

COMPARISON BETWEEN THE GnRH AGONIST AND ANTAGONIST PROTOCOL IN ASSISTED REPRODUCTION DURING CONTROLLED OVARIAN STIMULATION CYCLES

By

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ABSTRACT

Background: Although the question about the mechanism of GnRH agonists and GnRH antagonists' action is well answered, there is still no clear answer about which analogue gives better results in clinical practice. The reports are contradictory and often favor one type of the analogue.

Objective: To compare the impact of GnRH agonist and antagonist protocol during controlled ovarian stimulation cycles as regard the total number of oocytes retrieved and number of mature oocytes, fertilization rate, cleavage of embryos and their grading and pregnancy rate .

Patients and methods: This **prospective** randomized controlled study was conducted at Al-Azhar University Hospitals; including 80 women undergoing controlled ovarian stimulation for ICSI. Patients were assigned randomly into two equal groups : group 1 received GnRH agonist long protocol, and group 2 received GnRH antagonist protocol. Patients underwent GnRH agonist long protocol were processed for pituitary down-regulation on luteal peak period with triptorelin injection for 14 days. A basic evaluation was conducted by ultrasound examination and blood test for hormone levels. After 5 consecutive days of fixed dose of r FSH (Gonal-F) medication, transvaginal ultrasound examination was performed to monitor the development of follicles, and the dose of rFSH was optimally adjusted based on the number and size of developing follicles. In the GnRH antagonist protocol, at day 3 of a menstrual cycle, a basic evaluation was conducted and rFSH (Gonal-F) was initiated at same day the GnRH antagonist, cetrorelix, was administered after 5 days of fixed dose of stimulation drug , and continued to the day of human chorionic gonadotropin (HCG) administration. Oocytes were retrieved 34-38 h after HCG injection and were fertilized in vitro. Embryo transfer (ET) was carried out 72 h after oocyte retrieval. Outcome measures were the total number of oocytes retrieved and number of mature oocytes, fertilization rate, cleavage of embryos and their grading and pregnancy rate.

Results: There were no significant differences between two groups in the number of total oocytes retrieved, mature follicles, the number of embryos transferred, treatment duration and gonadotrophin consumption. Both groups showed similarities in the rate of chemical and clinical pregnancies. The rate of chemical pregnancy was higher (46.9%) in the GnRH antagonist protocol compared with long GnRH agonist group (40.6%). However, this rate did not reach a statistically significant level. The rate of clinical pregnancy was (31.3%) in antagonist group versus (28.1%) in agonist group.

Conclusion: On the basis of these results, we offered using GnRH antagonist as a patient friendly protocol in ART with immediate mode of action, similar pregnancy rate, time saving, more flexibility of treatment ,and it may be easier or more convenient to administer.

Keywords: GnRH agonist, GnRH antagonist protocol, Assisted reproduction, Controlled ovarian stimulation cycles.

INTRODUCTION

There are several ways how to perform the controlled ovarian hyperstimulation (COH) in patients included in the in vitro fertilization program and each one has its advantages and disadvantages (**Martin et al., 2015**).

The most important characteristic of GnRH agonists is prevention of premature LH surge in COH through desensitization of pituitary, which helps to increase the number of retrieved oocytes and decrease the number of cancelled cycles (**Martin et al., 2015**).

On one side, this is a good property, but, on the other side, it can lead to the ovarian hyperstimulation syndrome (OHSS). In addition to long duration of the widely used long GnRH-agonist protocol, some women may suffer hot flush and vaginal atrophy due to hypo-estrogenic state (**Grow et al., 2014**).

Due to these deficiencies of GnRH agonists, development of GnRH antagonists represented a major breakthrough because they cause fewer side effects. GnRH agonists bind to their receptor on pituitary and with maintaining the signal they cause desensitization of pituitary and consequently the downregulation of gonadotropin secretion after prolonged time. Also, GnRH antagonists bind to the receptor on a pituitary, but they block it almost straight away and consequently cause the suppression of gonadotropin secretion within a few hours (**Martin et al., 2015**).

