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Gonadotropin-releasing hormone agonist flare-up versus Gonadotropin-releasing hormone antagonist protocols in poor responders undergoing Intra Cytoplasmic Sperm Injection ICSI

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1. Abstract

Poor ovarian response (POR) is a multifactorial problem with less ovarian reserve and its incidence varies between 9% and 24%, therefore, early identification is It is better to reduce the risk of cycle cancellation as well as side effects. **Purpose:** To compare the use of Gonadotropin-releasing hormone GnRH flare-up versus GnRH antagonist protocol, in poor responders preparing for Intra Cytoplasmic Sperm Injection ICSI, as regards embryo quality, cycle parameters and clinical outcomes.

Patients and methods: RCT included one hundred and six qualified poor responders performing ICSI were divided into 2 groups each containing 53 patients. Group 1 received GnRH flare-up protocol and group 2 received GnRH antagonist protocol. Data were collected for both groups.

Results: No significant difference was found between both groups as regards patient age (p value 0.4), body mass index (p value 0.5), day 3 FSH level (p value 0.06), infertility cause, number of oocytes and MII oocytes and number of embryos transferred. Significant difference was found in the number of gonadotropin ampoules with less ampules in the flare-up group, 64 versus 76 ampules, peak estradiol level, which was higher in the flare-up group, 1192 versus 798 and the quality of embryos in favor of GnRH flare-up group (P-value= 0.017, 0.009 and 0.044) respectively. No significant difference was found in pregnancy and miscarriage rates (p value 0.90 and 0.87 respectively).

Conclusion: Flare-up protocol is more effective than GnRH antagonist protocol as regards the improved embryo quality, with more top-quality embryos in the flare-up protocol group.

2. Keywords: ICSI; Poor responders; Agonist; Antagonist

3. Introduction

In spite of the advancement of assisted reproductive

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technologies, the poor ovarian response is still a major problem. The type of stimulation protocol should be put into consideration when assessing the quality of ovarian response as marked pituitary down regulation increases the risk of poor ovarian response [1], therefore, the short down regulation protocol (Flare-up) is more likely to be used with either micro or regular dose of GnRH agonist in those patients [2].

Poor ovarian response (POR) generally means inadequate response of the ovary to adequate controlled ovarian stimulation [3]. It is mainly due to decreased ovarian reserve, which is affected by several factors as age, adnexal surgery, endometriosis, chemotherapy, irradiation, smoking, obesity and premature ovarian failure [4]. In a recent meta-analysis by Polyzos and Devroey, they found 41 variations in the definition of poor responders in 47 randomized controlled trials [5].

Poor ovarian response is associated with lower pregnancy rates [6], hence, it is important to assess ovarian reserve and identify low responders prior to IVF treatment, as this may reduce the side effects and cycle cancellation rate [7].

The incidence of poor ovarian response ranges between 9 and 26%, which is mainly due to wide variety of definitions between different studies [8]. The introduction of The Bologna Criteria [9], which defined poor ovarian response as having at least 2 of the following 3 criteria: "a) Advanced maternal age (40 years or more) or any other risk factor for poor ovarian response. b) Previous poor ovarian response (3 or less oocytes with a conventional stimulation protocol. c) Abnormal ovarian reserve test (antral follicle count less than 5 to 7 follicles or anti-Mullerian hormone less than 0.5 to 1.1 ng/ml)" is a forward step, in spite of that, some concerns are present as regards absent association with the oocytes' quality, pregnancy rate and over diagnosis of such cases [10].

Different strategies including different induction protocols were used in order to find protocols with acceptable pregnancy rates in those patients, however, more studies are needed in order to reach those protocols [11].

The aim of the current study is to compare between GnRH agonist flare up and GnRH antagonist treatment protocols in poor responder patients preparing for ICSI. The primary outcome is to improve the quality of embryos with the use of flare up protocol and this can improve the pregnancy rate. The secondary outcomes are; to reduce the number of gonadotrophin ampules used, to improve the cycle parameters and to improve the clinical outcome.

