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Journal:

Hum Reprod 2012

Human Reproduction, Vol.27, No.7 pp. 2036–2045, 2012
Advanced Access publication on May 4, 2012 doi:10.1093/humrep/des141

human reproduction

ORIGINAL ARTICLE *Infertility*

The duration of pre-ovulatory serum progesterone elevation before hCG administration affects the outcome of IVF/ICSI cycles

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Submitted on November 18, 2011; resubmitted on March 14, 2012; accepted on March 26, 2012

STUDY QUESTION: During controlled ovarian stimulation (COS), does the duration of premature serum progesterone (P) elevation before administration of hCG affect the outcomes of IVF/ICSI embryo transfer (-ET) cycles?

SUMMARY ANSWER: The duration of the premature serum P elevation is inversely related to the clinical pregnancy rate of IVF/ICSI-ET cycles.

WHAT IS KNOWN AND WHAT THIS PAPER ADDS: The majority of the previous studies only considered a single serum P measurement made on the day of hCG administration and the results of attempts to relate this to IVF/ICSI-ET outcomes were controversial. However, the effect of the duration of premature serum P elevation before the hCG administration on the outcomes of IVF/ICSI-ET cycles has not been studied well. Here we demonstrate that the duration of premature serum P elevation has a more significant inverse correlation than the absolute serum P concentration on the day of hCG administration with IVF/ICSI-ET outcomes.

DESIGN: It is a retrospective, single-centre cohort study. A total of 1784 IVF and/or ICSI-ET cycles were included from October 2005 to June 2011.

PARTICIPANTS AND SETTING: A total of 1784 patients underwent their IVF and/or ICSI-ET cycles in a university hospital IVF unit. The inclusion criteria include (i) age between 20 and 42 years and (ii) eligible indications for COS before IVF/ICSI.

MAIN RESULTS AND THE ROLE OF CHANCE: The duration of premature serum P elevation to >1 ng/ml is significantly inversely associated with the probability of clinical pregnancy (odds ratio = 0.773, 95% confidence interval: 0.660–0.891, $P < 0.001$), after adjustment for possible confounders with multivariate logistic regression analysis. However, the significance of inverse correlation between the absolute serum P concentration on the day of hCG administration with clinical pregnancy rate decreased after adjustment.

BIAS, CONFOUNDING AND OTHER REASONS FOR CAUTION: The cutoff value we chose to define premature serum P elevation (P > 1.0 ng/ml) might not be able to be applied to different immunoassay kits and study population. The retrospective nature of this study inevitably might be influenced by some selection bias.

GENERALIZABILITY TO OTHER POPULATIONS: Older patients (>42 years) are excluded from our study.

STUDY FUNDING/COMPETING INTEREST(S): This study was supported in part by grants from the National Science Council (100-2314-B-002-022-MY3) and National Taiwan University Hospital (NTUH 100-S1555), Taipei, Taiwan. No competing interests are declared.

TRIAL REGISTRATION NUMBER: nil.

Key words: progesterone / *in vitro* fertilization / pregnancy rate / ovarian stimulation / hCG

Introduction

For the past two decades, gonadotrophin-releasing hormone agonists (GnRHa) and antagonists have been used for pituitary down-regulation during controlled ovarian stimulation (COS) to prevent a premature luteinizing hormone (LH) surge (Smitz *et al.*, 1992). Despite the use of GnRH analogues, a subtle pre-ovulatory rise in the serum progesterone (P) concentration before the administration of hCG for final oocyte maturation still occurred in 5–30% of COS cycles (Melo *et al.*, 2006; Segal *et al.*, 2009; Elnashar, 2010). This phenomenon has been called premature luteinization (PL).

Several hypotheses have been proposed to explain the possible pathophysiology of PL, such as precocious elevation of follicular LH levels, serum accumulation of hCG or LH from hMG, and increased sensitivity of granulosa cell LH receptors to gonadotrophin (Lai *et al.*, 2009; Elnashar, 2010). However, so far no consensus has been reached. In recent years, it has been proposed that the term PL is inappropriate because premature serum P rise occurs when the serum LH concentration is low. Therefore, excess serum P is unlikely to be produced by the luteinization process and is more probably due to accumulation from a large number of follicles (Venetis *et al.*, 2007; Bosch *et al.*, 2010; Al-Azemi *et al.*, 2011).

Even the definition of a pre-hCG P rise remains controversial. Most studies arbitrarily selected an absolute cutoff value of serum P concentration on the hCG day to define premature elevation, with ranges from 0.8 to 2 ng/ml (2.5–6.4 nmol/l; Fanchin *et al.*, 1999; Ozçakir *et al.*, 2004). Younis *et al.* (2001) proposed a P/estradiol (P/E2) ratio >1 as the definition of pre-hCG P rise in order to define whether the serum P elevation results from pooled secretion from multiple mature follicles. A P/E2 ratio >1 was associated with lower ovarian reserve and poorer pregnancy outcomes (Younis *et al.*, 1998, 2001). However, this definition is not universally accepted.

