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The addition of GnRH antagonists in intrauterine insemination cycles: a pilot study

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Abstract. – AIM: This prospective study was designed to assess whether the use of GnRH antagonists can improve the success rate of controlled ovarian stimulation (COS) in intrauterine insemination (IUI) treatments.

PATIENTS AND METHODS: Eighty patients were divided into two groups: GnRH antagonist group (Group A, n=40) and control group (Group B, n=40). Patients in Group B underwent COS with recombinant Follicle Stimulating Hormone (r-FSH, 50-75 IU/d) only, while patients in Group A were administered r-FSH (50-75 IU/d) plus cetrorelix (0.25 mg/d, starting when ≥ 2 follicles ≥ 14 mm were detected on ultrasound scan). In both groups a single insemination was performed 36 hours after human Chorionic Gonadotropin (hCG, 250 mcg) administration. The primary outcome was clinical Pregnancy Rate (PR). Secondary outcomes were ongoing PR, incidence of Premature Luteinization (PL), number of follicles with mean diameter ≥ 16 mm and between 11 and 15 mm on the day of hCG administration, miscarriage rate, cycle cancellation rate, total amount of r-FSH used and duration of treatment. Student's t test and Chi-square test were used ($p < .05$ statistically significant).

RESULTS: A total of 146 cycles were performed (Group A: n=72; Group B: n=74). A trend towards higher PR in Group A was detected, although it was not statistically significant (Clinical PR: 18.05% vs 10.81%). The number of follicles ≥ 16 mm was significantly increased in Group A. The incidence of both premature LH surge and premature luteinization (PL) was significantly higher in Group B. No significant differences were found in the duration of the stimulation protocol, and in the total amount of r-FSH administered.

CONCLUSIONS: The addition of GnRH antagonist in COS/IUI protocol significantly increases the number of mature follicles. However, this multifollicular recruitment is not linked to a significantly higher PR.

Key Words:
GnRH antagonist, Cetrorelix, Pregnancy rates, Intrauterine insemination, Ovarian stimulation, Controlled ovarian stimulation, Infertility.

Introduction

The association of controlled ovarian stimulation (COS) with intrauterine insemination (IUI) may result in higher success rates[1-9]. Nevertheless, the multifollicular recruitment allowed by COS rapidly increases estradiol (E2) serum levels and leads to a Luteinizing Hormone (LH) surge while follicular growth is still in progress. Premature LH surge occurs in 24% of IUI cycles and it is held to be responsible for the luteinization and disruption of normal follicle and oocyte development[8,10], thus, possibly leading to cycle cancellation[11-14]. In order to avoid the risk of unexpected premature follicular luteinization, the physician should proceed to ovulation induction as soon as the leading follicle reaches 18 mm in diameter, regardless of the number and developmental status of the other recruited follicles[3,15-17]. Thus, most of the stimulated cycles would be monofollicular. This would reduce the chances of pregnancy because at least two mature follicles (≥ 16 mm) are needed to achieve a satisfactory pregnancy rate in IUI[1-5,18]. An alternative management could be the inclusion of GnRH antagonists (GnRH-ant) in IUI/COS regimens. These drugs successfully protect follicular development against unexpected luteinization (in *In Vitro* Fertilization, IVF), by preventing untimely LH release[19-23]. Furthermore, since GnRH antagonist postpone ovulation, they allow gonadotropin stimulation to be extended, enabling the appropriate development of more than one follicle. In fact several Authors have reported that a significant increase in PR is linked with a parallel increase in the number of mature follicles detected on the day of human Chorionic Gonadotropin (hCG) administration[11,16,17,24].

This study was aimed at assessing whether GnRH-ant can increase the pool of mature folli-

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cles and consequently the pregnancy rate (PR), comparing the traditional stimulation protocol based on the sole gonadotropins administration and a stimulation protocol associating gonadotropins and GnRH-antagonist.

Patients and Methods

Eighty couples presenting for infertility assessment at the Infertility Unit, University of Ferrara, between June 2010 and June 2012, were enrolled in this prospective study. The patients were divided into a GnRH antagonist group (Group A = 40 patients) and a control group (Group B = 40 patients).