4. Patients and Methods

One hundred and fifteen poor responder women with male factor of infertility attending the infertility clinic of The Assisted Reproductive Unit of Al-Azhar University, Cairo, Egypt and Air-Force Specialized Hospital between April and July 2017 were approached to take part in the study, 5 did not agree to take part in the study and 4 dropped out during investigation process. One hundred and six women agreed to take part in the study (56 from Al-Azhar & 50 from Air-Force Specialized Hospital). They were preparing for ICSI and fulfilled the inclusion criteria, which are The Bologna Criteria [9], regular menstrual cycle (21-35 days duration) and body mass index (BMI) less than 30 kg/m^2. Patients with systemic disease e.g. diabetes, those with endocrine disease e.g. Polycystic ovaries, those with uterine pathology e.g. submucous fibroid polyp or fibroid and those with severe male factor or azoospermia were excluded from the study. The ethical committee of Air-Force Specialized Hospital & Al-Azhar University approved the study and informed consents were obtained from all patients before entry in the study. The trial is registered in the Pan-African Clinical Trial Registry with the registration number PACTR201704002212265.

The details of each patient were recorded as age, menstrual history, baseline (day 3) pelvic ultrasound finding, FSH, LH, E2 and Prolactin levels at the

previous cycle, in addition to, a recent semen analysis of not more than 3 months.

All patients performed an ICSI cycle and the patients were randomized into 2 groups (53 each): Group 1 patients had GnRH agonist flare-up protocol for down-regulation, while group 2 had GnRH antagonist protocol for down regulation.

4.1 Stimulation regimens

GnRH flare-up group: Treatment was started with 10 IU subcutaneous Leuprolide acetate (Lucrin, Abbot) per day from cycle day one until the day of hCG administration. Exogenous gonadotropin stimulation started from day 2 with human menopausal gonadotrophins 75 IU ampoules (hMG Menogon, Ferring Germany) and the Leuprolide acetate dose was reduced to 5 IU only if patients had delayed response to HMG.

GnRH antagonist group: Exogenous gonadotropins were started on day 2 of the cycle with human menopausal gonadotrophins 75 IU ampoules (hMG Menogon, Ferring Germany) and 0.25 mg cetrorelix (Cetrotride; EMD Serono, Inc) were added when the lead follicle reached 14 mm. in diameter and continued until the day of hCG administration.

Both groups received 375-450 IU of human menopausal gonadotropin with individual adjustments according to the ovarian response measured by serial ultrasound scans and serum E2 levels from day 6-8 of gonadotropin stimulation. Human chorionic gonadotrophin (hCG) (Choriomon, IBSA) 10,000 IU was given when there were at least 2 follicles with a diameter of 18 mm. or more. Peak E2 level was measured on the same day. Seven women did not develop 2 follicles of at least 18mm size. The investigators were not blinded to patient's allocation.

Oocyte retrieval was performed 36 hours after hCG administration by transvaginal ultrasound guided follicular aspiration, mature (MII) oocytes were fertilized through ICSI, five women did not have oocytes on examination after oocyte retrieval.

Oocytes were examined 16-18 hours after ICSI for pronuclei (PN). Normal fertilization was defined as existence of two pronuclei (2PN). The embryos obtained will be categorized on day 2 or 3 into four categories depending on their morphologic appearance, zonal thickness, cytoplasmic fragmentation and blastomere size: Grade I (High quality): Equal blastomeres and no observed cytoplasmic fragmentation, grade II (good quality): Equal blastomeres and less than 20% cytoplasmic fragmentation, grade III (Fair quality): Unequal blastomeres and 20-50% cytoplasmic fragmentation, grade IV (poor quality): Unequal blastomeres and more than 50% cytoplasmic fragmentation [12].

One to three grade I-II embryos were transferred to the uterus 72 hours later according to the patient's age, embryo quality and the number of embryos available using a labotect Embryo Transfer Catheter (Labotect GmbH, Labor- Technik-Gottingen, Germany) guided by trans-abdominal ultrasound.

