Agonist hold Antagonist Protocol versus Antagonist Protocol in Intracytoplasmic Sperm Injection for Infertile Patients with Polycystic Ovary Syndrome

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Abstract
Background: Polycystic Ovary Syndrome (PCOS) is currently thought to emerge from a complex interaction of genetic and environmental traits.Polycystic Ovary Syndrome is the most common endocrinopathy among reproductive-aged women
Objectives: The aim of this study was to compare between agonist hold antagonist protocol and antagonist protocol in ICSI for Infertile patients with PCOs as regard the number of oocytes retrieved, fertilization rate, implantation rate and clinical pregnancy rate.
Patients and methods: This was a prospective comparative study, which was conducted at Assisted Reproduction Unit at Qena University Hospital.This work had been conducted on infertile women with PCOS undergoing ICSI in ART unit in obstetrics and gynecology department, Qena University hospital from January 2019 to June 2020.
Results: The number of implanted embryos was significantly higher in agonist hold antagonist protocol group compared to antagonist protocol group ($p<0.001$).Also, clinical pregnancy was found to be significantly higher in agonist hold antagonist protocol group compared to antagonist protocol group ($p=0.009$). On the other hand, prevalence of OHSS was significantly higher in antagonist protocol group compared to agonist hold antagonist protocol group ($p=0.023$).There was no statistically significant difference between the two groups regarding β-HCG ($p>0.05$).
Conclusion: GnRH agonis thold antagonist may be a preferred protocol for PCOS patients treated with ICSI in view of the reduction of the risk of OHSS, the shorter stimulation time and better pregnancy outcome.
Keywords: **Agonist hold Antagonist; Antagonist; ICSI; PCOS**

Introduction

Polycystic Ovary Syndrome (PCOS) is the most common endocrinopathy in reproductive-aged women in the United States, affecting around 7% of women. Although the specific cause of PCOS is unknown, it is assumed to be caused by a complex interplay of hereditary and environmental factors. Changes in luteinizing hormone (LH) action, insulin resistance, and a probable propensity to hyperandrogenism have all been related to the pathophysiology of PCOS **(Dafopoulos et al., 2009).**

The importance of ovarian stimulation in the success of in vitro fertilization and embryo transfer (IVF-ET) treatment has long been recognised. As a result, since the 1980s, a gonadotropin releasing hormone (GnRH) agonist protocol has been created and used in the context of IVF-ET treatment. By desensitising pituitary receptors, the GnRH agonist regimen is aimed to restrict the release of pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) **(Huirne et al., 2007).**

The introduction of a GnRH antagonist regimen, which blocks pituitary receptors, has recently provided another option for ovarian stimulation. The use of a GnRH antagonist strategy has been shown to minimize the length of the ovulatory stimulu sand the occurrence of ovarian hyperstimulation syndrome. The shorter time of analogue medication, the shorter duration of FSH stimulation, and the lesser chance of developing ovarian hyperstimulation syndrome (OHSS) are all advantages of antagonists **(Al-Inany et al. ,2016).**

Because the GnRH antagonist protocol is straightforward, convenient, and flexible, and because it does not cause functional ovarian cysts or "menopausal" symptoms like the agonist protocol, many doctors and patients like it. However, results from randomised clinical trials show that the antagonist protocol retrieves fewer oocytes and has lower pregnancy rates than the agonist long treatment (**Kim CH et al., 2011).**

The goal of this study was to assess the number of oocytes recovered, fertilization rate, implantation rate, and clinical pregnancy rate in PCOS patients undergoing ICSI cycles using agonist hold antagonist strategy against a standard antagonist approach.

Patients and methods

This was a prospective comparative study, which were conducted at Assisted Reproduction Unit of Obstetrics and Gynaecology Department, Qena University Hospital, This work had been conducted on infertile women with PCOS undergoing ICSI from January 2019 to June 2020.The women allocated into two groups: Group (A) where patients received agonist hold antagonist protocol. Group (B) where patients received antagonist protocol only.

Inclusion criteria: All PCOS patients with primary or secondary infertility undergoing ICSI in Qena University Hospital, assisted reproduction unit.

