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Article

Does the personalized controlled ovarian stimulation by highly purified hMG in ICSI cycles affect serum progesterone at day of trigger

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Abstract

Background: Premature progesterone elevation (PE) in late follicular phase is usually defined as progesterone levels of ≥ 1.5 ng/ml at the day of hCG trigger. The cause of PE is debatable. Recent studies suggested that PE maybe related to FSH exposure, number of oocytes retrieved, and estradiol level at the day of trigger as factors affecting the incidence of PE. The objective is to assess the progesterone rise in ICSI cycles using highly purified human menopausal gonadotropin (Hp-hMG) either sequential or combined with FSH. *Methods:* Prospective cohort study on consecutive 100 normal responder women undergoing ovarian stimulation for ICSI; 55 patients received combined protocol (FSH+ hp-hMG), 45 patients received the sequential protocol. *Outcome*: The primary outcome was serum progesterone (P4) at day of trigger of ovulation. Secondary outcomes were the number and percentage of metaphase II oocytes number of top quality embryos, and clinical pregnancy rate. *Results:* P4 at day of trigger was not significantly different between two groups. The range for P4 level at day of trigger in concomitant protocol was (min-max 0.05 – 2.58, median 0.63), while in sequential protocol was (min- max 0.09 – 2.43, with median 0.7), P value 0.667. Number of metaphase II oocytes was significantly higher in concomitant protocol; however, percentage of metaphase II was significantly higher in sequential protocol. No difference in top quality embryos or clinical pregnancy rate between two groups. *Conclusions:* Controlled ovarian stimulation protocols that contain hp-hMG seem to decrease the

incidence of premature progesterone rise, but no evidence that sequential protocol is superior to concomitant one in decreasing progesterone level.

Keywords: Hp-hMG, FSH, progesterone rise, GnRH agonist, GnRH antagonist, ICSI outcomes.

Introduction

Progesterone level (P4) has an important role in endometrial receptivity and continuation of pregnancy (1). Premature progesterone elevation (PE) in late follicular phase is usually defined as progesterone levels of ≥ 1.5 ng/ml at the day of hCG trigger (2). However the incidence of PE in studies varies from 0.8 to 2 ng/ml (3), Some authors defined PL as a P/E2 ratio of >1 (4).

In non-gonadotropin releasing hormone (GnRH) analogue cycles, premature PE can be explained by an early pre-ovulatory LH elevation, which results in endometrial asynchrony that ultimately affects implantation and pregnancy (1). However, follicular phase PE cannot be attributed to premature LH surge in GnRH analogue cycles, since the pituitary is suppressed (5). It was estimated to be about 35% in GnRH agonist cycles and 38% in GnRH antagonist cycles (6). The wide range of incidence of PE maybe due to heterogeneity of cut-off points and definitions of PE (7).

The incidence of such premature rise is more in high responders as it maybe correlated with larger number of follicles (8). Other possible etiologies for premature progesterone rise include exposure to higher doses of exogenous gonadotropins, the duration of stimulation or hypersensitivity of granulosa cells to LH (9).

The effect of premature progesterone elevation on intracytoplasmic sperm injection (ICSI) outcomes is contradictory. Venetis et al., in their large meta-analysis on PE had stratified the data according to different progesterone thresholds. They detected a significant negative correlation between progesterone elevation and pregnancy when progesterone levels are ≥0.8 ng/ml (10). Moreover, Griesinger et al., have reported that there is a detrimental effect of progesterone elevation above 1.5 ng/ml on the ongoing pregnancy rate in “low” and “normal” responders, no impairment of the pregnancy rate could be observed in high responder patients (11). Whereas, other studies reported that a significant rise in progesterone levels at the time of HCG triggering does not lead to decrease in pregnancy and implantation rates nor increase in the miscarriage rate (12). Such an effect if present, it is mainly expressed on endometrial receptivity, rather than embryos because the pregnancy rate was not affected if these cycles were managed by freeze all and embryo transfer of frozen-thawed embryos (2, 10).

There is still a possibility of affection of oocyte maturation; however, the majority of the literature does not support an adverse effect of premature elevation of progesterone on the quality of oocytes (13).

