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Serum progesterone concentration on day of human chorionic gonadotropin (HCG) injection in prediction of intracytoplasmic sperm injection (ICSI) outcome in normal responders

Mohamed A Ibrahem[1]*, Amira S Al-Karamany[2]
[1] Obstetrics and Gynecology Department, Faculty of Medicine, Zagazig University. Zagazig, Egypt
[2] Department of Medical Biochemistry, Faculty of Medicine, Zagazig University, Zagazig. Egypt

Abstract
Objective: The aim of the current study is to assess the presence of such an association between serum progesterone concentrations measured on day of human chorionic gonadotropin (hCG) injection to trigger ovulation and pregnancy outcomes in normal-responding women under ICSI after controlled ovarian hyperstimulation (COH) using the long gonadotropin releasing hormone agonist (GnRHa) protocol.
Methods: The study included infertile women who were planned to undergo ICSI for male factor or tubal factor, or for unexplained infertility. All women underwent COH after luteal phase down-regulation using GnRHa. Serum progesterone assay was performed to all included women on the same day before hCG injection. Study outcomes included: positive biochemical pregnancy, positive clinical pregnancy and positive ongoing clinical pregnancy.
Results: A total of 312 ICSI cycles in 289 women were included in the final analysis. Among the included cycles, the ongoing clinical pregnancy rate was 119/312 (38.1%). Serum progesterone measured on day of hCG injection was a significant predictor of positive biochemical, clinical and ongoing clinical pregnancy as shown the significantly large areas under the curve (AUCs). Binary logistic regression analysis showed that serum progesterone was the only significant independent variable that was associated with a positive ongoing clinical pregnancy.
Conclusion: Serum progesterone rise before hCG injection seems to have an adverse effect on pregnancy rates in women undergoing ICSI after COH using the long GnRHa protocol.

Keywords: serum progesterone–ICSI–pregnancy rate

Introduction
Progesterone has a well-known key role in implantation and maintenance of both spontaneous and assisted-reproduction-produced pregnancy [1, 2]. Progesterone is essential for inducing secretory changes in the endometrium as well as increased density and compaction; all of which are needed for successful implantation and pregnancy [1]. Premature serum progesterone rise has been proposed as a potential cause of failure in in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles; the issue, however, remains a matter of debate. Some authors claim such a deleterious effect on the endometrial receptivity when serum progesterone rises above a certain threshold; while others deny such an association [3-5]. Even those authors who suggested that claim have not found clear reasoning [6]. The most acceptable reason that might explain such as association, if it really exists, is accelerated endometrial maturation resulting in premature closure of the 'implantation window' which renders the endometrium unreceptive [7-8]. The aim of the current study is to assess the presence of such an association between serum progesterone concentrations measured on day of human chorionic gonadotropin (hCG) injection to trigger ovulation and pregnancy outcomes in normal-responding women under ICSI after controlled ovarian hyperstimulation (COH) using the long gonadotropin releasing hormone agonist (GnRHa) protocol.

Methods
The prospective study was conducted at a private fertility and IVF center in Zagazig, Egypt, during the period between July 2018 and August 2019. The study protocol agreed to the Helsinki declaration of Ethical Medical Research [last updated in Brazil, 2013]. All included women signed informed written consent before participation in the study. The study included infertile women who were planned to undergo ICSI for male factor or tubal factor, or for unexplained infertility. Women who have the potential for either high response (e.g. women with polycystic ovarian syndrome [PCOS]) or poor response (e.g. age above 37 years old, reduced ovarian reserve, ovarian endometriosis, and previous poor response) were not included in the study. All women underwent COH after luteal phase down-regulation using GnRHa [triptorelene acetate 0.1 IU, Decapeptyl®, Ferring Pharmaceuticals] in a daily subcutaneous dose from day 21 of the preceding cycle till the day of hCG trigger. COH was conducted using daily intramuscular injection of human menopausal gonadotropin (hMG) [Menogon® 75 IU, Ferring

Pharmaceuticals] from day 2 of menstruation. The initial dose was 225 IU/day. The dose was either maintained or readjusted according to the serum estradiol level and sonographic folliculometry conducted 6 days after onset of ovarian stimulation. When at least 3 dominant follicles reach a size above 18 mm in average dimension, 10,000 IU of hCG [Choriomon®, IBSA Pharmaceuticals] were given intramuscularly to trigger final maturation and ovulation. Serum progesterone assay was performed to all included women on the same day before hCG injection using the electrochemiluminescence method presented by the commercial Elecsys kits [Cobas® e-602 analyzer, Roche Diagnostics, Mannheim, Germany)] [9]. Oocyte retrieval was conducted 35 – 37 hours after hCG injection. Only oocytes who reached the MII phase were injected. All included women underwent embryo transfer at the blastocyst stage. Included women received luteal phase support in the form of vaginal progesterone (Prontogest® sup., IBSA 400 mg) twice per day till the day of quantitative serum hCG assay. Women who had unexpected hypo response (< 5 retrieved oocytes) and those who had no blastocyst-stage embryo transfer were excluded from the analysis.

