Original paper

A Higher Pregnancy Rate with GnRH Antagonist Stimulation Protocol During Intra Cytoplasmic Sperm Injection (ISCI).

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Abstract

Background: Controlled Ovarian Stimulation (COS) is a widely used method during assisted reproduction. There are two commonly used COS protocols: GnRH agonist and GnRH antagonist. Selecting the protocol for COS is the most critical decision could be made and there is no standard protocol which could be applied for all patients.
Aim: The aim of this study was to compare ICSI outcome between GnRH antagonist and agonist protocols represented by oocytes maturity, fertilization rate (FR), cleavage rate (CR), embryos quality and pregnancy rate (PR).
Materials and Methods: Fifty infertile females were included and they divided in to two groups according to the protocol: Group I: In which GnRH agonist was used (n=33) and Group II: In which GnRH antagonist was used (n=17). All were included in ICSI program. Following oocytes retrieval, microscopic assessment of oocytes' maturity and embryos' quality was done followed by calculation of FR, CR and PR. Results were compared between both groups.
Results: The study showed that GnRH agonist-stimulated females produced slightly more immature oocytes (1.40±2.01 VS 0.94±2.13) with a lower fertilization rate with no significant statistical difference regarding CR and embryo quality. Pregnancy rate tended to be lower 42.42% when compared with antagonist protocol 58.83%.
Conclusion: Both GnRH agonist and antagonist protocols have the same effect on ICSI outcome in form of oocyte maturity, FR, CR and embryo quality except PR which tends to be higher with the antagonist protocol.
Keywords: GnRH agonist, GnRH antagonist and ICSI outcome.

Introduction

Different treatment modalities have been suggested to improve assisted reproduction outcome in infertile couples. Two protocols became popular and widely used nowadays: GnRH agonist and GnRH antagonist protocols [1].

With the GnRH-agonist protocol, the administration in the early follicular phase will induce the initial release (flare) of endogenous follicle stimulating hormone (FSH) & luteinizing hormone (LH) which enhances the ovarian response to the subsequent administration of exogenous gonadotropins [1]. It leads to profound inhibition of endogenous gonadotropins during the follicular phase permitting the growth of the antral follicles in a co-ordinate manner when exposed to exogenous gonadotropin which ends in a simultaneous maturation and recruitment of mature follicles (2). Several studies have reported improved ovarian response and clinical outcomes using this protocol [3,4,5,6 and 7].

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While the GnRH antagonist protocol permits more natural recruitment of follicles during the follicular phase [8]. It interferes with the proper maturation of oocytes by a marked inhibition of LH release. It also negatively affects oocyte differentiation by its' receptor-mediated effect on granulose cell, reducing aromatase activity and inhibition of estrogen (E2) release [9].
However, the introduction of the GnRH antagonist during ovarian stimulation prevents premature LH surges [1]. So, the GnRH antagonist have the possibility of reducing the amount of gonadotropins, the duration of stimulation and the number of cancelled cycles [1]. GnRH antagonists have also been shown to directly influence the extra pituitary tissues, including oocytes, embryos and uterine endometrium [10]. It would decrease the effect of endometrial growth factors and reduce endometrial receptivity [10]. A detailed evaluation of uterine biophysical profile (uterine artery blood flow by Doppler and endometrial thickness pattern by TVUS) suggests that GnRH antagonist enhance uterine blood flow and endometrial thickness and hence improve implantation potential and pregnancy rate [11]. So, there is an argument about the effectiveness of both protocols and which induction protocol is superior one considered a matter of wide research.

Study design

A prospective cohort study that was done in the Fertility Center, Al- Sadr Medical City, Al- Najaf AL-Ashraf, Iraq. the statistical analysis by SPSS (24.0), the comparison by either independent sample t-test (for continuous data; mean ± SD) or Chi-square (for categorical data; percentage / number) at p-value ≤0,05.

