

## Conference Paper

# Comparative Study between Long Protocol with Antagonist Protocol on IVF Cycle

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## Abstract

**Objective.** Ovarian stimulation is one of important step in In Vitro Fertilization (IVF) program to produce numbers of good oocyte to achieve better pregnancy rate. Various methods of ovarian stimulation have been tried mostly now are long (agonist) and antagonist protocol. The aim of this study is to compare the efficacy and safety of both protocol. **Methods.** A retrospective comparative analytical study of all subfertile patients who underwent IVF program from January to December 2011 in Halim Fertility Centre that meets the inclusions and exclusions criteria was done. **Results.** Number of stimulation days in antagonist groups ( $9.3 \pm 1.34$  days) was similar with agonist group ( $9.8 \pm 1.57$  days). The total dosage of gonadotropin used in antagonist group ( $1279 \pm 805.65$ ) was significantly lower than agonist group ( $2196.3 \pm 931.99$ ). Pregnancy rates between both group was similar (34% in antagonist group vs 36% in agonist group). There was no severe Ovarian Hyperstimulation Syndrome (OHSS) case in antagonist group and there were 5 cases of severe OHSS found in agonist group. There was a significant higher severe OHSS cases in agonist group. **Conclusion.** Ovarian stimulation with agonist and antagonist protocol have similar efficacy in IVF outcome, but antagonist protocol appears to be safer than agonist protocol in term of incidence of severe OHSS.

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**Keywords:** protocols, controlled ovarian hyperstimulation, invitro fertilization

## 1. Introduction

Infertility is generally defined as one year of regular unprotected intercourse without conception [1]. Infertility in couples who wish to have children becomes a serious problem for themselves and their families, especially in Indonesia with eastern culture, that having offspring is something that is very important to continue the clan and descendant. Not infrequently they both be divorced and split up not being able to obtain offspring and even look for a new partner.

The incidence of infertility in Indonesia ranges from 10-15% of all couples of reproductive age [2]. Diverse causes of infertility in women include ovarian factors, peritoneal factors, tubal factors, uterine factors, cervical factors, and sperm abnormalities for male factor. Some of these cause couples not able to obtain offspring by natural means after efforts ranging from drugs, insemination, even surgery. Even several causes such as tubal damage, grade 3-4 endometriosis, and severe sperm abnormalities, can not be solved by surgery or drugs. Very fortunate that assisted reproductive

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technology is now present in the world of infertility treatment. Since the first successful IVF program with the birth of Louis Brown with the help of professor Robert Edward and professor Patrick Steptoe, has given hope for couples who unable to get child by the natural way [3].

At first IVF program done with natural cycles by monitoring the timing of ovulation, but this is very inconvenient because ovulation could happen anytime, even in the midnight. Furthermore, various protocols of ovarian stimulation have been developed to produce follicles and then pick up and process in the invitro fertilization laboratory [4].

GnRH agonist protocol or so called the long protocol with down regulation technique was the standar protocol in ovarian stimulation technique, by the use of GnRH agonist to supress pituitary to not produce FSH, and let the exogenous FSH and LH that stimulate and trigger ovulation on the cycle [5]. This long protocol is aimed to prevent premature luteinisation, and ovulation can be timed so that clinician could schedule the time of oocyte retrieval. But this long protocol takes a quite long time and the number of injections is pretty much. The agonist protocol or so called the short protocol offers less duration of injection, lower FSH dose, lower OHSS risk, with the same successful rate [6,7].

Various methods are used to simplify the IVF program, one of which is to modify the ovarian stimulation protocol. Short protocol with ovarian stimulation, and to control the premature luteinisation and ovulation by the GnRH agonist offers various facilities or simplicities, such as shorter time, fewer number of injections, and lower risk of OHSS. In this study we wanted to assess whether there were differences between the GnRH antagonist and GnRH agonist cycle in terms of effectiveness and side effects.

## 2. Materials and Methods

This is a retrospective comparative analytical study. The study conducted in Halim Fertility Centre IVF clinic, collaborate with FER Division, Obstetrics and Gynecology Departement of H. Adam Malik General Hospital, Medan, from January to December 2011.

