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Short Agonist and Antagonist Protocols in Normoresponding Patients Undergoing ICSI, a comparative study.

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ABSTRACT

Background: Subfertility is one of major problems worldwide, which led to continuous researches and advancements in the field of assisted reproductive technology. Both agonist and antagonist protocols are clinically used, hence comparison of both protocols is essential for clinical evaluation of their outcomes.
Aim: To compare of the outcomes of GnRH short agonist and GnRH antagonist protocols in normo-responder patients undergoing intracytoplasmic sperm injection ICSI.
Method: A prospective clinical trial conducted at Najaf Fertility Centre, during the period from 1st of March 2016 to 1st of February 2018. This trial Included 394 subfertility women aged less than 38 years, after obtaining of oral and written consent. All patients underwent intracytoplasmic sperm injection due to male factor except azoospermia, certain female factors, unexplained subfertility and mixed type to be assigned to short GnRH agonist protocol or GnRH antagonist protocol. The main outcomes measures were duration of stimulation days, numbers of 75 IU gonadotrophin ampules, estradiol levels on day of hCG, embryological parameters, fertilization rate, cleavage rate, chemical pregnancy rate and clinical pregnancy rate per cycle and per embryo transfer, multiple gestation, early pregnancy loss, and ovarian hyper stimulation syndrome, first trimester abortion.
Results: Out of the study sample, 19 patients were cancelled due to various reasons, and the remaining 375 subfertile women continued the ICSI cycles. Stimulation days and total numbers of gonadotrophins was significantly higher in GnRH-agonist protocol group, than GnRH-antagonist (10.13 vs 9.41and 28 vs 21, respectively, ($P<0.05$). Frequency of ovarian hyper stimulation syndrome was significantly higher in agonist than antagonist group 17.1% and 9.4%, respectively. No statistically significant differences had been found between both groups in all embryological data ($P>0.05$) except the mean number of good quality embryos it was significantly higher in agonist than antagonist group, 3.32and 3.02, respectively. The agonist group had significantly higher fertilization rate, Positive β-hCG, clinical pregnancy per cycle and clinical pregnancy per embryo transfer than antagonist group, in all comparisons, ($P<0.05$), no statistically significant difference found in cleavage rate, chemical pregnancy/cycle, multiple gestation or first trimester abortion, ($P>0.05$).
Conclusion: Shorter duration of stimulation days, fewer numbers of gonadotrophin injections and lower incidence of OHSS in GnRH-antagonist make the GnRH-antagonist more patient friendly protocol in ART. However, significant higher fertilization rate, good quality embryo and clinical pregnancy rate in agonist short protocol tips the balance in favor of the short protocol.
Keywords: ICSI, antagonist, normoresponding

INTRODUCTION

Sub fertility is defined as failure of the couple to conceive following 12 months of regular unprotected sexual intercourse[1]. Assisted reproduction is a process by which an egg is fertilized by sperm outside the body in vitro. The process involves monitoring and stimulating women's ovaries to achieve multiple follicles which then retrieved outside the ovary and letting sperm fertilize them in a fluid medium in a laboratory (in vitro fertilization)[17]. While Intracytoplasmic sperm injection is a technique include obligatory introduction of sperm inside the oocyte by micromanipulation to achieve fertilization[12].

Pituitary down regulation is an important step in multifollicular development during ART cycles for blockage of the positive estradiol (E2) feedback to the pituitary and the resulting untimely LH surges during follicular stimulation as this would result in inadequate follicular growth or rupture prior to egg retrieval. Treatment with GnRH agonists given daily prevents the natural LH surge and is continued throughout the treatment cycle. Alternatively, GnRH antagonists can be administered during the mid-and late follicular phase of a superovulation cycle to prevent the endogenous LH surge[25]. GnRH agonist administration leads to prolonged agonistic action on the GnRH receptors due to their higher affinity to the receptors and their higher biological stability. Agonist administration initially induces the liberation of high amounts of LH and FSH from the pituitary and an increase in the number of receptors (up-regulation) within 12th of administration, so called 'flare effect' leads to a five-fold increase of FSH, ten-fold rise in LH and four-fold elevation in estradiol. However, the prolonged administration of GnRH agonists with their chronic action on the pituitary gonadotropins subsequently suppresses pituitary function and gonadotropin secretion. This is due to down regulation of the GnRH receptors and the inhibition of post-receptor mechanisms (pituitary desensitization) with a long half-life, the dimer form of the receptor is favored and the receptors are incorporated into the cell but cannot return to the cell membrane. The cell cannot therefore respond to a subsequent pulse of GnRH that are responsible for the synthesis and release of gonadotropins so called "block effect" the pituitary store of

gonadotropins is depleted after a period of about 7-14 days[27].

