Flexible GnRH antagonist protocol vs. long GnRH agonist protocol in patients with polycystic ovary syndrome treated for IVF: comparison of clinical outcome and embryo quality

Protokół z antagonistą GnRH vs. długi protokół z agonistą GnRH u pacjentek z zespołem policystycznych jajników przygotowywanych do IVF: porównanie wyników klinicznych i jakości zarodków

Milan Trenkić[1], Jasmina Popović[1,2], Vesna Kopitović[3,4], Artur Bjelica[3,4], Radomir Živadinović[1,2], Sonja Pop-Trajković[1]

[1] Department of Gynecology and Obstetrics, Clinical Center of Niš, Serbia
[2] Faculty of Medicine, University of Niš, Niš, Serbia
[3] Department of Gynecology and Obstetrics, Clinical Center of Vojvodina, Novi Sad, Serbia
[4] Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia

Abstract

Objectives: *Polycystic ovary syndrome (PCOS) is a common endocrine disorder, primarily affecting women of the reproductive age. The aim of the study was to assess the clinical efficacy and embryo quality in flexible gonadotropin-releasing hormone (GnRH) antagonist protocol in comparison to the long GnRH agonist protocol in PCOS women undergoing in vitro fertilization (IVF).*

Material and methods: *This prospective, randomized study was conducted at the Department of Gynecology and Obstetrics, Clinical Center Niš, Serbia, between 2013 and 2014. The treatment included either a flexible GnRH antagonist protocol (n=45, antagonist group) or a long GnRH agonist protocol (n=45, agonist group).*

Results: *The length of the stimulation, total amount of gonadotropins used, as well as the average number of the aspirated and mature oocytes were higher in the agonists group. The endometrial thickness was also greater in the agonists group. A higher number of Class I and Class IV embryos were obtained after the agonist treatment and higher number of Class II and Class III embryos were obtained after the antagonist treatment. Pregnancy, implantation, and miscarriage rates were comparable between the groups.*

Conclusions: *The GnRH antagonist protocol in PCOS patients has a pregnancy rate comparable to that of the GnRH agonist protocol. Since this protocol has a lower rate of complications and is more convenient for patients, we believe that the GnRH antagonist protocol should be used as the first-line treatment for PCOS patients in an IVF program.*

Key words: **IVF** / **PCOS** / **GnRH agonist** / **GnRH antagonist** /

Corresponding author:
Milan Trenkić
Department of Gynecology and Obstetrics, Clinical Center of Niš
Sterijina 32, 18000 Niš, Serbia
tel. +381631095000
e-mail: trenkic@gmail.com

Otrzymano: **14.09.2015**
Zaakceptowano do druku: **15.03.2016**

DOI: 10.17772/gp/62205

Streszczenie

Cel pracy: *Zespół policystycznych jajników (PCOS) jest częstym zaburzeniem endokrynologicznym, głównie dotyczącym kobiet w wieku reprodukcyjnym. Celem badania była ocena skuteczności klinicznej oraz jakości zarodków uzyskanych w protokole flexible z antagonistą GnRH w porównaniu do protokołu długiego z agonistą GnRH u kobiet z zespołem PCO poddanych zapłodnieniu pozaustrojowemu (IVF).*

Materiał i metoda: *To prospektywne, randomizowane badanie przeprowadzono w Klinice Ginekologii i Położnictwa w Clinical Center Niš, w Serbii, w latach 2013 - 2014. Leczenie polegało na zastosowaniu protokołu flexible z antagonistą GnRH (n=45) lub długiego protokołu z agonistą GnRH (n=45).*

Wyniki: *Długość stymulacji, całkowita liczba użytych gonadotropin, jak również średnia liczba zaaspirowanych i dojrzałych oocytów była wyższa w grupie z agonistą. Grubość endometrium była również wyższa w grupie z agonistą. Wyższą ilość zarodków klasy I i IV uzyskano po podaniu agonisty natomiast a po leczeniu antagonistą uzyskano wyższą ilość zarodków klasy II i III. Liczba uzyskanych ciąż, implantacji i poronień była porównywalna w obu grupach.*

