Full-length article

Effect of a single dose of mifepristone on expression of pinopodes in endometrial surface of mice[1]

Dong-mei HUANG, Luciano G NARDO[2], Guang-ying HUANG[3], Fu-er LU, Yan-juan LIU

Institute of Integrated Traditional and Western Medicine, Affiliated Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China; [2]Department of Obstetrics and Gynaecology, Kingston University Hospital NHS Trust, Kingston upon Thames, Surrey, UK

Key words

mifepristone; endometrium; pinopode; embryo implantation; contraception

[1] Project supported by the National Natural Science Foundation of China (No 30171193).
[3] Correspondence to Dr Guang-ying HUANG.
Phn 86-27-8366-2577.
Fax 86-27-8364-0294.
E-mail GYhuang@tjh.tjmu.edu.cn

Received 2004-07-29
Accepted 2004-11-18

doi: 10.1111/j.1745-7254.2005.00036.x

Abstract

Aim: To investigate the effect of mifepristone (RU486) as a single dose on pinopodes expression in the endometrial surface of mice at the time of implantation. **Methods:** Pregnant mice in the treated four groups received mifepristone subcutaneously (0.1 mg) between 07:00 and 08:00 AM on Pd (day of pregnancy)1, Pd2, Pd3, and Pd4. Pregnant mice in the non-treated group were used as controls. The uterine horns were collected randomly from two mice in each group between 21:30 and 22:00 PM on Pd4, and from another two mice of the same group between 09:30 and 10:00 AM on Pd5. The specimens were examined by scanning electron microscopy for the detection of pinopodes. **Results:** When mifepristone was given on Pd1, developing and fully developed pinopodes were observed, but the expression was markedly reduced compared to the control group. When mifepristone was administered on Pd2, only a few developing pinopodes were present. When mifepristone was administered on Pd3, developing pinopodes were observed. When mifepristone was administered on Pd4, different development stage pinopodes were present in specimens collected between 21:30 and 22:00 PM on Pd4, but no pinopodes was observed in specimens taken between 09:30 and 10:00 AM on Pd5. **Conclusion:** These findings suggest that administration of a single dose of RU486 subcutaneously on Pd1, Pd2, Pd3, and Pd4 might play a role in inhibiting development and maturation of endometrium, hence affecting embryo implantation in mice.

Introduction

Mifepristone (RU486), a progesterone antagonist steroid, has been largely used for both planned and emergency contraception[1], as well as for termination of pregnancy[2]. Although the effect of RU486 for contraception has been described previously, its mechanism has not been completely elucidated. Anovulation, inhibition of fertilization and transportation of embryo through the Fallopian tube, and embryo implantation dysfunction have all been postulated. Recent data sustain the hypothesis that mifepristone reduces perivascular decidual haemostasis and increases extracellular matrix-degrading protease activity[3].

It is now well established that the cross-talk between the implanting embryo and the endometrial epithelium is a versatile and dynamic process, which requires a series of rather complicated and synchronous morphological and biochemical changes. Large and smooth membrane protrusions with pinocytotic function, known as pinopodes, have been observed in the endometrial surface during the window of implantation[4,5]. The formation and appearance of pinopodes appears to advance or regress, depending on hormonal milieu and other physiological modifications throughout the menstrual cycle[6]. Furthermore, a positive correlation between the pinopodes number and blastocyst implantation has been reported[7]. Fully developed pinopodes have been considered as the characteristic morphologic markers to assess endometrial receptivity and to locate the implantation win-

dow[8,9].

The aim of this experimental study was to investigate the timing of pinopodes expression in the endometrial surface epithelium of mice following a single dose administration of RU486.

Materials and methods

Animals and reagents Seventy female (weight: 28–30 g) and 15 male (weight: 40–45 g) adult Kunming mice (age 8–12 weeks) were provided by Sanitary Epidemic-Prevention Station of Hubei Province, China (Certificated No 19-082, China). Mifepristone tablets (Third Pharmaceutical Factory of Beijing, China) were ground carefully and dissolved in propylene glycol.

Animal treatment Female and male mice in a ratio of 2:1 were kept overnight in a cage for mating. The following morning, the display of a vaginal plug in female mice was designated as day 1 of pregnancy (Pd1). The pregnant mice were divided into five groups, as follows: a) non-treated group; b) treated group 1; c) treated group 2; d) treated group 3; and e) treated group 4. The non-treated group was considered the control. All mice in the treated groups received mifepristone subcutaneously (0.1 mL solution containing 0.1 mg mifepristone) between 07:00 and 08:00 AM on Pd1, Pd2, Pd3, and Pd4, respectively.

