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# MATERNAL SERUM PROGESTERONE, 17β-ESTRADIOL AND ESTRIOL ARE INCREASED IN PREGNANCIES WHICH FOLLOW TREATMENT WITH HUMAN MENOPAUSAL GONADOTROPINS: EFFECTS OF MULTIPLE GESTATION AND MATERNAL ENDOCRINE STATUS

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**Summary**—A high incidence of premature labor, incompetent cervix and fetal wastage occurs in multiple gestations which follow treatment with human menopausal gonadotropins (HMG). In order to determine the effect of treatment with HMG on hormone secretion in human pregnancy, progesterone (PROG), 17β-estradiol (E2), estriol (E3) and human chorionic gonadotropin (hCG) were determined by radioimmunoassay in 341 serum specimens from 229 normal singleton pregnancies and in 79 serum specimens from 20 pregnancies following induction of ovulation with HMG in women with either hypothalamic amenorrhea (HA) or the polycystic ovary syndrome (PCO). Fitting equations were found for the log transformed normal values and the residuals were obtained by subtraction of the predicted normal values from the log transformed values observed in the HMG pregnancies. In pregnancies which followed treatment with HMG, PROG and E2 were initially elevated above normal. As pregnancy progressed, the deviation from normal became proportionately less. PROG ($P < 0.025$) was lower and E2 ($P < 0.025$) and E3 ($P < 0.05$) were higher in PCO pregnancies than in HA pregnancies. Multiple gestation produced increases in PROG ($P < 0.005$), E2 ($P < 0.005$) and E3 ($P < 0.001$) in comparison to singleton pregnancies.

## INTRODUCTION

We and others [1] have observed a high incidence of incompetent cervix, premature delivery and fetal wastage in multiple gestations which followed treatment with human menopausal gonadotropins (HMG). We suspected that this might be due to the effects on the cervix and uterus of an altered ovarian and placental endocrinology. Accordingly, we compared samples from singleton and multiple pregnancies which followed treatment with HMG to values determined in normal spontaneous singleton pregnancies. Significant alterations in the pattern of hormonal secretion occurred following treatment with HMG. Both the antecedent endocrine disorder of the mother and the number of fetuses were found to influence the magnitude and nature of this effect.

## EXPERIMENTAL

### *Subjects*

Blood samples were obtained from volunteers and as residual samples from the clinical laboratories of the University of Wisconsin under protocols approved by the Human Subjects Committee of the University of Wisconsin. Days from the last menstrual period (LMP) and fetal number were determined for each specimen based on all information available, including an accurate LMP and one or more of the following: date of conception from a temperature chart, ultrasound evaluation of the fetal biparietal diameter and fetal number, and (in the case of patients undergoing termination of pregnancy used in the normal range) evaluation of the products of conception by the Department of Pathology. A total of 341 serum specimens from 229 pregnancies with a single fetus made up the normal population as described [2]. The samples from HMG pregnancies included a total of 57 serum samples from 14 singleton pregnancies, a total of 16 samples from 4 twin pregnancies, and a total of 6 samples from 2 triplet pregnancies. Three of the 14 women with single pregnancies who had HA contributed a total of 20 samples, while the 11 with PCO contributed a total of 37 samples. Three of the 6 women with multiple gestations who had PCO contributed a total of 9 samples (all twin pregnancies). The other 3 women with multiple gestations who had HA contributed a total of 13 samples (1 twin and 2 triplet pregnancies).

### *Chemical analysis*

hCG was determined by direct double antibody radioimmunoassay as described [3]. The assay was

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modified by use of $^{125}$hCG as tracer and an antiserum to hCG beta-subunit (H1-9/2/76) produced in this laboratory by immunizing a rabbit with hCG beta-subunit prepared and purified as described [4]. An hCG standard (Calbiochem, La Jolla, CA 92037) calibrated against the World Health Organization second international standard was utilized. The cross reaction of this antibody with hLH was 0.16%. Between and within assay variation were 3.8 and 3.7% respectively. The assay sensitivity was 0.5 mIU/ml.

