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OPINION

Characteristics and possible function of pinopodes seen on the surface of the receptive human endometrium

Anneli C. Stavreus-Evers, Ph.D.

Department of Clinical Science, Division of Obstetrics and Gynecology Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

ABSTRACT

A synchronized development of embryo and endometrium is a prerequisite for a successful implantation. During the time of implantation, pinopodes appear on the endometrial surface. The precise function of these structural markers of endometrial receptivity is not known, but it is generally believed that they play a role in the implantation process. Further understanding of the function of these biomarkers and their role in embryo implantation could aid in better diagnosis and treatment of infertile couples in the future.
Keywords: Implantation window, pinopodes, endometrium.

INTRODUCTION

The endometrium is changing throughout the menstrual cycle in order to prepare for the implantation of an embryo. The maturation of the endometrium is dependent on interplay between the steroid hormones estrogen and progesterone. The endometrium is only receptive for blastocyst implantation for a short period of time, so called "implantation window" before and after which implantation does not occur (1).

Pinopodes, believed to be ultra structural markers of endometrial receptivity, appear on the endometrial surface at the time of implantation (Figure 1). Pinopodes was first observed in mice and rats (2,3) but similar structures have also been found in other species (4). Johannisson and Nelson were the first to report pinopode-like structures in human endometrium (5).

Address: Department of Clinical Science, Division of Ob. Gyn. K57, Karolinska University Hospital, SE-141 86, Stockholm, Sweden, Phone: +46 8 585 875 08, Fax: +46 8 585 875 75, E-mail: Anneli.Stavreus-Evers@klinvet.ki.se

The morphological appearance of pinopodes differs between species. In rat and mouse, the pinopodes arise from the cell surface on stalks, which rise above the level of microvilli (6,7). In the rat, pinopodes are present in approximately 20% of the epithelial cells, whereas in human endometrium, pinopode structure seems to involve the majority of the non-ciliated epithelial cells (4,8).

Cellular organelles, such as mitochondria, have been found in pinopodes from several species (9,10) including human (11). Also considerable amount of glycogen is part of the content of pinopodes, which suggest that the formation of pinopodes is an energy consuming process (12).

THE IMPLANTATION WINDOW

Determination of the implantation window

Traditional dating according to the criteria of Noyes et al has been the standard method since the article was first published in 1950, and it still remains

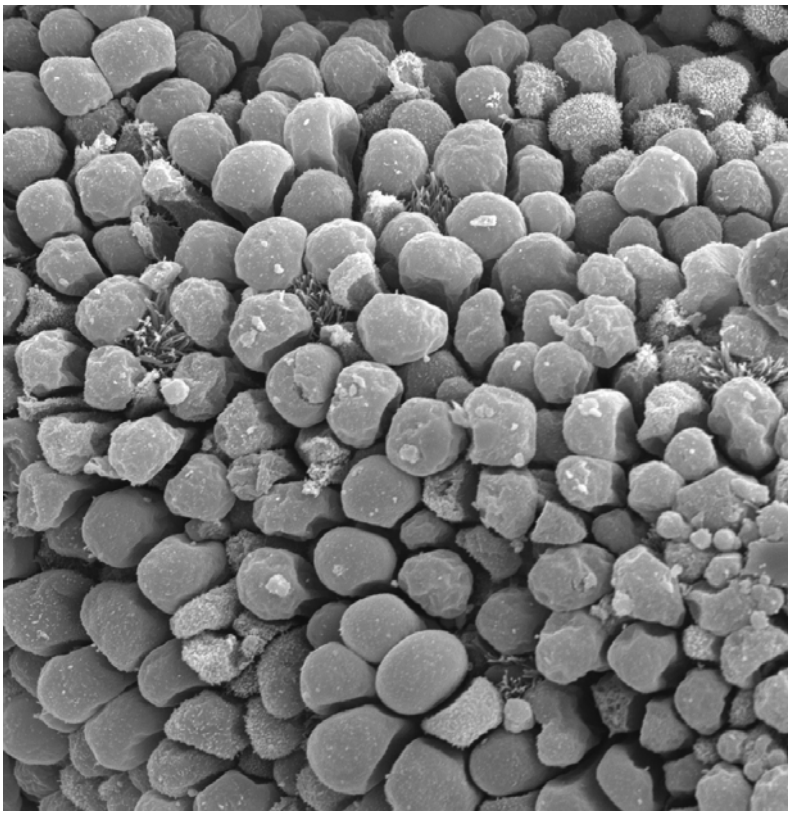

Figure 1. Pinopodes on the endometrial surface

the most frequently cited article in the literature on infertility (13). The method is not perfect and detailed quantitative histological techniques have been used to study endometrial receptivity and function (14,15). However, none of these techniques has proven optimal for determination of endometrial receptivity and function.