Materials and methods

Fifty infertile females were included and they divided in to two groups according to the stimulation protocol: Group I: In which GnRH agonist was used (n=33) and Group II: In which GnRH antagonist was used (n=17). All were included in ICSI program, following oocytes retrieval, microscopic assessment of oocytes maturity, FR, CR, embryos quality and calculation of PR was done and the results were compared between both groups.
The age of all females was less than 35 years old; they were normal ovulatory females whom attended the fertility clinic due to either tubal obstruction (normal male partners) or mild-moderate male factor infertility. Females with polycystic ovary syndrome (PCOS), endometriosis, uterine fibroid, sever male factor infertility and unexplained infertility had been excluded.
Male and female partners of both groups had been evaluated clinically depending on history, physical examination, anthropometric measures (weight, height and body mass index (BMI)), seminal fluid analysis (SFA), E2, LH, FSH, PROLACTIN and transvaginal ultrasound. Females of both groups had been subjected to pituitary down regulation using either gonadotropin releasing hormone (GnRH) antagonist; Cetroleix 0.25 mg*1 or agonist; Decapeptyle 0.1 mg*1 then controlled ovarian hyper stimulation by recombinant FSH (r-FSH); Follitrope 75 iu*2 for7-14 days which was done under a close supervision by serial trans-vaginal ultrasound (TVUS) and serum E2 level. Ovulation trigger was done by human chorionic gonadotropin (hCG) injection: Pregnyle 10000 iu*1 when the total number of the follicles 8-14 and their size are more than 17-mm

Oocytes pickup was done under general anesthesia and TVUS. Microscopic assessment of oocytes' maturity was done after denudation of the oocytes for better visualization and manipulation.
Mature oocytes are those that resumed their first meiotic division (MI) and reached second meiotic division (MII) and appropriate for injection [12]. The semen was concomitantly prepared by direct swim-up from a pellet. Following ICSI, assessment of embryo development represented by fertilization rate, cleavage rate and embryo quality was done. Embryos with many regular appearing cells with very little or no fragments had been considered as good quality (grade I and II), in contrast to bad quality embryos with little irregular shape cell with high percentage of fragments (grade II and III) [13]. Fertilization rate was calculated by dividing the number of zygotes (2PN) / the number of injected mature MII oocytes *100%. Cleavage rate was calculated by dividing the number of embryos/the number of zygotes*100%. Calculation of pregnancy rate by dividing the number of females with +ve pregnancy test on the number of females whom 3 good quality embryos were transferred *100%.

Results

Table (1) shows the demographic data of the studying subjects. There were no significant statistical differences regarding these parameters between both groups.
Table (2) compares hormonal profile, endometrial thickness (ET) and total dose of gonadotropins in both groups. There was no significant statistical variation regarding E2, LH, ET and total dose between females in both groups, the exceptions were serum FSH and prolactin which showed a significantly higher level in women in which antagonist protocol was used.

Table (3) shows a comparison between both groups regarding the total number of retrieved oocytes and their maturity. Although the mean total number of retrieved oocytes and the mean total number of immature ones were more in females in which GnRH agonist was used but with no significant statistical variation at p-value=0.53 and 0.45 respectively.

The effect on fertilization rate (FR), cleavage rate (CR) and embryo quality (good or bad) can be demonstrated in table (4). There was no significant statistical difference in both groups regarding FR despite being less in the females of the agonist group 74.38±24.34 VS 77.25±24.92 at p-value=0.69. Regarding the CR despite of being less in the females of the antagonist group 93.21±19.44 VS 97.13±11.8, there was no statistical significance at p-value = 0.38. There was no significant statistical variation regarding embryo quality with an equivalent mean total number of good and bad quality embryos.
Regarding pregnancy rate, it was higher in the group at which antagonist was used 58.82 VS 42.42% in the agonist group, p-value=027 as shown in table (5).

Discussion

The main purpose of this research is to assess the effectiveness and safety of both GnRH analogues during COS. Previously, GnRH agonist was the protocol of choice in any stimulated cycle during IVF/ICSI. Since the discovery of GnRH antagonist in 1990s, it made a breakthrough in reproductive medicine. It may result in an immediate and reversible arrest of gonadotropin release leads to create a stimulation cycle as close as natural, non-stimulated cycle so be more convenient and patients friendly [14].

