

The aptness of antagonistic protocol in intracytoplasmic sperm injection: Embryologic study

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Abstract

Background and objective: Infertility and poor ovarian response are serious problems in our society; fortunately the in vitro fertilization and intracytoplasmic sperm injection technique dissolve many of these problems. This study aimed to assess the effect of an individualized GnRH antagonist regimen on intracytoplasmic sperm injection process especially the aptness of it in given mature reiterative oocytes, normal fertilization, and acceptably zygote cleavage which enhance pregnancy.

Methods: The population of the study consisted of 877 couples attending the in vitro fertilization-infertility center in the maternity teaching hospital and chose to undergo intracytoplasmic sperm injection after using of antagonist protocol for stimulation of women ovaries. The women ages ranged between 24 and 44 years. The collected data included a number of reiterative oocytes; fertilization, embryo grades and lastly estimates the pregnancy results

Results: A significant association ($P < 0.001$) was found in the numbers of reiterative oocytes between the age groups of the study. A significant association ($P = 0.005$) was also found concerning to quality of fertilization process. The study of the zygote cleavage and blastomeres formation followed the fertilization show variable grade of embryos and analyzed data of embryo grades in this study indicate presence of significant association ($P = 0.003$). The rate of pregnancy showed significant association between the groups of the study with the <30 years age group have chance of pregnancy higher than other groups.

Conclusion: The antagonist protocol of ovary stimulation according to the results of this study is a qualified protocol; besides it is rapid and can be reversed. This protocol can give effective results in the intracytoplasmic sperm injection process especially for young women, and enhance the pregnancy.

Keywords: GnRH antagonist; Intracytoplasmic sperm injection stimulation; IVF protocol.

Introduction

Infertility and the problem of poor ovarian response in our century now a day is markedly increased due to an increase in the percentage of delayed marriage and advanced maternal age. Although the infertility is a source of profound psychological distress for those patients who choose to undergo in vitro fertilization (IVF), however often suffer additional anxiety and concern.¹⁻² However, a large number of IVF/ intracytoplasmic sperm injection (ICSI) cycles performed every year throughout the world. That means

a considerable number of patients are affected factors influencing the incidence of the ovarian hyperstimulation syndrome (OHSS) include age, polycystic ovary syndrome (PCOS), use of exogenous HCG to induce ovulation, luteal phase supplementation with HCG, and more importantly, the stimulation regimen.³ In ovarian stimulation protocols, because administering exogenous FSH achieves an adequate follicular response, however, growing follicles become increasingly sensitive to and ultimately depend on the presence of both LH and FSH for their

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normal development.⁴ Furthermore, clinical data show that LH is essential for oocyte maturation in the normal menstrual cycle.⁵ The gonadotropin-releasing hormone (GnRH) agonist protocol used in vitro fertilization (IVF) were reported and published for the first time in the 1980s. The GnRH agonists function depend on suppress luteinizing hormone (LH) and prevent premature LH surges allowing optimal timing of human chorionic gonadotropin (hCG) administration and ovum collection, which improved IVF outcomes.⁶ GnRH agonist long protocols have been considered as the standard and the most commonly used protocol during the last two decades. The GnRH agonist long protocol starting in the mid-luteal phase of the preceding cycle, typically involve about three weeks of GnRH analog treatment per cycle.⁷ However, the controlled ovarian hyperstimulation (COH) protocols for in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are stressful, invasive and can be associated with adverse pregnancy outcome, including miscarriage and ectopic pregnancy.⁸ More recently the GnRH antagonist s become available, which act in contrast to the agonist protocol. The antagonist protocol block pituitary GnRH receptors by competitive binding resulting in immediate and direct suppress of gonadotropin.⁹⁻¹⁰ GnRH antagonists can, therefore, be administered just before the expected LH surge that is mean between days 5-7 of stimulation when the risk of premature LH surge is most probable and need to be administered only for a few days (about 5-6 days per cycle.^{7,11,12} Because of increase using of antagonist protocol now a day in many of infertility and IVF center in Erbil city due to the conception that this type of stimulation protocol can give opportunity to control the endogenous LH surge more rapidly and conveniently in order to get of mature oocytes, success fertilization, and good grade of embryo which enhance pregnancy. This study tries to investigate the Quality of

antagonist's protocol in the ICSI process especially the part which concerning to early embryonic stage.

