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Outcome and recurrence risk of premature progesterone rise in IVF/ICSI cycles using GnRH antagonists for pituitary down-regulation

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Summary

Summary: To assess the outcome and recurrence risk of premature progesterone rise (PPRR) in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles using gonadotropin-releasing hormone (GnRH) antagonists. *Materials and Methods:* Two hundred and two patients undergoing IVF/ICSI using GnRH antagonists for pituitary down-regulation had measurements of luteinizing hormone (LH), progesterone (P), and estradiol (E2) on specific days of the first and subsequent stimulation cycle. *Results:* The overall clinical pregnancy rate including the total of 280 cycles was 29.3% (82/280). The incidence of PPRR on the day of triggering for P $\geq$ 1.5 ng/ml its was 20.8% (42/202). The risk of PPRR $\geq$ 1.5 ng/ml on the triggering day of the subsequent cycle was 40% if the previous cycle P was $\geq$ 1.5 ng/ml, 13.3% if the previous cycle P was $\geq$ 1.2 ng/ml and < 1.5 ng/ml, and 10% if the previous cycle P was < 1.2 ng/ml. *Conclusion:* The presence of PPRR in IVF/ICSI cycles using GnRH antagonists affects negatively the pregnancy rates and poses a significant risk of recurrence in a subsequent cycle.

Key words: GnRH antagonist; Ganirelix; Ovarian stimulation; Premature progesterone; IVF; ICSI.

Introduction

The introduction of gonadotropin-releasing hormone (GnRH) analogs into clinical practice has brought new consciousness in controlled ovarian hyperstimulation (COH) for assisted reproductive technology (ART) and a significantly decreased incidence of premature luteinizing hormone (LH) surge [1]. Despite pituitary down-regulation, however, several researchers have described a phenomenon reported as premature luteinization (PL) [2-5]. This refers to a rise in serum progesterone (P) levels on the day of human chorionic gonadotropin (hCG) administration for final oocyte maturation above a threshold level, which is usually arbitrarily defined. Premature progesterone rise (PPR) on triggering day occurs without increase in the levels of LH [6]. At present, there is no a unanimous opinion on whether progesterone elevation on the triggering day is affecting the achievement of pregnancy. Various studies have denied the presence of such an association [7-10], whereas others have confirmed the presence of a negative association [11, 12-14]. Most studies used an absolute P level on the day of hCG administration as an indicator of PL, and the cutoff level differed from 0.8 to 2 ng/ml [15, 16]. Antagonists analogs of GnRH have a direct inhibitory, reversible suppressive effect of gonadotropin secretion. Antagonistic molecules compete for and occupy pituitary GnRH receptors, thus competitively blocking the access of endogenous GnRH and precluding substantial receptor occupation and stimulation. Suppression attained by GnRH antagonists is immediate with no flare up effect and as receptor loss does not occur, a constant supply of antagonists to the gonadotroph is required to ensure that all GnRH receptors are continuously occupied [17]. LH acts directly on theca cells where LH receptors are constitutively present and ensure a tonic production of androgens during the whole follicular phase. Another question that has to be taken under consideration, is the cause of P rise during the follicular phase of assisted reproduction cycles. It is more likely that the elevated P levels might be attributed to an excess number of follicles that each produces normal amounts of P [18]. Moreover, in the late follicular phase, the secretion of P and the number of mature follicles correlate positively [19]. The raised peripheral concentrations of P in the late follicular phase are likely to influence the secretory changes of the endometrium, leading to impaired endometrial receptivity [20].

The aim of this prospective study was to evaluate the outcome of in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles with premature luteinization and to assess the risk of recurrence of PL on individuals that un-

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Table 1. — *Patients' characteristics.*

Mean age (years)	35.3 ± 4.7
Mean body mass index (BMI)	27.2 ± 2.6
Duration of infertility	3.3 ± 2.5
Cause of infertility	
Ovulatory disorders	19%
Tubal damage	18%
Male factor	38%
Idiopathic infertility	25%

derwent assisted reproduction regimens with antagonist down-regulation more than once.

Materials and Methods

This is a prospective study of women that underwent IVF/ICSI cycles with controlled ovarian stimulation (COS) and antagonist down-regulation between January 2009 and July 2015. A total of 202 patients were included and underwent 280 gonadotropin IVF/ICSI cycles. Patients were treated at the IVF unit of the Second Department of Obstetrics and Gynecology of University of Athens. Inclusion criteria were to have undergone IVF/ICSI cycle, BMI < 30, absence of endometriosis, and FSH < 12 IU/ L.

