ORIGINAL ARTICLE

Comparison Between Early and Short Follicular Antagonist (Sandwich), Short Gonadotropin-releasing Hormone Agonist and Flexible Antagonist Protocol in Poor Responders ICSI Programs

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ABSTRACT

Introduction: Poor response to COS protocols for variety of causes have less numbers of ovum picked up, embryos transferred and pregnancy outcome. Objective: To evaluate the usefulness and the for the most appropriate procedure in poor responder women: conventional antagonist, and short and early follicular antagonist (sandwich protocol) , short GnRH agonist protocol. **Methods:** Short and early follicular antagonist protocol (sandwich) used for thirty three ICSI patients. For conventional antagonist protocol, thirty one poor responder women used for ICSI cycle. Eighteen women undergone short Agonist protocol for ICSI. **Results:** : In poor responders, the average number of picked up oocyte was higher significantly in sandwich protocol and short GnRH agonist protocol (A) than antagonist (conventional protocol) B (P = 0.025). Average total embryos number transferred was higher significantly in sandwich than in both conventional antagonist and short GnRH agonist protocols (P = 0.024). In poor responders the women in sandwich protocol group has significantly higher pregnancy rate (11/33) 33.3 % than conventional antagonist group (3/31) 9.7 % with (p = 0.022). The pregnancy outcome also superior with sandwich 33.3 % than short GnRH agonist protocol 27.8 % although not significant. However, the difference in rate of pregnancy was insignificant between conventional antagonist and GnRH agonist (short protocol) (P=0.211) as well as between sandwich and GnRH agonist (short protocol) (P = 0.683). **Conclusion:** Sandwich protocols has potential to improve numbers of retrieved oocytes, transfer greater embryos numbers, also higher pregnancy rate.

Keywords: ICSI, Ovarian stimulation, Poor responders, Agonist,Antagonist.

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INTRODUCTION

A significant number (9% to 24%) of female undergoing IVF was poor ovarian response (POR) which is a major concern in which impairs the utility of the technique and subsequent pregnancy rate [1, 2].

The European Society of Human Reproduction and Embryology, define POR in 2011 by that devised predictive and prognostic criteria; Bologna criteria, which help establishing tailored management according to the severity of the condition. Bologna criteria has three main pillars which are; maternal age which correlates positively with severity, history of poor response and finally low ovarian reserve as assessed by radiological and laboratory methods [3].

Old age is associated with reduced ovarian reserve and progressive deterioration in the ovarian response to stimulation which necessities larger amounts of gonadotropins to obtain adequate response [4].

Functional ovarian reserve is routinely and effectively evaluated by hormonal level of anti-mullein hormone (AMH) and antral follicle count (AFC) and its low level is being clearly correlated with POR [5, 6].

Etiologically, it may, partly, due to a shortening in the follicular phase with reduce capability to recruit follicles, or early antral follicles sensitivity of to FSH stimulation [7] . Young “poor responders”, have different etiology that is not always known [8].

The principle of ovarian stimulation protocols relays on reducing the growth of follicular in the leuteal phase and fluctuation in hormonal during follicular phase, both will result in optimal follicular growth in response to the stimulation protocol [9].

During luteal phase, Gonadotropin-releasing hormone (GnRH) antagonist utilizes this principle, it is potent in stopping a premature LH surge, within six hours of administration for better follicular growth [10, 11]. In the mid-follicular phase, GnRH antagonist administration make the synchronization poor that is characterized by greater variation in the size of developing follicles and lower number of detectable follicles upon hCG with subsequent less retrievable oocytes [12]. Earlier administration of GnRH antagonist in COS may improve hypothetically synchrony between larger number of follicles with of good quality oocyte being obtained and minimize unwanted complications due to early exposure of follicular to E2 and LH [12].

Frankfurter et al, found that extend the follicular phase which elongate the recruitment phase of the cycle in poor responding patients due to administration of GnRH antagonist prior to ovarian stimulation would results in higher number of follicles when gonadotropin stimulation was started [13].

