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ARTICLE

High responders are not exempt from detrimental effects of prematurely rising progesterone levels in fresh embryo transfer cycles

BIOGRAPHY

Dr Oktem completed his residency in obstetrics and gynecology at Marmara University School of Medicine, Istanbul, in 2001, and his postdoctoral fellowship in reproductive endocrinology and infertility at the Center for Reproductive Medicine and Infertility at Weill Medical College and molecular/developmental biology at Memorial Sloan Kettering Cancer Center, New York, USA between the years 2003–2008. He is currently professor of obstetrics and gynecology at Koc University School of Medicine Department of Obstetrics and Gynecology, the division Reproductive Endocrinology and Infertility, Istanbul, Turkey.

Ozgur Oktem[1,2,*], Kayhan Yakin[1,2], Sule Yildiz Oguz[1], Aycan Isiklar[2], Basak Balaban[2], Bulent Urman[1,2]

KEY MESSAGE

High serum progesterone levels on the day of ovulation trigger are associated with declining clinical pregnancy rates in patients with all types of ovarian response, including high responders in fresh embryo transfer cycles in the GnRH agonist long protocol.

ABSTRACT

Research question: Are high-responder IVF patients protected from the deleterious effect of prematurely elevated serum progesterone level on the probability of pregnancy?

Design: In this retrospective cohort study, 2971 autologous fresh embryo transfer IVF cycles with gonadotrophin-releasing hormone agonist long protocol were analysed to investigate whether the detrimental effect of prematurely rising progesterone levels on clinical pregnancy rate (CPR) varies depending on the magnitude of ovarian response. Nine different evenly spaced intervals were constructed for serum progesterone level on the human chorionic gonadotrophin day (<0.5/0.5–0.9/1–1.4/1.5–1.9/2–2.4/2.5–2.9/3–3.4/3.5–3.9/>4 ng/ml). Then, IVF cycles in each of these intervals were further divided into low (≤3 oocytes), normal (4–15 oocytes) and high responders (≥16 oocytes).

Results: The progressive rise of serum progesterone from the <0.5 to the >4 ng/ml interval caused a gradual and continuous decline in the CPR of all three types of ovarian response. The absolute difference in the CPR between the lowest and the highest progesterone groups was not related to the magnitude of ovarian response (–26.6%, –37.7% and –40.7% for the low, normal and high responders, respectively). On multivariate logistic regression analysis, the detrimental effect of progesterone started at 1.5–1.9 ng/ml, 3.0–3.4 ng/ml and 4.0–4.4 ng/ml intervals for the low, normal and high responders, respectively.

Conclusion: High responders are not exempt from the detrimental effects of prematurely rising serum progesterone levels but the threshold interval where the detrimental effect begins is higher in the high responders compared with the low and normal responders.

[1] Koc University School of Medicine Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Istanbul, Turkey
[2] American Hospital Women's Health Centre, Assisted Reproduction Unit, Istanbul, Turkey

*Corresponding author. E-mail address: ooktem@ku.edu.tr (O Oktem). https://doi.org/10.1016/j.rbmo.2018.11.008
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KEYWORDS

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GnRH agonist protocol
HCG day
Premature progesterone rise

INTRODUCTION

Serum progesterone level may prematurely rise before ovulation trigger and reduce the success of IVF by impairing endometrial receptivity in fresh embryo transfer cycles. There is a growing body of evidence that serum progesterone levels at the time of human chorionic gonadotrophin (HCG) administration are closely related to the magnitude of ovarian response to gonadotrophin stimulation (*Griesinger et al., 2013; Kyrou et al., 2012; Martinez et al., 2016; Ochsenkuhn et al., 2012; Urman et al., 1999; Venetis et al., 2013,2015*). Taken together, these findings are highly suggestive that FSH stimulation itself and/or the degree of ovarian response to stimulation might be responsible for a premature increase in serum progesterone before ovulation. In line with these findings, it was recently shown that FSH stimulation promoted progesterone synthesis and output from human granulosa cells without luteinization in a dose-dependent manner by a direct stimulatory action on the expression and enzymatic activity of the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD), which converts pregnenolone to progesterone (*Oktem et al., 2017*). Therefore, it is likely that a premature increase in serum progesterone before ovulation trigger may result from the inability of the ovary to handle the increased output of precursor steroids generated during multifollicular development in FSH-stimulated IVF cycles. So far, a number of clinical studies, as well as a recent meta-analysis of over 60,000 cycles, have provided solid evidence for the negative impact of elevated pre-HCG serum progesterone levels on the probability of pregnancy in patients undergoing IVF cycles involving fresh embryo transfer (*Hill et al., 2015; Urman et al., 1999; Venetis et al., 2013,2015*). However, there is still controversy in the literature regarding the threshold of serum progesterone level at which its detrimental effect begins. Some studies have suggested that ovarian response itself may moderate the association of progesterone with the chance of pregnancy and the pregnancy rates of high responders are not compromised by premature increase in serum progesterone levels (*Fanchin et al., 1997; Griesinger et al., 2013; Requena et al., 2014; Urman et al., 1999; Xu et al., 2012*). The rationale behind this theory is that high responders are more capable of countering the detrimental effect of progesterone on implantation due to the availability of more good-quality embryos that can tolerate or neutralize the adverse effects of a less receptive endometrium. However, opponents hold that if a premature increase in serum progesterone advances the endometrium and changes its receptivity by altering the expression of endometrial genes within the window of implantation (*Labarta et al., 2011*), then it should affect all types of ovarian response and embryos regardless of their quality and developmental stages (*Bosch, 2015; Hill et al., 2015; Venetis et al., 2015*). This study therefore retrospectively analysed the impact of a wide range of pre-HCG administration serum progesterone levels on the chance of pregnancy in the fresh embryo transfer IVF cycles with the gonadotrophin-releasing hormone (GnRH) agonist long protocol. The primary outcome measure was to investigate whether high responders are protected from the deleterious effect of prematurely rising serum progesterone levels to a greater extent than the low and normal responders.