Scanning electron microscopy Two mice selected randomly from each group were killed by cervical dislocation between 21:30 and 22:00 PM on Pd4, and another two mice in the same group were killed between 09:30 and 10:00 AM on Pd5. All remaining mice were killed on Pd7 in order to observe the pregnancy and the average number of implanted embryos.

The murine uterine horns were excised and then cut open along the longitudinal axis. The endometrial tissue was rinsed in saline solution, fixed in 2.5% (w/v) glutaraldehyde solution in a sodium cacodylate buffer (0.15 mol/L, pH 7.3) and post-fixed in a 1% (w/v) osmium tetroxide solution in a sodium cacodylate buffer (0.15 mol/L, pH 7.3) containing sucrose (75 mmol/L). The specimens were dehydrated in a graded series of acetone, then dried in a critical-point drier with carbon dioxide, mounted on the specimen holder, coated with gold palladium, and observed by scanning electron microscopy (SEM) (S-520, Hitachi, Tokyo, Japan).

To avoid inter-observer bias, all specimens were analyzed by the same observer. Based on development stage and abundance, pinopodes were scored as developing, fully developed, or regressing, and then as few (<20%), moderate (20%–50%), or abundant (>50%), respectively. If different development stages were observed in the same specimen, only the commonest pattern was reported[6].

Statistical analysis Data were expressed as mean±SD. Unpaired *t*-test and χ^2-test were used. Software SPSS 11.0 for Windows was used. $P<0.05$ was considered to be statistically significant.

Results

Pregnancy rate and average implanted embryos No mice in treated groups 1, 2, or 3 conceived. In contrast, one mouse in group 4 achieved a pregnancy. Number of pregnant mice and average number of implanted embryos was significantly lower in treated group 4 as compared with controls (1 *vs* 9, $P<0.01$; 6 *vs* 14.67±1.35, $P<0.01$, respectively).

Pinopodes expression in endometrial surface In the control group, specimens collected between 21:30 and 22:00 PM on Pd4 showed abundant membrane projections widely distributed in the endometrial luminal surface. Clear, smooth, and slender projections were covered by short microvilli (developing pinopodes) (Figure 1). In specimens collected between 09:30 and 10:00 AM on Pd5, the endometrial surface was covered by membranous structures protruding and folding maximally (fully developed pinopodes). No microvilli were observed (Figure 2).

In treated group 1, the specimens collected between 21:30 and 22:00 PM on Pd4 showed smooth membrane projections. These structures, resembling developing pinopodes, were smaller, less abundant, and slightly lagged behind those present in controls (Figure 3). In specimens collected between 09:30 and 10:00 AM on Pd5, mainly fully developed pinopodes and developing pinopodes were present. Fully developed pinopodes were observed exclusively in epithelial cell depressions, while the neighboring surface was covered with short tips of microvilli (Figure 4).

In treated group 2, the specimens taken between 21:30 and 22:00 PM on Pd4 showed endometrial surface covered by scanty membrane projections. The endometrial luminal surface was relatively smooth with few and slender membranous projections (developing pinopodes) (Figure 5). These structures appeared slightly more pronounced in specimens collected between 09:30 and 10:00 AM on Pd5, but no fully developed pinopode was observed (Figure 6).

In treated group 3, no membrane projections were present in the endometrial surface of specimens collected between 21:30 and 22:00 PM on Pd4. The epithelial cells lining the luminal surface were rather smooth and covered by small tips of microvilli (Figure 7). The specimens taken between 09:30 and 10:00 AM on Pd5 showed smooth endometrial surface covered by normal epithelial cells and short microvilli in one case, while in the other there were slender membranous projections (developing pinopodes) which were much smaller