Samples were taken from all patients that underwent IVF program that fulfilled inclusions criteria and failed exclusions criteria in one year, which is all infertile patients that underwent IVF program in Halim Fertility Center that meets the inclusions and exclusions criteria within one year, from January to December 2011.

### **The inclusions criteria of this study were:**

1. 20-37 years old women.
2. Not showing menopausal symptoms and irregular menstrual disorders symptoms.
3. No history of oophorectomy or cycstectomy.
4. Both ovaries were seen on transvaginal ultrasound.

5. Willing to participate.
6. BMI below 30.
7. No history of previous IVF failure.
8. No systemic disease, severe endometriosis, or uterine abnormalities.

**The exclusions criteria of this study were:**

1. Congenital abnormalities of the uterus or uterine fibroid was found.
2. Ovarian cyst was found.
3. Allergy to gonadotropin and GnRH.

## 2.1. Sample Size Calculation

Sample size that used in this retrospective study is based on the period of 1 year. The formula for sample size:

$$N1 = n2 = \frac{(Z\alpha + Z\beta)^2 \pi}{(P1 - P2)} \quad (1)$$

$$N1 = n2 = \frac{(1.64 + 0.84)^2 0.05}{0.1} = 30.8 \quad (2)$$

Minimal number of sample in the study were 31 for each group.

## 2.2. Study Variable

The independent variable of this study are: age, duration of infertility, kind of infertility, causes of infertility, BMI, agonist and antagonist protocol. The dependent variable includes: the number of days of stimulation, total dose of stimulation, the number of days of GnRH usage, the number of follicles with more than 14 mm diameter, the number of follicles with more than 17 mm diameter, the number of subject that achieved ovum picked up (OPU), the number of subject that achieved embryo transfer (ET), the number of oocytes retrieved, number of oocytes, the number of 2PN embryos, the number of embryos with transferable quality, the number of subject that cryopreserved, the number of frozen embryos, the number of subject with severe OHSS, and the number of clinical pregnancies.

Data collected are processed and analyzed using SPSS 16 (Statistic Package for Social Science) software. Uni variat data to see the patient characteristics. The differences of stimulation outcomes and IVF-ICSI outcomes data are analyzed with Student t test and Mann Whithney U test, and categorical data by Chi square test.

### 3. Methods

Data were taken from medical records of all patients underwent IVF program, either using long protocol with GnRH agonist or short protocol with GnRH antagonist, in HFC IVF clinic, FER Division of Departement of Obstetrics and Gynecology Faculty of Medicine University of Sumatera Utara H. Adam Malik General Hospital Medan, as follows:

1. GnRH agonist protocol.
2. Oral contraceptive pills consumption start on day 1 (D1) untill day 21 (D21) of menstrual period.
3. 0.5 ml of Buserelin injection start on day 21 for about 14 days.
4. On the day 14 of suprefact injection, estradiol level was check and transvaginal ultrasound was perform to evaluate the endometrial thickness and the follicles. If the estradiol level was below 50 pg/ml then suprefact injection will perform for the next 7 days untill the estradiol level reach below 50 pg/ml, endometrial thickness below 6 mm, and follicles below 8 mm.
5. After that continued with rFSH injection with the dose of 225 IU (for patients below 37 years old) and 300 IU (for patients above 37 years old) in conjunction with 0.2 ml suprefact injection.
6. rFSH injection was perform for 7 days, and ultrasound was perform on day 8 before injection to evaluate the size of follicles, whether it is already reach the diameter of 17 mm, and the number of follicles that already reach the diameter of 17 mm is already more than 3 or not. If not, then the rFSH injection dose will be continued and increased in accordance with the follicular development.
7. USG examination will performed everyday start on day 8 of rFSH injection to evaluate the development of the follicles. Usually USG examination will performed untill day 10 or 11.
8. After we found at least 3 follicles that has reach the diameter of 17 mm, then 10.000 IU hCG injection will be given on the day that have been decided to finalize the maturation of the oocyte.
9. Ovum pick up (OPU) will performed in 36-40 hours after hCG injection.
10. On the same day of oocyte retrieval, husband's sperm also collected, at which time the patients have to be in 3-5 days of abstinencia condition.
11. For the luteal phase support, patients also recieve 50 mg progesterone injection on the night after OPU.
12. Embryo transfer (ET) was perform 3 days after OPU, which 2-3 embryos will transferred into the patients womb.

