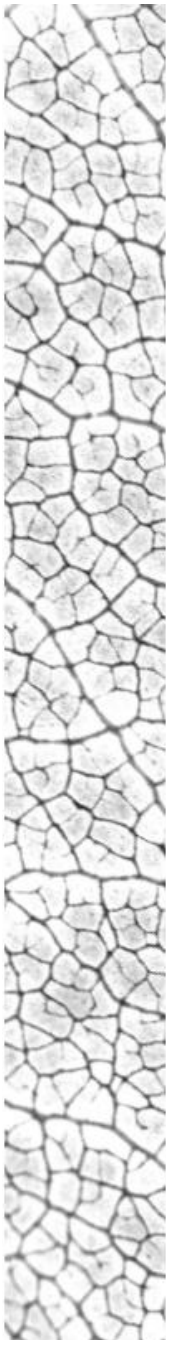

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An early and short gonadotropin releasing hormone (GnRH) antagonist (sandwich) protocol versus conventional gonadotropin releasing hormone (GnRH) antagonist in poor responders undergoing IVF/ICSI

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Abstract
In the field of IVF, the term 'poor responder' refers to the patients, with decreased ovarian reserve, that revealed as low retrieved oocytes in controlled ovarian hyperstimulation (COH) cycles which is a huge challenge in IVF. This study compares the effectiveness of an early and short gonadotropin releasing hormone (GnRH) antagonist (sandwich) protocol with conventional (GnRH) antagonist in poor ovarian responders. This randomized clinical trial consisted of 61 poor ovarian responder women enrolled in intra-cytoplasmic sperm injection (ICSI) cycles at High Institute for infertility Diagnosis and Assisted Reproductive Technologies, Baghdad, Iraq from January 2018 till February 2019. They were divided randomly in to two groups which are an early and short gonadotropin releasing hormone (GnRH) antagonist (sandwich) protocol group and conventional gonadotropin releasing hormone (GnRH) antagonist protocol group in order to compare between the two groups in the outcomes. The mean number of retrieved oocytes was significantly higher in sandwich than in conventional antagonist protocol (P = 0.026). Mean grade 1 embryo was significantly higher in sandwich protocol than in conventional protocol (P = 0.049). The rate of pregnancy was significantly higher in sandwich than that of conventional antagonist protocol (P = 0.026).
Conclusion: An early GnRH antagonist (sandwich) protocol can improve ovarian response in poor responders by stimulating and synchronizing follicle development

Keywords: IVF-ICSI, poor responders, sandwich protocol, antagonist protocol

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INTRODUCTION

Management of poor ovarian reserve (POR) is a compelling problem in assisted reproduction practice because of multiple definitions and various data regarding the optimal protocol (Mutlu et al, 2017).

The European Society of Human Reproduction and Embryology (ESHRE) introduced the Bologna criteria in (2011), according to these criteria, poor responders are identified by (i) advanced maternal age or any other risk factors for POR; (ii) a previous POR and (iii) an abnormal ovarian reserve test (Ferraretti et al, 2011).

A common aim of using antagonists is to prevent a premature LH surge (Yang et al, 2016). The GnRH antagonist action starts immediately after their administration in opposite to the long period required with GnRH agonists to achieve down regulation (Cédrin-Durnerin, Guivarc'h-Levêque and Hugues 2012).

GnRH antagonist cycles have the advantage of a shorter duration of COH, and lower incidence of OHSS than the GnRH agonist cycles. However, several studies have failed to show an advantage of the antagonist use in terms of pregnancy rates when compared with the GnRH agonist long protocol (Yang et al, 2016).

Poor responder patients generally have low numbers of retrieved oocytes and transferable embryos, and inevitably have low clinical pregnancy rates (Lee et al, 2018).

A physiological increase in the FSH level during the luteal follicular transition phase and premature selection of the dominant follicle after the rise of FSH in the late

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luteal phase may result in asynchrony in the follicular development, leading to slightly lower mature oocyte yield (Lee et al, 2018).

This heterogeneity of follicular growth in controlled ovarian stimulation cycles has been observed more frequently in poor responder patients, additionally, in poor responders the heterogeneity of follicular growth may partly result from a shortened follicular phase with limited ability to recruit a sizable cohort, or differential sensitivity of early antral follicles to FSH (Cakmak et al, 2014).

One of the strategies have been suggested in GnRH antagonist cycles to improve follicular synchronization is premenstrual administration of GnRH antagonist (Park et al, 2014). An early suppression of endogenous FSH by early GnRH antagonist administration may be advantageous for achieving follicular synchronization (Park et al, 2014).Earlier initiation of the GnRH antagonist has also been proposed to reduce exposure to FSH, LH and E2 in the early follicular phase during COH that if present, negatively affects the probability of pregnancy (Shin et al, 2018).

