Original Article

Study of Positive and Negative Consequences of Using GnRH Antagonist in Intrauterine Insemination Cycles

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Abstract

Background: To assess the usefulness of premature luteinization hormone (LH) surge prevention in an intrauterine insemination (IUI) cycle by GnRH antagonist administration.

Materials and Methods: Sixty patients with unexplained or mild male infertility or minimal to mild endometriosis were enrolled in this prospective randomized controlled trial. There were twenty patients in group A (with GnRH antagonist) and 40 patients in group B (without GnRH antagonist).

In all of the participants, clomiphene citrate and human menopausal gonadotropin (CC+HMG) were used for ovarian stimulation. When at least one follicle with ≥ 16 mm diameter was seen, LH surge was checked by a urinary LH kit. In patients with negative results, human chorionic gonadotropin was continued in both groups, but in group A 0.25 mg Ganirelix SQ was administered for two days, ,then in both groups human chorionic gonadotropin (HCG) was injected on the third day and IUI was done 36-40 hours later. Ongoing pregnancy was the primary outcome.

Results: Baseline characters and clinical parameters were similar in both groups with the exception of ≥14 mm follicles which were higher in group A (p value= 0.003). The pregnancy rate in both groups was not significantly different, although it was higher in group B (10% in group A and 15% in group B).

Conclusion: At least in CC+HMG stimulated cycles for IUI, the occurrence of premature LH surge could have a useful rule and GnRH antagonist administration could be an inappropriate intervention.

Keywords: GnRH Antagonist, Luteinizing Hormone, Artificial Insemination

Introduction

After presentation of intrauterine insemination as a technique for subfertility management by John Hunter around 200 years ago, proper patient selection became the first step for success.

Conditions which can be managed by intrauterine insemination (IUI) are as follows:

1. Ejaculatory dysfunction
2. Male subfertility (immunological and mild to moderate semen abnormality)
3. Cervical factors
4. Minimal or mild endometriosis
5. Ovulatory dysfunction
6. Idiopathic infertility.

Some prediction models use factors such as female age, duration of subfertility and number of IUI cycles to predict successful results after IUI (1). The cornerstone in IUI is the availability of better quality and quantity eggs in females. In males, laboratory washing and preparation of semen helps the availability of more motile and healthy sperm, thereby providing a higher chance of fertilization. In females, controlled ovarian stimulation enhances the chance of fertilization and pregnancy by producing more oocytes. By using more aggressive methods for ovulation induction such as increasing the dosage of drug; the number of recruited follicles may increase but this could result in unwanted conditions such as multiple pregnancy or ovarian hyperstimulation syndrome (OHSS) and therefore it is not advised. One method is the correct timing of IUI by avoiding a premature luteinization hormone (LH) surge which occurs in about 24% of the cycles (2). GnRH agonists have been used for more than a decade to reduce premature LH surge (3, 4). By avoiding premature LH surge in women with prior cycle failure, GnRH agonists can help

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increase pregnancy rates (5).
GnRH antagonists have only recently been used for this purpose due to GnRH agonist side effects. At first, their effectiveness was of interest in *in vitro* fertilization (IVF) cycles (6) but later, the role of GnRH antagonists was accepted in IUI cycles (7,8). GnRH antagonists have several advantages over GnRH agonists: immediate suppression without flare-up effects, short-term duration, simple method, good patient toleration, scheduling IUI after weekends (9) and a decreased risk of OHSS.
Theoretically, the goal of using GnRH antagonists is to prevent LH surge which is induced by elevated levels of estradiol (E2); therefore at least two follicles with a diameter of 16mm or more would be available without the fear of a premature LH surge. Several studies have shown the benefit of adding GnRH antagonists in improving follicular size and pregnancy rate by preventing LH surge (10-12), although there is some controversy (13).
In different studies in which ganirelix or cetrolix were used, beside pregnancy rate, costs and cycle duration were of importance.
On the other hand, the aim was to use fewer drugs at a lower cost to achieve a higher pregnancy rate. In most IUI studies, recombinant follicular stimulation hormone (FSH) was used for ovarian stimulation similar to IVF cycles. GnRH antagonists were applied when the follicular size was 13-14 mm in diameter and continued until follicles reached 18-20 mm in size and IUI was subsequently performed.
At first, the combination of clomiphene citrate and gonadotropins has been proposed in IVF cycles with a 20% chance of LH surge (14), but after GnRH antagonist marketing, the clinical efficacy of this treatment became important in IUI cycles. Their beneficial role has been noticed in letrozole/gonadorphin IUI stimulated cycles (15).
In this study, we attempted to achieve the lowest possible cost by using minimal stimulation (CC+HMG) plus a GnRH antagonist for only two days. A urinary LH kit for LH surge assay was used for the first time. By this method, we hoped to offer an inexpensive and easier technique for patients in order to attain their cooperation. Also, the positive and negative side effects of GnRH antagonist administration in IUI cycles were evaluated. From the positive aspect we looked at improving pregnancy rates and from the negative side, we looked for complexity in cycle management and increased cost.