Six hundred mg of micronized progesterone (Cyclogest or Prontogest) for luteal support was self-administered by the patient vaginally or rectally from the day of oocyte retrieval for 14 days and continued for further 8 weeks in pregnant cases.

Two weeks after embryo transfer serum beta hCG was measured for pregnancy diagnosis, while clinical pregnancy was diagnosed after visualization of fetal heart by transvaginal sonography.

The following data were collected for analysis: baseline clinical parameters as age, BMI, day-3 FSH and etiology of infertility, in addition to, ICSI cycle parameters as number of HMG ampoules used, peak E2 at the triggering day, number of oocytes retrieved, number of metaphase II oocytes, fertilization rate, embryo quality, number of embryos transferred, pregnancy rate and miscarriage rate.

Sample size calculation: A sample size of 53 per group, were estimated to improve the embryo quality and increase the number of top-quality embryos, the primary outcome, by 50% more, which achieves 80%

power to detect a mean of paired differences with a significance level (alpha) of 0.05.

Randomization: One hundred and six women fulfilling the inclusion criteria were randomized using computer generated numbers in a ratio 1:1. Distribution concealment was attained using closed opaque and sealed envelope method. An informed consent explaining the clinical trial and possible side effects was obtained from each woman prior to recruitment.

4.2 Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 18.0 Quantitative data were expressed as mean +/- standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

Independent-samples t-test of significance was used when comparing between 2 means.

Mann Whitney U in cases of two independent groups with non-parametric data.

Chi square (X^2) test of significance was used in order to compare proportions between two qualitative parameters.

Probability (P-value): P value less than 0.05 was considered significant and less than 0.001 was considered highly significant. P value more than 0.05 was considered not significant.

5. Results

Comparison between the two groups as regards mean and standard deviation of the basic patients' information of each group (age, BMI and day-3 FSH level).

Table 1: Showing comparison between the two groups as regards basic patient information (Age, BMI & day 3 FSH level).

Group characters	Antagonist Group (53)		Flare-up Group (53)		t-test	
	Mean	SD	Mean	SD	T	P-value
Age	35.71	4.18	36.33	3.23	0.830	0.409
BMI	23.71	1.68	23.11	2.11	0.711	0.533
FSH (mIU/ml)	10.20	1.81	10.82	2.01	1.912	0.061
LH	11.3	1.9	11.1	2.03	1.891	0.064
Estradiol	51.2	19.3	50.1	18.9	0.881	0.382
AMH	0.71	0.21	0.73	0.2	0.892	0.376
Previous ART cycles	2.7	CI (1-5)	2.8	CI (1-5)		0.421
Previous Pregnancy	0	0	0	0		
Oocytes retrieved in prev cycles	2	CI (0-3)	2	CI (0-3)		

The mean age was 35.71±4.28 in the antagonist group versus 36.33±3.33 in the flare-up group with non-significant difference (p value 0.4). Also, the BMI and mean FSH levels were not significantly different in both groups (p value 0.5 and 0.06 respectively).

The number of HMG ampoules used in the antagonist group were significantly more than the number used in the flare-up group (76.4 in comparison to 64.8 ampoules) and peak E2 level measured at the day of hCG stimulation was significantly higher in the flare-up group 1192.1 and 798.1.

Table 2: Showing comparison between the two groups as regards Number of HMG ampoules taken and peak E2.

Group characters	Antagonist Group		Flare-up Group		t-test	
	Mea	SD	Mea	SD	T	P-

	n		n			value
No. of HMG ampoules	76.40	15.01	64.80	22.59	3.024	0.003
Units of HMG	5730	1125	4860	1694		
Peak E2	798.10	275.60	1192.10	702.22	3.691	0.00005
No follicles	4		3			0.117

Comparison between the two groups as regards the median number of oocytes was non-significant, 2 and 3 in the antagonist and the flare-up group respectively. Moreover, the number of MII oocytes and the number of embryos in both groups were also non-significant.

Table 3: Showing comparison between the two groups as regards number of oocytes, number of MII oocytes and number of embryos.

