

# The Efficacy Of Mild Stimulation Protocol In In Vitro Fertilization In Haiphong Hospital Of Obstetrics And Gynecology From 1/2016 To 6/2017

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## Abstract

**Objectives:** To compare the efficacy of mild stimulation protocol versus short Antagonist protocol in in vitro fertilization in Haiphong Hospital of Obstetrics and Gynecology from 1/2016 to 6/2017.

**Subjects and method:** A randomized controlled clinical trial. A total of 100 patients in expected normal responders undergoing IVF/ICSI were divided into two groups: mild group (n=50) - receiving clomiphene citrate (50 - 100 mg/day for 5 days from the cycle day 2) with low doses of FSH daily (75-225IU/day from cycle day 7) and GnRH antagonist; Antagonist group (n=50).

**Results:** the duration of stimulation ( $7.98 \pm 1.42$  days vs  $8.56 \pm 1.43$  days) and the total dose of FSH ( $1551 \pm 399.14$  IU vs  $2683 \pm 590.45$  IU) were significantly less in the Mild group than in the Antagonist group ( $p < 0.01$ ). The number of retrieved oocytes ( $11.34 \pm 6$  oocytes vs  $11.72 \pm 6.31$  oocytes), fertilized oocytes (66.5% vs 73%) and clinical pregnancy rate (50 vs 42.6%) were similar in two groups ( $p > 0.05$ ).

**Conclusions:** Mild ovarian stimulation seems to constitute an equally effective method as compared to the Antagonist protocol in expected normal responders undergoing IVF/ICSI.

**Keywords:** Assisted reproductive techniques; clomiphene citrate; mild stimulation; Antagonist protocol.

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## 1. INTRODUCTION

Infertility affects not only every family but also the society. The birth of in vitro fertilization brings hope for infertile couples.

Why is it necessary to stimulate the ovaries at all in IVF programs? Recall that in 1977, after 102 unsuccessful attempts to obtain and transfer embryos P. Steptow and R. Edwards managed to achieve the first developing pregnancy. Louise Brown was born in 1978.

IVF was carried out in a natural cycle, but, undoubtedly, only the use of ovarian stimulation and the ability to control the induced cycle provided clinical effectiveness, and hence the appropriateness of the method.

The effectiveness of the IVF method for one treatment attempt averages 30-35% of pregnancies, has a pronounced variability, depending on the woman's age and the state of her ovarian reserve. This is quite satisfactory, bearing in mind that natural human fertility does not exceed 20% the onset of pregnancies in one menstrual cycle and drastically decreases with the age of the woman.

Physiological norm of the female reproductive system also applies to IVF programs. Everything happens within the framework of human physiology, and the principles of natural selection work both with the onset of natural pregnancy and with programs ECO. Moreover, the IVF method in its classical execution has reached its peak of effectiveness, and the ongoing statements about an increase in the frequency of pregnancy to sky-high heights — 50-60% per attempt are not credible. Indeed, if we conduct a program with 5 young promising women, then pregnancy may occur in 3 of them, but in the next 5, not so young and promising, pregnancy will not come at all. This is how the average treatment efficiency is formed, which is 30%, and these are real numbers.

Stimulation of the ovaries allows you to get a sufficient number of oocytes (8-15), choose the best of them, fertilize and get 4-6 blastocysts, transfer 1-2 blastocysts, remaining preserved. Such is classic IVF program, in which the cumulative pregnancy rate, taking into account cryo-transfers, can indeed reach 60% per patient.

There is practically no risk of complications, namely ovarian hyperstimulation syndrome (OHSS). Then does it make sense to modify something?

Minimal stimulation, modified, soft protocols. To whom, when and for what?

