

Elevated serum progesterone levels on the day of human chorionic gonadotropin administration in in vitro fertilization cycles do not adversely affect embryo quality*

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Objective: To assess the effect of an elevated serum P level on the day of hCG administration in an IVF cycle on resulting embryos by evaluating their performance at subsequent frozen ET.

Design: A retrospective study.

Participants: Ninety-six consecutive patients undergoing frozen ET cycles were studied in a tertiary care center.

Main Outcome Measures: Serum obtained on the day of hCG administration in an IVF cycle was assayed for E₂ and P by RIA. The main outcome measured was the development of a clinical pregnancy in a subsequent frozen ET cycle.

Results: Using a previously described breakpoint in serum P concentration of 0.9 ng/mL (2.86 nmol/L), 8 of 69 (11.6%) frozen ETs in which embryos from low P level IVF cycles were transferred and 7 of 27 (25.9%) frozen ETs of embryos from elevated P level IVF cycles were transferred resulted in the development of clinical pregnancies. Although this does not clearly demonstrate superiority of embryos obtained from elevated P cycles, employing a power calculation, the probability that the pregnancy rate in the elevated serum P group is at least equal to the observed rate in the low P group is 92.8%.

Conclusion: These data suggest that an elevated serum P level on the day of hCG administration does not adversely affect the quality of oocytes or resulting embryos.

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Key Words: In vitro fertilization, frozen embryo transfer, progesterone, embryo quality

We previously reported that a serum P level ≥ 0.9 ng/mL (2.86 nmol/L) on the day of hCG adminis-

tration is associated with a diminished pregnancy rate (PR) in IVF cycles stimulated with leuprolide acetate (LA) and hMG (1). Another published report concurred with this observation and suggested that the lower PRs seen in patients with elevated P levels might result from an adverse effect of P on the oocytes (2). Other possible sites of action for the adverse effect of the P elevation could be on the embryos or on the endometrium. This is a significant issue to address, because elevated P levels may precipitate different therapeutic intervention. If an early P rise (i.e., before an injection of hCG) adversely affects the oocyte or embryo, then one might consider either administering hCG earlier or

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canceling the oocyte retrieval. Conversely, if the P rise exerts its effect on the endometrium, then the timing of the hCG injection does not need to be changed, the retrieval can proceed as usual, and all embryos can be cryopreserved for subsequent frozen ET. To assess the effect of an elevated P level on the day of hCG administration on embryo quality, we retrospectively evaluated frozen ET cycles performed at our institution.

MATERIALS AND METHODS

Ninety-six consecutive patients undergoing frozen ET at the South Texas Fertility Center at The University of Texas Health Science Center at San Antonio were studied. All patients had undergone a previous IVF cycle at our center. During this cycle, all patients received SC injections of LA; (1 mg/d, Lupron; TAP Pharmaceuticals, Chicago, IL) beginning 10 days after spontaneous ovulation or 11 days after the detection of an LH surge. Ovulation was detected with transvaginal sonography, and the urinary LH surge was identified with a commercially available kit (Ovukit; Monoclonal Antibodies, Sunnysvale, CA). All patients had a qualitative serum pregnancy test performed before initiating LA therapy.

Two days after the onset of subsequent menses, patients underwent transvaginal sonography using an Acoustic Imaging 5200 equipped with a 7.5-MHz endovaginal probe (Acoustic Imaging Inc., Phoenix, AZ). Ovarian stimulation was initiated using hMG (Pergonal; Serono Laboratories, Inc., Randolph, MA) if the largest follicle was <10 mm in diameter. The daily LA dose was then decreased to 0.5 mg/d. Patients were followed with serial transvaginal sonography, and serum E₂ levels were drawn between 8:00 and 9:00 A.M. Daily serum samples were frozen for subsequent analysis. Human chorionic gonadotropin (Profasi; Serono Laboratories) was administered in a single IM dose of 10,000 IU when two follicles exceeded 15 mm in average diameter and serum E₂ concentrations were >250 pg/mL (918 pmol/L) per follicle > 15 mm.

Transvaginal follicular aspiration was performed 34 to 35 hours after hCG administration. Oocytes were cultured *in vitro* for 6 hours, and each mature oocyte was inseminated with 10,000 motile sperm. Immature oocytes were cultured *in vitro* for 22 to 24 hours before insemination. A maximum of four embryos were transferred fresh in all patients, and su-

pernumerary embryos were observed for possible cryopreservation.