Group characters	Antagonist Group		Flare-up Group		Z-Mann-Whitney Test	
	Median	Range	Median	Range	Z	P-value
No. of oocytes	2.0	0-4	3.0	0-5	1.101	0.270
No. of MII oocytes	2.0	0-3	2.0	0-4	1.161	0.246
No. of embryos	1	0-2	1	0-3	0.158	0.874
No oocytes	2		3			0.117

The mean and standard deviation of the fertilization rate for the flexible antagonist group were 78.14 and +/- 23.07 respectively, while the mean and standard deviation of the short agonist group were 88.89 and +/- 22.05 respectively. Using t-test t= -2.382and P-value 0.019.

In regard to embryo quality, there was more top-quality embryos in the flare-up group and this was significantly different (p value 0.044). On the other hand, there was no significant difference in pregnancy rate and miscarriage rate with p value 0.898 and 0.861 respectively.

Table 4: Showing comparison between the two groups as regards top-quality and poor-quality embryos, pregnancy rate and miscarriage rate.

Group characters	Antagonist Group		Flare-up Group		Chi-square Test	
	No.	%	No.	%	X^2	P-value
Top quality embryo	33	56.9	52	75.4	4.056	0.044
Poor embryo	25	43.1	17	24.6	4.056	0.044
Pregnancies	10	20.00	9	18.00	0.017	0.898

6. Discussion

The current study compared 2 treatment protocols (Flexible antagonist and short flare-up agonist) between 2 groups of poor responders with statistically non-significant difference in age, BMI, day 3 FSH and cause of infertility. A statistically significant difference was found between the 2 groups as regards the number of top-quality embryos, number of HMG ampoules used and peak E2 level in favor of the flare-up protocol, while no significant differences were found in pregnancy and miscarriage rates.

The current study agrees with the studies done by [13,14] as regards the number of gonadotropin ampoules required being significantly less and the peak E2 level being significantly higher in the flare-

up agonist protocol. Merviel et al. [15] studied 440 cases of POR and reached similar results, however, they added contraceptive pills to the flare-up protocol. The current study had different results from the study done by [16] who studied 112 POR and found significantly lower Gonadotrophin doses and significantly better oocyte and embryo parameters in the antagonist group in comparison to the flare-up group, however, clinical pregnancy rate and implantation rates were comparable with no significant differences.

The current study found no statistically significant difference between the 2 groups as regards the number of oocytes, the number of MII oocytes or the number of embryos transferred, which agrees with the results found by [14] who found no statistically significant difference as regards the number of oocytes and the number of embryos transferred between both groups, however, [13,17] found a statistically significant difference in 2 parameters (number of oocytes and number of MII oocytes) in favor of the flare-up agonist protocol, however, the latter study - which used a modified antagonist protocol - found no statistically significant difference as regards the number of embryos transferred.

The current study found statistically significant difference as regards the number of good quality embryos versus bad quality embryos in favor of the flare-up agonist protocol, however, insignificantly higher pregnancy rate and lower miscarriage rate were found in the antagonist group. Davar et al. [17] found similar results as regards the quality of embryos, while [14] found significantly higher ongoing pregnancy rate in the antagonist group, however, other studies as [18] who used the same modified antagonist protocol as [17] concluded that the agonist flare-up protocol had more positive results as regards pregnancy both chemically and clinically as compared to the antagonist group [19,20].

The drawback of the study is the number of participants. A larger study would give more significant results. Also, the study was not blinded to the investigators because of the different protocols which may have biased the results.

7. Conclusion

Flare-up protocol is more effective than GnRH antagonist protocol as regards number of top-quality embryos, number of needed gonadotropin ampoules and peak estradiol level. However, GnRH antagonist protocols did not show statistically significant better results in terms of both pregnancy and miscarriage rates.

Due to the inconsistency in the results of different studies there is a need to develop universally agreed criteria to define poor ovarian response which takes into consideration the quality of oocytes and embryos, clinical pregnancy rate and the different subgroups of poor ovarian response in order to optimize treatment for those patients.

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