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ASSISTED REPRODUCTION

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

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Abstract

Purpose Embryological and clinical efficacy of gonadotropin-releasing hormone (GnRH) antagonist and agonist stimulation protocols in non-obese women with polycystic ovarian syndrome (PCOS) were compared.

Methods A prospective randomized study. Setting: Medical University Hospital. Patients: 70 infertile PCOS patients; 33 in GnRH antagonist and 37 in GnRH agonist group.

Results Similar mature metaphase II oocyte rate (76% vs. 76%) was observed in both protocols. Optimal pronuclear morphology zygotes dominated in both groups (64% vs. 66%). Transferred embryo quality did not differ in both protocols. No significant differences between both protocols were found in delivery rate (p=0.481), pregnancy rate (p=0.810), multiple pregnancy rate (p=0.501), miscarriage rate (p=0.154), fertilization rate (p=0.388) and implantation rate (p=1.000). Duration of stimulation and total follicle-stimulating hormone (FSH) dose were significantly lower in GnRH antagonist protocol (p=0.0005).

Conclusions GnRH antagonist and agonist protocols in non-obese PCOS patients yield similar embryological and clinical outcomes. Shorter duration of treatment and lower FSH requirement in GnRH antagonist group may be financially beneficial and therefore attractive for patients.

Keywords GnRH agonist · GnRH antagonist · IVF · Polycystic ovary syndrome · Prospective randomized trial

Capsule GnRH antagonist and agonist protocols in non-obese PCOS patients yield similar embryological and clinical outcomes. GnRH antagonist protocols may be financially and clinically attractive for patients undergoing IVF.

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Introduction

The most common endocrine disorder among women of reproductive age and a leading cause of anovulatory infertility is polycystic ovarian syndrome (PCOS) [1, 2]. Because of development of multiple follicles and the associated risk of premature luteinization as well as ovarian hyperstimulation syndrome (OHSS), controlled ovarian hyperstimulation (COH) in patients with PCOS is individually tailored and precisely supervised. The ability of gonadotropin releasing hormone (GnRH) agonists to suppress luteinizing hormones (LH) before and during COH has earned them certain place in IVF treatment protocols in women with PCOS [3]. However, disadvantages of GnRH agonists such as loss of the endogenous feedback mechanism, multiple follicle development and risk of OHSS lead to search alternative ways of ovulation

stimulation in PCOS patients [4]. GnRH antagonists have several theoretical advantages over the GnRH agonists. They act by the mechanism of competitive binding to the GnRH receptors in pituitary which allows a modulation of the degree of hormonal suppression by adjustment of the dose [5, 6, 7]. Furthermore, GnRH antagonists suppress gonadotropin release within a few hours, have no flare-up effect and gonadal function resumes without a lag effect following their discontinuation [4].

Meta-analyses comparing effectiveness of GnRH antagonist and agonist in populace still demonstrate confusing results. Meta-analysis by Kolibianakis et al. [8] showed that the probability of the live birth does not differ between both protocols. On the other hand, the Al-Inany et al. [9] meta-analysis revealed that GnRH antagonist gives significantly worse results than GnRH agonist in the general IVF population. However, most analyses demonstrate that GnRH antagonist protocols have certain advantages over agonists such as reduction of the duration of stimulation as well as the lower incidence of severe OHSS [8, 9, 10]. Meta-analysis by Griesinger et al. showed similar benefits of GnRH analogs regarding PCOS populace [11]. On the other hand, meta-analyses mentioned above did not consider high body mass index (BMI) as an exclusion criteria, while current studies report obesity to be associated with relative gonadotropin resistance [12], lower number of collected oocytes [13, 14] as well as general poorer IVF outcome [15]. According to the latest consensus on infertility treatment related to PCOS, the optimal stimulation protocol is still under debate [4].

