Original Paper

GnRH Antagonist IVF Protocol in PCOS

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ABSTRACT Objective. The aim of the present study was to compare the GnRH agonist long protocol with the flexible GnRH antagonist protocol in infertile PCOS women undergoing COS in terms of clinical pregnancy rate (CPR), with special reference to the incidence of OHSS. Materials and Methods. The study was conducted at the Hospital Obstetrics and Gynecology Cuza Vodă Iaşi and Fertility Reproductive Medical Center Omini Clinic Iaşi from June 1, 2010, to September 31, 2012. PCOS as defined by the Rotterdam 2003 consensus, i.e. presence of two of the following three features: presence of oligo- and/ or anovulation, clinical and/or biochemical signs of hyperandrogenism, polycystic ovaries and exclusion of other endocrinopathies. Results. No differences were observed in clinical pregnancy rate (CPR) in the agonist and antagonist protocols, respectively. Incidence of OHSS was lower in the antagonist compared with agonist group (4% versus 28%). Duration of stimulation (13,80 + 1,4 vs 11,85 + 2,4 p < 0,001) and total gonadotrophin required (2435,5 + 884,5 versus 2005, 5 + 545,5 IU p < 0.003) were also lower in the antagonist compared with agonist protocol. Conclusions. The current study suggests that the flexible GnRH antagonist protocol is associated with a similar ongoing pregnancy rate, lower incidence of OHSS grade II, lower gonadotrophin requirement and shorter duration of stimulation, compared with GnRH agonist. The GnRH antagonist might be the treatment choice for patients with PCOS undergoing IVF.

KEY WORDS *polycystic ovary syndrome, GnRH antagonist, infertility*

Introduction</p>

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<p>The polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder in women of reproductive age and its diagnosis remains one of the most challenging issues in endocrinology, gynecology, and reproductive medicine. Polycystic ovary syndrome (PCOS) is a common endocrinopathy that affects 5– 10% of women of reproductive age.</p>

<p>Different combinations of clinical (irregular menstrual cycles, hirsutism, and acne), biological (elevated serum testosterone or androstendione levels or increased LH/FSH ratio), and ultrasound (U/S) criteria have been proposed, with very little international consensus. </p>

<p>Consensus conference held in Rotterdam in 2003 [<xref ref-type="bibr" rid="B1">1</xref>] has been proposed to include in the definition of PCOS the U/S criteria that are considered at the present time as the most specific, namely an increased ovarian volume (>10 ml) and/or the presence of 12 or more follicles in each ovary measuring 2–9 mm [<xref ref-type="bibr" rid="B1">1</xref>, <xref ref-type="bibr" rid="B2">2</xref>].</p>

<p>Using a threshold of 12 for the follicle number per ovary (FNPO), we showed that 75% of PCOS patients were diagnosed whereas 99% of the normal women were under this cut off value [<xref ref-type="bibr" rid="B3">3</xref>].</p>

<p>*In vitro* fertilization (IVF) remains a reasonable option in PCOS women who are refractory to conventional infertility treatment modalities or who have coexisting infertility factors. Many controlled ovarian stimulation (COS) strategies have been offered for the treatment of patients with PCOS undergoing IVF. However, there is no compelling evidence for the advantage of one stimulation protocol over the other. The ESHRE/ASRM consensus document has recently stressed the need to perform further randomized controlled trials (RCTs) comparing FSH stimulation protocols with the use of GnRH agonist versus GnRH antagonists [<xref ref-type="bibr" rid="B4">4</xref>].</p>

<p>PCOS patients undergoing COS have a high risk of developing ovarian hyperstimulation syndrome (OHSS), a serious iatrogenic complication of ovarian stimulation. It is a potentially life-threatening condition in its severe form, resulting in hospitalization in 1.9% of cases, and hCG, either exogenous or endogenous, is the triggering factor of the syndrome. OHSS is an exaggerated response to ovulation induction with FSH and hCG. OHSS is a self-limiting disorder with a broad spectrum of clinical manifestations related to increased capillary permeability and fluid retention mediated by many inflammatory mediators including VEGF. Patients need to be

informed that mild stages of OHSS are frequent and affect about 33% of the IVF cycles and 3-8% of the cycles become more complicated due to moderate or severe forms of OHSS.   </p>

