

# Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for *in vitro* fertilization: analysis of over 4000 cycles

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**BACKGROUND:** The influence of elevated serum progesterone levels during *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles on pregnancy rates is a matter of continued debate among fertility clinicians. Efforts to resolve this question have been impeded by the various assays used to measure progesterone and the different, arbitrary threshold values for defining 'high' progesterone levels.

**METHODS:** A non-interventional, retrospective, observational, single-centre cohort study evaluated the relationship between serum progesterone levels on the day of human chorionic gonadotrophin (hCG) administration and the ongoing pregnancy rate in 4032 patients undergoing IVF/ICSI cycles using gonadotrophin-releasing hormone (GnRH) analogues for pituitary down-regulation.

**RESULTS:** Ongoing pregnancy rates were inversely associated with serum progesterone levels on the day of hCG ( $P < 0.001$  for overall trend), irrespective of the GnRH analogue used for pituitary down-regulation. Patients with serum progesterone levels  $\leq 1.5$  ng/ml had significantly higher ongoing pregnancy rates than those with progesterone levels  $> 1.5$  ng/ml (31.0 versus 19.1%;  $P = 0.00006$ ); odds ratio, 0.53 (95% confidence interval, 0.38–0.72). Multivariate regression analysis showed that daily follicle-stimulating hormone dose, number of oocytes and estradiol values on the day of hCG administration were positively associated with progesterone levels ( $P < 0.0001$  for all). Serum progesterone levels were significantly greater in women treated with GnRH agonists ( $n = 1177$ ) versus antagonists ( $n = 2855$ ;  $0.84 \pm 0.67$  versus  $0.75 \pm 0.66$  ng/ml;  $P = 0.0003$ ).

**CONCLUSIONS:** Elevated serum progesterone levels on the day of hCG administration is associated with reduced ongoing pregnancy rates. In particular, serum progesterone levels of  $> 1.5$  ng/ml were associated with lower ongoing pregnancy rates following IVF/ICSI cycles irrespective of the GnRH analogue used for pituitary down-regulation.

**Key words:** progesterone / *in vitro* fertilization/intracytoplasmic sperm injection / GnRH analogues / ongoing pregnancy rate

## Introduction

During controlled ovarian stimulation (COS), progesterone levels rapidly increase following the administration of human chorionic gonadotrophin (hCG) that is given to induce final oocyte maturation (Huang *et al.*, 1986). However, premature luteinizing hormone (LH) surges, caused by the modulatory actions of estradiol ( $E_2$ ) levels induced by gonadotrophins, have led to premature luteinization and cancellation of treatment cycles in patients undergoing *in vitro* fertilization (IVF). By suppressing the release of endogenous gonadotrophins from the pituitary, the introduction of gonadotrophin-releasing

hormone (GnRH) agonists and antagonists has decreased the incidence of premature LH surge and these agents are now routinely used during IVF.

Despite the use of GnRH analogues, subtle increases in serum progesterone levels beyond an arbitrarily defined threshold value have been observed at the end of the follicular phase in COS cycles for IVF and intracytoplasmic sperm injection–embryo transfer (ICSI–ET). Although the frequency of elevated serum progesterone levels varies, incidences as high as 35% (5–35%) of stimulated cycles in individuals treated with GnRH agonists (Edelstein *et al.*, 1990; Silverberg *et al.*, 1991) and 38% (20–38%) of cycles in individuals treated with

GnRH antagonists (Ubaldi *et al.*, 1996; Bosch *et al.*, 2003) have been reported.

Although this pre-hCG progesterone increase has been referred to as ‘premature luteinization’ (Legro *et al.*, 1993), the term is misleading given that the increased levels of serum progesterone occur in the presence of GnRH analogues, i.e. they are taking place under low serum LH concentrations. Rather than excessive amounts of progesterone being produced by granulosa cells as part of early luteinization, it is more likely that the elevated progesterone levels might be attributed to an excess number of follicles, with each one producing a normal amount of progesterone consistent with the late follicular phase (Venetis *et al.*, 2007). In support of this, we have previously presented data suggesting that no relationship exists between LH and progesterone levels at the end of the follicular phase, since the observed increases in progesterone were not accompanied by increases in LH (Bosch *et al.*, 2003). We speculated in our previous report that the increases in progesterone may instead reflect the mature granulosa cell response to high FSH exposure.

The question of whether the presence of these increased serum progesterone levels on the day of hCG administration are associated with the ongoing pregnancy rate is a subject of much debate. Several studies suggest that there is no association between progesterone levels and pregnancy rates (Edelstein *et al.*, 1990; Silverberg *et al.*, 1991; Check, 1994; Check *et al.*, 1994; Givens *et al.*, 1994; Bustillo *et al.*, 1995; Levy *et al.*, 1995; Ubaldi *et al.*, 1995; Abuzeid and Sasy, 1996; Hofmann *et al.*, 1996; Miller *et al.*, 1996; Moffitt *et al.*, 1997; Doldi *et al.*, 1999; Urman *et al.*, 1999; Martinez *et al.*, 2004; Venetis *et al.*, 2007), whereas others have shown that the pregnancy rate is inversely related to serum progesterone levels on the day of hCG administration (Check *et al.*, 1993; Fanchin *et al.*, 1993; Harada *et al.*, 1995; Shulman *et al.*, 1996; Fanchin *et al.*, 1997a; Bosch *et al.*, 2003).