Ovarian follicle maturation and development of a competent oocyte depend on pituitary follicle-stimulating (FSH) and luteinizing hormones (LH) [16]. FSH initiates follicle recruitment and development of ovulatory follicles. LH establishes follicle selection, plays an important role in final stages of follicular maturation (resumption of oocyte meiosis), follicular rupture and oocyte expulsion with support of corpus luteum formation. Correct LH level stimulate androstendion production in theca cells to be followed by its aromatization to estradiol in the granulosa cells under the influence of FSH [17, 18]. Patients with PCOS may have elevated amplitude and frequency of LH pulses which can manifest with increased total LH serum concentration (60% of women) [19, 20]. Since LH determines the final stage of the oocyte nucleus maturation and the degeneration of gap junctions, the elevated levels of LH may also activate premature meiotic cleavage, damage the oocyte nucleus and lead to apoptosis [21]. High LH concentrations in patients with PCOS have been associated with an increased rate of miscarriage [22], although it has been questioned recently [23, 24]. Increased androgen concentration in follicular fluid induced by elevated LH may block the dominant follicle selection and cause the follicle degeneration. Failures of folliculogenesis manifest with irregular menstrual cycles and anovulatory infertility. Results of recent Pagán et al. [25] and other studies [26] suggest an inverse relationship between BMI and LH in PCOS patients. Therefore, since the relative importance and the role of monitoring of LH in follicular development are still disputable we decided to examine non-obese PCOS women with theoretically higher LH levels.

In the current study we decided to verify the embryological and clinical effectiveness of the GnRH antagonists protocols in comparison with GnRH agonist protocols in non-obese women with PCOS. In addition, we investigated correlation between LH levels and the treatment outcome in PCOS non-obese patients.

Materials and methods

Participants

This randomized, prospective, single center trial was conducted at the Department of Reproductive Medicine and Gynecology, Pomeranian University of Medicine in Szczecin in years 2004–2006. PCOS patients were considered eligible if they were scheduled for controlled ovarian stimulation and intracytoplasmic sperm injection (ICSI). Indications for ICSI included: male factor subfertility, several unsuccessful intrauterine inseminations, previous ineffective IVF (none or <30% of fertilizations). General inclusion criteria for all study participants included: (1) meeting of 2003 Rotterdam PCOS criteria (two of the following three manifestations: irregular or absent ovulation, elevated levels of androgenic hormones, and/or enlarged ovaries containing at least 12 follicles each; other conditions with similar signs, such as androgen-secreting tumors or Cushing's syndrome were ruled out); (2) age≤35 years; (3) body mass index<26 kg/m^2; (4) FSH<12 mIU/ml on the third day of the cycle; (5) negative screening for hepatitis B and C virus infection and human immunodeficiency virus (HIV) infection. Exclusion criteria included: ≥2 miscarriages, ≥3 unsuccessful IVF/ICSI cycles, anatomical abnormalities of the uterus on laparoscopy or hysteroscopy and existence of ovarian cysts.

Randomization

Each participant was allocated to one of two arms of the study according to a computer generated random letters (A for GnRH antagonists protocol or B for GnRH agonists protocol). Allocations were concealed in opaque sealed envelopes, opened once written informed consent according to ethical committee of the Pomeranian University of Medicine had been obtained. The patients were not blinded

to treatment group. In both protocols, only two clinicians and two embryologists, also not blinded to treatment group, were involved in the study. Trial registration: ACTRN12607000636459.

Outcome measures

Primary endpoints:
Embryological:

- Matured oocytes (M2) rate, defined as proportion of metaphase II to total number of retrieved oocytes
- Fertilization rate, defined as proportion of two pronuclei oocytes to number of injected oocytes
- Quality of zygotes on the first day of culture
- Quality of embryos on the third day of culture

Secondary endpoints:
Clinical:

- Delivery per attempt, defined as a live birth after 32 weeks of gestation
- Clinical pregnancy per attempt, defined as an ongoing pregnancy at 12 weeks of gestation
- Implantation rate; defined as gestational sacs per number of transferred embryos
- Multiple pregnancy per viable pregnancy
- Miscarriage per intrauterine pregnancy, defined as a miscarriage of an ongoing pregnancy after 12 weeks of gestation
- Occurrence of severe OHSS
- Number of days of gonadotropin treatment
- Gonadotropin consumption
- Correlation between serum LH level and IVF outcome