Although the question about the mechanism of GnRH agonists and action of GnRH antagonists is well answered, there is still no clear answer about which

analogue gives better results in clinical practice. The reports are contradictory and often favor one type of the analogue (**Grow et al., 2014**).

The present work aimed to compare between GnRH agonist and antagonist.

PATIENTS AND METHODS

This is a randomized comparative prospective study which was carried on 80 infertile women attended Assisted Reproductive Unit, Azhar University, and arranged for ICSI. The patients in this study were divided into two groups by random allocation using sealed envelope, the antagonist group (antagonist protocol) and the agonist group (standard long agonist protocol) with 40 patients in each group.

Inclusion criteria: Infertile women whether it is primary or secondary infertility, menstrual cycle from (27-33 days), male factor of infertility, tubal factor, endometriosis, unexplained infertility, and mixed factors.

Exclusion criteria: All patients did process ovarian stimulation 3 months prior to this cycle, and all patients received oral contraceptive pill (OCP) pretreatment before this cycle.

All the couples were subjected to detailed medical history including: the female age, parity, rhythm of menstrual cycles, duration of infertility, cause of infertility (male or female factor), type of infertility whether it is primary or secondary, previous ART attempts and their outcome, ovarian stimulation 3 months prior to this cycle, and oral

contraceptive pill (OCP) pretreatment before this cycle. General and local examination and assessment were done. Basal hormonal profile (FSH, LH, E2, and prolactin serum levels) on day 3 of the menstrual cycle was obtained in any cycles preceding ovarian stimulation. After informed consent, all the patients included underwent COH with either a GnRH long agonist or antagonist multiple dose protocol.

The GnRH agonist long protocol: Forty patients underwent GnRH agonist long protocol were processed for pituitary down-regulation on luteal peak period with triptorelin acetate (decapeptyl 0.1 mg; Ferring pharmaceuticals, Keil, Germany) injection for 14 days after confirmation of quiescent ovaries by transvaginal ultrasound and serum E2 on day 2/3 of the period. Medication was initiated with recombinant FSH (rFSH) (Gonal-F, EMD Serono) on day 3 of the next cycle after performing basic vaginal ultrasound evaluation, in which younger patients (< 35 years old) were prescribed for two ampoules (150 IU) of Gonal-F daily, and elder patients (\geq 35 years old) were administered for three ampoules (225 IU) of Gonal-F daily. The dose was fixed for the first 5 days of stimulation. After 5 consecutive days of medication, the dose was adjusted according to the ovarian response as detected by serial transvaginal folliculometry done day after day starting on day 7 or 9 till the leading follicle reaches a diameter of 16 mm, then daily TVS was done till three follicles reached \geq 17 mm and (the maximum

duration of rFSH administration was 16 days).

The GnRH antagonist protocol: The other 40 patients, on day 3 of a menstrual cycle, a basic evaluation was conducted by ultrasound examination. Medication was then initiated with recombinant FSH (rFSH) (Gonal-F, EMD Serono, Aubonne, Switzerland) at the day of ultrasound examination as described above, in which younger patients (< 35 years old) were advised to take two ampoules (150 IU) of Gonal-F daily, and elder patients (\geq 35 years old) were arranged to take three ampoules (225 IU) of Gonal-F daily. Similarly, the dose was fixed for the first 5 days of stimulation, and after 5 consecutive days of medication, transvaginal ultrasound B examination was carried out to monitor the development of follicles. The dose of rFSH was optimally adjusted based according to the ultrasound B results for the number and size of developing follicles. The GnRH antagonist, cetrorelix (Cetrotide, Serono Laboratories, Aubonne, Switzerland), was administered daily by s.c. injection (0.25 mg/d) in the morning (8:00-12:00 AM) from day 6 of the stimulation cycle to the day of human chorionic gonadotropin (HCG) administration. Serial transvaginal folliculometry were done day after day starting on day 7 or 9 till the leading follicle reached a diameter of 16 mm. Daily TVS was done till three follicles reached \geq 17 mm (The maximum duration of HMG administration is 16 days), and

endometrial thickness were also assessed on the day of HCG administration.

