

Subtle progesterone rise on the day of human chorionic gonadotropin administration is associated with lower live birth rates in women undergoing assisted reproductive technology: a retrospective study with 2,555 fresh embryo transfers

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Objective: To evaluate the association between serum P levels on the day of hCG administration and pregnancy outcome in women undergoing controlled ovarian hyperstimulation, prevention of premature ovulation by GnRH analogues, and fresh ET after 5 days of embryo culture.

Design: Retrospective, observational, cohort study.

Setting: Private IVF center.

Patient(s): A total of 2,555 women undergoing fresh ET on day 5 in 2,062 GnRH agonist and 493 GnRH antagonist cycles.

Intervention(s): None.

Main Outcome Measure(s): Live birth rate.

Result(s): Live birth rate in cycles with GnRH agonists was significantly lower in women with P levels ≥ 2.0 ng/mL (17.4%) on the day of hCG administration as compared with women with P levels < 1.5 ng/mL (24.6%) and 1.5–1.99 ng/mL (26.7%). No such significant differences in live birth rates in cycles with GnRH antagonist could be observed.

Conclusion(s): A rise of serum P levels ≥ 2.0 ng/mL on the day of hCG administration is associated with impaired early embryo implantation and reduced live birth rate in cycles with GnRH agonists after day-5 fresh ET.

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Key Words: IVF, pregnancy rate, progesterone, embryo transfer, GnRH agonist

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During controlled ovarian hyperstimulation (COH), premature luteinization, as detected by elevated serum P, is generally prevented by suppression of LH secretion with GnRH analogues (1). An inadvertent marked increase of P levels before final oocyte maturation with hCG would lead to cancellation of IVF cycles

in the majority of cases. Despite the use of GnRH analogues, a subtle rise in serum P levels is observed in a subgroup of women, particularly at the end of the stimulation cycle. Incidences have been described to be as high as 35% in GnRH agonist cycles (2–4) and 38% in GnRH antagonist cycles (5). An excess number of follicles, with each one producing a normal amount of P consistent with the late follicular phase rather than excessive amounts of P being produced by granulosa cells as part of early luteinization, has been suggested to account for this observation (6, 7).

A large number of clinical studies examined the effect of a subtle rise in serum P levels on the day of hCG administration in GnRH agonist cycles on pregnancy rates, but results remained controversial: effects on pregnancy rates were either positive (8, 9) or detrimental (7, 10–15), with variable P cutoffs. Other investigations failed to demonstrate any correlation between P levels and pregnancy outcome (2, 16–21). More recently an analysis of more than 4,000 cycles revealed a reduction of ongoing pregnancy rate if serum P levels on the day of hCG administration were elevated, in particular levels of >1.5 ng/mL (7).

There are a limited number of studies regarding a subtle P rise in GnRH antagonist cycles (5, 7, 22, 23), showing lower pregnancy rates (5, 7, 22) or no effect on pregnancy outcome (23).

The mechanism leading to lower pregnancy rates is unclear. Slightly elevated P levels may impair endometrial receptivity rather than oocyte quality (24, 25), given that both homologous transfer of frozen/thawed embryos as well as donor oocyte transfers from cycles with high P levels yield live birth rates comparable to fresh cycles with low serum P levels (9, 15).

We hypothesized that a subtle rise in serum P levels on the day of hCG administration adversely affects pregnancy outcome. We thus analyzed more than 2,500 IVF/ICSI cycles with COH, suppression of premature ovulation via GnRH agonists or antagonists, ovulation induction with hCG, and fresh ET 5 days after oocyte retrieval.

The primary objective was to determine the association between elevated serum P levels on the day of hCG administration and live birth rate after 23 completed gestational weeks.

MATERIALS AND METHODS

Trial Design

This noninterventive, retrospective, observational, single-center cohort study was conducted in women treated for infertility at a single private IVF center between January 2006 and January 2011.