All patients granted informed written consent for participation in the study. Institutional Ethics Committee approval was obtained for the treatment procedure.

The study included healthy women who met the following criteria: aged 18-40 years, Body Mass Index (BMI) 19-30 kg/m^2, regular menses (24-35 days), bilateral tubal patency at the hysterosonosalpingography performed within the last 2 years, normal thyroid function and prolactine (PRL) levels, no serum Follicle Stimulating Hormone (FSH) level > 10 IU/L during the early follicular phase. The patients enrolled in this study were affected by primary or secondary infertility lasting 1 year or more; idiopathic and mild male factor were taken into account as causes of infertility; women with endometriosis or polycystic ovarian syndrome were excluded.

In both groups ovarian stimulation started on day 3 with recombinant FSH (r-FSH) at a dose of 50-75 IU (Gonal-F, MerckSerono, Italy). In Group A the GnRH-antagonist Cetrorelix 0.25 mg per day was administered when two or more follicles reached a diameter of 14 mm until the day of hCG injection. Hormonal and ultrasound monitoring was started between the eighth and the tenth day of the menstrual cycle, depending on the menstrual characteristics of the patient and was then performed on alternative days. The ovarian response to the treatment was measured by serum Estradiol (E2), Progesterone (P) and LH levels using a commercially available kit (Elecsys, Roche Diagnostics). Follicular growth was monitored by transvaginal ultrasound using a 7.5-MHz transducer (Aloka Prosound SSD 3500sx, Tokyo, Japan). In both groups hCG, (Ovitrelle, MerckSerono) 250 mcg was administered intramuscularly (IM) when one or more follicles reached ≥18 mm diameter. P, E2 and LH levels were measured on the day of hCG administration. Premature LH rise was defined as LH ≥ 10 UI/L, while the combination of an LH level ≥ 10 IU/L and of a P level ≥ 1 ng/mL was indicated as premature luteinization.

IUI was performed 36 hours after hCG administration using a transcervical catheter (Gynetics Medical Products, Hamont-Achel, Belgium).

Semen for IUI was collected in the facilities of the laboratory after 3-4 days of abstinence and was elaborated using a standard swim-up technique. The inseminated volume was approximately 0.3 mL. After withdrawal of the insemination catheter, bed rest was prescribed for 10-15 minutes.

The luteal phase was supported by 200 mg per day of micronized vaginal Progesterone (Prometrium, Rottafarm, Milan, Italy). This was continued until a pregnancy test was done 2 weeks later and, in case of pregnancy, up to 10-12 weeks' gestation. Clinical pregnancies were confirmed by ultrasound visualization of at least one gestational sac at 7-8 weeks of gestation. Ongoing pregnancies were defined as pregnancies progressing beyond the first trimester.

All patients in both groups were allowed to undergo a maximum of three IUI cycles.

Cycles were cancelled when more than five or less than two follicles with a mean diameter ≥ 16 mm were detected on the day of hCG administration, in order to increase pregnancy chance and to reduce the risk of multiple pregnancy. Furthermore, some cycles were cancelled either owing to personal reasons, or to the absence of follicular development (no follicle > 10 mm observed on cycle day 21).

The primary outcome was clinical pregnancy rate. Secondary outcomes were ongoing pregnancy rate, incidence of premature luteinization (PL), number of follicles with mean diameter ≥ 16 mm and with mean diameter between 11 and 15 mm on the day of hCG administration, miscarriage rate, cycle cancellation rate, total amount of r-FSH used and duration of treatment. Finally E2, P, LH levels, and endometrial thickness on the day of hCG administration were evaluated.

Statistical Analysis

Data were analyzed using SPSS, version 20.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were compared with Student's *t* test. The Chi-square test and Fisher test were used to compare clinical outcomes between the two groups. p value of < 0.05 was considered statistically significant.

Results

A total of 80 patients were included in the study and were allocated to two groups: 40 in the GnRH antagonist group (Group A), and 40 in the control group (Group B).