Determination of the LH surge, measured in blood or urine, correlates well with histological dating according to Noyes et al (14). There has also been an attempt to date the endometrium by using integrins as biochemical markers (16). However the authors concluded that the morphological dating according to Noyes was superior.

The possibility to use pinopodes as markers of endometrial receptivity has been discussed from the early 1980s (17) and this was generally accepted in the mid 1990s (8,18). The embryo hatches on day 6 after the LH surge and is subsequently ready for implantation, probably within the nearest 24 hours. This corresponds to the formation of pinopodes in most women.

The length of the implantation window

The length of the implantation window is controversial. The implantation period is usually assumed to coincide with cycle day 20-22 in a standardized cycle of 28 days (19). However, subsequent evaluation of data based on highly sensitive hCG measurements in ART cycles estimated that the window of implantation lasts for approximately 5 days and extends from postovulatory days 6 to10 (cycle day 20-24 of an idealized 28 day cycle) (20).

The lifespan of pinopodes is also controversial. Our studies show that pinopodes are present in endometrium obtained on days LH+6 to LH+10 (21-24).

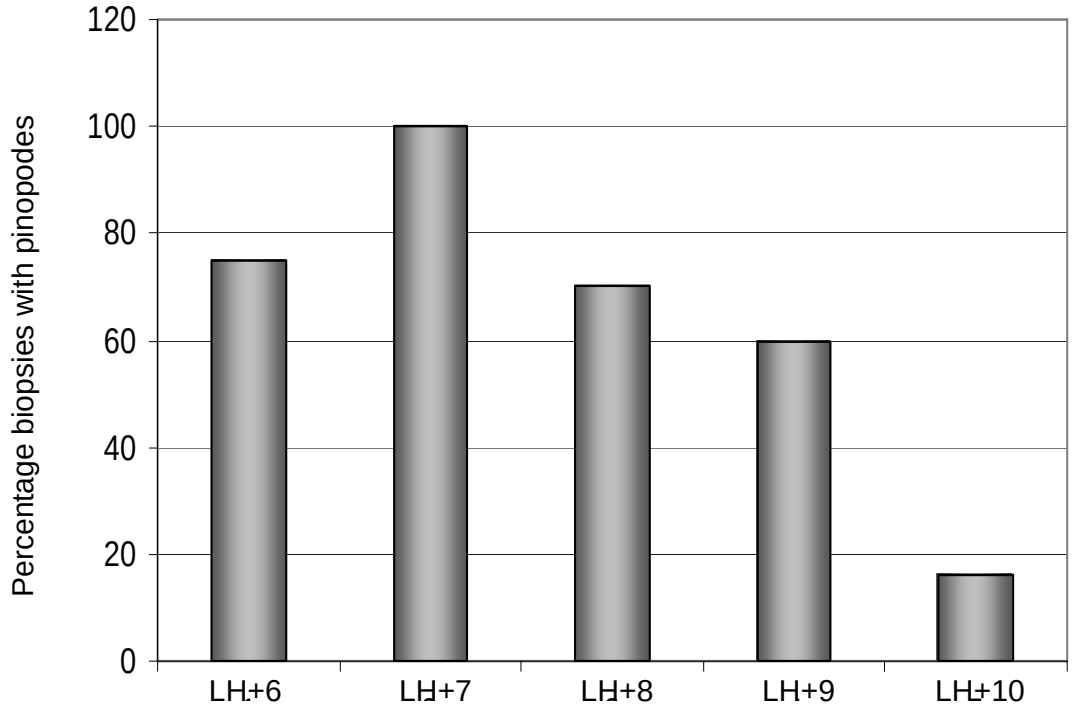

Figure 2. Percentage of endometrial biopsies containing pinopodes. Pinopodes was found in all biopsies obtained on day LH+7.

