Impact of gonadotropin-releasing hormone antagonist addition on pregnancy rates in gonadotropin-stimulated intrauterine insemination cycles

Shikha Jain,
Abha Majumdar
Centre of IVF and Human Reproduction, Sir Ganga Ram Hospital, New Delhi, India

Address for correspondence:
Dr. Shikha Jain,
88, Raj Nagar, Pitampura,
New Delhi - 110 034, India.
E-mail: dr.shikhajain@yahoo.com

Received: 30.11.2015
Review completed: 02.05.2016
Accepted: 15.06.2016

Access this article online

Quick Response Code:

Website:
www.jhrsonline.org

DOI:
10.4103/0974-1208.192053

ABSTRACT

OBJECTIVES: The objective of the study is to evaluate the efficacy of gonadotropin-releasing hormone (GnRH) antagonist in improving clinical pregnancy rate in gonadotropin-stimulated intrauterine insemination (IUI) cycles in patients of unexplained infertility. **STUDY DESIGN:** This was a prospective, randomized case–controlled study. **SETTINGS:** The study was conducted in the infertility clinic of a tertiary care center. **MATERIALS AND METHODS:** Four hundred twenty-seven women undergoing IUI following controlled ovarian stimulation with gonadotropins (recombinant follicle-stimulating hormone [r-FSH] 75 IU/day) were randomly divided into two groups. Women in Group I received GnRH antagonist (Cetrorelix 0.25 mg/day) in a multiple dose flexible protocol. Women in Group II received r-FSH alone. Ovulatory trigger was given with human chorionic gonadotropin 5000 IU when dominant follicle was ≥18 mm. IUI was performed within 44–48 h. Both groups received similar luteal phase support. Primary outcome measure was clinical pregnancy rate. The trial was powered to detect an absolute increase in clinical pregnancy rate by 13% from an assumed 20% clinical pregnancy rate in the control group, with an alpha error level of 0.05 and a beta error level of 0.20. **RESULTS:** Clinical pregnancy rate in Groups I and II was 27.6% ($n = 56$) and 26.5% ($n = 54$), respectively (P=0.800). Ongoing pregnancy and multiple pregnancy rates were likewise similar between the groups. **CONCLUSIONS:** Addition of GnRH antagonist to gonadotropin-stimulated IUI cycles results in no significant difference in clinical pregnancy rate.

KEY WORDS: Clinical pregnancy, gonadotropin, gonadotropin-releasing hormone antagonist, intrauterine insemination, unexplained infertility

INTRODUCTION

Unexplained infertility contributes to about 10–30% of subfertility, depending on diagnostic criteria.[1] Intrauterine insemination (IUI) combined with controlled ovarian stimulation (COS) has been established as a first-line treatment for couples with unexplained infertility.[2] The rationale of COS and IUI is to increase the number of available female and male gametes at the site of fertilization by achieving two to three dominant follicles, followed by a perfectly timed insemination.[2-4] The use of IUI with COS in a well-selected group of patients with unexplained infertility results in comparable cumulative pregnancy rate when compared to *in vitro* fertilization (IVF) and hence appears more cost-effective.[5,6]

To increase the chances of success in terms of pregnancy rate in COS-IUI cycles, various therapeutic approaches have been tried by various researchers, such as different ovarian stimulation protocols,[7] double insemination,[8,9] and prevention of premature luteinizing hormone (LH) surge.[10-12] According to the Cochrane review

How to cite this article: Jain S, Majumdar A. Impact of gonadotropin-releasing hormone antagonist addition on pregnancy rates in gonadotropin-stimulated intrauterine insemination cycles. J Hum Reprod Sci 2016;9:151-8.

on ovarian stimulation protocols for IUI in the women with subfertility, use of gonadotropins for COS in IUI results in higher pregnancy rate than clomiphene citrate-stimulated cycles (odds ratio [OR] 1.8, 95% confidence interval [CI] 1.2–2.7).[13] A recent meta-analysis clearly indicated that double insemination does not result in higher clinical pregnancy rate compared with single IUI in couples with unexplained infertility.[14] Double insemination has been suggested by researchers because of the hypothesis that capacitated sperms in the inseminate are active for only 2–3 h, so they may not be able to back up ovulation which takes place in between the next 20 and 24 h.[15] However, it appears that precise timing of insemination in relation to ovulation so as to enable active sperms to reach and fertilize the oocyte should obviate the need for double insemination.

