Journal of **R**eproduction & **I**nfertility
Volume 24, Issue no. 2
https://doi.org/10.18502/jri.v24i2.12499

Short Communication

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The Effect of Follitropin Alfa in Controlled Ovarian Stimulation Protocol for In Vitro Fertilization Cycles

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Received: Oct. 30, 2022
Accepted: Jan. 22, 2023

Abstract

Background: Follitropin alfa (FA) is one of the most widely used exogenous gonadotropins in both agonist and antagonist protocols for controlled ovarian stimulation (COS) and in vitro fertilization (IVF). However, reports of its effectiveness are limited, particularly in terms of its impact on overall IVF outcomes and ovarian hyperstimulation syndrome (OHSS). Therefore, in this study, FA competency was investigated by evaluating its effect on IVF outcomes and OHSS, administering agonist and antagonist COS protocols.

Methods: A retrospective study with 120 subjects was conducted. Outcomes comprising the number of retrieved and fertilized oocytes, quality of embryos, and clinical pregnancies were assessed. Statistical correlation between FA dose, IVF outcomes, and the incidence of OHSS was also analyzed. All statistical analyses were performed at 95% confidence level.

Results: There was no significant difference in both protocols regarding retrieved oocytes (p=0.604), fertilized oocytes (p=0.761), embryo quality including good, average, poor embryo (p=0.875, p=0.565, p=0.785), and clinical pregnancy (p= 0.844). However, FA doses in the agonist protocol were shown notably higher (p= 0.001). Negative correlations were also observed between FA dose and the number of retrieved oocytes (r=-0.255, p<0.01), fertilized oocytes (r=-0.296, p<0.01), and good quality embryos (r=-0.231, p<0.05).

Conclusion: Our study suggested that FA yields similar outcomes in both COS protocols, but agonist protocols require higher doses of FA and evaluation of its effect on OHSS is an important area of research for further investigation.

Keywords: Agonist, Antagonist, Follitropin alfa, Gonadotropin-releasing hormone (GnRH), In vitro fertilization, OHSS.
To cite this article: Prasetiawati N, Sundari AM, Supriyadi A, Sjarbaini H, Tarigan S, Suardana G, et al. The Effect of Follitropin Alfa in Controlled Ovarian Stimulation Protocol for In Vitro Fertilization Cycles. J Reprod Infertil. 2023;24(2):139-144. https://doi.org/10.18502/jri.v24i2.12499.

Introduction

Controlled ovarian stimulation (COS) is the main factor for the success of in vitro fertilization (IVF) which is intended to stimulate the growth of multiple ovarian follicles to yield abundant oocytes. Exogenous gonadotropins are usually employed to produce higher numbers of oocytes expected to increase embryo transfer success by initiating better folliculogenesis and steroidogenesis (1). Today, there are several available therapeutic gonadotropin products classified according to their source and ingredients, including urinary human menopausal gonadotropin

(hMG) and recombinant follicle-stimulating hormone (rFSH) (2, 3). Treatment by rFSH consists of follitropin alfa (FA), follitropin beta, and follitropin delta with FA as the most effective prescription as it is similar to endogenous FSH; moreover, it is easy-to-use, safe, and has suitable biochemical activity. However, Baart et al. and Friedler et al. highlighted that high doses of exogenous gonadotropin administration during COS decrease oocyte's number, quality, and maturation, and also implantation, clinical pregnancy, and live birth rate (4,5). Besides, it was reported that high gonadotropin doses increase the risk of life-threatening ovarian hyperstimulation syndrome (OHSS), and ultimately the risk of cardiovascular diseases and intellectual disability (6). However, there are limited available reports about FA in similar parameters.

COS protocol in IVF comprises administration of gonadotropin-releasing hormone (GnRH) agonist (GnRH-a) and antagonist (GnRH-ant). The initial reports of the use of long GnRH-a protocol have been prepared in the 1980s with the significant finding that GnRH-a long protocol administration at the luteal phase results in a better pregnancy rate compared to either short or ultra-short protocol administered at the follicular phase as it prevents premature luteinizing hormone (LH) surge by suppressing the endogenous pulsatile secretion of FSH and LH from the pituitary. Antral follicles development stimulated by exogenous gonadotropins subsequently results in enhanced number of retrieved oocytes with similar maturity for IVF treatment (7). Moreover, the GnRH-a protocol reduces embryo transfer failure and improves injection schedules for the patients (7). These reports then suggested the commencement of the wide use of the long GnRH-a protocol (8). However, several reports revealed its serious unpleasant side effects, including headaches, burning sensation, bleeding, and cyst formation (9). Besides, prolonged injection time is known to be responsible for OHSS occurrence, triggering various adverse complications, which are even life-threatening (10).