Wnioski: *Protokół z antagonistą GnRH u pacjentek z PCOS ma porównywalny odsetek ciąż jak protokół z agonistą GnRH. Ponieważ protokół z antagonistą GnRH ma mniejszą liczbę powikłań i jest wygodniejszy dla pacjentek, uważamy że powinien być stosowany jako leczenie pierwszego rzutu pacjentek z PCOS w programie zapłodnienia pozaustrojowego.*

Słowa kluczowe: **IVF** / **PCOS** / **agonista GnRH** / **antagonista GnRH** /

Introduction

Polycystic ovary syndrome (PCOS), which was first recognized in 1935, is a common endocrine disorder, primarily affecting women of the reproductive age. It is characterized by irregularity of the menstrual cycles, presence of polycystic ovaries detected by ultrasound, and hyperandrogenism (clinical or biochemical) [1, 2]. This syndrome can be diagnosed based on several different criteria. The Rotterdam criteria, which define PCOS by the presence of at least two out of three criteria: oligo-anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovaries (≥12 follicles measuring 2–9 mm in diameter, or ovarian volume >10 ml in at least one ovary), are the most widely accepted. According to these criteria, the prevalence of PCOS is 15% [3]. Chronic anovulation is the main problem of PCOS patients. Low progesterone levels, caused by missing selection of the dominant follicle and ovulation, lead to increased pulsatility of the gonadotropin-releasing hormone (GnRH) and, consequently, to elevated serum concentrations of the luteinizing hormone (LH) [4]. The increased concentration of LH can activate a premature meiotic cleavage, damage the oocyte nucleus, and lead to apoptosis [5]. It is also associated with higher miscarriage rate [6].

The optimal infertility treatment for PCOS patients is still a matter of debate, but in patients who are refractory to conventional modalities, *in vitro* fertilization (IVF) is a reasonable option. Patients with PCOS can undergo different IVF protocols. So far, no protocol has been chosen as the most effective in these patients [3].

The ability of GnRH agonists to suppress LH before and during controlled ovarian hyperstimulation (COH) has made the long GnRH protocol a reasonable option for women with PCOS [7]. On the other hand, GnRH antagonists have no flare-up effect and decrease the incidence of the ovarian hyperstimulation syndrome (OHSS), which is a serious iatrogenic complication during COH in PCOS patients [7, 8]. Furthermore, the GnRH antagonists protocol is shorter, less expensive, and more patient-friendly [9,10].

According to the consensus on infertility treatment related to PCOS, the optimal stimulation protocol is still under debate. For this reason, the ESHRE/ASRM consensus document has recently stressed the need to perform further randomized controlled trials, comparing follicle stimulating hormones (FSH) stimulation protocol with the use of GnRH agonist versus GnRH antagonist [3].

Objectives

The aim of the study was to assess the clinical efficacy and the quality of embryos in flexible GnRH antagonist protocol in comparison to the long GnRH agonist protocol in PCOS women undergoing IVF.

Material and methods

This prospective, randomized, single-center study was conducted at the Department of Gynecology and Obstetrics, Clinical Center Niš, Serbia, in 2013 and 2014. Each participant was randomly assigned to one of the two groups in a 1:1 ratio, according to a computer-generated randomization list. The treatment included either a long GnRH agonist (n=45, agonist group) or a flexible GnRH antagonist (n=45, antagonist group) protocol. Neither the patients nor the doctors were blinded to the treatment assignment. Written informed consent was obtained from all participants. Local Ethics Committee approved of the study.

Each patient could participate in the study only once. The inclusion criteria were as follows: previously diagnosed PCOS (the Rotterdam criteria), age 18-39 years, body mass index (BMI) of 18-30 kg/m². The exclusion criteria were: abnormalities of the uterine cavity, dysfunction of the thyroid or abnormal prolactin levels, ovarian cysts, as well as severe disturbances of spermatogenesis in the partner, requiring the ICSI method.

During the period of 21 days, all patients received an oral contraceptive pill (OCP) daily, starting on Day 2 of spontaneous menses of the cycle prior to the treatment cycle.

