Temporal expression pattern of progesterone receptor in the uterine luminal epithelium suggests its requirement during early events of implantation

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Objective: To determine the precise timing of progesterone receptor (PR) disappearing from the uterine luminal epithelium (LE) to help understand the significance of the dynamic PR expression in the LE during embryo implantation.
Design: Experimental rodent models.
Setting: University research laboratories.
Animal(s): Mice and hamsters.
Intervention(s): Pseudopregnancy and artificial decidualization.
Main Outcome Measure(s): Blue dye injection for detecting embryo attachment; immunohistochemistry, immunofluorescence, and in situ hybridization for detecting gene expression.
Result(s): Progesterone receptor remained expressed in the LE up to 6 hours after the initial detection of blue dye reaction in mice (day 3, 22:00 hours), but disappeared first from LE cells at the implantation site and subsequently from the entire LE layer by day 4, 06:00 hours, when uterine stromal decidualization had become obvious. Progesterone receptor remained highly expressed in the LE of day 4 at 11:00 hours in pseudopregnant mice, but it disappeared from the entire LE layer by day 4 at 06:00 hours in artificially decidualized pseudopregnant mice.
Conclusion(s): Progesterone receptor disappears from the LE after implantation has initiated and before the histologic decidualization manifests, suggesting an active role of continued PR expression in the LE for the initial implantation process. The disappearance of PR expression in the LE is regulated by uterine factor(s) produced upon embryo attachment. (Fertil Steril® 2011;95:2087–93. ©2011 by American Society for Reproductive Medicine.)

Key Words: Embryo attachment, embryo implantation, progesterone receptor, uterine luminal epithelium

Progesterone receptor (PR) has unique uterine expression patterns during early pregnancy in mice, especially during the periimplantation period: it increases in the uterine luminal epithelium (LE) from gestation day 0.5 to day 1.5 (mating night as day 0); it is up-regulated in both LE and stroma during preimplantation day 2.5 and day 3.5; and it disappears from the LE but is strongly expressed in the primary decidual zone in the postimplantation day 4.5 uterus (1, 2). Progesterone signaling mediated by PR is indispensable for embryo implantation in all mammals that have been studied (3–5). There are two main PR isoforms, PR-A and PR-B. The ratio of PR-A to PR-B is 3:1 in the mouse uterus (6). Studies from PR knockout mice have demonstrated that PR-A, but not PR-B, is critical for uterine function, including embryo implantation and decidualization (7–9).

Embryo implantation, which takes place between gestation days 3.5 and 4.5 in mice, is a multistep process that includes embryo apposition, attachment, and invasion (4, 10). Luminal epithelium is the first layer of cells that an embryo communicates with for implantation. Progesterone receptor disappears from the LE between gestation days 3.5 and day 4.5 in mice (1, 2). Multiple events happen during the hours between these two time points. When exactly does PR disappear from the LE? It is our objective to define the time of PR disappearance from the uterine LE during implantation. This will help us understand any potential role that PR plays in the LE during the implantation process and thus provide more insight into the molecular mechanism of embryo implantation.

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MATERIALS AND METHODS

Animals

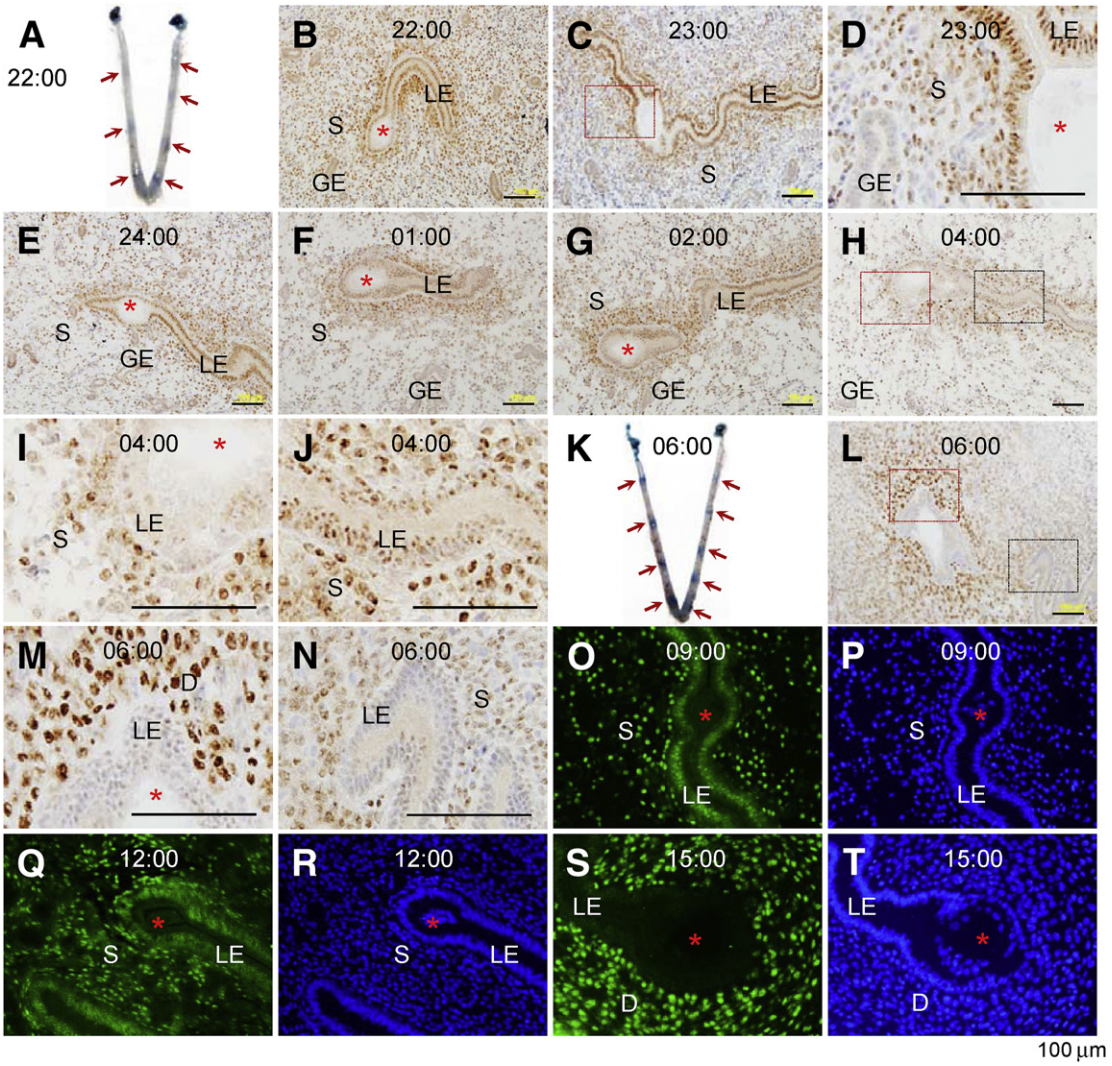
Young virgin mice (C57BL6) and hamsters (golden) were purchased from the Jackson and Charles River Laboratories (Bar Harbor, ME, and Wilmington, MA), respectively. They were housed in polypropylene cages with free access to regular food and water. The animal rooms were maintained on a 12-hour light-dark cycle (6:00 AM to 6:00 PM) at the University of Georgia and Vanderbilt University Medical Center. All methods used in this study were approved by the University of Georgia and Vanderbilt University Committees of Use and Care of animals and conform to National Institutes of Health guidelines and public law.

