Estrogen and progesterone receptor and hormone levels in human myometrium and placenta in term pregnancy

Firyal S. Khan-Dawood, Ph.D., and M. Yusoff Dawood, M.D.
Chicago, Illinois

Estradiol and progesterone receptors in the myometrium, decidua, placenta, chorion, and amnion of eight women who underwent elective cesarean section at term were determined by means of an exchange assay. The hormone levels in the peripheral plasma and cytosol of these tissues were measured by radioimmunoassays. Maternal plasma and the placenta had high concentrations of estradiol and progesterone, with the placenta having 12 times more progesterone than in maternal plasma but only half the concentrations of estradiol in maternal plasma. The decidua and placenta had detectable levels of cytosol and nuclear estradiol receptors, but the myometrium had no measurable cytosol estradiol receptors, whereas the chorion and amnion had neither cytosol nor nuclear estradiol receptors. However, the chorion and amnion had significantly higher concentrations of estradiol in the cytosol than those in the decidua and myometrium. Only the decidua and myometrium had cytosol and nuclear progesterone receptors, but the placenta, amnion, and chorion had neither cytosol nor nuclear progesterone receptors. In contrast, progesterone hormone levels were significantly higher in the placenta, amnion, and chorion than in the decidua and myometrium. The findings indicate that, in the term pregnant uterus, (1) the placenta, amnion, and chorion are rich in progesterone, estradiol, and nuclear estradiol receptors but have no progesterone receptors, (2) the decidua and myometrium have nuclear estradiol and progesterone receptors, and (3) the myometrium has a higher progesterone/estradiol ratio than that of the peripheral plasma, thus suggesting a highly progesterone-dominated uterus. (AM J OBSTET GYNECOL 1984;150:501-5.)

Considerable attention has been focused on the endocrine mechanism of uterine activation, but much of the attention has been directed to the endogenous uterotonic substances, such as prostaglandins and oxytocin. The human uterus becomes increasingly sensitive to oxytocin as pregnancy advances.[1] In the rabbit, estrogens increase uterine oxytocin receptors, whereas progesterone decreases them.[2] Therefore, the increasing sensitivity of the myometrium to oxytocin with advancing gestation may be related to the increase in estrogens or a combination of increasing levels of estrogen and declining levels of progesterone that result in an increase in uterine oxytocin receptors. In humans, a recent study suggested that oxytocin receptor binding activity is increased in the myometrium and decidua from the lower uterine segment in women after the onset of labor, compared to before the onset of labor.[3] However, Goren et al.[4] found that there was little or no correlation between oxytocin receptors and uterine activity.

From the Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Illinois College of Medicine, Chicago.
Presented in part at the Twenty-eighth Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 18-21, 1981.
Reprint requests: Firyal S. Khan-Dawood, Ph.D., Department of Obstetrics and Gynecology, University of Illinois College of Medicine, 840 South Wood St., Chicago, IL 60612.

Measurements of peripheral steroid and peptide hormones have not provided a satisfactory reflection of local hormonal changes which may be occurring in the uterus. Estrogens can induce both estrogen and progesterone receptors, whereas progesterone inhibits the formation of estrogen receptors.[5-7] Therefore, changes in estrogen and progesterone hormones and their receptor levels in the cells of uterine and placental tissue may mediate the sensitivity of the uterus as parturition is approached. As part of our studies on the endocrine mechanisms that regulate the onset of uterine activity and parturition, we examined the maternal plasma estradiol and progesterone levels in comparison with estradiol and progesterone concentration and cytosol and nuclear estradiol and progesterone receptor binding activity in the decidua, myometrium, chorion, amnion, and placental cotyledon. This article reports such a study carried out in normal term pregnancy prior to the onset of labor.