Furthermore, giving GnRH antagonist (Sandwich protocol) in the early follicular phase prior to ovarian stimulation resulted in substantial increase in yielded ova, zygote and embryo [11]. On the other hand, there will be decrease in the mature oocytes number and extra dose of the gonadotropins required which resulted from GnRH agonist long protocol which may cause desensitization of the ovary and more suppression of ovarian function [9]. Although, different protocols of stimulation in POR are used, unfortunately, the rates of pregnancy are still below expectations [14].

Belong to this work we tried to identify the best medication protocol that results in better ICSI outcome by comparison of among them during IVF .

MATERIALS AND METHODS

We conducted this prospective comparative study at the Al-Nahrain University / Higher Institute for Infertility Diagnosis and ART (Baghdad/Iraq) which granted the ethical approval for this work via Institute Medical Ethical Committee. An informed consent has been written and obtained from the enrolled patient. (25 in 7th of January 2019)

According to protocol of stimulation, the patients were separated in to three groups. 33 women were subjected to the short and early follicular Antagonist (sandwich group) protocol of ICSI cycle. 31 women underwent Antagonist (conventional protocol) of ICSI cycle (conventional antagonist group). Remaining 18 women followed the short Agonist protocol for ICSI.

The study inclusion criteria included poor responder patients who met the Bolongia criteria[3]. Exclusion criteria encompass patients with anatomical and pathological abnormalities in uterus and those with endocrine disorders such as thyroid dysfunction and diabetes mellitus.

Ovarian Stimulation:
It was done by of Recombinant FSH (rFSH) injection (Gonal f®, Merck Serono Company, Geneva, Switzerland) and 0.25 mg of Cetrorelix acetate as GnRH antagonist (Cetrotide®, Merck Serono Company, Geneva, Switzerland).

Starting dose of gonadotropin was personalized according to age of women, BMI, ovarian AFC, and previous history of response to ovarian stimulation.

Dose manipulation was done every 2 or 3 days according to response of the ovary as judged by serum Estradiol level in addition to trans vaginal ultrasonic follicular diameter assessment.

For the early and short follicular Antagonist protocol (sandwich group), 0.25 mg/day GnRH antagonist was given daily starting from day one, two and three of the menstrual cycle for thirty three patients assigned to the sandwich group. When the leading follicle was about 13 - 14 mm diameter, the drug was re-given and maintained till day of hCG.

The GnRH antagonist was administered to 31 women belong to conventional group when maturation of leading follicle (13 - 14mm) diameter and maintained till hCG injection day.

Eighteen women were subjected to the short agonist protocol for ICSI, they received 0.1 mg daily of short-acting Triptorelin (Decapeptyl®, Ipsen, Rome, Italy) on day two of menstrual cycle then hCG was started while gonadotropin injection given daily from day three of menstrual cycle.

When the trans vaginal ultrasound showed two or more follicles ≥18 mm in diameter, 6500IU /vial (250mg) Oviterlle® was injected (Merck -Serono® Company ,Geneva: Switzerland) [15]. Under ultrasound guide, ova pick up was carried on 34 - 36 hrs. following administration of hCG.

From the day of ova pick up, Cyclogest ® 400mg twice daily (Cox Pharmaceuticals ®, Barnstaple, UK) as luteal phase supported. Biochemical pregnancy was tested by measurement of blood Я-HCG on day 14 post embryo transfer. Later assessment of clinical pregnancy was done ultrasonically to identify the gestational sacs and their number and also to detect cardiac activity.

Laboratory Procedures
We applied the same standard techniques of handling of oocytes, sperm, zygotes, and embryos and their transfer

to all patients. In short, the cumulus oocyte complexes were incubated with hyaluronidase enzyme containing medium for denudation of cumulus and corona layers for 2 hours after retrieval with repeated pipetting. The presence of first polar body indicates that oocytes are in the second metaphase stage. Procedure of ICSI was performed as illustrated before by Pereira et al by Integra 3™ and Nikon ICSI Micromanipulators® using frozen or, preferably, fresh sperms [16].