In addition, to that starting GnRH antagonist in the follicular phase could lengthen the recruitment phase of the cycle, thereby permit the retrieve of more follicles by down regulation endogenous FSH and making the stimulation solely depending on exogenous FSH (Blockeel and Devroey 2012).

MATERIALS AND METHODS

This study was a prospective comparative study as a clinical trial that conducted in the High Institute for Infertility diagnosis and Assisted Reproductive Technologies / Al-Nahrain University (Baghdad/Iraq) from January 2018 till February 2019. The study was approved by the Local Medical Ethical Committee of the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University; a written Informed Consent has been taken from each patient who is involved in this study. Twenty-eight women undergone Early and short Follicular Antagonist protocol (sandwich) for ICSI. Thirty-three women undergone conventional flexible GnRH antagonist protocol for ICSI cycle.

According to the ESHRE agreement, at least two of the following three features indication must be present to define POR:

1) Advanced maternal age (≥40) or any other risk factor for the poor ovarian response,

2) Previous poor response (cycles canceled or ≤3 oocytes with a conventional stimulation protocol),

3) Abnormal ovarian reserve test (ORT) (AMH <0.5-1.1 ng/mL or AFC <5-7 follicles) (Ferraretti et al, 2011).

Patients with age >42 yr, endocrine or metabolic disorders, severe endometriosis were excluded from the study.

In the sandwich protocol group (n 28) The GnRH antagonist was administered (0.25 mg/d) on days 1, 2, and 3 of the menstrual cycle and stopped thereafter, to be re-administered when the leading follicle reaches a 13 to 14 mm diameter and continued until hCG day.

In the conventional antagonist protocol group (n 33) the GnRH antagonist was initiated as in the classic flexible protocol (i.e., whenever the leading follicle reached a 13 to 14mm diameter) and similarly continued until hCG day. In both groups, rFSH (gonal f, Merck - Serono Company, Geneva: Switzerland) was used for COH.

Oviterlle injections 6500IU /vial (250mg) of Human chorionic gonadotropin (HCG) (Merck-Serono Company, Geneva: Switzerland) was administered when the transvaginal scan showed two or more follicles with a diameter of ≥18 mm.

Oocytes were harvested via transvaginal ultrasound-guided follicular puncture 35–36 hours after hCG administration. Metaphase II oocytes were fertilized by an intracytoplasmic sperm injection (ICSI) technique within 4 hours after oocyte retrieval.

Embryos were scored according to the Istanbul consensus workshop (Alpha Scientist in Reproductive Medicine and ESHRE Special Interest Group of Embryology.2011)

Embryos were transferred on either post-fertilization day 2 or 3 under abdominal ultrasound guidance. Luteal phase was supported since the day of oocytes retrieval (Cyclogest®400mg twice: Cox Pharmaceuticals, Barnstaple, UK)

IVF outcomes included the total number of retrieved oocytes, the number of mature (metaphase II [MII]) oocytes, fertilization rate (percentage of 2PN stage zygotes approximately 16-18 hours after ICSI treatment) and the number of good embryos (above grade II according to the grading system). Serum beta hCG levels were measured 14 days after oocyte retrieval, and values above 5.0 IU were considered positive. Clinical pregnancy was defined as the presence of an intrauterine gestational sac with a yolk sac, a fetal pole, and fetal heart pulsations.

Statistical analysis

Data are statistically presented in terms of the mean± standard deviation, number of cases, and percentage when appropriate. We used Student's t-tests to evaluate continuous parameters. A P-value <0.05 was considered statistically significant. The statistical analysis was performed with SPSS version 23 and Microsoft Office Excel 2010.

RESULTS

Poor responders were categorized into women underwent conventional antagonist, early and women underwent short follicular antagonist (sandwich protocol).

Table 1. Demographic characteristics of poor responders

Characteristic	Total *n* = 61	Sandwich *n* = 28	Conventional *n* = 33	*P**
Age (years)	35.16± 6.17	36.42±6.20	34.09± 6.03	0.142NS
BMI (kg / m2)	30.24± 4.09	30.73±3.86	29.82± 4.30	0.39NS
Infertility duration (years)	8.26 ±5.09	7.85±5.26	8.60±5.01	0.57NS
FSH (IU/L)	8.03 ± 3.81	9.01± 4.03	7.20 ±3.46	0.064NS
LH(IU/L)	3.60 ± 1.42	3.81 ± 1.51	3.43± 1.34	0.30NS
FSH/LH	2.43 ± 1.38	2.62 ±1.49	2.28± 1.28	0.34 NS
E_2(pg/ml)	31.41 ± 12.92	31.89 ±12.70	31.009±13.28	0.79NS
Prolactin(ng/ml)	15.70± 6.31	16.06 ±5.81	15.37± 6.79	0.67NS
TSH (mIU/L)	1.92± 1.27	2.06 ±1.415	1.80± 1.14	0.42NS
AMH (ng/ml)	1.01 ± 0.56	0.96 ±0.59	1.06±0.54	0.48NS