Embryo cryopreservation was performed at the multicell stage in 1.5 M propylene glycol plus 0.1 M sucrose using a Planar (Middlesex, United Kingdom) R203 programmable freezer. Our embryo cryopreservation protocol employs six ramps that progressively lower the temperature to -40°C. During the second ramp, manual seeding is performed using aerosolized dichlorodifluoromethane (Accu Freeze; Baxter Scientific, McGaw Park, IL). Upon completion of the program, the embryo vials are removed and placed into liquid nitrogen, where they are maintained at -196°C. When the embryos are needed for subsequent transfer, the vials are placed into a 31°C water bath, where thawing occurs over a 3-minute period. Each embryo is placed into a series of human tubal fluid (HTF; Irvine Scientific, Irvine, CA) media droplets and gradually decreasing concentrations of propylene glycol, with a constant concentration of 0.2 M sucrose. A maximum of four embryos were subsequently transferred in 20 µL HTF plus 15% human serum using a Tomcat (Sherwood Medical, St. Louis, MO) catheter.

Frozen ETs of multicell embryos were performed in unstimulated cycles for those women with normal ovulatory function, as demonstrated by an in-phase endometrial biopsy performed during their routine infertility evaluation. The frozen ET was timed to occur 72 hours after a serum LH peak. Patients were seen daily for serial serum LH determinations, beginning 4 days before the date of expected ovulation, as determined from their normal menstrual cycle length. In addition, these patients underwent serial transvaginal ultrasound (US) examinations to confirm ovulation. Frozen ET cycles were canceled when follicle rupture could not be documented ultrasonographically. Oligo-ovulatory women received a combination of micronized E₂ (Estrace; Mead Johnson Laboratories, Evansville, IN) and IM P (50 mg/d; Eli Lilly, Indianapolis, IN), and all had in-phase endometrial biopsies in a simulated cycle before the frozen ET cycle on this hormonal regimen.

Stored serum was available for all patients from the day of hCG administration in the IVF cycle from which the cryopreserved embryos were obtained. These samples were all assayed for P and E₂ using commercially available ¹²⁵I RIA kits (Coat-A-Count; Diagnostic Products Corp., Los Angeles, CA) according to the manufacturer's directions.

Interassay and intra-assay coefficients of variation determined in our laboratory were 11.8% and 8.9%, respectively, for P, and 6.4% and 2.7%, respectively, for E₂. Specifically, at a P level of 0.9 ng/mL, the intra-assay coefficient of variation was 8.7%.

Data were analyzed using Fisher's Exact Test for all 2 × 2 categorical comparisons. Step-wise multiple logistic regression analysis was used to determine those predictor variables best correlated with cycle outcome (pregnancy). The power determination was made using a formula previously described (3).

Patients were categorized according to clinical outcome: nonpregnant or clinical pregnancy. A clinical pregnancy was defined as an ongoing intra-uterine pregnancy with documented cardiac activity, an ectopic pregnancy (EP), or a spontaneous miscarriage with chorionic villi identified in a pathology specimen. For purposes of this study, biochemical pregnancies were included in the non-pregnant group.

RESULTS

Uterine transfer of multicell embryos was performed in all 96 patients analyzed in this study. Fifteen patients subsequently developed clinical pregnancies (15.6%). Using a previously described breakpoint in serum P level on the day of hCG administration (0.9 ng/mL or 2.86 nmol/L), patients were divided into two groups: P < 0.9 ng/mL (group I, n = 69) and P ≥ 0.9 ng/mL (2.86 nmol/L; group II, n = 27). Neither etiology of infertility nor patient age was significantly different between the two groups of patients.

The characteristics of the IVF stimulation cycle are presented in Table 1. Group II patients received approximately 1 day more of hMG stimulation than did group I patients (P = 0.008). These patients also had significantly higher serum E₂ levels on the day of hCG administration (P = 0.01) and had more

Table 1 Initial IVF Stimulation Cycle Characteristics*

	Group I (n = 69)	Group II (n = 27)	P†
Days of hMG†	8.3 ± 0.2	9.1 ± 0.2	0.008
E ₂ at hCG (pg/mL)‡	1929.1 ± 115.0	2,661.4 ± 252.0	0.01
Oocytes obtained	16.3 ± 0.7	20.3 ± 0.9	0.002

* Values are means ± SEM.

† Group I versus group II.

