Reprinted from THE JOURNAL OF REPRODUCTIVE MEDICINE
Vol. 35 No. 11, November 1990

Progesterone and Estradiol Concentrations in Nonpregnant and Pregnant Human Myometrium

Effect of Progesterone and Estradiol on Cyclic Adenosine Monophosphate–Phosphodiesterase Activity

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We measured the concentration of progesterone and estradiol and calculated the progesterone:estradiol ratio in nonpregnant and pregnant human myometrium. Progesterone, estradiol and the progesterone:estradiol ratio were higher in pregnant than in nonpregnant myometrium. There was no difference in the concentration in the presence of labor. The progesterone:estradiol ratio showed a similar pattern. We also investigated the effect of the ovarian steroids on the activity of cyclic adenosine monophosphate–phosphodiesterase (cAMP-PDE). Progesterone in pharmacologic doses inhibited the activity of the high-affinity enzyme as much as 72% and the low-affinity form as much as 34%. High-affinity phosphodiesterase from nonpregnant myometrium was the least sensitive to inhibition, and the enzyme from pregnant myometrium obtained from laboring women was the most sensitive. Low-affinity phosphodiesterase from nonpregnant myometrium was less sensitive to inhibition than enzyme from pregnant women with or without labor. The degree of inhibition of the low-affinity enzyme in the two pregnant groups was not different. The type of inhibition was competitive in both the high- and low-affinity forms. Estradiol at similar concentrations did not have any effect on the activity of the enzyme. Progesterone in part may exert its effect on the human myometrium by its effect on cyclic adenosine monophosphate–PDE activity and the metabolism of cAMP.

Introduction

During human pregnancy a number of significant anatomic and functional changes take place. The purpose of these changes is to permit the normal and uninterrupted development of the human fetus. Complex hormonal interactions play a significant role in the development of the above changes.[1] Progesterone (P) and estradiol (E_2), by their opposing actions, are believed to be two of the principal hormones in the regulation of myometrial activity in humans as well as in other species.[2-5] E_2 promotes protein synthesis in the myometrial smooth muscle and exerts a stimulatory effect on the cell's contractility. P promotes relaxation, counteracting the effect of E_2.

A variety of explanations have been proposed to explain P's mechanism of action on the myometrial cell. Csapo et al[6] suggested that the effect of P is one of hyperpolarization. The higher resting potential of the myometrial cell during pregnancy prevents depolarization of the cell and spike generation. P was found to completely inhibit the estrogen-induced rise in oxytocin receptors in the pregnant rat.[7] Intrauterine infusion of prostaglandin $F_{2\alpha}$, which elicited a

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0024-7758/90/3511-01045/$1.50/0

marked mechanical response in the control animals, failed to stimulate the P-"blocked" uterus in pregnant ewes.[8] P is also thought to decrease the α-adrenergic myometrial receptors and to modify the ultrastructural organization of the myometrium by inhibiting the formation of gap junctions.[9]

We showed previously[10] that the activity of cyclic adenosine monophosphate–phosphodiesterase (cAMP-PDE) is 70–80% lower in pregnant than in nonpregnant human myometrium. A study was undertaken to investigate the changes in the human myometrial E_2 and P levels during pregnancy and to evaluate the *in vitro* effect of these hormones in the activity of human myometrial cAMP-PDE.

Materials and Methods

Subjects

Myometrium was obtained from the anterior wall of the uterine corpus of six nonpregnant women who underwent hysterectomy for benign gynecologic indications. All patients were operated on during the second part of the menstrual cycle. The specimen was obtained immediately after the uterine vessels were ligated. Myometrium was also obtained from the uteri of six women not in labor who underwent elective repeat lower segment transverse cesarean section at term and from six women in labor who underwent primary lower segment transverse cesarean section for various obstetric indications (high breech in labor, active labor with maternal herpetic genital lesions, cephalopelvic disproportion and fetal distress). All nonpregnant patients had an unremarkable medical history and did not receive any medication before hysterectomy. All six patients were operated on while under general anesthesia. All pregnant patients were healthy, and their pregnancies were uncomplicated until the time of delivery. None of the patients received uterotonic agents or other drugs. The procedure was performed with the pregnant patients under epidural anesthesia. The study was approved by the hospital's clinical research practices committee, and each patient gave informed consent.