Participants

A total of 2,555 women undergoing assisted reproduction (IVF or intracytoplasmic sperm injection [ICSI]) with COH, suppression of premature ovulation by GnRH agonists ($n = 2,062$) or antagonists ($n = 493$), and with simultaneous measurement of P on the day of hCG administration and fresh ET were included. The mean age was 36.8 years (range, 21–46

years). The primary or combined indications for fertility treatment were male subfertility (50.7%), tubal pathology (13.1%), endometriosis (10.7%), polycystic ovarian syndrome (PCOS) (5.9%), and other causes, including age (18.4%). Detailed patient characteristics are listed in Table 1. The study was approved by the Ethics Commission of the Medical Faculty of the Ludwig-Maximilians-University of Munich.

Assisted Reproductive Technology Procedures

For suppression of premature ovulation, patients were either treated with a GnRH agonist or a GnRH antagonist, as described elsewhere (26, 27). In brief, patients with the GnRH agonist regimen initially received the GnRH agonist nafarelin (Synarel; Pharmacia) in a twice-daily nasal spray application (0.4 mg/d) for at least 7 days before the onset of ovarian stimulation. When pituitary down-regulation was confirmed ($LH < 5$ mIU/mL, $E_2 < 50$ pg/mL), patients received daily SC injections of recombinant FSH (rFSH) (Puregon [MSD Sharp & Dohme] or Gonal-f [Merck Serono]). The dose of gonadotropin was individualized for each patient according to age, antimüllerian hormone level, FSH level on day 2–5 of a previous cycle, antral follicle count, body mass index, and previous response to ovarian stimulation. Dose adjustments were performed according to ovarian response as monitored by means of vaginal ultrasonographic scans and measurements of serum E_2 . Patients in the antagonist group received the GnRH antagonist ganirelix (Orgalutran, 0.25 mg/d SC; MSD Sharp & Dohme) or cetrorelix (Cetrotide, 0.25 mg/d SC; Merck Serono) starting on day 5–6 after the onset of stimulation with rFSH (Table 1).

When three follicles reached a mean diameter of 18 mm, 10,000 IU of hCG (Predalon; MSD Sharp & Dohme) were administered SC, and the oocytes were retrieved by transvaginal ultrasound-guided aspiration 36 hours later. Micronized P (Utrogest; Dr. Kade/Besins Pharma) at a dose of 600 mg/d was applied vaginally for luteal support for at least 14 days after oocyte retrieval. All transfers were carried out after 5 days of culture with a maximum of three embryos. Before transfer all blastocysts were graded according to an internal score: hatching blastocyst (score 1), fully expanded blastocyst (score 2), blastocyst with blastocystic cavity $\geq 50\%$ of volume of embryo (score 3), and all remaining blastocysts (score 4).

Hormone Measurements

Whole blood was collected between 7:00 and 12:00 AM on the day of hCG administration for the immediate measurement of P and E_2 and on days 11 and 14 after hCG administration for the measurement of β -hCG. Serum was separated by centrifugation, and further handling was carried out according to the manufacturer's instructions on an ADVIA Centaur XP auto-analyzer (Siemens Medical Solutions Diagnostics). Internal daily quality control was performed in our laboratory, and quarterly external quality control was carried out according to the guidelines of the German Federal Medical Association ("Richtlinien der Bundesärztekammer," <http://www.bundesaeztekammer.de/downloads/rilibaeklabor201111.pdf>).

TABLE 1

Patient characteristics.				
Characteristic	Group 1 (n = 1,835)	Group 2 (n = 484)	Group 3 (n = 236)	All groups (n = 2,555)
Serum P (ng/mL)	0.2–1.49	1.5–1.99	≥2.0	≥0.2
Age (y)				
Mean (SD)	37.0 (4.49)	36.2 (4.99)	36.5 (4.31)	36.8 (4.49)
Female fertility status (%)				
Primary infertility	74.3	74.2	70.8	74.0
Secondary infertility	25.7	25.8	29.2	26.0
Normal fertility status	51.9	56.8	59.3	53.5
Tubal pathology	13.1	13.6	15.3	13.4
Endometriosis	10.7	9.9	8.9	10.4
PCOS	5.9	5.4	6.4	5.9
Other causes	18.4	14.3	10.1	16.8
Male fertility status (%)				
Oligospermia	26.8	29.9	31.3	27.8
Asthenospermia	50.7	46.7	47.3	49.9
BMI (kg/m ²)				
Mean (SD)	22.9 (3.9)	22.0 (3.1)	22.2 (3.9)	22.7 (3.8)
ART (%)				
ICSI	67.8	64.9	71.2	67.6
IVF	14.2	14.5	12.3	14.1
IVF/ICSI	18.0	20.7	16.5	18.4
Stimulation protocol (%)				
Agonist (long) protocol	80.2	82.2	81.8	80.7
Antagonist protocol	19.8	17.8	18.2	19.3
Duration of stimulation (d)				
Mean (SD)	9.8 (2.1)	9.7 (1.8)	9.7 (1.8)	9.8 (2.0)
Range	6–23	6–17	6–16	6–23
Total FSH dosage (IU)				
Mean (SD)	1,889.4 (646.2)	1,868.8 (574.4)	1,888.3 (554.3)	1,885.4 (625.0)
Range	300–6,525	700–4,425	825–3,825	300–6,525