*Steroid extraction and chromatography*

One ml of serum was utilized for extraction except in pregnancies less than 100 days from the LMP where 2 ml was extracted if sufficient serum was available. Extraction [5] and chromatography [5] were performed as described with the modifications described [2]. Progesterone [6], 17$\beta$-estradiol [5] and estriol [5] were then determined by specific radioimmunoassay as described utilizing the appropriate fractions.

The assay for estriol was modified by use of an antibody (142-7/21/76) [5] produced by immunizing a sheep with a bovine serum albumin conjugate of estriol-16$\alpha$-[$\beta$-D-glucuronide]. The cross reactions of this antibody in a system utilizing estriol and [$^3$H]estriol as standard and tracer have been previously described [2]. All cross reacting steroids were separated from estriol by the column chromatography. Between and within assay variation were 15 and 6.6% respectively. A recovery experiment performed by adding estriol (X) in amounts from 0.3125 to 10 ng/ml in 2-fold increments to male serum in triplicate demonstrated linear recovery (Y) with $Y = -0.04 + 1.02X$, a slope not significantly different from 1. The assay sensitivity was 0.003 ng/ml.

*Statistical analysis*

The determinations of hCG, PROG, E2 and E3 for the normal controls with single fetuses have been previously published as scatter plots [2]. Since these data have been demonstrated to be log normal [2], the $\log_{10}$ transformed data were fitted to polynomal functions using 1 or more terms from the series: $X^{-2}$, $X^{-1}$, $X^0$, $X^1$, $X^2$, $X^3$, $X^4$ where X is days from the last menstrual period by the method of least squares using Minitab [7]. All values for $R^2$ were corrected for degrees of freedom. Prior to the 13th week, E3 was not measurable in a significant percentage of normal samples (ranging from 100% in the 5th week to 31% in the 12th week. In the 13th week, E3 was measurable in 12 of 13 samples (92%) and a value for E3 was available for 251 of the 255 samples beyond the 12th week. Accordingly, the data for E3 were fitted only from the 13th week (day 85) onward. The analysis of E3 residuals was also limited to the interval from day 85 onward.

In order to evaluate the significance, degree and pattern of deviation from normal in pregnancies which followed treatment with HMG, the predicted normal values derived from these fitting equations were subtracted from the individual values obtained for the samples from HMG pregnancies to produce the residual values. The residuals were then evaluated statistically by regression analysis and covariance analysis using Minitab [7] in the entire population of HMG pregnancies (including single, twin and triplet pregnancies).

Since most patients pregnant after induction of ovulation with HMG contributed multiple samples, the sum of squares attributable to the within subject effect was removed by creating a "dummy" (0.1) variable for each pregnancy and including all 20 such variables in each multiple linear regression performed on the residuals for the HMG pregnancies. For graphic presentation of the PROG, E2 and hCG data and in the table, the constant term was corrected to include the effects of the 20 subject "dummy" variables as well as that of the constant term from the original regression. This was done by calculating a modified residual for each point by subtracting the contributions of the 1/X, the HA and the IM terms from the observed value. The mean modified residual was used as the value for the modified constant term. Since there was no 1/X term for E3, the mean predicted value from the original regression was plotted for E3.

## RESULTS

The fitting equations for PROG, E2, E3 and hCG in the normal population, the scatter plots for the normal data and the superimposed fitted lines are shown in Figs 1a–4a. The data for the HMG pregnancies are presented in Figs 1b–4b and the residuals found by subtracting the predicted normal value are shown in Figs 1c–4c. Since the residuals were computed on the log-transformed variables, the process of subtracting the logs and then taking the antilog is equivalent to dividing the measured value by the predicted value (obtained by taking the antilog of the value given by the equations). Thus, the antilog of the residuals computed on the log-transforms is the factor which gives the observed value when multiplied by the antilog of the predicted value.