A meta-analysis suggests that the increase in circulating progesterone levels does not correlate with cycle outcome in terms of pregnancy rate (Venetis *et al.*, 2007). However, the results are conflicting owing to the different GnRH analogues administered and the different cut-off levels that were used to define ‘high’ progesterone serum levels (Venetis *et al.*, 2007). That is, the majority of studies that failed to demonstrate an association between serum progesterone levels and pregnancy rate used a threshold value of 0.9 ng/ml, which was mostly chosen arbitrarily without performing a trend analysis to identify an association between progesterone levels and pregnancy (Venetis *et al.*, 2007; Bosch, 2008).

Another point of controversy across these studies is the variation observed in the methodological assays used to assess the specific concentration of circulating progesterone. This is because progesterone assays are generally intended to identify large increases in progesterone to confirm ovulation and, therefore, are optimized for higher progesterone levels than required to identify small progesterone rises in the late follicular phase (Venetis *et al.*, 2007; Fleming, 2008). Future methods, therefore, need to be validated and must show greater consistency at the appropriate range of progesterone concentrations.

The mechanism by which these subtle increases in serum progesterone may impact on pregnancy rates is unclear, with data suggesting that elevated progesterone levels may impair endometrial receptivity rather than oocyte quality (Fanchin *et al.*, 1997b; Smitz *et al.*, 2007). Here, we report on a study that investigated the relationship

between serum progesterone levels on the day of hCG administration and the probability of ongoing pregnancy, in an unselected population of women undergoing COS for IVF/ICSI-ET.

## Materials and Methods

### Study population and design

This was a non-interventional, retrospective, observational, single-centre cohort study of patients undergoing routine practice. To reflect the broad range of patients typically encountered in clinical practice, no inclusion/exclusion criteria were applied on baseline characteristics such as age, body mass index (BMI) or ovarian response. Patients were treated at a single-centre at the Instituto Valenciano de Infertilidad during the period January 2003 to December 2007.

A total of 4032 IVF and/or ICSI-ET cycles (Table I) were performed in which serum progesterone levels were determined on the day of hCG administration. Patients underwent COS using either a GnRH agonist long protocol ( $n = 1177$ ) or a GnRH antagonist daily protocol ( $n = 2855$ ) for pituitary down-regulation. Ovarian stimulation was carried out with: (i) recombinant follicle-stimulating hormone (rFSH) alone (Gonal-F<sup>®</sup>, Laboratorios Serono, Madrid, Spain; Puregon<sup>®</sup>, Organon España S.A., Barcelona, Spain); (ii) rFSH combined with recombinant luteinizing hormone (rLH) (Luveris<sup>®</sup>, Laboratorios Serono, Madrid, Spain); (iii) highly purified human menopausal gonadotrophin (HP-hMG) (Menopur<sup>®</sup>, Ferring Pharmaceuticals, Geneva, Switzerland; alone); or (iv) rFSH combined with HP-hMG. As this was a retrospective study, no specific criteria for selection of stimulation protocol were defined; the choice of protocol was made on a case-by-case basis according to patient characteristics and clinician preference.

The initial dose of gonadotrophin was individualized for each patient according to age, basal FSH levels, antral follicle count, BMI and previous response to COS. Dose adjustments were performed according to ovarian response, which was monitored by means of vaginal scans and

**Table I** Baseline characteristics of the IVF/ICSI-ET population.

Parameter	$n = 4032$
Age (years)	$35.3 \pm 4.1$
Body mass index ( $\text{kg}/\text{m}^2$ )	$23.4 \pm 5.4$
Primary infertility cause [ $n$ (%)]	
Male factor	1237 (30.7)
Female age	713 (17.7)
Previous low response	433 (10.7)
Endometriosis	390 (9.7)
Tubal factor	312 (7.7)
Polycystic ovary	275 (6.8)
Recurrent pregnancy loss	138 (3.4)
Genetic	85 (2.1)
Unknown	449 (11.1)
Procedure [ $n$ (%)]	
ICSI	3447 (85.5)
IVF/ICSI	468 (11.6)
IVF	117 (2.9)

IVF, *in vitro* fertilization; ICSI-ET, intracytoplasmic sperm injection-embryo transfer.

E<sub>2</sub> determinations. As a part of routine clinical practice, a single determination of serum progesterone was performed on the day of hCG administration, which was indicated when three or more follicles reached mean diameter of 18 mm.

The primary objective was to determine the relationship between serum progesterone levels on the day of hCG administration and the ongoing pregnancy rate. Secondary objectives were to: (i) identify a serum progesterone threshold, if appropriate, which would define detrimental circulating progesterone levels for cycle outcomes; and (ii) examine factors related to progesterone elevation.