Note: ART = assisted reproductive technology; BMI = body mass index.
Ochsenkühn. Serum P and IVF outcome. *Fertil Steril* 2012.

The concentration of P was measured in serum by an automated quantitative immunoassay (ADVIA Centaur Progesterone Test; Siemens Medical Solutions Diagnostics) with a sensitivity of 0.2 ng/mL. Intraobserver and interobserver coefficients of variation were 9.8% and 5.8%, respectively.

The concentration of E₂ was measured by an automated quantitative immunoassay (ADVIA Centaur Enhanced Estradiol Test; Siemens Medical Solutions Diagnostics) with a limit of detection of 11.8 pg/mL. Intraobserver and interobserver coefficients of variation for a mean concentration of 1,619 pg/mL were 2.3% and 1.5%, respectively.

The serum concentration of total β-hCG was measured in a two-site sandwich immunoassay (ADVIA Centaur Total hCG Test; Siemens Medical Solutions Diagnostics). Concentrations are determined by a direct chemiluminometric approach using two antibodies raised against different specific epitopes on both the free β-subunit and the β-subunit of intact hCG. The detectable concentration of total β-hCG ranges from 2.0 mIU/mL to 1,000 mIU/mL. Serum samples with β-hCG levels >1,000 mIU/mL were diluted, and concentrations were determined by calculation with an appropriate dilution factor. Precision, as indicated by standard deviation to the mean, in our laboratory was 4.4 for an hCG level of 6.9 mIU/mL, 3.6 for an hCG level of 20.6 mIU/mL, and 3.1 for an hCG level of 638.5 mIU/mL. The ADVIA Centaur Total hCG Assay standardization is traceable to the World Health Organization (WHO) 3rd International Standard for hCG (IS 75/537). According

to the manufacturer, a comparison over the full assay range gave the following correlation: ADVIA Centaur Total hCG Assay = 0.92 (WHO) ($r = 1.0$).

Vaginal Sonography

Ultrasound (Voluson-e; GE Medical Systems) was performed with a vaginal micro-convex probe (4.0–10 MHz frequency range) for cycle monitoring and on day 21 after hCG administration in all patients with a positive pregnancy test for the eventual identification of a gestational sac. A second vaginal sonogram was performed on day 31 after hCG administration for the eventual identification of fetal heart activity.

Statistics

All statistical analysis was performed with the Statistical Package for Social Sciences (SPSS 18.0 for Windows). Rates for ET and rates for the occurrence of a biochemical pregnancy, an intrauterine gestational sac, positive fetal heart activity, and live birth were analyzed by the two-sided, exact χ^2 test (Fisher test). Hormone levels (E₂, β-hCG) and number of oocytes or pronucleus cells or transferred embryos were analyzed by one-way analysis of variance with least significant difference post hoc test. A *P* value of <.05 was considered to be statistically significant. The correlation between serum P on the day of hCG administration and E₂, number of oocytes, and age was analyzed by Spearman correlation.