In the antagonist group, on Day 2 of the cycle following discontinuation of OCP, we started a recombinant FSH (Gonal F, Merc Serono, Switzerland). Based on the ovarian response, as assessed by transvaginal ultrasound examinations and by measuring serum estradiol levels, the starting dose of 150 IU/day was individually adjusted. After 5 days of COH, the first control ultrasound examination was performed and estradiol concentration was measured. The moment the lead follicle reached the size of 14 mm in diameter and/or estradiol levels reached the value of >300 pg/ml, in the flexible GnRH antagonist protocol inj. cetrorelix 0.25 mg (Cetrotide; Merc Serono, Switzerland) was administered. Treatment with rFSH and GnRH antagonist was continued on a daily basis until and including the day of final oocyte maturation trigger.

In the agonist group, starting from Day 21 of the menstrual cycle, we began the treatment consisting of GnRH agonist 0.1 mg triptorelin (Diphereline, Ipsen Pharma Biotech, France), administered subcutaneously once a day. After pituitary desensitization had been confirmed (defined as serum estradiol levels of <20 pg/ml, serum LH of <2.0 mIU/ml), we started the administration of recombinant FSH. After 5 days of stimulation and based on the ovarian response, as assessed by transvaginal ultrasonography and serum estradiol levels, the initial dose of 150 IU/day was adjusted.

In both protocols, a dose of 10,000 IU of human chorionic gonadotropin (hCG) (Pregnyl, Organon, Holland) was used for final oocyte maturation. The patients received the dose either when a mean diameter of three follicles reached the size of ≥17 mm or when the dominant follicle reached the size of ≥18 mm, with the following two ≥16 mm. Next, 34-36h after the hCG injection, we performed transvaginal ultrasound-guided oocyte retrieval. The oocytes which reached metaphase II at retrieval were classified as mature (M2), while those in metaphase I (M1) or germinal vesicle stage (GV) were considered as immature. 38-40h after hCG injection, insemination was performed by the conventional IVF method. The appearance of two pronuclei (PN) 16-18h after insemination confirmed normal fertilization. The fertilization rate was expressed as the number of zygotes with two pronuclei over the total number of inseminated oocytes.

The assessment of the embryo scoring was made in accordance with the internal laboratory embryo score standards. Embryo quality was evaluated on the basis of morphological features and dynamics of embryo development. Embryonic morphological features are related to the blastomeres of equal or unequal size and present or absent fragmentation of cytoplasm. The assessment of the embryo development dynamics requires monitoring of the number of blastomeres every 24 hours until the day of ET, and the comparison of the actual number of blastomeres with their expected number. The embryo assessment system was created according to these two parameters and the embryos were classified into four groups. Class I embryos on Day 3 or 68±1 h after insemination, fulfil all three of the following criteria:

1) embryos have 6 to 8 blastomeres,

2) all blastomeres are equal,

3) there is no fragmentation in blastomeres.

Class II embryos do not meet one of the abovementioned three criteria – they have less than 6 blastomers or have 6-8 blastomers but unequal, or fragmentation is present. Class III embryos do not fulfil two criteria, whereas embryos in class IV do not meet any of the above stated criteria.

Embryos were transferred on Day 3. All patients received 600 mg of micronized progesterone per day (Utrogestan, Laboratories Besins-International S.A., France) for luteal phase support. For the assessment of pregnancy serum hCG assay was used 12 days from embryo transfer. Clinical pregnancy was confirmed by fetal cardiac activity visualized by transvaginal ultrasound at 6-7 weeks of gestation.

A modified classification system, based on several combined criteria reported by Golan et al., was used to establish the grades of OHSS severity [11]. Grade I included patients with abdominal distension and discomfort, which were recognized as symptoms of mild OHSS. Furthermore, the symptoms included nausea, vomiting, and diarrhea. In addition, ultrasound examination of the ovaries (5-12 cm) was also included in mild OHSS. Grade II, or moderate OHSS, included features of grade I, i.e. mild OHSS combined with the ultrasound evidence of ascites. Grade III, or severe OHSS, included patients who required hospitalization because they developed severe and critical OHSS, or because their medical condition fulfilled one or more of the hospital admission criteria. The admission criteria for hospitalization were established according to the presence of one of the following: ascites, hydrothorax, hematocrit ≥45%, oliguria, elevated liver enzymes, dyspnoea, anasarca, or acute renal failure.