Materials and Methods

This prospective clinical trial was performed between September, 2007 and September, 2008 in the Vali-e-Asr Reproductive Health Research Center which is affiliated with Tehran University of Medical Sciences. The study was approved by the University Medical Ethics Review Committee and with the informed consent of all participants. The criteria to participate in this experiment were:
1. age 18-38 years, 2. body mass index (BMI) 18-30 kg/m^2, 3. regular menstrual cycles (25-35 days), 4. normal basal FSH (≤10 mIu/L), prolactin (PRL) and thyroid stimulation hormone (TSH) levels, 5. normal uterine anatomy and patency of both fallopian tubes confirmed by laparoscopy/hysteroscopy or hysterosalpingography (HSG), 6. primary or secondary infertility of 1.5-6 years duration, 7. mild male factor, minimal to mild endometriosis and unexplained infertility as a cause of infertility, 8. at least 6 months interval since last ovulation induction or last IUI cycle, 9. at least 3 months interval after the last treatment for endometriosis, 10. total motile sperm ≥10 million/ml in the last semen analysis which was performed within the past 6 months.
According to the random allocation table, patients were divided into group A (GnRH antagonist administration, n=20 patients) and Group B (without GnRH antagonist administration, n=40 patients) with a proportion of 1:2.
In all patients, vaginal ultrasonography was performed on day 3 of their menstrual cycle. Patients with normal ultrasonography results underwent ovulation stimulation with clomiphene citrate 50mg TDS until day 7, followed by human menopausal gonadotropin (HMG) (Merional, IBSA, UK). Patients under 30 years of age received one ampoule a day and patients age 30 and above received two ampoules a day, intramuscularly. Follicle growth monitoring was started on the second or third day of HMG administration and repeated every other day until the appearance of at least one follicle ≥16 mm in diameter. Meanwhile, premature LH surge was checked with a mid day urine sample by using a urinary LH surge kit (FARAFAN Diagnostics Company, Iran under the license of CALPEX, UK) and those with positive results were excluded from the study. For confirmation, all patients with positive results plus half of those with negative results (randomly chosen) were sent to the laboratory on the same day to check for LH serum levels.
In Group A, patients with negative urinary LH results were administered 0.25 mg ganirelix (Orgalutran, Organon, Netherlands) subcutaneously for two days along with HMG. HMG was contin-

ued as before in Group B. On the third day, within 24-30 hours after the second dose of GnRH antagonist (16), 10000 IU HCG (Pregnyl, Organon, Netherlands) was injected intramuscularly in both groups. Prior to this, further sonographic evaluation was taken for both groups and patients with follicular diameters of ≥14 mm and ≥18 mm as well as endometrial wall thickness were assessed. A single IUI was performed 36-40 hours after HCG administration by using Wallace IUI catheter (Wallace, UK). There is no advantage in performing double insemination in a single cycle (17). All patients received 400mg of micronized progesterone vaginally twice a day for luteal phase support. Pregnancy was checked by serum BhCG levels two weeks after IUI. In case of pregnancy, transvaginal sonography was performed at 6-7 weeks of gestation to check for pregnancy outcome. Pregnancy was recorded as "ongoing" if fetal cardiac activity was observed in the first or second scan at 8-9 weeks of gestation.

