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Original Research Article

Early and short follicular GnRH antagonist (Sandwich) protocol versus conventional GnRH antagonist protocol in normal responders and poor responders

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Abstract

Gonadotropin-releasing hormone (GnRH) antagonists are among its analogues that were used to produce prompt down-regulation of pituitary gonadotropin secretion. During conventional antagonist protocol, exposure to high LH and E2 levels leads to worse clinical reproductive results. The current study aims to investigate how such a modification, a GnRH antagonist in the follicular phase (Sandwich protocol) affects. One hundred six women, 44 normal responders and 82 poor responders undergoing ICSI-ET cycles were randomized into two groups. The conventional GnRH antagonist group 30 normal responders and 31 poor responders to GnRH antagonist was administered as, flexible protocol. In the sandwich protocol group 14 normal responders and 33 poor responders, a GnRH antagonist was administered for three days started from day one of the menstrual cycle and continues as flexible protocol. In the sandwich protocol, mean number of follicles obtained by normal responders was significantly higher than that obtained by poor responders, the mean numbers of retrieved oocytes as well as that of MII oocytes were significantly higher in normal responders than in poor responders. Estradiol at trigger was significantly higher in normal responders than in poor responders. Mean total number of embryos and the mean number of frozen embryos were significantly higher in the normal than in the poor responders. The rate of pregnancy was significantly higher in the normal than in the poor responders. Within conventional GnRH antagonist protocol, the mean numbers of retrieved oocytes as well as that of MII oocytes were significantly higher in the normal responders. Mean total number of embryos, mean grade 1 embryo percentage and the mean number of frozen embryos were significantly higher in the normal than in the poor responders, the mean grade 1 embryo percentage was also significantly higher in normal than in poor responders, and the rate of pregnancy was significantly higher in the normal than in the poor responders. Early and short GnRH antagonists proved improvements in the synchronization of follicular development, in the number of mature oocytes retrieved, in the number of total embryos, in the number of frozen embryos, as well as the pregnancy rates.

Keywords: GnRH antagonist, follicular recruitment and mature oocytes.

INTRODUCTION

In the early years, for the in vitro fertilization (IVF), gonadotropin-releasing hormone (GnRH) agonist long protocol played a key role for poor ovarian responders and was used for ovarian stimulation to inhibit the premature surge of luteinizing hormone. Although it had a number of side effects, this method was widely accepted and used as a long duration protocol treatment, which also increased the pregnancy rate and the number of oocytes retrieved. With the administration of the agonist, follicule-stimulating hormone (FSH) and LH increases (Giri *et al.*, 2017). Different studies have characterized the ovarian hyperstimulation syndrome (OHSS) as complication associated with controlled ovarian stimulation (Kolibianakis *et al.*, 2006). Thus, to overcome

these complications, various studies were conducted using GnRH antagonists which had an immediate mode of action, shorter duration, decrease hospital stay and beneficial to patients undergoing ovarian stimulations (Devroey *et al*., 2009).After the introduction of GnRH antagonists it has proved and appreciated as an additional support to controlled ovarian stimulation in reproductive techniques on the basis of patient's benefits and the clinicians are taking advantage of these benefits (Giri *et al.,* 2017). In the IVF cycles to improve the outcome of the GnRH antagonist protocol, flexible rather than the fixed GnRH antagonist regimen (Ludwig *et al*. 2002), oral contraception pretreatment (Hwang 2004), initiation of GnRH antagonist from premenstrual period, with day 1 of stimulation until the day of HCG administration or earlier at the follicular phase (Kolibianakis *et al*., 2003; Fanchin *et al*., 2004; Lainas *et al*., 2005).

The presented study suggests that short pituitary down regulation in the early follicular phase would result in low gonadotropin levels and synchrony in the follicular developments before ovarian stimulation started, and in this way it would be comparable in idea to the long GnRH agonist protocol while maintains the benefits of the GnRH antagonist protocol. Because constant state of the GnRH antagonist level is reached after 2 days of treatment (Kolibianakis *et al*., 2004), GnRH antagonist will be started from menstrual cycle day 1 for 3 days.