It was suggested that the individualization of treatment according to patient characteristics may be the key to avoid premature progesterone elevation (14). Most of the previous studies have compared progesterone elevation between FSH and hMG treatment groups (15). To our

knowledge, no studies have compared more individualized protocols for controlled ovarian stimulation (COS).

Materials and methods

Study design and participants

One hundred patients were recruited from private IVF/ICSI Centers in Alexandria, Egypt, between May 2020 and July 2021. Group I (Conventional concomitant protocol); 55 consecutive patients readily available in IVF centers and matching inclusion criteria. Group II (Sequential protocol); 45 patients with cross matched criteria. Before the couples were enrolled into our study, they underwent a standard protocol of investigations including: semen analysis, ovarian reserve testing (AMH) and transvaginal ultrasound for assessment of uterine cavity and antral follicular count.

Inclusion criteria included: women age ≤ 37 years, regular ovulatory cycles, expected normal response if undergoing ICSI with pituitary down-regulation.

Exclusion criteria were: women with polycystic ovarian syndrome and poor responders according Bologna criteria.(16) Every patient was extensively counseled and gave an informed consent prior to participating in the study.

Ovarian stimulation protocol

Group 1 (concomitant protocol); 55 patients received concomitant FSH and highly purified human menopausal gonadotropin (Hp-hMG) from start of stimulation, in 2:1 ratio.

Group 2 (Sequential protocol); 45 patients received FSH only at the beginning of ovarian stimulation till day 6 of stimulation or when follicles reach 10-12mm, the dose of FSH was substituted by hp-hMG was continued till the day of triggering of ovulation. FSH: either recombinant FSH (rFSH), follitropin alfa (Gonal-F; Merck Serono, Geneva, Switzerland), (Gonapure; Mina Pharm Pharmaceuticals) or highly purified urofollitropin (Fostimon; IBSA Institut Biochimique SA). HP-hMG: either Meriofert (IBSA Institut Biochimique SA) or Menopur (FERRING Pharmaceuticals, Germany). The doses of gonadotropins were individualized according to patient's age, body mass index (BMI), AFC, AMH level and previous response to ovulation stimulation, ranged from 225 IU to 300 IU daily.

Pituitary down-regulation was done by either GnRH long agonist protocol using Triptorelin acetate 0.1mg (Decapeptyl; FERRING Pharmaceuticals, Germany) or by GnRH antagonist; daily dose of subcutaneous (Cetrolix®; Cetrotide 0.25 mg: Merck Serono, Aubonne, Switzerland) on Day 5-6 of stimulation.

Monitoring of ovarian response was done by the serum E2 concentration using COBAS e411 (Roche Diagnostics, Mannheim Germany) and measuring the diameter of follicles by transvaginal ultrasonography. At the day of trigger, morning serum sample was withdrawn for measuring LH, E2, P4 and hCG. Once the leading follicle reached 18 mm in diameter, trigger of ovulation was done using 1000 IU hCG.

Oocyte retrieval; was done using transvaginal ultrasound 36 hours after triggering of ovulation, followed by intracytoplasmic sperm injection (ICSI) procedure for mature oocytes 2-4 h later. Fertilization and cleavage was assessed and the embryos were classified according to their morphological appearance.

Embryos were transferred at day 4 or 5. The luteal phase was supported with a daily 100 mg of progesterone in oil intramuscularly and vaginal suppositories (400 mg twice daily) starting on the day of oocyte retrieval. Pregnancy was assessed 14 days after embryo transfer by analyzing the serum β-hCG. Clinical pregnancy was defined by the presence of gestational sac by transvaginal ultrasonography after 5–7 weeks embryo transfer.

Outcomes

The primary outcome was serum progesterone at day of trigger of ovulation. Secondary outcomes were the number and percentage of metaphase II oocytes, number of top quality embryos and clinical pregnancy rate.

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). For analysis, $p < 0.05$ was considered to be significant. Statistical analyses were carried out by the Mann– Whitney, Fisher's exact, Chi-square (x2) tests and Student's t-test. Pearson coefficient to correlate between two normally distributed quantitative variables.

Results

No significant differences were found in the baseline characteristics between two groups as shown in **Table 1**.

