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ASSISTED REPRODUCTION

Does subtle progesterone rise on the day of HCG affect pregnancy rate in long agonist ICSI cycles?

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Abstract
Purpose To evaluate the impact of subtle progesterone (P4) rise on the day of HCG on pregnancy outcome in ICSI patients stimulated with long agonist protocol.
Methods One hundred forty-nine consecutive controlled ovarian hyperstimulation cycles for ICSI using long luteal agonist protocol.
Results Mean serum progesterone on day of hCG was 0.88±0.51 ng/mL values ≥1 ng/mL were found in 34.2% of cycles. Serum E2 on day of hCG and number of oocytes retrieved were significantly higher in the group with P4≥ 1 ng/mL. The area under ROC for serum progesterone in prediction of pregnancy was 0.52, indicating that within the values studied, serum progesterone on day of hCG is not predictive of pregnancy outcome.
Conclusion P4 values ≥1 ng/mL on day of hCG are common in long agonist ICSI cycles particularly with high response. Within the P4 values encountered in this study, implantation and pregnancy rates are not adversely affected.

Capsule Pre-hCG, subtle progesterone rise is common in high response long agonist ICSI cycles, and is not associated with lower pregnancy rate.

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Introduction

Prior to the introduction of gonadotropin releasing hormone agonists (GnRHa), premature luteinization [1] was common, affecting up to 20% of controlled ovarian hyperstimulation cycles [2]. In non-agonist protocols, premature luteinization is common in cases with lower ovarian reserve [3, 4] where LH rise occurs despite lower response to stimulation and lower estradiol values. A deficiency of the so called gonadotropin surge attenuating factor (Gn-SAF) has been incriminated [5].

Nowadays when agonist and antagonist protocols are in routine use, genuine premature luteinization is rarely encountered. The term, however, despite being a misnomer, is still used to indicate the appreciable rise in serum progesterone which sometimes occurs before hCG administration [6–8]. Premature progesterone rise (PPR), a more precise term, is defined as serum progesterone ≥1.0 ng/mL (about 3.2 nmol/L) or P4/E2 ratio≥1 on the day of hCG administration [9, 10]. Such premature rise in serum progesterone was found to correlate with relatively high ovarian response to stimulation as indicated by large number of oocytes and high estradiol levels [11]. The reported incidence of PPR varies between 5–30% of long agonist cycles [12, 13]. A pituitary escape from the suppressive effect of GnRHa is unlikely because LH concentrations are invariably low. Exposure to large amounts of exogenous gonadotropins [14], increased LH sensitivity of granulosa cells, and pooled contribution of numerous mature follicles were suggested as causes for this PPR [15, 16].

The debate whether this subtle PPR negatively affects ICSI cycle outcome is still ongoing.

Table 1 Clinical and laboratory data classified by serum progesterone on day of HCG

Clinical data	P4<1	P4≥1	P value
Age	31.24±4.365	30.02±4.19	0.180
Stimulation days	11.9±1.7	11.7±1.1	0.193
No. of ampoules	47.6±12.6	44.5±11.8	0.073
E2 on day of hCG pg/mL	2,240±1,062	2,777±1,366	0.019*
No. of follicles	12.3±4.3	13.4±4.6	0.077
No. of oocytes	12.1±4.15	13.63±4.7	0.042*
% of mature oocytes	89.6±15.6	92.1±8.8	0.09
Fertilization rate	77.9±16.2	76.6±19.7	0.34
Cleavage rate	80.1±23.5	79.1±22.4	0.31
Embryos transferred	2.0±0.7	1.96±0.8	0.71
Implantation rate	25.2±31.1	24.6±32.9	0.41
Clinical pregnancy rate	44%	47%	0.63

*Significant $p<0.05$

Subjects and methods

One hundred and forty nine consecutive cycles of controlled ovarian hyperstimulation (COH) for ICSI because of male factor infertility were included. Criteria for inclusion were female age <37 years, regularly menstruating, and basal FSH <10 mIU/mL. Long luteal agonist protocol was used using Buserline acetate. Recombinant FSH injections in a fixed dose of 225 IU/day (Gonal-F 75 IU; Serono, Italy) were started once down regulation is confirmed (serum E2 <50 pg/mL). Ovarian response was monitored with vaginal ultrasonography as well as serum estradiol. Criteria for hCG were at least three follicles ≥18 mm in size with a serum E2 level of >150 pg/mL per follicle. Serum estradiol (E2) and progesterone (P) levels were assessed on the day of hCG administration. Oocyte retrieval (OR) was carried out 34–36 h after hCG using single lumen needle and automatic aspiration system. Cumulus removal was carried out after 2 h of pre-incubation by repeated pipetting in hyaluronidase (20 IU/mL), then corona was removed by repeated pipetting into 130 μ strippers. Semen was prepared by density gradient centrifugation and regular ICSI was carried out on metaphase II oocytes thereafter. Fertilization was assessed 16–18 h after ICSI and was confirmed by the presence of two pronuclei. Ultrasound-guided embryo transfer (ET) was done 52 h after OR. Class A embryos were those with equal blastomeres and less than 10% fragmentation. Vaginal micronized progesterone 800 mg/day was used for luteal support. Statistical analysis was done using SPSS software, version 14.0.