Premature LH surge is defined as the surge that precedes the triggering of ovulation iatrogenically. Prospective data have shown that premature LH surge occurs in almost 23% of COS cycles (95% CI 22–43%),[16] which appears quite significant and can interfere with the optimal timing of the insemination.[10,12] LH surge can be effectively prevented by administering a gonadotropin-releasing hormone (GnRH) agonist or GnRH antagonist.[12,17,18] Use of GnRH agonist is not recommended in IUI cycles because of prolonged administration of injections prior to and during stimulation to achieve complete downregulation of GnRH receptors, risk of excessive follicular stimulation, and higher cost and inconvenience to the patient.

On the other hand, GnRH antagonist competitively blocks the GnRH receptors and immediately causes pituitary suppression, thereby reduces LH and follicle-stimulating hormone (FSH) secretion within 2–4 h. The efficacy of GnRH antagonist in prevention of premature LH surge is well-established.[17,18] The inhibitory effect of GnRH antagonist is reversible, dose-dependent and is associated with the equilibrium between endogenous GnRH and GnRH antagonist concentration.[18] Cetrorelix (Cetrotide, EMD Serono) and Ganirelix (Antagon, Organon) are the two GnRH antagonists available for clinical use.

The protocols of GnRH antagonist administration in COS-IUI cycles are well-defined; however; the flexible regimen is the one which is used commonly in mild stimulation cycles.

Aims and objectives

- To assess whether the clinical pregnancy rate of the patients treated with recombinant-FSH (r-FSH) and IUI can be improved by addition of a GnRH antagonist
- To further assess whether the addition of a GnRH antagonist could affect the incidence of pregnancy loss, ongoing pregnancy, multiple pregnancies, and ovarian hyper-response in r-FSH/IUI cycles in patients with unexplained infertility.

MATERIALS AND METHODS

This was a prospective, randomized case–controlled study conducted at the tertiary care infertility center from October 2011 to September 2012. Three hundred thirty-one couples with unexplained subfertility meeting the inclusion criteria were included in the study. After counseling, written informed consent was obtained from all couples before randomization. Computer-generated randomization was done between study and control groups on the day of initiation of stimulation. The trial was not placebo-controlled as the outcome measures were objective. The approval of the Ethics Committee of the institution was obtained.

Inclusion criteria

- Unexplained infertility (primary/secondary)
- Postwash motile sperm counts ≥20 million/ml
- Bilateral tubal patency and normal uterine cavity (hysterosalpingography/laparoscopy)
- Regular menstrual cycle with basal FSH ≤10 IU/L
- Only first four cycles of IUI.

Exclusion criteria

Patients having one or more of the following were excluded from the study:

- Age ≥38 years
- Anovulatory infertility (polycystic ovary syndrome)
- Unilateral tubal patency or hydrosalpinx
- Intramural fibroid more than 4 cm or multiple fibroids or adenomyosis >12 weeks size
- Endometriosis Stage III/IV (if laparoscopy done)
- Artificial insemination donor.

Cancellation criteria

- No follicle ≥18 mm
- ≥3 follicles of ≥16 mm on the day of human chorionic gonadotropin (hCG)
- Endometrial thickness ≤6 mm on the day of IUI
- Spontaneous urine LH surge positive just before hCG.