n: number of cases; SD: standard deviation; BMI: body mass index; IVF: in vitro fertilization; FSH: follicle stimulating hormone; LHL luteinizing hormone; E2: estradiol; TSH: thyroid stimulating hormone; HS: highly significant at P ≤ 0.01; NS: not significant at P ≤ 0.05

Table 2. ovarian stimulation characteristics in Poor responders

Parameter	Total *n* = 61	Sandwich *n* = 28	Conventional *n* = 33	*P**
Stimulation days	8.77±1.44	8.78±1.66	8.75± 1.25	0.94NS
total r FSH (ampule75IU)	22.06 ±9.30	26.35±10.97	18.42± 5.57	<0.001HS
Day antagonist start	8.04 ±1.46	8.82±1.56	7.39±0 .99	<0.001HS
Number of antagonists (not including first 3 days)	3.86±0.90	3.64±0.91	4.06±0 .86	0.072NS
E_2 at trigger (pg/ml)	798.18 ±404.52	790.26±413.23	804.90± 403.28	0.889NS
Endometrial thickness at day of oocyte pickup	9.09 ±1.37	9.44±1.44	8.79± 1.25	0.064NS

n: number of cases; SD: standard deviation; FSH: follicle stimulating hormone; E2: estradiol; *: Independent samples t-test; HS: highly significant at *P* ≤ 0.01; NS: not significant at *P* ≤ 0.05

Hormonal status of poor responders with respect to type of protocol is shown in **Table 1**.The difference was not significant in mean serum FSH, Also, there was no significant difference in mean serum LH as well as FSH / LH ratio between patient undergoing conventional protocol and those undergoing sandwich antagonist protocol (*P* > 0.05). Mean serum E2, prolactin, TSH and AMH also have exhibited insignificant difference between conventional and sandwich antagonist groups in poor responders (*P* > 0.05), as shown in **Table 1**.

Ovarian stimulation characteristics include stimulation days, total rFSH, day of starting antagonist, number of antagonists (not including first 3 days), number of follicles, E2 level at trigger and endometrial thickness at day of oocyte pickup **Table 2**.There was no significant difference in duration of stimulation (*P* = 0.94) among sandwich protocol, conventional antagonist protocol.

There was highly significant difference in mean total rFSH (*P* < 0.001), being higher in sandwich protocol when compared to conventional antagonist protocol. Mean day of antagonist start was significantly higher in sandwich protocol than conventional antagonist protocol (*P* < 0.001). There was no significant difference in mean number ofantagonist (not including first 3 days) used for sandwich or conventional antagonist protocol (*P* = 0.072). Also, there was no significant difference in mean estradiol (E2) at trigger between sandwich and conventional antagonist protocols (*P* = 0.889). In addition, there was no significant difference in mean endometrial thickness among sandwich, conventional antagonist protocols (*P* = 0.064), as shown in **Table 2**.

Table 3. Oocyte characteristic in poor responders according to protocol

Parameter	Total *n* = 61	Sandwich *n* = 28	Conventional *n* = 33	*P**
Retrieved oocyte	3.98 ± 1.67	4.50±1.62	3.54± 1.62	**0.026 S**
MII oocyte	2.75 ± 1.52	2.78±1.49	2.72± 1.56	0.88 NS
MI oocyte	0.80 ± 0.83	1.07±0.85	0.57±0.75	0.019 S
GV oocyte	0.22 ± 0.61	0.35±0.82	0.1212± 0.33	0.13 NS

Data were expressed as mean ± standard deviation; n: number of cases; *: Independent samples t-test; HS: highly significant at *P* ≤ 0.01; NS: not significant at *P* ≤ 0.05

Table 4. Fertilization and embryos characteristics in poor responders according to protocol

Parameter	Total *n* = 61	Sandwich *n* = 28	Conventional *n* = 33	*P**
Fertilization rate	71.47± 37.23	79.64±33.66	64.53± 39.17	0.115
G1	0.80 ± 0.85	1.03±0.92	0.60±.74	**0.049 S**
G2	1.00± 0.81	0.96±0.88	1.03± 0.76	0.756 NS
G3	0.31± 0.56	0.32±0.61	0.30± 0.52	0.90 NS
Total embryos transfer	1.93 ± 1.12	2.14±1.04	1.75± 1.17	0.184 NS