Material and methods

Patients and tissues studied. Eight women with uncomplicated pregnancies who underwent repeat elective cesarean sections at 38 to 42 weeks of pregnancy were studied. All patients signed an informed consent for the study, which was approved by the Institutional Review Board at our institution. At the time of cesarean section, which was performed with the patient

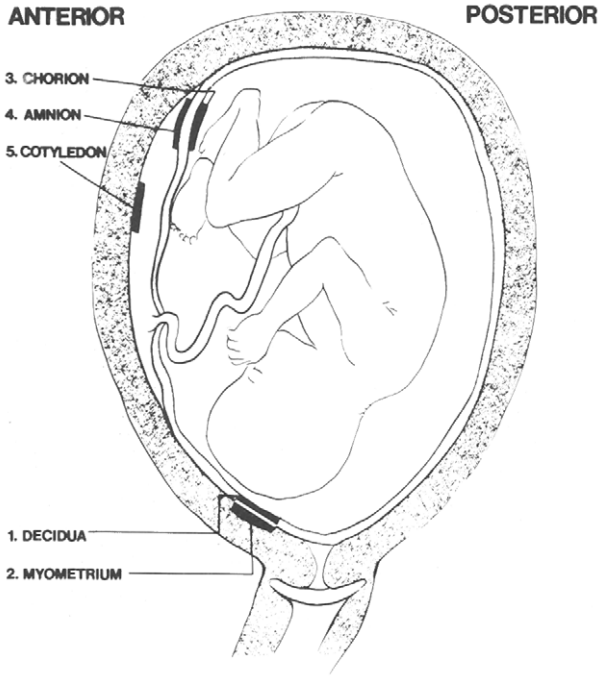

Fig. 1. Schematic representation of uterine and decidual tissues, chorion, amnion, and placental cotyledon removed at elective cesarean section for determination of progesterone and estradiol receptor and intracellular progesterone and estradiol hormone concentration.

under general anesthesia, a strip of full-thickness uterine muscle, including the decidua (about 1 gm), was removed along the edge of the uterine incision after the baby had been delivered. The placenta and the strip of uterine muscle were immediately placed in crushed ice and transported to the laboratory. The tissues were microdissected and divided into decidua, uterine muscle, chorion, amnion, and cotyledon (Fig. 1). They were weighed, washed in ice-cold physiologic saline solution, and frozen in liquid nitrogen immediately. The minimum amount of each tissue required for each receptor determination was 200 mg.

Preparation of cytosol and crude nuclei. The frozen tissue was pulverized and homogenized at 4° C in 5 volumes of Tris-glycerol buffer (10 mmol of Tris–hydrochloric acid, 1.5 mmol of ethylenediaminetetraacetic acid (EDTA), 1 mmol of dithiothreitol, and 10% v/v of glycerol, with a pH of 7.6) with a Polytron PT10 homogenizer at a setting of 6. Three to five 10-second bursts with 30-second cooling intervals were used for homogenization. The homogenates were filtered through three layers of cheesecloth, and aliquots were taken for determinations of protein[8] and DNA[9]. The remainder was centrifuged at 800 × g for 10 minutes at 4° C. The supernatant, after centrifugation at 100,000 × g for 60 minutes and treatment with dextran-coated charcoal pellet (12.5 mg of charcoal Norit A and 1.25 mg of dextran T70 per milliliter of cytosol) for 30 minutes at 4° C to remove endogenous unbound steroids, was used for the determination of cytosol receptors. The efficiency of the method to remove endogenous steroids was assessed by radioimmunoassay. This treatment removed 95% of the endogenous steroids. The 800 × g pellet was washed three times with 20 volumes of Tris-glycerol buffer, and the final pellet suspended in the same buffer was used for measurement of nuclear receptors. The cytosol protein concentration was determined by the method of Lowry et al.[8] and adjusted to a concentration of 1 mg/ml. The nuclear DNA concentration was determined by the method of Burton[9] and adjusted to a concentration of 150 to 200 μg of DNA per milliliter. For the cytosol and nuclear receptor assays, 100 μl of cytosol preparation and 500 μl of nuclear preparation, respectively, were used.