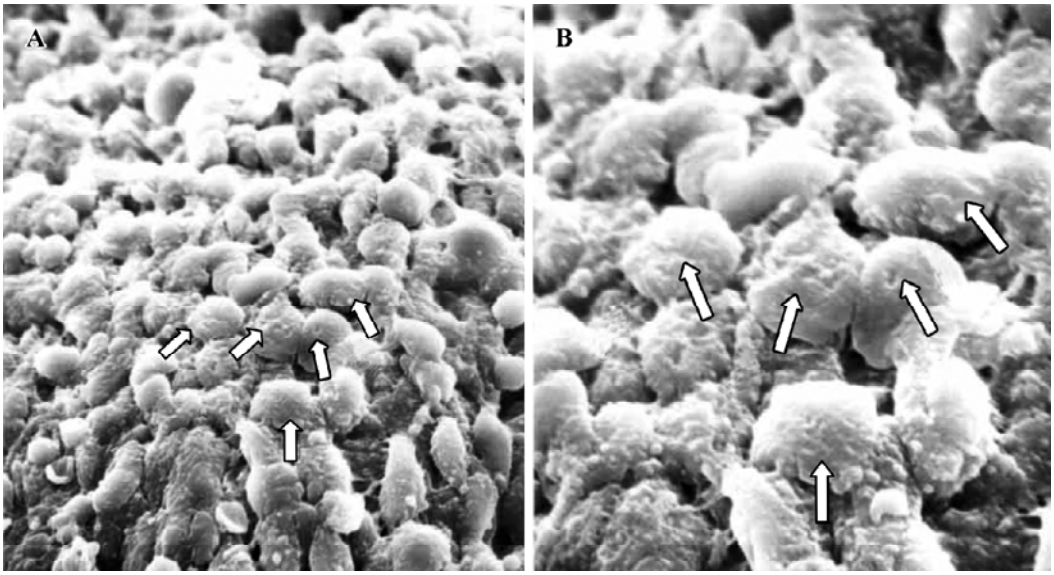

Figure 1. Pinopode expression in control group between 21:30 and 22:00 PM on Pd4. Smooth and slender membrane projections covered with short microvilli, almost the same shape and size, distributed over the whole endometrial luminal surface. (developing pinopodes: white arrow) (SEM, A×1500, B×3000).

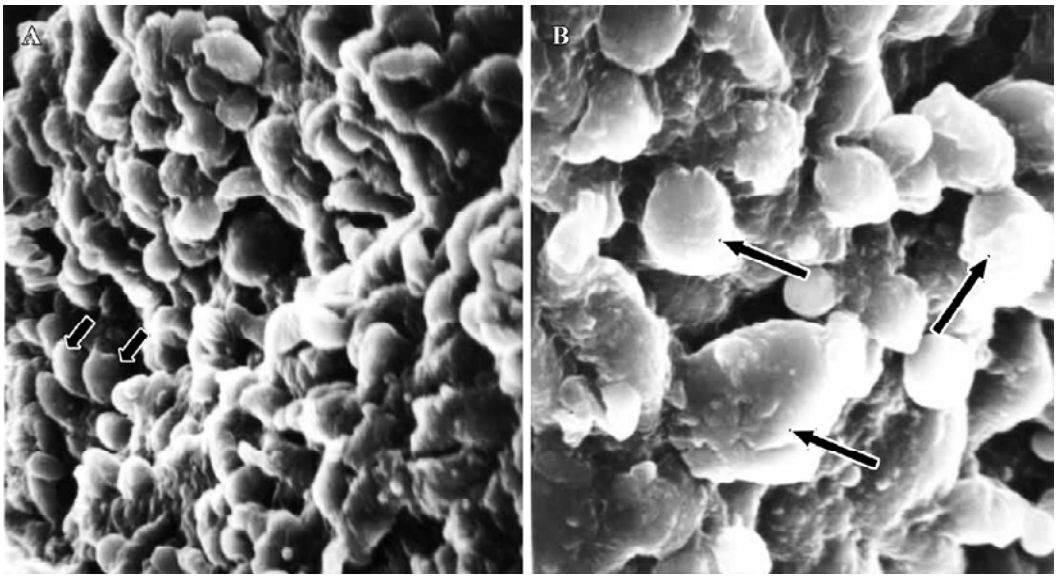

Figure 2. Pinopode expression in control group between 09:30 and 10:00 AM on Pd5. The endometrial surface was extensively covered with projections protruding and folding maximally (fully developed pinopodes: black arrow). No microvilli were observed. (SEM, A×1500, B×3000).

than those expressed in controls between 21:30 and 22:00 PM on Pd4 and between 09:30 and 10:00 AM on Pd5 (Figure 8).

Finally, in treated group 4, no synchronously developed structure was expressed in all specimens collected between 21:30 and 22:00 h on Pd4. Few pinopodes in different development stages were present. The majority of endometrial surface expressed no membrane projections and was covered by epithelial cells with short and thick microvilli. The boundaries of epithelial cells were not clear (Figure 9). Specimens collected between 09:30 and 10:00 AM on Pd5 had no pinopodes, but abundant microvilli and clear boundaries on the epithelial cells (Figure 10).