Data were expressed as mean ± standard deviation; n: number of cases, G: grade; ET: embryos transferred; *: Independent samples t-test; HS: highly significant at *P* ≤ 0.01; NS: not significant at *P* ≤ 0.05

Oocyte characteristics including number of retrieved oocyte, number of MII oocyte, MI oocyte, GV oocyte are revealed in **Table 3**; the mean number of retrieved oocyte was significantly higher in sandwich than in conventional antagonist protocol (*P* = 0.026), however, the mean number of MII oocyte was insignificantly different between sandwich and conventional antagonist protocol (*P* = 0.88); The mean number of MI oocyte was significantly different between two protocol with higher mean reported in sandwich protocol (*P* = 0.019) while the mean number of germinal vesicle (GV) oocyte was also insignificantly different between sandwich, conventional antagonist protocol (*P* = 0.13), as shown in **Table 3**.

There was no significant difference in the fertilization rate among sandwich and conventional antagonist protocols (*P* > 0.05). Mean grade 1 embryo was significantly higher in sandwich protocol than in conventional protocol (*P* = 0.049). However, there was insignificant difference in mean grade 2 and grade 3 embryo in poor responders between sandwich and conventional antagonist protocol (*P* > 0.05). There was also insignificant difference in mean transferred embryo numbers (*P* = 0.184) as shown in **Table 4**.

Table 5. Pregnancy rate according to ovarian response and protocol

Group	Protocol	Rate of biochemical pregnancy %	*p*-value
Poor	Sandwich	32.1%	0.026
	Conventional	9.1%	
	Total	19.7%	

The pregnancy rates were 32.1 %, 9.1 % for sandwich, conventional antagonist protocol, respectively, as shown in **Table 5**.

The rate of pregnancy was significantly higher in sandwich than that of conventional antagonist protocol (P = 0.026); as shown in **Table 5**.

DISCUSSION

There is a serious problem in poor responders to controlled ovarian stimulation due to a quantitative reduction in the follicular response and a consequent decrease in the retrieved oocyte number, transferable embryo number and pregnancy rate in comparison to normal responders (Davar et al, 2018).

Therefore, it is mandatory to identify an optimal stimulation protocol to improve the quality and number

of retrieved oocytes so that designated poor responders can achieve pregnancy.

The present study, applied a protocol that involve a short pituitary down regulation with GnRH antagonist in the early follicular phase immediately, followed by COH and compared with conventional GnRH flexible antagonist protocol.

Sandwich protocol had a statistical difference in oocytes retrieved, grade 1 embryos number in POR compared with conventional GnRH flexible antagonist protocol. Fertilization rate was higher in a sandwich protocol although not significant.

During controlled ovarian stimulation uncoordinated follicular growth may be a result of the size heterogeneities of early antral follicles during the early follicular phase (Davar et al, 2013). Therefore, diminished number of oocyte maturation and fertilization potential may be as result of difference in follicular size (Davar et al, 2018)

In the current study early GnRH antagonist administration may be resulted in more coordinated follicular growth and best IVF results.

Ashrafi et al. (2018) showed that the delayed start protocol (sandwich protocol) in poor responders can improve the fertilization rate, quality of embryos and reduce the cycle cancellation.

Lee et al. (2018) study showed that for patients with diminshed ovarian reserve, a low quantity and poor quality of oocytes have been major limitations on standard IVF cycles.An early GnRH antagonist administration protocol would improve the number of mature oocytes and transferable embryos and, consequently, better clinical pregnancy rates than conventional GnRH antagonist protocols.

In another clinical trial performed by Younis and coworkers among normal responders, 3 days early GnRH antagonist administration improved oocyte maturity and fertilization rates but did not change the pregnancy rates (Younis et al., 2010). Blockeel and colleagues study among women with normal ovarian reserve showed that early follicular phase GnRH antagonist administration for 3 days resulted in a higher number of retrieved oocytes but failed to yield significantly higher pregnancy rates as in our results in POR (Blockeel et al, 2011).

This study depends on assuming that by down regulation of the endogenous FSH will provide a hormonal environment for the follicles to express similar amounts of FSH receptors so by controlled ovarian stimulation more coordinated follicular growth and better IVF results.

ovarian stimulation more coordinated follicular growth and better IVF results.

CONCLUSION

During controlled ovarian stimulation, more synchronous follicular growth, more oocyte number and good quality embryos can be obtained by early follicular phase GnRH antagonist administration

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