MATERIALS AND METHODS

Study design

This study was a retrospective cohort analysis of fresh embryo transfer IVF cycles with GnRH agonist long protocol in which serum progesterone level at ovulation trigger was measured. The study was conducted in the IVF clinic of the American Hospital and approved by the Institutional Review Board of Koc University on 28 August 2015 (reference number: 2015.206.IRB2.076). In this clinic, serum progesterone level on the HCG day has been routinely measured since 2010 and so all women who underwent agonist cycles with day 3 fresh embryo transfer within a period of 5 years (2010–2015) were included. After excluding 121 women who had all their embryos cryopreserved (including two cases of moderate ovarian hyperstimulation syndrome in which fresh embryo transfer was not performed) and 45 women with no serum progesterone measurement on the day of HCG administration, the study was conducted with the remaining 2971 women. Each patient was included with only the first embryo transfer cycle to minimize the effect of individual differences on the outcome. Nine different evenly spaced intervals were constructed for serum progesterone level on the HCG day (<0.5/0.5–0.9/1–1.4/1.5–1.9/2–2.4/2.5–2.9/3–3.4/3.5–3.9/4–4.5 ng/ml). Then, the IVF cycles in each progesterone interval were further categorized depending upon ovarian response based on the number of collected oocytes (≤3 oocytes: low responder; 4–15 oocytes: normal responder; ≥16 oocytes: high responder) based on the previous classification (*Drakopoulos et al., 2016*).

Ovarian stimulation and ovulation trigger

Pituitary down-regulation was induced with GnRH agonist leuprolide acetate started 7 days prior to the anticipated day of menstrual bleeding and continued until the day of HCG administration. Recombinant FSH was started on cycle day 3 at a dose of 150–450 IU depending upon age, serum anti-Müllerian hormone level, antral follicle count (AFC), anticipated or documented previous ovarian response, and body mass index. Ovulation was triggered with 250 μg recombinant HCG (Ovitrelle; Merck-Serono, Istanbul, Turkey) when a leading follicle of ≥19 mm and two or more trailing follicles of ≥17 mm were recorded. Follicular aspiration was performed 36 h after ovulation trigger. Decision to proceed with oocyte retrieval and embryo transfers was not based on the serum progesterone levels at ovulation trigger.

Oocyte retrieval, embryo transfer and documentation of pregnancy

Oocyte retrieval was performed under general anaesthesia using a double lumen needle (Cook Ireland Ltd, Limerick, Ireland). Fertilization was achieved with intracytoplasmic sperm injection (ICSI) in all patients. Progesterone was started (Crinone 8% vaginal gel, once a day; Serono, Geneva, Switzerland) on the day of oocyte retrieval in fresh embryo transfer IVF cycles. Embryo culture was performed and cleavage-stage embryos were graded as described previously (*Balaban and Urman, 2005*). In brief, cleavage-stage embryos were graded as follows: grade 1 embryo: no fragmentation with equal-sized homogeneous blastomeres; grade 2 embryo: <20% fragmentation with equal-sized homogeneous blastomeres; grade 3 embryo: 20–50% fragmentation with equal or unequal-sized blastomeres; grade 4 embryo: >50% fragmentation

with equal or unequal-sized blastomeres. Embryos were transferred at cleavage stage (day 3), using soft embryo transfer catheters. Transfer of up to three embryos was allowed until 2010, when new Turkish legislation on assisted reproductive technologies limited the number of transferable embryos to one in the first two cycles in women younger than 35 years of age. A maximum of two embryos can be transferred in the third and subsequent cycles. In women 35 or older, a maximum of two embryos is allowed. Pregnancy test was performed 12 days after embryo transfer and repeated 48 h later when positive. Clinical pregnancies were documented with identification of gestational sac and a fetus with positive cardiac activity at 6–7 weeks of gestation by ultrasound. There were no significant changes in stimulation protocols, laboratory procedures, embryo transfer catheters or the providers during the study period.

Hormone assays

Serum samples for hormone assays were obtained by venepuncture and assessed using a validated electrochemiluminescence immunoassay (ECLIA method, Cobas® 6000; Roche, Basel, Switzerland). Analytical sensitivity (lower detection limit) for progesterone was 0.095 nmol/l (0.030 ng/ml) and the functional sensitivity (defined as the lowest analyte concentration that can be reproducibly measured with a between-run coefficient of variation [CV] of <20%) was 0.48 nmol/l (0.15 ng/ml). The day-to-day CV was 2.9% at 2.31 nmol/l (0.73 ng/ml), 1.4% at 9.57 nmol/l (3.1 ng/ml), and 0.9% at 103.00 nmol/l (32.4 ng/ml). Analytical sensitivity for oestradiol was 18.4 pmol/l (5 pg/ml). The day-to-day CV for oestradiol was 6.7% at 27.4 pg/ml, 1.1% at 1270 pg/ml and 1.9% at 2720 pg/ml. The same assay was used during the study period and was calibrated whenever a new reactive batch was used or whenever an outcome outside the normal range was observed.

Statistical analysis

Continuous variables in the baseline demographic and IVF characteristics were expressed as mean ± SD. Continuous variables of the IVF parameters among the subgroups categorized according to serum progesterone intervals were compared with ANOVA and multiple comparison post-hoc test. Categorical variables were compared using the chi-squared test. A two-tailed Pearson correlation test and linear regression analysis were used to identify the confounding variables that show significant association with serum progesterone level. Zero order, partial and part correlation coefficients and collinearity analysis were applied to determine the relative importance of significant predictors and their contribution to the model. Linear and quadratic regression models were applied to analyse the goodness of fit of slopes of CPR in relation to the intervals of serum progesterone level. The association of progesterone intervals with the probability of pregnancy was analysed using univariate and multivariate logistic regression models. Bivariate logistic regression analysis was used to determine the individual effects of the confounding variables on the odds ratio (OR) of the association of serum progesterone level with the chance of pregnancy that was reached on the univariate model. The significance level was set at 5% ($P < 0.05$). GraphPad Prism (Version 7) and SPSS (Version 23) statistical programs were used to analyse the data and create the figures.

RESULTS

Baseline demographic and IVF characteristics of the cycles are summarized in TABLE 1. The patient's age, day 3 levels of FSH and oestradiol, total dose of gonadotrophins, duration of stimulation, and the day and number of embryos transferred did not vary significantly across the progesterone intervals. The increases in serum progesterone intervals on the HCG day were associated with a better ovarian response to stimulation and higher oestradiol levels on HCG day and oocyte yield. Therefore, there were significant differences among the number of antral follicles >14 mm, oestradiol level on HCG day and the number of total and mature oocytes retrieved across the progesterone intervals (all $P < 0.001$; TABLE 1).

Clinical pregnancy rates according to serum progesterone levels on the day of HCG

CPR significantly decreased from 45.7% to 12.5% ($P = 0.0017$) with progressive rise of serum progesterone from <0.5 ng/ml to 4.0–4.4 ng/ml. There was a significant inverse relationship between the CPR and serum progesterone level on the HCG day in the correlation (r [95% confidence interval, CI]: –0.93 [–0.98 to –0.69], $P < 0.001$) and linear regression analyses ($R^2 = 0.86$, $P < 0.0001$). The quadratic regression model appears to explain this association better than the linear one based on R^2 statistics ($R^2 = 0.98$, $P < 0.0001$) (FIGURE 1). Univariate logistic regression analysis showed that serum progesterone level on the day of HCG was associated with a significant reduction in the chance of pregnancy (OR [95% CI]: 0.84 [0.76–0.92], $P < 0.001$).