When covariance analysis was performed on the entire population of HMG pregnancies using multiple linear regression by coding PCO as 0 and HA as 1 for the first "dummy" variable (IHA) and by coding singleton pregnancy as 0 and multiple pregnancy (twins and triplets) as 1 for the second "dummy" variable (IM), multiple linear regression on 1/X and both "dummy" variables as well as the "dummy" variables for the 20 individual pregnancies demonstrated that there was significant regression on 1/X for hCG ($P < 0.05$), PROG ($P < 0.001$) and E2 ($P < 0.001$) but not E3 ($P > 0.5$), indicating that

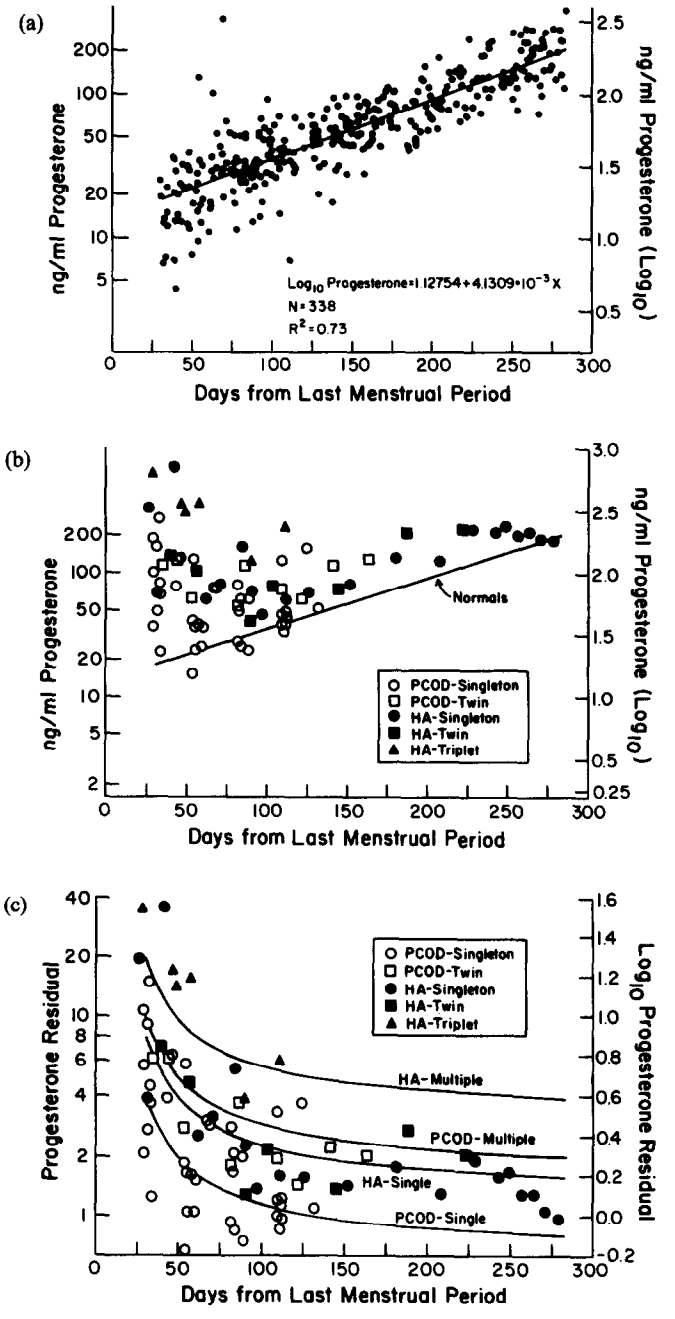


Fig. 1. Maternal serum progesterone in (a) normal singleton pregnancy and (b) pregnancy after menotropins. (c) Residuals for maternal serum progesterone in pregnancy after menotropins.

PROG and E2 were initially elevated. The effect of the regression on 1/X for hCG was relatively small. There was significant inhibition of PROG ($P < 0.025$) and a significant increase in E2 ($P < 0.025$) and E3 ($P < 0.05$) in PCO as compared to HA pregnancies. An increase in PROG ($P < 0.005$), E2 ($P < 0.005$) and E3 ($P < 0.001$) due to multiple pregnancy was also demonstrated.