Tissue

The tissue biopsy specimen was taken from the upper edge of the transverse uterine incision from pregnant patients and from the isthmic portion of the lower part of the anterior uterine wall from nonpregnant patients. One gram of tissue was removed from each patient. The tissue was rinsed in saline solution, blotted and immediately frozen in a dry ice–acetone bath (−65° C). The entire procedure was performed within 60 seconds. Subsequently the tissue was stored at −70° C until assayed.

A 10% (wt/vol) homogenate in 0.25 mol/L sucrose and 10 mmol/L TRIS HCl (pH 8) was prepared (homogenization buffer). The frozen tissue was weighed in the frozen state, thawed in a small amount of buffer and minced. It was then homogenized at 4° C. Part of the supernatant was assayed immediately, and the rest was stored at −70° C as a 1:1 solution in TRIS HCl–bovine serum albumin (0.5 mg bovine serum albumin per milliliter of 40 mmol/L TRIS HCl, pH 8). It was determined that freezing the tissue and the homogenate did not change the enzyme activity for up to six months. The amount of protein was measured with a rapid and sensitive method that used the principle of protein-dye binding.[11]

PDE Activity

PDE activity was measured in the supernatant with the two-step isotopic procedure described by Thompson et al.[12] All assays, conducted in duplicate, were carried out in conditions of linearity with respect to time and protein concentration, allowing measurements of the initial rate of reaction.

The assay mixture (0.4 mL) contained 5 mmol/L $MgCl_2$, 40 mmol/L TRIS HCl (pH 8), 4 mmol/L 2-mercaptoethanol, unlabeled cAMP at different concentrations, labeled and tritiated cAMP (containing at least 100,000 cpm) and 0.1 mL of uterine cytosol. Reactions were initiated by the addition of enzyme; incubations were for 10 minutes at 30° C and were terminated by boiling for 45 seconds. After the addition of 0.1 mL of snake venom nucleotidase (1 mg/mL) the mixture was incubated for 10 minutes at 30° C and then placed in an ice bath. The entire contents of the tubes were then transferred to Dowex 1-X8 columns (200–400 mesh) prepared in Pasteur pipettes (1 mL of 1:4 resin slurry used to make each column). The tritiated adenosine was then eluted with 2 mL of high-performance liquid-chromatography-grade methanol. The radioactivity of the eluate was determined in a liquid scintillation counter. In the range of substrate concentration from 0.125×10^{-6} to 100×10^{-6} mol/L, apparent Michaelis constant and maximum velocity values were calculated with Lineweaver-Burk plots by means of the computer program ENZPACK (Elsevier-BIOSOFT).

P and E_2 Measurements

P and E_2 were extracted from the supernatant solution with diethyl ether and assayed without chromatography using tritiated steroids in a radioim-

munoassay (RIA) procedure. The P RIA utilized a specific antiserum[13] that has a within-assay coefficient of variation (CV) and a between-assay CV of <10%. The E_2 antiserum was also specific[14] and had a within- and between-assay CV of <15%.

Inhibition Experiments

Commercially available reagent-grade P and E_2 were used. Both steroids were diluted in ethanol; the final concentration of ethanol in the assay mixture was 2.5%. Ethanol at concentrations ≤5% had no detectable effect on the activity of cAMP-PDE. At concentrations of >5%, ethanol decreased the activity of cAMP-PDE because of the denaturing effect on the protein molecule.

Statistical Analysis

The differences in the concentration of E_2 and P and the differences in the P:E_2 ratio between the three groups were analyzed with one-way analysis of variance and Tukey's least significant difference test. The same statistical method was used to compare the degree of inhibition of cAMP-PDE by P *in vitro*. A $\log_{10}$ transformation of the data was carried out to satisfy the prerequisite of homogeneity of variance when analysis of variance was used. The paired Student *t*-test was used to compare the control and P inhibition group.

Results

P and E_2 Concentrations and the P:E_2 Ratio

The P concentration was lowest in the nonpregnant myometrium (5.15±2.5 ng/g wet tissue, mean±SE) and highest in myometrium from pregnant women at term and not in labor (89.32±12.49 ng/g wet tissue). In myometrium from pregnant women in labor the concentration of P was similar to that in myometrium from nonlaboring women (63.52±16.59 ng/g wet tissue). The differences were significant between the nonpregnant and two pregnant groups (Table I). E_2 was lowest also in the nonpregnant myometrium (0.94±0.08 ng/g wet tissue) and highest in pregnant myometrium in labor (7.07±1.10 ng/g wet tissue). The differences were also significant only between the nonpregnant and two pregnant groups (Table I).