Study outcomes included: positive biochemical pregnancy [defined as positive serum hCG level above 25 IU/L 14 days after embryo transfer], positive clinical pregnancy [defined as sonographic detection of viable gestational sac(s)], and positive ongoing clinical pregnancy [defined as sonographic detection of viable gestational sac(s) beyond 12 weeks of gestation].

Statistical analysis was performed using MedCalc® version 7.0. Receiver operator characteristics (ROC) curves were constructed for estimating the association between serum progesterone and pregnancy outcomes. Binary multiple logistic regression analysis was performed to estimate the association between measured variables and the ongoing clinical pregnancy outcome. Significance level was set at 0.05.

Results

A total of 327 ICSI cycles in 294 women were recruited; of them, 15 (4.6%) were excluded [6 had unexpected hypo response; 7 underwent day-3 (cleavage-stage) embryo transfer and 2 had cancelled embryo transfer due to non-fertilization]. Therefore, 312 ICSI cycles in 289 women were included in the final analysis. The mean age was 29.48 ± 3.52 years (range: 24 – 35 years). The median duration of infertility was 6 years (range: 3 – 10 years). The median duration of ovarian stimulation was 15 days (range: 12 – 18 day). The mean total dose of gonadotropins was 4017.07 ± 795.56 IU (range: 2700 – 5400 IU). Table-1 shows the ICSI cycle outcomes in included women. Among the included cycles, the ongoing clinical pregnancy rate was 119/312 (38.1%).

The mean serum progesterone measured on day of hCG injection was 1.34 ± 0.44 ng/ml (range: 0.6 – 1.9 ng/ml). Serum progesterone measured on day of hCG injection was a significant predictor of positive biochemical, clinical and ongoing clinical pregnancy as shown the significantly large areas under the curve (AUCs). Serum progesterone levels ≤ 1.57 ng/ml, ≤ 1.03 ng/ml, and ≤ 0.9 ng/ml were significantly associated with positive biochemical, clinical and ongoing clinical pregnancy rates, at sensitivities and specificities of 89.84% and 73.6%; 80.77% and 96.6%; and 82.35% and 92.7%; respectively and respectively (figure 1).

Binary logistic regression analysis showed that serum progesterone was the only significant independent variable that was associated with a positive ongoing clinical pregnancy [OR 0.01, 95% CI (0.001 to 0.03), p<0001] (table 2).

Table 1: ICSI Cycles Outcomes in Included Women

No. of Retrieved Oocytes	19 (13 – 27)
Fertilization Rate	0.70 ± 0.13 (0.5 – 0.9)
No. of Transferred Embryos	2 (2 – 3)
Biochemical Pregnancy Rate	187 (59.9%)
Clinical Pregnancy Rate	130 (41.7%)
Ongoing Clinical Pregnancy Rate	119 (38.1%)

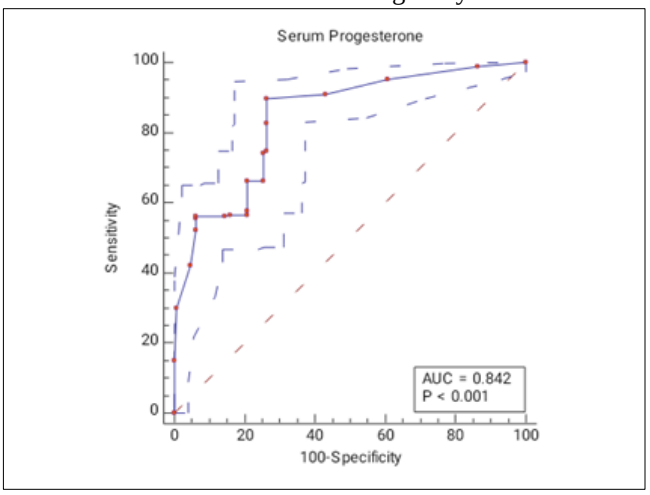
Data presented as median (range); mean ± SD (range); or frequency (percentage)