The ongoing pregnancy rate was defined as the presence of at least one viable fetus beyond Week 20 of pregnancy on ultrasound. A started cycle was considered when patients had their first injection of gonadotrophins.

## Progesterone measurement

Serum progesterone levels were measured on the day of hCG administration. Samples were tested with a microparticle enzyme immunoassay AxSYM System (Abbott Científica S.A., Madrid, Spain), which had a sensitivity of 0.2 ng/ml. Intraobserver and interobserver variation coefficients were 9.6 and 3.9%, respectively. This assay was used for the duration of the study. Besides the internal quality control checks performed daily by the institution laboratory, the assays were calibrated whenever a new reactive batch was used or whenever an outcome outside the normal range was observed. Furthermore, external quality control assessment of every hormone assay was performed monthly at the Spanish Society of Clinical Biochemistry and Molecular Pathology.

## Statistical analysis

To avoid bias of the results by assuming that any relationship between serum progesterone levels and ongoing pregnancy rates may be linear, patients were divided into six distinct groups according to their serum progesterone levels on the day of hCG administration:  $\leq 1.00$ , 1.01–1.25, 1.26–1.50, 1.51–1.75, 1.76–2.00 and  $> 2.00$  ng/ml. The cut-off levels used to determine the six groups were chosen to provide equal intervals focused around the different threshold values employed across previous studies (Andersen et al., 2006; Kilicdag et al., 2009; Lee et al., 2009; Saleh et al., 2009). Ongoing pregnancy rate was calculated for each progesterone interval for both GnRH protocols. Data were assessed for trend analysis using a Mantel–Haenszel test. To identify the progesterone threshold for a detrimental effect on cycle outcome, the odds ratio (OR) and 95% confidence interval (CI) of ongoing pregnancy rates for each progesterone interval, compared with the preceding interval, was calculated. To control for confounding factors, stratification was undertaken for: the cause of infertility (male factor, endometriosis, polycystic ovaries and tubal factor); age (5-year intervals of  $\leq 30$ ; 31–35; 36–40 and  $> 40$  years of age); BMI [normal ( $< 25$  kg/m<sup>2</sup>), overweight (25–30 kg/m<sup>2</sup>) and obese ( $> 30$  kg/m<sup>2</sup>) groups]; gonadotrophin consumption [low ( $< 1500$  IU), medium (1500–3000 IU) and high ( $> 3000$  IU) total dosage]; and serum E<sub>2</sub> levels (1000 pg/ml intervals of  $< 1000$ , 1000–1999, 2000–2999 and  $\geq 3000$  pg/ml). Factors related to progesterone elevation were assessed using a multivariate analysis that included the following potential related factors: age, period of stimulation, E<sub>2</sub> on the day of hCG administration, number of oocytes, daily FSH dose and daily LH dose.

## Results

### Patient characteristics

Baseline characteristics of the 4032 study participants are shown in Table I. The average age of the participants was 35.3 years (range 24–46 years). The primary indications for infertility were male

factor (31%), age (18%), low response (11%), unknown aetiology (11%) and endometriosis (10%).