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Table 1: Demographic characteristics between two protocols

Parameter	Concomitant FSH+HPHMG (n 55)	Sequential FSH then HPHMG (n= 45)	P value
Age (years)[a]	29.07 ± 4.08	27.89 ± 4.55	0.174[b]
BMI (Kg/m2)[c]	23.80 (22.1 – 26.0)	24.0 (22.1 – 26.6)	0.685[d]
Duration of infertility(years)[c]	4.50 (3.0 – 6.0)	3.50 (2.5 – 5.0)	0.205[d]
AMH (ng/dl)[a]	2.73 ± 0.73	2.67 ± 0.83	0.706[b]

a- Values are mean± S.D.

b- Independent t-test.

c- Values are median (range).

d- Mann–Whitney U-test.

The levels of hormones at day of trigger (LH, E2, P4, and HCG) were not significantly different between two groups (Table 2). The range for P4 level at day of trigger in concomitant protocol was (min-max 0.05 – 2.58, median 0.63), while in sequential protocol was (min- max 0.09 – 2.43, with median 0.7), P value 0.667.

By comparing the two groups regarding parameters of ICSI outcomes (**Table 2**); the total dose of hp-hMG was significantly higher in concomitant protocol than sequential. With sequential protocol, there was significantly lower number of oocyte retrieved but higher percentage of Metaphase II oocytes and fertilization rate.

With subgroup analysis according GnRH analogue, there was no difference in PE between the two protocols in agonist and antagonist protocols. The range for P4 level at day of trigger in concomitant protocol was (min-max 0.05 – 2.58, median 0.63), while in sequential protocol was (min- max 0.09 – 2.43, with median 0.7), P value 0.667. The incidence of PE was less than 1%, as in concomitant protocol; there were 4 cases with P4 ≥1.5 ng/ml, while there were 3 cases only in the sequential protocol. However, the incidence of PE was 1.6% when using P4 ≥1.2 ng/ml as a cutoff value.

Table 2: Endocrine profile and cycle characteristics and outcomes in both groups

Parameter	Group 1 (n= 55)	Group 2 (n= 45)	P value
Hormones at day of HCG			
LH[a]	2.25 (1.4 – 3.7)	2.37 (1.8 – 3.0)	0.887[b]
E2[a]	3000(2409.5–4100.5)	2982.0(2155.0–4115.5)	0.631[b]
P4[a]	0.63 (0.5 – 0.9)	0.70 (0.5 – 0.9)	0.667[b]
HCG[a]	0.48 (0.3 – 0.9)	0.60 (0.5 – 0.8)	0.089[b]
Duration of treatment[c]	10.35 ± 1.42	11.04 ± 1.48	0.018[d*]
Total dose FSH (IU)[a]	1350(900–2025)	1350.0(1125–1575)	1.000[b]
Total dose HP HMG (IU)[a]	825.0 (712.5 – 1237.5)	750.0 (600.0 – 900.0)	0.013*
No. of oocytes retrieved [a]	16.0 (12.0 – 20.0)	12.0 (10.0 – 15.0)	<0.001 [b*]
No. of metaphase II oocytes[a]	13.0 (8.5 – 18.0)	9.0 (8.0 – 13.0)	0.009[b*]
Percentage of MII[a]	83.33 (75.0 – 93.8)	90.91(80.0 – 100.0)	0.034[b*]
Fertilization rate[a]	77.78 (66.7 – 89.9)	86.67(76.9 – 100.0)	0.028[b*]
No. of top quality embryos[a]	7.0 (3.5 – 8.0)	6.0 (5.0 – 8.0)	0.693 [b]
Clinical pregnancy rate	34 (61.8%)	29 (65.9%)	0.064[e]

[a] Values are median, [b] Mann–Whitney U-test, [c] Values are mean± S.D, [d] Independent t-test, [e] χ^2 Chi square test,

* Significant p Value < 0.05

Discussion

The mechanism of PE is debatable. During follicular phase, the granulosa cells (GCs) are supra-physiologically stimulated by gonadotropins, which may result in increased serum progesterone levels (17). Opposing this theory, Kilani et al. stated that PE could not be entirely explained by more follicles as progesterone remained significantly different between treatment groups even when adjusting for the number of developed follicles (18). In our study, the progesterone level was significantly correlated with number of oocytes in concomitant protocol (P value 0.011), but this was not significant in sequential protocol (P value 0.18), may be due to lower number of oocytes in this group.