The aim of the current study was to investigate how such a modification affects the number of oocytes, the number of M2 oocytes, the number of embryos, the fertilization rates, the cleavage rates, the embryo grading and the pregnancy rates.

SUBJECTS, MATERIAL AND METHODS

The presented study was performed in the Higher Institute of Infertility diagnosis and Assisted Reproductive Techniques/Al-Nahrain University (Baghdad/Iraq) during the period from 2017 to 2019. One hundred twenty six women 44as normal responders and 82 as poor responders undergoing ICSI-embryo transfer cycles were randomized into two groups.

Inclusion Criteria

The patients who were normal responder, the patients, agreement to participate in the study, age group (18-44 years old),infertility due to male factors and couples with unexplained infertility.

Exclusion Criteria

Patient with endocrine disorders and anatomical and pathological abnormalities in uterus.

Ovarian Stimulation

Recombinant FSH (rFSH) (Gonal f, Merck Serono Company, Geneva, Switzerland), Menogon injections (Ferring, GmbH Company; Germany) 75 IU of both urinary FSH, LH and GnRH antagonist (Cetrorelix 0.25 mg) were used for controlled ovarian stimulation.

The gonadotropin dose was assessed for each women according to age, body mass index (BMI), antral follicle count, and/or previous responsiveness to ovarian stimulation. Further dose adjustments were performed on the basis of ovarian response, as evaluated using serum E2 measurement and follicular diameter by trans-vaginal ultrasound.

The conventional GnRH antagonist group30 normal responders and poor responders31, gonadotropin started from menstrual cycle day 2 or day 3 and continues until the day of HCG trigger, flexible GnRH antagonists (Cetrorelix 0.25 mg/d) administered according the follicular size (13-14 mm).

In the sandwich protocol group14 normal responders and 33 poor responders, a GnRH antagonist (Cetrorelix 0.25 mg/d) was administered for three days started from day one of the menstrual cycle and continues as flexible GnRH antagonists administered according the follicular size (13-14 mm). Gonadotropin started from the menstrual cycle day 3 and continued to day of HCG trigger.

According to the ovarian response, when transvaginal ultrasounds show 2 or more follicles with diameters ≥18 mm (Copperman and Benadiva2013) ovum pick up by transvaginal ultrasound was performed 35 hours HCG trigger.

Human chorionic gonadotropin (10,000 IU Pregnyl; NV Organon) or Oviterlle injections 6500IU/vial (250mg) of Human chorionic gonadotropin (HCG) (Merck-Serono Company, Geneva: Switzerland).

Luteal phase was supported since day of oocytes retrieval or the day after of oocytes retrieval by vaginal progesterone (Cyclogest®400mg twice: Cox Pharmaceuticals, Barnstaple, UK), or (Crinone,® 8% progesterone gel, MERK), and serum ß-HCG assay was done on day 14 after the embryo transfer indicative of biochemical pregnancy. A woman with positive result was indicated by an ultrasound examination later in order to objectify the existence of cardiac fetal activity that indicate of clinical pregnancy.

Procedures of ICSI Laboratory

The ICSI procedures will be performed similar in all women. The stage of oocytes will be assessed after denudation (enzymatic and mechanical). The oocytes

Table 1. Demographic characteristics of all study sample (normal and poor responders)

Characteristic	Statistic	Total cases *n* = 126	Normal responders *n* = 44	Poor responders *n* = 82	*P*
Age (years)	Mean ±SD	32.89 ± 6.89	28.77 ±4.90	35.10 ±6.81	<0.001* HS
BMI (kg / m2)	Mean ±SD	29.67 ± 4.96	28.53 ±4.41	30.28 ±5.16	0.059* NS
Infertility duration (years)	Median (IQR)	7.00 (8.00)	6.50 (6.50)	8.00 (9.00)	0.065† NS
Number of IVF cycles	Median (IQR)	0.00 (1.00)	0.00 (0.00)	0.00 (1.00)	0.009† NS
	Range	0-5	0.00 - 2.00	0.00 - 5.00	
Infertility cause	Female, *n* (%)	45 (35.7)	3 (6.8)	42 (51.2)	<0.001¥ HS
	Male, *n* (%)	28 (22.2)	28 (63.6)	0 (0.0)	
	Combined, *n* (%)	41 (32.5)	1 (2.3)	40 (48.8)	
	Unexplained, *n* (%)	12 (9.5)	12 (27.3)	0 (0.0)	
Type of infertility	Primary,	85 (67.5)	27 (61.4)	58 (70.7)	0.285 ¥ NS
	Secondary,	41 (32.5)	17 (38.6)	24 (29.3)	