RESULTS

Among all included IVF/ICSI cycles serum P on the day of hCG administration ranged from 0.2 ng/mL to 4.6 ng/mL. Cycles were assigned to group 1 (0.2–1.49 ng/mL), group 2 (1.50–1.99 ng/mL), or group 3 (≥ 2.0 ng/mL). Statistical distribution of the P levels in group 3 was as follows: mean, 2.5 ng/mL; median, 2.3 ng/mL; maximum, 4.6 ng/mL; 25th percentile, 2.1 ng/mL; 50th percentile, 2.3 ng/mL; and 75th percentile, 2.7 ng/mL. Group 2 and 3 were considered to show a subtle P rise.

The proportion of women with P levels ≥ 2.0 ng/mL on the day of hCG administration was similar in women with GnRH agonist and antagonist cycles (9.4% and 8.7%, respectively).

Age, female and male fertility status, mean number of stimulation days, ratio of GnRH agonist and antagonist cycles, total dose of administered rFSH, and number of transferred embryos did not differ between the three P groups (Tables 1 and 2). Serum E₂ and number of retrieved oocytes and pronucleus cells, however, significantly increased from group 1 to group 3 (Table 2). Progesterone on the day of hCG administration was positively and significantly correlated with E₂ (coefficient of correlation 0.465; $P < .01$) and number of oocytes (coefficient of correlation 0.394; $P < .01$). Progesterone was negatively correlated with age (coefficient of correlation -0.128 ; $P < .01$).

The mean number of transferred embryos was equal in all three groups, and the percentages of ETs with at least one or two blastocysts were not different between the three groups

(Table 2). The mean score of all transferred blastocysts was significantly better in the high-P group (Table 2).

Serum β -hCG levels on day 11 or day 14 after hCG administration were measured in 70% of all patients, statistically less often in the high-P groups. Mean serum β -hCG levels on day 14 were statistically lower in group 3 as compared with group 1 (Table 3). Significantly fewer women in group 3 as compared with group 2 and group 1 were identified with at least one intrauterine gestational sac (22% vs. 32% and 36%, respectively) and at least one positive heart activity (21% vs. 30% and 34%) (Table 3).

The overall live birth rate (GnRH agonist or antagonist) was 24.4%. The birth rates after transfer of one, two, or three embryos were 12.4%, 28.7%, and 15.3% respectively. The mean P levels in patients with a transfer of one, two, or three embryos were 1.08 pg/mL, 1.29 pg/mL, and 1.24 pg/mL respectively.

Live birth rate was significantly lower in group 3 (17.4%) as compared with group 1 (24.7%) and group 3 (26.7%) (Fig. 1A). After subgroup analysis, the same significant differences of live birth rates could also be observed in the GnRH agonist group (18.7% vs. 25.7% and 27.9%). In women with GnRH antagonists a trend for a lower live birth rate in group 3 could be observed without reaching statistical significance (Fig. 1B and C).

DISCUSSION

Our data suggest that women undergoing COH with pituitary down-regulation by GnRH agonists and ET 5 days after

TABLE 2

Results for hormone levels, oocyte and pronucleus cell numbers, and embryo quality.

Variable	Group 1 (n = 1,835)	Group 2 (n = 484)	Group 3 (n = 236)	All groups (n = 2,555)	P value
Serum P (ng/mL)	0.2–1.49	1.5–1.99	≥ 2.0	≥ 0.2	
Serum E ₂ (pg/mL)					
Mean (SD)	1,673 (1,007)	2,367 (1,051)	2,754 (1,363)	1,904 (1,120)	Group 1 vs. 2: $P < .001$ Group 1 vs. 3: $P < .001$ Group 2 vs. 3: $P < .001$
No. of oocytes					
Mean (SD)	9.8 (5.7)	12.8 (5.7)	13.9 (6.0)	10.7 (5.9)	Group 1 vs. 2: $P < .001$ Group 1 vs. 3: $P < .001$ Group 2 vs. 3: $P = .047$
Range	1–35	1–38	1–34	1–38	
Pronucleus cells (2PN) (n)					
Mean (SD)	5.7 (3.6)	7.3 (4.0)	7.9 (4.2)	6.2 (3.8)	Group 1 vs. 2: $P < .001$ Group 1 vs. 3: $P < .001$ Group 2 vs. 3: $P = .21$
Range	1–25	1–23	1–23	1–25	
Transferred embryos (n)					
Mean (SD)	2.0 (0.55)	2.0 (0.50)	2.0 (0.48)	2.0 (0.54)	Group 1 vs. 2: $P = .29$ Group 1 vs. 3: $P = .86$ Group 2 vs. 3: $P = .95$
Range	1–3	1–3	1–3	1–3	
ET with at least 1 blastocyst (%)	64.3	71.1	63.2	65.6	Group 1 vs. 2: $P = .06$ Group 1 vs. 3: $P = .84$ Group 2 vs. 3: $P = .14$
ET with at least 2 blastocysts (%)	27.4	37.5	34.2	29.6	Group 1 vs. 2: $P = .001$ Group 1 vs. 3: $P = .224$ Group 2 vs. 3: $P = .35$
Mean score of all transferred blastocysts (score 1–4)	3.20	3.12	2.92	3.16	Group 1 vs. 2: $P = .53$ Group 1 vs. 3: $P = .045$ Group 2 vs. 3: $P = .32$