The P:E_2 ratio demonstrated a similar pattern. In nonpregnant myometrium the ratio was 5.50±2.40 and in myometrium from nonlaboring pregnant women was 13.93±1.23 ($P<.05$). In myometrium from laboring women the ratio was 8.50±1.10. This was different from the nonpregnant myometrium ($P<.05$) but not significantly so from the myometrium obtained from nonlaboring women (Table I).

P and E_2 Effect on cAMP-PDE

P at concentrations ranging from 0.3 to 300 μmol inhibits the activity of human myometrial cAMP-PDE. The degree of inhibition of the high-affinity enzyme ranged from 10% to 70% and was found to be dose dependent with maximum P concentrations (300 μmol) (Figure 1). The high-affinity enzyme from nonpregnant myometrium was inhibited by 53%, while from pregnant myometrium not in labor it was inhibited by 63%. The enzyme from myometrium from laboring women was the most sensitive and was inhibited by 72% (Figure 2). P inhibited the activity of low-affinity enzyme to a significant degree, also (Figure 3).

The low-affinity enzyme was less sensitive to inhibition than was the high-affinity (Figures 4 and 5). The differences in the degree of inhibition between the three groups in the high-affinity enzyme were significant, while in the low-affinity enzyme only the difference between the nonpregnant myometrium and that from women pregnant and in labor was significant.

A kinetic analysis of the effect of P on the activity of cAMP-PDE, with the use of a Lineweaver-Burk plot, revealed a competitive type of inhibition. This

Table I *Progesterone, Estradiol and Progesterone:Estradiol Ratio in Nonpregnant and Pregnant Human Myometrium*

Myometrium	Progesterone (ng/g wet tissue) (mean±SE)[a]	Estradiol (ng/g wet tissue) (mean±SE)[b]	Progesterone: estradiol ratio[c]
Nonpregnant (n=6), group I	5.15±2.50	0.94±0.08	5.51±5.89
Pregnant, not in labor (n=6), group II	89.30±12.49	6.40±0.68	13.93±3.02
Pregnant, in labor (n=6), group III	63.50±16.59	7.07±1.10	8.52±2.74

[a] I vs. II ($P<.005$), I vs. III ($P<.01$), II vs. III ($P>.5$).
[b] I vs. II ($P<.001$), I vs. III ($P<.001$), II vs. III ($P>.5$).
[c] I vs. II ($P<.005$), I vs. III ($P<.05$), II vs. III ($P>.15$).

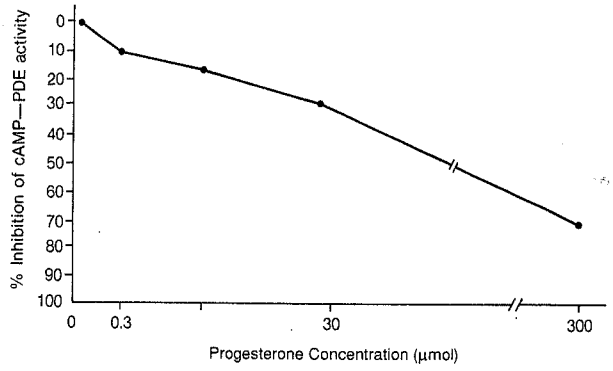

Figure 1
Representative dose-response curve of inhibition of cyclic adenosine monophosphate–phosphodiesterase (cAMP-PDE) by progesterone (myometrium from laboring women).

form of inhibition is similar to the effect of theophylline and papaverine (Figure 6).

E_2 (17β estradiol) at similar concentrations (250 μmol) had no effect on the activity of the enzyme. Similarly, the combination of P and E_2 at various ratios (2:1, 5:1 and 10:1) inhibited the enzyme activity to the degree that P alone could have achieved without evidence of synergism or antagonism.

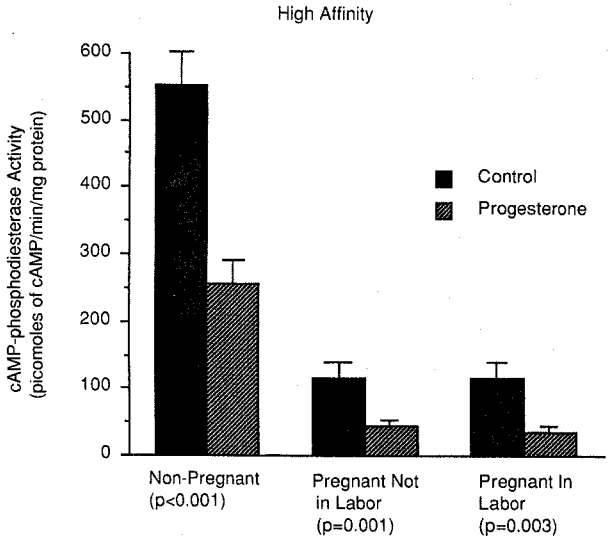

Figure 2
The effect of 300 μmol of progesterone on the activity of high-affinity cyclic adenosine monophosphate (cAMP)–phosphodiesterase.