### Hormone levels

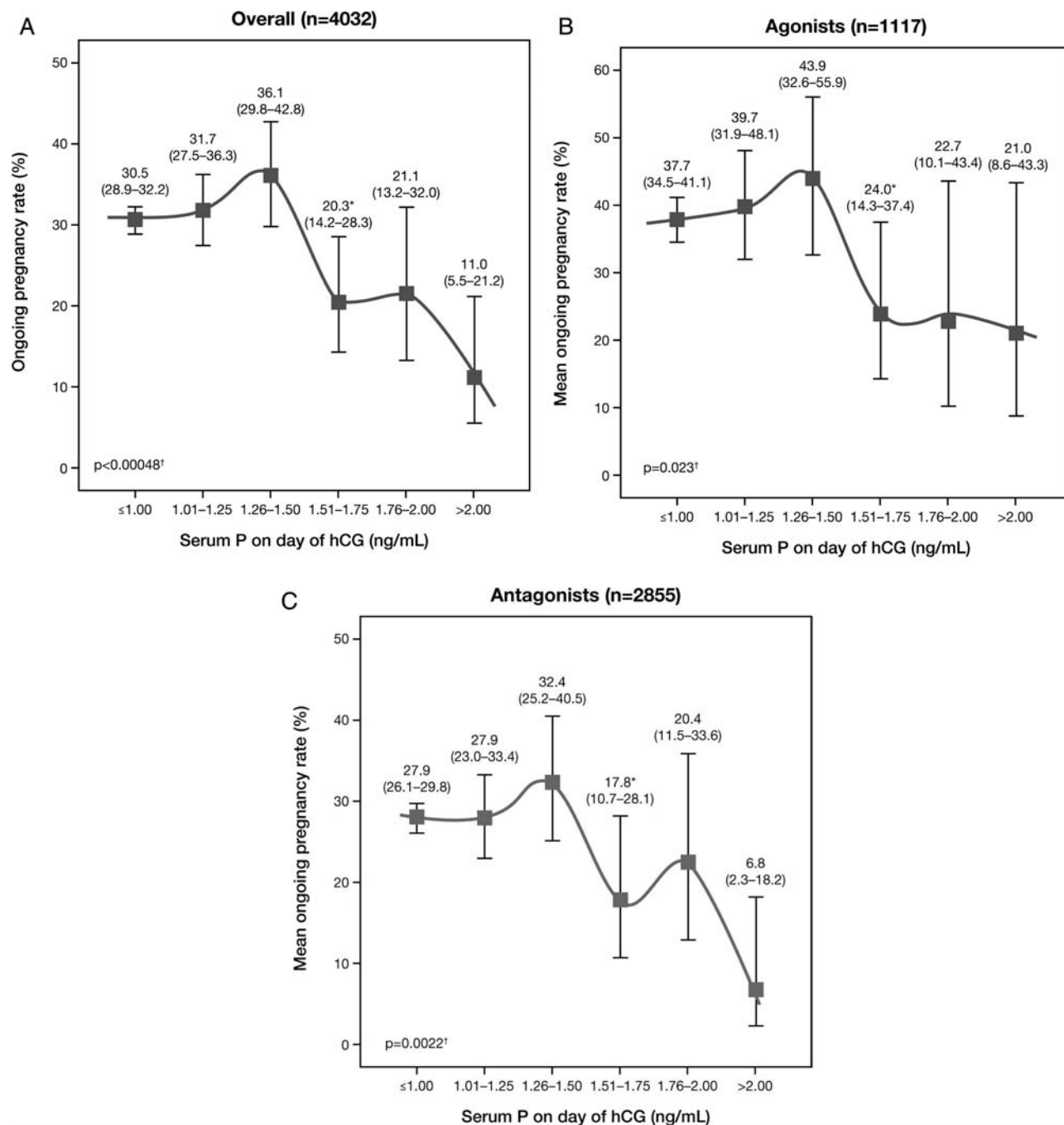
The mean ( $\pm$  standard deviation) level of serum progesterone on the day of hCG administration was  $0.77 \pm 0.66$  ng/ml (range 0.0–9.0 ng/ml; 95% CI: 0.75–0.80 ng/ml). When participants were analysed according to the GnRH analogue used for pituitary down-regulation, significantly higher serum progesterone levels were observed with GnRH agonists versus antagonists ( $0.84 \pm 0.67$  versus  $0.75 \pm 0.66$  ng/ml;  $P = 0.0003$ ; Supplementary data, Fig. S1). The incidences of high progesterone were found to be very similar during each year of data collection (5.6, 7.2, 6.6, 5.8 and 6.0%, respectively, for each year between 2003 and 2007), confirming that any changes to the equipment with which progesterone was measured, besides the batch-to-batch differences in reagents, did not impact the results.

### Relationship between pregnancy status and hormone levels

Figure 1A shows the overall association between ongoing pregnancy rates and serum progesterone levels. There was a reduction in ongoing pregnancy rates with progressively greater concentrations of serum progesterone  $> 1.5$  ng/ml ( $P = 0.00048$  for overall trend). Furthermore, this inverse relationship was maintained, irrespective of whether GnRH agonists ( $P = 0.023$ ) or antagonists ( $P = 0.0022$ ) were used for pituitary down-regulation (Fig. 1B and C).

Figure 2 shows the OR (95% CI) for ongoing pregnancy rate for each of the serum progesterone levels compared with the preceding progesterone group. The relative change in OR was very different between intervals, confirming the non-linear relationship between ongoing pregnancy rates and intervals of serum progesterone level. Furthermore, this difference was statistically significant only between the 1.26–1.50 and 1.51–1.75 ng/ml intervals in the overall study group ( $P = 0.003$ ), and in the GnRH agonist ( $P = 0.028$ ) or antagonist ( $P = 0.024$ ) subgroups (Fig. 2). These data suggest that a serum progesterone concentration of 1.5 ng/ml may represent the critical threshold level at which there is a negative impact of progesterone on ongoing pregnancy rate. That is, patients with serum progesterone levels  $\leq 1.5$  ng/ml have a better prognosis for achieving an ongoing pregnancy compared with patients with progesterone levels  $> 1.5$  ng/ml.