In 60 biopsies obtained during the luteal phase, we found pinopodes from day LH+6 to LH+10 and they were most abundant on LH+7 (Figure 2). The duration of the pinopode appearance in each individual woman cannot be determined by obtaining only one biopsy in a cycle, but it can be speculated that the pinopodes appear on days LH+6 and/or LH+7 and disappear on days LH+8 and/or LH+9.

There is only one study, where sequential endometrial biopsies have been obtained from healthy during the same cycle. The number of women included in the study is small, only 10 women where the biopsies were been obtained in a normal cycle (25,26). This study suggests a lifespan of pinopodes less than 48 hours. However, there are possibilities that serial biopsies could cause changes in the endometrium such as a healing process or inflammation process that might influence the results.

There are some studies suggesting the presence of pinopodes from day LH+5 to LH+11(27) or that pinopodes exist throughout the luteal phase (28-31). However, none of these studies have used additional methods to verify their findings. Therefore, the morphological appearance of pinopodes of great importance and is currently debated. The morphology of the protrusions seen on the surface is of importance, since it might not be appropriate to regard any protrusion on the endometrial surface as a pinopode. However, the morphology of the protrusions varies during the late luteal phase (31) and other events, such as apoptosis, might be mistaken for pinopodes.

PINOPODES

Hormonal regulation of pinopodes

In rats and mice, where endometrial receptivity requires both estrogen and progesterone, development of pinopodes is dependent on progesterone, and is inhibited by estrogen (32). In

humans, the highest frequency of pinopodes is found in response to progesterone alone (32,33)

The pinopodes are present on the endometrial surface, when the serum levels of progesterone are high (21). It is possible to inhibit pinopode formation by administration of antiprogestins (34).

Increased serum progesterone concentration and down regulation of glandular progesterone receptor B expression are concurrent with development of pinopodes at the time of implantation (21).

Function of pinopodes

So far, the morphology of pinopodes has been described in detail (21, 25), but the biological function is less understood. The number of pinopodes varies between women, and a correlation between number of pinopodes and implantation rate has been demonstrated (25)

The function of pinopodes was first demonstrated in mice. An electron-dense tracer (ferritin) was introduced into the uterine lumen and the tracer was taken up into large vacuoles in the epithelial cells, which demonstrated the pinocytotic function of these protrusions (6). This fact named these structures pinopodes, which in Greek means "drinking foot". The pinocytotic process seen in rats and mice has not been shown in other species (10).

Uterine secretion is reduced during the time of implantation (35), but in contrast to mice and rats, pinopodes in human endometrium do not display pinocytotic function (36). This suggests that other mechanisms are involved in the reduction of uterine fluid e.g. less secretion or increased uptake.

The pinopodes arise form apical surface of the epithelial cells, and are large enough to trap the cilia. The pinopode structures might thereby prevent the blastocyst to be "swept away" by the cilia. This would facilitate close contact between the embryo and the endometrial surface.

It is possible that the embryo adheres to the pinopodes. In an in vitro systems blastocysts adhered to pinopodes on the surface (37). However, later in vivo studies by the same group also showed adhesion of the embryo to epithelial cells not forming pinopodes (38).

BIOCHEMICAL MARKERS IN RELATION TO PINOPODES

There are several biochemical markers related to the implantation window but very few markers have been related to the presence of pinopodes.

Integrins

Integrins are surface glycoproteins composed of α and β subunits that serve as receptors for components of extra cellular matrix such as osteopontin, fibronectin and collagens. These components have the capacity to act as bridging molecules between the blastocyst and the endometrial surface during the adhesion phase of the implantation process. Integrins appear on the blastocyst as well as on the surface of glandular and luminal endometrial epithelium at the time of implantation (39). Fully developed pinopodes and strong intensity of alpha v beta 3 integrin is coinciding in endometrium obtained during the window of implantation (40).