Cytosol receptor assay for progesterone. Duplicate 100 μl aliquots of charcoal-treated cytosol were incubated for 16 hours at 4° C with increasing concentrations (0.1 to 20 nM) of [^{3}H]R5020 in the presence or absence of 2,500 nM of unlabeled progesterone and cortisol. Hormones were added in Tris-glycerol buffer. The final volume of the assay was 250 μl. After incubation, an equal volume of dextran-coated charcoal suspension (1.25% charcoal Norit A and 0.125% dextran T70 in Tris-glycerol buffer) was added and incubated for 10 minutes at 4° C. After centrifugation at 1600 × g for 10 minutes, the supernatant was processed for liquid scintillation counting. Specifically bound [^{3}H]R5020 was determined by Scatchard analysis with the use of a five-point assay.[10]

Cytosol receptor assay for estradiol. Duplicate aliquots (100 μl) of the charcoal-treated cytosol were incubated for 16 hours at 4° C with increasing concentrations of [^{3}H]estradiol (0.1 to 20 nmol in the presence or absence of a 100-fold excess of estradiol and androstanolone. After incubation, the cytosol was processed as described for the progesterone receptor, and specific binding was determined by Scatchard analysis with use of a five-point assay.[10]

Measurement of nuclear receptors. Concentrations of nuclear progesterone and estradiol receptors were determined by [^{3}H]R5020 and [^{3}H]estradiol exchange assays, respectively.[11] Triplicate aliquots (500 μl) of the nuclear suspension in Tris-glycerol buffer were incubated for 3 hours at 0° C to measure unoccupied receptors with either 10 nmol of [^{3}H]R5020 or 10 nmol of [^{3}H]estradiol. A 100-fold excess of nontritiated progesterone or estradiol was used to measure nonspecific binding. The final volume of assay was 700 μl. After incubation, the mixture was diluted with 1 ml of Tris-glycerol buffer that contained 10 mg of bovine serum albumin per milliliter (TGA buffer), and was centrifuged at 1600 × g for 10 minutes at 4° C. The pellet

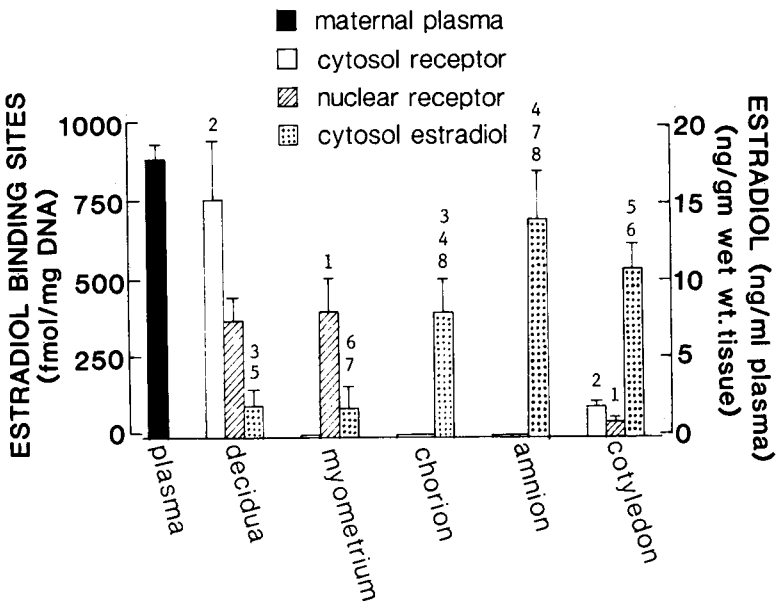

Fig. 2. Mean ± SEM maternal peripheral plasma estradiol level, and cytosol and nuclear estradiol receptor and cytosol estradiol concentrations in the decidual tissue, myometrium, chorion, amnion, and placental cotyledon removed at elective cesarean section in term pregnancies. The myometrium had no detectable cytosol estradiol receptor binding activity, whereas the chorion and amnion had no detectable cytosol or nuclear estradiol receptor binding activity. The numbers above the histogram indicate significant differences between the two levels having the same number. *1* = $p < 0.01$; *2* = $p < 0.001$; *3* = $p < 0.025$; *4* = $p < 0.05$; *5* = $p < 0.025$, *6* = $p < 0.005$; *7* = $p < 0.005$; *8* = $p < 0.05$ (with use of Student's *t* test).