The ongoing pregnancy rates in patients categorized according to a serum progesterone cut-off value of 1.5 ng/ml on the day hCG administration is shown in Table II. Patients with lower progesterone levels demonstrated significantly better ongoing pregnancy rates compared with those showing higher levels of progesterone, and this remained the case when pregnancy rates were corrected for confounding factors, including female age and BMI (Supplementary data, Figs S2 and S3). Regarding the aetiology of infertility, the same trend was observed in patients undergoing IVF/ICSI because of male factor infertility, and in patients with endometriosis, but not in patients with polycystic ovaries or tubal factor infertility (Supplementary data, Fig. S4). Furthermore, analysis of ongoing pregnancy rates, according to the number of oocytes retrieved and progesterone levels, show that the threshold value of 1.5 ng/ml is valid across all ranges of ovarian response (Fig. 3). The same was true when data were analysed in



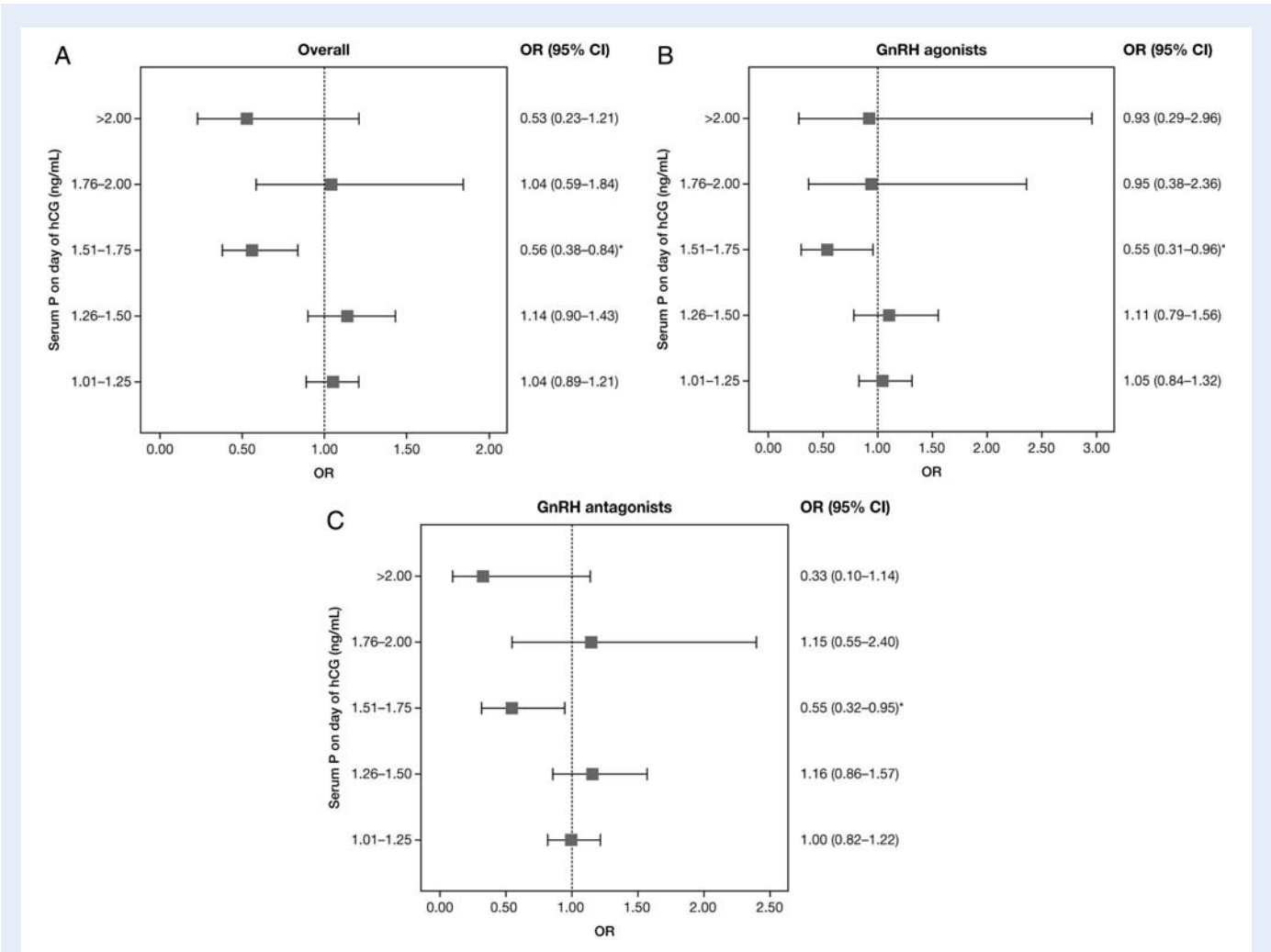
**Figure 1** Relationship between ongoing pregnancy rate and increasing serum progesterone levels in the overall study population (**A**) and in patients using GnRH agonists (**B**) or antagonists (**C**).

\* $P < 0.05$  for comparison with previous progesterone level interval;  $^\dagger$ trend analysed using Mantel–Haenszel test. Data are expressed as ongoing pregnancy rates (95% CI) for each of the serum progesterone levels; P, progesterone; hCG, human chorionic gonadotrophin; GnRH, gonadotrophin-releasing hormone.

terms of  $E_2$  levels (Supplementary data, Fig. S5) and total dose of gonadotrophins administered (Supplementary data, Fig. S6).