Figure 3. Pinopode expression in treated group 1 between 21:30 and 22:00 PM on Pd4. The specimens showed a lot of smooth membrane projections (developing pinopodes: white arrow) which were smaller, less abundant, and slightly lagged behind those in control group. (SEM, A×1500, B×3000).

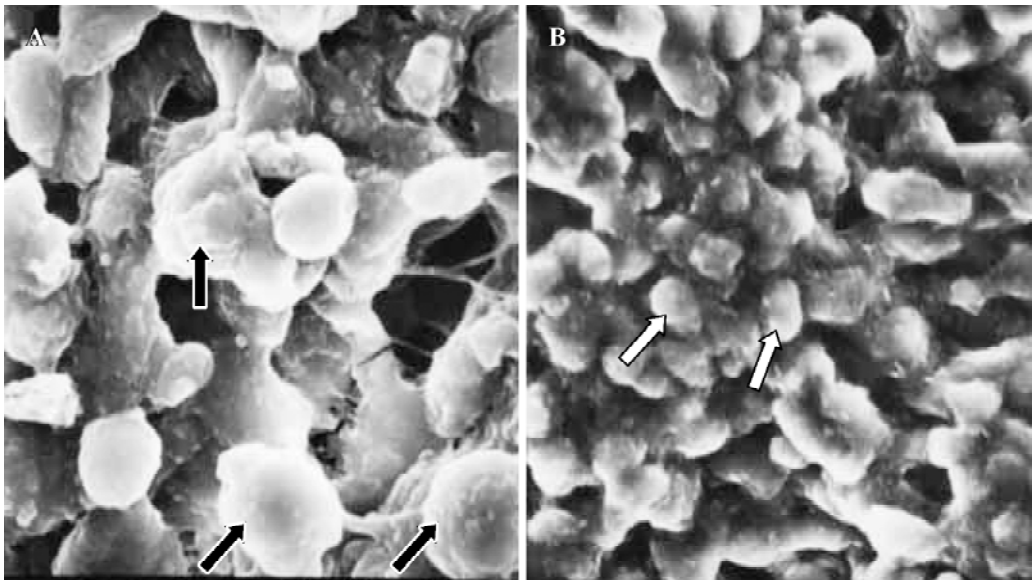

Figure 4. Pinopode expression in treated group 1 between 09:30 and 10:00 h on Pd5. Fully developed pinopodes (solid arrow) with many folds like a flower were observed in one specimen (SEM, A×3000), whereas another was mainly covered by abundant developing pinopodes (empty arrow) (SEM, B×1500).

Discussion

The use of mifepristone for emergency contraception has been widely accepted[1,3], however, a large number of prospective, randomized, double-blind studies are still in progress. Several clinical trials have shown that a single dose of mifepristone (10 mg) is effective for emergency contraception when given within 120 h from unprotected coitus, causing mild or