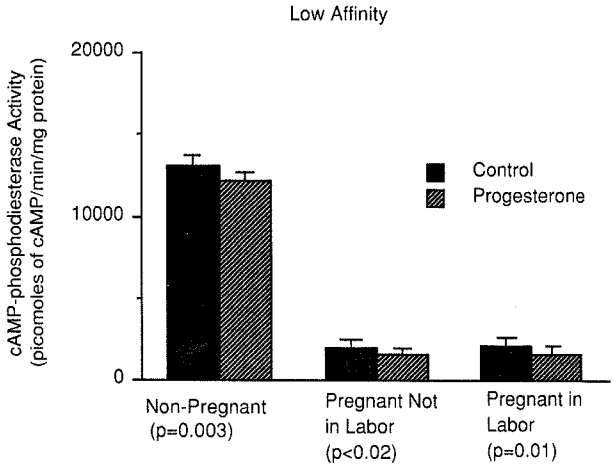

Figure 3
The effect of 300 μmol of progesterone on the activity of low-affinity cyclic adenosine monophosphate (cAMP)–phosphodiesterase.

Discussion

Previously we studied the changes in the activity of cAMP-PDE in human myometrium.[10] We found that the activity in nonpregnant myometrium is sever-

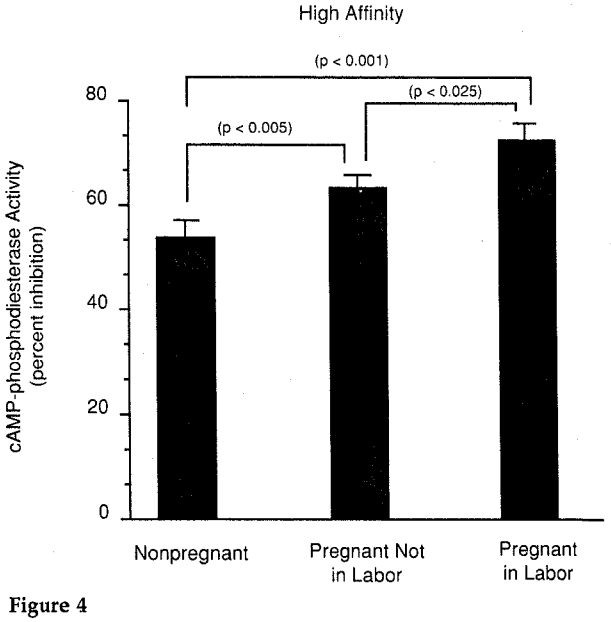

Figure 4
Percentage of inhibition of high-affinity cyclic adenosine monophosphate (cAMP)–phosphodiesterase activity by 300 μmol of progesterone. Comparisons are made between the three groups.

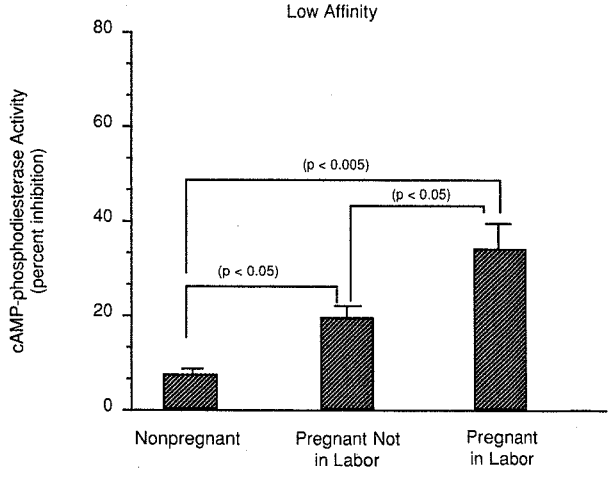

Figure 5
Percentage of inhibition of low-affinity cyclic adenosine monophosphate (cAMP)–phosphodiesterase activity by 300 μmol of progesterone. Comparisons are made between the three groups.

alfold higher than in pregnant myometrium. On the basis of our findings we speculated that P and E_2 changes during pregnancy might be responsible for this difference in the activity of the enzyme.