n: number of cases; SD: standard deviation; IQR: inter-quartile range; BMI: body mass index; IVF: in vitro fertilization; *: Independent samples t-test; †: Mann Whitney U test; ¥: Chi-square test; HS: highly significant at $p \leq 0.01$; NS: not significant at $p \leq 0.05$

classified as M2 with presence of the first polar body. The ICSI procedure was carried out as described by Pereira *et al.*(2015).By Integra 3™ and Nikon ICSI Micromanipulators, fresh ejaculated or frozen spermatozoa will be injected to the mature oocytes. Assessment of fertilization was performed 16–18 hours after ICSI. Embryo transfer was performed at day 2 or day 3 after the oocyte retrieval.

Embryos were scored according to the Istanbul consensus workshop (Alpha Scientist in Reproductive Medicine and ESHRE Special Interest Group of Embryology.2011) and classified into grade 1, 2, 3.according to (blastomere homogeneity, fragmentation and the degree of enucleated fragments) criteria.

RESULTS

Poor responder women were significantly older than normal responders ($p< 0.001$). However, there was no significant difference encountered neither in BMI nor in infertility duration between normal and poor responders ($p > 0.05$).Male factor was the predominant cause of infertility in normal responders whereas female factor was the predominant cause of infertility in poor responders and the difference in the distribution of women according to cause of infertility between normal and poor responders was highly significant ($p<0.001$). Infertile women were approximately equally distributed with respect to type of infertility, primary versus secondary, among normal and poor responders, since the difference in their distribution was statistically insignificant ($p = 0.285$), as shown in Table (1).

Baseline hormonal levels of all sub-fertile women are shown in Table (2). Serum FSH was significantly higher in poor responders in comparison with normal responders ($p = 0.002$), however, there was no significant difference in mean LH between poor and normal responders ($p = 0.883$), therefore mean serum FSH/LH ratio was significantly higher in poor responders in comparison with normal responders ($p = 0.005$). There was no significant difference in mean serum estradiol (E2), prolacatin and TSH levels between normal and poor responders. Mean anti-mullerian hormone (AMH) level was measured only for poor responders Table (2).

Comparison of ovarian stimulation characteristics between normal and poor responders within sandwich protocol is shown in Table (3) There was no significant difference in duration of stimulation between normal and poor responders ($p = 0.094$). Mean total rFSH and total HMG were significantly more in poor responders than normal responder ($p =0.015$) and ($p =0.010$), respectively. There was no significant difference in mean day of antagonist start between normal and poor responders ($p = 0.218$). There was no significant difference in mean number of antagonist (not including first 3 days) between normal and poor responders ($p = 0.607$). Mean number of follicles obtain by normal responders was significantly higher than that obtained by poor responders ($p< 0.001$). Estradiol at trigger was significantly higher in normal responders than in poor responders ($p = 0.017$), however, there was no significant difference in mean progesterone at trigger or progesterone to estrogen ratio between normal and poor responders ($p> 0.05$). There was also no significant difference in mean endometrial thickness between normal and poor responders ($p = 0.419$), as shown in Table (3).

Table 2. Hormonal status of all study sample (normal and poor responders)

Hormone	Statistic	Total cases *n* = 126	Normal responders *n* = 44	Poor responders *n* = 82	*P* *
FSH (IU/L)	Mean ±SD	7.93 ±3.87	6.52 ± 3.07	8.69 ±4.06	0.002 HS
LH (IU/L)	Mean ±SD	3.84 ±1.91	3.79 ± 1.65	3.86 ±2.04	0.833 NS
FSH/LH	Mean ±SD	2.40 ±1.42	1.92 ± 1.00	2.65 ±1.54	0.005 HS
E_2 (pg/ml)	Mean ±SD	31.07 ±14.88	29.41 ± 14.79	31.96 ±14.95	0.362 NS
Prolactin (ng/ml)	Mean ±SD	15.01 ±8.78	16.81 ± 11.70	14.05 ±6.60	0.092 NS
TSH (mIU/L)	Mean ±SD	1.87 ±0.96	1.72 ± 0.53	1.95 ±1.13	0.202 NS
AMH (ng/ml)	Mean ±SD	---	---	0.93 ± 0.69	---