Ochsenkühn. Serum P and IVF outcome. Fertil Steril 2012.

TABLE 3

Pregnancy outcome as referred to early serum β -hCG levels and vaginal sonography.

Variable	Group 1 (n = 1,835)	Group 2 (n = 484)	Group 3 (n = 236)	All groups (n = 2,555)	P value
Serum P (ng/mL)	0.2–1.49	1.5–1.99	≥ 2.0	≥ 0.2	
β -hCG tested on day 11 or day 14 n (%)	1,359 (74.1)	306 (63.2)	133 (56.4)	1,798 (70.4)	Group 1 vs. 2: $P < .001$ Group 1 vs. 3: $P < .001$ Group 2 vs. 3: $P = .09$
β -hCG tested on day 11 n (%)	1,249 (68.1)	280 (57.9)	124 (52.5)	1,653 (64.7)	Group 1 vs. 2: $P < .001$ Group 1 vs. 3: $P < .001$ Group 2 vs. 3: $P = .2$
95% CI	65.9–70.2	53.4–62.3	46.1–59.0	62.8–66.6	
β -hCG day 11 (mIU/mL) Mean (SD)	11.2 (15.7)	10.5 (13.6)	8.2 (13.9)	10.8 (15.2)	Group 1 vs. 2: $P = .85$ Group 1 vs. 3: $P = .076$ Group 2 vs. 3: $P = .32$
β -hCG tested on day 14 n (%)	1,087 (59.2)	253 (52.3)	97 (41.1)	1,437 (56.2)	Group 1 vs. 2: $P = .006$ Group 1 vs. 3: $P < .001$ Group 2 vs. 3: $P < .005$
95% CI	57.0–61.5	47.8–56.7	34.8–47.4	54.3–58.2	
β -hCG day 14 (mIU/mL) Mean (SD)	58.0 (87.1)	52.6 (78.7)	33.8 (82.9)	55.4 (85.6)	Group 1 vs. 2: $P = .71$ Group 1 vs. 3: $P = .02$ Group 2 vs. 3: $P = .16$
At least 1 IGS on day 21 n (%)	583 (31.8)	172 (35.5)	52 (22.0)	807 (31.6)	Group 1 vs. 2: $P = .13$ Group 1 vs. 3: $P = .002$ Group 2 vs. 3: $P < .001$
95% CI	29.6–33.9	31.2–39.8	16.7–27.4	29.8–33.4	
At least 1 IHA on day 31 n (%)	548 (29.9)	164 (33.9)	50 (21.2)	762 (29.8)	Group 1 vs. 2: $P = .96$ Group 1 vs. 3: $P = .006$ Group 2 vs. 3: $P < .001$
95% CI	27.8–40.0	29.7–38.1	15.9–26.4	28.1–31.6	

Note: IGS = intrauterine gestational sac; IHA = intrauterine fetal heart activity.

Ochsenkühn. Serum P and IVF outcome. *Fertil Steril* 2012.

oocyte retrieval exhibit a significant lower live birth rate in the case of a subtle P rise on the day of hCG administration. This observation is in line with earlier studies from the 1990s (10–13) and recent large-scale studies (7, 14, 15, 22). To the best of our knowledge, there exist only two other studies reporting on day-5 ET in GnRH agonist cycles, and they both show negative effects of elevated P levels on pregnancy outcome (15, 28).