‡ E₂ level on the day of hCG administration (conversion factor to SI unit, 3.108).

Table 2 Effect of Serum P Level on Oocyte Quality*

	Group I (n = 69)	Group II (n = 27)	P†
Mature oocytes (%)	91.4 ± 1.4	92.7 ± 1.4	0.50
Fertilization (%)	80.4 ± 1.8	78.3 ± 2.5	0.44
Polyspermy (%)	7.0 ± 1.7	4.1 ± 1.4	0.16
No. of embryos frozen	6.3 ± 0.5	10.5 ± 1.0	<0.001

* Values are means ± SEM. Serum P level on the day of hCG administration during the initial IVF stimulation cycle.

† Group I versus group II.

oocytes retrieved (P = 0.002) than did group I patients.

Oocyte maturity, fertilization rates, rates of polyspermy, and average number of embryos frozen per patient are shown in Table 2. Group II patients had significantly more embryos frozen than did group I patients (P < 0.001). No differences in oocyte maturity, fertilization rate, or polyspermic fertilization were noted between the groups.

There was no difference between the two groups in the method of endometrial preparation during the frozen ET cycle. Group II patients received significantly more embryos transferred during the frozen ET procedure than did group I patients (P = 0.002, Table 3). Using multiple logistic-regression analysis, however, the number of embryos transferred during the frozen ET procedure did not correlate with the establishment of a clinical pregnancy. There was a trend toward group II patients having a higher cycle fecundity rate than group I patients (P = 0.08), although this did not achieve statistical significance. A power calculation revealed that the probability that the PR in group II was at least equal to the observed rate in group was 92.8%.

DISCUSSION

Numerous published reports have confirmed our observation that a serum P level > 0.9 ng/mL (2.86

Table 3 Effect of Serum P Level on Subsequent Frozen ET*

	Group I (n = 69)	Group II (n = 27)	P†
Embryos transferred‡	2.6 ± 0.1	3.4 ± 0.2	0.002
Cycle fecundity	0.116	0.259	0.08

* Serum P level on the day of hCG administration during the initial IVF stimulation cycle.

† Group I versus group II.

‡ Values are means ± SEM.

nmol/L) on the day of hCG administration is associated with a diminished PR in IVF and ET cycles in which stimulation is performed with LA and hMG (2, 4–6). To date, only one published study has reported that serum P levels are not predictive of the establishment of pregnancy (7). Although no power calculation was presented in that report to establish the certainty of their findings, interpretation of the results of that study is difficult, due to the use of a P assay with a high (34%) coefficient of variation at a serum P level of 0.9 ng/mL.

This study was conducted to elucidate the possible mechanism by which premature P production adversely affects the establishment of pregnancy. Possible sites of action include the oocyte, the embryo, or the endometrium.

The model of IVF and ET allows us to investigate the effect of premature P production on the oocyte. If the adverse effect is exerted at this level, then one would expect to find either delayed oocyte maturity or postmaturity, poor rates of fertilization, or possibly an increased rate of polyspermic fertilization. Although high P levels induce mouse and frog oocyte atresia, leading to an increased number of meiotically inactive oocytes, no such effect has been observed in the human (8–10). As previously reported, we observed oocyte maturity rates of 85.0% and 87.9% for those patients with and without premature P production, respectively (1). Mio et al. (5) reported overall maturity rates of 96.9% and 98.3%, respectively, for similar groups. Fanchin et al. (4) concurred with this observation, finding no difference in oocyte maturity between patients with serum P levels < 0.9 ng/mL (2.86 nmol/L) and those with P levels > 0.9 ng/mL (2.86 nmol/L) on the day of hCG administration.

The literature evaluating the effect of P levels on oocyte fertilization is somewhat more divided. In our original study, we observed a significantly greater fertilization rate in oocytes obtained from cycles in which the P level was >0.9 ng/mL (2.86 nmol/L), compared with oocytes obtained from low P level cycles ($P < 0.05$) (1). With the smaller number of patients in this study, we were unable to demonstrate a significant difference in fertilization rates between the two groups of patients. Several investigators have reported that elevated serum P levels are associated with decreased oocyte fertilization rates (5, 6, 11). In the latter report, however, Kagawa et al. suggested that this might be due to marked elevations in serum LH concentrations (mean 15.9 mIU/mL) observed in their patients,

rather than to premature P production (6). Others have reported no adverse effect of premature P production on fertilization rates (4, 12, 13).