To further analyse the association between variables involved in increased progesterone levels, multivariate logistic regression was performed. Increases in daily FSH dose, number of oocytes collected and  $E_2$  values on the day of hCG administration were all associated with increased progesterone levels ( $P < 0.0001$  for all; Table III). The

correlation between serum progesterone levels on the day of hCG administration and daily FSH dose was also determined, and a significant positive correlation was found ( $P < 0.001$ ; Supplementary data, Fig. S7). Patients with progesterone levels  $\leq 1.5$  ng/ml had a significantly lower incidence of ovarian hyperstimulation syndrome compared with patients with progesterone levels  $> 1.5$  ng/ml (4.6 versus 13.7%;  $P < 0.0001$ ).



**Figure 2** Ongoing pregnancy rates according to serum progesterone levels in the overall study population (A) and in patients using GnRH agonists (B) or antagonists (C).

\**P* < 0.05 for comparison with previous progesterone level interval; data are expressed as OR (95% CI) for each of the serum progesterone levels compared with the lowest progesterone group (< 1.0 ng/ml); GnRH, gonadotrophin-releasing hormone; OR, odds ratio; CI, confidence interval; P, progesterone; hCG, human chorionic gonadotrophin.

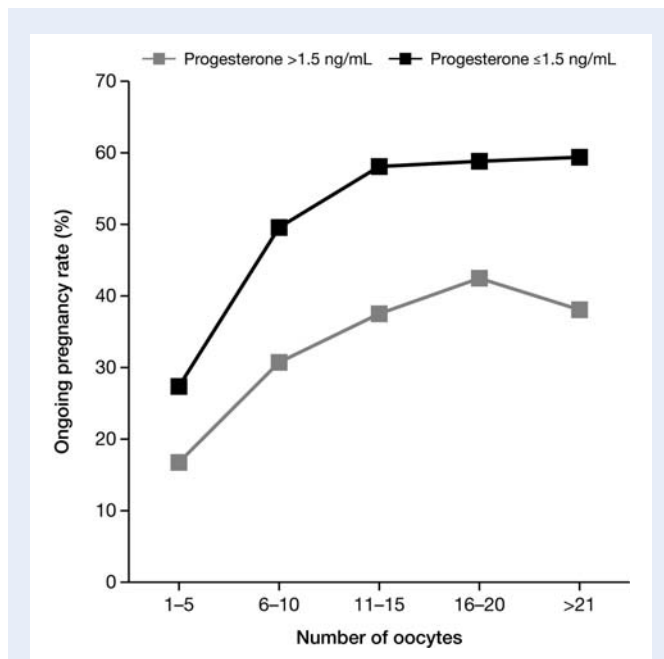
**Table II** Ongoing pregnancy rates for participants with serum progesterone levels ≤ 1.5 or > 1.5 ng/ml.

Serum progesterone level (ng/ml)	All (n = 4032)	GnRH agonist (n = 1177)	GnRH antagonist (n = 2855)
≤ 1.5	31.0 (29.5–32.5)	38.4 (35.4–41.5)	28.1 (26.4–29.9)
> 1.5	19.1 (14.4–24.4)	24.2 (15.8–34.3)	16.3 (11.0–22.8)
Difference in ongoing pregnancy rate	0.53 (0.38–0.72)*	0.51 (0.31–0.84)†	0.50 (0.33–0.76)‡

\**P* = 0.00006; †*P* = 0.007; ‡*P* = 0.0009; data are expressed as percentage (range) or OR (95% CI); GnRH, gonadotrophin-releasing hormone.

As patients were not randomly assigned to different stimulation protocols heterogeneity between groups may have introduced confounding factors that could mask the influence of the stimulation protocol. For example, stimulation protocols that use gonadotrophins with LH activity may have been favoured for those patients who were considered to be at risk of a greater elevation in progesterone level. Therefore, an unintended systematic bias may have been introduced in the analysis, such that protocols with LH activity could be over-represented in this particular group of patients, compared with rFSH-only stimulation protocols. Nevertheless it is interesting to note that of 1117 IVF/ICSI cycles with a GnRH agonist long protocol the progesterone level on the day of hCG administration was above 1.5 ng/ml in 8.7% of 138 treatment cycles using rFSH alone compared with 8.1% of 979 treatment cycles using a stimulation





**Figure 3** Ongoing pregnancy rate according to ovarian response and progesterone levels.

**Table III** Multivariate analysis of factors related to progesterone elevation.

Factor	OR (95% CI)	P-value
Daily FSH	1.44 (1.28–1.63)*	<0.0001
Number of oocytes	1.063 (1.041–1.085)	<0.0001
E <sub>2</sub> on day of hCG administration	1.0004 (1.0002–1.0010)	<0.0001

\*Increased OR per 75 IU; data are expressed as OR (95% CI); OR, odds ratio; CI, confidence interval; FSH, follicle-stimulating hormone; E<sub>2</sub>, estradiol; hCG, human chorionic gonadotrophin.

protocol with LH activity. Of even more interest in 2855 patients undergoing IVF/ICSI with a GnRH antagonist daily protocol the progesterone level on the day of hCG administration was above 1.5 ng/ml in 11.5% of 261 cycles using rFSH alone compared with 5.2% of 2594 cycles using a stimulation protocol with LH activity.