Exclusion criteria: age >35years, BMI>35 Kg/M2, patients with endometriotic ovarian cysts, patients with uterine abnormality, patients with abnormal endocrine function(DM, thyroid disorder, adrenal abnormality),chronic renal disease and chronic liver disease.

Study tools:

I-History taking: Including the duration of infertility, previous ovarian surgery as cystectomy and drilling and the male factor

II- Examination: General, abdominal and vaginal examination.

III- Investigations:

Laboratory investigations: Routine laboratory investigations, FSH,LHon day three (basal), AMH, prolactin, Estradiol (E2),serum Progesterone, thyroid Stimulating Hormone (TSH) and serum total Testosterone.

Transvaginal 3D ultrasound evaluation: (GE-Voluson P8 BT18)**:** Evaluate the uterus and the adnexae.

Patients participated, and fulfilling the inclusion criteria and after signing the informed consent form, were categorized in two groups. Group A (cases) received agonist hold antagonist protocol where as group B(controls) received antagonist protocol.

Since the primary endpoint was the number of oocytes retrieved and their quality, sample size calculation performed in order to detect a difference in the mean number of oocytes retrieved and their quality between the two groups. In group A (cases) draining of the pituitary from its hormones mainly LH using GnRH agonist; 1 mg leuprolide acetate (Lucrin, SANDOZ)was conducted 5 days before the end of COCS (Mid-luteal) and stopped with the end of COCS, after withdrawal bleeding occurred, ovarian stimulation was started at day 3 with a starting dose of 150 IU of recombinant FSH (Gonal pen,MERCK SERONO) and 75 IU of HMG(Menopure, FERRING). On day 6 of the stimulation an ultrasonic evaluation of the number and size of ovarian follicles was performed and the dose of recombinant FSH adjusted according to the individual reaction of the patient. Suppression of endogenous LH was performed by using 0.25 mg GnRH antagonist; cetrorelix acetate (Cetrotide, MERCK SERONO) when 2 or 3 follicles reached the size of 14 mm or more. In group B (controls) ovarian stimulation started on day 3 with the same doses and the same way as group A. Further ultrasonic and hormonal analysis performed every two days. Triggering of final oocyte maturation was performed as soon as three follicles of > = 18 mm diameter by injecting a 0.2 mg GnRH agonist triptorelin-acetate (Decapeptyl 0.1, FERRING). Thirty-six hours after triggering oocyte retrieval performed by transvaginal ultrasound guided follicle aspiration. Oocytes collected and embryos cultured in 30% protein enriched media (global total).In all patients' embryos were cryopreserved at stage of blastocyst by vitrification.

Preparation of the endometrium for embryo transfer: After 2 cycles of ovum pick up, patients with frozen embryos started preparation for transfer through the endometrial priming protocol (Hormonalreplacement therapy),received combined oral hormonal contraception (Gynera tablet, Schering ,0.03 mg Ethinyl estradiol and 0.075 mg

Gestodene) once daily starting from day 3 of the cycle then 0.1 mg GnRH agonist triptorelin-acetate (Decapeptyl 0.1, FERRING) was conducted 5 days before the end of COCS (Mid-luteal) and continued till the day of progesterone administration, after withdrawal bleeding, estrogen administration started on day 3 of the cycle in the form of daily estradiol valerate 6 mg orally (Progynova, Bayer Pharma AG-Germany) and daily progesterone 100 mg (Prontogest,IPSA) intramuscular daily starting when the endometrial thickness reached 8 mm and both continued till serum β HCG level was assessed on day 14 after ET and considered positive if >5 IU/L. At the end of the treatment cycle we recorded in each patient the number of oocytes retrieved, their quality, fertilized oocytes and cryopreserved embryos.

IV- Follow up: The number of oocytes retrieved their quality, fertilization rate and embryo and Serum β-HCG concentration 14 days after embryo transfer.

Statistical analysis: SPSS 24.0 for Windows was used to gather, tabulate, and statistically analyse all of the data (SPSS Inc., Chicago, IL, USA). The Shapiro Walk test was used to determine if the data had a normal distribution. Frequencies and relative percentages were used to depict qualitative data. The difference between qualitative variables was calculated using the Chi-square test and Fisher exact as specified. For parametric data, mean SD (standard deviation) was used, and for non-parametric data, median and range were used. The difference between quantitative variables in two groups was calculated using the independent t test and the Mann Whitney test for parametric and non-parametric variables, respectively. All statistical comparisons were done with a two-tailed significance level. P-values less than 0.05 indicate a significant difference, p0.001 indicates a highly significant difference, andP>0.05 shows no difference.

