±1.31	0.615
LH	4.40±2.69	3.97±1.46	0.479

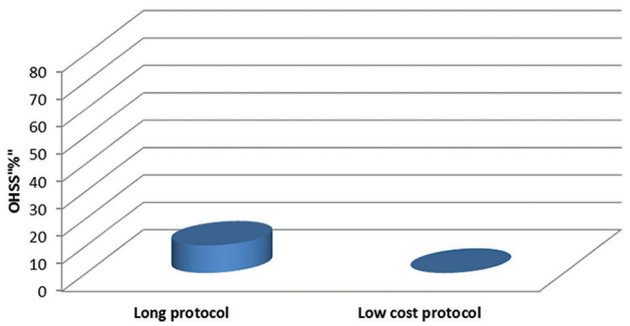
AFC, antral follicle count; AMH, anti-Müllerian hormone; E2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Figure 1



Consort flowchart of the participants. ART, Assisted Reproductive Technology; GnRH, gonadotropin-releasing hormone; IVF, in-vitro fertilization.

Figure 2



Bar chart showing the difference in OHSS incidence between protocols.

conducted as a noninferiority trial, but the results of LTZ were the lowest among the results of different induction protocols used in our university ART Unit through the study period as demonstrated by the periodic follow-up of the ART Unit results. Therefore, we performed an interim analysis because of the suboptimal results and the trial was stopped after enrolling half of the sample size. Details are shown in Fig. 1.

Both groups were comparable as regards age, BMI, duration of infertility, antral follicle count, and hormonal profile; estradiol (E2), AMH, FSH, and luteinizing hormone were also comparable, as shown in Tables 1 and 2. As shown in Table 3, women in the LTZ group significantly received lower doses of gonadotropins; approximately more than half of the dose was reduced in the LTZ group. In addition, women in the long protocol group achieved almost double the level of peak E2 measured at the day of trigger. The use of long protocol was also associated with a significant increase in the number of oocytes retrieved and fertilized. Endometrial thickness was higher also in the long protocol group. However, no significant difference ($P > 0.05$) was observed in oocyte maturation index between groups. There was no significant difference ($P > 0.05$) between the two protocols as regards the fertilization rate and the incidence of total fertilization failure, and canceled cycles. However, the incidence of OHSS was significantly higher in the long protocol group as reported in Table 4 and Fig. 2.

Table 3 *In-vitro* fertilization cycle characteristics of study groups

Item	Long protocol (*n*=40)	Letrozole protocol (*n*=21)	*P*
Total gonadotrophin dose	2573.58±274.45	1050.89±157.25	0.001**
Peak estradiol	2195.48±368.13	1075.27±197.20	0.001**
Endometrial thickness at day of trigger	11.10±1.30	9.21±1.24	0.001**
Oocytes retrieved	18.62±10.33	11.32±7.26	0.003**
Oocyte maturation index (*n* (%))	517/651 (79.4)	181/226 (80.0)	0.396

Table 4 Outcome of *in-vitro* fertilization in the study groups

Item	Long protocol (*n*=40)	Letrozole protocol (*n*=21)	*P*
Fertilization rate (%)	76.0	79.0	0.395
Total fertilization failure (*n* (%))	1 (2.50)	0.0	0.593
Canceled cycles (*n* (%))	2 (5.0)	1 (4.1)	0.447
Ovarian hyperstimulation syndrome (*n* (%))	4 (10)	0.0	0.031*

Discussion

The current work is basically a comparative effectiveness research comparing two interventional therapies in normal responder women as a part of controlled ovarian hyperstimulation. The study is aimed at evaluating the efficacy of LTZ as a modality for minimal ovarian stimulation aiming to decrease OHSS.

The two main complications associated with the use of assisted reproduction techniques, OHSS and multiple pregnancies, could be eliminated by milder ovarian stimulation protocols. In comparison with current approaches, the aim of mild stimulation is to develop safer and more patient-friendly protocols in which the risks of the treatment as a whole are minimized. 'Mini-IVF' has several advantages over conventional IVF protocols, including less medication and fewer injections, producing fewer eggs, but eggs of higher quality [9].

LTZ has been evaluated before for controlled ovarian stimulation with intrauterine insemination. Investigators found that the primary advantage of LTZ use was reduction in doses of gonadotropins, as well as in the chance of multiple pregnancies and OHSS [10].

LTZ has also been tried for ovarian stimulation in IVF/intracytoplasmic sperm injection cycles. However, very few trials with limited number of patients are available. Five randomized trials, with a total of 265 patients, dealt with poor responders. They were randomized to receive LTZ combined with gonadotropins or gonadotropins alone, in an antagonist or agonist protocol. The gonadotropin dose used was consistently lower in the LTZ cotreatment group in all trials [11,12].

The role of aromatase inhibitors during luteal phase of stimulated IVF cycle is to reduce circulating E2 levels, thus potentially leading to reducing OHSS risk [13,14].

We found in our results that women in the LTZ group had the advantages of LTZ use; they reported

reduction in total received dose and hence reduction in OHSS compared with the long agonist protocol group.

Women who received agonist protocol in the present work showed significantly increased incidence of OHSS (10 vs. 0%). It has been reported that the incidence of OHSS is much higher in patients who use GnRH agonists in a long protocol [15].

In accordance with our results, Mukherjee *et al.* [7] reported similar significant reduction in OHSS in the LTZ group. In addition to the danger of OHSS on patient life, this complication contributes in increasing the overall cost of the IVF cycle.

Conclusion

The use of LTZ in normal responders reduces unnecessary side effects such as OHSS and multiple pregnancies and reduces their cost implications.

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Conflicts of interest

There are no conflicts of interest.

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