Table I. Progesterone and estradiol levels in maternal plasma and cytosol of myometrium and placenta from eight pregnant women at term who underwent repeat cesarean section (mean ± SEM)

Tissue studied	*Hormone level (ng/ml of plasma or ng/gm wet weight of tissue)*		*Progesterone/ estradiol ratio*
	Progesterone	*Estradiol*	
Maternal plasma	170.1 ± 10.3	18.1 ± 0.9	16.5
Myometrium	82.2 ± 6.8	1.8 ± 0.2	45.7
Placenta	2100.1 ± 201.2	10.2 ± 1.5	205.9

was washed twice with 2 ml of TGA buffer and 2 ml of Tris-glycerol buffer. The washed pellet was suspended in Tris-glycerol buffer and transferred to a clean tube, centrifuged, and the pellet extracted with ethanol (2 ml). After centrifugation, the ethanol supernatant was counted.

Radioimmunoassay of estradiol and progesterone. Estradiol and progesterone hormone levels in the cytosol fraction were determined by specific radioimmunoassays as previously described,[12, 13] with the use of specific antibodies raised against estradiol-6-oxime bovine serum albumin conjugate and against progesterone-11-oxime bovine serum albumin conjugate for estradiol and progesterone, respectively.

Statistical analysis. Data were expressed as mean ± SEM (standard error of the mean). Means were compared by the nonpaired Student's *t* test with the use of a two-tailed p value; p values >0.05 were considered not significant.

Results

Table I summarizes the levels of estradiol and progesterone in the maternal plasma and in the cytosol of myometrium and placenta from eight pregnant women at term who underwent repeat elective cesarean section because of a previous cesarean section. Maternal plasma and placenta had high concentrations of both progesterone and estradiol, with the placenta having about 12 times more progesterone than that in maternal plasma, but only half the concentration of estradiol compared to that in maternal plasma. The mean ± SEM of concentrations of progesterone and estradiol in myometrial cytosol were 82.2 ± 6.8 and 1.8 ± 0.2 ng/gm wet weight of tissue, respectively. The myome-

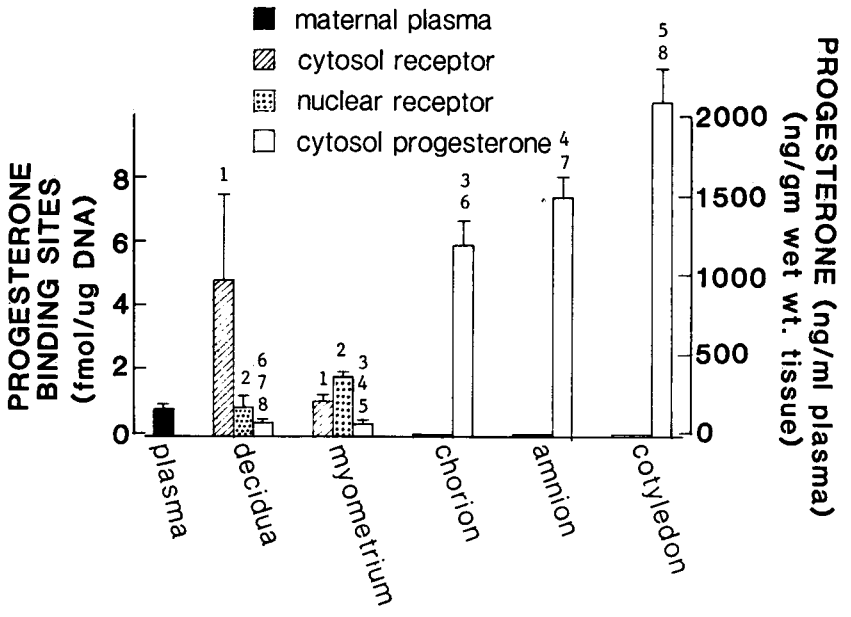

Fig. 3. Mean ± SEM maternal peripheral plasma progesterone level, cytosol, and nuclear progesterone receptor and cytosol progesterone concentrations in the decidual tissue, myometrium, chorion, amnion, and placental cotyledon removed at elective cesarean section in term pregnancies. The chorion, amnion, and cotyledon had no detectable cytosol or nuclear progesterone receptor binding activity. The numbers shown above the histogram indicate significant differences between the two levels having the same number. *1* = $p < 0.05$; *2* = $p < 0.005$; *3* = $p < 0.001$; *4* = $p < 0.001$; *5* = $p < 0.001$; *6* = $p < 0.001$; *7* = $p < 0.001$; *8* = $p < 0.001$ (with use of Student's *t* test).

trium had an almost three times higher ratio of progesterone to estradiol than that of the maternal peripheral plasma (45.7 versus 16.5).