The confounders of serum progesterone level on the HCG day and their impact on the effect of progesterone on the chance of pregnancy

Before assessing the impact of pre-HCG administration serum progesterone level on the chance of pregnancy using a multivariate logistic regression model, the variables that are significantly associated with serum progesterone level were first identified, and then analysed to see how these confounders change the effect of serum progesterone level on the chance of pregnancy on the bivariate regression model. It was found that serum progesterone level at the time of HCG administration was significantly associated with the patient's age ($R^2 = –0.15$, $P < 0.01$), AFC ≥14 mm ($R^2 = 0.56$, $P < 0.001$), oestradiol on the HCG day ($R^2 = 0.62$, $P < 0.001$) and the numbers of total ($R^2 = 0.58$, $P < 0.001$) and mature oocytes retrieved ($R^2 = 0.55$, $P < 0.001$), numbers of total ($R^2 = 0.41$, $P < 0.01$) and grade 1 ($R^2 = 0.34$, $P < 0.01$) embryos (FIGURE 2). Because this is a multivariable regression model, zero order, partial and part correlation coefficients and collinearity analysis, together with tolerance and variance inflation factor, were calculated to determine the relative importance of significant predictors and their contribution to the model. When all the confounders described above were included in the linear regression model, it appeared that they could only explain half of the serum progesterone elevations ($R^2 = 0.52$, $P < 0.001$). Among these confounders, oestradiol level on the HCG day, total and mature oocyte numbers and AFC ≥14 mm contributed more to the model because they had larger absolute standardized coefficients than total and grade 1 embryo numbers. The partial and part correlations for total and grade 1 embryo numbers

TABLE 1 CHARACTERISTICS OF THE FRESH EMBRYO TRANSFER IVF CYCLES CATEGORIZED ACCORDING TO NINE DIFFERENT THRESHOLD INTERVALS OF SERUM PROGESTERONE LEVEL ON THE HCG DAY

Progesterone (ng/ml)	Overall	<0.5	0.5–0.9	1–1.4	1.5–1.9	2–2.4	2.5–2.9	3–3.4	3.5–3.9	4–4.4
n	2971	186	626	933	612	320	149	81	40	24
Age (years)	31.2 ± 4.8	32.2 ± 3.1	31.6 ± 3.8	31.1 ± 2.8	31.0 ± 3.6	31.1 ± 2.7	31.5 ± 2.6	30.7 ± 1.9	30.6 ± 2.4	31.3 ± 3.7
FSH (mIU/ml), cycle day 3	5.8 ± 2.6	6.5 ± 2.5	6.1 ± 2.8	5.9 ± 2.5	5.7 ± 2.6	5.7 ± 2.3	5.3 ± 2.1	5.6 ± 2.1	5.7 ± 2.7	5.5 ± 1.5
Oestradiol (pg/ml), cycle day 3	45.3 ± 17.4	43.1 ± 19.0	42.1 ± 13.0	44.6 ± 20.0	45.8 ± 18.0	44.5 ± 13.0	42.3 ± 16.0	43.4 ± 30.0	51.5 ± 26.0	44.1 ± 33.0
Duration of stimulation (days)	10.3 ± 1.1	10.3 ± 1.2	10.2 ± 1.7	10.2 ± 1.5	10.4 ± 1.5	10.6 ± 1.5	10.5 ± 1.4	10.6 ± 1.7	10.6 ± 1.5	10.4 ± 1.3
Total FSH consumed (IU)	3292 ± 1323	3858 ± 1482	3336 ± 1248	3053 ± 1315	3082 ± 1050	3412 ± 1020	3282 ± 1260	3492 ± 1560	3356 ± 1580	3842 ± 1556
Number of follicles ≥14 mm[a]	12.8 ± 2.9	9.3 ± 2.3	9.2 ± 2.1	10.6 ± 2.1	11.3 ± 2.2	12.8 ± 3.4	13.6 ± 3.5	15.1 ± 3.1	18.1 ± 3.9	20.3 ± 3.5
Oestradiol (pg/ml), HCG day[a]	2884 ± 1422	1781 ± 1090	1863 ± 1241	2333 ± 1360	2620 ± 1435	2947 ± 1549	2981 ± 1508	2997 ± 1462	3520 ± 1736	3987 ± 1509
Progesterone (ng/ml), HCG day										
Mean ± SD	1.2 ± 0.76	0.3 ± 0.1	0.73 ± 0.1	1.17 ± 0.1	1.68 ± 0.1	2.16 ± 0.1	2.66 ± 0.1	3.16 ± 0.1	3.67 ± 0.1	4.2 ± 0.2
Median	1.1	0.29	0.7	1.2	1.7	2.2	2.6	3.1	3.7	4.2
Total oocyte number[a]	12.2 ± 2.8	9.6 ± 2.4	10.1 ± 3.2	11.7 ± 3.4	12.3 ± 2.9	13.1 ± 3.5	14.3 ± 2.5	14.8 ± 2.4	18.1 ± 2.8	20.4 ± 2.4
Mature oocyte number[a]	10.1 ± 3.2	7.1 ± 3.1	7.4 ± 2.1	8.4 ± 2.4	9.4 ± 2.2	9.8 ± 2.6	10.3 ± 2.3	11.2 ± 2.5	15.5 ± 2.7	17.1 ± 3.8
Cleavage rate (%)	99.5 ± 4.1	99.7 ± 2.6	99.7 ± 4.1	99.3 ± 4.7	99.5 ± 4.7	99.4 ± 3.1	99.1 ± 4.2	99.0 ± 0.6	99.6 ± 1.8	99.7 ± 2.8
Day of embryo transfer	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0
Number of embryos transferred	2.5 ± 0.4	2.5 ± 0.5	2.4 ± 0.7	2.5 ± 0.6	2.5 ± 0.4	2.3 ± 0.7	2.4 ± 0.5	2.8 ± 0.3	2.4 ± 0.2	2.5 ± 0.9
Clinical pregnancy rate, %[b]	42.2 (1255/2971)	45.7 (85/186)	45 (282/626)	44.1 (411/933)	42.6 (261/612)	38.4 (123/320)	38.9 (58/149)	29.6 (24/81)	20 (8/40)	12.5 (3/24)

HCG = human chorionic gonadotrophin;

[a] $P < 0.001$ (ANOVA and multiple comparison post hoc test); <0.5 vs 4.0–4.4 intervals: $P < 0.01$; <0.5 vs 3.5–3.9 intervals: $P < 0.01$; 0.5–0.9 vs 4.0–4.4 intervals: $P < 0.001$; 0.5–0.9 vs 3.5–3.9 intervals: $P < 0.001$; 1.0–1.4 vs 4.0–4.4 intervals: $P < 0.01$; 1.5–1.9 vs 4.0–4.4 intervals: $P < 0.01$.