The research results are systematized and analyzed using descriptive statistical and quantitative analysis software package SPSS [Statistical Package for Social Sciences (SPSS) 14.0 for Windows 2003]. The comparison of the arithmetic means of the two samples was performed using t-test for independent samples or Mann Whitney test, depending on data distribution. The comparison of the frequency of attributive features was performed by $\chi 2$-test or Fisher's test. The p-value of <0.05 was considered as statistically significant.

Results

The study included 90 women: 45 received the long GnRH agonist protocol treatment (50.00%) and 45 received the GnRH antagonist protocol treatment (50.00%). No statistically significant differences in age and BMI between the agonists and antagonists group were found (Table I).

Concentrations of FSH, LH, and AMH as well as the LH/FSH ratio did not differ significantly with regard to the stimulation protocol (Table II).

The length of the stimulation was significantly higher in the agonist group as compared to the antagonist group ($p<0.001$). The total amount of gonadotropins used for the stimulation was higher in the agonist group, but there was no statistically significant difference ($p=0.282$). The average number of the follicles ≥16 mm was statistically significantly higher in the agonist group ($p<0.001$). The average number of the aspirated oocytes was also statistically significantly higher in the agonist group ($p=0.005$). The number of mature oocytes was statistically significantly higher in the agonist group ($p=0.035$) (Table III). The number of immature oocytes was higher in the agonist group, but there was no statistically significant difference ($p=0.051$). The endometrial thickness on the day of HCG administration was statistically significantly greater in the group of agonists as compared to the group of antagonists ($p=0.021$).

The total number of the obtained embryos in the agonists and in the antagonists was 347 and 285, respectively. A statistically significantly higher number of Class I and Class IV embryos

DOI: 10.17772/gp/62205

Table I. Baseline characteristics of PCOS patients.

Parameter	Agonist group n=45	Antagonist group n=45	p†
Age, years	31.20±3.98	31.36±4.02	0.854
BMI (kg/m²)	23.16±3.03	23.22±3.16	0.919

† t test

Table II. Basal hormone levels.

Parameter	Agonist group n=45	Antagonist group n=45	p†
FSH(IU/L)	5.40±1.74	5.53±1.85	0.366
LH(IU/L)	7.44±3.28	6.84±1.96	0.504
AMH(ng/ml)	7.13±3.57	6.73±2.88	0.810
LH/FSH	1.52±0.80	1.24±0.33	0.197

† Mann-Whitney test

was obtained after the agonist treatment (p=0.006, respectively p=0.046). A statistically significantly higher number of Class II and Class III embryos was obtained after the antagonist treatment (p=0.008, respectively p=0.036) (Table IV).

The total number of the transferred embryos in the agonists was 115 and in the antagonists 115. A statistically significantly higher number of Class IV embryos were transferred in the group of agonists (p=0.003) (Table V).

The number of pregnancies, multiple pregnancies, miscarriages, the implantation rate, and OHSS are not significantly different as compared to the stimulation protocol (Table VI).

Discussion

Over the last two decades, the GnRH agonist protocol has been used as the first-choice treatment in COH for PCOS patients. Dealing with IVF patients, we also tried to take into consideration the psychological aspect of the treatment. We noticed that treatment duration in the GnRH agonist protocol and, primarily, the obligation to receive therapy every day at the same time over a long period of time, are considered very frustrating by most patients. On the other hand, although the GnRH antagonist protocol is more convenient for patients, it is still used in most centers as the second-line protocol. Our study has shown that this protocol is not only more patient-friendly but also much safer for patients with the same clinical pregnancy rate (CPR) as those in the long GnRH agonist protocol.