n: number of cases; SD: standard deviation; FSH: follicle stimulating hormone; LHL luteinizing hormone; E2: estradiol; TSH: thyroid stimulating hormone; *: Independent samples t-test; HS: highly significant at $p \leq 0.01$; NS: not significant at $p \leq 0.05$

Table 3. Ovarian stimulation characteristics between normal and poor responders within Sandwich protocol

Parameter	Normal		Poor		*P*
	n	Mean ±SD	*n*	Mean ±SD	
Stimulation days	14	8.29±1.44	33	9.15 ±1.64	0.094 NS
total r FSH (ampule75IU)	14	19.79±6.94	33	27.81 ±10.89	0.015 S
total HMG(ampule75IU)	14	4.36±3.77	33	11.58 ±9.63	0.010 S
Day antagonist start	14	8.71±1.20	33	9.24 ±1.37	0.218 NS
Number of antagonists(not including first 3 days)	14	3.71±1.27	33	3.55 ±0.90	0.607 NS
Number of follicles	14	17.93±6.50	33	9.79 ±4.05	<0.001 HS
E_2 at trigger(pg/ml)	14	1498.90±631.56	33	998.56 ±631.45	0.017 S
Progesterone at trigger day(ng/ml)	14	0.76±0.80	9	0.82 ±0.88	0.887 NS
Progesterone / Estrogen ratio	14	0.57±0.66	9	0.94 ±0.90	0.347 NS
Endometrial thickness at day of oocyte pickup	14	9.11±1.78	33	9.51 ±1.42	0.419 NS

n: number of cases; SD: standard deviation; FSH: follicle stimulating hormone; E2: estradiol; *: independent samples t-test; NS: not significant at $p \leq 0.05$; S: significant at $p \leq 0.01$ HS: highly significant at $p \leq 0.01$.

Comparison of ovarian stimulation characteristics between normal and poor responders within conventional GnRH antagonist protocol is shown in Table (4). There was no significant difference in duration of stimulation between normal and poor responders (p = 0.123). There was no significant difference in mean total rFSH between normal and poor responders (p = 0.443). Mean total HMG were significantly more in poor responders than normal responder (p =0.013). Mean day of antagonist start was significantly earlier in poor responders than normal responder (p =0.006). There was no significant difference in mean number of antagonist (not including first 3 days) between normal and poor responders (p = 0.801).

Mean number of follicles obtain by normal responders

Table 4. Ovarian stimulation characteristics between normal and poor responders within Conventional protocol

Parameter	Normal		Poor		*P*
	n	**Mean ±SD**	***n***	**Mean ±SD**	
Stimulation days	30	9.30±1.44	31	8.77 ± 1.18	0.123 NS
total r FSH(ampule75IU)	30	20.63±8.07	31	19.26 ± 5.68	0.443 NS
total HMG(ampule75IU)	30	2.60±3.83	31	9.26 ± 13.74	0.013 S
Day antagonist start	30	8.20±1.32	31	7.32 ± 1.05	0.006 HS
Number of antagonists(not including first 3 days)	30	3.73±1.34	31	3.81 ± 0.87	0.801 NS
Number of follicles	30	15.37±5.01	31	7.29 ± 3.08	<0.001 HS
E2 at trigger(pg/ml)	30	1400.60±812.21	31	819.58 ± 415.58	0.001 HS
Progesterone at trigger day(ng/ml)	30	0.71±0.41	8	0.57 ± 0.38	0.465 NS
Progesterone/Estrogen ratio	30	0.60±0.43	8	0.88 ± 0.87	0.366 NS
Endometrial thickness at day of oocyte pickup	30	9.07±1.47	31	8.90 ± 1.42	0.642 NS

n: number of cases; SD: standard deviation; FSH: follicle stimulating hormone; E2: estradiol; *: independent samples t-test; NS: not significant at $p \leq 0.05$; HS: highly significant at $p \leq 0.01$