Patients underwent COS with the use of a fixed GnRH-antagonist protocol. COS was started on days 2-3 of the menstrual cycle with 150-400 IU daily of recombinant FSH. Serum estradiol (E2), LH, and P were assessed on days 2-3, days 6-7, two to three days later depending on the levels of E2 and on the day of hCG administration. Starting dose of FSH was decided taking into consideration the patients age, BMI, response to a previous COS cycle, antral follicle count, and AMH if available. Management decision making of subsequent cycle was not affected by levels of P on triggering day of the previous cycle. FSH dose was adjusted according to ovarian response using a step-up or step-down protocol. The GnRH-antagonist ganirelix was administrated using a dose of 0.25 mg on a daily basis starting the 5th day of the stimulation cycle until the day of hCG administration. Patients were evaluated with ultrasonography examination and hormonal measurements every two to three days. hCG 5,000-10,000 IU subcutaneously was administrated when three or more follicles reached 18 mm in diameter and oocyte retrieval was scheduled 36 hours later. Embryo transfer was performed on the 3rd day after oocyte retrieval. The luteal phase was supported using natural P at a dose 200 mg tds vaginally from the date of oocyte retrieval. A measurement of β-HCG was done 14 days after the embryo transfer and ultrasound examination was performed at seven weeks of gestation to confirm the diagnosis of clinical pregnancy. Clinical pregnancy was defined as the presence of a gestational sac at about seven weeks of pregnancy with positive heartbeat. Ongoing pregnancy was defined a pregnancy more than 16 weeks with a positive heart beat confirmed by ultrasound. Written informed consent was obtained from all patients, and the study was approved by institutional ethics committee (Trial registration number: M94/22-12-2011).

D'Agostino–Pearson test was used for assessment of sample distribution. Chi-Square test was used for comparison of proportions and the *p* values were two-tailed. Statistical analysis was performed using the Medcalc software version 12.1.4.0 and a $p < 0.05$ was considered statistically significant.

Results

Two hundred and two patients were enrolled in the study and underwent a total of 280 IVF/ICSI cycles. There were 202 first IVF/ICSI attempt cycles and 78 repeat cycles. The mean age of the participants was 35.3 ± 4.7 years. The main causes of infertility in the present study were approximately: ovulatory disorders 19%, tubal damage 18%, male factor 38%, and 25% unexplained cases of infertility (Table 1).

The clinical pregnancy rate during their first cycle for the 202 patients was 29.7% (60/202). The overall clinical pregnancy rate including the total of 280 cycles was 30% (84/280). The clinical pregnancy rate according to the levels of P on the triggering day are presented in Table 2. Also, the P levels on the day of triggering for first IVF/ICSI cycle and the overall cycles (first and subsequent cycles) are presented in Table 2.

The risk of progesterone rise ≥ 1.5 ng/ml on the triggering day of the subsequent cycle if a) the first cycle P was ≥ 1.5 ng/ml (40%), b) if the first cycle P was ≥ 1.2 ng/ml and < 1.5 ng/ml (13.3%), and c) if the first cycle P was < 1.2 ng/ml was 10% (Table 3).

Discussion

The introduction of GnRH analogs for pituitary suppression in IVF significantly decreased the incidence of premature LH surge [1]. GnRH agonists are characterized by the

Table 2. — *Clinical pregnancy rate in relation to progesterone levels on the triggering day during the first IVF/ICSI cycle and including the total of examined cycles (280 cycles).*

Levels of progesterone on triggering day	First cycle (202 cycles)			Overall examined cycles (280 cycles)		
	Percentage of patients (%)	Clinical pregnancy rate	Ongoing pregnancy rate	Percentage of patients	Clinical pregnancy rate	Ongoing pregnancy rate
< 1.2 ng/ml	63.4% (128/202)	31.25%^{a1*} (40/128)	29.7% (38/128)	65% (182/280)	33% ^{a2}# (60/182)	31.3% (57/182)
≥ 1.2 ng/ml & < 1. 5ng/ml	15.8% (32/202)	43.7% ^{b1**} (14/32)	37.5% (12/32)	13.6% (38/280)	42.1%^{b2}## (16/38)	36.8% (12/38)
≥ 1.5 ng/ml	20.8% (42/202)	14.28%^{c1} (6/42)	9.5% (4/42)	21.4% (60/280)	13.3 %^{c2} (8/60)	6.6% (4/60)

*$p < 0.05$; **$p < 0.01$; #$p < 0.04$; ##$p < 0.002$.

*Statistical significant difference between a1 and c1. **Statistical significant difference between b1 and c1. #Statistical significant difference between a2 and c2. ##Statistical significant difference between b2 and c2.

