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Progesterone receptor isoform (A/B) ratio of human fetal membranes increases during term parturition

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Objective: The role of progesterone in the control of human parturition remains unsettled. Because there is no systemic progesterone withdrawal before the onset of labor, a 'functional progesterone withdrawal' has been proposed to be operative before human parturition. This may be accomplished by a change in the density of the progesterone receptor (PR) isoforms in myometrium and fetal membranes. The purpose of our study was to determine if spontaneous term labor is associated with changes of PR isoforms (PR-A and PR-B) in the fetal membranes.
Study design: Fetal membranes were obtained from women undergoing elective cesarean delivery at term (not in labor group), and from women with a vaginal delivery (labor group). The expression of PR isoforms was assessed by Western blot analysis of amnion and chorio-decidua. Densitometric analysis of PR-A/PR-B ratio was performed. Immunohistochemistry with specific antibodies to PR-A and PR-B was done. Nonparametric statistics were used for analysis.
Results: 1) The predominant isoform of PR in women not in labor was PR-B, and PR-A in patients in labor. The ratio of PR-A/PR-B in fetal membranes was significantly higher in women in labor than in those not in labor (for amnion, median 4.3, range [0.9-8.4] vs median 0.4, range [0.3-2.6], $P < .001$; for chorio-decidua, median 2.0, range [1.1-19.2] vs median 1.2, range [0.1-2.0], $P < .05$). 2) Fetal membranes expressed both types of PR. 3) Immunohistochemistry showed the presence of PR-A and PR-B in the cytoplasm of amnion epithelial cells, chorion trophoblast, and decidual cells.
Conclusion: Human parturition at term is associated with changes in PR isoforms in the fetal membranes and, thus, a local 'functional progesterone withdrawal' may operate in human parturition through this mechanism.

Progesterone is an essential hormone for the maintenance of uterine quiescence. In most mammals, maternal plasma progesterone decreases before the onset of labor. This is coupled with increased production of estrogens. This combination is responsible for the increased oxytocin receptor expression, gap junction formation, and

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production of prostaglandins.[1] In primates, including humans, there is no detectable decrease in maternal plasma progesterone concentration before the onset of labor. However, progesterone withdrawal is considered an important mechanism of human parturition because synthetic progesterone antagonists (RU486) or inhibitors of progesterone synthesis (Epostane) can induce labor and delivery.[2,3] Therefore, it has been proposed that a 'functional progesterone withdrawal' is operative in human parturition. Yet, the precise mechanism responsible for the suspension of progesterone action is unknown.

Progesterone receptors (PRs) exist as 2 isoforms, PR-A and PR-B. Structurally, PR-A is the truncated form of PR-B, lacking the 164 N-terminal residues. This region contains a unique activation domain (AF3) that is specific to the PR-B, but not the PR-A. There is evidence that PR-A acts as a potent repressor of PR-B mediated gene transcription in many tissues.[4] Recently, it has been proposed that a change in PR isoforms is one of the mechanisms responsible for a 'functional progesterone withdrawal' in human parturition. Most research has focused on the study of tissues in maternal compartments, such as myometrium.[5]

Whereas the myometrium is a crucial maternal tissue for labor, fetal membranes also play an important role. Evidence supports a role for the fetus in the timing of parturition in most species.[6] It is hypothesized that fetal membranes are central to the organ communication system that transfers information from the fetus to the mother.[7] However, little is known about the PR isoform changes in the fetal membranes during human parturition. The purpose of our study was to determine if spontaneous human term labor is associated with changes in PR isoforms (PR-A and PR-B) in fetal membranes.

Material and methods

Collection of fetal membranes

Fetal membranes were collected from women undergoing elective cesarean section at term (not in labor group, n = 14) or after uncomplicated vaginal delivery (labor group, n = 14). We also studied membranes from women in early labor with cervical dilatation ≤1 cm (early labor group, n = 8) obtained at the time of emergency cesarean section. The amnion layer was separated from underlying chorio-decidua and washed 3 times in phosphate-buffered normal saline. The indications for cesarean section in this early labor group were: previous cesarean section with labor (n = 4), previous cesarean section with uterine contractions (n = 2), failed induction (n = 1), and previous cesarean section with breech presentation and rupture of membranes (n = 1). All tissues were snap frozen with liquid nitrogen and stored at −70°C until studied. All specimens were collected after obtaining patient consent, and the collection of tissues was approved by the Institutional Review Board of the Seoul National University Hospital.

Table I Primary antibodies employed in Western blot and immunohistochemistry

Antibody	Source	Optimal dilution and condition
PR-AB52 (monoclonal)	Santa Cruz	1:500, 4°C, overnight
PR-A:hPRa7 (monoclonal)	Neomarkers (Calif)	1:100, 4°C, overnight
PR-B:hPRa6 (monoclonal)	Neomarkers (Calif)	1:20, 4°C, overnight

Western blot analysis

Western blot analysis for PR-A and PR-B was performed using amnion and chorio-decidual tissue. Protein derived from the T47D cell line (derived from a human breast carcinoma), which is known to express large amounts of human PR, was used as a positive control. After protein extraction and quantitation (Bradford assay), tissue extracts were diluted with sodium dodecyl sulfate (SDS)-loading buffer and boiled for 10 minutes at 100°C (60 μg-80 μg of protein were loaded). Samples were loaded on an 8% acrylamide gel and run at 100 V for 2 hours. After running, proteins were transferred by electrophoresis to nitrous cellulose membrane (Hybond ECL, Amersham Life Sciences, Sydney) at 150 mA for 2 hours. Membranes were blocked in a 5% milk protein solution at room temperature for 1 hour. After washing, the membranes were incubated with mouse antihuman PR monoclonal antibody (AB-52, Santa Cruz, CA) at 4°C overnight. Membranes were washed and incubated with a secondary antibody, goat antimouse alkaline phosphatase-labeled polyclonal antibody (Zymed, San Francisco, Calif) at room temperature for 1 hour. A BCIP/NBT tablet (Sigma, St Louis, Mo) diluted in distilled water was used for more sensitive development. All experiments were performed 3 times under the same conditions. Expressions of PR isoforms were assessed semiquantitatively by densitometric analysis and the mean ratio of PR-A to PR-B was calculated.

Immunohistochemistry

Paraffin blocks of fetal membranes from women not in labor and those in labor were sectioned (at 4 μm), and standard immunohistochemistry was performed using avidin-biotin procedure (LSAB2 system, DAKO, Glostrup, Denmark). In brief, the tissue sections were dewaxed and hydrated. Antigen retrieval was performed using heating in a microwave oven in 0.01 mol/L sodium citrate buffer (pH 6.0). After nonspecific endogenous peroxidase activity was blocked by incubation with 3%