Using different definitions, some studies reported no association between pre-hCG P rise and the pregnancy rate (Ubaldi *et al.*, 1995; Venetis *et al.*, 2007; Andersen *et al.*, 2011), while other studies including a recent meta-analysis showed that a pre-hCG P rise is associated with poorer pregnancy outcomes (Fanchin *et al.*, 1997a; Bosch *et al.*, 2003, 2010; Kolibianakis *et al.*, 2012). The pathophysiology of pre-hCG P rise and its impact on pregnancy outcomes remain inconclusive. Since successful implantation relies on a delicate interaction between the embryo and the endometrium, previous investigators have used oocyte-donation models (Hofmann *et al.*, 1993; Legro *et al.*, 1993), or embryo cryopreservation and thawed-transferred cycles (Silverberg *et al.*, 1994) to discriminate whether the presumed detrimental effect of pre-hCG P rise is exerted on the endometrium or the oocyte. It has been proposed that endometrial receptivity, which may indicate a change in the implantation window, is more likely to be affected than the oocyte quality (Melo *et al.*, 2006). Furthermore, recent studies have demonstrated that prematurely elevated serum P may cause an advanced endometrial secretory transformation resulting in the early closure of the implantation window (Labarta *et al.*, 2011).

The majority of the previous studies only analysed the serum P concentration on the day of hCG administration for oocyte maturation. Since the implantation window has been proposed to range from post-ovulatory Day 6 to Day 10, evaluating only 1 day of absolute serum P concentration might not accurately reflect the chronological change in the implantation window. Therefore, the aim of our study is to analyse the association between the duration of pre-ovulatory serum P elevation and the pregnancy outcomes of IVF/ICSI embryo transfer (ET) cycles.

Materials and Methods

Study population

This study was approved by the Ethics Committee of National Taiwan University Hospital. This was a retrospective, observational, single-centre cohort study. Patients were treated at the National Taiwan University Hospital from October 2005 to June 2011 with the routine practice of assisted reproductive technologies (Lee *et al.*, 2006; Ho *et al.*, 2008; Hugues *et al.*, 2011). A total of 1784 IVF and/or ICSI–ET cycles were included. Each patient entered the study only once. The data were collected from medical records and computerized databases.

The inclusion criteria included (i) age between 20 and 42 years and (ii) eligible indications for COS before IVF/ICSI. The exclusion criteria included (i) oocyte donation cycles and (ii) cancelled cycles that did not reach embryo transfer. There were 68 cancelled cycles within the study period. One cycle did not reach oocyte retrieval due to poor response to ovarian stimulation. Twenty-six cycles were cancelled due to fertilization failure. Eight cycles were cancelled because no oocytes were retrieved and four due to poor embryo cleavage. In 22 cycles all of the embryos were frozen due to an excessive number of follicles and the risk of ovarian hyperstimulation syndrome. In the remaining cycles all embryos were frozen without transfer because of other personal reasons. No cycles were cancelled due to a premature rise in serum P before hCG administration.

Patient characteristics were evaluated, including age, body mass index (BMI), gravidity, parity, basal FSH/LH/E2 levels and causes of infertility. Other measured parameters included the serial levels of E2/P/LH during the ovarian stimulation, the number of retrieved oocytes, the number of transferred embryos, fertilization rate, cleavage rate and good embryo rate. The major end-point was clinical pregnancy rate (i.e. presence of a fetal heart beat at a gestational age of 7 weeks).

Protocols for COS

Pituitary down-regulation was performed with either a GnRHa long protocol ($n = 358$), GnRHa short protocol ($n = 927$) or a GnRH antagonist protocol ($n = 499$), as shown in our previous studies (Lee *et al.*, 2006; Ho *et al.*, 2008; GnRH agonist: Supremon®, buserelin acetate; Hoechst AG, Frankfurt, Germany; GnRH antagonist: Orgalutran®, ganirelix; Organon, Dublin, Ireland, or Cetrotide®, cetrorelix acetate; Merck-Serono, Geneva, Switzerland). Ovarian stimulation was achieved with recombinant FSH (rFSH, Gonal-F®; Merck-Serono, Geneva, Switzerland or Puregon®; Organon Espanola S.A., Barcelona, Spain) or highly-purified hMG (hp-hMG, Menopur®; Ferring Pharmaceuticals, Geneva, Switzerland), containing 75 IU/ampules of FSH and LH activity.

The selection of the protocol and the type of gonadotrophin were individualized according to each patient's characteristics and clinician's preference. The mean starting dose of gonadotrophin was 213.6 IU and was individualized according to the patient's age, previous ovarian response and/or BMI. IVF/ICSI cycles were monitored by serial folliculometry and assessment of serum E2, P and LH levels every 1–2 days, starting from Day 6 in GnRH antagonist protocol or Day 7 in GnRH agonist protocol and continued until the day of hCG injection for the final oocyte maturation. A step-down regimen of gonadotrophin was applied if the serum E2 concentration was >300 pg/ml on Day 6 in the GnRH antagonist protocol or on Day 7 in the GnRHa short and long protocols.

Hormone assessment

Serum samples were analysed using Immulite 2000 reproductive hormone assays (Diagnostic Product Corporation, Siemens, Los Angeles, CA, USA). The sensitivity was 0.1 mlu/ml for FSH; 0.05 mlu/ml for LH; 15 pg/ml for E2 and 0.1 ng/ml for P. Intra-assay and inter-assay coefficients of variation were <3.6% and <4.3% for FSH; <4.8% and <10.7% for LH; <6.7% and <9.7% for E2 and <9.7% and <12.2% for P, respectively.