GnRH- antagonists competitively bind to pituitary GnRH receptors and block the ability of GnRH to initiate dimer formation (no flare-up effect) cause immediate and reversible suppression of gonadotropins[29]. 70% of LH and 30% of FSH suppression occurs in the initial 6 hours. As long as sufficient GnRH antagonist is present, suppression of FSH and LH will be sustained. Pituitary recovery starts after 12hours of administration of last dose of antagonist[30].

Short or flare-up antagonist protocol: It makes use of both the initial stimulatory and the subsequent inhibitory effects of GnRH agonists on pituitary gonadotropins[31]. Agonist started from the 2nd day of the cycle (CD2) and end at the maturation of follicles until human chorionic gonadotrophin (hCG) administration. Gonadotrophins started on cycle day 3.

Follicular growth takes 10-12 days, which is adequate to down-regulate the pituitary by the agonist and prevents a premature LH surge. The flare effect causing surge of LH in the initial phase of folliculogenesis and the agonist requirement for prolonged period for down-regulation[33,34]. It also associate with high Risk of severe OHSS[31]. In meta-analysis of randomized controlled trials has shown that the use of GnRH agonists has not only reduce cancellation rates but also increased the number of oocytes and embryos, allowing better selection so that , on average, the outcome in terms of pregnancy rates was improved[6]. So give this protocol to poor ovarian reserve[35].

Antagonist protocol: GnRH antagonists are generally preferred for IVF treatment in patients with low ovarian reserve or in those whom previous agonist protocols have been attempted and follicular development was poor. The most important reason for GnRH antagonists not having been preferred in the primary treatment is the published meta-analyses that report lower pregnancy rates[39,40]. In this protocol ovarian stimulation with gonadotropins is started on day 2 or 3 of cycle Then daily dose of 0.25mg cetrorelix or ganirelix is given subcutaneously (there is also a 3 mg dose of cetrorelix, which can last for several days). The antagonists given subcutaneously and are started either on a fixed or flexible protocol[42]. In the fixed protocol (French protocol), daily injection of small doses of antagonist 0.25mg are administered from day 6 of stimulation peroid. In the flexible protocol (Ludwig protocol), GnRH antagonist is started once the leading follicle is ≥14mm size or E2 level 500 pg/ml and more and is continued until and including the day of hCG trigger. The fixed protocol remains a simple approach and requires less monitoring of the cycle. On the other hand, the flexible protocol avoids unnecessary injections when risk of LH surge is minimal and hence uses less total antagonist ampules and less gonadotrophins[28]**.** The benefits of antagonists over agonists are: 1. No menopausal side-effects. 2. No cyst formation from the initial gonadotropin surge. 3. Shorter duration of treatment. 4. Less gonadotropin injections required in each cycle and thus lower drug costs[42]**.** 5. Reduces the incidence of OHSS[43]**.** However, the antagonist disadvantages have lower pregnancy and implantation rates because low LH level and impaired estrogen secretion[44]**.** So depending upon these scientific facts this study is designed to compare the outcomes of GnRH short agonist and GnRH antagonist protocols in normo-responder patients undergoing ICSI.

The objective of the study was to compare ICSI outcome in normoresponder patients undergoing ICSI by those using GnRH short agonist and those using GnRH antagonist protocols.

PATIENTS AND METHODS

This prospective clinical trial study was conducted at fertility center in Al-Sader medical city during the period from 1st of March 2016 to 1st of February 2018. A total of 394 subfertile couples undergoing ICSI were enrolled in the current study, after obtaining oral and written informed consent from all patients undergoing ICSI cycles.