Methods

This study was conducted in IVF –Infertility center in the Maternity Teaching Hospital in Erbil city, Iraq during the period from January 2013 to January 2016. The data of the couples attended the center and undergone ICSI were obtained which included a number of reiterative oocytes; fertilization, embryo grades (cleavages of the zygote for 3 or 5 days after fertilization) and lastly estimate the pregnancy rate. In this study, only the women who used the antagonistic program of ova stimulation was chosen in order to find the aptitude of the antagonist protocol in the formation of mature and good quality of ova which fertilize and divide equally to enhance pregnancy. In order to get most real results, some cases were excluded from this study included the teratospermia, necrospermia, asthinospermia, azoospermia and frozen sperms of both cases ejaculated sperms and testicular sperms aspiration (TESA) to limits the failure ratio. The population of the study consisted of 877 couples. The women ages ranged between 24 and 44 years. The cases classified according to age, reiterative oocytes (<10, 10-15, >15 oocytes), fertilization which was checked after 18 hours of sperm injection (ICSI) using the inverted microscope to check the presence of two pronuclei (PN). The Good fertilization represents efficient fertilization that means the presence of 2 PN. The Poor fertilization represents incomplete fertilization or rapid fertilization; in this case, only one PN was seen. The not fertilized case reported when no PN was seen. Also, the embryo grad assayed according to the checked of fertilized ova after 24 hours of ICSI. Embryos are graded in the following manner. Grade 1 characterized by even number, regularly shaped blastomeres, intact zona pellucida and less than 10% fragmentation. Grade 2

characterized by uneven number, irregular shape blastomeres, intact zona no more than 10% of fragmentation. Grade 3 which was similar to grade 2 with no more than 50% fragmentation. Grade 4 differs from grade 3 because containing more than 50% fragmentation and the last one degenerates' embryo which was characterized by dark appearance and shrieked shape. Data were coded and analyzed using the statistical package for the social sciences (version 18). Categorical variables were presented as the percentage. Chi-square test of association was used to compare proportions. A *P* value of ≤ 0.05 was considered statistically significant.

Results

The study included 877 cases of couples attended the IVF center of maternity teaching hospital in different months of the last three years, and the participated

women undergone the antagonist program for ovary stimulation and then ICSI process. The ages of participated women ranged between 24- 44 years and the highest percentage of participated women include the age less than 30 years by (36.2%) followed by the age group ranged between 30-34 years old with (34.9%) whereas the low number of participating represent the older women who have age more than 40 years old with (7.1%) as shown in Table 1. The effect of antagonist's protocol on the reiterative oocytes, fertilization, grades of embryo and pregnancy rate is shown in Table 2. This stimulation program can give the highest percentage (51.7%) for the group of less than ten reiterative oocytes in contrast to the group which gives more than 15 ova (15.4%). Also, according to data, we can see the high fertilization percentage (87.8%) for this type of stimulation.

Table 1: Distribution of women by age.

Age (Years)	No.	%
<30	318	36.2
30-34	306	34.9
35-39	191	21.8
≥ 40	62	7.1
Total	877	100

Table 2: study data of reiterative oocytes, fertilization, embryo grads, and pregnancy ration.

Variable	Group	No	%
Reiterative oocytes	< 10	453	51.7
	10-15	289	33
	> 15	135	15.4
Fertilization	Not Fertilized	39	4.4
	Good Fertilization	770	87.8
	Poor Fertilization	68	7.8
Embryo grades	Grade1	39	4.4
	Grade2	128	14.6
	Grade 3	139	15.8
	Grade 4	201	22.9
	degenerated	370	42.2
Pregnancy	Negative	493	56.2
	Positive	384	43.8
Total		877	100

Regarding the response of the different age groups to the antagonist protocol of stimulation, a very high significant association ($P < 0.001$) was found in the reiterative oocytes. The group of women less than 30 year gives a higher percentage (22.9%) of reiterative Oocytes, and highly significant association ($P = 0.005$) of fertilization also found in this age group (89.3%). A highly significant association ($P = 0.003$) in grades of the embryo was found between the different

age groups (Table 3) which depend on the zygote cleavage, appearance of blastomeres and amount of fragmentation insides, as it explained in the methods of the study (Figures 1, 2, 3 and 4). The study found a very high significant association ($P < 0.001$) in the pregnancy rate as it is illustrated in Table 3 and Figure 5 between the ages groups, the younger women had more chance to get pregnant than other ages (51.9%).