The baseline characteristics of the patients are presented in Table I: there were no significant differences between the two groups. A total of 146 cycles were started: 72 cycles with combined r-FSH/GnRH-antagonist stimulation protocol and 74 with only r-FSH (Group A: n = 72, Group B: n = 74). Patient data during stimulation are shown in Table II. No statistically significant differences could be noticed in terms of total dose of r-FSH used, duration of r-FSH administration, endometrial thickness on the day of hCG administration and number of follicles with mean diameter of 11-15 mm. Conversely, a significant increase in the number of mature follicles (diameter ≥16 mm) was detected in GnRH antagonist group (Group A: 2.01 ± 0.83 *vs* Group B: 1.62 ± 0.65, $p < 0.05$). Regarding hormonal parameters on the day of hCG administration, E2 serum levels did not differ significantly between the groups (Group A: 733.88 ± 127.52 vs Group B: 697.90 ± 102.59, Not Significant, NS), while the mean value of LH and P were significantly higher in the control group (LH levels: Group A: 5.71 ± 2.05 vs Group B: 7.79 ± 1.88, $p < 0.05$; *p* levels: Group A: 0.64 ± 0.35 vs Group B: 0.84 ± 0.33, $p < 0.05$, respectively).

A premature LH surge occurred in 4 cycles in the GnRH antagonist group and in 13 cycles in the control group (respectively, 5.55% and 17.56%, $p < 0.05$). Premature luteinization (PL) was observed in 1 cycle in the GnRH antagonist group and in 8 cycles in the control group (respectively, 1.38% and 10.81%, $p < 0.05$). None of the women developed an ovarian hyperstimulation syndrome (OHSS). Among the 146 cycles started, 10 (6.84%) were cancelled (Group A: n = 4, Group B: n = 6, NS) for several reasons: insufficient ovarian response (Group A: n = 1, Group B: n = 1, NS), development of too many follicles (Group A: n = 2, Group B: n = 2, NS), or personal reasons (Group A: n = 1, Group B: n = 3, NS).

The data regarding pregnancy rates in the two groups are presented in Table III. Both the clinical pregnancy rate (respectively, 32.5% *vs* 20% per couple, and 18.5% *vs* 10.81%, per cycle) and the ongoing pregnancy rate (respectively, 25% *vs* 15% per couple, and 16.21% *vs* 8.10%, per cycle) were higher in the GnRH antagonist group, but these findings did not reach statistical significance. One twin pregnancy occurred in the GnRH antagonist group (respectively, 2.5% per couple, and 1.38% per cycle); no high order pregnancies were observed in the two groups. Three spontaneous first-trimester miscarriages were observed: 1 in the GnRH antagonist group, 2 in the control group (respectively 1.38% vs 2.70%, NS).

Discussion

The present study was aimed at proving that the additional administration of a GnRH antagonist in a gonadotropin superovulated protocol is linked with higher PR. Furthermore, we speculated that a combined r-FSH/GnRH-Ant protocol would reduce the rate of premature luteinization and increase the number of mature follicles on the day of hCG administration.

The results of the study did not completely confirm our initial assumptions. In the study group a significantly higher number of mature follicles could be observed, however this occurrence wasn't linked to a significant increase in PR. In fact, a trend towards higher PR could be

Table I. Baseline characteristics of patients in each group before initiation of treatment.

	Group A (n = 40)	Group B (n = 40)	*p*
Age (y)	32.97 ± 3.29	32.10 ± 2.93	NS
BMI (kg/m^2)	24.23 ± 2.46	25.16 ± 2.72	NS
Basal FSH (IU/l)	6.88 ± 1.45	7.22 ± 1.21	NS
Basal LH (IU/l)	5.24 ± 1.28	5.36 ± 1.19	NS
Basal estradiol (pg/ml)	44.52 ± 14.87	48.60 ± 15.42	NS
IUI cycles started (n)	72	74	–
Primary infertility n (%)	34 (85%)	35 (87.5%)	NS
Male infertility n (%)	15 (37.5%)	13 (32.5%)	NS
Idiopathic infertility n (%)	18 (45.0%)	19 (47.5%)	NS
Duration of infertility (y)	1.65 ± 0.62	1.72 ± 0.59	NS

Table II. Patient data during stimulation.