LIF and LIF receptor

Leukemia inhibitory factor plays an important role in the implantation process. In the mouse it has been shown that maternal endometrial LIF is an absolute pre-requisite for embryo implantation. (41). It has been shown that infertile women have less LIF in the uterine secretion than women with proven fertility (42). Increase of LIF in the glandular epithelium and upregulation of LIF receptor expression in the luminal epithelium has been demonstrated in the presence of pinopodes (24).

HB-EGF

HB-EGF is a stimulator of cell proliferation, cell migration and cell motility (43). HB-EGF is highly expressed in the glandular and luminal epithelial cells at the time of implantation (23,44,45). HB-EGF is found both in the luminal epithelial cells of the endometrium and on the surface of the pinopodes (23). There are several possible functions of HB-EGF in the implantation process such as signaling between the

endometrium and the blastocyst, to facilitate adhesion of the blastocyst to the luminal epithelial surface, and to heal the luminal epithelial surface after the invasion of the embryo.

Glutaredoxin

Glutaredoxin is expressed in the pinopodes, suggesting a role of these markers for the implantation process (22). The function is not known, but it can be speculated that these markers protect the endometrium and pre-implantation embryo from oxidative stress.

CLINICAL RELEVANCE

The importance of being able to determine when the endometrium is receptive is essential during IVF treatment. There are women, which do not become pregnant even after receiving a number of good quality embryos. For those women, with repeated implantation failure, there is reason to suspect a defect in endometrial development.

Two main types of defect endometrial receptivity have been described. The first is a delayed receptivity, accompanied by histological retardation (46,47). The second is the endometrium histologically in place but with a defect biochemical alteration (48).

The possibility to detect when the endometrium is receptive would be of great clinical value. However, there are still only few studies, where attempts to predict the endometrial receptivity have been performed. In one study, endometrial biopsies were obtained in one cycle before the embryo transfer (ET) and the ET time was estimated and according to the appearance of pinopodes. The study showed a possibility to predict the implantation window, and thereby increase the implantation rate (49)

CONCLUSION

Pinopodes are present on the endometrial surface at the time of implantation. The formation of pinopodes correlated to the increase in progesterone and decrease on PRB. However, further studies are needed to determine the function of pinopodes.

Further unravelling of molecules involved in the implantation process is needed for a better comprehension of the link between endometrial development and endometrial receptivity. Understanding the function of pinopodes and their role in implantation would result in more efficient diagnosis and treatment of infertile couples in the future.

REFERENCES

1. Navot D, Bergh P. Preparation of the human endometrium for implantation. Ann N Y Acad Sci. 1991;622:212-9.
2. Nilsson O Ultrastructure of mouse uterine surface epithelium under different estrogenic influences. 1. Spayed animals and oestrous animals. J Ultrastruct Res. 1958; 1:375-96.
3. Warren RH, Enders AC. An electron microscope study of the rat endometrium during delayed implantation. Anat rec. 1964;148:177-95
4. Murphy CR Understanding the apical surface markers of uterine receptivity: pinopods-or uterodomes? Hum Reprod. 2000;15:2451-4
5. Johannisson E, Nilsson L Scanning electron microscopic study of the human endometrium. Fertil Steril. 1972;23:613-25.
6. Enders AC, Nelson DM. Pinocytotic activity of the uterus of the rat. Am J Anat 1973; 138:277-300
7. Parr MB, Parr EL. Uterine luminal epithelium: protrusions mediate endocytosis, not apocrine secretion in the rat. Biol Reprod 1974;11:220-233
8. Nikas G, Drakakis P, Loutradis D, Mara-Skoufari C, Koumantakis E, Michalas S, Psychoyos A. Uterine pinopodes as markers of the 'nidation window' in cycling women receiving exogenous oestradiol and progesterone. Hum Reprod. 1995;10:1208-13.
9. Guillmont M, Guay P Ultrastructural features of the cell surfaces of uterine and tropohblast epithelia during embryo attachment of the cow. Anat Rec 1982; 204:315-322
10. Parr MB, Parr EL. Relationship of apical domes in the rabbit uterine epithelium during the peri-implantation period to endocytosis, apocrine secretion and fixation. J Reprod Fertil. 1982;66:739-44
11. Dockery P, Ismail RMJ, LiTC et al The effect of single dose od mifepristone (RU486) on the fine structure of the human endometrium during the early luteal phase Hum Reprod 1997; 12:1778-1784
12. Novotny R, Malinsky J, Oborna I, Dostal J. Ultrastructure of endometrial surface relief in normal menstrual cycle and after hormonal stimulation. Acta Univ Palacki Olomuc Fac Med. 1999;142:47-55.