Sample size calculation

Sample size calculation was carried out to meet statistical eligibility to verify primary embryological end points. The sample size analysis assuming comparison of two groups (with at least 40% patients in one of them) using Mann–Whitney test showed that 70 women in total will need to be recruited for a power of 80% and an alpha of 5% to detect the hypothetical true difference between the groups equal to 15% of mature oocyte (M2) when the estimated standard deviation of the parameter is 20% (M2% was approximately 75±20% according to our laboratory results), 66 women in total will need to be recruited for a power of 80% and an alpha of 5% to detect the hypothetical true difference between the groups equal to 2 of quality of zygotes on the first day of the culture when the estimated standard deviation of the parameter is 2.5 (optimal pronuclear morphology of all embryos (Z1+Z2) was approximately 5±2.5 according to our laboratory results), 42 women in total will need to be recruited for a power of 80% and an alpha of 5% to detect the hypothetical true difference between the groups equal to 0.5 of quality of zygotes on the 3rd day of the culture when the estimated standard deviation of the parameter is 0.5 (optimal pronuclear morphology of transferred on day 3 embryos (Z1+Z2) was approximately 2±0.5 according to our laboratory results).

Protocol for controlled ovarian hyperstimulation

All patients received oral contraceptives pills (Cilest; Janssen-Cilag, Belgium) for a month before starting COH. None of the patients used oral antidiabetic medications (biguanides or thiazolidinediones).

GnRH antagonist protocol

From the second day of the cycle women were given regular daily recombinant human FSH (Gonal F; Merck Serono, Switzerland) subcutaneous injections (usually between 1800 and 2000 hours). Starting dose of a 150 IU/day was adjusted individually depending on an ovarian response measured by transvaginal ultrasonography and the level of estradiol. Ultrasound and estradiol level monitoring started from the seventh day of the cycle (sixth day of COH) after five doses of rFSH and was continued every second day until the day of human chorionic gonadotropin (hCG) administration. A GnRH antagonist—cetrorelix (Cetrotide; Merck Serono, Germany) was administered subcutaneously between 900 and 1200 hours when at least two ovarian follicles reached 14 mm in diameter. The protocol consisted of daily Cetrotide 0.25 mg subcutaneous injections, average 4, until the criteria for recombinant hCG administration were met.

GnRH agonist protocol

During oral contraception (OC) on days 16–18 of the preceding cycle, after transvaginal ultrasonographic screening of ovaries, an intramuscular injection of GnRH agonist triptorelin (Diphereline SR 3.75; Boufor Ibsen Pharma, France) was given. After confirmation of pituitary desensitization (LH <2 mIU/mL and estradiol <40 pg/mL) the administration of FSH was commenced. Women were given regular daily recombinant human FSH (Gonal F; Merck Serono, Switzerland) subcutaneous injections (usually between 1800 and 2000 hours). Also in this protocol starting dose was 150 IU/day, adjusted individually depending on ovarian response. Ultrasound and estradiol level monitoring started after five doses of rFSH and was continued every second day until the day of hCG administration.

Final follicular maturation

The final oocyte maturation in both protocols was induced by an intramuscular (Pregnyl; Organon, Holland) injection of 10,000 IU hCG or subcutaneous (Ovitrelle; Merck Serono, France) injection of 250 μg hCG when the dominant follicle reached ≥18 mm with the following two ≥16 mm and estradiol level between 1,000 and 4,000 pg/mL.

Hormonal assessment

LH and estradiol (E2) levels were determined from a single serum sample collected from each woman between 800 and 900 hours. Serum LH and estradiol levels were measured by an electrochemiluminescence immunoassay 'ECLIA' (Roche Diagnostics Inc., Germany) using the Roche Elecsys 2010 automated immunoassay analyzer by the local laboratory. LH_1 in GnRH antagonist protocol refers to LH level at the beginning of the COH and to the LH level 12–14 days after triptorelin injection in the GnRH agonist protocol. LH_2 corresponds to the LH level on the sixth to seventh day of the stimulation in both protocols. On the day of ultrasonographic and estradiol indication for hCG administration LH_3 was assessed.