Oocytes were retrieved 34-38 h after HCG injection by transvaginal ultrasound-guided needle aspiration under general anesthesia.

Embryos were transferred on 3rd day after oocyte retrieval, depending on the woman's age and the embryo quality one to three embryos were transferred. After 48 hours, embryos that had cleaved were identified and embryos grading was done as follow: **Grade A:** Even equally sized spherical cells (blastomeres) with no cellular fragmentation. **Grade B:** Embryos have uneven or irregularly shaped blastomeres, and less than 10% fragmentation of blastomeres. **Grade C:** Embryos have up to 25% fragmentation. Blastomeres appeared viable (although may be granular). **Grade D:** Embryos have 25-50 % fragmentation. Blastomeres appeared viable (although may be granular).

Grade A embryos were transferred to the uterus under sonographic guidance.

Luteal-phase support by progesterone (in oil) i.m. daily (80 mg/day) was given starting at the day of oocytes retrieval till occurrence of biochemical pregnancy confirmed by serum B-HCG concentration when it was >25 IU/L on day 14 after embryo transfer and was continued till 11 weeks gestation unless there was any other indication. Clinical pregnancy was defined as an ultrasound evidence of presence of an intrauterine gestational sac \pm fetal heart (*Kucuk, 2008*).

Statistical Methods: The collected data were revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (**SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001**). $P < 0.05$ was considered significant. Data were presented and suitable analysis was done according to the type of data obtained for each parameter. Descriptive statistics: mean, standard deviation (\pm SD) and range for numerical data, frequency and percentage of non-numerical data. Analytical statistics: Student's t- test was used to assess the statistical significance of the difference between two study group means. ANOVA test was used to assess the statistical significance of the difference between more than two study group means. Correlation analysis (using Pearson's method) to assess the strength of association between two quantitative variables. The correlation coefficient denoted symbolically (r) defined the strength and direction of the linear relationship between two variables. Chi-Square test was used to examine the relationship between two qualitative variables. Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count was less than 5 in more than 20% of cells.

RESULTS

Figure (1) showed the study flow chart and patient outcomes. A total of 80 patients were recruited to the study, with 40 randomized to each treatment arm.