In assessing polyspermic fertilization, Edwards (14) reported that high P levels increase the incidence of polyspermy. He suggested that this might be due to postmaturity of the oocyte. Numerous reports, including ours, have since failed to confirm this observation (1, 11, 13, 15). In summary, therefore, it appears that premature P production does not exert its adverse effect at the level of the oocyte. An elevated serum P level results in neither altered oocyte maturity nor an increase in the rate of polyspermic fertilization. The overall effect on monospermic oocyte fertilization cannot be definitively determined, either from the present study or from a review of the literature.

There are several possible ways to assess the effect of premature P production on the embryo. First, one could examine cleavage rates from IVF cases, with the underlying null hypothesis being that premature P production does not result in diminished cleavage rates. Several recent reports support this conclusion (1, 2, 4, 12, 16). In the largest and most recent prospective series, Fanchin et al. observed a 59% cleavage rate in 485 IVF cycles in which serum P levels were <0.9 ng/mL (2.86 nmol/L) on the day of hCG administration, compared with a 57% cleavage rate in 100 IVF cycles in which P levels were elevated.

Although these data tend to suggest that the adverse effect of premature P production is not exerted at the level of the embryo, this may not be an appropriate conclusion, in light of the observation that unfertilized oocytes may cleave and divide to at least the four-cell stage (17).

There are, however, two other models that one can use to address this question. The first example is that of oocyte donation. In their recent retrospective series, Hoffman et al. (18) observed no significant difference in implantation or delivery rates between 47 patients who received oocytes donated from women who had serum P levels < 1.1 ng/mL (3.5 nmol/L) on the day of hCG administration and 21 patients who received oocytes donated from women who had serum P levels > 1.1 ng/mL (3.5 nmol/L) on the day of hCG administration in their LA- and hMG-stimulated cycles. There were no demographic differences between the two groups of oocytes donors nor were there differences in length of stimulation, number of ampules of hMG used, or peak serum E_2 levels. Oocyte maturity, fertilization

rates, and the incidence of polyspermic fertilization were also similar between the two groups of donors. There were no demographic differences between the oocyte recipients, and all recipients had their endometrium prepared artificially using exogenous hormone replacement. The authors therefore concluded that the adverse effect of premature P production is not exerted at the level of the embryo.

Frozen ET is another model that can be used to assess the effect of premature P production on the embryo. This study specifically examines this issue, by evaluating supernumerary embryos that had been cryopreserved and subsequently transferred into an endometrium that had not been exposed to the supraphysiologic levels of E_2 and P commonly encountered in hyperstimulation cycles. Inasmuch as the endometria were similarly prepared in these two groups of patients and because all patients had received a fresh transfer of their four morphologically best embryos during their previous failed IVF cycle, this method of study design allows a direct comparison of the performance of the embryos obtained from the original IVF cycles. The only demonstrable differences between the IVF cycles that resulted in these embryos were that group II embryos were exposed to 1 additional day of hMG stimulation, as well as higher serum E_2 and P levels. Of these parameters, using multiple logistic-regression analysis, the only one that was predictive of the establishment of pregnancy was the serum P level on the day of hCG administration.

There were, likewise, no differences in parameters that could be evaluated in the frozen ET cycle between the two groups, other than the number of embryos that were transferred. As previously stated, however, again using multiple logistic-regression analysis, the number of embryos transferred did not correlate with the establishment of pregnancy. Therefore, this study allowed us to assess directly the effect of premature P production on the embryo. We observed that embryos obtained from IVF cycles in which premature P production occurred resulted in pregnancy at least as often as did embryos obtained from IVF cycles in which premature P production did not occur. Although there was a trend toward a higher PR with premature P production, this difference was not statistically significant ($P = 0.08$). These data, combined with the relatively large number of patients in this study, demonstrate with a power of 92.8% that these two groups of embryos performed similarly. This sug-

gests that the adverse effect of premature P production is not exerted at the level of the embryo.

Based on this information, it seems likely that the lower PR observed in IVF cycles in which premature P production occurs is due to an adverse effect upon the endometrium. It may therefore be advisable to avoid fresh embryo transfer in patients who have a serum P level > 0.9 ng/mL (2.86 nmol/L) on the day of hCG administration in an LA, hMG, and hCG IVF cycle. All embryos may be cryopreserved for subsequent frozen ET. Definitive evidence of a direct effect of premature P production on the endometrium, which impairs the establishment of pregnancy, awaits further studies.

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