[b] $P < 0.0001$ (contingency table analysis).

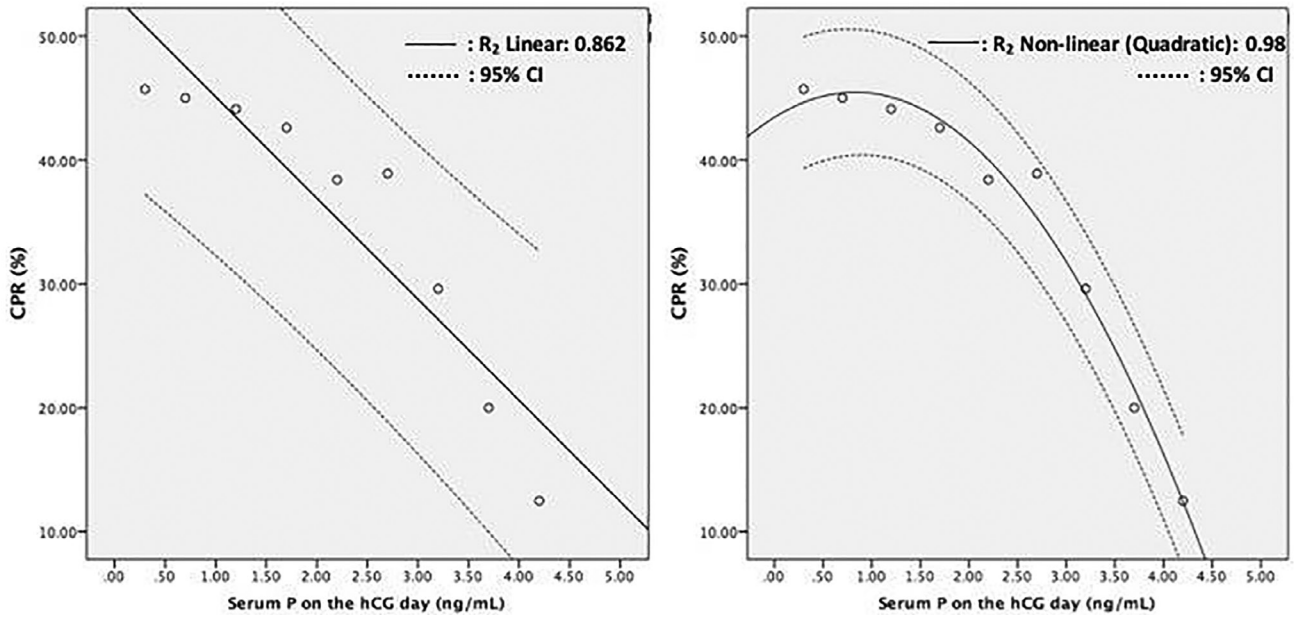

FIGURE 1 The clinical pregnancy rates (CPR) across the intervals of serum progesterone level on the human chorionic gonadotrophin (HCG) day. The non-linear quadratic regression ($R^2 = 0.98$, $P < 0.0001$) explains this association better than the linear model ($R^2 = 0.86$, $P < 0.001$) based on R^2 statistics.

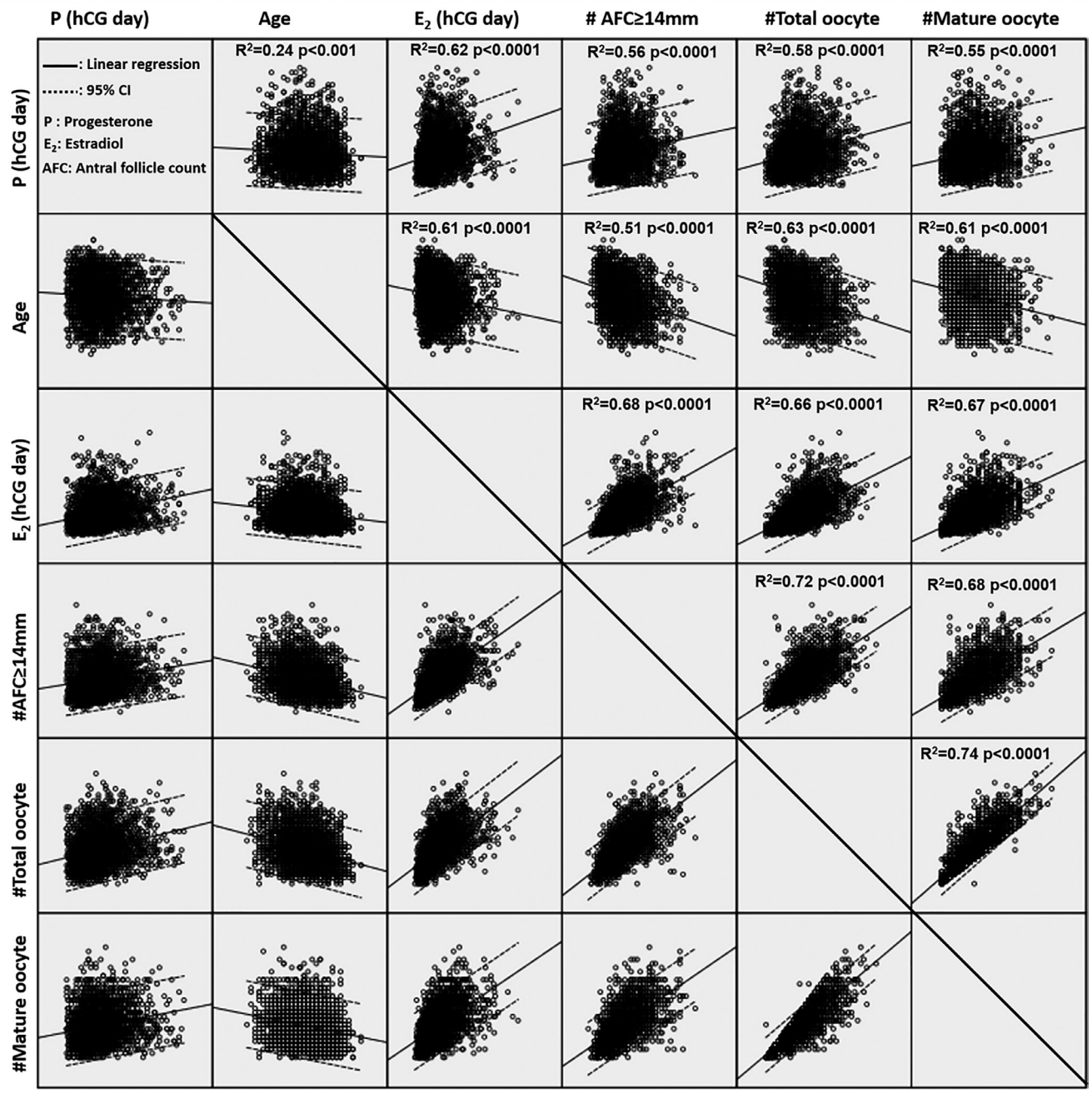

FIGURE 2 Scatter matrix diagram showing the variables that are significantly associated with serum progesterone level on the HCG day in the linear regression analysis. R^2: the coefficient of determination in the linear regression analysis. Solid line: linear regression. Dotted line: 95% confidence interval (CI).