Oocytes retrieval

Oocytes were retrieved with transvaginal ultrasound guided aspiration needle (OPS Single lumen: without tap; Laboratoire C.C.D, France) 36 h after hCG administration to a collection tubes containing HEPES-buffered medium (FertiCult Flushing medium, FertiPro, Belgium), washed, and then cultured in ISM1 medium (Medicult, Denmark). Before ICSI, a 10% hyaluronidase solution (Hyaluronidase solution in Flushing Medium, FertiPro, Belgium) dose was used to remove cumulus cells. After that, oocytes were washed in flushing medium (FertiCult Flushing medium, FertiPro, Belgium) until the ICSI. Retrieved oocytes in metaphase II (M2) were classified as mature and in metaphase I (M1) or germinal vesicle stage (GV) as immature. Only mature M2 oocytes underwent ICSI.

Sperm preparation

Semen samples in all cases were obtained by masturbation. Sperm was extracted from seminal plasma by density gradient technique on gradient system for semen preparation (Sil-Select-Plus; FertiPro, Belgium) or in cases of severe oligospermy by wash technique in combination with double centrifugation (Washing/insemination medium; FertiPro, Belgium).

Sperm assessment

Semen parameters were assessed according to the WHO (motility and concentration) [27] and Krugers (morphology) [28] criteria. Based on the results sperm parameters were classified as normal in case of semen concentration over 20 mln/ml, 50% motility A+B in semen and at least 14% normal spermatozoa, as mild oligoasthenoteratozoospermy (OAT) in cases with 10–20 mln/ml sperm concentration or decreased sperm motility (20–40% motility A+B in semen) or 10–14% of normal spermatozoa, moderate OAT in case of 3–10 mln/ml sperm concentration or 5–19% of motility A+B in semen or 5–10% of normal cells and severe OAT for up to 3 mln/ml concentration or below 5% of motility A+B in semen or below 5% of normal sperm cells (Table 1).

Intracytoplasmic sperm injection

During ICSI micro-manipulation holding pipette (K-HPIP-3335; Cook, USA) and injection pipette (K-MPIP-1035; Cook, USA) were used. Injected oocytes were placed in ISM1 medium (Medicult, Denmark) immediately after the procedure. This medium was used for the first 48 h of the culture and for the following 24 h ISM 2 medium (Medicult, Denmark) was used.

Table 1 Clinical characteristics and the history of treatment of the patients in the treatment protocols

	GnRH antagonist	GnRH agonist
Number of patients	33	37
Age (years)	31.33±3.91	30.36±3.40
BMI (kg/m^2)	23.1±1.3	22.3±1.6
Male cause of infertility (number of patients)		
Normal semen parameters	9	6
Coexisting male factor	24	31
Mild OAT	10	13
Moderate OAT	8	11
Severe OAT	6	7
Previous treatment history (number of patients)		
Laparoscopy	19	31
IUI	17	21
IVF/ET	5	6
ICSI/ET	16	25
Previous obstetrics history (number of patients)		
Pregnancies	8	6
Deliveries	2	2
Extrauterine pregnancies	3	1
Miscarriages	3	3

When appropriate values are means (±SD)
BMI Body mass index, *OAT* oligoasthenoteratozoospermy, *IUI* intrauterine insemination, *IVF* in vitro fertilization, *ICSI* intracytoplasmic sperm injection, *ET* embryo transfer

Embryo evaluation

Fertilizations were checked during routine non-invasive examination 16–18 h after ICSI [29, 30]. Embryo grading was assessed on the pronuclear stage according to Scott et al. [31] criteria and on the day of the embryo transfer according to the internal laboratory embryo score standards [32]. We also distinguished 'class A' embryos on the third day of the culture defined as an embryo with ≥8 symmetrical, non-fragmented blastomers, without cytoplasmic defects.