13. On day 19, beta hCG and progesterone level will be checked, and if the beta hCG level was above 7 mIU/ml then the patients is stated as pregnant.
14. GnRH antagonist protocol.
15. Patients were recommend to come on day 2 of menstrual period, to perform baseline ultrasound examination to rule out the presence of follicular cyst or pregnancy.
16. If the criteria was fulfill, then it is continued by rFSH injection with the dose of 225 IU (for patients below 37 years old) and 300 IU (for patients above 37 years old) in conjunction with 0.2 ml superfact injection.
17. Gonal F or puregon injection were perform for 4 days, and on day 6 of menstrual period, ultrasound examination were perform before injection to evaluate the diameter of the follicle whether it has reach 14 mm or not, if it has reach above 14 mm diameter then GnRH antagonist was given in conjunction with FSH, until there was at least 3 follicles that has reach above 17 mm diameter. If not, then rFSH injection dose will continued and increased in accordance with the follicular development.
18. USG examination will performed everyday start on day 8 of rFSH injection to evaluate the development of the follicles. Usually USG examination will performed untill day 10 or 11.
19. After we found at least 3 follicles that has reach the diameter of 17 mm, then 10.000 IU hCG injection will be given on the day that have been decided to finalize the maturation of the oocyte. This injection is usually performed at night, at around 8-10 pm.
20. Ovum pick up (OPU) will performed in 36-40 hours after hCG injection.
21. On the same day of oocyte retrieval, husband's sperm also collected, at which time the patients have to be in 3-5 days of abstinencia condition. And on the same day, ICSI also will performed.
22. For the luteal phase support, patients also recieve 50 mg progesterone injection on the night after OPU (Do and D1).
23. Embryo transfer (ET) was perform 3 days after OPU, which 2-3 embryos will transferred into the patients womb.
24. On day 19, beta hCG and progesterone level will be checked, and if the beta hCG level was above 7 mIU/ml then the patients is stated as pregnant.

## 4. Results

Data in this study were taken retrospectively from the medical records of all patients that underwent IVF program in HFC clinic, FER Division, Obstetric and Gynecology of

	GnRH antagonist group	GnRH agonist group	P
Number of patients (n)	50	50	
Age (years)	34.06 ± 3.54	33.26 ± 3.05	0.33
BMI (kg/m <sup>2</sup> )	23.53 ± 3.12	24.68 ± 3.05	0.68
Primary Infertility (n)	43	41	0.28
Secondary Infertility (n)	7	9	0.28
Length of Infertility (years)	6.38 ± 1.8	6.34 ± 1.8	0.913
Base FSH level (U/L)	7.13 ± 2.02	7.04 ± 4.32	0.89
Tubal Factor (n)	10 (20%)	11 (22%)	0.48
Ovarian Factor (n)	5 (10%)	6 (12%)	0.61
Endometriosis (n)	7 (14%)	6 (12%)	0.54
Male Factor (n)	20 (40%)	17 (34%)	0.41
Uterine Factor (n)	3 (6%)	4 (8%)	0.59
Unexplained Infertility (n)	5 (10%)	6 (12%)	0.57

TABLE 1: Clinical characteristic of both group.

Faculty of Medicine H. Adam Malik General Hospital, 50 cases of each group either GnRH agonist and GnRH antagonist protocol.

In this study we can see the data about demographic characteristic in both group were not significantly different in terms of age and BMI. Mean age were 34 and 33 years old in the antagonist and in the agonist group, respectively. Mean BMI were  $23.53 \pm 3.12$  and  $24.68 \pm 3.05$  in the antagonist and agonist group, respectively.

In terms of cases characteristic, based on primary and secondary infertility, we found 43 and 41 cases of primary infertility in antagonist and agonist group, respectively; 7 and 9 cases of secondary infertility in antagonist and agonist group, respectively. Both group were not significantly different. In terms of length of infertility, we found the average of  $6.38 \pm 1.8$  and  $6.34 \pm 1.8$  years in antagonist and agonist group, respectively. Both group were not significantly different. Based on FSH basal level, we found the average of  $7.13 \pm 2.02$  and  $7.04 \pm 4.32$  U/L in antagonist and agonist group. Both group were not significantly different.