We hypothesized that when the serum P concentration is above a critical value during COS, it might mimic the condition of the LH surge in natural ovulation cycles and induce a change in implantation window. This idea is supported by the observations that gene expression profiles and the expression of estrogen and P receptors on the endometrium in stimulated cycles are different from that in natural cycles, and may cause a change of endometrial receptivity (Bourgain *et al.*, 2002; Papanikolaou *et al.*, 2005; Horcajadas *et al.*, 2008; Liu *et al.*, 2008; Haouzi *et al.*, 2009). Therefore, we defined high P as a value greater than the 97.5th centile of the reference range of the serum P level in natural cycles measured by Immulite 2000 immunoassays in a multinational study (Vankrieken, 2000). The median P level of the mid-follicular day (Days 5–11) was 0.43 ng/ml, and the central 95% ranged from non-detectable to 0.98 ng/ml. Therefore, serum P of 1.0 ng/ml was defined as the cut-off point for abnormally high P levels during the follicular phase.

Oocyte retrieval, fertilization and embryo grading

A 6500–13000 IU dose of hCG (Ovidrel®; Merck-Serono) was administered when two or more than two follicles reached 18 mm in diameter. Oocyte retrieval was scheduled 34–36 h later. The embryos were classified according to the shape of the blastomeres and the amount of detached anuclear fragments. A good day 3 embryo had at least 6–10 cells with <20% fragmentation. A good day 5 blastocyst had distinct trophectoderm and inner cell mass.

Embryo transfer and luteal support

Embryo transfer was performed from 2 to 5 days after oocyte retrieval. A maximum of four embryos were transferred. Luteal support was started 2 days after oocyte retrieval and continued until 7 weeks of gestation. The adequacy of P supplementation was evaluated by testing the serum P level at the mid-luteal phase. A serum beta-hCG pregnancy test was performed 16 days after oocyte retrieval. The presence of a fetal heart beat was confirmed at 7 weeks of gestation by transvaginal ultrasonography.

Statistical analysis

Statistical analysis was performed using SPSS version 17.0 software. The patients were divided into three groups according to the duration of P elevation (0, 1–2 and ≥3 days). Associations between demographic and clinical characteristics of the patients according to the outcome of IVF/ICSI cycles in three groups of P elevation duration were assessed using one-way analysis of variance (ANOVA) for continuous variables and χ^2 or Fisher's exact tests for categorical variables. Fisher's least significant difference *post hoc* test was applied when ANOVA revealed statistical significance.

The duration of P elevation was treated as a continuous variable since we wanted to assess the linear effect of the duration of P elevation in the following regression models. Univariate logistic regression analysis was used to determine whether age (years), BMI (kg/m^2), gravidity (%), parity (%), basal FSH (mlu/ml)/LH (mlu/ml)/E2 (pg/ml) levels, duration of COS (day), total gonadotrophin dose (IU) and E2 (pg/ml)/P (ng/ml)/LH (mlu/ml) levels on the day of hCG administration, duration of pre-hCG P rise (day), number of retrieved oocytes, number of transferred embryos and good embryo rate (%) were associated with the success of clinical pregnancy. And the factors that had significant association with clinical pregnancy rate by univariate analysis were further analysed by multivariate logistic regression models. These variables include the duration of pre-hCG P rise (days), age (years), serum P concentration on the day of hCG administration (ng/ml), number of retrieved oocytes, good embryo rate (%) and number of transferred embryos all were presented as continuous variables.

Univariate and multivariate logistic regression analysis were also used to assess the correlation of clinical pregnancy rate and the duration of premature serum P elevation between different protocols (Fig. 1) and different ovarian responses (Fig. 2) of the participants. The duration of premature serum P elevation (day) is presented in three groups as in Table II. The adjusted variables included age (years), the number of transferred embryos, the number of oocytes retrieved and good embryo rate (%). Values are expressed as mean ± standard error of the mean (SEM). All tests were two-tailed with a confidence level of 95% ($P < 0.05$).

Results

Baseline characteristics

The patients' data and cycle characteristics are summarized in Table I. The average age of participants was 34.9 years (range, 20–42 years). The overall incidence of serum P elevation before hCG administration was 36.0% (643/1784). The serum P concentration (mean ± SEM) on the day of hCG administration was 0.95 ± 0.02 ng/ml (range, 0.2–14.5 ng/ml).

Table II shows the patients' characteristics and cycle outcomes according to the duration of pre-ovulatory serum P elevation to >1 ng/ml. The age, BMI, body weight, implantation rate and clinical pregnancy rate are significantly lower in patients with a longer duration of premature serum P elevation. The number of retrieved oocytes and the E2 level on the day of hCG administration are significantly higher in the patients with a longer duration of premature serum P elevation. No differences were detected in the LH level on the day of hCG administration, the total dosage of gonadotrophin, the fertilization rate, the number of transferred embryos or the good embryo rate.

The percentage ETs done on Day 2 is significantly higher in the group without pre-hCG P elevation. This might because we preferred earlier ET when fewer oocytes were retrieved and the group without pre-hCG P elevation had significantly fewer oocytes retrieved on average. For similar reasons the percentage of Day 5 ETs is significantly lower in the group without pre-hCG P elevation.

