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Establishment of *In Vitro* 3-Dimensional Culture System of Mouse Endometrial Cells

I. Cytohistological Study on Mouse Endometrium

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마우스 자궁내막 세포를 이용한 3차원적 배양시스템 확립에 관한 연구

I. 마우스 자궁내막에 관한 세포조직학적 연구

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남화경 · 김은영 · 이금실 · 박세영 · 박은미 · 권중균[1] · 윤산현[2] · 박세필 · 임진호[2]

연구목적: 본 연구는 마우스의 자궁에 있어 착상시 일어나는 자궁내막의 변화를 미세구조적 측면에서 관찰하고, 이러한 기초 자료를 바탕으로 마우스 자궁내막세포를 이용한 3차원적 체외 배양시스템을 확립하는데 있다. 실험동물은 임신이 유도된 6~8 주령의 ICR을 이용하였다.

연구재료 및 방법: 생검 조직은 hCG 접종 후 D1과 D5에 자궁 전체를 적출하여 획득하였다. 채취된 조직은 2.5% glutaraldehyde와 1% osmium tetroxide를 이용하여 고정시킨 후, 탈수, 포매 및 절편 과정을 거쳐 염색시키고, 광학현미경, 투과전자현미경을 이용하여 관찰하였으며, 주사전자현미경을 이용한 관찰을 위한 표본은 고정된 조직을 탈수, 건조 및 코팅 과정을 거쳐 획득하였다.

결과: 1) 광학현미경상에서, 후기 분비기인 D5의 생검 조직은 초기 분비기인 D1의 조직에 비해 결합조직의 증가에 따른 기질층의 확장이 두드러졌으며, 이와 함께 자궁내막선과 혈관이 크게 발달되어 있었다. 2) 투과전자현미경상에서, 마우스 자궁내막의 미세구조는 단층원주형의 표면상피세포층, 기저층 및 기질층의 3층을 이루고 있었다. 또한, D5에서는 미세융모, 소포체, 골지체, 지질, 글리코겐 및 분비 과립 그리고 기저층의 표면적이 크게 확장되어 있었다. 3) 주사전자현미경상에서, 분비기가 진행될수록 마우스 표면상피세포의 주름의 정도와 미세융모의 분포가 크게 증가함을 알 수 있었으며, 특히 마우스의 착상시기인 D5에서는 자궁 수용성의 표지자인 자궁돌기의 출현이 두드러졌다. 마우스의 자궁돌기는 자궁내벽을 따라 불규칙적으로 좁은 지역에 분포하고 있었으며, 동일 표본 내에서도 서로 다른 발달 단계를 보여주고 있었다.

결론: 마우스 자궁내막의 형태학적 변화에 관한 이러한 관찰 결과는 마우스의 자궁내막이 발정주기 중 특히 착상직전의 시기에 커다란 형태학적 변화를 경험함을 보여주었다.

Key Words: Mouse, Implantation, Endometrium, Pinopodes, Ultrastructure

For successful pregnancy in IVF program, it is necessary to study interactions between the embryo and maternal tissue at implantation as well as to improve quality of embryos at pre-implantation.[1] However, the study of these early stages of human implantation *in vivo* for practical purposes is impossible, several culture systems *in vitro* have therefore been developed.

But the previous studies on 3-dimensional culture systems *in vitro* fully imitation of the normal structure and function of the endometrium *in vivo* were few.[2,3] Also, in order to assess exactitude of the established culture system, there is a necessity for identification *in vivo* situations.

In mouse, there is no attempt to establish about 3-dimensional culture system *in vitro* reflection the true situation *in vivo*, although some authors, who have studied implantation mechanisms or aspects, have reported and commented on monolayer cultures.[4~6] Since Nilsson (1958) had described on the ultrastructure of mouse uterine surface epithelium under different hormone level, none of one investigated the uterine endometrial morphology *in vivo* during peri-implantation period in mouse.[7]

The present study was to obtain the fundamental information for the establishment of 3-dimensional culture system of mouse endometrial cells *in vitro*, and identify the ultrastructural changes of mouse endometrium during peri-implantation period.

MATERIALS AND METHODS

1. Experimental animals

Female ICR mice at 6~8 weeks of age were superovulated by the intraperitoneal injection of 7.5 IU of PMSG (Sigma), followed 48 hr later by the injection of 7.5 IU of hCG (LG Chem.). Immediately thereafter, in order to conduct on pregnant, the mice were mated overnight with males of the same strain. As the day of hCG injection was designated as day 0, the mouse endometrial tissues were obtained at days (D1) and 5 (D5), early secretory phase and late secretory phase, implantation time, respectively.

2. Endometrial tissue collection and preparation for electron microscopy

For transmission electron microscopy (TEM) and scanning electron microscopy (SEM), the fresh biopsied materials were commonly fixed in 2.5% glutaraldehyde (v/v, WAKO) in a phosphate buffer (0.1 M, pH 7.4) for 3 hr at 4℃. For LM and TEM, the specimens were washed in 0.1 M phosphate buffer and fixed in 1% osmium tetroxide added 0.1 M phosphate buffer for 1 hr at 4℃. Subsequently, they were dehydrated and embedded in Epon. The embedded biopsies were sectioned into semi-thin and ultra-thin sections. Semi-thin sections were stained with 1% toluidine blue and were observed under a Olympus IMT2-NA2, while ultra-thin sections were contrasted with uranyl acetate and lead citrate and examined under a Hitachi H-600. For SEM, the specimens were washed in 0.1 M phosphate buffer, fixed in 1% osmium tetroxide and dehydrated in graded ethanols. After drying to the critical point, the specimens were sputter-coated with gold and examined under a Hitachi S-2680N at 15 kV.