Embryo transfer (ET)

Decision on number of transferred embryos was taken according to ASRM embryo-transfer guidelines [33]. Embryos were transferred to uterine cavity in the catheter (FRYDMAN SOFT 4.5, Laboratoire C.C.D., France) 72 h after ICSI.

Luteal support

Luteal phase support included simultaneous oral 30 mg/day of dydrogesterone (Duphaston; Solvay Pharma, Belgium) and intravaginal 150 mg/day of progesterone (Luteina; Adamed, Poland).

Pregnancy confirmation

Pregnancy was checked by pregnancy test in serum 14 days after ET and confirmed by vaginal ultrasound scan at 12 weeks of gestation. Biochemical gestation was not taken into consideration at any stage of the trial.

Statistical tests

Since the majority of quantitative variables were non-normally distributed, they were analyzed by non-parametric Mann–Whitney test. Chi-square test or Fisher's exact test for 2×2 tables were used to analyse statistical significance for qualitative variables. Statistical significance of LH levels variability in each group was verified by Friedman's ANOVA followed by Wilcoxon's matched-pair test. Spearman's rank correlation coefficient was used to measure the correlations between the quantitative variables. p value<0.05 was considered as significant. All calculations were performed using Statistica for Windows 7.1 (StatSoft Inc., Tulsa; USA).

Results

A total of 74 women were randomized, 37 were allocated to the GnRH antagonist protocol arm and 37 to the GnRH agonist arm. 4 women in the GnRH antagonist group were excluded after randomization, two of them because of insufficient compliance with medication as established by the respective protocol. Further two patients quit the preparations for the treatment without notice. Thus, group I consisted of 33 patients treated with the GnRH antagonist cetrorelix (Cetrotide; Merck Serono, Germany). Group II included 37 patients treated with the GnRH agonist triptorelin (Diphereline SR 3.75; Boufor Ibsen Pharma, France). All 70 women included in the study underwent embryo transfer and none was lost to follow-up. The clinical characteristics and the history of treatment of the patients in both examined groups are shown in Table 1. Baseline patient characteristics did not differ.

Number of oocytes, mature metaphase II oocyte rate and fertilization rate were similar in both protocols. Zygotes with optimal pronuclear morphology classified as Z1 and Z2 dominated in both groups with no significant differences between studied protocols (Table 2). Transferred embryo quality did not differ in both protocols. An average transferred embryo had seven symmetrical, non-fragmented blastomers (Table 3).

There were no differences in GnRH antagonist vs. GnRH agonist in delivery rate, implantation rate, pregnancy rate, multiple pregnancy rate, miscarriage rate (Table 4). Two cases of severe OHSS were identified in GnRH agonist group.

The number of stimulation days and total FSH dose were significantly lower in the group stimulated with GnRH antagonist. Serum LH levels at the beginning, during and on the day of hCG administration were significantly higher in the group stimulated with GnRH antagonist. Serum E2 levels on the day of hCG administration were similar in both studied protocols (Table 5).

We found a negative correlation in GnRH antagonist and agonist protocol between duration of the COH and serum LH level at the beginning ($r=-0.41$ and $r=-0.49$), during COH ($r=-0.43$ and $r=-0.58$) and on the day of hCG administration ($r=-0.44$ and $r=-0.59$), respectively. There were no correlations between serum LH concentration and delivery rate, implantation rate, pregnancy rate, miscarriage rate, mature metaphase II oocyte and fertilization rate in both studied protocols.

