Original Article

Serum progesterone concentrations before and after human chorionic gonadotropin triggering and invitro fertilization : Intracytoplasmic sperm injection cycle outcome in long gonadotrophin releasing hormone agonist protocol

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ABSTRACT

Objective: To assess the impact of serum progesterone concentrations before and after HCG triggering on IVF/ICSIcycle outcomes in long GnRHa protocol.

Patients and Methods: This is a single-center prospective observational cohort study, in which 102 IVF/ICSI patients with normal cycle day 2 basal hormones were recruited and underwent ovarian stimulation using the long GnRHa protocol. Serum progesterone on the day of HCG trigger; one day after, progesterone/estradiol was assessed and correlated with pregnancy rates, the total dose of used gonadotropins, serum estradiol level on day of trigger, the number of collected oocytes, the number of metaphase II oocytes and the quality of transferred embryos.

Results: 96 women were included in the analysis. In our study, the receiver-operating characteristic (ROC) analysis was used to gain more diagnostic accuracy. Our data according to ROC analysis showed that the P levels on the day of hCG trigger, and its levels one day after and P/E2 on the day of hCG trigger had no role in prediction of clinical pregnancy rates. There was significant correlation between the serum progesterone levels before and after HCG triggering with the total dose of gonadotropins used. They also correlated strongly with serum estradiol level on day of trigger, the number of collected oocytes and the number of metaphase II oocytes.

Conclusion: Peri-ovulatory serum progesterone concentrations cannot predict pregnancy rates in IVF/ICSI cycles using long GnRHa protocol.

Key Words: Gonadotrophin releasing hormone agonist, IVF/ICSI, pregnancy rate, periovulatory, serum progesterone concentrations.

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INTRODUCTION

In humans, ovarian steroidogensis occurs according to the two-cell/two-gonadotropin theory; cholesterol is converted to progesterone (P) in the granulosa cells under the effect of follicular stimulating hormone (FSH) which passes to theca cells to be converted to androgens under the effect of luteinizing hormone (LH). Then, androgens pass to the granulosa cells to be converted to estradiol (E2)[1].

Premature luteinization (PL) is defined as premature elevation of serum P in the late follicular phase before the human chorionic gonadotropin (HCG) administration. The introduction of gonadotropin-releasing hormone agonist (GnRHa) in the controlled ovarian stimulation (COS) for IVF/ICSI cycles prevents both immunoactive and bioactive LH surges in 95-98% of patients[2]. Therefore, the term PL for subtle P rise in COS for IVF/ICSI cycles has proven to be misleading because it is not associated with LH rise, although it was reported to occur in 2-40% of the cycles[3]. Many hypotheses were postulated to explain the premature subtle P rise in COS, primarily the increase in the number of follicles with increased production of P without the balancing effect of LH activity to convert it to E2 in the granulose cells, secondly the accumulation of LH from human menopausal gonadotropin (hMG) and finally increased sensitivity of granulosa LH receptors to gonadotropins[4].

The cut of value of subtle P rise on the day of HCG is arbitral in most of the studies ranging from 0.8-2 ng/ml ; Younis *et al* 2001 defined PL as progesterone/estradiol (P/E2) >1 on the day of HCG administration to differentiate between P from immature follicles and that from healthy mature follicles[5].

The impact of subtle P rise on the day of HCG administration on IVF/ICSI outcome is controversial; some studies showed that it has no association with

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pregnancy rates (PRs)[4, 6-10], whereas others showed that it has negative impact[11-14].

The aim of our study is to assess the relation of peri-ovulatory serum P concentration, to assess the impact of an extended window of progesterone elevation and P/E2 on day of HCG injection in IVF/ICSI cycle outcomes with long GnRHa protocol.

PATIENTS AND METHODS

A single center prospective observational cohort study which was conducted in the IVF unit of Cairo University Hospital, Egypt. One hundred and two infertile women eligible for IVF/ICSI and fulfilling the inclusion criteria were included.

The study protocol was approved by the Institutional Review Board of Cairo University and consent forms were obtained from the patients after they were informed about the study and its outcomes.

Inclusion criteria:

Woman's age from 20-35 years, normal hormonal assay on cycle day 2 (FSH<10 mIU/ml, LH<10 mIU/ml, E2 level<60 pg/mL) and eligible for IVF/ICSI.

Exclusion criteria:

History of previous adenexal masses, endometriosis, PCOS (polycystic ovary syndrome).