Inclusion criteria: age < 38 years old, BMI 19-30 kg/m$^{2.}$, regular menstrual cycle, normal cycle day 2 FSH levels <10 mIU/ml, LH < 10 mIU/ml and estradiol <50 pg/ml.

Exclusion criteria: polycystic ovary syndrome (PCOS), antral follicle count (AFC) ≤ 4, history of poor response in previous treatment cycle, history of repeated ICSI failure (≥ 3 failed cycles), azoospermia and confirmed endometriosis.

All women were asked to be seen at cycle day 2 (CD2) to evaluate their fitness for ICSI program, the history was taken from the patients included name, age, menstrual history, type of subfertility whether primary or secondary, duration and causes of subfertility, history of previous IVF and its outcome, family history, drug history. Examination had been done to each patient including body weight and height.

The Body mass index (BMI) measured as individual body weight in Kg divided by square of her height in meters. Trans-vaginal ultrasound (TVUS) scan was performed to all patients using real-time ultrasound device (Philips 11, E, Netherland) by using vaginal probe (5-7 MHZ). The baseline scan done to measure the endometrial thickness(mm) , number of antral follicles (AFC), check the uterus and ovaries for any pathologies like fibroid, polyp, or ovarian cysts.

All women sent for hormonal assay include FSH, LH, estradiol, Thyroid stimulating hormone (TSH) and prolactin. Also routine screening blood tests of both partners for human immune deficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV). Before treatment, all women partners sent for seminal fluid analysis.

Ovarian stimulation: The 394 patients were divided into two groups according to the stimulation protocol they received. Group I (number 204) short GnRH agonist protocol (flare-up protocol) and group II (number 190) GnRH antagonist protocol group.

In group I short agonist protocol: Triptorelin (decapeptyl, Ferring, Germany) 0.1mg daily subcutaneous injections were started from cycle day 2 till the day of hCG injection, and on cycle day 3 the gonadotropins were administered in the form of recombinant FSH (rFSH) (Gonal F, Serono, Switzerland), and/or HMG (Menogon, Ferring, Germany) in a dose of 150- 225 IU/day depending on age, BMI and history of patient (previous response).

In group II antagonist protocol: On CD2 gonadotropin was initiated, the starting dose varied according to patient's age and history. Flexible antagonist protocol was used in this study, when the dominant follicle reached 14mm in

diameter, cetrorelix (Cetrotide, Serono, Switzerland) 0.25mg subcutaneous daily injection was administered till and including the day of hCG.

Patients were monitored by trans-vaginal ultrasonography repeated done at stimulation day 6 then every other day according to the response, the gonadotropins dose adjusted on the base of follicle graph. Patients also monitored by serum estradiol levels. Ovulation was triggered when at least 3 follicles ≥ 17mm in diameter were observed by trans-vaginal ultrasound by using 10,000 IU hCG (pregnyl, Organon, Netherlands) or GnRH-agonist 0.2 mg subcutaneous.

Cycle cancellation (no ovum pick up) was considered when less than 3 follicles were found during monitoring and high responders (>20 follicles and/or E2 levels >4000iu/l in our study) discussed with the patients for either continue or cancel their cycle to prevent OHSS.

Ova pick up (OPU): Oocyte collection was performed 34 to 36 hours after hCG injection; the procedure of oocyte retrieval was performed under general anesthesia. Under ultrasound guidance, aspiration of follicles done by using thin needle (REPRO Line, Germany) the follicular fluid was aspirated by gentle suction. The aspirated fluid then passed immediately on to laboratory where it examined under a microscopy by embryologist in order to identify the oocytes and then the collected oocytes were transferred into culture medium in incubator. After that denudation process was performed by phenol red, the oocytes were scored by embryologist in the inverted microscope and their maturation stages were noted.

Meanwhile sperm preparation was done; semen specimen was obtained after 3-5 days of the sexual abstinence in labeled standard sterile disposable plastic container at the day of pickup. Semen analysis was assessed according to World Health Organization criteria before and after sperm preparation. Sperm preparation involves washing from seminal plasma, leukocytes and bacteria, this method can remove prostaglandins that cause uterine contraction.