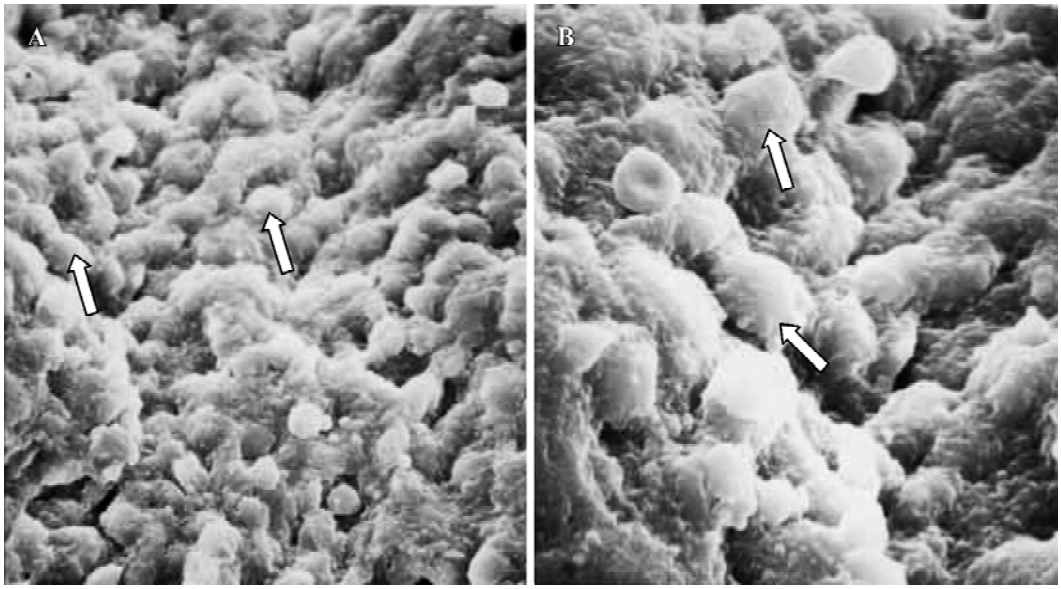

Figure 5. Pinopode expression in treated group 2 between 21:30 and 22:00 PM on Pd4. The endometrial luminal surfaces were relatively smooth with few and slender membranous projections (developing pinopodes: white arrow). (SEM, A×1500, B×3000).

Figure 6. Pinopode expression in treated group 2 between 09:30 and 10:00 AM on Pd5. These membrane projections (white arrow) appeared a little more pronounced in specimens, but no fully developed pinopodes were observed. (SEM, ×1500).

no side-effects[10,11]. However the biological mechanism of RU486 remains a matter of much debate.

Wang *et al*[12] investigated the effects of a single dose of mifepristone (10 mg) on the endometrial expressions of HOXA-11, progesterone receptors (PR), and leukaemia inhibitory factor (LIF). The found that following oral administration of mifepristone on d 2 post-ovulation (LH+2) the development of endometrium was quite delayed. In the glandular epithelium, the expressions of HOXA-11 and PR increased significantly and that of LIF decreased. Conversely, in the stromal epithelium, the expressions of these markers remained unchange.

More recently, it has been proved that mifepristone could inhibit the establishment of uterine receptivity in some animals[13,14]. Marions *et al*[15] reported that following mifepristone administration during early luteal phase in fertile women, down-regulation of PR was inhibited and no significant modifications of the remaining markers of endometrial receptivity, such as pinopodes, integrin dimmers α_4 and β_3, cyclooxygenase-1 and -2 were found. These authors[15] have also observed that the endometrial changes happened irrespective of embryo; however, its influence on implantation could not be ignored. Furthermore, they observed the influence of RU486 on endometrium only after RU486 was given on LH+2 whereas RU486 is effective for emergency contraception when given within 120 h from unprotected coitus.

A large consensus of opinion sustains that fully developed pinopodes represent specific morphological markers of endometrial receptivity[6,8,9]. The appearance and disappearance of fully developed pinopodes coincide with the nidation window both in humans and animals. Synchronous expressions of fully developed pinopodes and other markers of uterine receptivity such as integrins ($\alpha_v\beta_3$)[16], heparin-binding epidermal growth factor (HB-EGF)[17], LIF and

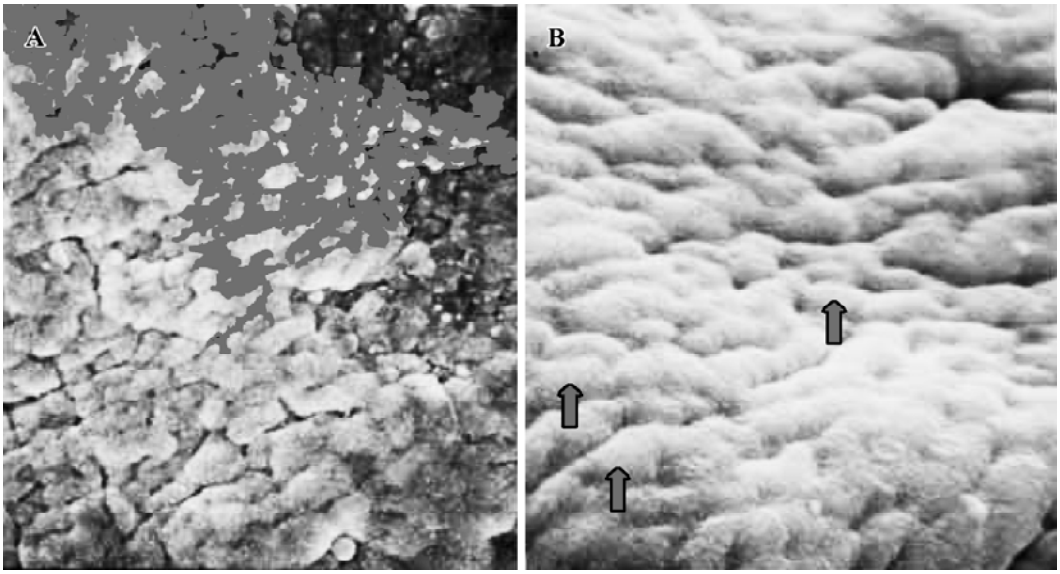

Figure 7. Pinopode expression in treated group 3 between 21:30 and 22:00 PM on Pd4. No membrane projections appeared. The epithelial cells (grey arrow) lining the luminal surface were rather smooth and covered by small tips of microvilli. (SEM, A×1500 B×3000).