E_2 at concentrations ranging from 0.25 to 250 μmol did not have any effect on the enzyme; this finding is in agreement with that of Ferre et al,[15] who used 600 μmol of E_2 and found negligible or no inhibition. Beatty et al[16] reported that myometrial cAMP-PDE activity from spayed monkeys treated with E_2 was significantly higher than that of monkeys treated with E_2 plus P. Vallet-Strouve et al[17] demonstrated that cAMP-PDE activity in cultured sheep myometrial cells was inhibited similarly by both E_2 and P at a 10^{-8}- and 10^{-6}-mol concentration, respectively. These differences may be due to the fact that the three studies were performed on three different species and/or to the fact that the conditions of the experiments were different (*in vivo*, *in vitro* cultured cells and the current study).

The higher levels of P during pregnancy, along with the higher sensitivity of the enzyme, certainly are the best combination for the achievement of cAMP-PDE inhibition, which in turn may promote myometrial relaxation by its effect on the cAMP concentration in the myometrial cell. It appears that nonlaboring myometrium with high P concentrations is saturated to some extent and the additional P has less of an effect, as supported by the fact that increasing levels of P were also found by Csapo et al[2] to be associated with decreasing myometrial contractility. The lack of difference in the P concentration between the two pregnant groups is consistent with the activity of the enzyme in pregnant myometrium with and without labor.[10]

The type of cAMP-PDE inhibition that occurs with pharmacologic doses of P is competitive, while the natural inhibition during the course of pregnancy is noncompetitive. It is possible that P at physiologic concentrations *in vivo* exerts an inhibitory effect on cAMP-PDE activity by its effect on protein synthesis, influencing, by this means, the quantity of enzyme available and/or its structure. In contrast, increasing pharmacologic doses of P *in vitro* alter the properties of the existing enzyme and exert competitive inhibition similar to that exerted by theophylline and papaverine at similar concentrations.[15]

Darne et al[18] found a lower P:E_2 ratio in the saliva of women in premature labor with intact membranes. Others[2,5] measured P and E_2 in term human myometrium, and their results revealed trends similar to ours, although the absolute values differ.

This is the first report that compares pregnant and nonpregnant myometrium and pregnant myometrium in the presence and absence of labor. Our data indicate that there is a significant increase in the myometrial concentrations of both ovarian steroids during pregnancy. In addition, they indicate that the concentration of P and E_2 is not different in pregnant myometrium during labor than in the absence of labor. The P:E_2 ratio increased significantly during

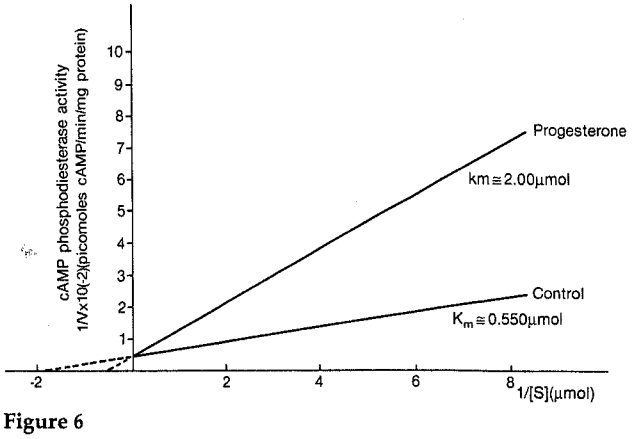

Figure 6
Competitive inhibition of high-affinity human myometrial cyclic adenosine monophosphate (cAMP)–phosphodiesterase by 300 μmol of progesterone (representative Lineweaver-Burk plot).

pregnancy, also, but did not demonstrate any significant change during labor. Our data demonstrate the absence of significant P withdrawal in human myometrium during labor. However, we have also demonstrated that cAMP-PDE is more sensitive to inhibition by P during labor. This last finding leads us to speculate that P in pharmacologic doses may indeed be a good tocolytic agent.