It has been suggested that a decline in HCG/LH activity may lead to premature PE (15), Andresen et al. 2006 (MERIT trial) found that progesterone at the end of stimulation was significantly higher with rFSH compared with Hp-hMG, even after adjusting for ovarian response ($p < 0.001$). The threshold value for defining serum PE in this study was 1.25 ng /ml (19). Thus, the difference between hp-hMG and rFSH in progesterone could be hypothetically attributed to an FSH action in granulosa cells through paracrine signals that modify the enzymes involved in progesterone and androgen synthesis. In the relative absence of LH activity, the function of these enzymes may be affected resulting in higher levels of progesterone. (20).

However, another study suggested that hCG/LH does not protect against PE, but it instead enhances progesterone production in the follicular phase (21). Opposing the hCG/LH theory, Filicori et al. have proposed that the cause of premature progesterone elevation might be due to enhanced FSH stimulation in ART cycles (22).

In another study, Filicori et al. (23), added an increasing doses of hCG as source of LH activity from day 8 of stimulation (ranged from 0 IU to 50 IU, 100 IU and 200 IU) with declining FSH and found that follicular progesterone levels were significantly lower in rFSH only group than rFSH plus HCG groups (p value < 0.01).

Kolibianakis et al. (24) pooled data from five COS-IVF trials using either GnRH antagonists or agonists that evaluated the impact of the type of gonadotropin, rFSH alone, rFSH combined with rLH, HP-hMG alone, and rFSH combined with HP-hMG on PE. The authors found that progesterone levels in the late follicular phase were associated with the number of oocytes retrieved and serum estradiol levels, irrespective of type of GnRH analogue, but not with the type of gonadotropin administered. Furthermore, there was no association between PE and duration of stimulation or FSH requirement. In contrast, our study had showed that there was significant correlation between progesterone and FSH dose in concomitant protocol (P value 0.008), however, there was no significant relation in the sequential protocol, and this may be attributed to lower doses of FSH used

Requena et al. (25) had reported that the mean serum P4 levels did not differ significantly with respect to the type of gonadotropin used for COS: rFSH + rLH (P: 1.01 ng/ml), rFSH alone (P: 1.06 ng/ml), rFSH + HP-hMG (P: 1.30 ng/ml), and HP-hMG alone (P: 1.10 ng/ml). In agreement with

them, O.Kan et al. have found that serum progesterone levels on the day of trigger administration were similar in rFSH and rFSH plus hp-hMG group (26).

In contradiction with previous results, Fleming and Jenkins, have found that the type of gonadotropin used for ovarian stimulation impacts progesterone production. They have further suggested that LH is responsible for increased progesterone catabolism in the theca cells by the 17 α-hydroxylase enzyme. This reduces the amount of progesterone entering the general circulation (27).

In agreement with them, Shu et al. 2019 (28) have found that in rFSH group, the P level on the day of HCG trigger were significantly higher than that of hp-hMG+ rFSH group (4.3±2.2 vs. 3.8±1.7 nmol/L, P<0.001).

So the data about the relation between PE and type of gonadotropin is conflicting, but it seems that the reduction of FSH dose towards the end of stimulation may result in a lower incidence of progesterone elevation. As the incidence of PE is much lower than previous studies (less than 1% with P4 ≥1.5 ng/ml), this could be attributed to hCG content in hp-hMG as source of LH bioactivity, hCG that drives steroidogenesis away from P4 production.

Conclusion

Controlled ovarian stimulation protocols that contain hp-hMG seem to decrease the incidence of premature progesterone rise, but no evidence that sequential protocol is superior to concomitant one in decreasing progesterone level.

Statement of ethics

Written informed consent was obtained from all participating patients. Consent forms and protocols were approved by the local Ethics Committee, Alexandria University.

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Author contributions

HM is the designer of the study protocol, implementation of research. OY was responsible for collection and analysis of data, writing the paper.

Declaration of Competing Interest

The authors report no conflicts of interest.

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