<p>Hormonal markers are, therefore, being investigated as potential predictors of ovarian response, with anti-Mullerian hormone (AMH) being a promising candidate. AMH is expressed in granulosa cells from preantral and small antral follicles and is a measure of ovarian reserve. Initial studies suggest that AMH is a reliable predictor of ovarian response, able to differentiate normal (more than 4 oocytes) responders (using a cut-off level of 1.26 ng/mL AMH) to ovarian stimulation with a success rate of 98% [<xref ref-type="bibr" rid="B5">5</xref>]. </p>

<p>Data also suggest that AMH is a more accurate predictor of normal ovarian response than age, FSH, or inhibin-B alone or in combination. One study noted that all cycle cancellations due to OHSS risk were in patients in the highest AMH quartile (>7 ng/mL), suggesting that AMH levels might be a predictor of direct risk of OHSS. These findings were substantiated by a recent cohort study of 262 IVF cycles with 21 cases (8%) of moderate or severe OHSS in which baseline serum AMH levels 3 days before ovarian stimulation were found to be significantly correlated with subsequent development of OHSS [<xref ref-type="bibr" rid="B6">6</xref>]. </p>

<p>AMH predicted OHSS better than age and body mass index, and an AMH cut-off value of 3.36 ng/mL gave a sensitivity of 90.5% and a specificity of 81.3%.</p>

<p>Gonadotropin releasing hormone antagonists have been shown to offer an advantage over standard long agonist protocol in terms of decreasing incidence of OHSS, short duration of treatment, lower cost, lesser dose of gonadotropins required and being more patient friendly. Although there are some RCTs comparing GnRH agonists versus antagonists in the PCOS population, there is still a lack of consensus as to which protocol is better [<xref ref-type="bibr" rid="B7">7</xref> - [<xref ref-type="bibr" rid="B9">9</xref>].</p>

<p>The aim of the present study was to compare the GnRH agonist long protocol with the flexible GnRH antagonist protocol in infertile PCOS women undergoing COS in terms of clinical pregnancy rate (CPR), with special reference to the incidence of OHSS.</p>

<p>Materials and Methods.</p>

<p>The study was approved and was conducted at the Hospital Obstetrics and Gynecology Cuza Vodă Iaşi and Fertility Reproductive Medical Center Omini Clinic Iaşi from June 1, 2010, to September 31, 2012. Patients who complained of infertility, menstrual irregularity, and hyperandrogenism were recruited. </p>

<p>PCOS as defined by the Rotterdam 2003 consensus, i.e. presence of two of the following three features: presence of oligo- and/ or anovulation, clinical and/or biochemical signs of hyperandrogenism, polycystic ovaries and exclusion of other endocrinopathies. We performed transvaginal scan (using 7.5 MHz vaginal probe, Voluson 730 Pro, GE Healthcare and Sonoace - Medison SA-8000 Live)</p>

<p>Age 18–35 years, body mass index of 18–30 kg/m^2, baseline Follicle Stimulating Hormone <10 IU/L, normal uterine cavity as assessed by hysteroscopy and no evidence of thyroid or prolactin dysfunction. Patients falling out of the above criteria, with presence of congenital uterine malformations, Asherman syndrome, genital tuberculosis, surgical retrieved sperms, hydrosalpinx and those showing poor response in previous IVF cycles were excluded from the study.   </p>

<p>Each of the studied subjects gave informed consent. </p>

<p>The following subjects were excluded from the study and control populations: 1) women who had been diagnosed with other etiologies that should be excluded when diagnosing PCOS [<xref ref-type="bibr" rid="B10">10</xref>]; 2) women who received hormones or drugs for major medical diseases; 3) women who presented ovarian cysts or ovarian tumors; and 4) women who were >40 years old. In total, 200 women were recruited.</p>

<p>The subjects' medical histories were obtained, and the number of menstrual cycles during the previous year was recorded. Obesity was defined as a BMI more 25 kg/m2.</p>

<p>The following components were measured and calculated: </p>

<p>1) total testosterone (T), DHEAS, levels and the free androgen index (FAI); The FAI was calculated by using the formula FAI = T (nmol/L) x 100/SHBG (nmol). Hyperandrogenism was defined as a total serum T levels more 2.98 mmol/L. (Diagnostic Systems Synevo Laboratories).</p>