Discussion

The concept to employ GnRH antagonist along with the administration of exogenous FSH to induce development of multiple follicles constitutes an interesting strategy for controlled ovarian stimulation in PCOS patients. However, we still do not have an indisputable proof of effectiveness

Table 2 Embryological outcome measures: characteristics of the oocytes and pronuclear morphology in GnRH antagonist and agonist protocols

	GnRH antagonist median (min–max)	GnRH agonist median (min–max)	*p* value[a]
Number of retrieved oocytes	13 (5–28)	14 (2–23)	0.903
Mature metaphase II oocyte (%)	82.3 (31.3–100)	80 (33.3–100)	0.903
Fertilization rate (%)	73.9 (33.3–100)	85.7 (25–100)	0.388
Pronuclear morphology of all embryos			
Z1	1 (0–6)	1 (0–5)	0.551
Z2	3 (1–7)	3 (0–7)	0.263
Z1+Z2	4 (1–10)	4 (1–12)	0.672
Z3	2 (0–9)	2 (0–7)	0.794
Z4	0 (0–2)	0 (0–6)	0.195
Pronuclear morphology of transferred on the third day of culture embryos			
Z1	0.5 (0–3)	1 (0–2)	0.885
Z2	1 (0–2)	1 (0–3)	0.689
Z1+Z2	2 (1–3)	2 (0–3)	0.498
Z3	0 (0–2)	0 (0–2)	0.949
Z4	0 (0–1)	0 (0–1)	0.195

Z1+Z2=optimal pronuclei
[a] Mann–Whitney test

and safety of GnRH antagonists in PCOS patients and more studies concerning PCOS patients and GnRH antagonist are needed [3]. In our study we managed to gather a homogeneous group of 70 BMI<26 PCOS women. By including only non-obese women with PCOS we decreased the risk of possible influence of metabolical disturbances and negative impact of the obesity on the oocyte number and the duration of the stimulation [13, 14]. Furthermore, to study LH dynamics during COH in both protocols we decided to exclude BMI inhibitory effect on LH [25, 26]. Current study presents detailed embryological portrayal of both studied protocols, includes clinical outcome measures as well as correlations of LH levels and overall outcomes at different stages of the COH.

The median number of retrieved oocytes, mature oocyte rate and fertilization rate did not differ between two studied groups, and consequently the median number of obtained embryos was comparable. It was postulated that oocytes retrieved from patients with PCOS are often immature and of poorer quality with lower fertilization capacity [34, 35], although it has been disputed in our previous comparison [36]. In current study we have also demonstrated that the mature oocytes dominated in retrieved cohort of oocytes with no significant differences between both studied protocols. Also, in contrast to mentioned studies [24, 35], fertilization capacity was high in both groups and the zygotes had mainly optimal pronuclear morphology with no significant differences between studied protocols. Also Griesinger et al. [11] in his meta-analysis concerning 3 studies [37, 38, 29] and abstract by Kim et al. [40] reveals no significant differences between two studied protocols regarding the number of cumulus oocyte complexes in PCOS populace.

Generally, the clinical outcome measures were similar in both studied groups. We have shown pregnancy rate ~60%, implantation rate ~30% and delivery rate >40% per attempt

Table 3 Embryological outcome measures: characteristics of the embryos on the third day of culture in GnRH antagonist and agonist protocols

	GnRH antagonist median (min–max)	GnRH agonist median (min–max)	*p* value[a]
Number of embryos	6 (3–18)	8 (1–14)	0.448
Class A embryos among all embryos	2 (0–9)	2 (0–6)	0.830
Number of transferred embryos	2 (2–3)	2 (1–3)	0.520
Mean number of blastomers in transferred embryo	7.75 (4–9)	8 (4–9)	0.912
Quality of blastomers in transferred embryos			
Symmetry			
A	2 (0–3)	2 (0–3)	0.785
B	0 (0–3)	0 (0–3)	0.672
C	0 (0–0)	0 (0–0)	–
Fragmentation			
None	2 (0–3)	2 (0–3)	0.263
<20%	0 (0–3)	0 (0–3)	0.455
20–50%	0 (0–0)	0 (0–1)	0.732
>50%	0 (0–0)	0 (0–0)	–

Class A embryo An embryo with ≥8 symmetrical, non-fragmented blastomers, without cytoplasmic defects.
[a] Mann–Whitney test

Table 4 Comparison of clinical outcome measures in GnRH antagonist and agonist protocols