By including only patients with BMI <30, we excluded the possibility of the negative effect of high BMI on LH surge. LH is known to play an important role during COH. However, the issue of optimal LH concentration necessary for the most favorable COH is still under debate, and remains a subject of different studies [12]. A study by Lainas et al., showed significantly lower basal LH levels among antagonist group women [13]. However, that study did not examine the relation between the LH level and the IVF outcome. Kurzawa et al., demonstrated significantly higher LH levels at the beginning and during COH, as well as on the day of hCG administration in the antagonist group, but found no correlation between serum LH concentration and the IVF outcome [14]. In our study, there were no statistically significant differences in basal serum LH concentration between the agonist and the antagonist group.

Our results have shown that a shorter duration of gonadotropin stimulation and a lower total amount of rFSH are required in the antagonist group. These results are similar to those published by other studies (Bahceci et al., 2005; Ragni et al., 2005; Griesinger et al., 2006; Lainas et al., 2007). We are of the opinion that after the desensitization period in the long protocol, there is a great decrease in serum LH level, which correlates to a lower FSH sensibility and, consequently, leads to longer stimulation and requires a higher amount of gonadotropin.

A meta-analysis by Griesinger et al., showed no significant differences between the obtained oocytes from GnRH agonist and GnRH antagonist group in patients with PCOS [10]. Although, according to many studies, oocytes retrieved from patients with PCOS are immature and of poorer quality with a lower fertilization capacity, a study by Kurzawa showed no differences between the two groups regarding fertilization capacity [15, 16, 17]. In our study, the total number of the aspirated oocytes was higher in the agonist group, with a statistical difference regarding mature and no statistical difference regarding immature oocytes. Endometrial thickness on the hCG day was statistically smaller in the group of antagonists as compared to the agonist group. Our results are similar to the results of Huang SY et al., whose study included unselected patients undergoing IVF and embryo transfer [18]. We have not been able to prove that the reduced endometrial thickness has any impact on the CPR. The fertilization rate showed no differences between the groups.

The total number of the obtained embryos was higher in the agonist group. However, those patients had more class I and class IV embryos, whereas the antagonist group had more class II and class III embryos. Since the usual policy of the clinic is to transfer two (and never more than three) embryos, there were no differences between the number of the transferred embryos. We know that class IV embryos are of poor quality and, as such, are not suitable for embryo transfer, especially for single transfer. In our study, 9 of these embryos were used for embryo transfer in the agonist group, exclusively as the third embryo together with the two other class I and class II embryos. In our opinion, they could not have influenced the CPR. To the best of our knowledge, this study is the first to examine embryo quality differences between the agonist and the antagonist groups. We hypothesize that since PCOS patients receive more oocytes during COH and, consequently, more embryos, the selection of the best-quality embryo for transfer can overcome the lack of quality in all embryos. More studies concerning this theory are needed.

Literature reports on the IVF outcome in these two protocols remain confusing. A meta-analysis by Kolibianakis et al., showed that the probability of a live birth does not differ between the GnRH agonist and antagonist protocol [19]. On the other hand, a meta-analysis by Al-Inany et al., revealed that GnRH antagonist gives significantly worse results than agonist in the general IVF population [20]. A meta-analysis by Griesinger et al., showed similar benefits of GnRH analogs regarding PCOS populace [10]. The implantation rate as well as the pregnancy rate in our study revealed no differences between the two groups. The number of multiple pregnancies as well as OHSS was higher in the GnRH agonist group. The reason for the higher incidence of OHSS in

Table III. Ovarian stimulation characteristics.

Parameter	Agonist group n=45	Antagonist group n=45	p
Length of stimulation (days)	10.91±2.09	9.44±2.40	<0.001†
Total amount of gonadotropins used (IU)	1804.51±710.24	1580.53±415.86	0.282†
Number of follicles ≥16mm	14.16±6.53	9.24±4.83	<0.001†
Number of retrieved oocytes	13.71±6.69	10.11±6.46	0.005†
Number of mature oocytes	9.80±6.08	7.29±4.95	0.035†
Number of mature/aspirated oocytes (%)	71.01%	72.08%	0.700‡
Number of immature oocytes	4.00±2.82	2.82±2.27	0.051†
Number of immature/aspirated oocytes (%)	28.98%	27.91%	0.700‡
Endometrial thickness on the day of HCG administration (mm)	10.38±1.54	9.71±1.12	0.021#
Percentage of fertilization	67.85%	68.13%	0.849‡

† Mann-Whitney test, ‡ Chi-square test, # t test,

Table IV. Characteristics of embryos on Day 3 in protocols (N, %).