The use of P in an oral, micronized preparation was found to be successful in inhibiting active labor in >80% of patients.[19] This success rate is comparable to that with β agonists. The use of β agonists has been associated with severe cardiovascular complications and maternal death, while the use of P orally is free of any untoward effects. It is possible that the combination of P and lower doses of β agonists might prove to be a useful and safer tocolytic regimen. The use of progestational agents instead of P, however, will not be appropriate because those agents bind very poorly to the cytosolic receptors, and their efficacy is only a fraction of P's.[20]

Conclusion

In this study, the concentration of P and E_2 in human myometrium increased dramatically during pregnancy. The P:E_2 ratio also increased about threefold during the same period. During labor, however, there was no change in the concentration of P and E_2. As one would expect, the P:E_2 ratio followed the same pattern. P at pharmacologic doses inhibits cAMP-PDE activity competitively, while E_2 has no effect. Our study, however, cannot exclude a possible effect on protein synthesis that E_2 may exert *in vivo* on the quality and quantity of the enzyme by its interaction with P.

Acknowledgments

Joseph Thompson and Samuel Strada provided guidance with the laboratory and theoretical aspects of cAMP-PDE activity and enzyme kinetic analysis.

References

1. Takahashi K, Burd L: Initiation of labor. *In* Gynecology and Obstetrics. Third volume. Edited by JJ Sciarra. New York, Harper & Row, 1981, pp 1–22
2. Csapo AI, Pulkkinen M: Regulatory and functional asymmetry of the pregnant human uterus. Perinatol Neonatol 1:12, 1977
3. Turnbull AC, Anderson BM: Endocrine control of human parturition. Acta Obstet Gynaecol 34:1094, 1982
4. Williams LT, Lefkowitz RJ: Regulation of rabbit myometrial alpha adrenergic receptors by estrogen and progesterone. J Clin Invest 60:815, 1977
5. Khan-Dawood FS, Dawood MY: Estrogen and progesterone receptor and hormone levels in human myometrium and placenta in term pregnancy. Am J Obstet Gynecol 150:501, 1984
6. Csapo A: Brook Lodge Symposium on Progesterone. Augusta, MI, Brook Lodge Press, 1961, pp 7–23
7. Fuchs A-R, Periyasamy S, Alexandrova M, et al: Correlation between oxytocin receptor concentration and responsiveness to oxytocin in pregnant rat myometrium: Effects of ovarian steroids. Endocrinology 113:742, 1983
8. Lye SJ, Porter DG: Demonstration that progesterone 'blocks' uterine activity in the ewe in vivo by a direct action on the myometrium. J Reprod Fertil 52:87, 1978
9. Garfield RE, Puri CP, Csapo AI: Endocrine, structural, and functional changes in the uterus during premature labor. Am J Obstet Gynecol 142:21, 1982
10. Kofinas AD, Rose JC, Meis PJ: Changes in cyclic adenosine monophosphate-phosphodiesterase activity in nonpregnant and pregnant human myometrium. Am J Obstet Gynecol 157:733, 1987
11. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248, 1976
12. Thompson WJ, Terasaki WL, Epstein PM, et al: Assay of cyclic nucleotide phosphodiesterase and resolution of multiple molecular forms of the enzyme. *In* Advances in Cyclic Nucleotide Research. Tenth volume. Edited by G Brooker, P Greengard, GA Robinson. New York, Raven Press, 1979, pp 69–92
13. Thau RB, Lanman JT, Brinson AO: Metabolic clearance rates, production rates and concentrations of progesterone in pregnant Rhesus monkeys. Biol Reprod 16:678, 1977
14. Goodman AL, Descalzi CD, Johnson DK, et al: Composite pattern of circulating LH, FSH, estradiol and progesterone during the menstrual cycle of cynomolgus monkeys. Proc Soc Exp Biol Med 155:479, 1977
15. Ferre F, De Pariente D, Breuille M, et al: Inhibition of human myometrial cyclic AMP phosphodiesterase by uterine relaxant drugs. Biochem Pharmacol 22:1292, 1978
16. Beatty CH, Bocek RM, Herrington PT: Regulation of cyclic nucleotide phosphodiesterase activity in myometrium from pregnant and spayed rhesus monkeys. J Reprod Fertil 55:391, 1979
17. Vallet-Strouve C, Ferre F, Breuiller M: Evolution of cAMP phosphodiesterase activity in cultured myometrial cells: Effects of steroids and of successive subcultures. J Cell Physiol 120:391, 1984
18. Darne J, McGarrigle HHG, Lachelin GCL: Increased saliva oestriol to progesterone ratio before idiopathic preterm delivery: A possible predictor for preterm labour? Br Med J 294:270, 1987
19. Erny R, Pigne A, Prouvost C, et al: The effects of oral administration of progesterone for premature labor. Am J Obstet Gynecol 154:525, 1986
20. Haukkamaa M: High affinity progesterone binding sites of human uterine microsomal membranes. J Steroid Biochem 20:569, 1984