13. Noyes RW, Hertig AT, Rock J Dating the endometrial biopsy Fertil Steril 1951;1;3-25
14. Johannisson E, Parker RA, Landgren BM, Diczfalusy E. Morphometric analysis of the human endometrium in relation to peripheral hormone levels. Fertil Steril. 1982;38:564-71.
15. Li TC, Rogers AW, Dockery P, Lenton EA, Cooke ID A new method of histologic dating of human endometrium in the luteal phase. Fertil Steril. 1988;50:52-60.
16. Lessey BA, Castelbaum AJ, Wolf L, Greene W, Paulson M, Meyer WR, Fritz MA (2000) Use of integrins to date the endometrium Fertil Steril 73 ;779-787
17. Martel D, Malet C, Gautray JP, Psychyos A. Surface changes of the luminal uterine epithelioum during the human menstrual cycle: a scanning electron microscopic study. In: de Brux J, Morrtel R, Gautray JP eds. The endomentrium: Hormonal Impacts. New York Plenum Press 1981:15-20
18. Nikas G, Psychoyos A. Uterine pinopodes in peri-implantation human endometrium. Clinical relevance. Ann N Y Acad Sci. 1997 Jun 17;816:129-42
19. Davis OK, Rosenwaks Z. Preparation of the endometrium for oocyte donation. J Assist Reprod Genet. 1993;10:457-9
20. Bergh PA, Navot D The impact of embryonic development and endometrial maturity on the timing of implantation Fertil Steril. 1992;58:537-42.
21. Stavreus-Evers A Nikas G, Sahlin L, Eriksson H, Landgren BM. Formation of pinopodes in human endometrium is associated with the concentrations of progesterone and progesterone receptors. Fertil Steril. 2001;76:782-91.
22. Stavreus-Evers A, Masironi B, Landgren BM, Holmgren A, Eriksson H, Sahlin L. Immunohistochemical localization of glutaredoxin and thioredoxin in human endometrium: a possible association with pinopodes. Mol Hum Reprod. 2002;8:546-51
23. Stavreus-Evers A, Aghajanova L, Brismar H, Eriksson H, Landgren BM, Hovatta O. Co-existence 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.
24. Aghajanova L, Stavreus-Evers A, Nikas Y, Hovatta O, Landgren BM. Coexpression of pinopodes and leukemia inhibitory factor, as well as its receptor, in human endometrium. Fertil Steril. 2003;79 Suppl 1:808-14.
25. Nikas G. Pinopodes as markers of endometrial receptrivity in clinical practice. Hum Reprod 1999;14:99-106
26. Develioglu OH, Hsiu JG, Nikas G, Toner JP, Oeninger S, Jones HWJ. Endometrial estrogen and progesterone receptor and pinopode expression in stimulated cycles of oocyte donors. Fertil Steril 1999;71:1040-1047
27. Lopata A, Bentin-Ley U, Enders A. "Pinopodes" and implantation. Rev Endocr Metab Disord. 2002;3:77-86
28. Acosta AA, Elberger L, Borghi M, Calamara JC, Chemez H, Doncel GF, Kliman H, Lema B, Lustig L, Papier S (2000) Endometrial dating and determination of the window of implantation in healthy fertile women. Fertil Steril 73, 788-798.
29. Creus M, Ordi J, Febergues F, Casamitjana R, Ferrer B, Coll E, Vanrell JA, Balash J (2002) αvβ3 integrin expression and pinopode formation in normal and out of phase endometria of fertile and infertile women. Hum Reprod 17, 2279-2286
30. Creus M, Ordi J, Fabregues F, Casamitjana R, Carmina F, Cardesa A, Vanrell JA, Balash J (2003) The effect of different hormone expression and pinopode formation in the human endometrium: a controlled study. Hum Reprod 18, 683-693
31. Usadi RS, Murray MJ, Bagnell RC, Fritz MA, Kowalik AI, Meyer WR, Lessey BA (2003) Temporal and morphologic characteristics of pinopod expression across the secretory phase of the endometrial cycle in normally cycling women with proven fertility. Fertil Steril 79, 970-974
32. Martel D, Monier MN, Roche D, Psychyos A. Hormonal control of pinopode formation at the uterine luminal surface. Hum Reprod. 1991;6:597-603
33. Murphy CR The cytoskeleton of uterine epithelial cells: a new player in uterine receptivity and the plasma membrane transformation. Hum Reprod Update. 1995; 1:567-80
34. Paulson RJ, Sauer MV, Lobo RA. Potential enhancment of endometrial receptivity in cycles using controlled ovarian hyperstimulation with antiprogestins: a hypothesis. Fertil Steril 1997;68:185-188
35. Gemzell-Danielsson K, Hamberg M The effect of antiprogestin (RU 486) and prostaglandin biosynthesis inhibitor (naproxen) on uterine fluid prostaglandin F2 alpha concentrations Human Reproduction, 1994;9:1626-1630,
36. Adams SM, Gayer N, Hosie MJ, Murphy CR. Human uterodomes (pinopods) do not display pinocytotic function. Hum Reprod 2002;17:1980-1986
37. Bentin-Ley U, Sjogren A, Nilsson L, Hamberger L, Larsen JF, Horn T. Presence of uterine pinopodes at the embryo-endometrial interface during human implantation in vitro Hum Reprod. 1999 Feb;14(2):515-20.
38. Bentin-Ley U, Horn T, Sjogren A, Sorensen S, Falck Larsen J, Hamberger L. Ultrastructure of human blastocyst-endometrial interactions in vitro J Reprod Fertil. 2000 Nov;120(2):337-50.
39. Lessey BA, Castelbaum AJ, Buck CA. Further characterization of endometrial itegrins during menstrual cycle and pregnanct. Fertil Steril 1994 ;62 :497-506
40. 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.
41. Stewart CL Kaspar P, Brunet LJ, Bhatt H, Gadi I, Kontgen F, Abbondanzo SJ. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature. 1992;359:76-9.
42. Laird SM, Tuckerman EM, Dalton CF, Dunphy BC, Li TC, Zhang X. The production of leukaemia inhibitory factor by human endometrium: presence in uterine flushings and production by cells in culture. Hum Reprod. 1997;12:569-74.