The odds ratios (ORs) and 95% confidence intervals (CI) of each possible explanatory factor for the probability of clinical pregnancy analysed with univariate and multivariate logistic regression analysis are shown in Table III. By univariate analysis, factors associated with decreased clinical pregnancy rate include age, the duration of pre-hCG P rise to >1 ng/ml (days) and the serum P concentration on the day of hCG administration. The inverse association between the duration of pre-hCG P elevation and clinical pregnancy rate remained significant after further adjustment for other confounding factors, including age, serum P concentration on the day of hCG administration, good embryo rate, number of retrieved oocytes and number of transferred embryos in the multivariate logistic regression analysis. The OR of the duration (days) of pre-hCG P rise is 0.773 (95% CI: 0.660–0.891, $P < 0.001$), which means that on average 1 day increase in pre-hCG P rise will result in 22.7% decrease in the

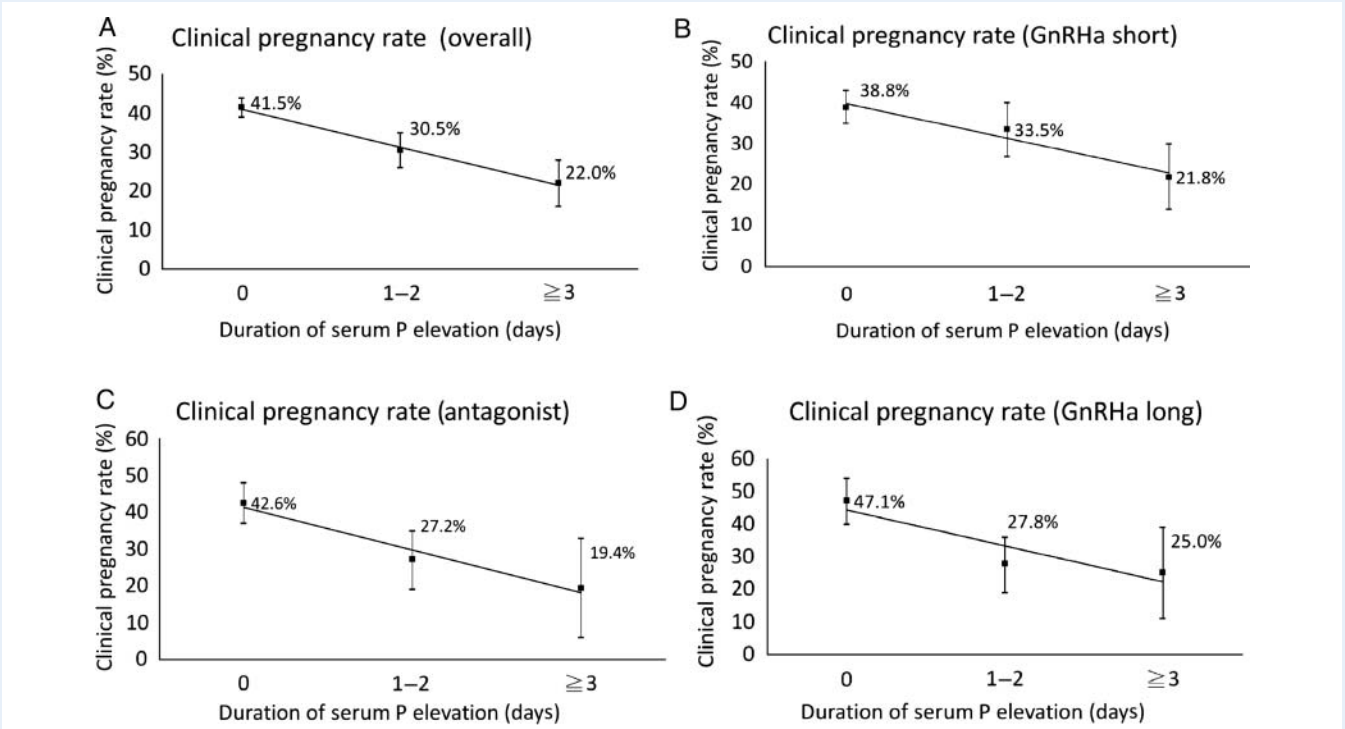

Figure 1 The association between the duration of pre-hCG P elevation (>1 ng/ml) and clinical pregnancy rate calculated by multivariate logistic regression analysis. The duration of pre-hCG P elevation was divided into three groups (0 day, 1–2 and ≥3 days). The confounding factors adjusted for include age (years), the number of retrieved oocytes, the number of transferred embryos and good embryo rate (%). (A) The overall clinical pregnancy rate is significantly decreased with a longer duration of serum P elevation before hCG administration ($P < 0.001$). (B–D) This remains true irrespective of the down-regulation protocol. (B) GnRHa short protocol: $P < 0.001$, $n = 927$. (C) GnRHa antagonist protocol: $P < 0.001$, $n = 499$. (D) GnRHa long protocol: $P = 0.001$, $n = 358$.