dropped sharply from the zero order correlation, meaning that much of the variance in serum progesterone level that is explained by total and grade 1 embryo numbers is also explained by other variables. In line with these results tolerance values were very low and variance inflation factors were greater than 3 for the total and mature oocyte numbers (Supplementary **TABLE 1**).

Serum progesterone level on the HCG day was found to be associated with a significant reduction in the chance of pregnancy (OR [95% CI]: (0.84 [0.76–0.92], $P < 0.001$) on univariate analysis. Among the confounding variables analysed above, total oocyte number appeared to be the strongest confounder as it caused the greatest reduction in the OR (–17.8%) on bivariate analysis. This was followed by the mature oocyte number (–10.7%), the number of embryos transferred (–9.5%), AFC ≥14 mm (–4.76%), oestradiol on the day of HCG (–3.57%), age (–2.38%) and the FSH dose (–2.38%). Year, duration of stimulation and aetiology of infertility did not change the OR. Total and grade 1 embryo numbers increased the OR by +1.19% and +4.76%, respectively.

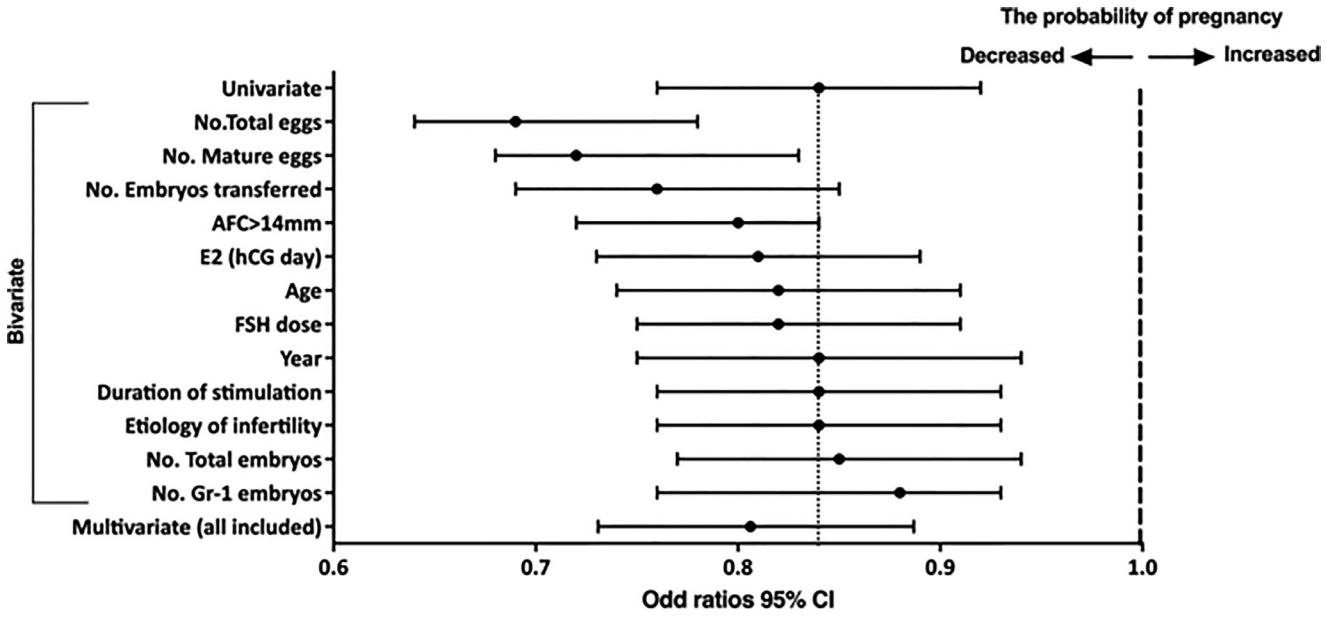

FIGURE 3 The individual effect of each confounding variable on the association of serum progesterone level with the chance of pregnancy on bivariate logistic regression analysis.

When multivariate analysis was applied after including all of the confounders described above, the OR further declined to 0.80 (0.73–0.88, $P < 0.001$) (FIGURE 3).

The effect of serum progesterone on the chance of pregnancy according to the ovarian response

Baseline demographic and IVF characteristics of these cycles are provided in TABLE 2. As can be expected certain variables such as oocyte yield, mean serum progesterone level and the rates of clinical pregnancy showed significant variations according to the

TABLE 2 THE CHARACTERISTICS OF THE IVF CYCLES CATEGORIZED ACCORDING TO THE TYPE OF OVARIAN RESPONSE AS LOW (≤3 OOCYTES), NORMAL (4–15 OOCYTES) AND HIGH RESPONDERS (≥16 OOCYTES)

Progesterone (ng/ml)	≤3 oocytes	4–15 oocytes	≥16 oocytes
n	98	2141	732
Age (years)[a]	31.9 ± 4.8	32.7 ± 4.6	29.5 ± 4.7
FSH (mIU/ml), cycle day 3	6.8 ± 2.7	5.9 ± 2.5	5.3 ± 2.3
Oestradiol (pg/ml), cycle day 3	46.6 ± 16.6	49.1 ± 19.0	48.6 ± 17.0
Duration of stimulation (days)	10.1 ± 1.8	10.3 ± 1.2	10.2 ± 1.7
Total FSH consumed (IU)	3626 ± 1509	3417 ± 1424	3220 ± 1278
Number of follicles ≥14 mm[b]	4.1 ± 2.2	9.3 ± 2.3	20.2 ± 2.1
Oestradiol (pg/ml) HCG day[b]	934 ± 514	2015 ± 1131	3633 ± 1477
Progesterone (ng/ml) HCG day[b]			
Mean ± SD	0.95 ± 0.5	1.2 ± 0.6	1.6 ± 0.7
Median	0.9	1.1	1.6
Total oocyte number[b]	2.5 ± 0.6	9.5 ± 4.1	20.1 ± 3.8
Mature oocyte number[b]	2.1 ± 1.1	7.2 ± 2.7	13.8 ± 3.8
Cleavage rate (%)	99.4 ± 5.1	99.7 ± 2.6	99.7 ± 4.1
Day of embryo transfer	3 ± 0	3 ± 0	3 ± 0
Number of embryos transferred[c]	1.7 ± 0.8	2.7 ± 0.8	2.4 ± 0.9
Clinical pregnancy rate (CPR), %[d]	21.2	41.4	46.9

[a,b]Multiple comparison post hoc test after ANOVA.

