Pinopodes are present in *Lif* null and *Hoxa10* null mice

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Objective: To assess pinopode formation in *Lif* null and *Hoxa10* null mice with infertility secondary to failed implantation.
Design: Controlled animal experiment.
Setting: Animal research and laboratory facility.
Animal(s): *Lif* null, *Hoxa10* null, and ICR mice and Sprague-Dawley rats.
Intervention(s): Endometrial tissue was collected during the peri-implantation period and after ovariectomy.
Main Outcome Measure(s): Endometrial epithelial tissue was examined under scanning-electron microscopy and assigned a score depending on the number of pinopodes present.
Result(s): Pinopode scores in ICR, *Lif* null, and *Hoxa10* null mice were comparable throughout the peri-implantation period, rising on day 3.5 of pregnancy and remaining elevated through to day 7.5, suggesting that pinopodes are not a good indicator of receptivity in mice. In contrast, pinopode scores in rats clearly demarcated the window of receptivity, appearing on day 4 of pregnancy and declining sharply on day 6. Pinopode scores were low in E_2-treated ovariectomized mice, but unexpectedly, pinopode scores in vehicle-injected ovariectomized ICR mice were markedly elevated.
Conclusion(s): *Lif* null and *Hoxa10* null mice, in which implantation is impaired, have a similar number of pinopodes to fertile ICR mice. Pinopodes do not define a window of implantation in mice. (Fertil Steril® 2007; 88(Suppl 2):1021–8. ©2007 by American Society for Reproductive Medicine.)

Key Words: Endometrium, pinopode, pregnancy, mouse, *Lif*, *Hoxa10*, Sprague-Dawley rat, implantation window, scanning electron microscopy

In the rat, after mating, endometrial exposure to 48 hours of progesterone (P_4), followed by Estradiol (E_2), is required for successful embryo implantation to occur (1). Hormonal preparation results in a transient state of receptivity that lasts for <24 hours on day 5 of pregnancy in the rat (2) and is associated with several molecular (3) and morphological markers (4, 5). One proposed marker is the presence of pinopodes (4, 6), which are smooth mushroom-like projections that arise from the apical surface of the endometrium (Fig. 1A). The function of pinopodes is unknown, but one speculation is that blastocysts attach to them during the initial processes of implantation, on the basis of observations of embryo attachment to endometrial epithelial cell cultures in vitro (7).

Pinopode-like structures were first described in the rodent endometrium >30 years ago (4, 5, 8–10). The described projections lacked membrane-bound organelles and were not covered in microvilli (8). They were attached to the apical surface of the cell by a stalk or pedicle (9, 10) (Fig. 1B) and often contained one or two large vacuoles thought to be used in the pinocytotic uptake of fluid from the uterine lumen (9). In comparison, human pinopodes extend from the whole surface of the cell (11) (Fig. 1B) and are not pinocytotic (12).

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Pinopodes and pinopode-like structures have been identified in a wide variety of animals including gerbils, hamsters, cows, rabbits, pigs, deer, sheep, camels, monkeys, and viviparous lizards (13). In the literature, endometrial structures with a wide range of morphological features have been termed *pinopodes*. We have therefore assigned them to the following four categories, according to diameter: [1] microvilli and blebs (diameter, <1 μm), [2] pinopodes (diameter, 1 to 2 μm), [3] pinopodes (diameter, >2 to 12 μm), and [4] macropodes (diameter, >12 μm; Fig. 1A).

Publications elsewhere have suggested that leukemia inhibitory factor (*Lif*) and homeobox A10 (*Hoxa10*) are involved in pinopode formation in the mouse (14, 15). Null females of both strains have infertility secondary to failed implantation, and lack of pinopodes has been suggested as a contributing factor to their phenotype. Both of the aforementioned studies were conducted by using transmission electron microscopy (EM), which gives two-dimensional pictures. We have repeated the study by using scanning EM, which gives three-dimensional pictures and is considered the gold standard for pinopode visualization (16).