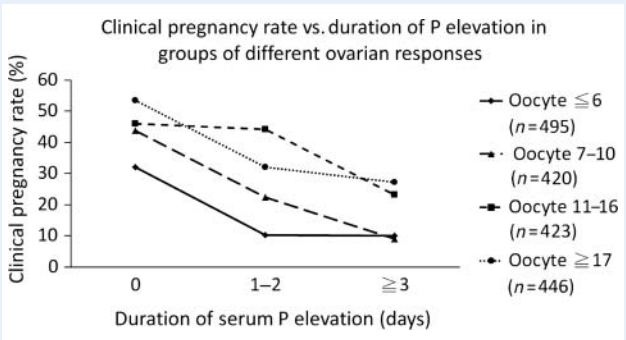

Figure 2 The clinical pregnancy rate is significantly decreased with longer durations of premature P elevation to >1 ng/ml in patients grouped according to their ovarian responses, as expressed by the amount of retrieved oocytes. The duration of pre-hCG P elevation was divided into three groups (0, 1–2 and ≥3 days). Multivariate logistic regression analysis showed that in each ovarian response group clinical pregnancy rate significantly decreased with longer durations of serum P elevation. The confounding factors adjusted for include age (years), the number of transferred embryos, the number of oocytes retrieved and good embryo rate (%). The *P*-values of test for trend were <0.001 in Group 1 (1–6 retrieved oocytes), <0.001 in Group 2 (7–10 retrieved oocytes), 0.021 in Group 3 (11–16 retrieved oocytes), <0.001 in Group 4 (≥17 retrieved oocytes).

probability to achieve clinical pregnancy. However, the association between serum P concentration on the day of hCG administration and the clinical pregnancy rate decreased after adjusting for the effect of the duration of serum P elevation and other possible confounders previously mentioned.

We examined the variance inflation factor (VIF) for six variables in multiple regression analysis, including the duration of pre-hCG P rise (day), serum P concentration on the day of hCG administration (ng/ml), age (years), number of retrieved oocytes, good embryo rate (%) and number of transferred embryos. And values of VIF exceeding 10 are often regarded as indicating multicollinearity. The range of values of VIF was from 1.01 to 1.84, indicating that there were no multicollinearity problems in this study.

The duration of pre-hCG P rise is inversely associated with the clinical pregnancy rate independent of the protocol utilized for pituitary down-regulation (Fig. 1A–D).

The correlation between clinical pregnancy rate and the duration of premature P elevation in patients with different ovarian responses is shown in Fig. 2. The patients were divided into four subgroups according to the quartiles of the number of oocytes retrieved (Group 1: 0–25th ≤6 oocytes; Group 2: 25–50th, 7–10 oocytes; Group 3: 50–75th, 11–16 oocytes; Group 4: 75–100th, ≥17 oocytes). After adjusting for confounding factors, including age, the number of retrieved oocytes, the number of transferred embryos and good embryo rate, with multivariate logistic regression analysis, the clinical

Table I Basic characteristics of the IVF/ICSI-embryo transfer population.

Parameter	
Age (years), mean ± SEM (range)	34.9 ± 0.10 (20–42)
BMI (kg/m^2), mean ± SEM (range)	21.4 ± 0.08 (15.1–34.9)
Infertility cause	
Male factors	891 (49.9%)
Tubal factors	490 (27.5%)
Ovulatory factors	258 (14.5%)
Endometriosis	228 (12.8%)
Unknown	155 (8.7%)
Procedure	
ICSI	1145 (64.2%)
ICSI + IVF	278 (15.6%)
IVF	361 (20.2%)
Day of embryo transfer	
Day 2	256 (13.6%)
Day 3	1142 (60.7%)
Day 5	456 (24.3%)
Protocol	
GnRH antagonist	499 (28.0%)
GnRH agonist short	927 (52.0%)
GnRH agonist long	358 (20.1%)
No P elevation before hCG administration	1141 (64.0%)
P elevation (>1 ng/ml) before hCG administration	643 (36.0%)
P elevation (>1 ng/ml) in GnRH antagonist protocol	161/499 (32.1%)
P elevation (>1 ng/ml) in GnRH agonist short protocol	334/927 (36.1%)
P elevation(>1 ng/ml) in GnRH agonist long protocol	148/358 (41.2%)

Values are number (%) unless stated otherwise ($n = 1784$).

pregnancy rates within each subgroup were all significantly inversely correlated with the duration of premature serum P elevation.

Discussion

To the best of our knowledge, this is the first large study which demonstrates that the duration of prematurely elevated serum P concentration is associated with an adverse effect on the outcomes of IVF/ICSI cycles. Our results with 1784 IVF/ICSI cycles indicate that the clinical pregnancy rate was significantly decreased in women with longer durations of serum P elevation, independent of the protocol used and the ovarian response.

The majority of previous studies only analysed the absolute serum P concentration on the day of hCG administration. However, some researchers proposed that P supplementation for luteal phase support starting on the day of hCG administration did not affect the pregnancy outcomes (Howles *et al.*, 1988; BenNun *et al.*, 1990; Elnashar, 2010), although definition of the optimal scheme of luteal phase support remained inconclusive and controversial (Sohn *et al.*, 1999; Fatemi *et al.*, 2007). Furthermore, de Ziegler *et al.* (1995) postulated that the duration of exposure to P rather than the P concentration at a given time was crucial for the proper triggering of the implantation window (a limited period with highest endometrial receptivity for embryos; Bourgain and Devroey, 2003). Therefore, analysing only one absolute serum P concentration on the day of hCG administration might not accurately reflect the chronological change in endometrial receptivity.