	Group A (n = 72)	Group B (n = 74)	p
rFSH dose (U)	711.29 ± 167.71	654.31 ± 218.15	NS
rFSH treatment (d)	7.69 ± 1.35	7.27 ± 1.41	NS
Endometrial thickness (mm)	9.75 ± 1.31	9.31 ± 1.47	NS
Follicle 11-15 mm (n)	1.34 ± 0.77	1.50 ± 0.57	NS
Follicles ≥ 16 mm (n)	2.01 ± 0.83	1.62 ± 0.65	< 0.05
E2 on hCG day (pg/ml)	733.88 ± 127.52	697.90 ± 102.59	NS
P on hCG day (ng/ml)	0.64 ± 0.35	0.84 ± 0.33	< 0.05
LH on hCG day (IU/l)	5.71 ± 2.05	7.79 ± 1.88	< 0.05
LH surge (%)	4/72 (5.55%)	13/74 (17.56%)	< 0.05
PL (%)	1/72 (1.38%)	8/74 (10.81%)	< 0.05
OHSS (%)	0 (0%)	0 (0%)	NS
Cancellation cycle (%)	4/72 (5.55%)	6/74 (8.10%)	NS

detected in the GnRH antagonist group, though it did not reach statistical significance. This finding is in accordance with Steward et al study[25], in which a higher number of mature follicles on the day of hCG administration is not linked to a significant increase in PR. Even if this assumption is confirmed by further studies, it should not lead to abandon the GnRH-ant. In fact, a stimulation protocol based on GnRH-ant could be successful in specific categories of patients, such as women with demonstrated premature luteinization in previous cycles[13,25,26]. Furthermore, a combined GnRH-ant/r-FSH stimulation protocol is more flexible, because it enables the clinicians to adapt hormonal stimulation to ultrasound findings and ovarian response[11,13] and prevents insemination at weekends[13,27,28]. Altough our study failed to prove a significant increase in PR, it demonstrated the efficacy of these drugs both in the reduction of PL incidence and in the increase of multifollicular development.

As to the reduction of PL occurrence, it has been widely demonstrated that a premature LH peak may eventually lead to lower oocyte quality and fertilization rates, poorer embryo quality; thus, it could be related to worse PR in IVF cycles[29,30]. According to literature, the addition of a GnRH-antagonist to a gonadotropine superovulated protocol successfully prevents premature luteinization and premature LH surge[11,31]. However, in most of the studies, like Lambalk et al, for instance, the reduction of PL ensured by GnRH antagonists is not linked to a significant increase in PR[13]. Only few reports showed a direct connection between the lower PL and the increase in PR[11,16,17]. Furthermore, previous studies reported that PL is linked to a higher miscarriage rate[32]. Since GnRH-antagonists successfully prevent PL, we expected a higher abortion rate in the control group. Nevertheless, in accordance with previous works[17,25], no significant difference could be noticed between the two groups. These results are difficult to explain; they suggest that achievement of pregnancy in COS-IUI is only modestly affected by premature luteinization in comparison with other factors, such as the timing of ovulation-triggering and insemination[13]. An alternative explanation could be that the advantages related to the prevention of premature luteinization are balanced by not-well-understood detrimental effects of GnRH antagonists[33].

Table III. Pregnancy rates after stimulation.

	Group A (n = 72)	Group B (n = 74)	p
Clinical pregnancy rate per couple n (%)	13/40 (32.5%)	8/40 (20%)	NS
Clinical pregnancy rate per cycle n (%)	13/72 (18.05%)	8/74 (10.81%)	NS
Ongoing pregnancy rate per couple n (%)	12/40 (25%)	6/40 (15%)	NS
Ongoing pregnancy rate per cycle n (%)	12/72 (16.21%)	6/74 (8.10%)	NS
Twins per couple n (%)	1 (2.5%)	0 (0%)	–
Twins per cycle n (%)	1 (1.38%)	0 (0%)	–
High-order pregnancies n (%)	0 (0%)	0 (0%)	–
Abortion rate per cycle n (%)	1/72 (1.38%)	2/74 (2.7%)	NS

As to the increase of multifollicular development, we proved that the use of GnRH antagonist is associated with a higher number of follicles on the day of hCG administration. As it has been suggested in literature, a multifollicular recruitment is always preferable, since the number of mature follicles affects the chances of pregnancy during IUI[1-3,5]. A wide retrospective study dealing with predictive factors for pregnancy after IUI states that: "The 'ideal' stimulation cycle enables the recruitment of two follicles measuring > 16 mm with an E2 concentration > 500 pg/mL on the day of hCG administration"[18].