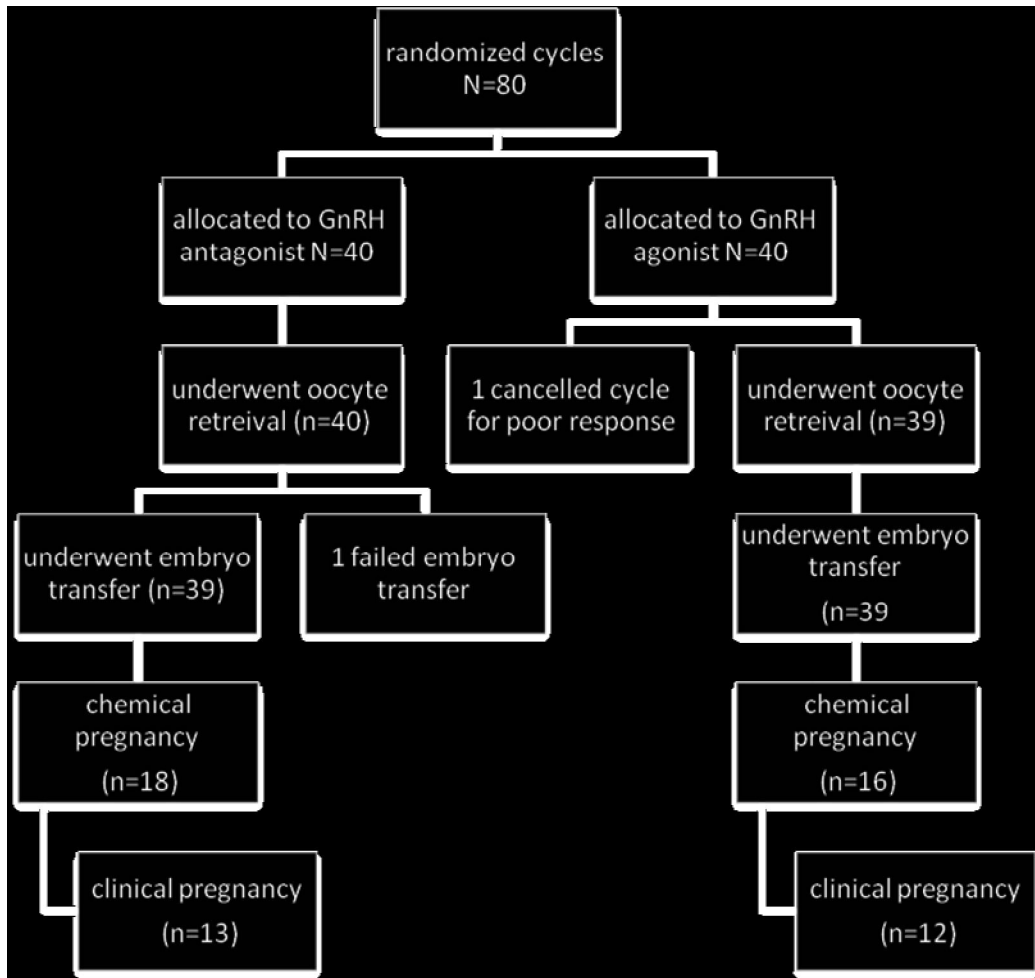


Figure (1): Study flow chart and patients outcomes

One cycle was cancelled in the long GnRH agonist group because no oocytes were obtained. In antagonist group, only 39 patients underwent embryo transfer, while one case of failed embryo transfer was recorded due to cervical stenosis.

No significant difference as regard number of oocytes retrieved, number of mature oocytes, number of fertilized oocyte, embryo number and number of

transferred embryo between Long agonist protocol and antagonist protocol. It also showed no significant difference as regard incidence of chemical and clinical pregnancy between Long agonist protocol and antagonist protocol. Analysis for good quality embryo in agonist and antagonist protocol revealed no statistical difference, 67, 5% (27\40) vs 70,0% (28\40) (Table 1).

Table (1): Comparison between agonist and antagonist groups as regard outcome of stimulation, embryo quality, and pregnancy rate (Mean±SD).

Groups		Agonist		Antagonist		P value
		N	%	N	%	
Outcomes						
Oocytes retrieved		9.1	5.6	9.9	4.1	0.479
Mature oocytes		6.8	4.0	7.6	3.6	0.396
Fertilized oocytes		5.4	3.5	5.5	3.0	0.879
Embryo number		4.8	3.5	5.3	2.8	0.525
Embryo Transfer		2.5	.8	2.8	.5	0.117
		N	%	N	%	
Chemical pregnancy	Negative (n %)	24	60.0%	21	52.5%	0.801*
	Positive (n %)	16	40.0%	19	47.5%	
Clinical pregnancy	Zero sac (n %)	29	72.5%	28	70.0%	0.961**
	One sac (n %)	10	25%	9	22.5%	
	Two sacs (n %)	1	2.5%	2	5.0%	
	Three sacs (n %)	0	.0%	1	2.5%	
Total no of Grade (1) Embryos		27	67.5%	28	70%	

*Chi square test

**Fisher exact test

DISCUSSION

GnRH antagonists with high potency and fewer side effects have been introduced into IVF and have emerged as an alternative in preventing premature LH surges. Unlike GnRH agonists, these potent GnRH antagonists cause immediate rapid gonadotropin suppression (Copperman and Benadiva, 2013).