After 16-18 hours following ICSI, the existence of 2 pronuclear and polar bodies define normal fertilization. Embryo transfer was done after 48 or 72hours post retrieval of oocyte.

Depending on the Istanbul consensus workshop, the morphology embryos were scored [17] and classified into grades; 1, 2 and 3 depending on blastomere homogeneity, fragmentation and the anucleated fragmentation degree. Examples of the three grades are shown in figure 1.

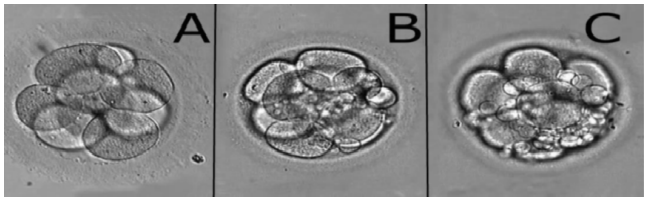

Figure 1: Examples of the three grades

Hormonal assays

We collected peripheral venous blood from the patients on the second day of menstrual cycle for measurement of Serum LH,FSH, E2, AMH, prolactin and TSH and on day of trigger for measurment of E2, progesterone. The hormone were analysed using Vidas® machine(Biomerux, France) using its costume kit.

Imaging studies: Ultrasound work for measurements of follicular growth done every 2-3 days

Statistical:

By using Statistical Package for Social Sciences version 23 (SPSS, PSS Inc., Chicago, Ill., USA), the statistical analysis was performed. We used one way ANOVA for correlation studies, While for differences between numerical variables, Fischer Exact test and student T- test. Statistically significant was considered when p <0.05.

RESULT

Clinic pathological characteristics

The poor responders clinic-demographic characteristics of are illustrated in table I,. According to the stimulation protocol, Patients were divided into three groups; conventional group, sandwich group and short GnRH group. Numerical details are shown in table I.

Table I : Demographic characteristics of poor responders

Characteristic	Total n = 82	Sandwich n = 33	Conventional n = 31	Short n = 18	P*
Age (years)	35.10 ± 6.81	35.36 ± 7.18 B	32.77 ± 6.8 C	38.61 ± 4.5 A	0.013, S
BMI (kg / m2)	30.3 ± 5.16	30.97 ± 5.12	30.12 ± 5.00	29.29 ± 5.60	0.534,NS
FSH (IU/L)	8.7 ± 4.06	9.16 ± 3.92	7.55 ± 3.71	9.80 ± 4.60	0.121, NS
LH (IU/L)	3.9 ± 2.04	4.19 ± 2.48	3.24 ± 1.4	4.33 ± 1.9	0.097,NS
FSH/LH	2.65 ± 1.54	2.70 ± 1.59	2.57 ± 1.4	2.71 ± 1.80	0.934, NS
E_2 (pg/ml)	32 ± 14.9	34.15 ± 15.14	31.34 ± 14.30	29.01 ± 15.91	0.487, NS
Prolactin (ng/ml)	14.0 ± 6.60	13.47 ± 5.52	15.4 ± 7.4	12.82 ± 6.8	0.353, NS
TSH (mIU/L)	1.95 ± 1.2	2.10 ± 1.29	1.9 ± 1.17	1.81 ± 0.7	0.614,NS
AMH (ng/ml)	0.96 ± 0.7	0.94 ± 0.74	1.0 ± 0.7	0.87 ± 0.50	0.657,NS

n= number of cases; SD= standard deviation; BMI= body mass index; ; FSH= follicle stimulating hormone; LH= luteinizing hormone; E2= estradiol; TSH= thyroid stimulating hormone; *: one way ANOVA; significant $p \leq 0.05$;NS= not significant $p \geq 0.05$.

Poor responders undergoing short GnRH agonist protocol were significantly older than both sandwich and conventional antagonist categories A (P=0.013), also women undergoing sandwich protocol were significantly older than conventional antagonist categories B (table 1). Poor responders hormonal status (LH, FSH, E2, Prolactin, AMH and TSH) according to protocol type is revealed in table I with no significant difference among them. However, AMH was low in all cases.