Protocol for ovarian stimulation:

The standard long GnRHa protocol was used for all patients; triptorelin acetate 0.1 mg (Decapeptyl, Ferring Pharmaceuticals) was given once daily SC starting from day 21 of the preceding cycle till the day of HCG injection. Gonadotropins in the form of human menopausal gonadotrophin (hMG) (Merional, IBSA, Institut Biochimique SA) or purified FSH (follitropin beta, Puregon; NV Organon, Oss, the Netherlands) were given from the 2nd day of menstruation after checking down regulation. The starting dose ranged from 150-225 IU depending on basal FSH level, antral follicle count (AFC), maternal age, BMI and previous response to COS.

Serial trans-vaginal ultrasound and serum E2 were done to assess follicular growth starting from day 6 of the cycle and onward, with adjustments of gonadotropin dose based on patient's response. Once 3 or more leading follicles reach 18mm, HCG 10,000IU (Choriomon, IBSA, Institut Biochimique SA) was given IM. Oocyte retrieval was performed 35 hours from HCG injection followed by IVF/ICSI.

Assessment of embryo quality:

The assessment was done after 42 to 48 hrs after IVF/ICSI according to embryo morphology (shape of blastomere and degree of fragmentation).

Grade I: excellent embryos (intact and symmetrical blastomere with no fragmentation).

Grade II: good embryos (asymmetrical cleavage or < 20% embryo fragmentation). Grade III: fair embryos (embryo fragmentation 20-50%).

Grade IV: poor embryos (embryo fragmentation > 50%).

Embryo transfer (ET) was done 3 days following ovum pick up using labotec catheter (Labotec, Gottingen Germany) with ultrasound guidance.

All patients were given progesterone vaginal pessary (Cyclogest, Alpharma, UK) 400 mg twice daily from the day of egg collection till the day of the pregnancy test. Serum ß-HCG was done 14 days after ET and ultra sound imaging was done 2 weeks after positive ß-HCG to determine viability and number of gestational sacs.

Progesterone measurement:

Serum progestrone was measured on the day of HCG administration and one day after. Samples were tested with electrochemiluminescence immunoassay (ECLIA) (Cobas e411, Roche Diagnostics, Indianapolis, IN), which had a sensitivity of 0.3 ng/dl progesterone. Instructions in the Operator's Manual were followed for preparation, set-up, dilutions, and adjustment, assay and quality control procedures. The inter-assay coefficient of variation was less than 10% and the intra-assay variation was less than 15%.

In our study P measurements had no impact on patient management or decision making. P/E2 ratio was calculated as P (ng/mL) × 1,000/E2 (pg/mL)[10].

Primary outcome:

Identification of the serum progesterone threshold measured on the day of HCG administration and one day after which may be associated with detrimental cycle outcome. Clinical pregnancy was considered with a positive serum ß-HCG with an ultrasound evidence of a gestational sac.

Secondary outcome:

To examine other factors related to P elevation as dose of gonadotropins used for COS, number of oocytes retrieved, number of metaphase II (MII) oocytes, E2 level on day of HCG administration and quality of transferred embryos.

Statistical methods:

Data were statistically described in terms of mean ±standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Correlation between various variables was done using Pearson moment correlation equation for linear relation in normally distributed variables and Spearman rank correlation equation for non-normal variables. Accuracy was represented using the terms sensitivity, and specificity. Receiver operator characteristic (ROC) analysis was used to determine the optimum cut off value for the studied

diagnostic markers. p values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

RESULTS

One hundred and two patients were enrolled in our study, 6 patients were excluded because of cancelled ET. One case for failure of fertilization, the rest 5 cases because of the risk of ovarian hyper stimulation syndrome (OHSS) and all embryos were cryopreserved for transfer in a future cycle; thus 96 patients had ET and were enrolled in the analysis (figure A).