In addition to positive results in the urinary LH kit, IUI could be cancelled if less than 2 or greater than 5 follicles with a diameter of 14-15 mm were observed during cycle monitoring.

Data were entered into SPSS version 11.5 computer software and subsequently analyzed by using statistical tests. Student's t test was used for unpaired data and chi-square and Fisher tests were used to assess frequency distribution and clinical outcome. Also, ANOVA and regression tests were used for comparison between the two groups. A p value < 0.05 was considered as significant and power of study was 80%.

Results

A total of 60 patients were randomly selected to participate in this clinical trial; 20 patients in Group A (with GnRH antagonist) and 40 patients in Group B (without GnRH antagonist). No statistical difference was found between the basic clinical characteristics of each group (Table 1).

Table 1: Basic characters

Characters	Group A	Group B
Age (year)	28.6 ± 5	28.7 ± 4.5
Duration of infertility (year)	3.5 ± 1.5	4.1 ± 1.5
BMI (kg/m^2)	24.1 ± 3.9	24.01 ± 2.9
Basal LH (mIU/ml)	5.6 ± 2.6	5.3 ± 2.6

Primary infertility was detected in 39 patients (13 patients in Group A and 26 patients in Group B) and secondary infertility was found in 21 patients (7 patients in Group A and 14 patients in Group B).

The causes of infertility were somewhat similar in both groups: unexplained infertility was 60% in Group A and 57.7% in Group B, male factor infertility was 30% in both groups, and endometriosis was 10% in Group A and 12.5% in Group B.

Various clinical parameters associated with ovarian stimulation are presented in Table 2.

Table 2: clinical parameters

Parameters	Group A	Group B
HMG dose (IU)	622.5 ± 213.6	558.7 ± 191.3
Endometrial thickness (mm)	8.2 ± 1.9	7.9 ± 1.5
Follicles ≥ 14 mm	2.7 ± 0.73	2.2 ± 0.83
≥18 mm	1.6 ± 0.69	1.6 ± 0.78

There was no significant association between using HMG, endometrial wall thickness or the number of follicles with a diameter of ≥18 mm in both groups. However, the number of follicles with a diameter of ≥14 mm was higher in Group A (p value: 0.003).

The pregnancy rate was 10% in Group A and 15% in Group B, which was not statistically significant. Overall, three patients had chemically positive pregnancy test results and three patients had ongoing pregnancy, all of them had singleton and only one patient had an ectopic pregnancy (Fig 1).

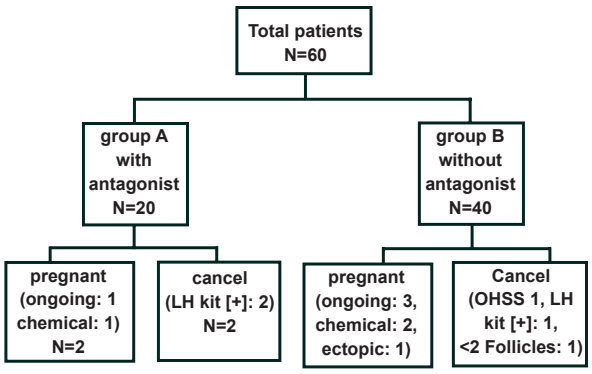

Fig 1: Trial profile

Patients with positive urinary LH results had serum LH levels >20 mIU/ml and half of the patients with a negative LH result had serum LH levels between 0.9-12 mIU/ml. The serum LH level was less than 10 mIU/ml in all women with a subsequent pregnancy.

Discussion

In accordance with the latest review from the Cochrane database (18), injection of a stimulant drug in IUI is more effective than an orally prescribed drug. This treatment provides better

utilization of endogenous FSH, exogenous FSH and milder stimulation. The addition of a GnRH antagonist in the stimulation cycle may induce a rapid reduction in LH levels with negative effects on the endometrium and oocyte quality. Although there is doubt about the necessity to administer exogenous LH, HMG is prescribed instead of GONAL-F in order to reduce cost (19).