Characteristics of stimulation protocols

sandwich protocol utilize higher amount of rFSH when compared to both conventional antagonist and short GnRH agonist (B) protocols (P < 0.001). In sandwich protocol, the GnRH antagonist start day was relatively late comparative to conventional antagonist protocol (P < 0.001).

The resultant follicles number obtained by sandwich and short GnRH agonist stimulation protocols (A) was higher significantly if judged against conventional protocol (B) (P = 0.037). Estradiol (E2) at trigger was significantly higher in short GnRH agonist protocol (A) than both sandwich and conventional antagonist protocol (B) (P = 0.001). Endometria thickness and progesterone level was not influenced by the protocol.

The numerical data of the above parameters are shown in table II.

Table II : ovarian stimulation characteristics in Poor responders

Parameter	Total		Sandwich		Conventional		Short		*P value*
	n	Mean	n	Mean	n	Mean	n	Mean	
Stimulation days	82	9.0 ±1.65	33	9.2 ±1.6	31	8.8 ± 1.2	18	9.2 ± 2.3	0.601 NS
total FSH (ampule75IU)	64	23.6 ±9.7	33	27.8 ±10.9 A	31	19.3 ± 5.7 B	18	19.56 ±8.32 B	<0.001,HS
Day antagonist start	64	8.31 ±1.55	33	9.24 ±1.4	31	7.3 ± 1.0	0	---	<0.001, HS
Number of antagonists (not including first 3 days)	64	3.7 ±0.9	33	3.6 ±0.90	31	3.81 ± 0.9	0	---	0.245 NS
Number of follicles	82	8.7 ±4.1	33	9.8 ±4.0 A	31	7.3 ± 3.0 B	18	9.33 ± 5.03 A	0.037, S
E_2 at trigger (pg/ml)	82	1036.20 ±596.65	33	998.56 ±631.45 B	31	819.6 ± 415.6 B	18	1478 ± 587.7 A	0.001, HS
Progesterone at trigger Day(ng/ml)	17	0.70 ±0.7	9	0.82 ±0.9	8	0.57 ± 0.4	0	---	0.471, NS
Progesterone / Estrogen Ratio	17	0.91 ±0.9	9	0.94 ±0.90	8	0.88 ± 0.87	0	---	0.874, NS
Endometrial thickness (mm) at day of oocyte pickup	82	9.2 ±1.4	33	9.51 ±1.4	31	8.90 ± 1.4	18	9.35 ± 1.32	0.216, NS

n= number of cases; SD= standard deviation; FSH= follicle stimulating hormone; E2= estradiol; *: one way ANOVA; NS= not significant at $P \leq 0.05$; HS= highly significant at $P \leq 0.01$

Oocyte characteristics

The number of retrieved oocyte was higher significantly in sandwich and short GnRH agonist protocols (A) when compared to conventional antagonist protocol B (P = 0.025). The mean abnormal oocytes was higher significantly in sandwich (A) followed by short GnRH agonist protocol and conventional protocols (P = 0.037) respectively as exposed in table III. All the protocols showed similar maturation rate MI, MII and GV oocytes as shown in table III.

Table III : Oocyte characteristic in poor responders according to protocol

Parameter	Total n = 82	Sandwich n = 33	Conventional n = 31	Short n = 18	*P**
Retrieved oocyte	5.54 ± 3.6	6.67 ± 3.7 A	4.26 ± 2.8 B	5.67 ± 4.00 A	0.025 S
MII oocytes	3.39 ± 2.4	4.06 ± 2.6	2.84 ± 2.2	3.11 ± 2.4	0.111 NS
Maturation rate	63.95 ±25.2	60.63 ±21.1	71.86 ± 28.6	56.42 ± 23.6	0.071 NS
MI oocytes	1.18 ± 1.2	1.42 ± 1.25	0.87 ± 1.2	1.28 ± 1.1	0.165 NS
GV oocytes	0.35 ± 0.8	0.27 ± 0.7	0.32 ± 0.8	0.56 ± 1.10	0.503 NS
Abnormal oocytes	0.54 ± 1.2	0.91 ± 1.6 A	0.16 ± 0.45 C	0.50 ± 0.8 B	0.037 S

