Serum Progesterone in Predicting Pregnancy Outcome After Assisted Reproductive Technology¹

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Purpose: Our purpose was to determine whether serum progesterone predicts pregnancy outcome after superovulation.

Methods: One hundred twenty-three consecutively pregnant patients were divided into three groups: group I, 55 patients following superovulation for assisted reproductive technologies; group II, 23 patients after correction of oligoovulation; and group III, 45 patients who conceived spontaneously. When β -human chorionic gonadotropin was positive, progesterone was measured on the same serum sample. A serum progesterone level of 45 $\mu\text{m/L}$ was set to differentiate between nonviable pregnancy and ongoing pregnancy.

Results: In group I, zero (0%) of 38 ongoing pregnancies and 10 (59%) of 17 nonviable pregnancies were observed with a progesterone level of $<45 \mu\text{m/L}$ [14.2 ng/ml ($P < 0.001$)]. In group II, 4 (27%) of 15 ongoing pregnancies and 5 (63%) of 8 nonviable pregnancies had a progesterone level of $<45 \mu\text{m/L}$ ($P = \text{NS}$). In group III, 10 (42%) of 24 ongoing pregnancies and 15 (71%) of 21 nonviable pregnancies were observed with a progesterone level of $<45 \mu\text{m/L}$ (14.2 ng/ml) ($P = \text{NS}$).

Conclusions: A serum progesterone level of $<45 \text{ nM}$ predicts nonviable pregnancy after superovulation for assisted reproductive technology.

KEY WORDS: assisted reproductive technology; in vitro fertilization; pregnancy; progesterone; superovulation.

INTRODUCTION

Progesterone (P) levels in early pregnancy reflect the interrelationship between the embryo and the corpus luteum. Low P values are less an indication of corpus luteum function than of embryonic viability. This information has been used clinically to predict pregnancy outcome (1–4). The influence of the number of corpora lutei on serum P values is unclear. It is important to determine whether different P threshold levels should be set in women who conceive after assisted reproductive technology (ART) treatment who have multiple corpora lutei and may also be taking P supplements in early pregnancy. Multiple corpora lutei may lead to high serum P levels even with a nonviable pregnancy. Our study aims to determine the P threshold level below which viable pregnancy is unlikely in an early ART pregnancy. To answer this question we studied serum P levels in three groups of pregnant women: group I, patients pregnant after superovulation for in vitro fertilization (IVF) or intrauterine insemination (IUI), all of whom had multiple corpora lutei—many of these patients were also taking P supplements; group 2, patients pregnant after correction of oligoovulation and who were presumed to have a smaller number of corpora lutei; and group 3, patients who were spontaneously pregnant, presumably with only one corpus luteum.

MATERIALS AND METHODS

The charts of all patients with a positive β -human chorionic gonadotropin (hCG) level who attended the Fertility Center at the University of Ottawa between January 1997 and December 1997 were reviewed.

Patients were assigned to one of three groups. Group I consisted of 55 patients who conceived after controlled ovarian hyperstimulation with human meno-

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pausal gonadotropin (hMG) for IVF or IUI. For IVF, patients were given hMG after gonadotropin-releasing hormone analogue down regulation as described previously (5). Progesterone luteal support was given routinely to all IVF-embryo transfer ET (ET) patients using micronized P, 200 mg twice daily, vaginally (Prometrium; Upjohn), starting from the day of ET. Progesterone administration was stopped after the identification of fetal heart activity on ultrasound at 4 to 5 weeks after ET. Superovulation for intrauterine insemination (SO-IUI) was generally achieved with hMG alone at a starting dose of 2 ampoules daily and titrated for two to five follicles reaching a diameter of 18–21 mm at the time of hCG administration. Occasional SO-IUI patients were pretreated with gonadotropin-releasing hormone agonist down regulation before hMG as was done in IVF-ET patients. Luteal support with P was used only in patients who were pretreated with gonadotropin-releasing hormone analogue down regulation. In these SO-IUI patients, micronized P, 200 mg twice daily, vaginally (Prometrium; Upjohn), was given starting on the day of IUI and continued until identification of fetal heart activity on ultrasound. Seventeen days after ET or IUI serum β -hCG was measured. If positive, the same serum sample was tested for P. Group II included 23 oligoovulatory patients who conceived after induction of ovulation with hMG or clomiphene citrate. After missed menses a β -hCG was measured. If positive, serum P estimation was performed using the same sample. Group III comprised 45 patients who conceived spontaneously. Assays were performed in this group when pregnancy was suspected. All blood samples were drawn into Becton Dickinson SST Vacutainers (Becton Dickinson, Franklin Lakes, NJ) using standard phlebotomy technique and were transported to the laboratory within 1 hr of collection. Specimens were allowed to clot and were centrifuged at 3000g for 10 min to separate serum. Specimens were analyzed for β -hCG, with all positive samples analyzed for P. Both β -hCG and P were determined by microparticle enzyme immunoassay (MEIA) on the Abbott AxSym using the manufacturer's reagents (Abbott Diagnostics, Abbott Park, IL). The analytical performance characteristics of the AxSym P assay are as follows: sensitivity, 0.6 nmol/L; precision (CV), 5.4% at 2.7 μ m/L, 4.1% at 23.7 μ m/L, and 5.1% at 65.3 μ m/L; and linearity, 0–127 μ m/L.