Table 2: Logistic Regression Analysis of Measured Variables in Prediction of Positive Ongoing Clinical Pregnancy in Included Women

	Coefficient	**SE**	**Wald**	**OR 95% CI**	***P***
Age	0.011	0.045	0.064	1.01 (0.92 to 1.10)	0.800
Duration of Infertility	0.086	0.073	1.377	1.08 (0.94 to 1.25)	0.241
Total Gonadotropin Dose	-0.0006	0.003	0.042	0.99 (0.99 to 1.01)	0.837
Duration of Ovarian Stimulation	-0.073	0.104	0.494	0.93 (0.75 to 1.14)	0.482
No. of Retrieved Oocytes	0.195	0.596	0.107	1.21 (0.37 to 3.91)	0.743
Fertilization Rate	-0.715	1.222	0.342	0.48 (0.04 to 5.37)	0.559
Serum Progesterone	-4.341	0.447	94.289	0.01 (0.001 to 0.03)	<0.001
Constant	4.123	2.155	3.657		0.056

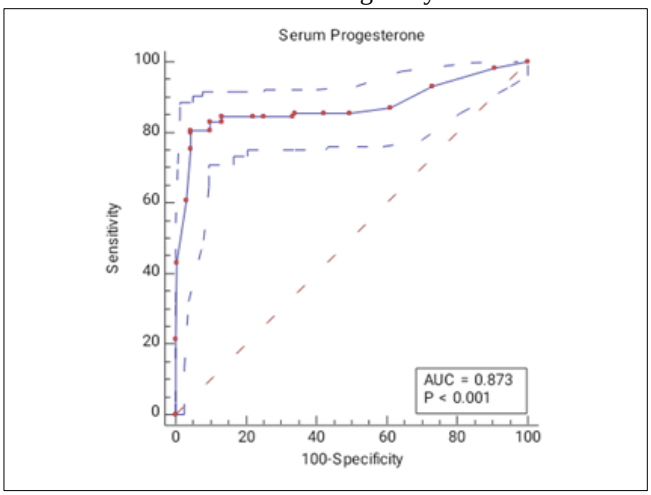
SE standard error OR (95% CI) odds ratio and its 95% confidence interval

Biochemical Pregnancy



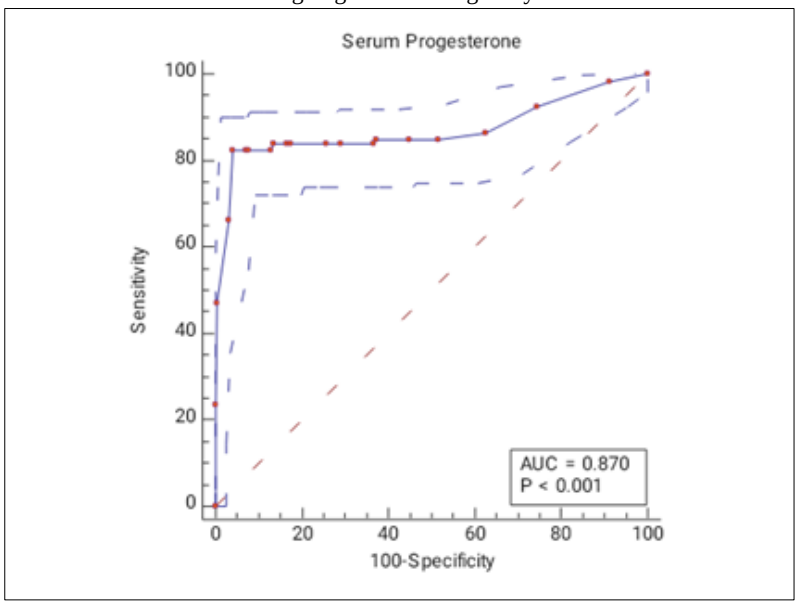
AUC = 0.842, 95% CI (0.797 to 0.881)
Cutoff Value = ≤ 1.57 ng/ml
Sensitivity 89.84% (84.6 – 93.8)
Specificity 73.60% (65.0 – 81.1)
LR+ 3.4 (2.5 – 4.6)
LR- 0.14 (0.09 – 0.2)

Clinical Pregnancy



AUC = 0.873, 95% CI (0.830 to 0.908)
Cutoff Value = ≤ 1.03 ng/ml
Sensitivity 80.77% (72.9 – 87.2)
Specificity 95.6% (91.5 – 98.1)
LR+ 18.4 (9.3 – 36.4)
LR- 0.2 (0.1 – 0.3)