Since an additive model was used for the log transformed data, the antilogs gave a multiplicative model for the actual data. The magnitudes of the multiplicative effects were estimated by taking the antilogs of the coefficients presented in Table 1. Thus the value (and 95% confidence limits) for PROG was approx. 2.0 (1.2–3.2)-fold higher in *HA* pregnancies than in *PCO* pregnancies. In contrast, the values for E2 were 2.6 (1.2–5.4) fold and those for E3 were 2.5 (1.03–6.3)-fold higher in *PCO* pregnancies than in *HA* pregnancies. Multiple gestation increased values for PROG 2.5 (1.4–4.3)-fold, E2 4.1 (1.6–10.1)-fold, and E3 4.3 (2.2–8.4)-fold in comparison to singleton pregnancies.

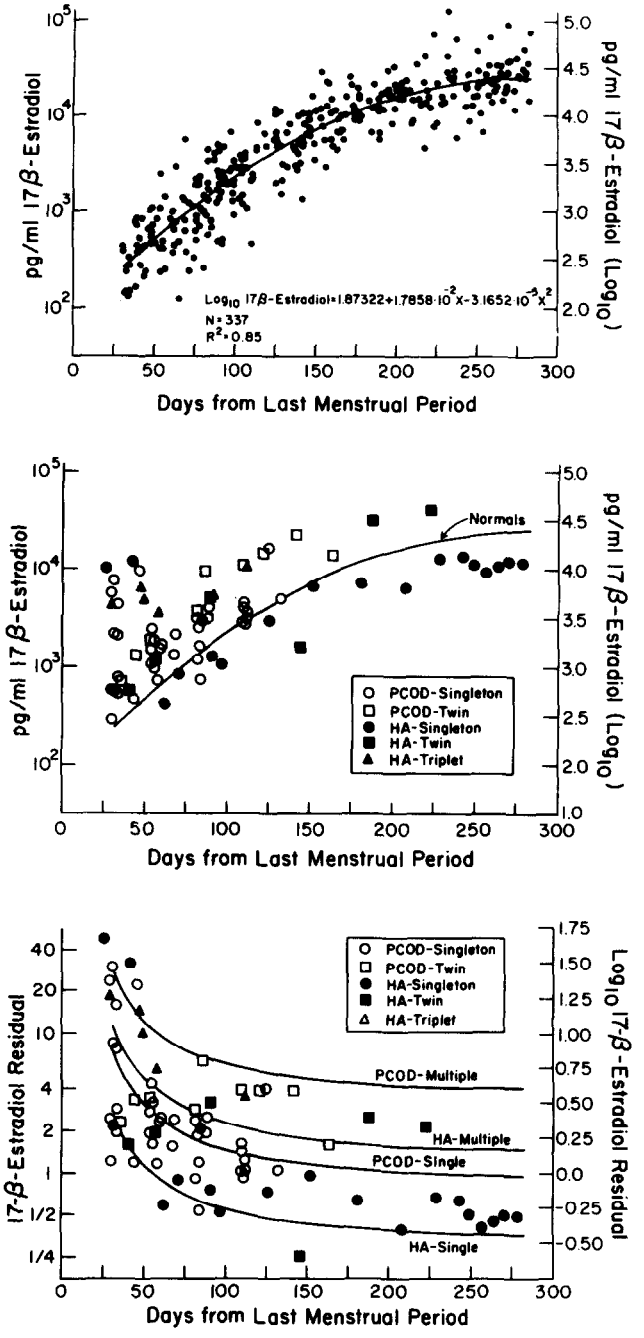


Fig. 2. Maternal serum 17β-estradiol in (a) normal singleton pregnancy and (b) pregnancy after menotropins. (c) Residuals for maternal serum 17β-estradiol in pregnancy after menotropins.

## DISCUSSION

Our demonstration of excessive plasma levels of both PROG and E2 in the first trimester appears to represent the excessive activity of the multiple corpus luteums present in pregnancies which follow induction of ovulation with HMG. This suggests the possibility that other corpus luteum products such as relaxin [8] may also be produced in excess. The early excess production of PROG and E2 becomes proportionately less during pregnancy, leading to an abnormal pattern of steroid secretion.