Results

The age in agonist hold antagonist protocol group ranged from 20 to 34 years with mean ±SD was 28.9± 4.05 years while in antagonist protocol group the age ranged from 20 to 34 years with mean ±SD was 29.1± 3.73 years with no statistical significant difference (p=0.923) between the two groups. Likewise, there was no statistically significant difference between the two groups regarding age groups (p=0.962).**Table 1.**

There was no statistical significant difference between the two groups regarding FSH, LH, AMH (P>0.05). Likewise, there was no statistically significant difference between the two groups regarding basal E2, prolactin and testosterone (p>0.05). **Table 2.**

M2 Oocyte was significantly higher in agonist hold antagonist protocol group compared to antagonist protocol group (p= 0.023). Likewise, Fertilization rate was significantly higher in agonist hold antagonist protocol group compared to antagonist protocol group (p= 0.001).There was no statistically significant difference between the two groups regarding antral follicle count, duration of stimulation and endometrial thickness (p>0.05). Also, there was no statistically significant difference between the two groups regarding number of mature follicles (p>0.05). **Table 3.**

The number of good quality embryos was significantly higher in agonist hold antagonist protocol group compared to antagonist protocol group (p<0.001).Transferred embryos were significantly higher in agonist hold antagonist protocol group compared to antagonist protocol group (p=0.01). There was no statistically significant difference between the two groups regarding injected Oocyte (p>0.05). **Table 4.**

The number of implanted embryos was significantly higher in agonist hold antagonist protocol group compared to antagonist protocol group (p<0.001). Also, clinical pregnancy was found to be significantly higher in agonist hold antagonist protocol group compared to antagonist protocol group (p=0.009). on the other hand, prevalence of OHSS was significantly higher in antagonist protocol group compared to agonist hold antagonist protocol group (p=0.023). There was no statistically significant difference between the two groups regarding β-HCG (p>0.05). **Table 5.**

Table 1. Comparison between the two groups regarding the age

Variables		Mixed protocol group (No. = 50)		Conventional protocol group (No. = 50)		P-value
		No.	%	No.	%	
Age (years)	Mean± SD	28.9± 4.05		29.1± 3.73		0.999
	Median	29.5		29.0		
	Range	20.0 – 34.0		20.0 – 34.0		
Age groups	20- <25 years	7	14.0 %	7	14.0%	0.910
	25- <30 years	18	36.0 %	20	40.0%	
	30- 35 years	25	50.0 %	23	46.0%	

Table 2. Comparison between the two groups regarding the hormonal parameters

Variables		Mixed protocol group (No. = 50)		Conventional protocol group (No. = 50)		P-value
		No.	%	No.	%	
FSH (mIU/ml)	Mean± SD	3.3± 2.5		4.8± 1.4		0.753
	Median	3.5		4.5		
	Range	3.0 – 4.5		3.0 - 5.0		
LH (mIU/ml)	Mean± SD	7.8± 3.4		6.9± 2.1		0.125
	Median	8.0		7.0		
	Range	8.0 – 10.0		6.0 - 9.0		
AMH (ng/dl)	Mean± SD	6.7± 0.3		5.9± 0.4		0.414
	Median	6.5		6.0		
	Range	4.0 – 10.0		3.0 - 9.0		
Basal E2 (Pg/ml)	Mean± SD	47.1± 14.5		46.7± 18.7		0.056
	Median	47.0		46.5		
	Range	30.0 – 49.0		38.0 - 50.0		
Prolactin (ng/ml)	Mean± SD	13.5± 6.5		12.9± 5.9		0.823
	Median	13.5		13		
	Range	5.0 – 15.0		6.0 - 14.0		
Testosteron e (ng/ml)	Mean± SD	0.2± 0.1		0.1± 0.3		0.09
	Median	0.2		0.1		1
	Range	0.0 – 1.0		0.0 - 2.0		