Intervention

COS was started with r-FSH on the day 2/3 of menses after a baseline transvaginal ultrasound scan to ensure ovarian quiescence. The daily r-FSH dose ranged from 75 IU upward, depending on the body mass index (BMI), age of the women, and anticipated ovarian response. Monitoring was performed from day 7 to day 8 of cycle with Transvaginal sonography (TVS) and dose adjustment was done according to the follicular development. In the study group, when a dominant follicle of 15 mm or

more was detected, a GnRH antagonist (cetrorelix) was started at a dose of 0.25 mg/day subcutaneously until hCG administration [Figure 1]. Women in control group received only r-FSH and monitoring was done with urine LH surge along with TVS until hCG administration. Ovulatory trigger with urinary hCG 5000 IU was administered intramuscularly when there was at least one follicle of 18 mm or more. Semen was prepared for insemination by double density gradient technique. A single IUI was performed between 44 and 48 h after hCG injection. Couples were allowed to have natural intercourse in periovulatory period. Luteal phase support was given to all women in both groups with vaginal-micronized progesterone 400 mg twice daily starting from day after IUI and GnRH agonist (lupride 1 mg SC) on the 8th, 9th, and 10th day after IUI. Serum β-hCG was estimated 2 weeks after IUI. Ultrasonography for the gestational sac and cardiac activity was done 2–3 weeks after the positive β-hCG. Luteal phase support with vaginal progesterone continued until the 10th week of gestation in women with a positive pregnancy test.

Outcome measures

- Primary outcome measures
  - Clinical pregnancy rate.

- Secondary outcome measures
  - Pregnancy loss rate
  - Ongoing pregnancy rate
  - Multiple pregnancy rate
  - Ovarian hyper-response rate.

Clinical pregnancy: Pregnancy diagnosed by ultrasound visualization of at least one gestational sac with or without cardiac activity (multiple gestational sacs are counted as one clinical pregnancy).[19]

Pregnancy loss: It includes abortion (up to 12 weeks) and ectopic pregnancy.

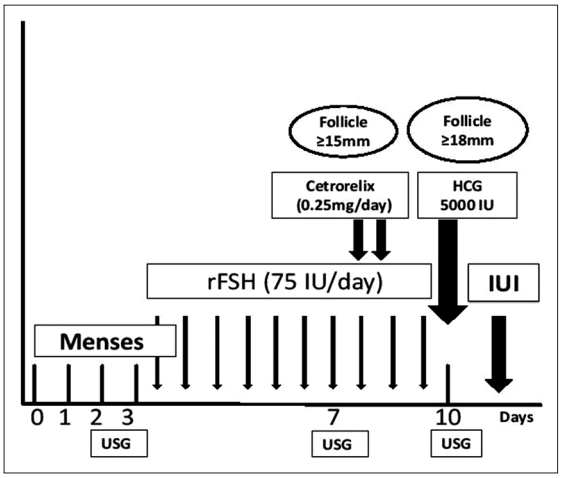

Figure 1: Controlled ovarian stimulation protocol

Ongoing pregnancy: Pregnancy continuing beyond the 12th gestational week.

Multiple pregnancies: Ultrasound visualization of more than one gestational sac with or without cardiac activity.

Ovarian hyper-response: ≥4 follicles >14 mm on the day of hCG.

Statistical analysis

Sample size calculation

Clinical pregnancy rate was the primary outcome measure. The power analysis was based on pregnancy rate per cycle from published randomized studies comparing GnRH antagonist with placebo or no GnRH antagonist. More than 2500 treatment cycles would have been required to detect a statistically significant difference of 5% in clinical pregnancy rate which was not feasible for a single-center trial planned to be completed in 1 year. Therefore, the trial was designed to include 400 patients, which would enable detection of an absolute increase in clinical pregnancy rate by 13% from an assumed 20% clinical pregnancy rate in the control group, with an alpha error level of 0.05 and a beta error level of 0.20.

The difference of 13% was arbitrarily defined to complete the trial in a year. However, 13% was compatible with the 95% CI of the difference between clinical pregnancy rates per cycle in the previous trial.[10]

Statistical tests

Statistical analyses were performed using the SPSS version 17.0 program (SPSSInc.Chicago, IL, USA) for Windows. We conducted a Shapiro–Wilk test to verify the distribution of the data. All data were summarized as the mean ± standard deviation while those with a skewed distribution were described as a median (interquartile range). The Chi-square test was used to compare the differences in variables between the two groups. Student's *t*-test was used for continuous normal variables. The Mann–Whitney test was used to test independent relationships between the variables that did not demonstrate normality. A two-sided $P < 0.05$ was considered statistically significant.