ICSI:

Intracytoplasmic sperm injection was carried out on all morphologically intact oocytes that have extruded the first polar body (metaphase II). In Intracytoplasmic sperm injection (ICSI) procedure, a single motile spermatozoon is selected and immobilized by pressing its tail between micro needle and the bottom of the dish. The sperm cell is then aspirated tail- first into the injection pipette, a mature oocyte is fixed by holding pipette with the first polar body at the 6 o'clock position. The plan of oocyte at 3 o'clock position, the injection pipette is introduce at 3 o'clock and rupture of the oolemma is ascertained by slight suction. The sperm cell is delivered into the oocyte with minimal volume of medium and the pipette can be withdrawn carefully, the procedure carried out in a plastic microinjection dish containing 10-µl droplets of (ferticult TM-HEPES, Belgium) buffered medium covered with mineral oil. After injection procedure, oocytes rinsed and cultured in micro-droplets covered with lightweight paraffin oil. They are incubated at 37º C in an atmosphere of 5% O2, 5% CO2 and PH of 7.2-7.4.

Next is fertilization and embryo cleavage, the oocytes were examined for fertilization next day after ICSI. Oocytes are considered to be normally fertilized when two polar bodies are presented together with two clearly visible pronuclei (two PN).

In embryo culture (Gain medium, fertipro, Belgium), the embryos are examined for cleavage 48 hours after ICSI and cleaved embryos are assessed for quality according to equality of size of the blastomeres and proportion of anucleated fragments. Four categories are distinguished within this scoring system.

Grade 1: cells are of equal size; no fragmentation seen.
Grade 2: cells are of equal size; minor fragmentation only (1-20%). Grade 3: cells are of unequal size; no fragmentation to moderate fragmentation (21-50%).
Grade 4: cells are of unequal size; fragmentation is moderate to heavy (over 50%) [63].

Cleavage rate (CR): is defined as the ratio between the number of embryo to the number of diploid zygotes [64].

Embryo transfer ET: Embryo transfer was performed at 2nd or 3rd day after OPU depending on individual circumstances. Up to three selected embryos were transferred by using trans-cervical catheter (Cook catheter Ob/Gyn, USA) to the uterus under abdominal ultrasound guidance.

In both groups, the luteal phase was supported with progesterone in the form of vaginal suppository cyclogest (cyclogest, Actaves) 400mg twice a day and oral duphastone 20mg/day (duphastone by Abbott) until pregnancy test.

Pregnancy test by serum β-HCG performed 14 days after embryo transfer and ultrasound was performed 3 weeks after positive hCG for the presence of gestational sac(s) and fetal heart (clinical pregnancy was diagnosed by presence of gestational sac with positive fetal heart). All pregnant women followed up to 12 weeks.

Statistical analysis: Data were entered and analyzed using the statistical package for social sciences (SPSS) version 24. Descriptive statistics of variables expressed as mean, standard deviation, frequencies (No.) and simple percentages (%). Chi square test was used to assess the significance of differences in frequencies of qualitative variables, Fisher"s exact test statistical test was used as an alternative when chi squared was inapplicable (in any table, if more than 20% of the cells have expected value < 5, chi square couldn't be applied), Student's t test was used to compare quantitative variables mean between both protocols groups . Level of significance of ≤ 0.05, considered as significant difference. Finally results and findings presented in tables and figure using the MS word software, 2010.

RESULTS

A total of 394 subfertile women enrolled in the study, from them 204 women treated with agonist short protocol namely "Agonist group" compared with 190 women treated with the antagonist protocol namely "Antagonist group". From the 394 total number 9 women (4 from agonist and 5 from antagonist group) lost from the study for different causes and 10 women the ICSI cycle was cancelled for poor or hyper-response, (Table 3.1). No statistically differences between the two groups were found regarding cycle cancellation. In agonist group a total of 6 cycles were

cancelled (2 due to poor folliculogenesis and 4 to hyper-response). While in antagonist group 4 cycles were cancelled due to poor folliculogenesis.