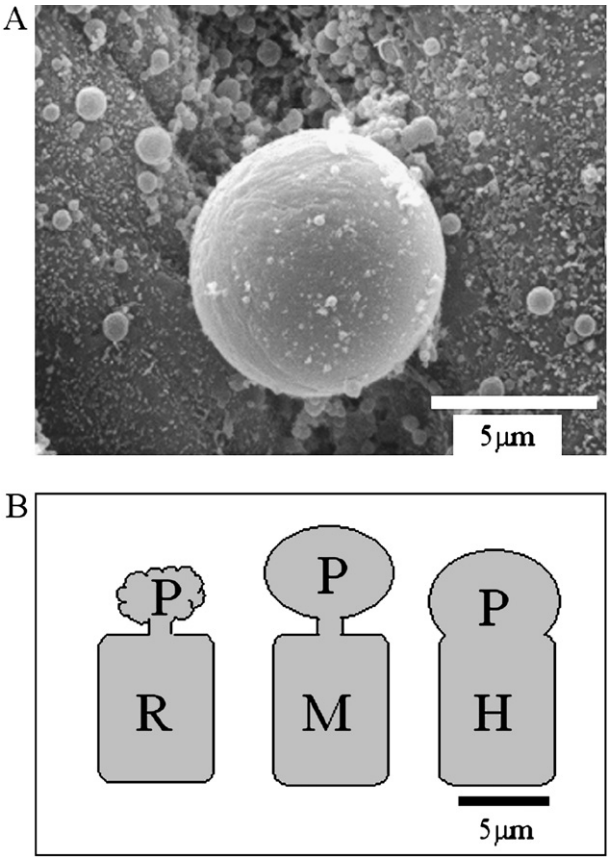
MATERIALS AND METHODS

Animals

All animals (Sprague-Dawley rats [Charles River, St. Constant, Quebec, Canada], ICR mice [Harlan, Indianapolis, IN],

FIGURE 1

(**A**) Pinopode (~7-μm diameter) in the ICR mouse endometrium, surrounded by smaller microvilli and blebs and by pinopodes. (**B**) Cartoon of morphological differences between pinopodes (P) extending from the apical surface of luminal epithelium cells in the rat (R), mouse (M), and human (H), as seen during their respective windows of receptivity. **A** and **B,** bar = 5 μm.



Quinn. Pinopodes in the rodent endometrium. Fertil Steril 2007.

Lif null mice [National Cancer Institute, Frederick, MD: Stewart Lab, mixed BALB/cXC57BL6 (17)], and *Hoxa10* null mice [Harvard Medical School, Boston, MA: Maas lab, 129/SvJ (18)]) were given free access to food and water and housed under 12:12-hour light–dark conditions. For matings, ICR female mice were mated to ICR males, *Lif* null females were mated to *Lif* null males, and *Hoxa10* null females were mated to *Hoxa10* heterozygote or homozygous wild-type males (*Hoxa10* null males have impaired fertility). Presence of a vaginal plug the morning after mating was considered day 0.5 of pregnancy in mice and was considered day 1 of pregnancy in rats. Ovariectomized (OVX) mice were allowed a minimum of 4 days to recover from surgery. Ovariectomized mice treated with E_2 were administered a daily SC injection of vehicle or of mid- (0.15 μg per animal per day) or high-dose (1.0 μg per animal per day) 17β-E_2 (Sigma Chemical Co., St. Louis, MO) dissolved in corn oil. Rats were killed by intracardiac potassium chloride injection, and mice were killed by cervical dislocation between 10 AM and 12 PM. All animals were handled in accordance with the guidelines of the Canadian Council on Animal Care and the Mount Sinai Hospital Research Institute Research Annex Council on Animal Care.

The endometrium was examined by using full-thickness tissue collected from the uterine horns of both *Lif* null and *Hoxa10* null mice during the peri-implantation period (days 0.5 to 7.5 of pregnancy; n = 3 at each time-point) and visualized under the scanning EM. Pinopode formation was compared with ICR mice and Sprague-Dawley rats. Ovariectomized ICR mice with or without E_2 treatment were used as negative controls because pinopode formation has been shown elsewhere to be P_4 dependent (19, 20).

Scanning Electron Microscopy

Samples were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer, rinsed in buffer, postfixed in 1% osmium tetroxide in buffer, dehydrated in a graded ethanol series, critical-point dried, and sputter coated with gold. The samples then were examined with an FEI XL30 (FEI Systems Canada Inc., Toronto, Ontario, Canada) scanning electron microscope.

Pinopode Scoring

Fifty fields of endometrial epithelium were examined per sample at ×3,500 magnification (the apical surface of ~250 cells can be visualized per field: 250 × 50 = ~12,500 cells visualized per sample). The total number of pinopodes seen in all 50 fields was added up and used as the final sample score. In uteri collected after embryo implantation, endometrial epithelium was scored in areas between implantation sites.

Statistical Analysis

We used SAS statistical software (version 9.1; Statistical Analysis Systems Institute Inc., Cary, NC) for data analysis, and *P* values of <.05 were considered statistically significant. Data for Figure 2 were analyzed by using multivariate modeling, with mouse strain and day of pregnancy modeled as fixed events. Data for Figures 3, 4, and 5 were analyzed by within-blocks analysis of variance, with experiments being considered blocks.

RESULTS

Pinopodes in ICR Mice, *Lif* Null Mice, and *Hoxa10* Null Mice

Pinopodes were present in intact ICR, *Lif* null, and *Hoxa10* null mice from day 0.5 to day 7.5 of pregnancy, with scores ranging from 4 to 132 (Fig. 2). Pinopode scores in all three lines of mice were comparable throughout the peri-implantation period. In the intact ICR mice, the pinopode score rose on day 3.5 of pregnancy (average score of 70: 0.56% of cells