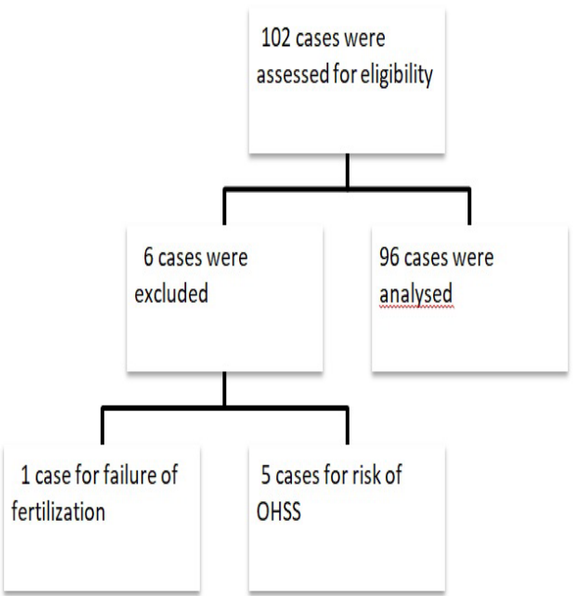

Fig. a: The recruitment flow chart of patients who were included in the study.

The mean patient's age was 28.9 ± 5.7 years; the mean duration of infertility was 4.8 ± 3.6 years. Sixty nine cases (71.9%) had 1ry infertility and 27 cases (28.1%) had 2ry infertility. Indications for ICSI were: mild to moderate male factor in 72 cases (75%), tubal factor in 13 cases (13.5%) and unexplained infertility in 11 cases (11.4%).

The mean serum P level on the day of HCG trigger was 0.7 ± 0.6 ng/ml and the mean serum P on the day after was 3.6 ± 2.9 ng/ml.

Receiver-operating characteristic (ROC) curve analysis for the values of serum P on the day of HCG injection, one day after triggering and P/E2 on the day of HCG injection in conception, non conception cycles is shown in figure (b, c, d).The area under the curve was 0.906 with 95% CI: 0.391-0.623, 0.599 with 95% CI: 0.41-0.64 and 0.979 with 95% CI: 0.383-0.620, respectively which was not predictive of clinical pregnancy.

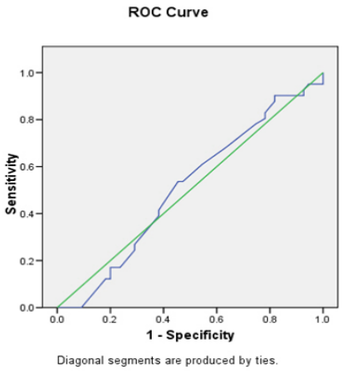

Fig. b: Receiver operating characteristic curve for serum progesterone concentration on the day of HCG for the prediction of clinical pregnancy rate.

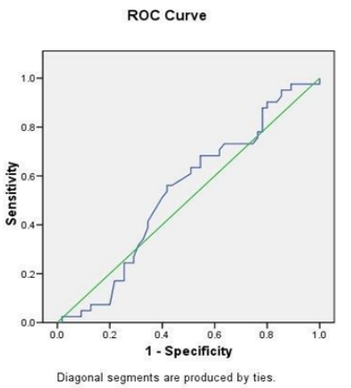

Fig. c: Receiver operating characteristic curve for serum progesterone concentration on the day after HCG for the prediction of clinical pregnancy rate.

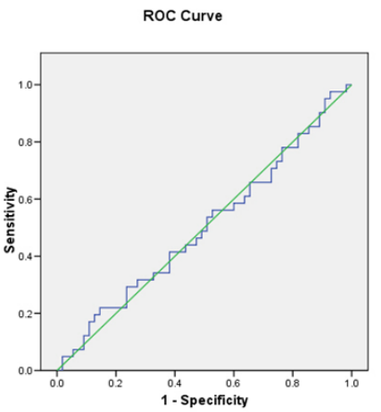

Fig. d: Receiver operating characteristic curve for serum progesterone /estradiol ratio on the day of HCG for the prediction of clinical pregnancy rate.

The cycle characteristics and outcomes are presented in table 1.

Table 1: Cycle characteristics and outcomes in our group of patients

Characteristic/Outcome	Mean	Standard deviation
Duration of hMG treatment (days)	11.5	1.5
Total doses of gonadotropin (IU)	2860.5	668.3
E2 levels on HCG day (pg/mL)	4864.8	4784.9
Progesterone levels before triggering (ng/ml)	0.7	0.6
Progesterone levels after triggering (ng/ml)	3.6	2.9
Endometrial thickness (mm)	10.9	1.5
Number of collected oocytes	9.6	5.7
Number of MII oocytes	6.6	4.4
Number of fertilized oocytes	5.7	3.6
Number of good embryos transferred	2.4	1.1
Clinical pregnancy rate (n/n, %)	41/96, 42.7	
Early miscarriage rate (n/n, %)	6/96, 6.3	

E2: Estradiol; HCG: human chorionic gonadotropin; hMG: human menopausal gonadotropin.