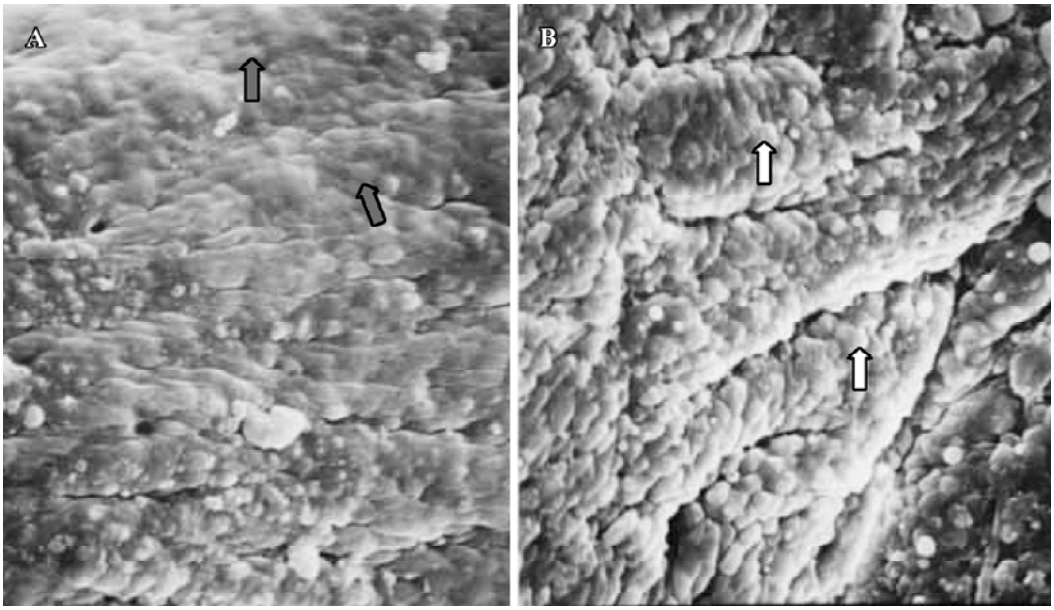

Figure 8. Pinopode expression in treated group 3 between 09:30 and 10:00 AM on Pd5. Smooth endometrial surface was covered by normal epithelial cells (grey arrow) and short microvilli in one case (SEM, A×1500), while in the other there were very slender membranous projections (developing pinopodes: white arrow) (SEM, B×1500).

LIF-receptor[18] have been noted. Therefore, it is plausible that morphological and biochemical changes during the menstrual cycle may be reliable in evaluating endometrial function and receptivity.

In this study, mifepristone as a single dose was administered straight away after the murine successful coitus was confirmed. When RU486 was given on Pd4, only one mouse conceived and the number of implanted embryos was significantly lower compared to controls (P<0.01). No mice conceived when RU486 was given on Pd1, Pd2, and Pd3. These data show that a single dose of RU486 is effective when given on Pd1, Pd2, Pd3, and Pd4.