RESULTS

In our study, 331 eligible couples were recruited and randomized into study and control groups. The participant flow through the trial is displayed in Figure 2. The baseline demographic characteristics of patients in two groups were similar and are shown in Table 1. The characteristics of IUI treatment cycles were also comparable in patients of two groups [Table 2]. The total dose of r-FSH was significantly higher in GnRH antagonist group compared with control group (900 IU vs. 750 IU; $P = 0.001$).

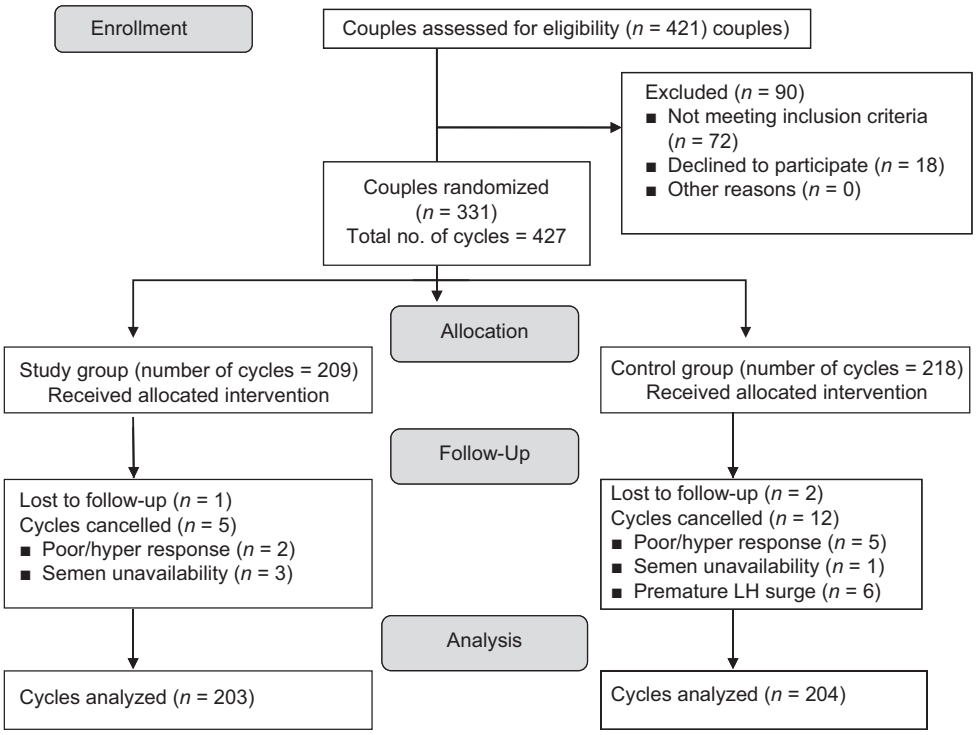

Figure 2: CONSORT flow diagram

Table 1: Baseline characteristics

Variable	Study group (*n*=203)	Control group (*n*=204)	*P*
Age (years)	30.5±3.69	30.0±3.46	0.111
BMI (kg/m^2)	24.9±3.91	25.0±4.39	0.885
Duration of infertility (years)	3.0 (2.0-4.5)	3.0 (2.0-5.0)	0.605
Baseline FSH (IU/L)	6.8 (5.6-8.0)	6.8 (5.6-8.0)	0.819
Baseline LH (IU/L)	5.0 (3.5-6.2)	5.1 (4.0-6.3)	0.391
Baseline E2 (pg/ml)	44.0 (30.0-55.0)	44.5 (34.0-55.0)	0.527

Data are presented as mean±SD or median (IQR) as applicable. BMI=Body mass index, FSH=Follicle-stimulating hormone, LH=Luteinizing hormone, E2=Estradiol, IQR=Interquartile range, SD=Standard deviation

The results of the study are shown in Tables 3 and 4. Clinical pregnancy rate was 27.6% in GnRH antagonist group and 26.5% in the control group (P = 0.800). Spontaneous pregnancy loss was observed in 17.9% cases in GnRH antagonist group and 13.0% cases in the control group (P = 0.666). Ongoing pregnancy rate was 22.7% in GnRH antagonist group and 22.5% in the control group, with no clinically significant difference in two treatment groups (P = 0.979). Similarly, multiple pregnancy rates were similar in two groups, 14.3% in GnRH antagonist group and 9.2% in control group (P = 0.414). Incidence of ovarian hyper-response was significantly higher in control group, 16.7% when compared to GnRH antagonist group, 8.9% (P = 0.018).

