

Effectiveness of Gonadotropin-Releasing Hormone Antagonists in the Management of Multifollicular Recruitment in Intrauterine Insemination Cycles

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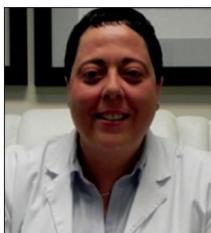
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ABSTRACT

Introduction: To evaluate the efficacy of GnRH antagonists in terms of increasing the pregnancy rates in intrauterine insemination (IUI) after controlled ovarian stimulation (COS) when more than one dominant follicle is recruited. **Methods:** This is a prospective and randomized clinical trial that included 300 couples with primary or secondary infertility that underwent their first or second COS-IUI cycle with recombinant FSH. In all of these patients two or three leading follicles > 14 mm of mean diameter were detected by vaginal ultrasound (US) and were randomized into two groups. In group A the patients received rFSH+GnRH antagonists until the day that the hCG was given, while in group B the patients followed a standard COS received rFSH only. **Results:** Total amount of rFSH units (620.8+245.1 vs 575.5+296.4) and clinical pregnancy rates (31.16% vs 19.15%) were statistically significantly higher in patients who were treated with GnRH antagonists. A similar number of twin pregnancies and miscarriages occurred in both groups. **Conclusion:** Multiple doses of GnRH antagonists in COS-IUI significantly increase pregnancy rates in multifollicular cycles.

INTRODUCTION

Intrauterine insemination (IUI) is one of the first therapeutic steps in assisted reproduction. Many studies have shown that the use of this technique in association with ovulation-inducing treatments improves results and that the use of gonadotropins increases pregnancy rates (PR) compared with the use of clomiphene citrate.¹⁻⁴

When combined with controlled ovarian stimulation (COS) protocols, intrauterine insemination is relatively

affordable for all public and private centres and has an effectiveness rate that varies between 8% and 18% of gestations per treatment cycle.^{1,2,5-7} Many factors influence the results of these treatments, but one factor, the appearance of premature peaks in luteinising hormone (LH) during the course of controlled ovarian stimulation, significantly reduces the possibilities for success with this technique.⁸

In clinical practice, spontaneous LH peaks during in vitro fertilisation or sperm microinjection cycles are prevented by administering analogues of gonadotropin-releasing hormone (GnRH). However, during artificial insemination treatments, pituitary suppression is not conducted routinely, although the published non-negligible percentages of spontaneous ovulation range from 24% to 35% of initiated cycles.⁹⁻¹¹

Agonist and antagonist GnRH analogues inhibit pituitary activity. However, the action of GnRH

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antagonists is immediate, blocking GnRH receptors without the flare-up phenomenon of the agonists and producing rapid and marked reductions of serum LH levels.^{12,13} The immediate pituitary inhibition caused by GnRH antagonists has been used during intrauterine insemination cycles to reduce the percentage of spontaneous LH peaks¹⁴⁻¹⁶ and has allowed many study groups to improve their gestation rates with new treatment protocols that include one of the antagonists presently available on the market: cetrorelix (Cetrotide[®], Merck-Spain) or ganirelix (Orgalutran[®], MSD-Spain).^{11,15,17}

There are two advantages offered by inhibiting the LH peak during artificial inseminations. One of them is the possibility of improving flexibility, thus reducing the treatment cancelation rate resulting from a lack of available reproductive health personnel and reducing any overload on the hospital's emergency team.¹⁸⁻²⁰ The second advantage is that GnRH antagonist allows gonadotropin stimulation to be extended, enabling the appropriate development of more than one follicle and increasing pregnancy rates in intrauterine insemination.²¹

The objective of this study was to evaluate the effectiveness in terms of pregnancy rate of antagonists of GnRH (cetrorelix and/or ganirelix) in COS-IUI cycles with multifollicular recruitment.

METHODS

Design and Inclusion Criteria

This study was a prospective, randomized clinical trial that was performed between October 2011 and October 2015 in Instituto FIVIR, a private and specialized clinic of reproductive medicine in Carcaixent, Valencia, Spain and in University Hospital of La Ribera in Alzira, a general public hospital in Valencia, Spain.