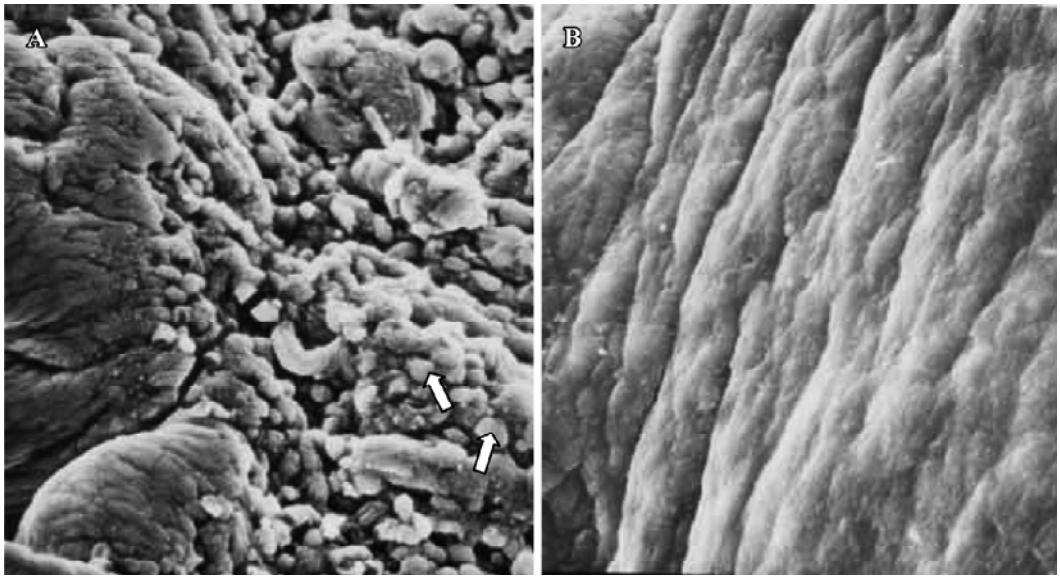

Figure 9. Pinopode expression in treated group 4 between 21:30 and 22:00 PM on Pd4. No synchronously developed structures were expressed in the samples. The pinopodes (white arrow) were few and with different development stages (SEM, A×1000). The majority of endometrial surface expressed no membrane projections and was covered by epithelial cells with short and thick microvilli. (SEM, B×1500).

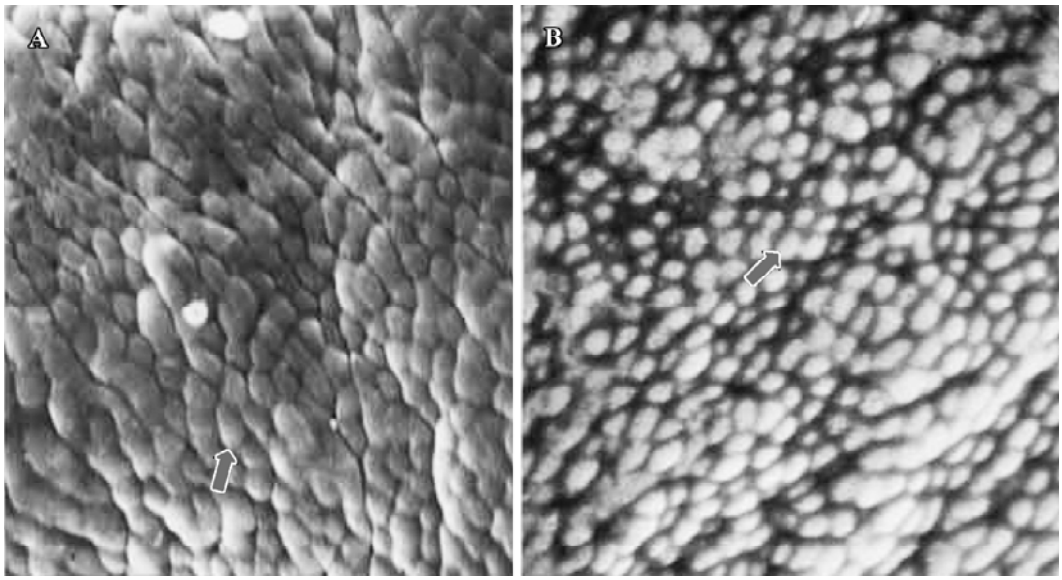

Figure 10. Pinopode expression in treated group 4 between 09:30 and 10:00 AM on Pd5. Specimens had no pinopodes, but abundant microvilli and clear boundaries on the epithelial cells (grey arrow) (SEM, ×1500).

When RU486 was administered on Pd1, developing and fully developed pinopodes were observed in the endometrial surface, but its expression was reduced compared to that of controls. It is therefore likely that mifepristone administration on Pd1 inhibits zygote development or embryo transportation through the Fallopian tube rather than having an effect on the endometrial surface. When mifepristone was given on Pd2, the uterine receptivity was significantly impaired, hence elucidating the effects of this antiprogestogenic steroid on the endometrium and its rationale for contraception. Likewise, administration of RU486 on Pd3 and Pd4 appeared to impair development and matura-

tion of pinopodes, so affecting endometrial receptivity and embryo implantation.

The effect of RU486 on the endometrium on Pd3 is stronger than that on Pd2 and Pd4. Conversely, the effect is relatively weak when this compound is given on Pd1. To our knowledge, this is the first report in the literature assessing pinopode expression as morphological marker of implantation window in mice following administration of mifepristone. Therefore, comparison with other data is not possible.