Based on causes of infertility, we found 10 cases of tubal factor, 5 cases of ovarian factor, 7 cases of endometriosis, 20 cases of male factor, 3 cases of uterine factors, and 5 cases of unexplained infertility in antagonist group, whereas in the agonist group we found 11 cases of tubal factor, 6 cases of ovarian factor, 6 cases of endometriosis, 17 cases of male factor, 4 cases of uterine factor, and 6 cases of unexplained infertility. Both group were not significantly different in terms of the causes of infertility. Based on demographic and clinical characteristic investigation, as seen on Table 1, there were no significant different found between both group, so both groups are considered homogenous.

From this study we found that the average of total number of stimulation days in antagonist group was 9.3, and 9.8 in agonist group. Although it looks a little more time in the second group, but not significantly different statistically. The total dose of

	GnRH antagonist group	GnRH agonist group	P
Length of stimulation (days)	9.3 ± 1.34	9.78 ± 1.57	0.104
Total dose of rFSH used (IU)	1279 ± 805.65	2196.3 ± 931.99	0.000
The number of follicles with diameter of more than 17 mm (n)	4.28 ± 2.56	5.8 ± 4.7	0.050
The number of follicles with diameter of more than 14 mm (n)	3.34 ± 2.62	3.84 ± 3.07	0.380

TABLE 2: Stimulation outcome data.

rFSH used was  $1279 \pm 805.65$  and  $2196.3 \pm 931.99$  IU in antagonist and agonist group, respectively. We found an increase in the use of rFSH that were significantly more in the agonist group compare to the antagonist group. Table 2 shows the outcome of stimulation.

The same results obtained in the North American Ganirelix [8] study group, where they found that the antagonist group use less rFSH compared to the agonist group significantly.

This implies that we can save costs in the antagonist group by the use of fewer rFSH, considering the price is quite expensive in IVF program.

In terms of stimulation results, we found the average number of follicles with a diameter of more than 17 mm as much as 4 and 5 in the antagonist and agonist group, respectively. Although a little more in the second group, but it was not significantly different statistically. And also we found the average number of follicles with a diameter of more than 14 mm as much as 3 and almost 4 in the antagonist and agonist group, respectively. But it was not significantly different statistically. While Ho et al found that the number of follicles with a diameter of more than 10 mm was significantly more in the antagonist group compared to the agonist group.

Based on the number of stimulated follicles, either with the diameter of more than 14 mm and 17 mm, although it looks a little more in the second group, but it was not significantly different statistically. So stimulation result in both group were considered equally good.

In terms of stimulation outcome either using agonist and antagonist protocol, we found that all cases in this study reached the step of ovum pick up and also embryo transfer. This is may be because all group of sample included in the study were in the reproductive age and tend to have adequate ovarian reserve.

The average number of oocytes that has been obtained was 11 and 12 in the antagonist and agonist group, respectively. Although it looks a little more in the second group, but it was not significantly different statistically. While the number of metaphase II oocytes which is mean that the oocyte has been ready to fertilize was almost 9 and 9 in the antagonist group and agonist group, respectively. There were no significant difference between both group.

Fertilization result also showing no significant difference between both group, whereas 7 embryos in average successfully fertilized in the antagonist group and reached the 2 PN zygotes, while in the agonist group also have 7 embryos in average.

	GnRH antagonist group (n)	GnRH agonist group (n)	P
Number of patients reached the step of OPU	50	50	1
Number of patients reached the step of ET	50	50	1
Number of oocytes	11.16 ± 6.63	12.48 ± 9.69	0.428
Number of M II oocytes	8.76 ± 4.82	9.3 ± 6.29	0.631
Number of fertilization	7.38 ± 4.89	7.22 ± 4.32	0.863
Number of good quality embryos	6.66 ± 3.94	6.62 ± 4.35	0.962
Number of patients with frozen embryos	27	28	0.841
Number of severe OHSS cases	0	4 (8%)	0.017
Clinical pregnancy rates	17 (34%)	18 (36%)	0.832

TABLE 3: Outcome data of OPU-IVF-ICSI.