However, the number of mature follicles is also associated with the risk of multiple pregnancy. Some studies show a direct connection between the number of mature follicles on the day of hCG administration and the higher rate of multiple pregnancy[34]. Furthermore, according to a recent meta-analysis[35], the higher PR achieved is connected with an increased, though not significant rate of multiple pregnancies. Since we observed only one twin in the GnRH antagonist group, our findings are in accordance with other reportss asserting that GnRH-ant/r-FSH stimulation protocol increases PR and it is associated with an acceptable rate of multiple pregnancy[11,14,17]. Thus GnRH-ant/r-FSH can be considered an effective and safe regimen for COS/IUI[11,14]. There was no statistically significant difference concerning endometrial thickness, and this is in disagreement with other works[28]. Furthermore, the study group did not differ significantly either in the IU of r-FSH administered or in the total duration of stimulation. Thus, in case further studies should prove a significant increase in pregnancy rate, an advantageous cost-effectiveness of this protocol can be speculated.

In accordance with previous works[17,25,36], our results showed an overall cycle cancellation rate of 6.84%. In particular, 40% of cancelled cycles were due to the development of too many follicles, increasing the risk of both OHSS and multiple pregnancies. The conversion to In Vitro Fertilization (IVF), could be considered as an alternative to cycle cancellation, in order to improve pregnancy rates and reduce patients' emotional distress. This technique shows promising results[37-41]. Quaas et al study, in particular, demonstrated that the use of GnRH antagonist in cycles converted from COS-IUI to IVF is linked with a higher number of follicles, mature oocytes, retrived oocytes and fertilization rate[41].

The current study findings should be interpreted considering some methodological limitations. At first, hormonal levels were not taken into account to determine the correct timing of GnRH-antagonist administration. The importance of hormonal assessment has been pointed out by Allegra et al[14]. The Authors state that GnRH-ant should be administered following precise cut-off values for estradiol (> 200 pg/ml), LH (< 10 mUI/ml) and *p* (< 2 ng/ml) together with a mean diameter of the leading follicle > 16 mm. Observance of these conditions allows to extend the effect of endogenous LH on follicular growth, before stopping it through GnRH-ant administration[14]. According to Allegra et al the results obtained in Lambalk et al multicenter trial (no significant increase in pregnancy rate in patients treated with GnRH-ant and r-FSH)[13] should be examined in the light of these assumptions. Furthermore, Cantineau et al[42] report that a single-measure assessment of hormonal levels on the day of hCG administration could have affected the results of his study negatively, since hCG administration could have been belated (i.e. premature LH and P surge could have already occurred). Besides, the sample was not selected on the grounds of its response to hormonal stimulation. Since the addition of GnRH-ant to the traditional stimulation protocol was meant to increase pregnancy rate through a multifollicular recruitment, it would have been preferable to enroll normal-high responder patients (on the grounds of age and FSH value on the third day of the cycle) to optimize the effect of the drug. Finally the sample size was small and the study was not blinded or placebo controlled. Additional large, well-designed controlled trials are needed before more meaningful deductions can be drawn.

Conclusions

The current study demonstrated that the addition of a GnRH antagonist in a gonadotropin superovulated protocol significantly reduces the incidence of premature luteinization and increases the number of mature follicles.

Multifollicular recruitment is associated with an acceptable number of multiple gestations and with a trend towards higher PR, although not statistically significant. The higher PR in the study group should be attributed to both the larger pool of mature follicles and to the prevention of premature LH surge, protecting follicular develop-

ment and oocyte quality. Further studies are needed to clarify the mechanism of action of GnRH-ant as well as their effect on the prevention of premature LH surge and follicular development, so as to set the appropriate indications for a combined r-FSH/GnRH-ant stimulation protocol.

Competing Interest

The Authors declare that they have no competing interests.

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