Table 5. Comparison of oocyte characteristics between normal and poor responders in sandwich protocol

Parameter	Normal ***n* = 14**	Poor ***n* = 33**	***P***
Retrieved oocyte	13.00 ± 5.79	6.67 ± 3.70	<0.001 HS
MII oocyte	8.36 ± 4.60	4.06 ± 2.56	<0.001 HS
Maturation rate	64.63 ±22.21	60.63 ±21.11	0.561 NS
MI oocyte	1.64 ± 2.02	1.42 ± 1.25	0.653 NS
GV oocyte	1.71 ± 2.84	0.27 ± 0.67	0.008 HS
Abnormal oocyte	0.71 ± 1.14	0.91 ± 1.65	0.689 NS
Ruptured oocyte	0.43 ± 0.65	0.12 ± 0.33	0.036 S

Data were expressed as mean ± standard deviation; n: number of cases; *: one way ANOVA; NS: not significant at P ≤ 0.05; S: significant at $p \leq 0.05$; HS: highly significant at $p \leq 0.01$

was significantly higher than that obtained by poor responders ($p< 0.001$). Estradiol at trigger was significantly higher in normal responders than in poor responders ($p = 0.001$), however, there was no significant difference in mean progesterone at trigger or progesterone to estrogen ratio between normal and poor responders ($p> 0.05$). There was also no significant difference in mean endometrial thickness between normal and poor responders ($p = 0.419$), as shown in Table (4).

Table (5) shows the comparison of oocyte characteristics between normal and poor responders within sandwich protocol. The mean numbers of retrieved oocyte as well as that of MII oocyte were significantly higher in normal responders than in poor responders ($p< 0.001$); however, there was insignificant difference in mean number of MI oocyte between normal and poor responders ($p = 0.653$). The mean number of GV oocyte

Table 6. Comparison of oocyte characteristics between normal and poor responders in conventional antagonist protocol

Parameter	Normal *n* = 30	Poor *n* = 31	*P*
Retrieved oocyte	8.63 ± 3.99	4.26 ± 2.85	<0.001 HS
MII oocyte	5.50 ± 3.36	2.84 ± 2.19	<0.001 HS
Maturation rate	62.59 ±17.01	71.86 ± 28.56	0.130 NS
MI oocyte	1.90 ± 1.21	0.87 ± 1.15	0.001 HS
GV oocyte	0.50 ± 0.90	0.32 ± 0.83	0.427 NS
Abnormal oocyte	0.40 ± 0.72	0.16 ± 0.45	0.127 NS
Ruptured oocyte	0.20 ± 0.41	0.13 ± 0.43	0.509 NS

Data were expressed as mean ± standard deviation; n: number of cases; *: independent samples t-test; NS: not significant at $p \leq 0.05$; HS: highly significant at $p \leq 0.01$

Table 7. Comparison of fertilization and cleavage characteristics between normal and poor responders in sandwich protocol

Parameter	Normal n = 14	Poor n = 33	*P*
2PN percent	54.66 ± 15.72	53.85 ± 28.59	0.922 NS
Cleavage rate	85.94 ± 21.35	74.15 ± 35.92	0.260 NS
G1percent	48.51 ± 24.88	49.35 ± 35.43	0.936 NS
G2percent	44.69 ± 18.56	37.99 ± 32.18	0.472 NS
G3percent	6.81 ± 14.08	6.60 ± 16.32	0.967 NS
Total embryos	6.29 ± 3.36	3.00 ± 1.87	<0.001 HS
ET percent	61.39 ± 36.60	84.91 ± 28.31	0.021 S
Number of frozen embryos	2.29 ± 3.07	0.30 ±0.92	0.001 HS
Fertilization rate	72.36 ± 9.29	66.03 ± 30.18	0.448 NS
Abortion (n %)	3/4 (75.0 %)	1/5 (20.0 %)	0.524 NS
OHSS (n %)	1 (7.1 %)	0 (0.0 %)	0.298 NS

Data were expressed as mean ± standard deviation; n: number of cases; 2 PN: fertilized oocytes (2 pronuclei); G: grade; ET: embryos transferred; OHSS: ovarian hyperstimulation syndrome; †: Independent samples t-test; ¥: Fischer exact test; NS: not significant at $p \leq 0.05$; HS: highly significant at $p \leq 0.01$.

was significantly higher in normal responders than in poor responders ($p = 0.008$), however, there was no significant difference in abnormal oocyte between them ($p = 0.689$). The mean number of ruptured oocyte was significantly higher in normal responders than in poor responders ($p = 0.036$), as shown in Table (5).