The mean ± SEM of cytosol and nuclear estrogen receptors, together with the cytosol estradiol concentrations of myometrium, decidua, chorion, amnion, and placental cotyledon, are also shown in Fig. 2. The decidua and placental cotyledon had detectable levels of both cytosol and nuclear estradiol receptors or binding sites, whereas the myometrium had no detectable cytosol estradiol receptors. In contrast, the chorion and amnion had neither cytosol nor nuclear estradiol binding sites. However, both chorion and amnion had significantly higher concentrations of estradiol in the cytosol than those in the decidua and the myometrium ($p < 0.025$).

Fig. 3 shows the mean ± SEM of progesterone cytosol and nuclear receptor or binding sites, together with the cytosol progesterone concentrations of myometrium, decidua, chorion, amnion, and placental cotyledon. Both the myometrium and the decidua gave detectable levels of cytosol and nuclear progesterone receptor or binding sites. In contrast, the placental cotyledon, amnion, and chorion did not have any progesterone binding in either their cytosol or nuclear fractions. However, cytosol progesterone concentrations were significantly higher in the placenta, amnion, and chorion than in the myometrium and decidua ($p < 0.001$).

Comment

In a previous study,[11] progesterone receptor levels were found to be markedly lower in the uterus of term pregnancy than during the menstrual cycle. In the present study, cytosol and nuclear receptors of both estradiol and progesterone, as well as the cytosol concentrations of both hormones, were measured in the same samples of myometrial and decidual tissue. The myometrial cytosol and nuclear progesterone receptor levels obtained by us were similar to those reported by Giannopoulos and Tulchinsky.[11] In the myometrium, the nucleus had higher levels of progesterone receptors than those in the cytosol. In contrast, the decidua had more progesterone receptors in the cytosol than in the nucleus, with the cytosol progesterone receptor levels being significantly higher and the nuclear progesterone receptor levels being significantly lower than those in the myometrium.

Since nuclear receptors are obligatory intermediates in steroid hormone action, the higher proportion of myometrial progesterone receptors in the nucleus than in the cytosol may account for the relative quiescence of the myometrium at term when the woman is not in labor. In the term pregnant uterus, the high concentrations of maternal circulating progesterone will mediate a higher proportion of nuclear receptors in the total cellular receptor content. In addition, the threefold higher progesterone-to-estradiol ratio in myometrial tissues than in the peripheral plasma should

provide a stronger modulation for a higher proportion of nuclear progesterone receptors, as well as a highly progesterone-dominated local uterine environment. However, whether myometrial progesterone receptors undergo significant changes just prior to or with the onset of spontaneous labor is not known. With the decidua, the significantly higher level of cytosol receptors appears to be due to the fact that the high concentrations of estradiol and progesterone in the fetal membranes (amnion and chorion) and maternal plasma can induce progesterone receptors, which are under estrogen and progesterone control.[5-7]

Nuclear estradiol receptors are present in both the decidua and myometrium, but cytosol estradiol receptors are present in the former and not detectable in the latter. The concentrations of estradiol receptors in the myometrium and decidua are much lower than those of progesterone receptors, thus reflecting the progesterone-dominated uterus at term.

Neither cytosol nor nuclear progesterone receptors were measurable in the placenta and its membranes in term pregnancy. Similarly, cytosol and nuclear estradiol receptors were not detectable in the chorion and amnion. Although the placental cotyledon had detectable concentrations of both cytosol and nuclear estradiol receptors, these were significantly lower than the corresponding concentrations in the myometrium and decidua. Our finding of an absence of progesterone receptors in the placental tissue is similar to the observation in a recent report.[14] The striking absence of detectable progesterone receptors in the placenta and its membranes in the quiescent term uterus may be due to the hundred-fold higher placental and membrane concentrations of progesterone than of estradiol. Such a highly progesterone-dominated local environment would tend to inhibit the induction of both estrogen and progesterone receptors. In the absence of readily available binding sites in the placenta and its membranes, placental estrogen and progesterone are more readily available to reach the myometrium and bind with their receptors to produce the necessary biologic effect.