The main aim of this study was to show a simple way of detecting LH surge in the new IUI protocol, in which a GnRH antagonist was also added. For the first time, LH levels were detected by using urine samples instead of routine serum control. Although the efficacy of the urinary LH kit in a minimal stimulation IUI cycle has been shown in one study (20), such studies are lacking in the case of more complex stimulation regimens. Ovulation mostly occurs 35-44 hours after the onset of plasma LH surge. Urinary LH could be detected about 24 hours after maximum serum LH levels because it needs time to be extracted from the blood and collected in urine. This action may show some variation due to differences in the sensitivity of urinary LH kits. Therefore it is more accurate if the test is performed twice rather than once a day. Also, we took early morning samples from all women in because the morning time showed better results for LH surge (21). This inexpensive and easy test could replace blood sampling.

GnRH antagonists were initially used in ovulation induction cycles but high cost was a big issue. To reduce expenses, some researchers stated that alternative day administration is as effective as once daily administration in order to prevent premature leutinization (22). Others mentioned that a half-dose GnRH antagonist could be used if pre-treatment with OCP is performed (23). Also, a single depot form of the drug could induce effective suppression in 75%-90% of patients until the day of HCG injection (24).

Controversial evidence exists about the adverse effects of GnRH antagonists on endometrium and oocyte quality. Some studies show that the administrations of GnRH antagonist dose not impose adverse effects on the endometrium (25), while others show that endometrial maturation may be expedited by three days through genetic changes (26). At the oocyte level, studies show controversy with a positive effect on the number of oocytes and embryo quality (27) and a reduction in the number of M2 oocytes (28). In our study the lower pregnancy rate, which was not statistically significant in the treatment group, could be justified.

Based on a large meta-analysis study, one premature LH surge in an IUI cycle can be prevented by adding GnRH antagonist to four patients and only one clinical pregnancy will be achieved with its administration in more than 20 cycles (29), therefore GnRH antagonist application must not be a routine method in all IUI cycles and it should only be considered in special situations, such as: patient age <35 years, 5-9 antral follicles, absence of poly cystic ovary sndrome (PCOS) or endometriosis and no history of a poor response (16). Also, recent findings show the effectiveness of GnRH antagonist administration in patients with PCOS (28, 30).

Interestingly, some evidence exists about the benefit of LH surge on pregnancy rate in clomiphene citrate-stimulated cycles in contrast to FSH-stimulated cycles. Also, this favourable effect remains when FSH is combined with clomiphene citrate (31). In FSH-stimulated cycles, rapidly rising estradiol levels induce premature LH surge in immature follicles but in milder stimulated cycles, the process of natural LH surge allows better follicle maturation and a higher chance of pregnancy. Therefore, the administration of GnRH antagonist could be useful in these patients. Furthermore, because LH surge could last up to two days in some women, it is better to trigger ovulation by HCG after onset of the surge, thereby increasing the chance of pregnancy (32), but it has to be at the right time to eliminate any intervention (synchronous, not before or after an endogenous LH surge). HCG is injected regardless of whether the cycles were treated with a GnRH antagonist following LH confirmation. As with our study, pregnancy rates were higher in non-treated patients and HCG was injected on the third day after urinary LH testing. In this study, GnRH antagonist was administrated for only two days, but it would have been better if the urinary LH test was repeated after administration, on the day of the last sonography control before HMG injection.

Conclusion

GnRH antagonist administration should be considered only for some patients. The negative and positive aspects must be justified at first. Also, the urinary LH kit is a useful tool to simplify cycle monitoring, prevent painful sampling methods and minimize costs. We did not find any benefit from suppression of LH surge due to the small sample size in our study. It seems that natural events such as LH surge do not have any detrimental effect on IUI cycles and the importance of these events should be considered before any inappropriate intervention.

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