During induction of ovulation with HMG, women with PCO have been demonstrated to have higher concentrations of DHEAS, testosterone, and androstenedione than do women with HA [9]. The observed suppression of placental progesterone production in women with PCO in comparison to those with HA may be due to the known inhibition of 3β-hydroxy-steroid-dehydrogenase by androstenedione [10]. It appears that the increased production of E2 and E3 in women with PCO in comparison to those with HA is due to the increased

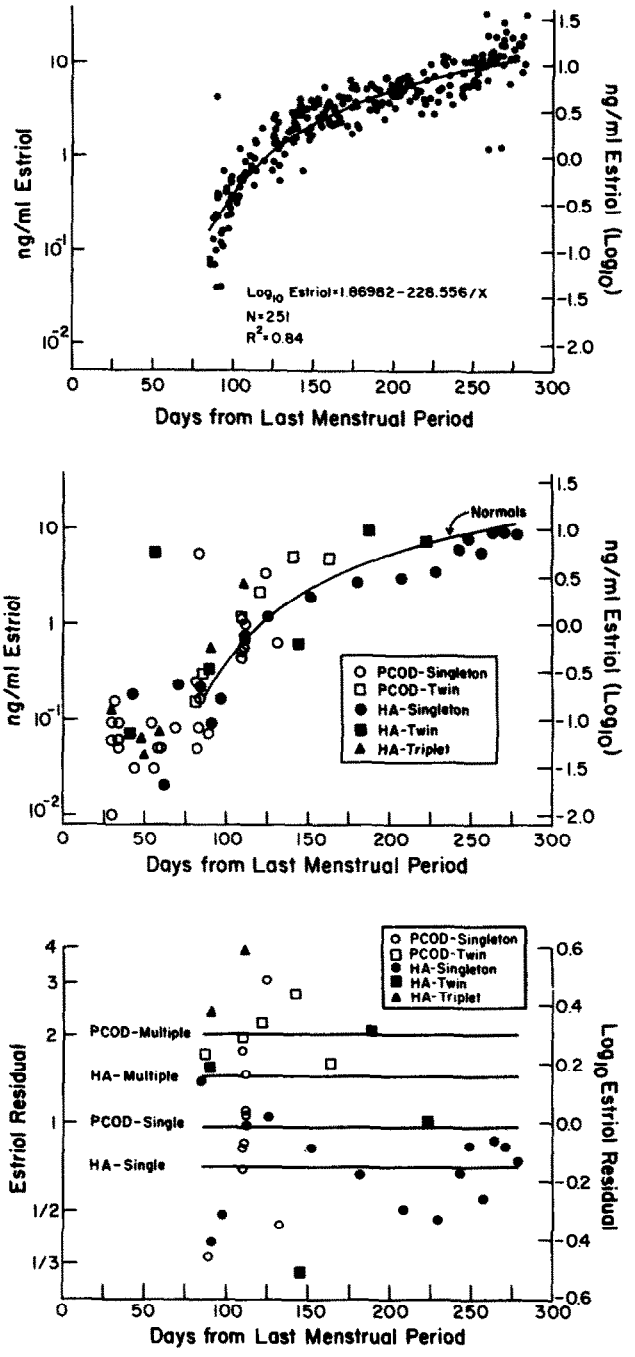


Fig. 3. Maternal serum estriol in (a) normal singleton pregnancy and (b) pregnancy after menotropins. (c) Residuals for maternal serum estriol in pregnancy after menotropins.