Table 3. Comparison between the two groups regarding ICSI characteristics

Variables		Mixed protocol group (No. = 50)	Conventional protocol group (No. = 50)	P-value
Antral follicle count	Mean± SD	21.23± 4.94	22.47± 5.76	0.413
	Median	20.0	22.0	
	Range	14.0 – 35.0	14.0 - 35.0	
Duration of stimulation	Mean± SD	11.97± 2.30	11.48± 1.70	0.137
	Median	12.0	11.0	
	Range	7.0 - 16.0	9.0 - 14.0	
Endometrial thickness (mm)	Mean± SD	0.7± 0.09	0.8± 0.07	0.557
	Median	0.7	0.8	
	Range	0.1 - 1.0	0.1 - 1.0	
Number of mature follicles	Mean± SD	21.23± 4.94	22.47± 5.76	0.413
	Median	20.0	22.0	
	Range	14.0 – 35.0	14.0 – 35.0	
M2 oocyte	Mean± SD	5.30± 1.75	4.47± 2.83	**0.023**
	Median	4.0	3.5	
	Range	1.0 - 9.0	0.0 - 10.0	
Fertilization rate	Mean± SD	10.70± 3.85	7.40± 3.65	**0.001**
	Median	10.0	7.0	
	Range	4.0 – 20.0	2.0 – 15.0	

Table 4. Comparison between the two groups regarding ICSI process

Variables		Mixed protocol group (No. = 50)	Conventional protocol group (No. = 50)	P-value
Injected Oocyte	Mean± SD	12.90± 4.85	11.53± 5.47	0.254
	Median	12.0	12.0	
	Range	4.0 – 25.0	3.0 – 24.0	
Rate of good quality embryos	Mean± SD	8.50± 3.09	4.17± 2.28	<0.001
	Median	8.0	3.5	
	Range	3.0 – 15.0	1.0 - 10.0	
Transferred embryo	Mean± SD	2.93± 0.25	2.63± 0.56	0.01
	Median	3.0	3.0	
	Range	2.0 – 3.0	1.0 - 3.0	

Table (5): Comparison between the two groups regardingβ-HCG, embryos implanted, clinical pregnancy and cancellation

		Mixed protocol group (No. = 50)		Conventional protocol group (No. = 50)		P-value
		No.	%	No.	%	
β-HCG	Positive	26	52.0%	20	40.0 %	0.065
	Negative	24	48.0%	30	60.0 %	
Embryos implanted	1	15	30.0%	40	80.0 %	<0.001
	2	30	60.0%	8	16.0 %	
	3	5	10.0%	2	4.0%	
Clinical pregnancy	Negative	20	40.0%	33	66.0 %	0.009
	Positive (cardiac pulsation)	30	60.0%	17	34.0 %	
OHSS	No	48	96.0%	45	90.0 %	0.023
	Yes	2	4.0%	5	10.0 %	

Discussion

Regarding the demographic characteristics of the studied groups, we found that the age in agonist hold antagonist protocol group ranged from 20 to 34 years with mean ±SD was 28.9± 4.05 years while in antagonist protocol group the age ranged from 20 to 34 years with mean ±SD was 29.1± 3.73 years with no statistically significant difference (p=0.923) between the two groups.

As well we found that the duration of infertility in agonist hold antagonist protocol group ranged from 3 to 8 years with mean ±SD was 6.23± 1.89 while in antagonist protocol group the it ranged from 3 to 9 years with mean ±SD was 6.37± 1.63 years with no statistically significant difference (p=0.753) between the two groups.

According to **Lai et al., 2013**, the age ranged from 24 to 43 years old, with an average age of 33.2 4.0 years. The lowest body mass index (BMI) was only 16 kg/M2while the highest was 28.7 kg/M2(average 20.9 2.1). Infertility lasted an average of 6years.

A cross-sectional study by **Behery et al.(2020)** found that group 1 had a mean age of 29. 4.2 years and a mean BMI of 27.2 2.05 kg/m2, while group 2 had a mean age of 28.5 4.3 years and a mean BMI of 26.9 2.06 kg/m2.

This study results showed that there was no statistically significant difference between the two groups regarding FSH, LH, AMH (P>0.05). Likewise, there was no statistically significant difference between the two groups regarding basal E2, prolactin and total testosterone (p>0.05).