The remaining 375 women (194 from agonist and 181 from antagonist group) continue their cycles and complete the study. Table 2 demonstrates the baseline characteristics of the studied groups, there were not statistically significant differences had been reported between both groups with respect to women's age, body mass index (BMI), causes, type and duration of subfertility and the number of previous attempts, (P> 0.05)

Regarding baseline hormonal levels of the studied groups, the mean FSH, LH and E2 levels were statistically not significant between both groups, (P>0.05), Table 3

A comparison of ovarian stimulation outcomes between the two groups is summarized in Table 4. The mean number of gonadotropins ampules was significantly higher in agonist group (28) compared to antagonist[21], ampules, (P= 0.001). The mean duration of stimulation days in agonist group was 10.13 days which was significantly longer than that in antagonist group which was 9.41 days, (P= 0.001). The mean E2 level on day of hCG was 2452.84 ± 1628.34 in agonist and 2261.19 ± 927.18 in antagonist group, the difference did not reach the statistical significance (P>0.05). Endometrial thickness on day of hCG and mean number of follicles ≥ 17mm in diameter were also insignificantly different between both groups, (P>0.05).

Regarding the OHSS it was reported in 33 patients (17.1%) in agonist group and in 17 patients (9.4%) in antagonist group. The difference was statistically significant. (P=0.043). All were mild and moderate types of OHSS, there were no severe OHSS. in our study (Table 5).

The comparison of embryological data of the studied groups are shown in (Table 6), there were no statistically significant differences had been found between both groups in the mean number of oocyte retrieved, MII oocyte, 2PN oocyte, total number of embryos and the number of embryos transferred, (P>0.05). Except the mean number of good quality embryos, it was significantly higher in agonist than antagonist group, (3.32 ± 1.12) vs. (3.02 ± 1.20) respectively, (P=0.005).

***Values are mean ± standard deviation unless mentioned:** However, fertilization rate was significantly different between both groups; in agonist group it was 69.6% versus antagonist group it was 64.4%, (P=0.008). Conversely the Cleavage rate was 53.2% in agonist group which was insignificantly different than the 51.1% in antagonist group, (P=0.23), (Table 7)

Regarding the pregnancy outcomes of the protocols (Table 3.8) , from 194 OPU cycles 183 did embryo transfer in agonist group and 165 from 181 cycles in antagonist group (because no embryo for transfer or freeze all the embryos). Higher proportion of women in agonist group had positive B-hCG, 94/194 (48.5%) which was significantly higher than that in antagonist group , 67/181, (37%), (P=0.033).

The chemical pregnancy/cycle was insignificantly different between agonist and antagonist group, 6.7% vs. 8.3%, respectively, (P=0.70). On the other hand, clinical pregnancy rate/cycle was higher (41.8%) in agonist group than that of antagonist group (28.7%) and the difference was statistically significant, (P=0.012). Moreover, the rate of clinical pregnancy per ET was significantly higher in agonist group (44.3%) than that in antagonist group (31.5%), (P=0.020). Regarding the multiple gestation 18 (22.2%) women in agonist group and 10 (19.2%) women in antagonist group had multiple gestation, however, the difference was statistically insignificant, (P>0.05). Unfortunately, 15 (18.5%) women in agonist and 17 (32.7%) in antagonist group had first trimester abortions, with no statistically significant difference between both groups, (P>0.05).

Table 1: Causes of cancelling the cycles according to the type of protocol.

Cause of cancelling	**Agonist**	**Antagonist**	**Total (n = 10)**	*Statistics*
Hypo-response	2	4	6	sher"s exact test = 0.076
Hyper-response	4	0	4	P. value = 0.14

Table 2: Baseline characteristics of the studied group

Variable		**Agonist group (n=194)**	**Antagonist group (n=181)**	**P. value**
Age (mean ± SD*)		27.9 ± 5.44	27.1 ± 4.36	0.12
BMI		24.93 ± 2.49	24.61 ± 2.06	0.18
Cause of Subfertility	Male n (%)	82 (42.3)	85 (48.6)	0.65
	Female n (%)	53 (27.3)	43 (24.6)	
	Unexplained n (%)	42 (21.6)	35 (20.0)	
	Mixed n (%)	17 (8.8)	12 (6.9)	
Type of Subfertility	Primary n (%)	161 (83.0)	149 (82.3)	0.97
	Secondary n (%)	33 (17.0)	32 (17.7)	
Duration of Subfertility (mean ± SD*)		7.7 ± 3.9	7.9 ± 4.0	0.62
No. of attempts	None n (%)	89 (45.9)	93 (51.4)	0.37
	One n (%)	91 (46.9)	72(39.8)	
	Two n (%)	14 (7.2)	16 (8.8)	

Table 3: Comparison of baseline hormonal levels of the studied group.