The objective of our study was to compare the advantages of using fixed, multi-dose GnRH antagonist to long GnRH agonists in patients undergoing ICSI.

Our current study showed that there was no significant difference in the number of oocytes retrieved and mature oocytes retrieved in both the GnRH-ant and GnRH-a protocols, which was similar to the results of previous studies by *Danhua et al .(2011) and Xiao et al. (2014)*.

However, *Kolibianakis et al. (2006)* and *Kaur et al. (2012)* reported that the number of oocytes retrieved and mature oocytes retrieved in GnRH-a group was significantly greater than that in the GnRH-ant group.

According to our study, there were no significant differences in mean numbers of fertilized oocytes between the GnRH antagonist and GnRH agonist protocols. This agreed with **Trenkić et al. (2016)**.

In this study, there were no significant differences in mean numbers of embryos obtained between the GnRH antagonist and GnRH agonist protocols. This might be attributed to the insignificant difference in the total number of recruited oocytes between both protocols. This agreed with **Cheung et al. (2005)**.

The results of our study showed no significant differences between both groups in the quality of embryos. In contrast to these results, **Trenkić et al. (2016)** analysis showed that the GnRH-agonist protocol was associated with higher number of Class I and Class IV embryos were obtained after the agonist treatment and higher number of Class II and Class III embryos were obtained after the antagonist treatment. However **Vengetesh et al. (2015)** analysis showed that antagonist protocol had favorable outcomes compared with the agonist protocol and the yield of high grade embryos were found higher.

Our study suggested that the pregnancy rate was higher in GnRH antagonist protocol compared with long GnRH agonist group. However, this rate did not reach a statistically significant level. This was in agreement with a meta-analytic review by **Al-Inany et al. (2005)**. The analysis concluded that there was no statistically significant difference in pregnancy rate per woman randomized, although there was a trend towards a higher pregnancy rate with the fixed

antagonist protocol, especially with delayed administration beyond day 8.

The results of our study regarding clinical pregnancy rate disagreed with a meta-analysis by **Siristatidis et al. (2015)** which showed a moderate quality evidence of lower clinical pregnancy rate in patients treated with GnRH antagonists compared with patients treated with long agonist protocols. The lower pregnancy rate resulting from treatment with GnRH antagonists was attributed to an effect on oocyte quality and/or the endometrium. On the contrary; **Hosseini et al. (2010)** observed higher significant chemical and clinical pregnancy rates in patients treated with GnRH antagonist.

CONCLUSION

The results of this study showed that a protocol including GnRH antagonist appeared at least as effective as one using a GnRH agonist in patients undergoing ICSI and resulted in outcome nearly equal to those obtained by standard long GnRH agonist protocol. On the basis of these results, we offer using the "GnRH antagonist" as a patient friendly protocol in ART with immediate mode of action, similar pregnancy rate, time saving, more flexibility of treatment and it may be easier or more convenient to administer.

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مقارنة بين برنامج مثيل هرمون GnRH وبرنامج ضد هرمون GnRH في المساعدة على الإنجاب خلال دورات تنشيط المبيض المراقبة

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خلفية البحث: بالرغم من معرفة طريقة عمل مثيل هرمون GnRH و ضد هرمون GnRH ، إلا أنه لم يمكن معرفة أيهما أفضل بالنسبة للإستخدام الإكلينيكي و هناك كثير من الدراسات المتضادة و التي تفضل أحدهما على الآخر.