In line with our findings, a study by **Behery et al.(2020**) found no statistically significant differences in hormonal markers between the tested groups.

According to our findings, a study by **Singh et al.(2014**) found no statistically significant differences in hormonal markers across the tested groups.

When an ICSI features were compared between the two groups, it was discovered that M2 oocyte was substantially greater in the agonist hold antagonist protocol group than in the antagonist

protocol group (p= 0.023). Similarly, the fertilization rate in the agonist hold antagonist protocol group was considerably greater than in the antagonist protocol group (p= 0.001). In terms of antral follicle count, stimulation time, and endometrial thickness, there was no statistically significant difference between the two groups (p>0.05). In addition, there was no statistically significant difference in the number of mature follicles between the two groups (p>0.05).

The mean rFSH timeframe (days) of the GnRh agonist and antagonist groups were 8.99 1.58 and 7.94 1.47 respectively, the mean rFSH dosage (amp) of the GnRh agonist and antagonist groups were 31.94 12.04 and 26.48 11.54 respectively, and the mean Endometrial thickness (mm) of the GnRh agonist and antagonist groups were 10.86 2.27 and 10.40 2.29 respectively, according to the study by Lai et al. They also discovered that the fertilization rate (2PN) (percent) for the GnRh agonist and antagonist groups was 53.9 and 53.7, respectively.

Behery et al., 2020 found that the mean length of stimulation was 11.3 0.8 and 10.3 1.1 days, and the fertilization rate (percent) was 51.1 and 49.9% for the GnRH agonist and antagonist groups, respectively. They also discovered that while there was a statistically significant difference in the length of stimulation across the examined groups, there was no statistically significant variation in the Fertilization rate.

Singh et al., 2014 found that for the agonist and antagonist groups, the mean number of days of stimulation was 9.71.7 and 9.51.8, the mean number of follicles on the day of HCG was 16.58.2 and 14.76.5, and the Fertilization rate was 77.918.2 and 78.516.9, respectively. They found no statistically significant difference between the groups when it came to the duration of stimulation, but they backed up our findings by finding no statistically significant difference between the groups when it came to the number of follicles on the day of HCG and the rate of fertilization.

Regarding the difference of OHSS among the studied groups, we found that there was statistically significant difference between groups regarding OHSS.

According to our findings, Behery et al., 2020 found significant differences between group 1 and group 2 in terms of mild, moderate, and severe OHSS (15 percent, 6 percent, and 1 percent vs. 4.5 percent, 2.5 percent, and 0.55 with p =.04) in mild, moderate, and severe OHSS (15 percent, 6 percent, and 1 percent vs. 4.5 percent, 2.5 percent, and 0.55 with p =.04).

Also, according to **Ashrafi et al., 2005**, there was a statistically significant difference between groups in terms of OHSS, however the antagonist group had 7 cases (30 percent) at risk of developing OHSS whereas the agonist group had none.

In contrast to **Ashrafi et al., 2005**'s findings, **Singh et al.(2014**) found no statistically significant difference between groups in terms of OHSS, however there were two patients (2%) at risk of developing OHSS in the agonist group and none in the antagonist group.

In terms of pregnancy outcomes, we discovered that there was a statistically significant difference between groups in terms of positive HCG/cycle, Embryos implanted, and clinical pregnancy among the analyzed groups.

According to study by **Lai et al.,2013** the GnRH antagonist strategy may be more effective in increasing pregnancy outcomes in patients who have had recurrent IVF-ET treatment failures.

Behery et al.(2020), on the other hand, found no significant difference between the two groups in terms of pregnancy result (37 percent vs. 32 percent, p =.125).

In addition, according to **Singh et al., 2014**, there was no statistically significant difference between the studied groups in terms of clinical pregnancy.

Toftager et al.(2016) reported that when spontaneously conceived pregnancies were eliminated from both groups, the short GnRH antagonist group had slightly higher pregnancy rates, though not statistically significant.

Out et al.(2004) and **Bodri et al.(2006**), both using meta-analyses, found no significant difference in birth outcomes between these two protocols.

Conclusion

GnRH agonist hold antagonist may be a favored protocol for PCOS cases where ICSI is used to treat these patients in order to reduce the risk of OHSS, the shorter stimulation time and better pregnancy outcome.

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