In women with GnRH antagonist cycles pregnancy outcome was also lower in the high-P group, but this observation did not reach statistical significance, probably because of small sample size. Four other studies examined the effect of a subtle P rise on the day of hCG administration on pregnancy outcome in women treated with GnRH antagonist cycles (5, 7, 22, 23). In the majority of these studies reduced pregnancy rates were reported, at least in women with day-3 ET (5, 7, 22). In one study pregnancy rates after day-3 or day-5 ET were reported (22). Interestingly, P rise on the day of hCG administration impaired pregnancy outcome after day-3 ET but had no effect after day-5 ET (22).

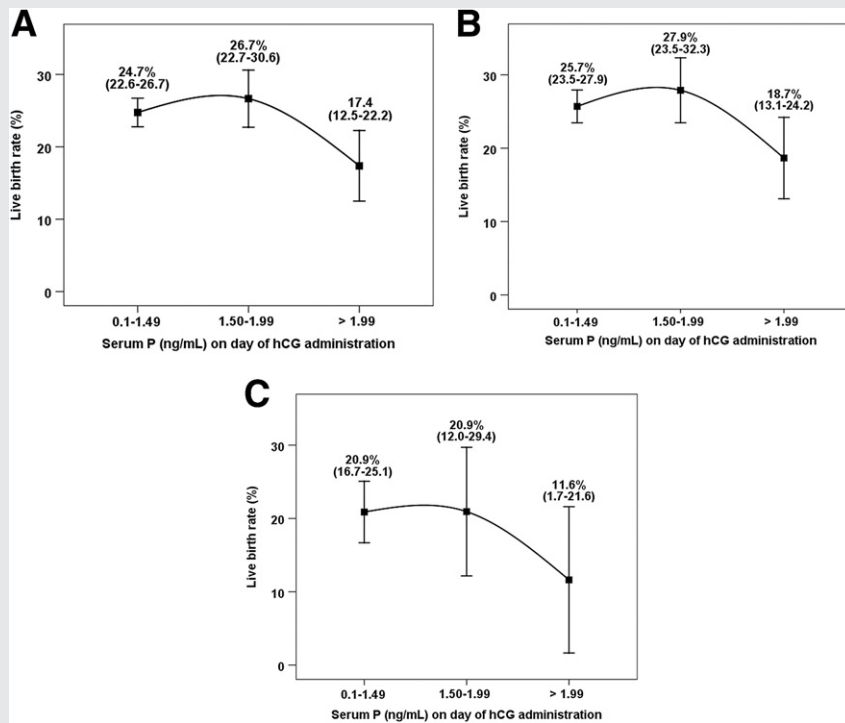
It has been proposed that high follicular P advances the endometrium, and therefore the replacement of a day-3 embryo in an asynchronous endometrium results in failure of establishing embryo–endometrium cross-dialogue, this leading to embryo demise and failure of implantation (22). Papanikolaou et al. suggested that on the fifth luteal day the endometrium has already significantly recovered from the violation induced from the supraphysiologic steroid concentrations

(22). Because we did not examine pregnancy outcome after day-3 ET, it cannot be excluded that an earlier ET may have shown different results.

In our study, the serum P cutoff level for a significant negative effect on live birth rates was 2.0 ng/mL. In both GnRH agonist and antagonist cycles a P rise > 1.99 ng/mL occurred in 9% of women. Previous studies with negative effects on pregnancy outcome found lower threshold levels for P: 0.9 ng/mL (12), 1.0 ng/mL (10), 1.1 ng/mL (14), 1.5 ng/mL (7, 11), and 1.7 ng/mL (15). It cannot be excluded that the use of a large variety of available P assays may account for different thresholds (6, 29).