Reports on GnRH-a's side effects led to the development of a novel protocol, GnRH antagonist (GnRH-ant), that is considered safer and friendlier. In contrast to the previous protocol, it directly suppresses endogenous FSH and LH through the receptors of the pituitary. Studies revealed that GnRH-ant significantly reduced the duration of controlled ovarian stimulation (COS) which improves patients' comfort, prevents acute induction of endogenous gonadotropins, and controls cyst formation and development. Additionally, the absence of hypoestrogenism during antagonist injection dispelled burning sensations (10). According to these findings, the competencies of FA as exogenous gonadotropin in both COS protocols are emerging fields which need further investigation. Although numerous studies have elucidated the effect of various variables on GnRH-a/-ant, there is still a matter of debate and no definite answer regarding the prime protocol for obtaining optimal IVF outcomes. Besides, as one of the most used drugs, FA's effect on OHSS is rarely investigated. Therefore, this study was conducted to examine the competency of FA as an exogenous gonadotropin in GnRH-a/-ant COS protocol. The finding is expected to enrich input for practitioners in deciding which prescription is to be used to yield optimal outcomes in the population of Indonesia.

Methods

Study design and patients' selection: The study was conducted using retrospective analysis with data extracted from patients' medical records who underwent in vitro fertilization/ICSI in Harapan Kita Women and Children Hospital, Jakarta, Indonesia between July 2007 to June 2016 after obtaining the approval of the Ethics Committee of the hospital (No: IRB/33/11/2020). Data for agonist and antagonist protocol were taken in 2007-2013 and 2014-2016, respectively selected by its completeness for all observed parameters. In total, 120 participants were recruited to meet the minimum sample size as described previously (11). Enrolled subjects were then divided into two groups based on the protocol of controlled ovarian stimulation: 1) long protocol of GnRH agonist (n=60) and 2) short protocol of GnRH antagonist (n=60), both with FA as an exogenous gonadotropin. Studied participants were 28-43 years old with clinical criteria, including tubal pathology, male infertility, unexplained infertility, and a combination of these factors.

Studied participants who had polycystic ovary syndrome (PCOS) and endometriosis were excluded because they might have altered oocyte quality leading to data bias. Participants who underwent natural and modified stimulation and did not have complete data were also excluded.

Long GnRH agonist protocol: GnRH-a (Suprefact; Hoesct AG, Germany) at the dose of 0.25 *ml* (250

pg) was injected daily starting from day 21 of the menstrual cycle (luteal phase) continuously for 14 days. After this period, blood tests and transvaginal ultrasound were taken. Stimulation by exogenous gonadotropin, rFSH at a dose of 150-225 *IU*, (Follitropin alfa, Gonal F, Merck Serono, Italy) was subsequently performed if there were follicles <5 *mm* in size and levels of E2 were <20 *nmol/L*. GnRH-a administration was prolonged until day of HCG injection.

Short GnRH antagonist protocol: The medication was initiated by rFSH (FA, Gonal F, Merck Serono, Italy) injection at dose of 150-225 IU on the second day of the menstrual cycle. GnRH-ant (Cetrotide; Asta Medica, Germany) was subsequently injected until HCG injection day when a dominant follicle ≤14 *mm* in size was observed. When at least three ≥18 *mm* follicles were found, hCG at the dose of 10.000 was administered for both protocols. Oocyte retrieval was then carried out 34-36 *hr* later with transvaginal ultrasound.