الهدف من الدراسة : تهدف هذه الدراسة إلى مقارنة برنامج مثيل هرمون GnRH وبرنامج ضد هرمون GnRH في المساعدة على الإنجاب خلال دورات تنشيط المبيض المراقبة من حيث العدد الكلي للبويضات التي تم سحبها و عدد البويضات الناضجة و معدل الإخصاب و إنقسام الأجنة و درجاتها و معدل حدوث حمل. **المرضى و طريقة البحث:** هذه دراسة مستقبلية عشوائية تم اجرائها في مستشفيات جامعة الأزهر ؛ و فيها تم دعوة ٨٠ امرأة من اللاتي سيتم عمل تنشيط مراقب للمبيض لهن استعدادا لعمل أطفال أنابيب للمشاركة في هذه الدراسة . و تم تعيين المرضى عشوائيا إلى مجموعتين متساويتين: مجموعة ١ تلقت هرمون مثيل GnRH لبرنامج طويل ' و المجموعة ٢ تلقت هرمون ضد GnRH. و قد تم تجهيز ٤٠ مريضة خاضعات لبرنامج مثيل GnRH الطويل حيث يقل تنظيم الغدة النخامية في دورة إرتفاع هرمون LH مع حقن التيربتوريلين لمدة ١٤ يوم . و تم التقييم عن طريق الفحص بالموجات فوق الصوتية و فحص الدم لمستويات الهرمونات. و بعد ٥ أيام متتالية من تناول جرعة ثابتة من عقار جونال اف، حيث تم إجراء الفحص بالموجات فوق الصوتية عبر المهبل لرصد تطور البويضات و عمل تحاليل الدم اللازمة، و جرعة الجونال اف تم تعديلها على النحو الأمثل إستنادا إلى عدد و حجم البويضات النامية و التحاليل اللازمة. أما بالنسبة لبرنامج هرمون ضد GnRH في اليوم الثالث من الدورة الشهرية ، تم إجراء تقييم ٤٠ مريضة جديدة عن طريق الفحص بالموجات فوق الصوتية. و تم الحقن بأمبولات جونال اف بجرعة ثابتة لمدة ٥ أيام بدءا من يوم الفحص بالموجات فوق الصوتية. و تم إجراء الفحص بالموجات فوق الصوتية عبر المهبل لرصد تطور البويضات، مع الأخذ في الإعتبار أن جرعة الجونال اف تم تعديلها على النحو الأمثل إستنادا إلى نتائج الموجات فوق الصوتية وفقا لعدد و حجم البويضات النامية و تحاليل الدم اللازمة. و تم بدء إعطاء عقار سيتروريليكس (ضد هرمون GnRH) عن طريق الحقن تحت الجلد من اليوم السادس من دورة التنشيط ليوم تناول عقار HCG. و تمت المتابعة حتى تصل ثلاث بويضات ≤ 17 مم. ١٠,٠٠٠ وحدة دولية من عقار HCG تم حقنها. كما تم إسترجاع البويضات ٣٤-٣٨ ساعة بعد حقن HCG و تم الإخصاب في المختبر وفقا للإجراءات القياسية. و قد تم نقل الأجنة بعد إسترجاع البويضة بإثنين و سبعين ساعة و تم نقل كحد أقصى ثلاثة أجنة إلى كل مريض.

النتائج: لم يكن هناك فرق بين البرنامجين من ناحية عدد البويضات المستخلصة و البويضات الناضجة. وذلك بمقارنة جودة الأجنة و عدد الأجنة الناتجة التي تم نقلها و لم يكن هناك فرق بين البرنامجين من حيث حدوث حمل كيميائي أو إكلينيكي و هذا يؤكد أن هرمون ضد GnRH له نفس فعالية هرمون مثيل GnRH في منع حساسية الغدة النخامية.

الإستنتاج: برنامج هرمون ضد GnRH أكثر أمانا و أقل إستهلاكا للوقت، و أكثر مرونة و سهولة في الإستخدام و نتائجها متساوية لبروتوكول هرمون مثيل GnRH.