Parameter	Stimulation protocol				p†
	Agonist group		Antagonist group		
	N	%	N	%	
Total number of the obtained embryos	347	100.00	285	100.00	
Class I	116	34.43	67	23.51	0.006
Class II	72	20.75	85	29.82	0.008
Class III	77	22.19	84	29.47	0.036
Class IV	82	23.63	49	17.19	0.046

† Chi-square test

Table V. Characteristics of transferred embryos (N, %).

Parameter	The stimulation protocol				p
	Agonist group		Antagonist group		
	N	%	N	%	
Total number of transferred embryos	115	100.00	115	100.00	
Class I	64	55.65	63	54.78	0.894†
Class II	27	23.48	38	33.04	0.143†
Class III	15	13.04	14	12.17	0.842†
Class IV	9	7.83	0	0	0.003‡
Average number of transferred embryos	2.56±0.92		2.56±0.87		0.853#

† Chi-square test, ‡ Fisher's test, # Mann-Whitney test

Table VI. Comparison of the clinical efficacy of antagonist and agonist protocols.

Parameter	Agonist group n=45	Antagonist group n=45	p
Implantation rate	24.35%	20.87%	0,636‡
Number of clinical pregnancies	20 (44.40%)	21 (46.70%)	0.832†
Number of biochemical pregnancies	2 (4.40%)	1 (2.20%)	0.557†
Number of multiple pregnancies	7 (15.56%)	2 (4.44%)	0.156‡
Twins	6 (13.33%)	1 (2.22%)	0.115‡
Triples	1 (2.22%)	1 (2.22%)	1.000‡
Number of cancelled cycles/embryo transfer	3 (6.67%)	3 (6.67%)	1.000‡
OHSS total number	7 (15.56%)	3 (6.70%)	0.314‡
OHSS Grade I	5 (11.10%)	3 (6.70%)	0.241‡
OHSS Grade II	2 (4.40%)	0	0.494‡

† Chi-square test, ‡ Fisher's test

DOI: 10.17772/gp/62205

the agonist group is related to a greater number of follicles and oocytes, and additionally, to a greater number of small follicles, which leads to high estradiol levels [21]. Kaur et al, stated that for every 25 women undergoing downregulation by an agonist, we should expect one extra case of severe OHSS [22]. In our study, there were seven patients with OHSS in the agonist group and three patients in the antagonist group. There were only two patients with moderate OHSS in the agonist group, and no such patients in the antagonist group. A small sample size may explain the lack of statistically significant differences. However, we believe that three times higher number of multiple pregnancies and over twice as many cases of OHSS in the agonist group, as compared to the antagonist group, cannot exclude a clinically significant difference.

Bearing in mind the risk of developing OHSS, preventive measures should be undertaken before, during, and after stimulation. The strategy for the prevention and management of OHSS among PCOS patients can be divided into three levels. The primary preventive measures, which have been used in our study, include the choice of the starting dose of gonadotropins, which does not exceed 150 IU/day. Furthermore, a primary strategy is to decrease the dose of gonadotropins during stimulation – a step-down protocol [23]. Close ultrasound and estradiol level monitoring is also effective in reducing the incidence of OHSS, with a sensitivity and specificity of about 80% [24]. Secondary prevention measures, which were not used in our study, include coasting, administration of GnRH antagonists in GnRH agonists cycles, and administration of a GnRH agonist instead of hCG in GnRH antagonist cycles as a trigger for final oocyte maturation [25, 26]. Coasting is withholding of gonadotropin use until safe levels of estradiol are obtained. A recent meta-analysis has not confirmed this approach to OHSS prevention [27]. The third level of prevention of OHSS includes the use of albumin, dopamine agonist, and vitrification of embryos, or cycle cancellation. We applied only albumin in our study. The Cochrane review showed only limited evidence of benefit from intra-venous albumin administration at the time of oocyte retrieval in prevention or reduction of the incidence of severe OHSS in high-risk women undergoing IVF [28].