***In vitro* fertilization:** After oocyte retrieval, cumulus-oocyte complexes (COCs) were briefly washed with MOPS buffer medium supplemented with human serum albumin (GMOPS-plus; Vitrolife, Sweden), then incubated in 0.5 *ml* GIVF medium (Vitrolife, Sweden) under paraffin oil at 37°*C* and 6% CO_2 for 2 *hr*. Shortly, COCs were denudated by 0.1 *IU* hyaluronidase (Hyase; Vitrolife, Sweden) to remove cumulus cells. Metaphase II oocytes with distinct polar bodies were subsequently inseminated by intracytoplasmic sperm injection (ICSI). After ICSI, fertilized oocytes were incubated into 30 *µl* culture media (G1 Plus, Vitrolife, Sweden) under paraffin oil (Ovoil, Vitrolife, Sweden). Within 18-20 *hr* later, zygotes were collectively transferred to 30 *µl* of new culture media (G1 Plus, Vitrolife, Sweden), and each drop consisted of 2-3 embryos for three days. Prior to ICSI, semen was collected, analyzed, and motile and normal sperm was separated using density gradient centrifugation (DGC).

Morphological assessment of embryo: Fertilization was assessed 17±1 *hr* after ICSI by evaluating two pronuclei and polar bodies. Meanwhile, day-3 embryos were assessed at 68±1 *hr* after ICSI and graded as good, average, and poor based on the number of blastomeres, cell fragmentations, and the presence or absence of multinucleate cells according to consensus standard of ALPHA Scientists in Reproductive Medicine (2017) (12).

OHSS assessment: OHSS was assessed according to the category described by Golan and Weissman (2009) as presented in table 1 (13).

Statistical analysis: Data were analyzed using SPSS statistics *vs*. 20 (SPSS Inc., USA). The continuous and categorical variables are presented as mean±standard deviation and percentages, respectively. Mann-Whitney test was performed on continuous variables, and Chi-Square tests were performed for categorical data. In order to determine the relationship between variables, Spearman correlation was utilized at $p<0.05$ significance level.

Table 2. Characteristics of participants in the study

Variables	Short P antagonist n=60	Long P agonist n=60
Age	(35.07±3.62)	(35.73±3.45)
Indication of treatment (%)		
Tubal pathology	18	32
Male factor	42	50
Unexplained	40	18

* values given in mean ± standard deviation or in percentages

Results

A total of 120 participants who underwent either GnRH-a/-ant protocol with FA were investigated and results were summarized in tables 2 and 3. As shown in the tables, studied participants were of similar age and the number of retrieved oocytes, mature oocytes, immature oocytes, fertilized oocytes, and clinical pregnancy in both protocols were not different (p-value was 0.604, 0.126, 0.891, 0.592, and 0.844, respectively). Similarly, no differences were observed in the maturation and fertilization rate (p=0.866 and p=0.761, respectively). Inconclusive results were also noted in terms of embryo quality. Statistical significance

Table 1. Classification of OHSS

No	Severity	Symptoms
1	Mild	Abdominal distension/discomfort, nausea, vomiting
2	Moderate	Ultrasonic evidence of ascites
3	Severe	Clinical evidence of ascites, oliguria, thrombosis, pleural effusion

Table 3. IVF outcomes of agonist and antagonist protocol with follitropin alfa

Variables	Short-P antagonist (n=60)	Long-P agonist (n=60)	p-value
Retrieved oocytes	556 (9.23±6.92)	549 (9.15±5.06)	0.604
Immature oocytes	69 (1.15±1.67)	82 (1.37±1.41)	0.126
Mature oocytes	487 (8.12±5.91)	467(7.78±4.47)	0.891
Fertilized oocytes	312 (5.20±3.85)	277 (4.62±3.09)	0.592
Maturation rate (%)	88	85	0.866
Fertilization rate (%)	64	59	0.761
Embryos			
Good	139 (2.32±2.26)	107 (2.32±2.01)	0.875
Average	82 (1.37±1.33)	79 (1.32±1.43)	0.565
Poor	91 (1.52±1.94)	90 (1.50±1.88)	0.785
Clinical pregnancy	19 (0.32±0.47)	18 (0.30±0.46)	0.844
OHSS			
Mild (%)	3	-	-
Severe (%)	-	8	-
Follitropin alfa dose (ampoule)	33.42±8.83	41.82±12.42	0.001

* values given in total number (mean ± standard deviation) or in percentages.
* p-value was analyzed by Mann-Whitney analysis

Table 4. The correlation between follitropin alfa dose and IVF outcome

Variables	Retrieved oocytes	Fertilized oocytes	Embryo's quality			Clinical pregnancy
			Good	Average	Poor	
Follitropin alfa dose	r= -0.255 (p<0.01)	r= -0.296 (p<0.01)	r= -0.231 (p<0.05)	r= -0.188 (p=0.647)	r= -0.126 (p=0.750)	r= -0.069 (p=0.684)