Results

Among the 149 included cycles, serum progesterone (P4) on day of hCG ranged between 0.08 and 2.9 (0.88±0.51). P4 level was <1 ng/mL in 98 cycles (65.8%) while values ≥1 ng/mL were found in 51 cycles (34.2%) among them values ≥2 ng/mL were found only in five cases (3%) within which one pregnancy occurred. Cycles were classified using P4 values ≥1 as cut-off into two groups. The group with P4≥1was considered to have progesterone rise. Age, stimulation days, number of ampoules, number of embryos transferred, implantation rates and pregnancy rates were not different between the two groups. Serum E2 on day of hCG, and number of oocytes retrieved, however, were significantly higher in the group with P4≥1 ng/mL. (P values 0.019 and 0.042 respectively) (Table 1). The area under ROC for serum progesterone in prediction of pregnancy was 0.52, 95% confidence interval 0.427 and 0.616 indicating that within the values studied, serum

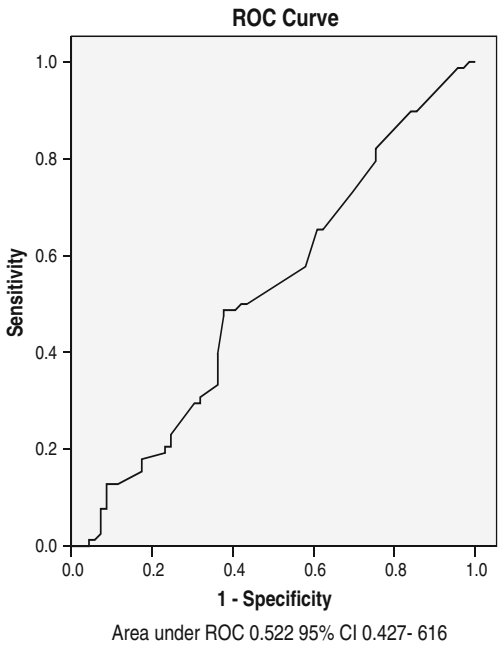

Fig. 1 Receiver-operating characteristic curve for the value of serum progesterone on the day of hCG in prediction of pregnancy

progesterone on day of hCG is not predictive of pregnancy outcome.(Fig. 1)

Discussion

The subject is not new but the issue is still unresolved. Schoolcraft et al [8] started the debate in 1991 when they reported that pregnancy rate is adversely affected by this progesterone rise. Shortly after this report Silverberg and colleagues [17], and Mio et al [18] reported similar results. Contemporary to these early studies, several other reports fueled the debate by refuting the finding and denying any effect of PPR on cycle outcome [19–21].

Because embryo quality was found to be improved rather than impaired in these cases, as tested in donor oocytes [22], and frozen-thawed ET cycles [23], impaired endometrial receptivity was suspected as a primary cause of decreased pregnancy rates in these cycles.

In this study, serum levels of progesterone on day of hCG were ≥1 ng/mL in 51 cycles (34.2%), while values ≥2 ng/mL were found only in five cases (3%), among which one pregnancy occurred. According to most of the literature, serum progesterone ≥1.0 ng/mL (about 3.2 nmol/L) or P4/E2 ratio≥1 on the day of hCG is defined as premature progesterone rise [9, 10]. Therefore, cycles in this study were classified using P4 values ≥1 ng/mL as cut-off into two groups, one with PPR defined as values ≥1 ng/mL, and one with values <1 ng/mL. Age, stimulation days, number of ampoules, number of embryos transferred, implantation rates and pregnancy rates, were not different between the two groups. Serum E2 on day of hCG, and number of oocytes retrieved however, were significantly higher in the group with P4≥1. The area under the receiver operating characteristic (ROC) curve was calculated to assess the predictive value of the progesterone for the probability of pregnancy. The area under the ROC curve was 0.522 [95% confidence interval (CI) 0.427–0.616] which favors the null hypothesis meaning that progesterone values are not predictive of pregnancy. Indeed, data are still conflicting regarding the issue; while Li et al [24] in 2008 reported a negative association between serum progesterone levels ≥3.97 nmol/L and pregnancy rate, a recent meta-analysis in 2007 [25] considered that the best available evidence does not support any association between progesterone elevation on the day of hCG administration and the probability of clinical pregnancy in women undergoing ovarian stimulation with GnRH analogues and gonadotrophins for IVF. An even more confusing report was that of Papanikolaou et al [26] in which progesterone rise on the day of hCG was found to impair pregnancy outcome in day 3 single embryo transfer, while having no effect on day 5 single blastocyst transfer. In a recent trial to explain and evaluate PPR done by Nikolettos et al [27], patients with serum progesterone above 0.9 ng/mL on day of hCG had elevated serum concentrations of IL-6, VEGF, and bFGF, as well as elevated intrafollicular concentrations of IL-6. They suggested that these cytokines might influence certain enzymes in steroidogenic pathway. In their report, the outcome of ICSI cycles was not associated with premature elevation of progesterone when the cut-off value is set at 0.9 ng/mL.

We believe that using different methodologies for hormone measurement, different progesterone cut-off levels in different patient populations with different stimulation protocols and different responses. The issue would remain unresolved. We also agree with de-Ziegler in his letter [28] that until proven otherwise, the clinical consequence of pre-hCG progesterone elevation should be analyzed within the context of the ovarian response to COH in which it is encountered. Only when pre-hCG progesterone elevation is observed in case of low response to COH, poor prognosis might be predicted.

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Contribution to authorship Design of the study, acquisition of data, analysis of data, reviewing and finalizing the manuscript: Hisham Ali Saleh. Participation in acquisition and analysis of data, drafting and editing the manuscript: Mervat sheikh Elarab. Contribution to collection and analysis of data Mohamed Draz. All authors have approved the final version of manuscript.

Details of ethics approval The trial was performed in accordance with the declaration of Helsinki, and subsequent revisions, and approved by the ethical institutional review board. Written informed consent was obtained from all included cases before entering in study.

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