Treatments

In natural pregnancy, the females were mated with fertile males and checked for a vaginal plug in mice or the presence of sperm in a vaginal smear in

FIGURE 1

Time-course expression of progesterone receptor (PR) during the early hours of embryo implantation in the mouse uterus A–N as detected by immunohistochemical (IHC) analysis and the hamster uterus O–T as detected by immunofluorescence (IF). Frozen sections (10 μm) through an embryo were used. (**A**) Representative uterine image on day 3 at 22:00 hours. (**B**) PR IHC of an implantation site on A. (**C**) PR IHC, day 3 at 23:00 hours. (**D**) Enlarged view of the red boxed area in C. (**E**) PR IHC, day 3 at 24:00 hours. (**F**) PR IHC, day 4 at 01:00 hour. (**G**) PR IHC, day 4 at 02:00 hours. (**H**) PR IHC, day 4 at 04:00 hours. (**I**) Enlarged view of red boxed area in H. (**J**) An enlarged view of black boxed area in H. (**K**) Representative uterine image on day 4 at 06:00 hours. (**L**) PR IHC of an implantation site on K. (**M**) Enlarged view of red boxed area in L. (**N**) Enlarged view of black boxed area in L. (**O**) PR IF, day 3 at 09:00 hours. (**P**) DAPI stain of the section on O. (**Q**) PR IF, day 3 at 12:00 hours. (**R**) DAPI stain of the section on Q. (**S**) PR IF, day 3 at 15:00 hours. (**T**) DAPI stain of the section on S. Red arrows indicate implantation sites detected by blue dye reaction (**A, K**). LE = luminal epithelium; S = stroma; GE = glandular epithelium; D = decidual zone; red asterisk: embryo. Scale bar: 100 μm. N = 3–4. No specific staining was detected in the negative control (data not shown).



Diao. PR in LE around implantation initiation. Fertil Steril 2011.

hamsters the next morning (midnight of mating night as day 0, 00:00). Implantation sites were detected by intravenous injection of blue dye, as previously described elsewhere (11). Mouse uterine tissues were collected on day 3 at 11:00, 22:00, 23:00, and 24:00 hours, and day 4 at 01:00, 02:00, 04:00, 06:00, and 11:00 hours. Hamster uterine tissues were collected on day 3 at 09:00, 12:00, and 15:00 hours. Pseudopregnancy was induced by mating female mice with vasectomized males, and the uteri were dissected on day 3 at 11:00 hours, and day 4 at 11:00 hours. Artificial decidualization was induced by oil injection in one uterine horn and two pinches with a hemostat in the contralateral horn in pseudopregnant mice on day 3 at 10:00 hours; the blue dye reaction was determined before uterine collection on day 3 at 24:00 hours, and day 4 at 06:00 hours. At least three pregnant mice and hamsters were included in each time point in each study.

Immunohistochemistry

Frozen uterine sections (10 μm) from different groups in the same study set were mounted on the same slide to keep the staining consistent, then were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), washed in PBS, and subjected to antigen retrieval in 0.01 M sodium citrate buffer, pH 6.0, for 20 minutes. Endogenous peroxidase was inactivated with 3% H_2O_2, and nonspecific staining was subsequently blocked using 10% goat