In our study, prolonged serum P elevation may reflect an inappropriately advanced endometrium leading to an early closure of the implantation window, and therefore, significantly decreased clinical pregnancy rates. Literature review suggested that the histological dating of endometrium showed a prematurely secretory transformation on the day of oocyte retrieval in both GnRH agonist and antagonist protocols, in as many as 48–100% of COS cycles (Chetkowski *et al.*, 1997; Ubaldi *et al.*, 1997; Kolibianakis *et al.*, 2002; Saadat *et al.*, 2004). Such advanced endometrium may still be potentially receptive to rapidly cleaving embryos but less receptive to slower developing embryos (i.e. embryo-endometrial dyssynchrony; Chetkowski *et al.*, 1997).

Multivariate regression analysis was applied to adjust for the influence of possible confounders. Using univariate logistic analysis, both the duration of serum P elevation and the serum P level on the day of hCG administration were inversely associated with the probability of clinical pregnancy. However, the significance of the serum P level on the day of hCG administration disappeared after adjustment for the duration of premature serum P elevation and other confounding factors. Meanwhile, the correlation of the duration of serum P elevation remains significant after the adjustment for the effect of the absolute P-value on the day of hCG administration. Therefore, the inverse correlation between the clinical pregnancy rate and the duration of pre-ovulatory P elevation might be more significant than that with the absolute P value on the day of hCG administration. This finding might partially explain why there are conflicting results reported by previous studies, which mostly considered just the absolute serum P concentration on the hCG administration day.

We and other researchers consistently have found that the E2 level on the day of hCG administration and the number of retrieved oocytes are significantly higher in patients with a longer duration of serum P elevation before hCG administration (Ubaldi *et al.*, 1996; Fanchin *et al.*, 1997b; Filicori *et al.*, 2002; Venetis *et al.*, 2007), adding further evidence to the hypothesis that the elevated P level is caused by the physiological accumulation from multiple follicles during the late follicular phase (Fanchin *et al.*, 1997b; Venetis *et al.*, 2007; Bosch *et al.*, 2010). Besides, increased sensitivity to P, endometrial secretory advancement might be induced by a supra-physiologically elevated estrogen concentration (Kyrou *et al.*, 2009; Al-Azemi *et al.*, 2011). Additionally, the patients tend to be younger and to have lower baseline FSH levels with longer durations of premature P elevation. Prolonged durations of premature serum P elevation may therefore occur more easily in the patients with better ovarian reserve.

As for the effect of pre-hCG P rise on the oocyte or embryo, our findings are in agreement with the previously reported literature. The prematurely elevated P concentration during the follicular phase does not significantly affect the oocyte or embryo quality, with respect to

Table II Patients' characteristics and cycle outcomes according to the duration of serum progesterone elevation >1 ng/ml before hCG administration (pre-hCG P rise).

	Duration of pre-hCG P rise			*P*-value
	0 day	1–2 days	≥3 days	
Number of cases	1141	466	177	
Age (years), mean ± SEM	35.2 ± 0.1[ac]	34.8 ± 0.2[ab]	33.7 ± 0.3[bc]	<0.001
BMI (kg/m^2), mean ± SEM	21.7 ± 0.1[ab]	21.1 ± 0.1[a]	20.7 ± 0.2[b]	<0.001
Body weight (kg), mean ± SEM	56.0 ± 0.3[ab]	54.1 ± 0.4[ac]	52.9 ± 0.6[bc]	<0.001
Gravidity (Yes/No)	521 (46%)	199 (43%)	80 (46%)	NS
Parity (Yes/No)	186 (16%)	59 (13%)	21 (12%)	NS
Male factor	562 (49%)	239 (51%)	90 (51%)	NS
Tubal factor	341 (28%)	131 (28%)	45 (25%)	NS
Ovulatory factor	180 (16%)	56 (12%)	22 (12%)	NS
Endometriosis factor	146 (13%)	56 (13%)	26 (15%)	NS
Unexplained factor	106 (10%)	63 (12%)	4 (13%)	NS
GnRH antagonist protocol	338 (30%)[a]	125 (27%)	36 (20%)[a]	0.031
GnRHa short protocol	653 (54%)	241 (51%)	105 (57%)	NS
GnRHa long protocol	210 (18%)	108 (23%)	40 (23%)	NS
Day 2 ET	185 (16%)[ab]	40 (9%)[a]	11 (6%)[b]	<0.001
Day 3 ET	714 (63%)	267 (57%)	102 (58%)	NS
Day 5 ET	229 (20%)[ab]	148 (32%)[a]	63 (36%)[b]	<0.001
Baseline FSH level (mIU/ml)	8.45 ± 0.11[ab]	7.39 ± 0.14[a]	6.93 ± 0.19[b]	<0.001
Baseline LH level (mIU/ml)	4.46 ± 0.29	4.48 ± 0.14	4.53 ± 0.19	NS
Baseline E2 level (pg/ml)	35.84 ± 0.44[a]	35.86 ± 0.65[b]	41.04 ± 1.46[ab]	<0.001
Total rFSH dose (IU)	1707 ± 19	1762 ± 33	1666 ± 43	NS
Duration of COS (days)	9.77 ± 0.06[ab]	10.11 ± 0.08[a]	10.37 ± 0.13[b]	<0.001
E2 level on hCG day (pg/ml)	2036.3 ± 47.1[ab]	3435.1 ± 99.6[ac]	4343.0 ± 205.3[bc]	<0.001
P level on hCG day (ng/ml)	0.62 ± 0.01[ab]	1.36 ± 0.02[ac]	1.97 ± 0.09[bc]	<0.001
LH level on hCG day (mIU/ml)	3.59 ± 0.10	3.84 ± 0.18	3.15 ± 0.18	NS
No of retrieved oocytes	9.9 ± 0.2[ab]	14.8 ± 0.3[ac]	18.3 ± 0.8[bc]	<0.001
Fertilization rate	78.6 ± 0.6%	77.1 ± 0.8%	76.0 ± 1.2%	NS
Cleavage rate	98.2 ± 0.3%	98.2 ± 1.3%	97.8 ± 0.6%	NS
No. of transferred embryos	2.94 ± 0.03[ab]	3.17 ± 0.04[a]	3.07 ± 0.06[b]	<0.001
Good embryo rate	32.3 ± 1.0%	29.2 ± 1.4%	29.9 ± 2.2%	NS
Implantation rate	24.3 ± 0.9%[ab]	17.8 ± 1.3%[ac]	12.0 ± 1.9%[bc]	<0.001
Clinical pregnancy rate	41.5 ± 1.5%[ab]	30.5 ± 2.1%[ac]	22.0 ± 3.1%[bc]	<0.001