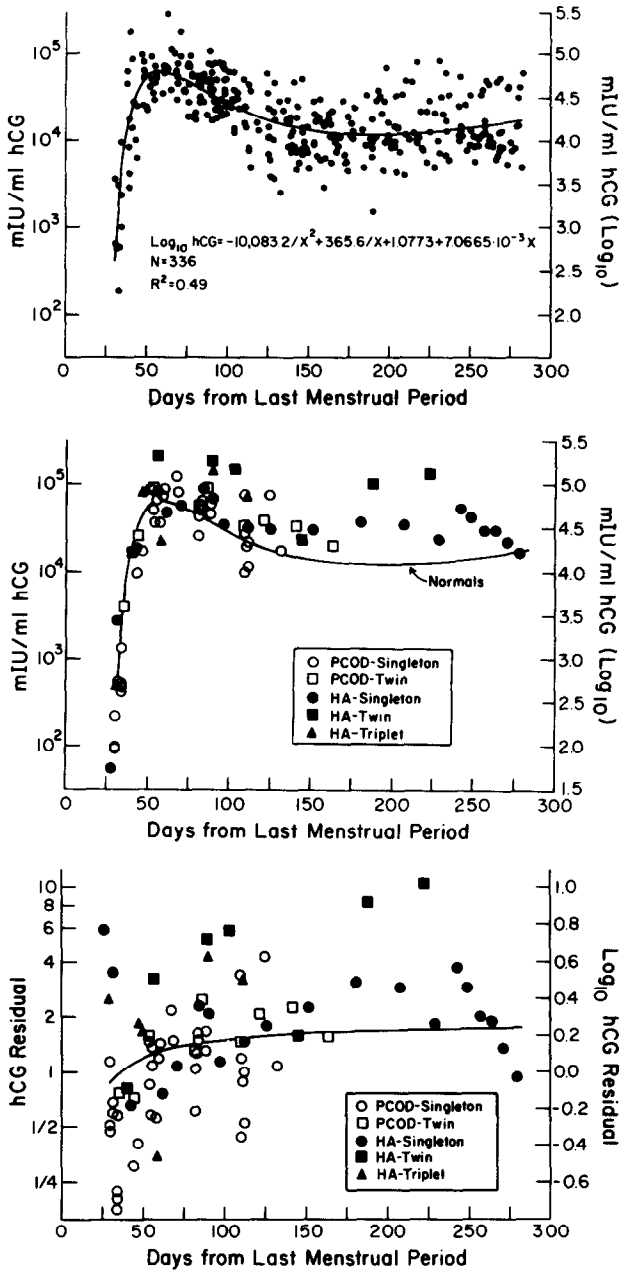


Fig. 4. Maternal serum hCG in (a) normal singleton pregnancy and (b) pregnancy after menotropins. (c) Residuals for maternal serum hCG in pregnancy after menotropins.

Table 1. Evaluation of the residuals using multiple linear regression: effects of regression on 1/X, multiple gestation versus singleton gestation, and hypothalamic amenorrhea versus polycystic ovary disease

| Residuals ($log_{10}$) | Coefficients Constant | 1/X | IHA | IM | $R^2$ | N |
|---|---|---|---|---|---|---|
| Progesterone | −0.1887 | 24.537[4] | 0.2907[2] | 0.3927[3] | 0.83 | 79 |
| 17β-Estradiol | −0.1549 | 32.050[4] | −0.4216[2] | 0.6089[3] | 0.71 | 78 |
| Estriol | 0.0434 | NS | −0.4037[1] | 0.6383[4] | 0.58 | 36 |
| hCG | 0.2724 | −9.812[1] | NS | NS | 0.45 | 79 |

The numbers entered in each column represent the coefficients found by multiple linear regression for the terms shown. Where X = days from the LMP, IHA = the "dummy" variable for HA vs PCOD and IM = the "dummy" variable for single vs multiple gestation. Where the initial regression showed non-significance (NS) for a term, the regression was recomputed without the term. The superscripts represent the significance for the regression on the term shown: $^1P < 0.05$, $^2P < 0.025$, $^3P < 0.005$, and $^4P < 0.001$.

availability of maternal substrates for placental aromatase of ovarian and adrenal origin in women with PCO (testosterone, androstenedione, and DHEAS). It is possible that the disordered endocrinology of early pregnancy may be responsible for the increased incidence of premature labor which we and others [1] have observed in multiple gestations which follow induction of ovulation with HMG.

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