The clinical pregnancy rate was higher in multifollicular cycles in comparison to monofollicular cycles (P = 0.02); however, both groups were comparable. Furthermore, the incidence of multiple pregnancies in multifollicular cycles in study group (16.3%) and control group (9.5%) was comparable (P = 0.354).

DISCUSSION

This prospective, randomized controlled study aims to prove the role of GnRH antagonist in increasing pregnancy rates in gonadotropin-stimulated IUI cycles. The role of GnRH antagonist in prevention of premature LH surge is well-established.[18,20] In IVF-embryo transfer cycles, GnRH antagonist gives comparable pregnancy rates to GnRH agonists with significant reduction in the ovarian hyperstimulation syndrome (OHSS) incidence.[21]

The present study takes into account the suppressive effect of GnRH antagonist on premature LH surge in timing of IUI so that it may help in improving pregnancy rate in gonadotropin-stimulated cycles. However, the effect of GnRH antagonist on clinical pregnancy rate could not be confirmed in our study, probably due to certain limitations.

The present study being time bound for 12 months, we could not take a larger sample size to reach a statistically significant difference in clinical pregnancy rate.

From the available evidence, we found that premature LH surge did not start before follicle size of 14–15 mm

Table 2: Characteristics of intrauterine insemination treatment cycles

Variable	Study group (*n*=203)	Control group (*n*=204)	*P*
Duration of stimulation (days)	8.0 (7.0-10.0)	8.0 (7.0-10.0)	0.138
Duration of GnRH antagonist (days)	2.12±0.96	-	-
Number of dominant follicles (>18 mm)	2.0 (1.0-3.0)	2.0 (1.0-3.0)	0.378
Endometrial thickness (mm)	8.2 (7.5-9.0)	8.3 (7.6-9.2)	0.264
Total dose of gonadotropins (IU)	900.0 (675.0-1125.0)	750 (531.2-1050.0)	**0.001 (S)**
Serum E2 (pg/ml)	271.0 (176.0-453.0)	269.5 (162.2-521.0)	0.957

Data are presented as mean±SD or median (IQR) as applicable. GnRH=Gonadotropin-releasing hormone, E2=Estradiol, IQR=Interquartile range, SD=Standard deviation

Table 3: Primary and secondary outcomes of intrauterine insemination cycles

Outcome measures	Study group (*n*=203)	Control group (*n*=204)	*P*
Clinical pregnancy rate (%)	27.6 (*n*=56)	26.5 (*n*=54)	0.800
Pregnancy loss rate (%)	17.9 (*n*=10)	14.8 (*n*=8)	0.666
Abortion rate	17.9 (*n*=10)	13.0 (*n*=7)	0.478
Ectopic pregnancy	0 (*n*=0)	1.9 (*n*=1)	0.306
Ongoing pregnancy rate (%)	22.7 (*n*=46)	22.5 (*n*=46)	0.979
Multiple pregnancy rate (%)	14.3 (*n*=8)	9.2 (*n*=5)	0.414
Ovarian hyper response rate (%)	8.9 (*n*=18)	16.7 (*n*=34)	**0.018 (S)**

Data are presented as percentage and absolute numbers. S=Significant

Table 4: Comparison of clinical pregnancy rate in monofollicular and multifollicular cycles

Outcome	Study group (*n*=203)	Control group (*n*=204)	*P*
Clinical pregnancy rate in monofollicular cycles (%)	19.7 (13/66)	19.7 (12/61)	0.997
Clinical pregnancy rate in multifollicular cycles (%)	31.4 (43/137)	29.4 (42/143)	0.714

Data are presented as percentage and absolute numbers

in majority of cases.[22,23] Hence, we have taken follicle size >15 mm as the cutoff limit to start GnRH antagonist so as to reduce the task of monitoring to minimum. In the control group, we measured urinary LH surge beyond the follicle size of 15 mm until the ovulatory trigger. In control group, premature LH surge started in six cycles which were excluded from the analysis. Had these cases been included, it would not have affected the outcome.