	GnRH antagonist	GnRH agonist	*p* value	OR (95% CI)[c]
Delivery per attempt (%)	14/33 (42%)	18/37 (49%)	0.481[a]	1.43 (0.55–3.71)
Implantation rate (%)	23/76 (30%)	27/89 (30%)	1.000[b]	1.02 (0.52–1.98)
Pregnancy per attempt (%)	20/33 (61%)	21/37 (57%)	0.810[a]	0.85 (0.33–2.22)
Multiple pregnancy rate (%)	3/33 (9%)	5/37 (13%)	0.501[b]	1.85 (0.44–7.85)
Miscarriages rate (%)	6/33 (18%)	3/37 (8%)	0.154[b]	0.32 (0.07–1.38)
Severe OHSS occurrence	None	2/37 (5%)	–	–

OHSS Ovarian hyperstimulation syndrome
[a] Chi squared test
[b] Fisher exact test
[c] Odds ratio (95% confidence interval) for GnRH agonist vs. GnRH antagonist

in our PCOS patients. These results are comparable to the percentages reported in other studies of PCOS patients. Bahceci et al. [38] reported pregnancy rates of 57.6% and 58.5%, implantation rates of 34.0% and 34.6% in GnRH antagonist and agonist arms of the study, respectively. Hwang et al. [39] among PCOS patients stimulated with GnRH antagonist and GnRH agonist reported pregnancy rate per started cycle of 37% and 34.5%, respectively. While meta-analyse by Kolibianakis et al. suggests that the probability of live birth is not dependent on the type of GnRH analogue used for suppression of premature LH rise/surge [8], Al-Inany et al. [9] showed significant lower the ongoing pregnancy/live-birth rate in the antagonist group. We observed a trend towards lower rates of delivery for GnRH antagonist group. Prior reports also mentioned higher multiple gestations rate in women with PCOS [41]. In our investigation pregnancies were mainly singleton, there were three sets of twins in GnRH antagonist group and five sets of twins plus one set of triples in GnRH agonist group.

Some studies previously reported lower conception rates in PCOS women undergoing ovulation induction [42]. This could not be confirmed in the current study, especially when compared with mean pregnancy rates after ICSI per transfer in the European (28.7%) and American studies (41%) [43, 44]. Although miscarriage rate in our study did not differ between both studied groups, we observed tendency to higher number of miscarriages in GnRH antagonist than in GnRH agonist group (18% vs. 8%, respectively). Conversely, Hwang et al. [39] reported lower percentage of miscarriages in GnRH antagonist group (10% vs. 20%, respectively). Previous studies suggest increased risk of OHSS in women with PCOS [41, 42]. In current study, two cases (5%) of severe OHSS were observed in GnRH agonist group. One of them was found in a patient with twin pregnancy and one in a patient with triplet pregnancy. Similar occurrence of ovarian hyperstimulation syndrome in both studied groups was reported by Bahceci et al. (5% vs. 7.1%) [38] and Hwang et al. (8% vs. 8.3%) [39]. On the contrary, Ashrafi et al. [37] reported significantly higher number of PCOS patients at risk of developing OHSS (more than 20 follicles visited in each ovary or E_2>3,000 pg/ml) in patients in GnRH antagonist arm than GnRH agonist arm of the study (0% vs. 30.4%, p=0.004)

Undoubtedly, LH plays an important role during ovarian stimulation [45]. Decreased LH concentrations result in

Table 5 Comparison of clinical outcome measures: hormone evaluation in GnRH antagonist and agonist protocols

	GnRH antagonist median (min–max)	GnRH agonist median (min–max)	*p* value [a]	mean difference (95% CI) [b]
Number of FSH stimulation days	9 (6–21)	11 (6–19)	0.0005	−2.0 (−3.4 to −0.6)
Total FSH dose (IU)	1,087.5 (937,5–3,150)	1,275 (1,050–2,550)	0.005	−413 (−764 to −62)
Serum LH_1 level (IU/L)	3.7 (0.47–15.7)	0.71 (0.2–4.1)	<0.0001	+3.5 (+2.3 to +4.7)
Serum LH_2 level (IU/L)	1.27 (0.14–4.4)	0.75 (0.3–3.3)	0.002	+0.6 (+0.3 to +0.9)
Serum LH_3 level (IU/L)	1.4 (0.14–2.6)	0.7 (0.1–3.8)	0.002	+0.5 (+0.1 to +0.9)
Serum E2 level on hCG day (pg/mL)	2,200 (752–8,930)	2,037 (426–7,000)	0.322	+188 (−431 to +807)