Pregnancy outcome was recorded as nonviable (ectopic pregnancy or spontaneous abortion) or ongoing as documented by the presence of fetal heart activity beyond the twelfth week of gestation.

After exploring different threshold levels, two levels, 45 μ m/L (14.2 ng/ml) and 70 μ m/L (22 ng/ml), were examined to assess their sensitivity and specificity for differentiating nonviable from viable ongoing pregnancies. Statistical analyses were performed using χ^2 and Fisher's exact tests when appropriate.

Clinical sensitivity, specificity, positive predictive value, and negative predictive value were determined for each group at a P decision of 45 μ m/L (14.2 ng/ml) and 70 μ m/L (22 ng/ml) using the following definitions:

True positives: Nonviable intrauterine or ectopic pregnancy; $P < 45 \mu\text{m/L}$ (14.2 ng/ml) or $< 70 \mu\text{m/L}$ (22 ng/ml).

False positives: Viable intrauterine pregnancy; $P < 45 \mu\text{m/L}$ (14.2 ng/ml) or $< 70 \mu\text{m/L}$ (22 ng/ml).

True negatives: Viable intrauterine pregnancy; $P > 45 \mu\text{m/L}$ (14.2 ng/ml) or $> 70 \mu\text{m/L}$ (22 ng/ml).

False negatives: Nonviable intrauterine pregnancy or ectopic pregnancy; $P > 45 \mu\text{m/L}$ (14.2 ng/ml) or $> 70 \mu\text{m/L}$ (22 ng/ml).

RESULTS

When the threshold value for P was set at 70 μ m/L (22 ng/ml), we observed the following results. In group I, 7(23%) of 31 ongoing pregnancies and 6(40%) of 15 nonviable pregnancies were observed with a P level $< 70 \mu\text{m/L}$ (22 ng/ml), with a sensitivity of 40% and a specificity of 77% ($P = \text{NS}$). In group II, 4(27%) of 15 ongoing pregnancies and 6(75%) of 8 nonviable pregnancies were observed with a P level $< 70 \mu\text{m/L}$ (22 ng/ml), with a sensitivity of 75% and a specificity of 73% ($P = \text{NS}$). In group III, 18(75%) of 24 ongoing pregnancies and 20(95%) of 21 nonviable pregnancies were observed with a P level $< 70 \mu\text{m/L}$ (22 ng/ml), with a sensitivity of 95% and a specificity of 25% ($P = \text{NS}$) (Table I).

When the threshold value for P was set at $P < 45 \mu\text{m/L}$ (14.2 ng/ml), we observed the following results. In group I, zero (0%) of 38 ongoing pregnancies and 10(59%) of 17 nonviable pregnancies were observed with a P level of $< 45 \mu\text{m/L}$, with a sensitivity of 59% and a specificity of 100% ($P = 0.0000007$). In group II, 4(27%) of 15 ongoing pregnancies and 5(63%) of 8 nonviable pregnancies had a P level of $< 45 \mu\text{m/L}$, with a sensitivity of 63% and a specificity of 73% ($P = \text{NS}$). In group III, 10(42%) of 24 ongoing pregnancies and 15(71%) of 21 nonviable pregnancies were observed with a P level $< 45 \mu\text{m/L}$ (14.2 ng/ml), with

Table I. Pregnancy Outcome with P <70 nM (22 ng/ml) in All Groups

Group	No. of pts.	Ongoing pregnancy (%)	Nonviable pregnancy (%)	P value	Sensitivity (%)	Specificity (%)	PPV ^a (%)	NPV ^b (%)
I	55	23 (7/31)	40 (6/15)	NS	40	77	46	73
II	23	27 (4/15)	75 (6/8)	NS	75	73	60	85
III	45	75 (18/24)	95 (20/21)	NS	95	25	53	86

^a Positive predictive value.^b Negative predictive value.

a sensitivity of 71% and a specificity of 58% ($P = \text{NS}$) (Table II).