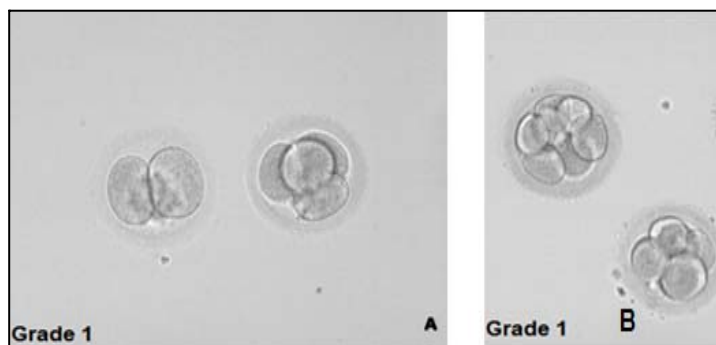


Figure 1: A & B represent grade 1 of embryo (cleavage of blastomeres in the 2nd and third day of fertilization successional).

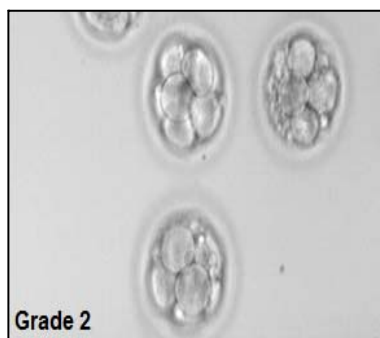


Figure 2: Represent grade 2 of the embryo.

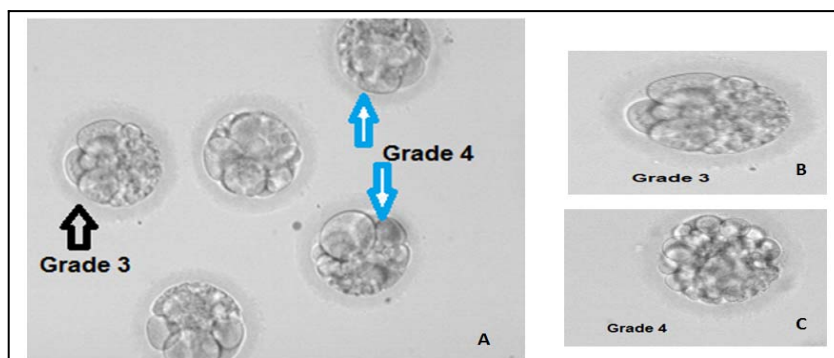


Figure 3: A- Represent grade 3 and 4 of embryo, B- Represent grade 3 and C- Represent grade 4.

Table 3: Data of reiterative oocytes, fertilization, embryo grades and pregnancy ration in different age groups.

Variable		Age groups								P value
		<30		30-34		35-39		≥40		
		No	%	No	%	No	%	No	%	
Reiterative oocytes	<10	106	33.3	171	55.8	123	64.4	53	85.5	<0.001
	10-15	139	43.7	92	30.1	50	26.2	8	12.9	
	>15	73	22.9	43	14.1	18	9.4	1	1.6	
Fertilization	Not fertilized	10	3.1	8	2.6	13	6.8	8	12.9	0.005
	Good fertilized	284	89.3	273	89.2	161	84.3	52	83.9	
	Poor fertilized	24	7.6	25	8.2	17	8.9	2	3.2	
Grade of embryos	Grade 1	10	3.1	8	2.6	13	6.8	8	12.9	0.003
	Grade 2	45	14.1	49	16.0	26	13.6	8	12.9	
	Grade 3	45	14.1	48	15.7	29	15.2	16	25.8	
	Grade 4	67	21.1	72	23.5	53	27.7	9	14.5	
	Degenerated	151	47.5	129	42.2	70	36.6	21	33.9	
Pregnancy	Negative	153	48.1	166	54.2	132	69.1	42	67.7	<0.001
	Positive	165	51.9	140	45.8	59	30.9	20	32.3	