The study included 300 couples, with primary or secondary infertility that underwent their first or second COS-IUI cycle with recombinant FSH. In all of these women, two or three leading follicles > 14 mm of mean diameter were detected by vaginal ultrasound (US) during the ovarian stimulation with recombinant FSH (Gonal F[®], Merck-Spain or Puregon[®], MSD-Spain).

The patients were randomly divided into a treatment group (Group A: 150 patients) and a control group (Group B: 150 patients) using an online research randomiser software (www.randomizer.org). Group A maintained the COS treatment and received a subcutaneous daily dose of a GnRH antagonist (0.25 mg, Cetrotide[®] or Orgalutran[®]) until the day that recombinant hCG was given, while Group B followed controlled ovarian stimulation treatment until the hCG day.

The following inclusion criteria were applied in women: age between 18 and 40 years old, basal ovarian hormonal profile with FSH < 10 IU/L and estradiol < 80 pg/ml, normal levels of prolactin and thyroid-stimulating hormone, body mass index < 35 kg/m², normal uterine cavity and bilateral tubal patency assessed by hysterosalpingography. Moreover, only patients with partners with normal seminal parameters according to World Health Organization criteria and whose total motile sperm count (TMSC) after sperm washing by swim-up was equal to or greater than 10 million/ml were accepted.

Patients who had clinically significant systemic disease, evidence of endometriosis by surgery or ultrasound, or diagnosis of intramural or submucous uterine fibroids, were excluded from the present study.

Controlled Ovarian Stimulation

The patients received 25 to 150 IU/d of recombinant FSH (rFSH) (Gonal-F[®], Merck-Spain; Puregon[®], MSD-Spain) beginning on the 2nd-5th day of the menstrual period after assessing ovarian quiescence via vaginal US. The response to treatment was evaluated using ultrasonography, beginning on the 5th day of stimulation. Both the controls and the doses were adjusted individually, and the daily quantity of recombinant FSH was increased if no response was evident after 7 days of stimulation.

Eligible patients whose ultrasound images revealed 2 or 3 follicles measuring at least 14 mm of mean diameter were included in the study and they were randomized to receive GnRH antagonists or following standard COS treatment.

When the follicular size of the leading follicles were between 18 and 20 mm, recombinant hCG (Ovitrelle[®], 250 µg/0.5 ml, Merck-Spain) administration was recommended to induce ovulation.

Sperm Preparation

Sperm samples for intrauterine insemination were obtained by masturbation in a sterile jar after 3- 5 days of sexual abstinence and were delivered for processing within 30-60 minutes after their collection. The semen was incubated for 30 minutes at 37°C, and after liquefaction, the volume and viscosity were determined. The motility and initial concentration of the spermatozoa were calculated using a Makler chamber (Sefi Medical Instruments, Ltd.).

The swim-up technique was used to prepare the semen. Sperm Preparation Medium (60 ML; Medicult[®]) was used to wash the semen. The samples were incubated at 37°C in 5% CO₂ for 45 minutes on an inclined rack using the same culture medium. Finally, the concentration of motile

spermatozoa in the preparation was determined using the Makler chamber.

Insemination Technique and Luteal Phase Support

A single intrauterine insemination was delivered 36-40 hours after recombinant hCG was administered. Semen was deposited using a disposable flexible catheter (Gynetics[®]) that instilled 0.3-0.5 ml in the uterus. The patients remained at rest for 10 minutes after the technique.

The same day after the IUI, all of the patients started treatment with natural micronized progesterone to supplement the luteal phase (Utrogestan[®], Seid, Spain; or Progeffik[®], Effik, Spain). A dose of 200 mg/day was vaginally administered over 15 days, and treatment was maintained until the 12th week of gestation or discontinued if the urine pregnancy test was negative.

Pregnancy

Pregnancy was diagnosed via the qualitative determination of beta HCG in the urine 2 weeks after insemination. If positive, a transvaginal ultrasound was programmed for 2 weeks later. Clinical pregnancy was defined as the presence of an intrauterine gestational sac with positive embryo heartbeat.