Conclusions

Since GnRH antagonist protocol has a pregnancy rate comparable to that of the GnRH agonist protocol and, is correlated with a lower rate of complications such as multiple pregnancies and OHSS, and since it is more convenient for the patients, we believe that the mild stimulation ovarian protocol should be used as the first-line treatment for PCOS patients in the IVF program.

Authors' contribution:

1. Milan S. Trenkić – concept and design of study, analysis and interpretation of data, article draft, acquisition of data, corresponding author.
2. Jasmina Popović – acquisition of data, concept and design of study.
3. Vesna Kopitović – revising the manuscript critically.
4. Artur Bjelica – analysis and interpretation of data, revising the manuscript critically.
5. Radomir Živadinović – acquisition of data, analysis and interpretation of data.
6. Sonja Pop-Trajković – acquisition of data.

Authors' statement:

- This is to certify, that the publication will not violate the copyrights of a third party, as understood according to the Act in the matter of copyright and related rights of 14 February 1994, Official Journal 2006, No. 90, Clause 63, with respect to the text, data, tables and illustrations (graphs, figures, photographs);
- there is no 'conflict of interests' which occurs when the author remains in a financial or personal relationship which unjustly affects his/her actions associated with the publication of the manuscript;
- any possible relationship(s) of the author(s) with the party/parties interested in the publication of the manuscript are revealed in the text of the article;
- the manuscript has not been published in or submitted to any other journal.

Source of financing:

The authors certify that have NO affiliations with or involvement in any organization or entity with any financial interest, and have non-financial interest in the subject matter or materials discussed in the manuscript.