Variable	Agonist group (n = 194)	Antagonist group (n = 181)	P. value
FSH	5.34 ± 2.06	5.15 ± 1.70	0.34
LH	3.88 ± 2.10	4.21 ± 2.31	0.15
E2	37.51 ± 16.79	37.86 ± 15.21	0.83

Table 4: Ovarian stimulation outcome comparison of among study group.

Variable	Agonist group (n = 194)	Antagonist group(n = 181)	P. value
	mean ± SD	mean ± SD	
Number of gonadotropins ampules	28 ± 11	21±6	< 0.001
Duration of stimulation days	10.13 ±1.67	9.41±1.73	< 0.001
E2 levels on day of hCG	2452.84±1628.34	2261.19±927.18	0.17
Endometrial thickness on day of hCG	9.05 ± 2.26	9.31±1.72	0.22
No. of mature follicles ≥ 17mm	10.48 ± 4.66	10.03±3.19	0.28

Table 5: OHSS distribution in the studied groups.

	Agonist group (n = 194)	Antagonist group (n = 181)	P-value
OHSS	33 (17.1%)	17(9.4%)	0.043
No OHSS	161 (82.9%)	164(90.6%)	

Table 6. Embryological data of the studied groups

Parameter *	Agonist group (n = 194)	Antagonist group(n = 181)	*P* Value
No. of oocyte retrieved	9.20 ± 4.79	8.70 ± 2.98	0.23
No. MII oocyte	7.49 ± 4.16	7.33 ± 3.04	0.67
No. 2PN oocyte	4.98 ± 3.13	4.72 ± 1.67	0.32
No. of embryos	3.81 ± 1.59	3.64 ± 1.05	0.46
No. of good quality embryo	3.32 ± 1.12	3.02 ± 1.20	0.005
No. of transferred Embryos	2.72 ± 0.92	2.65 ± 0.64	0.67

Table 7: Comparison of Fertilization and cleavage rates among the studied groups

Parameter	**Agonist group**		**Antagonist group**		***P* Value**
	Mean	SD	Mean	SD	
Fertilization rate	69.6%	21.4%	64.4%	14.2%	**0.008**
Cleavage rate	53.2%	18.3%	51.1%	13.4%	0.23

Table 8: The main pregnancy outcomes of the studied groups

Pregnancy outcomes	**Agonist group (n = 194) * No. (%)**	**agonist group = 181) * No. (%)**	**P. value**
Positive β-hCG / cycle	94 (48.5)	67 (37.0)	**0.033**
Chemical pregnancy/cycle	13 (6.7)	15 (8.3)	0.70
Clinical pregnancy/ cycle	81/194 (41.8)	52/181 (28.7)	**0.012**
Clinical pregnancy/ ET	81/183 (44.3)	52/165 (31.5)	**0.020**
Multiple gestation	18 (22.2)[$]	10 (19.2)[$]	0.85
Clinical first trimester abortion	15 (18.5)[$]	17 (32.7)[$]	0.10
[$] Percentages calculated from the total number of clinical pregnancy in each Group			

DISCUSSION

There is a debate regarding the best stimulation protocol which should be the first choice in normal responder women. It has been over 19 years since GnRH antagonists were first applied in clinical practice in 1999. Since there several randomized controlled trials (RCTs) have been designed to compare the efficacy of the GnRH-antagonist with that of GnRH agonist protocol continues even today, but these studies often show conflicting results.

Results of the current study mainly focused on the effectiveness and safety of GnRH agonist short protocol and GnRH antagonist protocol in COH in normo-responder patients undergoing ICSI cycle.

Considering cycle cancelation the current study did not observe any significant differences among the two protocols. (Mohammed , et al. 2015 and Schoolcraft , et al. 2008) [65, 66] also found no differences in the cycle cancellation rates among short agonist and antagonist protocols.