Table 3. — *Recurrence risk of progesterone > 1.5 ng/ml in the subsequent cycle in relation to progesterone levels on the triggering day of the first cycle.*

Levels of progesterone on the 1st and subsequent cycle.	Percentage of recurrence risk/ in brackets number of cases
1st P ≥1.5 ng/ml then 2nd > 1.5 ng/ml	40% (8/20)
1st P < 1.5 ng/ml then 2nd > 1.5 ng/ml	13.3% (4/30)
1st P ≥ 1.2 and < 1.5 ng/ml then 2nd > 1.5 ng/ml	20% (2/10)
1st P < 1.2 ng/ml then 2nd > 1.5 ng/ml	10% (2/20)

1st: first IVF/ICSI cycle, P: progesterone, 2nd: second IVF/ICSI cycle.

presence of a flare-up effect on the secretion of FSH and LH from the pituitary, while suppression attained by GnRH antagonists is immediate with no flare-up effect and because there is no receptor loss, a constant supply of antagonists to the pituitary is required to ensure that all GnRH receptors are continuously occupied [17]. Concerns have been raised in the literature by different researchers concerning a phenomenon reported as PL [2, 5], which refers to a inappropriate rise of P on the day of oocyte triggering. PPRR on triggering day occurs without increase in the levels of LH [6]. Furthermore it has been proposed that major components of this PPR are: a) the number of follicles (granulosa cells), b) the level of FSH stimulating the ovaries, and c) the amount of available LH, which by acting on theca cells will promote conversion of P to androgens and estrogens [18].

In the present study, the incidence of P levels between ≥ 1.2 and < 1.5 ng/ml and > 1.5 ng/ml was 15.8% (32/202) and 20.8%, respectively, for the first cycle and 13.6% (38/280) and 21.4%, respectively, for the overall cycles examined, and there was no statistically significant difference concerning the incidence of P rise between first and subsequent cycle. The clinical pregnancy rate for P levels < 1.2 ng/ml was 31% (40/128) for the first cycle and 33% (60/182) for overall cycles, P between ≥ 1.2 and < 1.5 ng/ml and > 1.5 ng/ml was 44% (14/32) for first cycle and 42% for overall cycles (16/38), and for P ≥ 1.5ng/ml was 14% for first cycle and 13% for overall cycles, respectively. There was no statistically significant difference ($p < 0.05$) concerning the corresponding clinical pregnancy rates between first and overall cycles. The results concerning incidence of PPR and clinical pregnancy rates in the first cycle are comparable with other studies [21]. Also, there was a statistically significant reduction of clinical pregnancy rate of total (overall) cycles with P ≥ 1.5 ng/ml (13.3%) compared with cycles of P ≥ 1.2 and < 1.5 ng/ml (42%) ($p < 0.002$, Table 2) and compared with cycles with P < 1.2 ng/ml (33%) ($p < 0.04$). There was no statistically significant difference concerning clinical pregnancy rates between P levels < 1.2 ng/ml and ≥ 1.2 and < 1.5 ng/ml, which probably signifies that values of P < 1.5 ng/ml do not affect clinical pregnancy rate and is similar with other studies [22].

At present, there is no unanimous opinion on whether P elevation on the triggering day is affecting the achievement of pregnancy. Various studies have denied the presence of such an association [8,-10], whereas others have confirmed the presence of a negative association [11, 14, 15]. Most studies used an absolute P level on the day of hCG administration as an indicator of PL, and the cutoff level differed from 0.8 to 2 ng/ml [15,16]. Probably a crucial point for the determination of effect of raised P on clinical pregnancy rate is the cut-off point used as reference value, and if it is too low or too high, probably no difference will be detected. In the present study, it seems that values of P > 1.5 ng/ml are associated with a statistically significant reduction of clinical pregnancy rate and it would be wiser for the patients benefit to freeze all embryos and do the embryo transfer in another cycle [22], or to do embryo transfer at blastocyst stage (if possible) [21].

Concerning the risk of recurrence of P rise (≥ 1.5 ng/ml) as presented in Table 3, if in the first cycle P was ≥ 1.5ng/ml, the risk of recurrence in a subsequent cycle was 40%. If in the first cycle P was < 1.5ng/ml, the risk to have P ≥ 1.5ng/ml in a subsequent cycle was 13.3%. If in the first cycle P was ≥ 1.2 and < 1.5 ng/ml, the risk to have P ≥ 1.5 ng/ml in a subsequent cycle was 20%, and if in the first cycle P was < 1.2 ng/ml, the risk to have P ≥ 1.5ng/ml in a subsequent cycle was 10%. These findings have not been reported by others studies according to the present authors'. knowledge. It seems that values of P < 1.5ng/ml are associated with a relatively reduced risk of recurrence of raised P in the subsequent cycle, nonetheless it should be advised that the levels of P on the triggering day to be determined in order to decide if there is rise of P ≥ 1.5 ng/ml between embryo transfer or embryo freezing [22], having fully informed the patient about the available data.

In conclusion, values of P ≥ 1.5 ng/ml are associated with a statistically significant reduction of clinical pregnancy rate and carry a 40% risk of recurrence in a subsequent cycle. Therefore, P should be determined on the triggering day and such P's values could give the option of either a) embryo freezing or b) embryo transfer at blastocyst stage.

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