<p>2) fasting insulin, fasting glucose, 2-hour oral glucose tolerance test (OGTT) and the presence of diabetes; World Health Organization 2006 diagnostic criteria for diabetes were used (fasting plasma glucose FPG >126 mg/dL or 2-

hour plasma glucose > 200 mg/dL). Impaired glucose tolerance (IGT) was defined as 2-hour glucose levels of 140–199 mg/dL in the 75-g oral glucose tolerance test. In women with IGT, the FPG level should be <126 mg/dL [<xref ref-type="bibr" rid="B11">11</xref>].</p>

<p>3) serum FSH, LH; AMH levels were measured with an ELISA kit (Diagnostic Systems Synevo Laboratories).</p>

<p>4) total cholesterol, triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL); Metabolic syndrome was defined (2005 National Cholesterol Education Program—Adult Treatment Panel III - ATP III) as the presence of at least three of the following criteria: abdominal obesity (waist circumference >80 cm in women), serum TG >150 mg/dL, serum HDL <50, blood pressure (BP) >130/85 mm Hg, and FPG >100 mg/dL and </p>

<p>5) glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT).</p>

<p>Patients were assigned to two groups on Day 2 of the menstrual cycle: 25 cases received standard GnRH agonist long protocol and 25 were allocated to the GnRH antagonist protocol.</p>

<p>All patients underwent baseline transvaginal sonography on Day 2/3 of the menstrual cycle to check for antral follicle count and endometrial thickness and to rule out the presence of ovarian cyst. Patients were assigned to the agonist or antagonist group non-randomly. In the agonist group, treatment was started from Day 21 of the menstrual cycle with inj. Diphereline 0.1 mg (Ipsen) subcutaneously once daily till downregulation was achieved/Day 2 of menstrual cycle (defined as serum estradiol <50 pg/mL, endometrial thickness <5 mm, no cyst in the ovaries, serum Leutinising hormone <2.0 IU/L). Once downregulation was achieved, the inj. Diphereline dosage reduced to 0.05 mg daily and recombinant FSH (inj. (Gonal F, Merck-Serono, Switzerland) was started. The starting dose of recombinant FSH was 75–200 IU. The dose was adjusted after 4 days of stimulation depending on the ovarian response, assessed by transvaginal scan (using 7.5 MHz vaginal probe, Voluson 730 pro, GE Healthcare) and serum estradiol levels. Follicular growth was monitored by serial ultrasonographyand. The dose of rFSH was adjusted according to serum estradiol levels and dynamics of ovarian follicular growth.</p>

<p>In the flexible antagonist protocol, inj. recombinant FSH (inj. Gonal-F, Merck Serono) was started on Day 2 of the cycle (75–200 IU daily). GnRH antagonist inj. *Cetrorelix acetate (Cetrotide* - Merck-Serono, Switzerland) 0.25 mg s/c. Treatment was started when the lead follicle reached a diameter of 14 mm and/or the estradiol levels were >400 pg/mL. Treatment with rFSH and antagonist was continued till the day of final oocyte maturation trigger. When three or more follicles of size 18 mm or more were seen, final oocyte maturation trigger was given with Pregnyl inj. hCG 5000 IU intramuscular or inj. Ovidrel - Choriogonadotropin alfa (r-hCG) 250mcg/0.5mL; liq for SC inj.(Merck-Serono, Switzerland). Transvaginal ultrasound-guided oocyte aspiration (OPU) was performed approximately 35–36 hours after hCG injection under i.v. anaesthesia. </p>

<p>Oocyte assessment was performed by standard morphology criteria proposed by Lin *et al*., [<xref ref-type="bibr" rid="B12">12</xref>] and nuclear maturity assessment was performed in cases subjected to intracytoplasmic sperm injection (ICSI). Conventional IVF or ICSI was performed depending on the semen parameters and previous fertilization history. Culture media used was vitrolife (Vitrolife Sweden AB, Goteborg, Sweden). Fertilization was defined as presence of pronuclei 16–18 h post-insemination/injection. Embryo grading was done by standard morphology assessment. Embryo Transfer was done on Day 2/3 following oocyte retrieval. Luteal phase support with 600 mg of micronized progesterone (Utrogestan Laboratoires Besins-International S.A., France) was initiated 2 days after oocyte retrieval. Serum estradiol, LH and progesterone levels were measured on the day of hCG administration and compared in the two groups. Measurement of estradiol, progesterone, LH, FSH and βhCG was done by fully automated electro-chemiluminscence technology (Roche Cobas analyzer - Diagnostic Systems Synevo Laboratories).</p>