[d]Contingency table analysis and Fisher's exact test.

[a] $P < 0.001$ low vs high and normal vs high responders.

[b] $P < 0.001$ low vs normal, low vs high, and normal vs high responders.

[c] $P < 0.01$ low vs normal and low vs high responders.

[d] $P < 0.0001$.

TABLE 3 MULTIVARIATE LOGISTIC REGRESSION ANALYSIS OF THE IMPACT OF THE SERUM PROGESTERONE INTERVALS ON THE CLINICAL PREGNANCY RATES IN THE FRESH IVF CYCLES ACCORDING TO THE OVARIAN RESPONSE CATEGORIZED AS LOW (≤3 OOCYTES), NORMAL (4–15 OOCYTES) AND HIGH RESPONDERS (≥16 OOCYTES)

Progesterone interval (ng/ml)	Low responders ≤3 oocytes	Normal responders 4–15 oocytes	High responders ≥16 oocytes
Overall	**0.64 (0.55–0.82)**	**0.72 (0.57–0.95)**	**0.78 (0.51–0.92)**
<0.5	0.80 (0.54–1.13)	0.82 (0.55–1.21)	0.78 (0.52–1.26)
0.5–0.9	0.73 (0.55–1.2)	0.81 (0.66–1.23)	0.68 (0.45–1.24)
1–1.4	0.89 (0.58–1.22)	0.85 (0.66–1.26)	1.06 (0.44–1.33)
1.5–1.9	**0.56 (0.43–0.76)**	0.79 (0.51–1.18)	1.20 (0.29–1.14)
2–2.4	–	0.86 (0.63–1.19)	0.98 (0.53–1.32)
2.5–2.9	–	0.77 (0.54–1.18)	0.85 (0.44–1.28)
3–3.4	–	**0.66 (0.52–0.85)**	0.85 (0.44–1.21)
3.5–3.9	–	**0.52 (0.36–0.74)**	**0.78 (0.58–0.91)**
4–4.4	–	**0.46 (0.25–0.65)**	**0.67 (0.55–0.85)**

Values shown are odds ratio (OR) and 95% confidence interval. The OR were calculated by multivariate logistic regression analysis after including all the covariates that were found to have an impact on the association of serum progesterone level on the chance of pregnancy on bivariate analysis (age, AFC ≥14 mm, oestradiol on the HCG day and the numbers of total and mature oocytes retrieved and the numbers of total and grade 1 embryos produced). The OR written in bold show statistical significance at 0.001 level. No statistical analysis was conducted in the low responders after progesterone ≥2 ng/ml because there were only two and one cases in the 2–2.4 and 2.5–2.9 ng/ml intervals, respectively, and there were no cases when progesterone exceeds ≥3 ng/ml. The OR for a specific progesterone interval was calculated for each ovarian response type. Comparison was not made between the ratio of the odds of pregnancy in a specific interval group vs the odds of pregnancy in another group for each specific ovarian response category.

type of ovarian response. Frequency distribution of serum progesterone levels at ovulation trigger according to the IVF cycles achieving pregnancy versus no pregnancy are illustrated in Supplementary FIGURE 1. The number of IVF cycles with pregnancy was disproportionately low at each progesterone interval and further decreased with rising serum progesterone in women with the low ovarian response group (≤3 oocytes) compared with the normal (4–15 oocytes) and high responders (≥16 oocytes). With higher ovarian response and oocyte yield the number of IVF cycles with pregnancy increases with rising serum progesterone level at ovulation trigger (Supplementary FIGURE 1).

The association of nine different intervals of serum progesterone level on the probability of pregnancy was analysed for each ovarian response type using multivariate regression model after including the confounders described above. The OR and 95% CI were provided for each progesterone interval and ovarian response type in TABLE 3. Increasing progesterone levels were associated with a better response to controlled ovarian stimulation. There were no cases of low response when progesterone levels exceeded 3 ng/ml. Overall, the mean CPR was significantly lower in the low responders compared with the normal (21.2% versus 41.4%, respectively; $P < 0.0001$) and high responders (21.2% versus 46.9%, respectively; $P < 0.0001$). In all types of ovarian response there was a gradual decline in the CPR with increasing serum progesterone level on the HCG day. The rise of serum progesterone level from <0.5 to 4.0–4.4 ng/ml interval caused a significant decline in the CPR of the normal responders (44.8% [64/143] to 7.1% [1/14], $P = 0.0083$). The decline in the high responders did not show statistical significance (60.7% [17/28] to 20% [2/10]). In the low responders, the number of IVF cycles in each progesterone interval was gradually diminished with rising serum progesterone level. Therefore, it was not possible to conduct a reliable statistical analysis at progesterone intervals >2 ng/ml. However, as can be seen in FIGURE 4A, there was still a gradual and continuing decline in the pregnancy rate with rising serum progesterone level in this group as well (FIGURE 4A). However, the absolute difference in the CPR between the lowest and the highest progesterone groups was not related to the magnitude of ovarian response (–26.6%, –37.7% and –40.7% for the low, normal and high responders, respectively). The association of serum progesterone intervals with the chance of pregnancy were separately analysed for each type of ovarian response in the following section.

Low responders

On overall analysis without considering the progesterone intervals, serum progesterone level on the HCG day was associated with a significant reduction in the probability of pregnancy (OR [95% CI]: 0.64 [0.55–0.82], $P < 0.001$) in the low responders. The negative impact of progesterone on the chance of pregnancy first appeared in the 1.5–1.9 ng/ml interval (OR [95% CI]: 0.56 [0.43–0.76], $P < 0.001$). The effect of serum progesterone ≥2 ng/ml could not be assessed because there were only two and one IVF cycles in the 2.0–2.4 and 2.5–2.9 ng/ml intervals, respectively, and no pregnancy was achieved in those cycles. Also, there were no cases in the 3.0–3.4, 3.5–3.9 and 4–4.4 ng/ml intervals (FIGURE 4B).