Natural intercourse was not restricted in any of the groups, therefore excluding the bias of spontaneous conception; however, the inclusion of subfertile couples minimizes that effect. To avoid a hypothetical effect of antagonist on function of corpus luteum, we routinely gave luteal phase support to all women in both groups.

Both groups were comparable in demographic characteristics regarding age, BMI, cause, and duration of infertility as well as baseline FSH, LH, estradiol (E2) levels, which rule out the selection bias. Similarly, there was no significant difference in most of the treatment variables between the two groups, except the total dose of gonadotropin which was significantly higher in GnRH antagonist group (900 IU vs. 750 IU).

There is conflicting evidence regarding multifollicular development and increased pregnancy rates with the use of GnRH antagonist in gonadotropin-stimulated IUI cycles. Gómez-Palomares *et al*. and Bakas *et al*. found a significant increase in the number of dominant follicles with GnRH antagonist compared to no GnRH antagonist (2.4 vs. 1.3 and 2.1 vs. 1.4), and they concluded that addition of GnRH antagonist leads to increased pregnancy rates in multifollicular cycles.[24,25] On the other hand, Allegra *et al*. found no difference in number of dominant follicles with the addition of GnRH antagonist (3.08 vs. 3.20).[10] Similarly, the number of dominant follicles was not different between study and control groups (2.0 vs. 2.0) in our study. On comparing the clinical pregnancy rates in multifollicular cycles with monofollicular cycles, there was a statistically significant difference favoring multifollicular cycles in both the groups; however, there was no significant difference between the study and control groups [Table 4].

Ovarian follicles are most LH sensitive between 11 and 15 mm sizes.[22] While the growth and maturation of a dominant follicle are not affected by sudden LH withdrawal induced by GnRH antagonist, there is arrest in growth of intermediate size follicles,[22] i.e., when one follicle takes lead and GnRH antagonist is added to suppress LH, the rest of the follicles having higher LH threshold get atretic and there are ultimately lesser follicles achieving dominance. This hypothesis also forms the basis of our secondary outcome, i.e. ovarian hyper-response rate which was not addressed in any of the previous trials. The significant reduction in ovarian hyper-response rate with the addition of GnRH antagonist (8.9% vs. 16.7%) proves its role in reduction of OHSS, which is not acceptable in an IUI cycle. There was no case of OHSS in our study. More number of mature follicles also increases the risk of multiple gestations. There was no statistically significant difference in multiple pregnancy rates in study and control groups (14.3% vs. 9.2%). There was no case of high-order multiple pregnancies (HOMP) in our study. No increase in multiple pregnancies, OHSS, or HOMP is attributable to the strict cancellation criteria in

our study. Pregnancy loss rate was also similar between the study and control groups (17.9% vs. 14.8%).

In our study, the clinical pregnancy rate was 27.6% and 26.5% and ongoing pregnancy rate was 22.7% and 22.5% in the study and control groups, respectively. We owe such high success in IUI cycles to perfect timing of ovulation and insemination. After hCG injection, ovulation starts around 36 h later and sequential thereafter. Therefore, at 44–48 h post-hCG, the oocyte should be somewhere in the fallopian tube and <12 h old thus, should be able to give best results in terms of pregnancy if IUI is performed at that time. In addition, washed sperms in prepared semen can fertilize oocyte only within the next 2–3 h because removal of seminal plasma initiates sperm capacitation by changes in the acrosome. Therefore, best time for insemination to maximize the chances of fertilization is within 2–3 h of semen preparation.[26,27] These results suggest that success rate of IUI mainly depends on timing of hCG injection and single well-timed insemination, that too within 12 h postovulation as the fertilizable life span of a mature oocyte is only 12–24 h[28] and of washed sperms is 2–3 h.[15] Hence, too early or too late, an insemination would be futile in terms of achieving a pregnancy.