Data are mean ± SEM for continuous variables and number (%) for categorical variables. *P*-value from one-way ANOVA or χ^2 test as appropriate. Figures sharing the same superscript (a, b or c) are statistically significantly different at $P < 0.05$ by LSD analysis *post hoc*. COS, controlled ovarian stimulation; ET, embryo transfer; No, number; NS, not significant.

the fertilization rate, cleavage rate or good embryo rate (BenNun *et al.*, 1990; Hofmann *et al.*, 1993; Fanchin *et al.*, 1997a; Ubaldi *et al.*, 1997; Andersen *et al.*, 2006; Melo *et al.*, 2006). However, the clinical pregnancy rate remain significantly decreased in the patients with a longer duration of pre-ovulatory P elevation, suggesting that the detrimental effect of the premature P rise on the endometrial receptivity might outweigh the positive effects of younger age, better ovarian reserve and comparable oocyte or embryo quality.

We also found that the duration of rFSH stimulation to be significantly longer in the patients with a longer duration of premature P rise, which is consistent with the results from previous studies (Fanchin *et al.*, 1997b; Bosch *et al.*, 2003, 2010). Kolibianakis *et al.* (2005) reported a significantly higher incidence of premature endometrial secretory transformation on the day of oocyte retrieval when they deliberately prolonged the follicular phase by delaying hCG administration for 2 days. However, in contrast to other studies which reported a greater dose of rFSH administration in patients that exhibited a pre-hCG P rise (Fanchin *et al.*, 1997b; Bosch *et al.*, 2003, 2010), we failed to identify a correlation between the total dose of rFSH administration and premature serum P elevation. This is probably due to a general 'step-down' policy of gonadotrophin utilized in our hospital. Since the longer duration of serum P elevation is associated with the patients with better ovarian reserve and more follicles, those daily rFSH dosages were

Table III ORs and 95% CIs for associations of parameters with clinical pregnancy from logistic regression.

Parameter	Univariate		Multivariate adjusted*	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age (years)	0.927 (0.904–0.951)	<0.001	0.918 (0.990–0.946)	<0.001
BMI (kg/m^2)	1.010 (0.971–1.052)	0.612		
Gravidity (Yes/No)	1.033 (0.851–1.251)	0.740		
Parity (Yes/No)	1.173 (0.898–1.531)	0.242		
Baseline FSH level (mIu/ml)	0.967 (0.935–1.000)	0.051		
Baseline LH level (mIu/ml)	0.993 (0.971–1.016)	0.549		
Baseline E2 level (pg/ml)	1.004 (0.997–1.010)	0.244		
Duration of pre-hCG P rise (days)	0.781 (0.714–0.854)	<0.001	0.773 (0.660–0.891)	<0.001
P level on hCG day (ng/ml)	0.654 (0.540–0.790)	<0.001	0.729 (0.529–1.006)	0.054
E2 level on hCG day (pg/ml)	1.000 (1.000–1.000)	0.241		
LH level on hCG day (mIu/ml)	0.976 (0.946–1.007)	0.126		
rFSH dose (IU)	1.000 (1.000–1.000)	0.853		
COS duration (days)	1.006 (0.956–1.060)	0.812		
Number of retrieved oocytes	1.019 (1.007–1.032)	0.002	1.024 (1.007–1.040)	0.004
Number of transferred embryos	1.463 (1.299–1.649)	<0.001	1.524 (1.321–1.759)	<0.001
Good embryo rate (%)	3.117 (2.204–4.409)	<0.001	3.497 (2.418–5.058)	<0.001

P-value represents the significance value of the studied comparison. $P < 0.05$ is significant. The adjusted factors were as shown in the table. COS, controlled ovarian stimulation; P, progesterone.
*The adjusted $R^2 = 0.138$.

usually tapered in order to prevent ovarian hyperstimulation syndrome. Therefore, even though the duration of rFSH stimulation is significantly longer in patients with longer durations of pre-ovulatory serum P elevation, the total dosage was not significantly different.