Statistical Analysis

The statistical analyses were performed using Minitab Statistical Software 16.1.0 (Minitab Inc.). The data were expressed as mean +standard deviation SD. The mean of the normal quantitative variables (level of basal serum FSH and estradiol) were compared using the Student's *t*-test, and the rest of variables, non-normal variables, were compared using the Mann-Whitney *U*-test. The chi-square test was used for the qualitative variables. Significant differences were considered when $p < 0.05$.

RESULTS

Among the 300 couples eligible and randomized, 12 were excluded in the group treated with antagonists of GnRH (Group A), and 9 in the control group (Group B). After the exclusion, the remaining patients for analysis were 138 in group A and 141 in group B (Figure 1).

The basal characteristics of both groups were similar, and we did not find statistically significant differences when comparing the mean value of the following parameters: age of both members of the couple; the woman's basal serum FSH and estradiol levels; the parameters in diagnostic fresh semen analysis and diagnostic TMSC (Table 1).

The total amount of rFSH units was higher in the group of patients receiving the antagonist with respect to the group of patients receiving rFSH alone, and these parameters reached

significance (620.8+245.1 vs 575.5+296.4. Mann-Whitney *U*-test: $p=0,0073^*$) (Table 2).

By the day that hCG was indicated, no significant differences were observed in the number of follicles > 18 mm, endometrial thickness or TMSC post-swim up between the antagonist group (Group A) and the control group (Group B) (Table 2).

The total number of pregnancies achieved in the rFSH+GnRH antagonist group was 43 (31.16%), while 27 gestations (19.15%) were achieved in Group B. The difference reached statistical significance (X^2 : $p = 0,021$) (Table 3).

No differences were statistically significant when we compared the rate of miscarriages (16.28% vs 11.11%. X^2 : $p = 0,548$) or multiple gestations (twins) (6.98% vs 3.70%. X^2 : $p = 0,566$). No triplets were diagnosed in any of the groups (Table 3).

DISCUSSION

There is no accord among researchers regarding the effectiveness of GnRH antagonists in COS-IUI cycles

Table 1: Basal characteristics of the study population

	Group A (n=138)		Group B (n=141)		p
	Mean	SD	Mean	SD	
Age, females (years)	31.26	4.06	32.19	3.95	p=0,0898
Age, males (years)	33.38	4.40	34.18	5.14	p=0,2471
Body mass index, females (kg/m ²)	23.58	3.89	23.56	4.22	p=0,6227
FSH (IU/l)	7.19	1.74	7.15	1.52	p=0,837
Estradiol (pg/ml)	46.43	15.22	46.47	14.64	p=0,980
Fresh sperm count (mill/ml)	110.19	71.94	109.84	67.85	p=0,7760
Sperm motility a+b (%)	68.30	10.78	66.91	12.51	p=0,2497
Normal sperm (%)	13.65	7.37	14.22	9.26	p=0,6771
Diagnostic TMSC (mill/ml)	36.07	24.59	37.82	30.39	p=0,9864

Table 2: COS-IUI characteristics

	Group A (n=138)		Group B (n=141)		p
	Mean	SD	Mean	SD	
rFSH (total units)	620.8	245.1	575.5	296.4	p=0,0073*
Total number of follicles >18 mm	2.22	0.42	2.18	0.38	p=0,3251
Endometrial thickness (mm)	9.19	1.68	9.39	1.72	p=0,3865
TMSC (mill/ml)	47.00	38.19	46.71	35.96	p=0,8503

Table 3: Outcomes

	Group A (n=138) (%)	Group B (n=141) (%)	p
Clinical pregnancy	31.16 (43/138)	19.15 (27/141)	p=0,021
Twins	6.98 (3/43)	3.70 (1/27)	p=0,566
Miscarriages	16.28 (7/43)	11.11 (3/27)	p=0,548