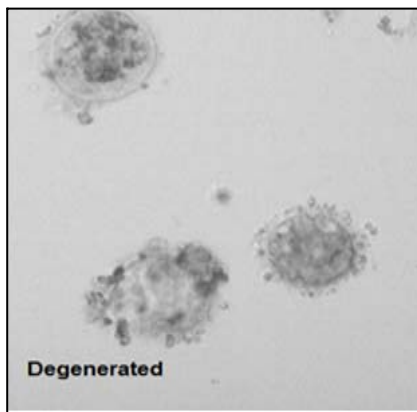


Figure 4: Represent degenerated embryo

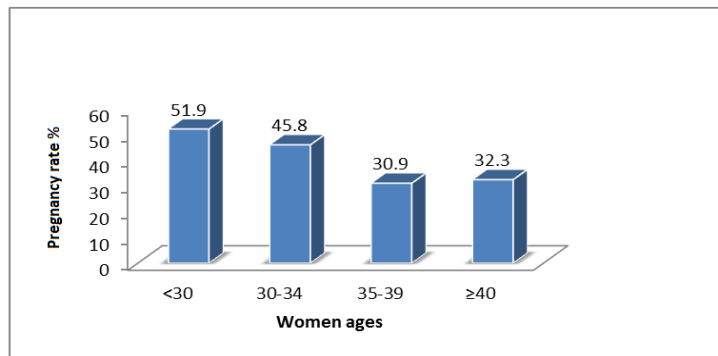


Figure 5: Pregnancy rate in different age group.

Discussion

The antagonistic program of ovary stimulation is commonly used recently in many of infertility and IVF centers. GnRH antagonists competitively block pituitary GnRH receptors; including a rapid, reversible suppression of gonadotropin secretion.¹³ Pharmacological modes of GnRH antagonist's action can be administrated at mid-cycle to prevent a premature LH surge while not causing any suppression in the early follicular phase, which is a crucial time for follicular recruitment. The results of this study show qualified effect of this type of ovary stimulation protocols in the number of Reiterative oocytes (Table 2) when the reiterative oocytes mostly less than 10. Therefore, this type of stimulation can be considered safer than other types. On the other hand, this stimulation program can be controlled by reversing its effect through the administration of GnRH agonist as it is reported by many studies¹⁴⁻¹⁶. The lower number of retrieved oocytes and subsequently lower estradiol levels might have a beneficial effect on the occurrence of ovarian hyperstimulation. Concerning to the effect of the antagonist on quality of retrieved oocytes; the results of fertilization and grades of the embryo which depend on the cleavages of zygote after fertilization that follow the ICSI process shows significant differences between the study groups as shown in Table 3. The younger aged of the women (age <30, and 30-35) give a higher percentage of fertilization (89.3%, 89.2%) and a higher percentage of grade 1, grade 2 embryos which considered as a perfect embryo to be transferred to the women uterus than the other groups. This agrees with other studies which suggested that the antagonist administration which is flexible regimen would lead to better clinical results and improve clinical outcome.¹⁷⁻¹⁸ Also, the study shows very high significant association ($P < 0.001$) between the study groups in the ratio of pregnancy (Figure 5). Although the younger ages (<30 years)

have the chance of pregnancy higher than other age the other ages also give a good percentage of outcome (positive percentage of pregnancy) if we compare the results of this study with expected results of IVF/ICSI. Also, that agrees with the conception which believed that antagonist protocol is acceptable to program mainly for the patient with unfavorable prognoses (e.g., older patient) or those whom previous cycles have been unsuccessful.¹⁹ A wide variety of GnRH antagonist protocols have been proposed, reflecting the fact that the protocol is still undergoing refinement.²⁰⁻²¹

Conclusion

According to the results of this study, the antagonist program of ovary stimulation is a qualified or aptness protocol for IVF/ICSI process due to its results and its mode of action which was rapid and reversible. This protocol considered a safer program to use.

Competing interests

The author declares no competing interests.

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