<p>Pregnancy was assessed by serum hCG assay after 15 days from embryo transfer and then confirmed when a gestational sac was visualized at vaginal US after two further weeks. Only cases with US confirmation of pregnancy were counted in the calculation of pregnancy and implantation rates, whereas biochemical pregnancies were not considered.</p>

Results

<p>Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented as mean ± SD (min–max) and results on categorical measurements are presented as number (%). Significance is assessed at the 5% level of

significance. Student's t test (two-tailed, independent) has been used to determine the significance of the study parameters on a continuous scale between the two groups [intergroup analysis] on metric parameters and Chi-square/Fisher Exact test has been used to find the significance of the study parameters on a categorical scale between two or more groups. *P*-value of <0.05 was taken as significant.</p>

<p>The statistical software, namely SAS 9.2, SPSS 19.0, were used for the analysis of the data and Microsoft Word and Excel have been used to generate graphs, tables, etc. Although the sample size is small looking at the baseline pregnancy rate for PCOS patients being 35–40%. However, we undertook the study to compare the response of </p>

<p>PCOS patients to GnRH antagonists and agonists.</p>

<p>Baseline characteristics are depicted in Table 1. There was no significant difference in the baseline parameters in the two groups. </p>

<p>Table 1 - Baseline parameters of patients in the agonist and antagonist groups

	Agonist protocol N = 25	Antagonist protocol N = 25	p
Age în years	27,3 ± 3,2	28,1 ± 2,8	NS
BMI (kg/m²)	25,35 ± 4,7	25, 83 ± 5,2	NS
Iregular cycle (% of cases)	12 (48%)	13 (52%)	NS
Primary infertility (% of cases)	17 (68%)	18 (72%)	NS
FSH (UI/L)	5,35 ± 0,7	5,26 ± 1,3	NS
LH (UI/L)	6,39 ± 2,2	6,85 ± 3,1	NS
AFC in D2 (mean number)	17,31 ± 5,7	16,15 ± 5,6	NS

Table 2 - Comparasion of two groups regarding stimulation characteristics

	Agonist protocol N=25	Antagonist protocol N=25	p
Stimulation duration	13,80 ± 1,4 (11-15 days)	11,85 ± 2,4 (10-14 days)	0,001
Dose of gonadotrophins (IU)	2435,5 ± 884,5	2005, 5 ± 545,5	0,003
No. of follicles on hCG day	17,35 ± 6,7	16,80 ± 5,1	NS
E2 on the hCG day	2760 ± 911,7 (1265-4570)	2550 ± 145 1,7 (1387 - 4450	NS
Progesterone on hCG day (nmol/L)	3,15 ± 1,0	3,16 ± 1,1	NS
Endometrial thickness on day of embrio trasfer	10,38 ± 1,2	9,85 ± 1,4	NS

Table 3 - Embryology parameters are depicted

	Agonist protocol N = 25	Antagonist protocol N= 25	p
IVF (%)	10 cases (40%)	11 cases (44%)	NS
IVF and ICSI (%)	15 cases (60%)	14 cases (56%)	NS
Mean no. of mature oocytes	15,35 ± 6,0	14,90 ± 4,8	NS
Mean no. of oocytes fertilized	9,37 ± 5,1	8,58 ± 5,4	NS
Mean no. of oocytes cleaved	8,15 ± 4,0	8,36 ± 5,1	NS
Mean no. of embryos transferred	3,38 ± 0,8	3,85 ± 0,7	NS

Table 4 - OHSS rate was significantly more in agonist group

	Agonist protocol N = 25	Antagonist protocol N=25	p
Clinical Pregnancy Rate	8 (32%)	9 (36%)	NS
Multiple Pregnancy Rate	1 (4%)	1 (4%)	NS
Miscarriage Rate	1 (4%)	1 (4%)	NS
Ectopic Pregnancy Rate	1 (4%)	0	NS
Live Birth Rate	6	8	NS
OHSS Rate	7 (28%)	1 (4%)	0,003 HS

Discussion</p></p>

<p>For more than 20 years, GnRH agonists have been the "gold standard" protocol in COS. The vast majority of IVF treatment cycles are still performed using the GnRH agonist long protocol.</p>