Ongoing Clinical Pregnancy



AUC = 0.870, 95% CI (0.827 to 0.905)
Cutoff Value = ≤ 0.9 ng/ml
Sensitivity 82.35% (74.3 – 88.7)
Specificity 95.85% (92.0 – 98.2)
LR+ 19.87 (10.0 – 39.4)
LR- 0.18 (0.1 – 0.3)

Data presented as value (95% CI), AUC area under the curve – LR+ positive likelihood ratio – LR- negative likelihood rate

Fig 1: ROC Curves for Serum Progesterone on Day of hCG Injection as Predictor of Pregnancy Outcomes in Included Women

Discussion

The current study has shown a significant association between a lower serum progesterone level on day of hCG injection and a successful pregnancy outcome. The results of previous similar studies have been conflicting, however. In a large systematic review and meta-analysis of 63 studies including 3,296 women, Venetis *et al.* found that elevation in late follicular phase progesterone in long GnRH agonist cycles (0.8 – 1.1 ng/ml) was significantly associated with lower pregnancy rates [OR 0.79, 95% CI (0.67 to 0.95)]; and further rise (≥ 1.2 ng/ml) was

associated with even lower pregnancy rate [OR 0.67, 95% CI (0.53 to 0.84)] [10]. In the same direction, a retrospective analysis of 4,032 fresh IVF/ICSI cycles using the long GnRH agonist protocol, revealed that ongoing pregnancy rates were 31% and 19.1% in women who had a late follicular progesterone level < 1.5 and ≥ 1.5 ng/ml, respectively ($p<0.001$) [11]. In the other direction, a prospective study on 158 fresh IVF/ICSI cycles found a relatively high incidence (13.3%) of progesterone elevation (> 1.5 ng/ml). The authors found a higher (but not to a statistically significant level) pregnancy rates in women who had a progesterone level < 1.5 ng/ml [27% vs. 19%, $p>0.05$] [12]. Similarly, in a multicenter retrospective analysis of 475 cycles, Andersen *et al*. found a significant positive association between late follicular serum progesterone and the no. of retrieved oocytes. The authors, however, found no significant association between serum progesterone level and clinical pregnancy rates [13]. These conflicting results have been partially explained by a systematic review of 6 trials (including 1,866 cycles). In this review, cycles were stratified into three categories: low responders (no. of retrieved oocytes 1 – 5), normal responders (6 – 18 oocytes) and high responders (> 18 oocytes). The prevalence of late follicular phase progesterone elevation was 4.5% and 19% in low responders and high responders, respectively. The overall analysis showed a significantly lower pregnancy rates in women who had high serum progesterone (≥ 1.5 ng/ml). The subgroup analysis, however, showed that such adverse effect was encountered in women with low and normal response, but not in those with high response [14]. Like this latter analysis, Wu Z *et al*. analyzed 2,351 patients receiving fresh IVF embryo transfer and found detrimental effect of high serum progesterone in low and intermediate ovarian responders but not in high responders 15. Similar Requena *et al*. analyzed 2,850 cycles in high responders (defined as retrieval ≥ 20 oocytes and/or peak serum estradiol ≥ 3,000 pg/ml). The authors found no significant adverse impact of high serum progesterone level (up to 1.8 ng/ml) on embryo quality, endometrial receptivity or pregnancy rates. Only when levels got above 1.8 ng/ml, marginal reduction in clinical pregnancy rates was noticed [OR 0.73, 95% CI (0.61 to 0.99)] [16]. Similar conclusion was reached by a retrospective analysis of 1,800 cycles in high responding women. The authors found no significant difference between women who had a serum progesterone > 1.5 ng/ml and those who had it < 1.5 ng/ml (54.6% vs. 59.9%, respectively) [17].

These latter findings suggest that presence of high-quality embryos might overcome the potential detrimental effect of high serum progesterone on endometrial receptivity [12, 18]. This assumption has been rather confirmed by a study which compared the association between serum progesterone and pregnancy rates in women who underwent single ET at both cleavage-stage and blastocyst-stage. The authors found that high serum progesterone significantly reduced pregnancy rates in women who underwent cleavage-stage ET but not in those who underwent blastocyst-stage ET [19].

In conclusion, serum progesterone rise on day of hCG trigger seems to have an adverse effect on pregnancy rates in normal-responding women undergoing ICSI after COH using the long GnRHa protocol. Further studies are, however, needed to evaluate the value of canceling fresh ET and thawed/frozen ET in subsequent cycles for such cycles when serum progesterone prematurely rise.

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