In our study, an inverse relationship between the duration of pre-ovulatory serum P elevation and pregnancy outcome exists in patients of all different ovarian responses, as measured by the number of retrieved oocytes. This finding is in contrast to the results shown by Fanchin *et al.* (1997b), who indicated a modulating effect of ovarian response on the association between premature P elevation and the ongoing pregnancy rate. An elevated P level (>0.9 ng/ml) was negatively associated with the pregnancy outcome only in patients with poor ovarian response because the adverse effect of premature P elevation could be compensated by the better oocyte quality in those patients with good ovarian responses. The different outcomes probably result from the heterogeneity in study populations, study methodologies, COS protocols and the accepted definitions of pre-hCG P rise. However, the adverse effect of pre-ovulatory P elevation on the pregnancy outcome has been shown to exist in all ovarian responses (Bosch *et al.*, 2010), and in selected patients with good ovarian response in other studies (Kiliçdag *et al.*, 2010). Our findings were consistent with those studies.

Interestingly, our results showed that the body weight or BMI is significantly lower in patients with longer durations of serum P elevation. The possible explanation might be a higher peak serum rFSH concentration in thinner patients. It has been postulated that the initial intense rFSH stimulation may increase granulosa cell steroidogenesis, resulting in early serum P elevation (Fanchin *et al.*, 1997b; Bosch *et al.*, 2003, 2010). Another possible mechanism may be the higher serum leptin concentration in heavier patients, which has been reported to reduce the ovarian response during COS (Fedorcsák *et al.*, 2004; Erel *et al.*, 2009) and to suppress gonadotrophin-stimulated estrogen and P production by human granulosa cells in some *in vitro* studies (Agarwal *et al.*, 1999; Brannian *et al.*, 1999; Karamouti *et al.*, 2008; Erel *et al.*, 2009). However, more studies are needed to clarify these hypotheses.

Recent advances in molecular biotechnology (e.g. immunohistochemical studies, scanning electron microscopy, DNA microarrays and polymerase chain reaction) are being used to investigate the possible effects of abnormally high ovarian steroids on endometrial receptivity during ovarian stimulation (Bourgain *et al.*, 2002; Papanikolaou *et al.*, 2005; Horcajadas *et al.*, 2008; Haouzi *et al.*, 2009; Labarta *et al.*, 2011). Oocyte donors had also been good study subjects to analyse the endometrium and the results were promising (Horcajadas *et al.*, 2008; Labarta *et al.*, 2011). However, these methods often rely on endometrial biopsy, which can limit their application and utility in daily clinical practice because of its invasiveness. Therefore, a simpler and less invasive method to predict the pregnancy outcomes and help clinicians individualize treatment is certainly needed. Our results suggest it is still worthwhile to monitor the serum P concentration continuously during COS cycles, considering the deleterious effect of a prolonged duration of pre-ovulatory P elevation on the pregnancy outcomes of IVF/ICSI treatments.

Some strategies have been proposed to compensate for the deleterious effect of pre-hCG P rise. Earlier administration of hCG for the final oocyte maturation when the serum P level is approaching the cutoff value might improve pregnancy outcomes (Elnashar, 2010; Kiliçdag *et al.*, 2010). Additionally, cryopreserving the resulting embryos and

transferring them in the subsequent thawed cycles has been suggested (Elnashar, 2010; Kiliçdag *et al.*, 2010). Shapiro *et al.* (2010) reported that cryopreserving all the embryos at two pronuclear (2PN) stage and transferring them at the blastocyst stage in a thawed cycle could improve the implantation rate and the ongoing pregnancy rate. Moreover, since the prematurely elevated serum P had been proposed to be associated with the stronger intensity and longer duration of gonadotrophin stimulation and an excess amount of retrieved oocytes (Devroey *et al.*, 2004; Kiliçdag *et al.*, 2010), milder stimulation protocols might offer more natural and balanced endocrine and endometrial environments, and therefore better IVF outcomes. However, it has also been proposed that a higher ovarian response does not compromise the chance of pregnancy (Fatemi *et al.*, 2011). Therefore, more studies are still needed to determine whether these aforementioned methods can really improve pregnancy outcomes in the patients with premature P elevation.

In conclusion, our study provides new insight into the correlation of premature P elevation and pregnancy outcomes of IVF/ICSI cycles. The clinical pregnancy rate is significantly inversely correlated with the duration of premature serum P elevation. When serum P level is elevating and approaching the cutoff value (1.0 ng/ml in our study) during ovarian stimulation, modification of the clinical treatment on a case-by-case basis might be considered to improve the outcomes.

Acknowledgements

The authors would like to thank Ms Li-Jung Chang and Ms Yi-Yi Tsai for their technical assistance, and also thank the National Translational Medicine and Clinical Trial Resource Center and the Department of Medical Research in National Taiwan University Hospital for the statistical assistance.

Authors' roles

C.C.H. interpreted the data, and drafted the article. S.U.C. contributed to the design of the study and numerous important revisions for the content. C.C.H., M.J.C. and C.H.C. conducted the statistical analysis. C.J.S. and Y.L.Y. provided laboratory support. Y.R.L., H.F.C. and Y.S.Y. provided important comments to the manuscript.

Funding

This study was supported in part by grants from the National Science Council (100-2314-B-002-022-MY3) and National Taiwan University Hospital (NTUH 100-S1555), Taipei, Taiwan.

Conflict of interest

None declared.

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