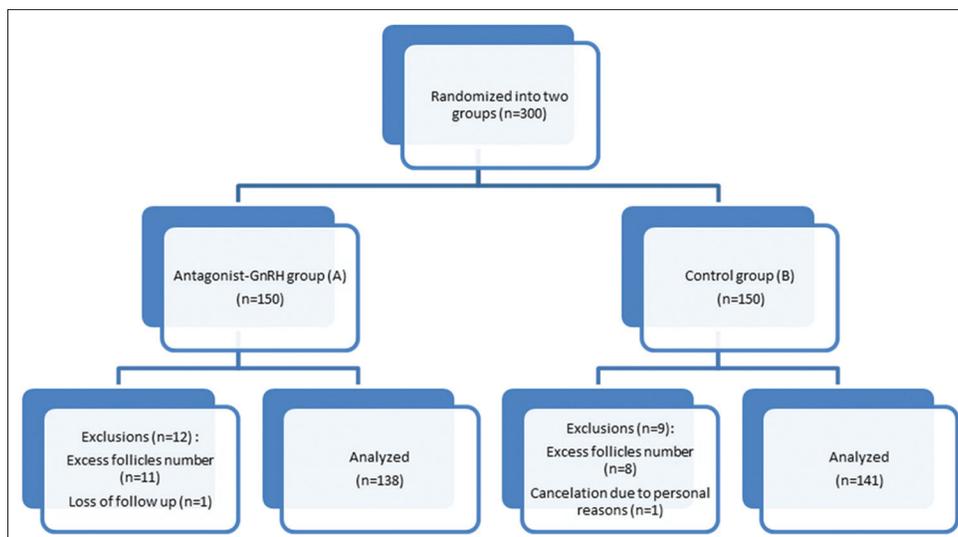


Figure 1: Flowchart of the study

in terms of increasing pregnancy rates, although recent meta-analysis suggested that GnRH antagonists increase the clinical pregnancy rate when used in COS-IUI.²²

Several authors have taken advantage of antagonists' quick action to avoid weekend inseminations for logistical reasons and have found higher gestation rates per cycle among patients treated with GnRH antagonists, although the difference was not statistically significant.^{18,20}

Other authors, however, have published gestation rates per cycle that were significantly higher when they introduced GnRH antagonists into COS-IUI cycles.^{21,23} Those authors concluded that this positive effect was caused less by the inhibition of spontaneous LH peaks than by the fact that pituitary suppression would allow an increased number of mature follicles on the day of hCG treatment.

Effectiveness of COS with rFSH in IUI is based on the ability to increase the number of available oocytes at the moment of ovulation by ensuring more than one dominant follicle.²³ However, the more follicles are recruited, the higher serum estradiol level we find, and it is known that serum estradiol level is the trigger of the surge of luteinizing hormone (LH) and it may lead to a premature luteinización in detrimental to oocyte quality, fertilization, embryo implantation and pregnancy rate.^{24,25}

GnRH antagonists have the advantage of inducing deep and immediate pituitary suppression that is much more pronounced for LH than for FSH²⁶ and one of the most useful indications of these drugs in COS-IUI is to avoid premature luteinisation when more than 1 follicle is recruited during ovarian stimulation.

Gómez-Palomares and colleagues published two interesting studies that demonstrated that when a single preovulatory follicle of size >18 mm is recruited in a COS-IUI, no differences in pregnancy rates were observed between patients treated with rFSH plus GnRH antagonists or patients with rFSH alone.^{21,27} Conversely, when two or three preovulatory follicles were present at the time of hCG administration, pregnancy rates significantly increased comparing with the group of patients who did not receive the antagonists of GnRH.²¹

The published data agree with our results. We have demonstrated a significant increase in the number of gestations achieved when two or three follicles are recruited and antagonists of GnRH are introduced in the protocol of controlled ovarian stimulation combined with intrauterine insemination.

One of the great problems in reproductive medicine and its treatments is the number of multiple (high-order) pregnancies. It may be considered that if we promote the development and maturation of more than one oocyte, the proportion of multiple pregnancies may be higher. In fact, literature describes an average for multiple gestations in protocols of COS-IUI that introduce antagonists of GnRH increased.^{21,28-30}

When advices of Dickey and colleagues are followed and less than four follicles >15 mm or less than six follicles >12 mm are measured, triplet and higher-order implantations occurred in less than 1% of cycles and in less than 5% of pregnancies.²⁸

The rate of multiple pregnancies in our study is similar to what is described in the literature¹⁸⁻²¹ with no triplets in our study population.

In conclusion, our study demonstrates that protocols of treatment with multiple doses of GnRH antagonists in COS-IUI when two or three follicles are recruited are safe in terms of number of multiple gestations and improve significantly the outcome of the treatment raising of the chances of achieving a pregnancy.

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