Normal responders

Analysis of the normal responders in the same manner revealed that serum progesterone was negatively associated with the chance of pregnancy (OR [95% CI]: 0.72 [0.57–0.95], $P < 0.001$). The detrimental effect began at 3.0–3.4 ng/ml interval (OR [95% CI]: 0.66 [0.52–0.85], $P < 0.001$) and continued at 3.5–3.9 (0.77 [0.43–0.88], $P < 0.01$) and 4.0–4.4 ng/ml interval (0.46 [0.25–0.65], $P < 0.0001$). Serum progesterone level in any other intervals <3 ng/ml was not associated with any reduction in the chance of pregnancy (FIGURE 4B).

High responders

High responders were also affected by the deleterious effects of elevated serum

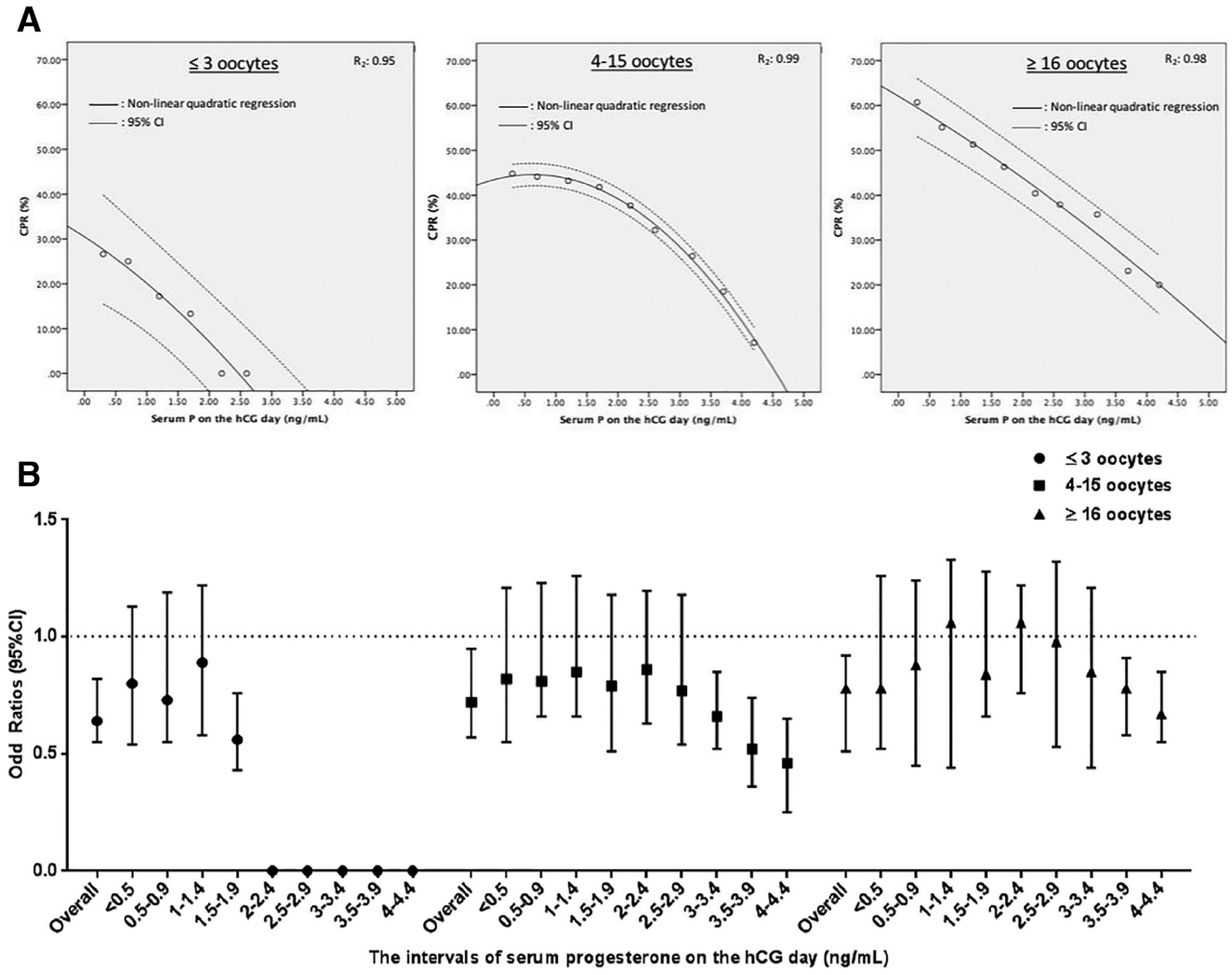

FIGURE 4 (A) The clinical pregnancy rates (CPR) of the IVF cycles across the progesterone intervals and goodness of fit of the slopes in quadratic regression analysis categorized according to the types of ovarian response. (B) The association of the progesterone intervals with the probability of pregnancy according to the types of ovarian response in multivariate regression analysis.

progesterone levels. There was a trend for a negative impact of progesterone on the chance of pregnancy in the 3.5–3.9 ng/l interval (0.78 [0.58–0.91], $P = 0.052$). It became significant with further rise of serum progesterone to the 4.0–4.4 ng/ml (0.67 [0.55–0.85], $P = 0.001$). On overall analysis without considering the progesterone intervals, serum progesterone showed a significant negative association with the chance of pregnancy in this group (0.78 [0.51–0.92], $P < 0.01$) (FIGURE 4B).

We also divided progesterone levels into three different thresholds (<0.5, 0.5–1.5 and ≥1.6 ng/ml) as many previous studies did and then categorized ovarian response types according to these intervals. We observed that CPR gradually decline in all three types of ovarian response with the rise of progesterone from <0.5 to ≥1.6 ng/ml. While the decline in CPR was significant for the low ($P = 0.002$) and normal responders ($P = 0.04$) it was not significant for the high responders (Supplementary FIGURE 2A). On multivariate regression analysis deleterious effect of progesterone on the probability of pregnancy occurred at ≥1.6 ng/ml cut-off value in both the low and normal responders but was not present in the high responders (Supplementary FIGURE 2B). These results suggest that these cut-off values of progesterone were not able to identify detrimental progesterone level in the high responders and discriminate it from the low and normal responders.

DISCUSSION

It was shown in this study that a premature rise in serum progesterone was associated with a significant reduction in the probability of pregnancy after fresh embryo transfers and high responders were not exempt from this negative impact. The absolute difference in the CPR between the lowest and the highest progesterone subgroups was similar in low, normal and high responders (–26.6%, –37.7% and –40.7%, respectively). However, the detrimental effect of progesterone was not evident until the serum progesterone level reached the 4.0–4.4 ng/ml interval in the high responders, in contrast to the levels of 3 ng/ml and 1.5 ng/ml in the normal and low responders, respectively.