Table (6) shows the comparison of oocyte characteristics between normal and poor responders within conventional GnRH antagonist protocol. The mean numbers of retrieved oocyte as well as that of MII oocyte were significantly higher in normal responders than in poor responders ($p< 0.001$).The mean number of MI oocyte was significantly higher in normal responders than in poor responders ($p = 0.001$). There was also insignificant difference in mean number of GV, abnormal and ruptured oocyte between normal and poor

Table 8. Comparison of fertilization and cleavage characteristics between normal and poor responders in conventional antagonist protocol

Parameter	Normal n = 30	Poor n = 31	*P*
2PN percent	46.90 ± 21.42	51.60 ± 35.51	0.535 NS
Cleavage rate	89.06 ± 29.41	67.22 ± 45.18	0.030 S
G1percent	39.97 ± 29.15	22.31 ± 28.01	0.019 S
G2percent	51.96 ± 31.43	43.55 ± 37.93	0.351 NS
G3percent	4.74 ± 14.76	11.56 ± 24.78	0.199 NS
Total embryos	3.80 ± 2.04	1.90 ± 1.30	<0.001 HS
ET percent	77.33 ± 31.01	69.89 ± 45.83	0.462 NS
Number of frozen embryos	0.43 ± 0.94	0.00 ± 0.00	0.012 S
Fertilization rate	65.06 ± 27.59	58.42 ± 36.51	0.428 NS
Abortion (n %)	0/4 (0.0 %)	1/3 (33.3 %)	0.429 NS
OHSS (n %)	4 (13.3 %)	0 (0.0 %)	0.053 NS

Data were expressed as mean ± standard deviation; n: number of cases; 2 PN: fertilized oocytes (2 pronuclei); G: grade; ET: embryos transferred; OHSS: ovarian hyperstimulation syndrome; †: Independent samples t-test; ¥: Fischer exact test; NS: not significant at $p \leq 0.05$; HS: highly significant at $p \leq 0.01$

Table 9. Pregnancy rate according to ovarian response and protocol

Group	Protocol	Rate of biochemical pregnancy %
Normal	Sandwich	9/14 (64.3 %)
	Conventional	12/30 (40.0 %)
Poor	Sandwich	11/33 (33.3 %)
	Conventional	3/31 (9.7 %)

responders ($p > 0.05$), as shown in Table(6).

Table (7) shows comparison of fertilization and cleavage characteristics normal and poor responders in sandwich protocol. There was no significant difference in mean 2PN percentage as well as in cleavage rate in sandwich protocol between normal and poor responders ($p > 0.05$). Mean total number of embryos was significantly higher in normal than in poor responders ($p<0.001$). However, there was insignificant difference in mean grade I, grade 2 and grade 3 embryo percentage between normal and poor responders ($p > 0.05$). The mean transferred embryo number was significantly lower in normal than in poor responders ($p = 0.021$); however, the mean number of frozen embryos was significantly higher in normal than in poor responders ($p = 0.001$). In addition, there was insignificant difference in mean abortion rate and rate of ovarian hyper stimulation syndrome (OHSS) between normal and poor responders ($p > 0.05$), as shown in Table (7).

Table (8) shows comparison of fertilization and cleavage characteristics normal and poor responders in conventional GnRH antagonist protocol. There was no significant difference in mean 2PN percentage in conventional GnRH antagonist protocol between normal and poor responders ($p = 0.535$). The mean cleavage rate was significantly higher in normal responders than that in poor responders ($p = 0.030$).Mean total number of embryos was significantly higher in normal than in poor responders ($p< 0.001$); mean grade 1 embryo percentage was also significantly higher in normal than in poor responders ($p = 0.019$); however, there was insignificant difference in mean grade 2 and grade 3 embryo percentage between normal and poor responders ($p > 0.05$).