In the present study, compared ovarian stimulation outcome between the agonist and antagonist protocols. The duration of stimulation days were higher in agonist group, therefore, requiring larger number of gonadotropins. The number of injections in total that the patient takes is also increased. These advantages make the antagonist protocol more patients friendly. (P > 0.05) which was significant.

These findings are consistent with that reported by Arruda, et al in 2013[67] in a retrospective study found that the total gonadotropin doses and stimulation days were significantly higher in agonist short protocol and Moez, et al in 2014[68] also found significant longer duration of stimulation in agonist flare-up group. (Olivennes, et al. 2000 and Tehraninejad, et al.2011)[69, 70] results also consistent with our findings but they used long agonist

protocol. Conversely, the study conducted by Murber, et al in 2009 [71] found the mean
days for stimulation in antagonist and agonist groups was not significantly different, another study by Gordts, et al. in 2012[72] concluded that no differences in duration of the stimulation although a higher doses of gonadotrophins was needed in the antagonist group.

Our study did not find a difference in term of the number of mature follicles (10.48 vs. 10.03, P-value=0.28). This finding confirmed by the study reported by Xavier , et al in 2005 [73]. Nevertheless, Minaretzis , et al in1998 [74] in his study reported the number of matures follicles was higher with antagonist protocol. While Arruad, et al in 2013 study[67] showed significant higher number of day hCG follicles in agonist than antagonist group.

Regarding endometrial thickness on day of hCG, in the present study, there is no significant differences in both groups, this finding supported by studies conducted by (Haouzi, et al. 2010 and Kuc, et al 2011)[75,76]. But disagreed with (Tehraninejad et al 2011 and Prasad et al.2016)[70,77] showed statistically higher in agonist group than antagonist, the difference may be they used agonist long protocol vs. antagonist protocol not flare-up protocol.

The difference in E2 level on day of hCG was not significant in both groups in this study which disagree with study performed by Mohammed , et al in 2015[65] that showed significant increase E2 level on day of hCG in agonist group than antagonist group, while Blockeel, et al in 2011[78] showed higher E2 level on day of hCG with antagonist than agonist group. This may attributed to the criteria of patients in the current study, as they were normo-responders, while other researches in the previous studies was general patients.

Ovarian hyperstimulation syndrome, a serious life threatening complication is an exaggeration of normal ovarian physiology. Its intensity related to the degree of ovarian response to ovulation induction therapy. The results of the present study regarding OHSS statistically significant differences between agonist and antagonist protocols, it is higher in agonist group than antagonist group (P-value= 0.043). All were mild and moderate types of OHSS, there was no sever OHSS. The most likely explanation because of rapid suppression of gonadotrophin in antagonist protocol (i.e. short cycle length and less gonadotropins used) and the use of agonist as a trigger for the final maturation. The findings of the current study is similar to the studies conducted by (Prasad, et al. 2016, Ludwing and et al. 2000, Al- Inany , et al. 2011)[77,79,80]. Meanwhile, the current study disagreed with study designed by Punder , et al in 2012[81] that showed no differences in the overall OHSS prevention between the two protocols, the difference was limited to sever OHSS.

The embryological data of both groups (agonist and antagonist) in the current study, there was no difference had been found in both groups regarding the mean No. of oocyte retrieved, MII oocyte, 2PN oocytes, total number of embryos and No. of the embryo transferred (P value < 0.05) though higher the number of oocytes retrieved in GnRH agonist group was not significant. Except the mean No. of good quality embryos was significantly higher in agonist than antagonist group, (P-value=0.005).

(Arruda, et al.2013, Gordts , et al. 2012, Minaretzis , et al. 1998, Bodri, et al. 2006 and Rahman , et al 2017)[67, 2,74,82,83] found a similar mean number of oocytes retrieved between the two protocols in their studies. While studies conducted by (Al-Inany , et al. 2002, Olivennes , et al. 2000, Albano , et al. 2000) [34,69 84] showed significantly lower oocyte retrieved with antagonist than agonist group, the heterogeneity between findings of the different studies are attributed to the different types of protocol they used (short agonist, long agonist, fixed antagonist, flexible antagonist).

Regarding to mean No. of MII and 2PN oocytes, previous study conducted by Lainas, et al in2008[85] had similar findings to the current study. Conversely, the findings of the current study disagreed with study conducted by Olivennenes , et al in 2000[69] that showed MII oocytes were seen to be greater in the agonist than antagonist group.