Pregnancy rates gradually declined in a linear fashion with progressive rise of serum progesterone levels on the day of HCG in all three types of ovarian response in this cohort, suggesting that

the endometrial receptivity is gradually perturbed by rising progesterone level. In fact, altered expression of the receptivity genes in the endometrium has been shown in autologous fresh embryo transfer IVF cycles with elevated progesterone levels. The rise of progesterone from <0.9 to 1–1.5 ng/ml caused alterations in the expression of a small number of the endometrial genes (28 genes) whereas its increase to a level >1.5 ng/ml from 1–1.5 ng/ml was associated with alterations in a larger number of genes (819 genes) (*Van Vaerenbergh et al., 2011*). Another study with a similar methodology analysed the changes in the expression of endometrial genes with micro-array in 12 oocyte donors after stimulation with either GnRH agonist or antagonist protocols. The study identified 140 genes significantly dysregulated (64 up- and 76 down-regulated) in the study group (six patients with serum progesterone >1.5 ng/ml) compared with the other half whose serum progesterone level at ovulation trigger was <1.5 ng/ml (*Labarta et al., 2011*). In support of these findings, one study examined the effect of premature progesterone rise on the genomic profile of peri-implantation endometrium in 20 IVF patients with normal and elevated serum progesterone level on the day of HCG. The study analysed transcriptome profiles of the peri-implantation endometrium in stimulated cycles and identified 197 genes differentially expressed (26 up-regulated and 171 down-regulated with a fold-change value of ≥1.5) in endometrial biopsy samples of the patients with elevated serum progesterone >1.7 ng/ml on the HCG day compared with those with progesterone <1.7 ng/ml. Interestingly, some of the changes in the expression profiles of the genes were involved in the natural killer cell mediated cytotoxicity pathway (*Liu et al., 2017*). Although these studies had small sample size their findings may provide molecular evidence for the altered expression of genes involved in endometrial receptivity when serum progesterone level prematurely elevates before ovulation trigger.

There are conflicting reports in the literature regarding the impact of prematurely elevated serum progesterone levels on pregnancy rates. While a group of studies, all considering different cut-off values for progesterone levels, showed that elevated serum progesterone levels on the day of trigger were not associated with poor clinical outcome, *Xu et al. (2012)* reported that high progesterone levels had a negative impact on the chance of pregnancy and the threshold where pregnancy rates were impaired was higher (>2.25 ng/ml) for high responders, compared with poor and normal responders (*Fanchin et al., 1997; Griesinger et al., 2013; Urman et al., 1999; Xu et al., 2012*). Of note, high responders continued to produce good-quality embryos in increasing numbers despite high serum progesterone levels. But it is unclear whether the transfer of good-quality embryos may override this adverse effect of high progesterone to a certain extent, until it reaches a threshold level where implantation is prevented due to gross perturbations in endometrial receptivity. When deciding to cancel fresh embryo transfer, patients should be individually counselled regarding the threshold effect of progesterone according to ovarian response.

In fact, a recent study showed that premature progesterone elevation at early follicular phase may also be associated with decreased fresh and cumulative live birth rates by increasing embryo wastage when over 3400 GnRH antagonist ICSI cycles with fresh embryo transfer were stratified according to the following progesterone levels on the day of ovulation triggering: ≤0.50, 0.51–1.49 and ≥1.50 ng/ml (*Racca et al., 2018*). Similar retrospective data on GnRH antagonist cycles showed that elevated serum progesterone is associated with a decrease in the number of top-quality day 5 embryos. Based on the ROC curve analysis the study identified progesterone level >1.49 ng/ml as the best cut-off for identification of patients at risk for the absence of top-quality blastocysts (AUC 0.55, $P < 0.01$) (*Vanni et al., 2017*). Although these results were obtained from retrospective cohorts and need to be substantiated in prospective studies, they suggest that elevated serum progesterone before ovulation may reduce the chance of pregnancy by not only impairing endometrial receptivity but also decreasing embryo quality and increasing embryo wastage.

It may be speculated that higher progesterone levels are due to an increased output from the granulosa cells of multiple stimulated follicles while the mechanism is perturbed in some other way in low responders. Intra-ovarian actions of FSH and/or the degree of ovarian stimulation might be responsible for premature progesterone output from granulosa cells without luteinization. In line with this notion, our recent work has demonstrated that FSH has a direct stimulatory action on the expression and enzymatic activity of the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD), which converts pregnenolone to progesterone in human granulosa cells and ovarian tissue samples. This FSH stimulation resulted in a dose-dependent increase in the synthesis and secretion of progesterone from granulosa cells without luteinization (*Oktem et al., 2017*). It was shown that serum progesterone may prematurely rise in up to 28% of the natural cycles and adversely impact pregnancy rates if its elevations persist for two or more days. The underlying pathogenetic mechanism of progesterone rise in natural cycles might be different from stimulated IVF cycles given that there is no gonadotrophin use or multifollicular development in the former (*Lee et al., 2014*).

The major limitation of this study is the lack of data regarding ongoing pregnancy and live birth rates. Despite the occurrence of implantation, pregnancies in a high progesterone environment may result in miscarriage. Another limitation is that the limited number of cases with high serum progesterone levels (serum progesterone >3.5 ng/ml) may decrease the reliability of the results of the logistic regression test by causing the regression coefficients to be biased in both positive and negative directions (*Peduzzi et al., 1996*). The strengths, however, are the large number of subjects included from a single centre and a relatively homogenous patient population (long protocol, recombinant FSH stimulation, no significant change in the laboratory protocols and embryo transfer providers), and all patients undergoing cleavage-stage embryo transfer. Another major strength of the study is that the cycles were not cancelled based on progesterone levels.

High serum progesterone levels on the day of ovulation trigger with HCG is associated with declining CPR in patients with all types of ovarian response, including high responders in fresh embryo transfer GnRH agonist IVF cycles. The question that remains to be answered is whether alterations in endometrial receptivity or perturbations

in embryo quality or both are responsible for the decline in CPR. While the former could be managed with frozen–thawed embryo transfer strategy, poor embryo quality will undoubtedly have a negative impact on the probability of pregnancy in both fresh and frozen embryo transfer cycles. Measuring serum progesterone level before ovulation trigger could be particularly important for IVF patients who continue to have unexplained repeated implantation failures despite a high or good ovarian response and transfer of good-quality embryos. When deciding to cancel fresh embryo transfer, patients should be individually counselled regarding the threshold effect of progesterone according to ovarian response. Other strategies that have been recently outlined, such as avoidance of overt and prolonged ovarian stimulation or freeze-all strategies (*Lawrenz et al., 2018*) should be considered.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2018.11.008.

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