There was no significant difference in mean transferred embryo number between normal and poor responders ($p = 0.462$); however, the mean number of frozen embryos was significantly higher in normal than in

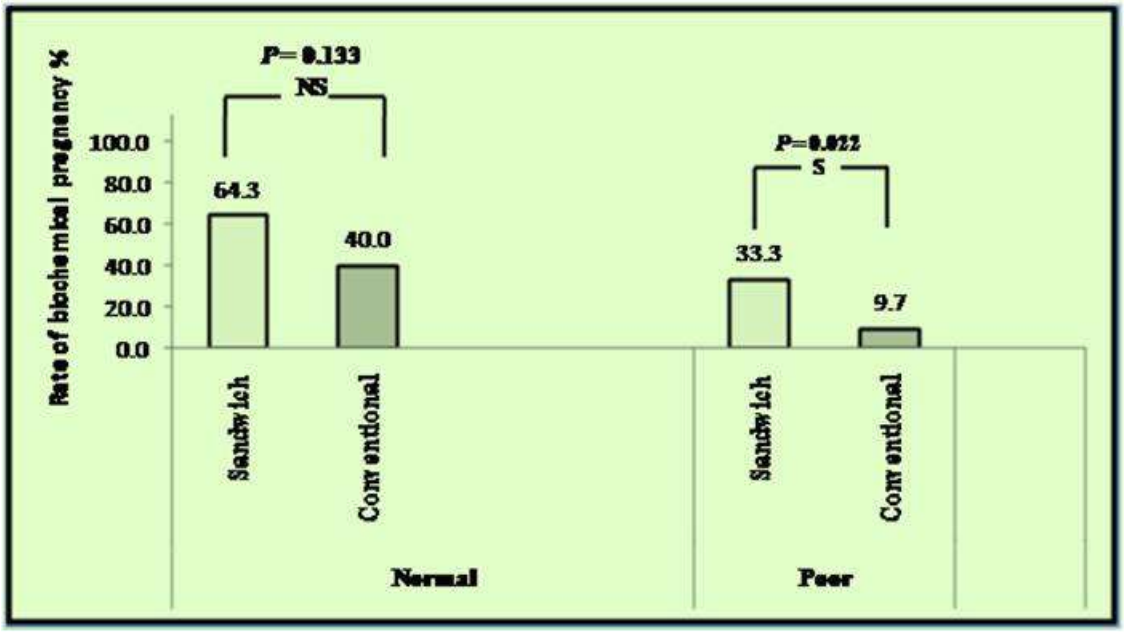

Figure 1. Pregnancy rate according to group of women as normal versus poor responder