Regarding the total No. of embryos, this study was in agreement with Arruda ,et al in2013[67] that found same number of embryos in both protocols, but disagree with Mohammed ,et al in2015[65] that stated total No. of embryos were higher in agonist than antagonist group and they attributed this result to the increased number of oocytes retrieved.

(Moez, et al.2014, Prasad, et al. 2016 and Lainas, et al.2008)[68,77,85] showed no statistically differences in the No. of embryo transferred between agonist and antagonist groups that goes with our current study. But Mohammed. et al in 2015 [65] findings disagree with results of the present study as he illustrated that No. of embryo transferred was significantly higher in agonist than antagonist group.

Regarding the mean No. of good quality embryo the results of the current study are similar to findings conducted by Malmusi , et al in 2005[86] that showed the mean No. of good quality embryo was higher in agonist than antagonist group. Conversely, current result disagree with Lainas, et al in 2008[85] study who showed no differences in No. of good quality embryo between both groups. Also, disagreed with Minaretzis, et al in 1998 [74] and Yarali , et al in 2008 [87] that showed No. of good quality embryo is higher in antagonist than agonist group. The discrepancy between these studies may be related to various designs of studies (randomized controlled trial, retrospective, case-control) and type of patient response (poor responders) while in present study included normoresponder patients in prospective clinical trial.

Our study, compared the fertilization and cleavage rates between the short agonist and antagonist groups, the fertilization rate was significantly higher in agonist group than the antagonist one (P value = 0.008).

Malsumi et al in 2005[86] also demonstrated the fertilization rate was significantly higher in agonist group than antagonist group, but another study reported by (Moez et al. 2014 and Lainas, et al. 2008)[68,85] showed no statistically differences in fertilization rate between both groups. However, the researchers in the previous studies used poor responders while used normo- responders in the current study may be the causes of this discrepancy. Unlike the fertilization rate, the cleavage rate was not significantly different between the two groups, (P-value=0.23). Lia, et al in 2013[88] found similar result although he used long agonist

protocol.

The important outcome of ICSI is the pregnancy results. Chemical pregnancy rate per cycle was not different between agonist and antagonist groups in this study (P-value=0.70), similar finding with Arruda, et al in 2013[67]. Conversely, disagreed by study performed by Bahceci, et al in 2009[89] that reported the rate of chemical pregnancy /cycle was higher in antagonist than agonist group.

Rate of clinical pregnancy per cycle and per ET was higher in agonist group than antagonist group, (P-value=0.012, P-value 0.020 respectively, was significant) the current study goes with study conducted by(Moez et al. 2014 and Mohammed, et al. 2005)[68,90], conversely the current study disagreed with study performed by (Arruda et al. 2013, Gordts et al 2012, Bodri et al 2006 and Bahceci et al. 2009)[67,72,82,89] that showed the clinical pregnancy/cycle was similar in both groups. About multiple pregnancy, there was statistically not significant between the two groups (P>0.05).

In the current study, observed that the first trimester abortion was slightly higher in antagonist than agonist group but statistically not significant (P>0.05). This finding consistent with (Arruda, et al. 2013 and Bahceci, et al[67,89].

GnRH-antagonist molecules are potent inhibitors of cell cycle because they decrease the synthesis of locally produced growth factors. They can exhibit this activity in all tissues presenting GnRH receptors like ovary, uterus, and endometrium and consequently influence blastomere formation and endometrial development (Moez et al in 2014)[68].The GnRH antagonist decreases the production of E2 by the granulosa cells; hence, may be insufficient to develop an ideal endometrium to maintain the life of the embryos(olivennes ,et al in 2002)[91]. These effects may explain the decreased pregnancy rates and slight increased abortion rate in antagonist group.

CONCLUSIONS

Shorter duration of stimulation days, fewer numbers of gonadotropin injections and lower incidence of OHSS, these advantages make the GnRH-antagonist therefore seems to be more patient friendly protocol in ART cycle with lower incidence of side effects and time saving.
However, a significant higher fertilization rate, good quality embryos and clinical pregnancy rate in the agonist short protocol tips the balance in favor of the short protocol

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