There is strong evidence from histologic observations and expression analysis of implantation window markers that ovarian hyperstimulation in IVF cycles profoundly alters the luteal-phase endometrium (30). Patients with elevated P levels > 1.5 ng/mL on the day of hCG administration display a significantly different gene expression profile of the endometrium as early as 36 hours later when compared with patients with P levels < 1.5 ng/mL (31). Similar effects were observed 7 days after hCG administration, corresponding to the window of implantation, when a total of 370 analyzed genes were dysregulated by more than twofold in patients with P levels > 1.5 ng/mL (32). Both studies support the concept of an endometrial impairment in the presence of elevated P and accordingly lower ongoing pregnancy rates (7, 14, 15). Consistent with these observations, elevated P levels > 0.9 ng/mL on the day of

FIGURE 1



(A) All cycles ($n = 2,055$): live birth rates are significantly lower in group 3 as compared with group 1 ($P=.012$) and group 2 ($P=.007$). (B) Gonadotropin-releasing hormone agonist cycles ($n = 2,062$): live birth rates are significantly lower in group 3 as compared with group 1 ($P=.034$) and group 2 ($P=.01$). (C) Gonadotropin-releasing hormone antagonist cycles ($n = 493$): There are no significant differences between the P groups.

Ochsenkühn. Serum P and IVF outcome. *Fertil Steril* 2012.

hCG administration are associated with an accelerated increase of the endometrial echogenicity during the early luteal phase of COH cycles (33).

Our data show significantly lower β -hCG levels on days 11 and 14 and significantly less intact intrauterine pregnancies on days 21 and 31 after hCG administration in the high-P group, suggesting that the lower live birth rates corresponded to a failure of very early implantation (i.e., before the fourth gestational week). Because early β -hCG levels were only measured in some of the women, this conclusion may be attenuated. However, early vaginal ultrasound has been applied in all patients and can therefore narrow down the detrimental effects of a subtle P rise to early pregnancy (i.e., before the fifth gestational week).

In our study the birth rates after transfer of one, two, or three embryos were 12.4%, 28.7%, and 15.3%, respectively. The possible reason for the lower live birth rate in the group of patients with transfer of three embryos is the higher mean age: 40.0 years in patients with three transferred embryos vs. 37.2 years and 36.1 years in patients with one or two transferred embryos, respectively.

According to our data the P rise was not associated with an increased number of transferred embryos, although oocyte number increased in accordance with E_2 and P levels. Our data show that high P levels are associated with a significant increase in blastocyst quality. The theory that premature P

exposure does not reduce the quality of embryos is also supported by a study in patients with transfer of frozen-thawed embryos from cycles with raised P showing similar live-birth rates when compared with those embryos from natural cycles (15). We therefore suggest cryopreservation of pronuclear or cleavage-stage embryos in patients with elevated P levels ≥ 2.0 ng/mL on the day of hCG administration in GnRH agonist cycles for later ET with optimal endometrial receptivity.

A limitation of our study is that no generally accepted score for embryo grading was applied (e.g., Gardner score) (34). It cannot be excluded that the application of a more reliable, internationally accepted score may have altered the results regarding different embryo quality. This study is also limited because data on cumulative pregnancy rates after transfer of frozen-thawed embryos from COH cycles with known P levels are missing. Data on pregnancy rates after cryotransfer of embryos in a natural or hormonally substituted cycle with low P levels before ovulation may have given more insight into the possible detrimental effects of P on implantation and would have possibly allowed pinpointing lower pregnancy rates to the hyperstimulated endometrium.

The results of our study may not be generalizable for different reasons. First, the observed cutoff limit of 2.0 ng/mL may depend on the used P assays. It has been reviewed that a large number of assays for P are available on the market

(6). It cannot be excluded that different institutes may have different P thresholds. Second, only embryos after 5 days of in vitro culture, in many cases blastocysts, have been transferred. It cannot be excluded that day-3 ET may have impaired pregnancy rates even more. Last, the conclusion of this study may not be applicable to a population with a different allocation of fertility cause. In our study only 6% of women had PCOS, and subgroup analysis was therefore not reasonable. In a study of exclusively patients with PCOS, elevated serum P on the day of hCG administration in IVF was associated with a higher pregnancy rate (8).

We conclude that a subtle rise of serum P levels on the day of hCG administration is associated with impaired early embryo implantation and reduced live birth rate in GnRH agonist cycles after day-5 fresh ET. The threshold for detrimental P levels should be individualized for each IVF institute because of the diversity of available P assays and duration of embryo culture.

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