43. Raab G, Klagsbrun M. Heparin-binding EGF-like growth factor. Biochim Biophys Acta. 1997 Dec 9;1333(3):F179-99
44. Leach RE, Khalifa R, Ramirez ND, Das SK, Wang J, Dey SK, Romero R, Armant DR. Multiple roles for heparin-binding epidermal growth factor-like growth factor are suggested by its cell-specific expression during the human endometrial cycle and early placentation J Clin Endocrinol Metab. 1999 Sep;84(9):3355-63.
45. Yoo HJ, Barlow DH, Mardon HJ. Temporal and spatial regulation of expression of heparin-binding epidermal growth factor-like growth factor in the human endometrium: a possible role in blastocyst implantation. Dev Genet. 1997;21(1):102-8.
46. Lessey BA, Damjanovich L, Coutifaris C, Castelbaum A, Albelda SM, Buck CA. Integrin adhesion molecules in the human endometrium. Correlation with the normal and abnormal menstrual cycle. J Clin Invest. 1992 Jul;90(1):188-95.
47. Kao Kao LC, Germeyer A, Tulac S, Lobo S, Yang JP, Taylor RN, Osteen K, Lessey BA, Giudice LC. Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. Endocrinology. 2003 Jul;144(7):2870-81.
48. Klentzeris LD, Li TC, Dockery P, Cooke ID. The endometrial biopsy as a predictive factor of pregnancy rate in women with unexplained infertility. Eur J Obstet Gynecol Reprod Biol. 1992 Jul 3;45(2):119-24.
49. Pantos K, Nikas G, Makrakis E, Stavrou D, Karantzis P, Grammatis M. Clinical value of endometrial pinopodes detection in artificial donation cycles. Reprod Biomed Online. 2004 Jul;9(1):86-90.

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