* p-value was analyzed by Spearman test

value derived from both protocols for good, average, and poor embryos was 0.875, 0.565, and 0.785, respectively. However, the dose of FA in the agonist protocol was significantly higher than the agonist protocol (33.42±8.83 *vs*. 41.82±12.42, p=0.001). In terms of OHSS, 5 out of 60 patients (8%) had a severe condition in agonist protocol, whereas 2 out of 60 patients (3%) exhibited mild symptoms in antagonist protocol. Moreover, the Spearman test showed a negative correlation between FA dose and retrieved oocytes (r=-0.255 (p<0.01), fertilized oocytes (r=-0.296, p<0.01), and both good and average embryos (r=-0.231, p<0.05; r=-0.188, p=0.647) as presented in table 4.

Discussion

Our study was conducted to unravel the effect of FA on IVF outcomes by administering agonist and antagonist COS protocols in Indonesian population. Our findings indicate that FA yields similar outcomes in both GnRH-a/-ant in terms of the number of oocytes, maturity, embryo quality, and clinical pregnancy rate. These findings are consistent with previous reports of Lunenfeld et al. and Al-Inany et al. Although they did not work on FA, their findings implied that the use of exogenous gonadotropin provided similar results regarding embryo quality and clinical pregnancy rate in both COS protocols. Mochtar et al. particularly highlighted that the number of oocytes retrieved from both protocols was not different. Furthermore, Al-Inany et al. Reported that both GnRH-a/-ant resulted in similar pregnancy rates. Furthermore, Stimpfel et al. In 2015 reported that FA in both protocols resulted in similar fertilized oocytes and pregnancy rates in patients with normal responses. It is suggested that administration of FA provides similar outcomes in GnRH-a/-ant (9, 14-16).

In terms of OHSS, our study indicated 8% of severe cases in agonist protocol and 3% of mild cases in the antagonist protocol. This result is in line with Hsieh et al.'s (2008) findings which showed that OHSS incidence in both GnRH-a/-ant protocols utilizing FA was 5.8% and 8.6%, respectively. Another study employing FA also revealed that OHSS was lower in antagonists than agonists (17). This finding should be interpreted with caution as patients with severe OHSS require special supervision at the hospital, which is relatively costly. The OHSS severity itself was classified according to the patient's complaints (13) as shown in table 1. The risks of OHSS then could be prevented through several options, including administration of <150 *IU* of initial dose of FA, increasing by 12.5-25 *IU* daily according to ultrasound monitoring and hormonal examinations or utilizing a low dose of hCG. Delayed embryo transfer and cryopreservation of all oocytes and embryos are also designated alternatives against OHSS (18). Meanwhile, the difference in the frequency of OHSS is thought to be due to the differences in FA dose. Compared to the agonists, the antagonist protocol required smaller doses of FA with faster COS duration, which reduces the possibility of OHSS occurrence (19-21). In fact, GnRH-ant has the competence to rapidly bind into pituitary GnRH receptors, which makes COS duration shorter (9, 21).

Utilizing correlation analysis, a negative correlation was observed between the FA doses and the number of total retrieved oocytes and embryo quality. These results are based on Friedler et al.'s (2016) research using another rFSH, which manifested a negative correlation between exogenous gonadotropin dose with oocytes number and clinical pregnancy rates (5). Additionally, Iaconelli et al. performed a research on other types of follitropins and reported that lower doses lead to increased number of mature retrieved oocytes, embryo quality, and blastocyst formation (22). It was also noted that even though it might produce similar clinical pregnancy outcomes, a higher dose of follitropin could exert adverse effects such as a decrease in fertilized oocytes and embryo quality (23).

Conclusion

In conclusion, our study suggests that FA produces similar outcomes in both agonist and antagonist COS protocols, but administering a lower dose in the antagonist is preferable. Additionally, the rate of OHSS occurrence was higher in agonist protocol implying that further investigations are required in assessing the protocols and evaluating the effect of FA on OHSS.

Acknowledgement

This research was funded by the Directorate of Research and Community Service, University of Indonesia through Hibah Publikasi Terindeks Internasional (PUTI) Q2 Tahun Anggaran 2022-2023 (NKB-641/UN2.RST/HKP.05.00/2022).

Conflict of Interest

Authors declare no conflict of interest.

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