FSH follicle-stimulating hormone, *LH* luteinizing hormone, *E2* estradiol, *hCG* human chorionic gonadotropin, LH_1 LH level at the beginning of the hyperstimulation in GnRH antagonist protocol and LH level 12–14 days after triptorelin injection in the GnRH agonist protocol, LH_2 LH level on the sixth to seventh day of the controlled ovarian hyperstimulation, LH_3 LH level assess on the day of hCG administration
[a] Mann–Whitney test
[b] Mean difference between the GnRH antagonist and agonist group and its 95% confidence interval

estradiol production impairment, follicle selection derangement, inability of luteinization and ovulation after hCG administration [46]. Increased LH concentrations are believed to be responsible for poor oocytes quality, low fertilization rate and high miscarriage percentage in patients with PCOS [47, 48]. Optimal concentration of LH needed to most favorable COH became the subject of many studies and discussions. In our study serum LH levels at the beginning and during COH as well as on the day of hCG administration were substantially higher in a group stimulated with GnRH antagonist. Nevertheless, there were no correlations between serum LH concentration and delivery rate per attempt, pregnancy rate per attempt, retrieved mature M2 oocyte rate and fertilization rate in both studied protocols. We also did not find relationship between serum LH levels and miscarriages among all patients and among pregnant women. Even though other studies reveal lack of association between IVF outcome and the LH level on the beginning [49, 50] and during [51–54] COH, they did not concern PCOS patients. Study by Lainas et al. [55] described significantly lower LH levels on days 3 and 5 and significantly higher LH levels on days 1, 7 and 8 of stimulation in the antagonist when compared with the agonist group among PCOS women. But these study did not consider the influence of LH on IVF outcome.

Al-Inany et al. [10] after evaluation of 27 randomized controlled trials that compared GnRH antagonist with long GnRH agonist protocol in all types of patients reported reduced stimulation and gonadotropin consumption with the antagonist protocol. Griesinger et al. [11] including only PCOS patients in his meta-analysis indicates that the number of stimulation days were significantly decreased with GnRH antagonists multiple dose in comparison with GnRH agonist long protocol, though it was not associated with a significant reduction in gonadotropin total dose. We found a negative correlation between serum LH level and duration of the COH in GnRH antagonist and agonist protocol. However, duration of stimulation and the FSH consumption in the current study were significantly reduced in a group stimulated with GnRH antagonist in comparison to GnRH agonist group. We hypothesise that increased levels of LH during first period of GnRH antagonist protocol could be associated with higher FSH sensibility and consequently shorter stimulation length and lesser gonadotropin utilization, but more studies concerning this theory are needed.

Until recently it was clear that the stable suppression of luteinizing hormone level during ovarian hyperstimulation should be achieved in order to avoid LH negative effect. However, LH level in patients prepared for COH is already decreased with oral contraceptive (OC) on the preceding cycle [39]. Subsequently, according to our results, pulsative LH secretion in GnRH antagonist protocol within the normal physiological range did not have effect on overall outcome. On the contrary, higher LH levels were associated with shorter duration of stimulation.

Acceptance of GnRH antagonist co-treatment during COH for in vitro fertilization is relatively slow [56]. The issue of which analogue is the best for PCOS patients is still open and more comparative studies between agonists and antagonists are necessary. Present study demonstrates comparable embryological and clinical outcomes in GnRH antagonist and agonist protocols in non-obese PCOS patients. Shorter duration of treatment and lower FSH requirement observed with GnRH antagonist may be financially beneficial and therefore attractive for PCOS non-obese patients. LH levels in PCOS patients during COH have no negative effect on the overall COH efficiency.

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