<p>The present study showed that clinical pregnancy rate and live birth rate were not significantly different in the agonist versus the antagonist groups. Fertilization rate and clearage rate did not show any significant difference in the two groups. But, the OHSS rate was significantly lower in the antagonist protocol. Severe OHSS is a life-threatening complication of ovulation induction and should be an important consideration when deciding the treatment plan for PCOS patients.</p>

<p>Therefore, in patients with a high risk of OHSS, GnRH antagonist should be the preferred protocol. It enables the use of GnRH agonist instead of hCG as ovulation trigger, which markedly decreased the incidence of OHSS. Therefore, use of GnRH antagonists in PCOS patients results in a safer way of performing ovarian stimulation for IVF.</p>

<p>In the present study, it was shown that the flexible GnRH antagonist protocol was associated with a significantly lower probability of moderate–severe OHSS (consequently the need for hospitalization) compared with the long agonist protocol. These results are in corroboration with previous studies in the general population [<xref ref-type="bibr" rid="B13">13</xref> - [<xref ref-type="bibr" rid="B15">15</xref>].</p>

According to the Cochrane 2011 review of 45 RCTs, use of antagonist compared with long GnRH agonist protocols has been shown to be associated with a large reduction in OHSS, and there was no evidence of a difference in live birth rates. When only the women with PCOS were compared, there was no significant difference in the ongoing pregnancy rate. Regarding the safety, a GnRH antagonist significantly reduced the incidence of OHSS by 50%. In addition, with GnRH antagonist treatment, the chance of cancellation or coasting due to high risk to develop OHSS was only 53% of that with the GnRH agonist treatment. The corresponding number needed to harm (NNH) was 25 (95% CI 19–36), with an absolute risk reduction of 4% (95% CI 2.79–5.13). This means that for every 25 women undergoing downregulation by an agonist, you would expect one more case of severe OHSS. In addition, the cancellation rate due to the high risk of developing OHSS was significantly higher in the GnRH agonist group. This indicates that the difference would be highly significant without cancellation, suggesting that GnRH antagonist is safer than GnRH agonist. Therefore, in patients at high risk of OHSS, the GnRH antagonist should be the preferred protocol during their first IVF attempt, because it enables the use of GnRH agonist, instead of hCG, to trigger ovulation, with the consequent elimination of severe OHSS. These benefits would justify a change from the standard long agonist protocol to antagonist regimens [<xref ref-type="bibr" rid="B15">15</xref>].

The meta-analysis of Griesinger *et al.* compared agonist and antagonist protocol in a total of 305 patients with PCOS, and included four studies. In agreement with the results of the present study, pregnancy rates were not significantly different in the agonist and antagonist groups. But, while analyzing the patient at high risk of OHSS, the incidence of severe OHSS was significantly lower in the antagonist group [<xref ref-type="bibr" rid="B16">16</xref>].

Similar results have been shown by Ragini *et. al.* [<xref ref-type="bibr" rid="B17">17</xref>]

The other main advantage of antagonist is that they are more patient friendly. Duration of treatment is short by at least 14 days in the antagonist, and dose of gonadotrophins administered may be low. Although this might not lead to direct reduction in the cost of treatment, but, if we take into consideration the cost of treatment per pregnancy including the cost of hospitalization due to OHSS, number of working hours lost due to prolonged treatment and inconvenience of multiple injections for more days, the final cost may be higher in the agonist protocol. Although there are no studies on economic comparison in the two groups, according to the Cochrane review 2011, significant reduction in the incidence of severe OHSS in the antagonist group could have a direct impact on the reduction of cost of cycle.

There is no risk of withdrawal symptoms, risk of cyst formation and accidental administration of GnRH analogues during early pregnancy. Today, there is an eager desire to shift to more patient-friendly mild ovarian stimulation protocols globally in which use of GnRH antagonists may be a suitable solution [<xref ref-type="bibr" rid="B18">18</xref>-[<xref ref-type="bibr" rid="B20">20</xref>].

The current study suggests that the flexible GnRH antagonist protocol is associated with a similar ongoing pregnancy rate, lower incidence of OHSS, lower gonadotrophin requirement and shorter duration of stimulation, compared with GnRH agonist. Considering, in addition, that the antagonist protocol is more patient friendly as compared with the agonist, GnRH antagonists might be the protocol of choice for patients with PCOS. This, however, remains to be verified by a future meta-analysis.

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