Stimulation protocols are considered standard, not without reason, in which 8-15 oocytes are obtained, 4-6 blastocysts, transfer and preserve the excess. It's classic IVF, providing the best results as in treatment cycle, and the cumulative frequency of pregnancies. Nevertheless, there is a large group of specialists who advocate "soft" stimulation schemes. It got to the point that an organization of adherents was created "Soft" stimulation - ISMAAR [1]. Its supporters believe that "mild" stimulation can reduce the risk development of OHSS, hormonal load, to ensure the physiological course of the induced cycle, to reduce the cost of treatment while maintaining its effectiveness [1,9-10]. Many experts do not share this opinion, they believe that obtaining a small number of oocytes reduces the frequency the onset of pregnancy both in the treatment cycle and cumulative, since there are no embryos left for conservation, as a result of which the number of IVF attempts increases, which only increases the cost of treatment.

Ferraretti et al. [1] used the scheme of stimulation of clomiphene citrate + small doses of gonadotropins: pregnancy rate (PNB) for embryo transfer was 40.4%, cryopreservation - 22.6%, CNB for cryo transfer - 27.3%, cumulative CNB -43.5%. Great results! But here's the data of N. Gleicher [11] showed that when using the same protocol in comparison with standard stimulation: CNB for transfer embryos accounted for 21.4 and 50%, cryopreservation - 0 and 60%, PNB for cryotransfer - 0 and 28.5%, cumulative CNB - 21.4 and 64.3%. The results are drastically different from those given earlier!

The analysis of indicators of other clinics demonstrate a direct dependence of the pregnancy rate on the number of oocytes. The highest rates were obtained when receiving 8-16 oocytes - 32.2 and 33.4% of pregnancies per treatment cycle, with it was possible to cryopreserve embryos in 40.2 and 61.7% of patients, which undoubtedly provides a high cumulative pregnancy rate. Quite often, "soft" stimulation and the so-called double stimulation are carried out with the aim of "accumulation" of embryos, their cryopreservation and subsequent [8].

The meaning of "double stimulation" (duostim) is stimulation of the ovaries in the follicular phase of the cycle (classically), using as a trigger of ovulation a GnRH, ovarian puncture, a break of 3-4 days, re-stimulation of the ovaries and re-puncture [12]. The purpose of "double" stimulation, as, in part, "soft" of protocols is the accumulation of embryos with reduced ovarian reserve. How much is this tactic effective? Once again, we give an opinion specialist of the POSEIDON group [7] who believe that it is quite effective in young women with reduced ovarian reserve indices, but ineffective in older patients due to the high incidence of aneuploidies.

So, what, then, to do with older patients if they insist on achieving pregnancy with your own oocyte? Probably, use all possible modifications, warning about the low effectiveness of treatment. "Soft" modified protocols are used in young patients with multifollicular ovaries, when obtaining 3-4 oocytes is able to ensure pregnancy due to the high quality of embryos, at the same time, the risk of developing OHSS is low, less cost of treatment [13].

Ovarian stimulation is one basic stage and playing an important role in IVF. Today, with the goal of personalization of treatment and towards the cycle of ovarian stimulation that is similar to nature, mild stimulation of the ovary is gradually becoming a trend in clinical practice of IVF. However, the effectiveness of this regimen is controversial.

That's why I conducted this research to assess the effectiveness of mild stimulation regimen by comparing this regimen with Antagonist regimen in patients who were in-vitro inseminated at Hai Phong Obstetrics Hospital since 1/2016 to 6/2017.

## 2. METHODS

This is a prospective, controlled clinical trials including 100 patients expected normal responders undergoing IVF/ICSI in Haiphong Hospital of Obstetrics and Gynecology from 1/2016 to 6/2017.

Normal responders were defined as  $\leq 39$  years old, AMH level of  $> 1.1$  ng/ml, AFC  $> 5$ , FSH level of  $< 10$  IU/L and body mass index  $< 29$  kg/m<sup>2</sup>.

Patients in this study were randomized to received one of two treatment protocols: a mild stimulation protocol and an Antagonist stimulation protocol.

- **Patients in group 1 received a mild stimulation protocol (50 patients):** An oral dose of 50 -100 mg/day Clomiphene citrate was taken from day 2 to day 6 of the cycle. By the 7th day of injecting FSH at a dose of 75 - 225UI / day, patients got ultrasound to re-evaluated the development of follicular follicles. If follicular reached 14 mm, starting daily administration of GnRHant at a dose of 0.25 mg / day until there were at least 2 follicles  $\geq 18$  mm. 10000 IU hCG was intramuscularly injected once and oocyte were collected after 36 hours.