Mean ± standard deviation were used to express data ; n= number of cases; *: one way ANOVA; A, B and C referred by pos hoc LCD test ; NS= not significant at $p \geq 0.05$; S= significant at $p \leq 0.05$

Fertilization and cleavage characteristics

The embryos total number was higher significantly patients underwent sandwich (A) protocol than those obtained by the conventional antagonist and short GnRH agonist protocols (B) (P = 0.024). The percentage of embryo grade one was higher significantly in patients of sandwich protocol (A) compared to both conventional antagonist and short GnRH agonist protocols (P = 0.004) respectively. Embryo transfer and abortion rate failed to show any statistical difference among the protocols. Detailed data are presented in table IV.

The pregnancy rates

The pregnancy rate were 33.3 %, 9.7 % and 27.8 % for sandwich, conventional antagonist and short GnRH agonist protocol, sequentially, as detailed numerically in Figure 2.

In sandwich the pregnancy rate was higher significantly compared to that of conventional antagonist protocol (P = 0.022); however, the difference in rate of pregnancy was insignificant between short GnRH agonist protocol and conventional antagonist (P = 0.211) as well as between sandwich and short GnRH agonist protocol (P = 0.683), as shown in (figure 2).

Table IV : Fertilization and cleavage characteristics in poor responders according to protocol

Parameter	Total n = 82	Sand-wich n = 33	Conven-tional n = 31	Short n = 18	P
Fertilization rate	61.33 ± 33.34	66.03 ± 30.18	58.42 ± 36.51	57.70 ± 34.04	0.581¥ NS
Cleavage rate	72.63 ± 40.00	74.15 ± 35.92	67.22 ± 45.18	79.17 ± 38.59	0.584† NS
G1percent	35.53 ± 33.66	49.35 ± 35.43 A	22.31 ± 28.01 C	32.96 ± 30.94 B	0.004† HS
G2percent	42.51 ± 35.50	37.99 ± 32.18	43.55 ± 37.93	48.98 ± 37.82	0.566† NS
G3percent	8.55 ± 20.10	6.60 ± 16.32	11.56 ± 24.78	6.94 ± 17.68	0.578† NS
Total em-bryos	2.43 ± 1.65	3.00 ± 1.87 A	1.90 ± 1.30 B	2.28 ± 1.49 B	0.024† S
Embryo transfer percent	79.50 ± 37.24	84.91 ± 28.31	69.89 ± 45.83	86.11 ± 33.46	0.191† NS
Abortion (n %)	4/13 (30.8 %)	1/5 (20.0 %)	1/3 (33.3 %)	2/5 (40.0 %)	0.786¥ NS
OHSS (n %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	----

Mean ± standard deviation were used to expressed data; n= number of cases; G= grade; OHSS= ovarian hyperstimulation syndrome; †= one way ANOVA;A,B and C referred by pos hoc LSD test; ¥: Fischer exact test ; NS= not significant at $p \geq 0.05$; HS= highly significant at $p \leq 0.01$.

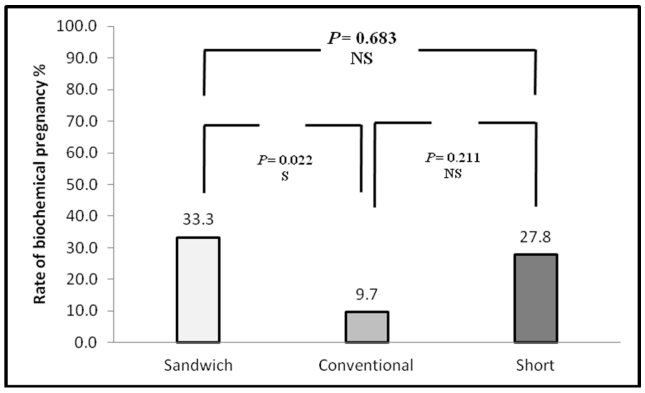

Figure. 2: The difference in rate of pregnancy was insignificant between short GnRH agonist protocol and conventional antagonist (P = 0.211) as well as between sandwich and short GnRH agonist protocol (P = 0.683)