## Discussion

The results of our study in 4032 IVF/ICSI-ET cycles suggest that increased circulating progesterone levels at the end of COS are related to poorer ongoing pregnancy rates, irrespective of the GnRH analogue used. Using a trend analysis, a serum progesterone level of 1.5 ng/ml on the day of hCG administration was identified as the most appropriate threshold to define detrimental levels of progesterone for the outcome of IVF/ICSI-ET cycles when utilizing the microparticle enzyme immunoassay AxSYM System (Abbott Cientifica S.A., Madrid, Spain). Our results are in contrast to those of a recent meta-analysis, which suggested that increased progesterone levels do not correlate with clinical outcomes, in terms of pregnancy rates (Venetis *et al.*, 2007). However,

the value of this meta-analysis may be limited by the heterogeneity of the studies included, such as arbitrarily defined serum progesterone threshold values using various different assays.

The results of the present study are also in contrast to those of a smaller study that used receiver operating characteristic (ROC) curve analysis to assess the impact of progesterone levels of <1 or ≥1 ng/ml on pregnancy outcome, where the area under the ROC curve for serum progesterone on the day of hCG (0.52) was not predictive of pregnancy outcome (Saleh *et al.*, 2009). We believe that the area under the ROC curve may not be the most suitable test for this analysis because the relationship between serum progesterone levels and pregnancy outcomes is not linear. Indeed, ROC curve analysis in the present study is in agreement with the previous study, as it was unable to predict ongoing pregnancy (AUC = 0.499), although the data presented here comparing intermediate serum progesterone levels did demonstrate a significant, but non-linear, relationship between these two parameters. Thus, the differences between the analyses used in both studies may represent a possible reason for the discordance between the respective findings. Overall, the strength of the association observed here may be attenuated by the approach used to classify patients according to progesterone levels. Because the assay used had a sensitivity of 0.2 ng/ml, it is possible that some patients close to each cut-off value may be misclassified. In order to minimize this possibility, our analysis used discrete and regular intervals for circulating progesterone levels close to the sensitivity limit of the assay (0.24 ng/ml). In addition, the large sample size in this study would be expected to compensate for any misclassified patients. Nonetheless, when interpreting results in the clinical setting, the decision on how to manage patients close to each cut-off value will inevitably fall to individual clinical judgement.

We showed that the progesterone threshold of 1.5 ng/ml can be applied to all ovarian responses, as measured by the number of oocytes retrieved. In contrast, a previous study by Fanchin *et al.* (1997b), showed a modifying effect of ovarian response on the association between progesterone elevation and the probability of pregnancy. Again, however, the serum progesterone cut-off value for detrimental effects of pregnancy outcome, as well as the criteria for classifying the ovarian response, in that study was based on arbitrarily chosen values. The variation across these studies emphasizes the importance of using appropriate methodological approaches (Fleming, 2008). The progesterone assay used in our study showed consistency and limited variability across control samples. Furthermore, the internal and external quality control of the assays utilized in our laboratory ensured that these standards were maintained. It should also be noted that the identified cut-off progesterone value in our study reflects the specific assay used to measure serum progesterone. Therefore, further studies are required to determine whether the same threshold level of 1.5 ng/ml applies to different assays.

The rise in progesterone levels seen during COS for IVF/ICSI cycles cannot be explained by luteinization of granulosa cells, since this occurs in the presence of GnRH analogues and, hence, low LH levels. Indeed, results of our multivariate analysis showed that higher daily FSH dose was the factor most related to the occurrence of serum progesterone elevation, whereas serum E<sub>2</sub> levels on the day of hCG administration and the number of oocytes collected also showed statistically significant relationships with progesterone elevation. These data support the findings from a previous study,

which showed that GnRH antagonist cycles with high progesterone levels on the day of hCG administration ( $\geq 1.2$  ng/ml) required higher doses of FSH and a longer stimulation period than cycles with lower progesterone levels (Bosch et al., 2003). Crucially, however, the numbers of mature follicles at the end of stimulation were similar for both groups of patients (Bosch et al., 2003). Therefore, the explanation that we had previously proposed for the early increase in progesterone levels—that it results from an initial intense FSH stimulation, leading to increased granulosa cell steroidogenic activity (Bosch et al., 2003)—remains plausible considering the present findings. In support of this observation, studies have also shown a positive correlation between follicular phase progesterone levels with the administered FSH dose (Filicori et al., 2002), as well as with circulating FSH concentrations (Adonakis et al., 1998). Consistent with this observation, higher serum progesterone levels have been related to greater FSH administration in both GnRH agonist long protocol (Andersen et al., 2006) and GnRH antagonist cycles (Bosch et al., 2005, 2008). Follicle-stimulating hormone acts on granulosa cells to promote conversion of cholesterol to progesterone, which is passed to the thecal cells to be converted to androgens under the influence of LH. The androgens are then passed to the granulosa cells to be converted to estradiol; the classical ‘2-cell, two-gonadotrophin’ hypothesis (Moon et al., 1978). Prior to luteinization, LH acts to reduce circulating progesterone by promoting conversion to androgens, which are then further metabolized to estrogens by granulosa cells. Therefore, in an ovary with multiple follicles stimulated by high FSH concentrations, greater progesterone production could be anticipated than with a single follicle in the normal mid-follicular phase, particularly if the FSH action is not balanced by LH activity.