References

1. Norman RJ, Davies MJ, Lord J, [et al.]. The role of lifestyle modification in polycystic ovary syndrome. *Trends Endocrinol Metab.* 2002,13 (6), 251–257.
2. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. *Am J Obstet Gynecol.* 1935, 29, 181–191.
3. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril.* 2004, 81 (1), 19-25.
4. Taylor AE, McCourt B, Martin K, [et al.]. Determinants of abnormal gonadotropin secretion in clinically defined women with PCOS. *J Clin Endocrinol Metab.* 1997, 82 (7), 2248–2256.
5. Yoshimura Y, Wallach EE. Studies of the mechanism(s) of mammalian ovulation. *Fertil Steril.* 1987, 47 (1), 22–34.
6. Balen AH, Tan SL, MacDougall J, [et al.]. Miscarriage rates following in-vitro fertilization are increased in women with polycystic ovaries and reduced by pituitary desensitization with buserelin. *Hum Reprod.* 1993, 8 (6), 959–964.
7. Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. *Hum Reprod.* 2008, 23 (3), 462–477.
8. Homburg R. Management of infertility and prevention of ovarian hyperstimulation in women with polycystic ovary syndrome. *Best Pract Res Clin Obstet Gynaecol.* 2004,18 (5), 773–788.
9. Ragni G, Vegetti W, Riccaboni A, [et al.]. Comparison of GnRH agonists and antagonists in assisted reproduction cycles of patients at high risk of ovarian hyperstimulation syndrome. *Hum Reprod.* 2005, 20 (9), 2421-2425.
10. Griesinger G, Diedrich K, Tarlatzis BC, [et al.]. GnRH-antagonists in ovarian stimulation for IVF in patients with poor response to gonadotrophins, polycystic ovary syndrome, and risk of ovarian hyperstimulation: A meta-analysis. *Reprod BioMed Online.* 2006, 13 (5), 628–638.
11. Golan A, Ron-el R, Herman A, [et al.]. Ovarian hyperstimulation syndrome: an update review. *Obstet Gynecol Surv.* 1989, 44 (6), 430-440.
12. Filicori M. The role of luteinizing hormone in folliculogenesis and ovulation induction. *Fertil Steril.* 1999, 71 (3), 405–414.
13. Lainas TG, Petsas GK, Zorzovilis IZ, [et al.]. Initiation of GnRH antagonist on Day 1 of stimulation as compared to the long agonist protocol in PCOS patients. A randomized controlled trial: effect on hormonal levels and follicular development. *Hum Reprod.* 2007, 22 (6), 1540–1546.
14. Kurzawa R, Ciepiela P, Baczkowski T, [et al.]. Comparison of embryological and clinical outcome in GnRH antagonist vs. GnRH agonist protocols for in vitro fertilization in PCOS non-obese patients. A prospective randomized study. *J Assist Reprod Genet.* 2008, 25 (8), 365-374.
15. Nardo LG, Rai R, Backos M, [et al.]. High serum luteinizing hormone and testosterone concentrations do not predict pregnancy outcome in women with recurrent miscarriage. *Fertil Steril.* 2002, 77 (2), 348–352.
16. Balen AH, Tan SL, Jacobs HS. Hypersecretion of luteinizing hormone: a significant cause of infertility and miscarriage. *Br J Obstet Gynaecol.* 1993, 100 (12), 1082–1089.
17. Homburg R, Berkowitz D, Levy T, [et al.]. In vitro fertilization and embryo transfer for the treatment of infertility associated with polycystic ovary syndrome. *Fertil Steril.* 1993, 60 (5), 858–863.
18. Huang SY, Huang HY, Yu HT, [et al.]. Low-dose GnRH antagonist protocol is as effective as the long GnRH agonist protocol in unselected patients undergoing in vitro fertilization and embryo transfer. *Taiwan J Obstet Gynecol.* 2011, 50 (4), 432-435.
19. Kolibianakis EM, Collins J, Tarlatzis BC, [et al.]. Among patients treated for IVF with gonadotrophins and GnRH analogues, is the probability of live birth dependent on the type of analogue used? A systematic review and meta-analysis. *Hum Reprod Update.* 2006, 12 (6), 651–671.
20. Al-Inany HG, Abou-Setta AM, Aboulghar M. Gonadotrophin-releasing hormone antagonists for assisted conception. *Cochrane Database of Systematic Reviews* 2006; (3), CD001750.
21. Navot D, Bergh PA, Laufer N. Ovarian hyperstimulation syndrome in novel reproductive technologies: prevention and treatment. Fertil Steril. 1992, 58 (2), 249-261.
22. Kaur H, Krishna D, Shetty N, [et al.]. A prospective study of GnRH long agonist versus flexible GnRH antagonist protocol in PCOS: Indian experience. *J Hum Reprod Sci.* 2012, 5 (2), 181-186.
23. Busso CE, Garcia-Velasco JA, Simon C, [et al.]. Prevention of OHSS: Current strategies and new insights. *Middle East Fert Soc J.* 2010,15 (4), 223-230.
24. Kahnberg A, Enskog A, Brännström M, [et al.]. Prediction of ovarian hyperstimulation syndrome in women undergoing in vitro fertilization. *Acta Obstet Gynecol Scand.* 2009, 88 (12),1373–1381.
25. Gustofson RL, Segars JH, Larsen FW. Ganirelix acetate causes a rapid reduction in estradiol levels without adversely affecting oocyte maturation in women pretreated with leuprolide acetate who are at risk of ovarian hyperstimulation syndrome. *Hum Reprod.* 2006, 21 (11), 2830–2837.
26. Griesinger G, von Otte S, A. Schroer A, [et al.]. Elective cryopreservation of all pronuclear oocytes after GnRH agonist triggering of final oocyte maturation in patients at risk of developing OHSS: a prospective, observational proof-of-concept study. *Hum Reprod.* 2007, 2 (5),1348–1352
27. D'Angelo A, Brown J, Amso NN. Coasting (withholding gonadotrophins) for preventing ovarian hyperstimulation syndrome. *Cochrane Database Syst Rev.* 2011, 15 (6),CD002811.
28. Youssef MA, Al-Inany HG, Evers JL, [et al.]. Intra-venous fluids for the prevention of severe ovarian hyperstimulation syndrome. *Cochrane Database Syst Rev.* 2011, 16, (2),CD001302.