In conclusion, these findings suggest that mifepristone might inhibit endometrial receptivity and, to some extent, prevent embryo implantation as a result of morphological modifications of the luminal epithelial cells. Since the establishment of endometrial receptivity includes morphological immunological changes, further studies exploring the effects of RU486 on the expressions of cytokines, adhesion molecules and other immunological factors during the implantation window would provide new insights and improve our knowledge of implantation and early pregnancy.

References

1 Cameron ST, Critchley HO, Thong KJ, Buckley CH, Williams AR. Effects of daily low dose mifepristone on endometrial maturation and proliferation. Hum Reprod 1996; 11: 2518–26.

2 Basu R, Gundlach T, Tasker M. Mifepristone and misoprostol for medical termination of pregnancy: the effectiveness of a flexible regimen. J Fam Plann Reprod Health Care 2003; 29: 139–41.

3 Papp C, Schatz F, Krikun G, Hausknecht V, Lockwood CJ. Biological mechanisms underlying the clinical effects of mifepristone (RU486) on the endometrium. Early Pregnancy 2000; 4: 230–9.

4 Psychoyos A, Mandon P. Study of the surface of the uterine epithelium by scanning electron microscopy: observation in the rat at the 4th and 5th day of pregnancy. Crit Rev Acad Sci Paris 1971; 272: 2723–9.

5 Enders AC, Nelson DM. Pinocytotic activity of the uterus of the rat. Am J Anat 1973; 138: 277–99.

6 Nardo LG, Sabatini L, Rai R, Nardo F. Pinopode expression during human implantation. Eur J Obstet Gynecol Reprod Biol 2002; 10: 104–8.

7 Nikas G, Makrigiannakis A, Hovatta O, Jones HW Jr. Surface morphology of the human endometrium: basic and clinical aspects. Ann N Y Acad Sci 2000; 900: 316–24.

8 Psychoyos A, Nikas G. Uterine pinopodes as markers of uterine receptivity. Ass Reprod Rev 1994; 4: 26–32.

9 Bentin-Ley U, Sjogren A, Nilsson L, Hamberger L, Larsen JF. Presence of uterine pinopodes at the embryo-endometrial interface during human implantation *in vitro*. Hum Reprod 1999; 14: 515–20.

10 Von Hertzen H, Piaggio G, Ding J, Chen J, Song S, Bartfai G. Low dose mifepristone and two regimens of levonorgestrel for emergency contraception: a WHO multicentre randomized trial. Lancet 2002; 360: 1803–10.

11 Xiao BL, Von Hertzen H, Zhao H, Piaggio G. A randomized double-blind comparison of two single doses of mifepristone for emergency contraception. Hum Reprod 2002; 17: 3084–9.

12 Wang L, Wang H, Wu J, Luo HZ, Zhu ZM, Wang JD. The effect of low dose RU486 on the expression of HOXA11 in human endometrium during midluteal phase. J Reprod Med 2000; 9: 165–70. Chinese.

13 Gao F, Xu FH, Zhou XC, Han XB, Liu YX. Mifepristone regulates expression of apoptosis related genes fas and fasL in mouse endometrium. Acta Pharmacol Sin 2001; 22: 524–9.

14 Liu CQ, Wang ZX, Yuan Y. Effect of mifepristone on uterine receptivity in guinea pigs. Acta Pharmacol Sin 2002; 23: 177–82.

15 Marions L, Hultenby K, Lindell I, Sun X, Stabi B, Gemzell Danielsson K. Emergency contraception with mifepristone and levonorgestrel: mechanism of action. Obstet Gynecol 2002; 100: 65–71.

16 Nardo LG, Nikas G, Makrigiannakis A, Sinatra F, Nardo F. Synchronous expression of pinopodes and alpha v beta 3 and alpha 4 beta 1 integrins in the endometrial surface epithelium of normally menstruating women during the implantation window. J Reprod Med 2003; 48: 355–61.

17 Stavreus-Evers A, Aghajanova L, Brismar H, Eriksson H, Landgren BM, Hovatta O. Co-expression of heparin-binding epidermal growth factor-like growth factor and pinopodes in human endometrium at the time of implantation. Mol Hum Reprod 2002; 8: 765–9.

18 Aghajanova L, Stavreus-Evers A, Nikas Y, Hovatta O, Landgren BM. Co-expression of pinopodes and leukemia inhibitory factor, as well as its receptor, in human endometrium. Fertil Steril 2003; 79 (Suppl 1): 808–14.