In addition to greater FSH stimulation, there are further factors influencing progesterone levels, including the increase in follicle number that occurs as a result of COS, and the suppression of LH itself, which prevents the LH-driven conversion to  $E_2$  by thecal cells. The increase in progesterone that results from the combination of these factors has the potential to advance the endometrium, without influencing the embryo. This can, in turn, lead to a state of asynchrony between embryo and endometrial dating, which may result in reduced implantation (Bourgain and Devroey, 2003) and, consequently, a reduced pregnancy rate. This may have been the case in the MEnotrophin versus Recombinant FSH *in vitro* fertilization Trial (MERiT) where, despite increases in oocyte production, the implantation rate was lower when progesterone levels were  $>4$  nmol/l on the day of hCG than when progesterone levels were  $\leq 4$  nmol/l (Andersen et al., 2006). Interestingly, the cut-off level of progesterone in MERiT correlates well with the cut-off level presented in our study (the threshold level of progesterone of 4 nmol/l in MERiT is equivalent to 1.26 ng/ml, similar to the 1.5 ng/ml threshold in our study, which is equivalent to 4.77 nmol/l). In the MERiT trial, the importance of balanced LH activity may also be reflected by the fact that there was a higher incidence of patients with elevated circulating progesterone at the end of stimulation in the rFSH treatment group versus the HP-hMG treatment group (24.1 versus 11.8%;  $P < 0.001$ ; Andersen et al., 2006).

Studies with GnRH agonists suggest that elevated progesterone levels may act at the level of the endometrium, since adverse effects on the oocyte and embryo quality have not been observed (Hofmann et al., 1993; Fanchin et al., 1997a; Ubaldi et al., 1997; Fanchin et al., 1999;

Andersen et al., 2006). Moreover, a study performed in an oocyte donation programme suggested that pregnancy rates of recipients were not influenced by progesterone levels of the donors at the end of stimulation (Melo et al., 2006). Advancement of histological dating after oocyte retrieval with use of a GnRH antagonist compared with GnRH agonist has been described previously, although there was no difference in the progesterone level between groups in this study (Kolibianakis et al., 2002). Whether serum progesterone levels affect pregnancy outcome as a result of an adverse effect on the endometrium or the oocyte requires further investigation.

The negative association between progesterone elevation on the day of hCG administration and the probability of pregnancy could be used to optimize the treatment of patients undergoing IVF/ICSI–ET. For example, the threshold value may help physicians to decide whether to continue to ET in a fresh cycle or cryopreserve the embryos and transfer in a subsequent frozen-thawed cycle (Legro et al., 1993; Silverberg et al., 1994). Alternatively, administering hCG at an earlier timepoint in the follicular phase, prior to progesterone elevation, might be beneficial in patients who have previously exhibited elevated progesterone levels after COS (Harada et al., 1996). When choosing the type of gonadotrophin used for COS, caution should be exercised when considering our findings owing to the lack of randomization in the drug allocation by clinicians. Accordingly, there is the possibility of inadvertent bias and, thus, it is only possible to speculate on the significance of our results pertaining to gonadotrophin choice. Nonetheless, significantly higher serum progesterone levels were observed in patients treated with rFSH than those treated with HP-hMG for COS in GnRH antagonist cycles (Bosch et al., 2008) and GnRH agonist cycles (Andersen et al., 2006). Considering the findings from the present study, gonadotrophin treatments that are associated with lower serum progesterone levels on the day of hCG administration should demonstrate better clinical outcome. Although in both these earlier studies the pregnancy rates were not significantly higher in patients treated with HP-hMG compared with patients treated with rFSH (Andersen et al., 2006; Bosch et al., 2008), the number of patients in these studies was limited. A greater statistical power for such a comparison was achieved with a recent meta-analysis of all studies comparing HP-hMG with FSH, which showed superior ongoing pregnancy/live birth rates with HP-hMG treatment in IVF cycles (Al-Inany et al., 2009).

In conclusion, our study shows that high serum progesterone levels on the day of hCG administration is a frequent event in GnRH agonist and antagonist IVF/ICSI–ET cycles and is associated with a decreased pregnancy rate. Its occurrence seems to be directly related to the total FSH dose used during COS and the number of oocytes obtained.

## Supplementary data

Supplementary data are available at <http://humrep.oxfordjournals.org/>.

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