- **Patients in group 2 received an Antagonist protocol (50 patients):** Recombinant FSH had taken since day 2 of the cycle with an average dose of 150-300 IU / day depending on the patient. After 6 days of FSH injection, patients got ultrasound to re-evaluated the development of oocyte follicles. If follicular reached 14 mm, starting daily administration of GnRHant at a dose of 0.25 mg / day until there were at least 2 follicles  $\geq 18$  mm. 10000 IU hCG was intramuscularly injected once and oocyte were collected after 36 hours.

### 3. RESULTS

#### 3.1. The uniformity of the two research groups

**Table 1.** The uniformity of the two groups on the clinical characteristics of patients

Characteristics	Group 1	Group 2	P
Age (years)	28.56 $\pm$ 3.66 (20 – 39)	30.12 $\pm$ 4.25 (19 – 39)	> 0.05
Duration of infertility (years)	3.78 $\pm$ 2.62 (1 – 10)	4.24 $\pm$ 2.65 (1 – 11)	
BMI (kg/m <sup>2</sup> )	20.72 $\pm$ 1.85 (17.35 – 24.22)	20.28 $\pm$ 1.92 (16.33 – 24.6)	
FSH (IU/L)	5.79 $\pm$ 1.25 (2.3 – 9.02)	6.15 $\pm$ 1.26 (3.6 – 9.47)	
AMH	6.09 $\pm$ 2.81	5.47 $\pm$ 3.43	

(ng/ml)	(1.34 – 15)	(1.5 – 15)	
AFC	14.58 ± 4.03	14.72 ± 4.13	
(follicles)	(7 – 26)	(8 – 28)	

### 3.2. Comparison of the dose of FSH, the number of days of FSH stimulation, the treatment outcomes, OHSS rates and the results of embryos transferred in this research

**Table 2.** Characteristics of ovarian stimulation of 2 research groups

Characteristics	Group 1	Group 2	P
FSH starting dose	197 ± 48.88	312 ± 35.8	< 0.01
Total FSH doses (IU)	1351 ± 399.14 (525 – 2025)	2683 ± 590.45 (1500 – 4000)	< 0.01

**Table 3.** Comparison of the treatment outcomes

Characteristics	Group 1	Group 2	P
Number of oocytes	11.34 ± 6 (1 – 28)	11.72 ± 6.31 (2 – 32)	> 0.05
Number of MII oocytes obtained	8.34 ± 4.91 (1 – 21)	9.26 ± 5.01 (1 – 24)	> 0.05
Fertilization rate (%)	66.5% ± 20.8%	73% ± 15%	> 0.05
Number of embryos obtained (min – max)	6.82 ± 4.67 (0 – 18)	7.98 ± 5.01 (0 – 24)	> 0.05

**Table 4.** Ovarian hyperstimulation syndrome rates of two groups

Characteristics	Group 1		Group 2		P
	Frequency	Rate %	Frequency	Rate %	
OHSS	4	8%	8	16%	> 0.05

**Table 5.** Results of embryo transferred of two groups

Characteristics	Group 1		Group 2		P
	Frequency	Rate %	Frequency	Rate %	

Clinical pregnancy rate / ET	23/46	50	20/47	42,6	> 0.05
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## 4. DISCUSSION

### 4.1. Ovarian stimulation characteristics of two study groups

#### 4.1.1. Comparison of FSH starting dose of two group (Table 2)

The starting dose of FSH injected at the beginning of the menstrual cycle will raise the level of FSH in the blood above the "max value" causing the recruiting activity of follicle. Subsequent doses only help maintain the development of recruited follicles without increasing the number of follicles [3][4].

In this research, group 1 used much lower FSH starting dose than Antagonist group ( $197 \pm 48.88$  IU vs  $312 \pm 35.8$  IU)  $p < 0.01$ .