And next, on day three of embryos cleavage, the quality of the embryos were evaluate, and we found that the average number of embryos that are at the stage of quality that can be transferred is 6, either in the antagonist and agonist group. Both groups were statistically equal. Number of oocytes, good quality oocytes, 2 PN zygotes, and number of embryos that are at the stage of quality that can be transferred were similar in the antagonist and agonist group.

As a result of excess embryos after embryos transferred, we still have some embryos to be frozen in some cases. In the antagonist group there were 27 cases that having the opportunity to freeze the embryos, and 28 cases in the agonist group. There were no significant difference between both group. This means that both group have the same opportunity to utilize the extra embryos, in case of failure of the first IVF program or if the patient want to be pregnant again in the future, without having to undergo the injection process anymore, and also cost-effective medicines.

In terms of clinically succesful pregnancy rates, the number of pregnant patients was 34% and 36% in the antagonist and agonist group, respectively. Although it looks a little more in the second group, but it was not significantly different statistically. Barmat et al [9] have the same result, that there were no significant difference in terms of pregnancy rates in both protocols, either antagonist and agonist. Details of the data can be seen on Table 3.

In terms of complication rates of severe OHSS, we did not encounter any case in the antagonist group, while we found 4 cases of severe OHSS in the agonist group. There were significant difference between both group. Ludwig et al [10] also found increased incidence of moderate to severe OHSS in agonist group compared to antagonist group, but not significantly different statistically. And also in the group using cetrorelix, they found decreased incidence of severe OHSS, but not in the group using ganirelix.



With the acquisition of all of the above data, we can assess that using antagonist protocol have some advantages, which is relatively fewer number of injection compared to agonist protocol that having at least 14 extra days of GnRH agonist injection, and patient discomfort caused by the large number of injections.

Based on the fewer number of rFSH used in antagonist protocol compared to agonist protocol, clearly we can save cost considering the rFSH price is quite expensive. So we can say that the antagonist protocol was cheaper compared to agonist protocol.

By using the antagonist protocol with this lower cost, we obtained same number of follicles, same number of oocytes, same number of embryos, and have the same opportunities to freeze the extra embryos, and also no different in pregnancy rates statistically, compared to the agonist protocol.

In terms of severe OHSS rates (some of the complications of OHSS can lead to death such as thromboembolism, stroke, pleural effusion, ascites, and shock), we found significant difference between antagonist and agonist group, whereas we did not encounter any severe OHSS case in the antagonist group. So we considered that the antagonist group is safer compared to the agonist group.

## 5. Conclusion and Suggestion

### 5.1. Conclusion

1. There were no significant difference in terms of the length of stimulation days between antagonist and agonist group.
2. There were significant different in terms of the total dose of rFSH used, whereas the agonist protocol need higher dose compared to the antagonist protocol.
3. There were no significant difference in terms of the stimulation results (either the number of follicles with diameter of more than 14 mm and more than 17 mm) between antagonist and agonist group.
4. There were no significant different in terms of the number of oocytes and also the number of metaphase II oocytes that successfully obtained between antagonist and agonist group.
5. There were no significant different in terms of the number of 2 PN zygotes as a result of fertilization between antagonist and agonist group.
6. There were no significant different in terms of the number of embryos that are at the stage of quality that can be transferred between antagonist and agonist group.
7. There were no significant different in terms of the number of cases that having extra embryos to freeze between antagonist and agonist group.
8. There were significant different in terms of the number of severe OHSS incidence as a complication of ovarian stimulation between antagonist and agonist protocol,

whereas we did not encounter any severe OHSS case in the antagonist group, but we found 4 cases of severe OHSS in the agonist group.

## 5.2. Suggestions

Antagonist protocol can be recommended to replace the conventional agonist protocol, because the same effectiveness by offering convenience with which fewer number of injections needed, fewer number of days of injections, more comfortable for the patient, less expensive, and the incidence of OHSS as complications was smaller compared to the agonist protocol.

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