DISCUSSION

Progesterone is secreted from a single corpus luteum in spontaneous pregnancies. Patients undergoing controlled ovarian hyperstimulation for IVF or IUI will have multifollicular development. If pregnancy occurs, there will be multiple corpora lutea. It might be expected that this group of patients would have higher levels of serum P than would be found during a spontaneous pregnancy. Furthermore, many ART patients receive P supplements during the luteal phase and in early pregnancy. This might lead to yet higher levels of serum P even with a nonviable pregnancy. However, our data show that serum P levels of <45 $\mu\text{M/L}$ are very predictive (100% specificity) of nonviable pregnancy after ART (Table II).

Surprisingly, unlike others (1–4), we did not find serum P levels as useful for the prediction of pregnancy outcome with respect to spontaneous or simple ovulation induction pregnancies. We had only 29 nonviable pregnancies in these two groups, which may not have the necessary statistical power to show a relationship between a low P level and a poor pregnancy outcome. McCord *et al.* (3) had 1828 nonviable pregnancies in their series. They studied the outcome of 3674 pregnancies diagnosed in an emergency room setting without differentiating between spontaneous pregnancy and

those resulting from fertility treatments. Their series may have included ART pregnancies similar to our group I, which may have contributed to their findings showing that viable pregnancies rarely occur when serum P levels are <15.9 $\mu\text{M/L}$ (5 ng/ml).

Due to these small numbers, we were also unable to use a receiver operator curve to determine the optimal serum P value for differentiating between ongoing and nonviable ART pregnancies. However, after studying our data, we found that a serum P level of <45 $\mu\text{M/L}$ (14.2 g/ml) is a suitable value to select as a cutoff for prediction of nonviable pregnancy after superovulation. We did not find that a higher threshold value of serum P <70 $\mu\text{M/L}$ (22 ng/ml) to be predictive of pregnancy outcome in any of the groups (Table I). Furthermore, we found a serum P level of <45 $\mu\text{M/L}$ (14.2 g/ml) to be the mean of the cutoff levels reported by others (2,3).

A reliable test for the prediction of nonviable pregnancy after ART would be helpful, especially when ectopic pregnancy is suspected. Yamashita *et al.* (6) evaluated 220 IVF pregnancies for outcome by means of β -hCG, estradiol, and P between 2 and 4 weeks after oocyte collection. They concluded that these tests provide useful data for predicting ongoing or ectopic pregnancy. However, Bustillo *et al.* (7), analyzing 282 pregnancies resulting from IVF, were unable to demonstrate that serum P and estradiol values could predict pregnancy outcome using receiver–operator characteristic curves.

Table II. Pregnancy Outcome with P <45 nM (14.2 ng/ml) in All Groups

Group	No. of pts.	Ongoing pregnancy (%)	Nonviable pregnancy (%)	P value	Sensitivity (%)	Specificity (%)	PPV ^a (%)	NPV ^b (%)
I	55	0 (0/38)	59 (10/17)	0.0000007	59	100	100	84
II	23	27 (4/15)	63 (5/8)	NS	63	73	55	78
III	45	42 (10/24)	71 (15/21)	NS	71	58	60	70

^a Positive predictive value.^b Negative predictive value.

Based on our data we cannot recommend a P threshold level for the prediction of outcome in spontaneous or simple ovulation induction pregnancies. We have, however, demonstrated that a P level of $<45 \mu\text{m/L}$ predicts the presence of a nonviable pregnancy following superovulation for ART.

The predictive value of serum P levels may be different for different groups of patients. Further research into the value of serum P for the prediction of pregnancy outcome in various groups of patients such as those presenting with symptoms of threatened abortion or ectopic pregnancy is warranted.

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