Tissue concentrations of both estradiol and progesterone were significantly higher in the placenta and its membranes than in the myometrium and decidua, thus reflecting the placental production of these hormones. Although progesterone concentration was highest in the placenta, estradiol concentration was highest in the amnion, which had a significantly higher estradiol concentration than that of the chorion. Since the radioimmunoassay procedure used detects only unconjugated estradiol, and the placental membranes readily hydrolyze estrogen sulfate to unconjugated estrogen,[15] the amnion can be expected, therefore, to have higher concentrations of unconjugated estradiol.

The present study has established estrogen and progesterone receptor and hormone levels in the uterine, placental, and fetal membrane tissues at term before the onset of labor. Further studies will be necessary to determine whether changes in these endocrine parameters occur in the uterine, placental, and fetal membrane tissues with the onset of labor, which may account for a more sensitive uterus readily activated when the necessary cascade of events leads to the onset of labor.

REFERENCES

1. Caldeyro-Barcia R, Sereno JA. The response of the human uterus to oxytocin throughout pregnancy. In: Caldeyro-Barcia R, Heller H, eds. Oxytocin. Elmsford, NY: Pergamon Press, 1961:177.
2. Nissenson R, Flouret G, Hechter O. Opposing effects of estradiol and progesterone on oxytocin receptors in rabbit uterus. Proc Natl Acad Sci USA 1978;75:2044.
3. Fuchs A-R, Fuchs F, Husslein P, Soloff MS, Fernstrom MJ. Oxytocin receptors and human parturition: a dual role for oxytocin in the initiation of labor. Science 1982;215:1396.
4. Goren HJ, Geonzon RM, Hollenberg MD, Lederis K, Morgan DO. Oxytocin action: lack of correlation between receptor number and tissue responsiveness. J Supramol Struct 1980;14:129.
5. Illingworth DV, Wood GP, Flickinger GL, Mikhail G. Progesterone receptor of the human myometrium. J Clin Endocrinol Metab 1975;40:1001.
6. Bayard FS, Damilano S, Robel P, Baulieu EF. Cytoplasmic and nuclear estradiol and progesterone receptors in human endometrium. J Clin Endocrinol Metab 1978;46:635.
7. Milgrom E, Luu Thi M, Atger M, Baulieu EE. Mechanisms regulating the concentration and the conformation of progesterone receptor(s) in the uterus. J Biol Chem 1973;248:6366.
8. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265.
9. Burton KH: A study of the conditions and mechanism of diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 1956;62:315.
10. Scatchard G. The attraction of proteins for small molecules and ions. Ann NY Acad Sci 1949;51:660.
11. Giannopoulos G, Tulchinsky D. Cytoplasmic and nuclear progestin receptors in human myometrium during the menstrual cycle and in pregnancy. J Clin Endocrinol Metab 1976;49:100.
12. Mikhail G, Wu CH, Ferin M, Vande Wiele RR. Radioimmunoassay of plasma estrone and estradiol. Steroids 1970;15:333.
13. Abraham GW, Swerdloff R, Tulchinsky D, Odell WD. Radioimmunoassay of plasma progesterone. J Clin Endocrinol Metab 1971;32:619.
14. McCormick PD, Razel AJ, Spelsberg TC, Coulam CB. Absence of high-affinity binding of progesterone (R5020) in human placenta and fetal membranes. Placenta 1981;3 (suppl):123.
15. Goebelsmann U, Wiqvist N, Diczfalusy E, Levitz M, Condon GP, Dancis J. Fate of intra-amniotically administered oestriol-16-^{3}H-3-sulphate and oestriol-16-^{14}C-16-glucosiduronate in pregnant women at midterm. Acta Endocrinol (Copenh) 1966;52:550.