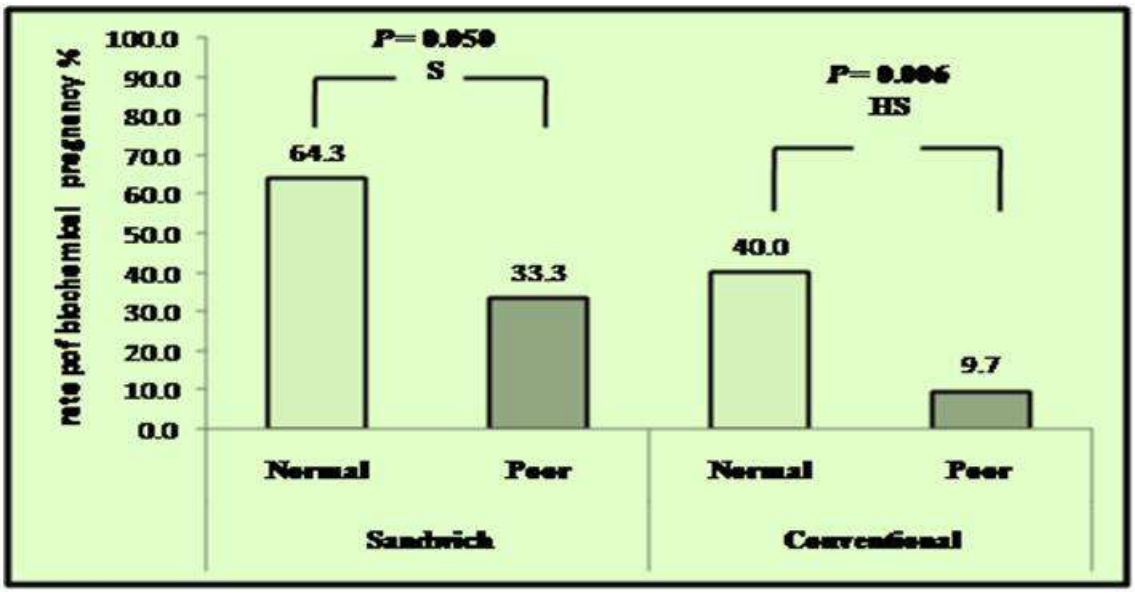

Figure 2. Pregnancy rate according to protocol

poor responders (p = 0.012). In addition, there was insignificant difference in abortion rate and rate of OHSS between normal and poor responders (p> 0.05), as shown in Table (8).

On the other hand, in sandwich protocol, the rate of pregnancy was significantly higher in normal than in poor responders (p= 0.050); in conventional antagonist protocol the rate of pregnancy was also significantly higher in normal than in poor responders (p = 0.006). As shown in Table (9), Figure (1) and Figure (2).

DISCUSSION

Poor ovarian response is clinically displayed by a shortened follicular phase (Badawy *et al*., 2011). Early and short follicular GnRH antagonist prolonged the duration of stimulation (Younis *et al*., 2010).

The data of sandwich protocol in the present study show; duration of stimulation was higher in poor responders than normal responders, although not significant. There was also no significant difference in

mean day of antagonist start between normal and poor responders. However mean total rFSH and total HMG were significantly more in poor responders than normal responder.

While in conventional GnRH antagonist protocol; duration of stimulation was higher in normal responders than poor responders, although not significant. However mean day of antagonist start was significantly earlier in poor responders than normal responder and mean total total HMG were significantly more in poor responders than normal responder .But there was no significant difference in mean total rFSH between normal and poor responders (p = 0.443).

In order to increase the oocytes yield in female with advanced age, a high FSH dose is needed and thus a significantly higher total FSH dosage than normal responders (Borges *et al.,* 2017).

In normogonadotropic patients LH addition does not appear to be beneficial (Lehert *et al*., 2014). However LH addition have benefit of in women with advanced age. (Bosch, Labarta and Munoz 2018).

The number of follicles, estradiol at trigger, the mean numbers of retrieved oocyte, MII oocyte, means total number of embryos and the mean number of frozen embryos was significantly higher in normal responders when compared with poor responders in both sandwich protocols and conventional GnRH antagonist. But the mean transferred embryo number was significantly lower in normal than in poor responders in sandwich protocols. This agrees with Nichi *et al*. (2011) and Younis *et al*. (2005) studies that showed number of total oocytes and matured oocyte was lower in poor responder women.

Lee *et al*. (2018) study showed that for women with poor ovarian reserve that undergo ICSI protocols, poor quality and a low quantity of oocytes have been major parameters that determine the reproductive outcome. The luteal E2 administration and GnRH antagonist pretreatment protocol would improve the number of mature oocytes, total embryos and pregnancy rates than conventional GnRH antagonist IVF protocols.

In addition, the mean numbers of GV oocyte and ruptured oocyte were significantly higher in normal responders than in poor responders in the present study. The explanation for these results showed by Angle (2010) study, the number of mature oocytes retrieved and the estradiol levels on day of hCG negatively correlated with degeneration.

On the other hand, in sandwich protocol, the rate of pregnancy was significantly higher in normal than in poor responders (p = 0.050). In the GnRH conventional antagonist protocol the rate of pregnancy was also significantly higher in normal than in poor responders (p = 0.006). Oudendijk *et al.* (2012) confirmed that poor responders have a diminished pregnancy rate compared with normal responders.

CONCLUSIONS

It seems ovarian responsiveness during COS get better with the use of the early and short GnRH antagonistic protocol and may result in more coordination in follicular development, more mature oocyte retrieved, higher numbers of embryos, and improved pregnancy rates in normal and poor responders.

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