Table 1. Demographic data comparison between both groups.

Parameters	Agonist (n=33) Mean ±SD	Antagonist (n=17) Mean ±SD	P-value
Age (years)	27.87±4.05	28.11±4.19	0.84
BMI (Kg/m2)	28.75±5.11	26.04±3.91	0.06
Duration(years)	8.27±3.99	6.70±4.13	0.20
Primary infertility	20	14	0.11
Secondary infertility	13	3	
Spermiogram			
Normal SFA	13	6	0.77
Abnormal SFA	20	11	

Table 2. A comparison of cycle day 2 hormones, endometrial thickness and total dose of gonadotropins between the studied groups.

Parameter	Agonist (n=33) Mean±SD	Antagonist (n=17) Mean±SD	P-value
E2 (pg/ml)	34.78±15.13	34.69±17.32	0.98
LH (IU/L)	2.70±1.02	6.88±13.36	0.07
FSH (IU/L)	4.59±1.68	7.62±8.36	0.04
Prolactin (ng/l)	15.48±8.40	25.97±18.12	0.007
ET (mm)	3.60±0.93	3.23±0.66	0.15
To. Dose of gonadotropin(IU)	1629.54±498.64	1705.00±683.90	0.65

Table 3. A comparison between both groups regarding the total number of retrieved oocytes and oocytes maturity.

Parameters	Agonist(n=33) Mean±SD	Antagonist(n=17) Mean±SD	P-value
Total No. of oocytes	9.12±6.28	8.05±4.03	0.53
Mature (MII)	7.65±5.54	7.05±3.68	0.69
Immature (GV+MI)	1.40±2.01	0.94±2.13	0.45

Table 4. A comparison of FR, CR and embryos quality between our studied groups.

Parameter	Agonist Mean±SD	Antagonist Mean±SD	P-value
FR	74.38±24.34	77.25±24.92	0.69
CR	97.13±11.8	93.21±19.44	0.38
Total No. of good quality embryos	4.75 ±3.8	4.47±2.5	0.78
Total No. of bad quality embryos	0.59±1.18	0.24±0.56	0.24

Table 5. Pregnancy rate comparison between he studied groups.

Parameter	Agonist	Antagonist	Total	P-value
Pregnant	14(42.42%)	10(58.82%)	24	0.27
Not pregnant	19	7	16	
Total	33	17	50	

So, whether GnRH agonist has been replaced by GnRH antagonist is a contraversial topic and a comparison between them is necessary to assess their efficacy. In the current study, both treatment protocols have been found to be effective with a satisfactory ovulation rate; both of them were produced a desired number of oocytes with an acceptable maturity Many studies were in agreement with this result [1,15,16]. Some disagreed and showed that the total number of retrieved oocytes and the total number of mature oocytes were significantly less when using GnRH antagonist [17].

Regarding FR and CR, the study was showed that both of them were comparable in both groups without significant difference which consistent with many studies that showed both FR and CR are same in both protocols [1, 18, 19]. Regarding pregnancy rate it was lower in the GnRH agonist 42.42% in comparison with 58.82%. Researches agreed with this results and states that better implantation and higher pregnancy rates had been found when the stimulated protocol was GnRH antagonist [1, 15, 16] while others showed that pregnancy rate is insignificantly less with GnRH antagonist [17].

The study also showed that both protocols produced a desirable good quality embryo without a significant difference. Similar results were obtained from different studies [16,17].

So, from these results we can conclude that both protocols have the same efficacy and producing a desired number of mature oocytes, acceptable FR, CR and embryo quality except pregnancy rate. So, the possible impact of GnRH agonist on endometrial receptivity should be fully elucidated and other factors which might directly or indirectly affect implantation with GnRH agonist must be researched.

Conflict of interest: the authors declare that there was no any conflict of interest.

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