The serum P level before trigger and one day after trigger was significantly correlated with the total dose of gonadotropins used for COS (r= 0.233, *p=0.023* and r= 0.241, *p=0.018* respectively).

There was a strong correlation between serum P level before trigger and serum E2 level on day of trigger (r= 0.340, *p=0.001*), number of collected oocytes (r= 0.318, *p=0.002*) and number of MII oocytes (r= 0.305, *p=0.002*).
There also was a strong correlation between serum P level one day after trigger and serum E2 level on day of trigger (r= 0.472, *p=<0.001*), number of collected oocytes (r= 0.399, *p=<0.001*) and number of MII oocytes (r= 0.312, *p=0.002*).

There was no correlation between serum P level before trigger or one day after trigger with the quality of transferred embryos [(p = 0.246, r= 0.097) (p =0.291, r=0.088)] respectively.

There was no relation of statistical significance between serum P level before trigger or one day after trigger and the type of used gonadotropins (hMG or purified FSH) (p= 0.739, 0.674 respectively) or the miscarriage rate (p 0.739, 0.674 respectively).

DISCUSSION

The pathogenesis of the subtle P in the late follicular phase of cycles stimulated with GnRHa and gonadotropins is still poorly understood and its impact on pregnancy outcome is still debatable. The studies which showed there is deleterious effect on PRs attributed that to the impairment of implantation due to endometrial secretory advancement rather than the affection of oocyte and embryo quality. Other studies showed that it had no impact on pregnancy rate.[4, 6-10].

Pre HCG progesterone rise was defined by many studies that serum P more than 0.8-2 ng/ml[3, 6, 13, 14], however in most of these studies, the threshold for P was demonstrated arbitrary.

The relation of peri-ovulatory serum progesterone and PRs is little studied, however Prien *et al.* 1994 stated that the threefold rise in serum P following HCG administration can predict pregnancy rates in IVF/ICSI cycles[15]. The aim of our work is to study the impact of an extended window of elevated serum P on IVF/ICSI cycle outcome.

In our study, the receiver-operating characteristic

(ROC) analysis was used to gain more diagnostic accuracy; which were used in a very few number of similar studies.

Our data according to ROC analysis showed that the P levels on the day of HCG trigger, and levels one day after ; and P/E2 on the day of HCG trigger had no effect in the prediction of clinical pregnancy rates which is in agreement with many previous studies which studied either P levels on the day of hCG trigger or one day after[4, 6, 7, 10, 16].

Other studies showed that there is an inverse relationship between elevated levels of serum P on day of HCG administration and pregnancy rates[5, 11, 16-18], this difference may be attributed to different methods of P assessment, different cutoff values of progesterone, different patients' responses and different stimulation protocols.

Doldi *et al.* showed that elevated serum P is associated with increased PRs, they explained this by stating that elevated serum P is associated with increased ovarian response and increased number of available embryos for transfer[9].

The serum P level before trigger and on the day after was strongly correlated with serum E2 level on day of trigger, the number of collected oocytes and the number of MII oocytes which goes in agreement with many studies [4,19], because the more the recruited follicles the more the production of P from the granulosa cells. There was no correlation between serum P before trigger or one day after with quality of transferred embryos this agrees with many previous studies[7, 8, 18].

Legro *et al.* and Silverberg *et al.* found that there was even improved embryo quality with elevated serum P levels on the day of HCG administration in donor oocytes cycles and also in frozen thawed ET cycles[20, 21]. These findings favor that serum P levels have no role in oocyte or embryo quality.

In our study, serum P levels before trigger and one day after were significantly correlated with the total dose of gonadotropins used for COS which is consistent with Filicori *et al.*, 2002[22]. While, Huang *et al.* 2012 found no correlation between serum P on day of HCG administration and the total dose of gonadotropins used for COS; but they used step down policy of gonadotropins in their study, which may explain this finding[18].

Several studies showed no statistical significance between P levels on the day of HCG administration or one day after and the type of used gonadotropins[12], which is similar to our findings.

CONCLUSION

In conclusion, peri-ovulatory serum P levels and P/E2 on day of HCG injection cannot predict PRs in IVF/ICSI cycles using GnRHa long protocol; but correlate strongly with serum E2 level on day of trigger, the number of collected oocytes and the number of MII oocytes. They should not be used to alter physicians' decision making in treatment IVF/ICSI cycles.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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