#### 4.1.2. Comparison the duration of FSH stimulation in two groups (Table 3.

About FSH stimulation duration of mild group was also shorter than Antagonist group ( $7.98 \pm 1.42$  compared to  $8.56 \pm 1.43$ ) with  $p < 0.05$ . The fact that the pressure on prolonged treatment is one of the factors that makes the patient not comply with the treatment, on the other hand, the injection of ovarian stimulation is an invasive procedure that can cause many side effects and cause uncomfortable for patients. The mild stimulation protocol has achieved the goal of reducing the duration of treatment and the exogenous FSH dose to patients, on the other hand, it is less annoying to take home-based CC medication than to go to ART center to have daily FSH injection.

#### 4.1.3. Comparison of total FSH doses in two groups (Table 2)

The total dose of FSH is a combination of the number of days of ovarian stimulation by FSH, FSH starting dose and dose adjustment during follicular monitoring. The total dose of FSH not only affects the results of ovarian stimulation but also helps assess the cost of a cycle of ovarian stimulation. The higher the total dose of FSH, the greater the cost.

The study results showed that the average total FSH dose used in the mild stimulus group was also lower than that of the Antagonist group with statistical significance  $p < 0.01$  ( $1551 \pm 399.14$  IU versus  $2683 \pm 590.45$  IU ).

Thus, with a reduction in the total dose of FSH, the mild stimulation protocol reduced the economic burden for a cycle of ovarian stimulation. This is one of the strengths of this protocol compared with the currently used ovarian stimulation protocol, especially when the cost of infertility treatment and assisted reproductive techniques in Vietnam is not covered by insurance.

#### **4.2. The number of oocytes obtained from the two protocols (Table 3)**

The mild stimulation protocol used in this study has the same combination of antagonist GnRH as Antagonist protocol, which helped prevent early LH peaks in patients that have ovarian stimulation in IVF. Since, the follicles are developed synchronously, the quality of oocytes obtained is also better.

The number of oocytes obtained after aspiration in the mild stimulating group was  $11.34 \pm 6$ , equivalent to the Antagonist group of  $11.72 \pm 6.31$  with  $p > 0.05$ , in which the rate of mature ovule of the two regimens was same (72.57% and 77.79%)  $p > 0.05$ . Thus, although the number of days of ovarian stimulation by FSH and the total FSH dose was lower, the quantity and quality of oocytes obtained in the mild stimulation group did not differ from that of the Antagonist group.

#### **4.3. Results of embryo culture (Table 5)**

The number of fertilized oocytes and the rate of fertilized oocytes are the combined results of the quality of ovule, sperm and fertilization method. Both research groups used the method of fertilization, oocyte intramuscular injection (ICSI - Intracytoplasmic Sperm Injection), which helps to reduce the impact of the fertilization method to the research results of the two groups. In addition, ICSI is now commonly implemented to produce more embryos, to have excess embryos to store cold and to help increase the incidence of cumulative pregnancy for patients [2].

The average number of fertilized oocytes of the Antagonist group in my study was  $8.7 \pm 5$  higher than the mild stimulus group of  $7.68 \pm 4.69$  and the insemination rate of the mild stimulus group was 66.5%, lower than 73% of the Antagonist group, however, the difference is not statistically significant  $p > 0.05$ .

#### **4.4. Result of embryo transfer (Table 5).**

The mild stimulation group has a clinical pregnancy rate of 50% of the number of embryos, while other studies around the world only fall in the range of 25 - 40% [1] [5]. This rate was also higher than Antagonist group 42.6% but the difference was not statistically significant with  $p > 0.05$ .

### **5. CONCLUSION**

- The mild stimulation protocol is more effective than the Antagonist protocol: The duration of ovarian stimulation with FSH is shorter and the total amount of exogenous FSH is lower
- The mild stimulation protocol is similar to Antagonist protocol: The number of oocytes obtained, number of mature oocytes, average number of embryos, fertilization rate, rate of clinical pregnancy / embryo transfer.

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