DISCUSSION

According to our results, the most prevalent ages were (5-13 year) which disagree with (13), study that showed that most of the patient was under 5 years. Also, the most abended grade in T patients group was +1 (85%), and that agrees with the study of (14) in which his result showed hyperplasia plus one of the other chronic pathological features and confirmed a strong correlation between the clinical diagnosis and histological examination of patients. Also, the predominant grade in the H patients group was +3 (50%) and +2 (45%) respectively and this is similar to the result of (15, 16).

The explanation for hypertrophy may due to inflammation as a site of being the proliferation of lymphoid follicles or as a part of generalized lymphoid hypertrophy as well as cervical vascular congestion (17). According to (18), result grade, +3, and grade +2 was the most appended grade and that agrees with our result. Because the results of the clinical pathology tests were predominantly lymphoid hypertrophies, and hypertrophy of the palatine tonsils may be related to recurrent tonsillitis, it suggests that the classification of hypertrophic tonsils was determined to be useful in grading.We can consider tonsillar enlargement as a guide for prognostic evaluation.

The study (19), investigates the factor that affects the success of tonsillectomy and he supposed that tonsillar size is one of the most affecting factors, as a result, the study has been showing that a higher tonsil grade was related to the higher success rate. In this study, the grading was investigated between the two groups to determine if there is a certain difference between the two groups. According to the result, there was a difference between T and H patient groups as well as result showed that the most dominant grade in the T patient group was +1 and in the H patients group was grade +3 and +2 respectively.

The increase in the ASO titer in our result agrees with (20) study which assumed the increase in the ASO level was due to tonsillitis caused by streptococcal infections or its complication (glomerulonephritis, reactive arthritis, or rheumatic fever). The result disagrees with the study (21), a result which doesn't show a significant correlation between the ASO level so it suggests that the determination of the ASO titer does not have any value in acute and recurrent tonsillitis and thus should not be performed. In the current study, the result showed a high increase in the ASO titer in patients that corresponding with the result of (22), which reported an elevate in ASO titer in patients and in contrast with (23) study which reported a decrease in the ASO titer in (86%) of the patient involving in the study.

Our result agreed with the results of another previous study (11) presented a significant change which according to his result the group of patients showed a lower vitamin D level is compared with the control group. On the other hand, study results of (24-26) showed patients with tonsil hypertrophy significantly have a low level of vitamin D and its deficiency. In contrast to our results, the study of (7,27) showed no significant change. The study (28-29) indicated significant connotations

between low levels of vitamin D and tonsillar diseases, this independent of the vitamin D deficiency may suggest the importance of hypovitaminosis in the initial stages or the complementary role of inflammation.

Many studies convened that children with allergy symptoms appeared to be more disposed to tonsillar disease. Conversely, other studies dose not found a direct relationship between tonsil volume and allergies. The argument stays concerning the relationship between tonsil and allergies (30-31) also showed an elevation in the level of serum IgE in his result in the children from H patient group. In our study, the presence of allergy in children from the T patients group and H patients group was investigated, and the result agrees with (32) result which showed an increase in the level of IgE in children with H disease which state that there is a correlation between IgE elevation and allergy. The tonsils contain many immunological tissues which have a humoral immunity by synthesis and secretion of immunoglobulins (IgE, IgA, IgG), and cellular immunity by T-lymphocyte penetrating the epithelial barrier (14). According to (33) in children, systemic atopy may not be caused by tonsillar tissues, thus, anti-allergy medication is still required for children with atrophy following tonsil ectomy.

CONCLUSION

Sandwich protocols has the potential to improve numbers and competence of retrieved oocytes, as well embryos number, in addition to that sandwich protocols improves the coordination of multifollicular development in poor responders. Short GnRH agonist protocol better results than conventional GnRH antagonist for poor responders.

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