Most studies performed so far were heterogeneous in sample size, selection criteria, stimulation protocols, timing of administration of hCG, timing of IUI, number of inseminations, luteal phase support and so were the pregnancy rates.[29-35] The timing of insemination was between 32 and 42 h in most of the studies, and in some studies, double insemination was performed, which could have led to difference in pregnancy rate. Further, very few studies compared pregnancy loss rate, multiple pregnancy rate, ovarian hyper-response rate, and incidence of OHSS.

If we compare large randomized controlled trials (RCTs), almost half of them do not demonstrate any significant beneficial effect of GnRH antagonist addition in gonadotropin-stimulated IUI cycles; however, most of the studies are underpowered in terms of sample size [Table 5].

The meta-analysis done by Kosmas *et al*. included six RCTs up to 2006 and showed a beneficial effect in favor of GnRH antagonist (OR 1.56, 95% CI 1.05–2.33). Based on OR derived from meta-analysis, the number of patients needed to prevent one additional LH rise was 4 (95% CI 3–6) and to achieve one additional clinical pregnancy, with the addition of GnRH antagonist was 19 (95% CI 10–81). In addition, it showed a parallel trend for multiple pregnancies.[32]

In the last Cochrane systematic review, regarding optimal stimulation protocols for IUI, five RCTs up to 2007 were included and it was concluded that adding a GnRH antagonist does not increase pregnancy rate (OR 1.5, 95% CI 0.83–2.8).[13]

Table 5: Review of literature

Study	Number of cases	PR in Study group (%)	PR in Control group (%)	*P*
Williams *et al*.[29]	120	12	7	0.29
Gómez-Palomares *et al*.[30]	82	38	14	**0.01 (S)**
Lambalk *et al*.[12]	204	13.6	13	1.00
Crosignani and Somigliana[11]	261	12.2	12.6	1.00
Allegra *et al*.[10]	302	53.8	30.8	**0.02 (S)**
Gómez-Palomares[24]	367	23	11	**<0.05 (S)**
Bakas *et al*.[25]	234	22	11	**<0.05 (S)**
Cantineau *et al*.[4]	572	11	14	0.31
Steward *et al*.[31]	80	23	20	0.80
Our study (2012)	407	27.6	26.5	0.824

S=Significant, PR=Pregnancy rate

So far only two large double-blinded, placebo-controlled, multicenter trials have been done on this issue by Lambalk *et al*. and Cantineau *et al*., both of which proved that GnRH antagonist addition does not increase pregnancy rate and live birth rate, respectively, in gonadotropin-stimulated IUI cycles.[4,12]

The results of our study are in line with the available evidence. Further meta-analysis or a Cochrane review update is definitely required to disentangle this issue.

CONCLUSIONS

Addition of GnRH antagonist to gonadotropin-stimulated IUI cycles results in no significant difference in clinical pregnancy rate, pregnancy loss rate, ongoing pregnancy rate, or multiple pregnancy rates, but it significantly lowers the ovarian hyper-response rate.

These results suggest that most important determinant of achieving optimal pregnancy rates in IUI cycles is perfect timing of insemination in relation to ovulatory trigger (44–48 h) and semen preparation (2–3 h). Other factors such as choice of stimulation regimen, prevention of premature luteinization with the use of GnRH antagonist can only marginally add on to the success.

Clearly, this study does not support the routine use of GnRH antagonist in COS-IUI protocols in terms of chances of success, but we strongly believe that some points need to be better defined. The sample size recruited in this study is not sufficient to disentangle this issue. Nevertheless, GnRH antagonist treatment could allow flexible timing of hCG injection and insemination, thereby decreasing

the need of extensive cycle monitoring and avoiding IUI during weekends. The role of GnRH antagonist in preventing OHSS in COS-IUI cycles is also worth consideration.

Acknowledgment

The authors are thankful to Dr. Mohinder Kochar, Dr. Shweta Mittal, Dr. Neeti Tiwari, Dr. Ruma Satwik, Dr. Tejshree Singh, and Dr. Dattaprasad Inamdar for providing subjects and inputs for the study. Dr. Ajay Jain is appreciated for his generous help and constant inspiration. We express gratitude to the staff of Centre of IVF and Human Reproduction, Sir Ganga Ram Hospital, and all the patients who participated in the study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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