RESULTS

1. The morphology of mouse biopsied endometrium by LM

At D1 (early secretory phase), the mouse biopsied endometrium was consisted of a monolayer epithelial cells and stromal cells, which included a few glands and vessels (Figure 1A). And at D5 (late secretory phase), the stromal layer was extended by increased connective tissues and the endometrial glands and vessels were fully developed compared with D1 (Figure 1B).

2. The ultrastructure of mouse biopsied endometrium by TEM

The mouse biopsied endometrial cells were consisted of 3-layers, a simple columnar epithelial cells, basement membrane and stromal cells filled into the extracellular matrix. At D1, the epithelial cells were polarized, with microvilli on the surface (Figure 2A), apical tight junctions and desmosomes between epithelial cells (Figure 2B). And at D5, in epithelial cells, the distribution of microvilli, endoplasmic re-

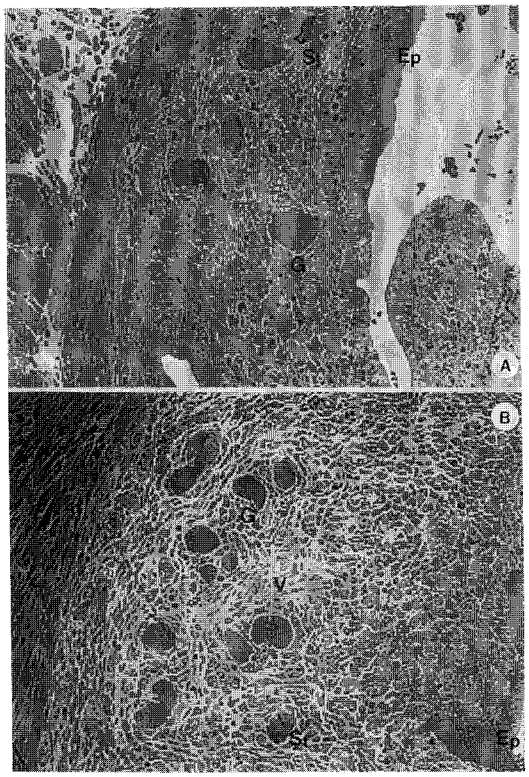

Figure 1. Light micrographs of the mouse biopsied endometrum. (**A**) At D1, the endometrium was consisted of a monolayer epithelial cells (Ep) and stromal cells (St), which included a few glands (G) and vessels. ×200 (**B**) At D5, the stromal layer was extended by increased connective tissues and the endometrial glands and vessels (V) were fully developed. ×200.

ticulum, Golgi body, lipid and glycogen deposits, secretory granules and surface areas were increased. And in stromal layer the extracellular matrix was extended (Figure 2C, D).

3. The ultrastructure of mouse biopsied endometrium by SEM

The mouse uterine luminal apical surface was showed accelated maturation. At D1, there were many graves and foldings (Figure 3A) and the cells were bulging and covered with well-developed microvilli. These patterns were positioned at folding areas (Figure 3B). At D5, the cell bulging was increased and the microvilli appeared more longer (developing pinopodes). Wide areas were covered by these shapes in same sample (Figure 3C). And at the same time, the microvilli were virtually absent in perfect protrusions (fully developed pinopodes) so that they were seemed the cells with smooth naked membrane. These patterns distributed at narrow sites some folding areas (Figure 3D). At D5, cell membrane was wrinkled, with few small tips of microvilli (regressing pinopodes) (Figure 3E, F).

DISCUSSION

In this study, we confirmed that the mouse endometrium at oestrus cycle was experienced the dramatic morphological changes using LM, TEM and SEM. There were characteristics found in the human endometrium at implantation time; apical junctional complexes, vesicles at the apical cell membrane, interdigitation of the lateral cell membranes, invagination of the nucleus, a rough endoplasmic reticulum, a supranuclear Golgi system, glycogen deposits in the basal and apical cytoplasm, giant mitochondria and nucleolar channel system.[8,9] Our morphological findings in mouse referring to the human *in vivo* material were in accordance with the literature. However, in rabbit and human, it has known that there were the cilia with microvilli on the apical epithelial cells,[10,11] while in the specimens of the mouse, we could not demonstrate the cilia. This result was similar to that of the rat.[12] Maybe, it is thought to result from the peristaltic movements of the uterus of the mouse for multiple pregnancy.[13] It demonstrated that the morphology of uterine endometrium was corresponded to the difference of implantation processes (blastocysts attachment and trophoblastic outgrowth) between the species.[5]

The pinopodes as a specific marker for uterine receptivity had described the ectoplasmic projections by Psychoyos and Mandon[14] and have named for pinocytotic activity.[12] In the present study, the mouse pinopodes only localized in narrow sites within the antimesometrial

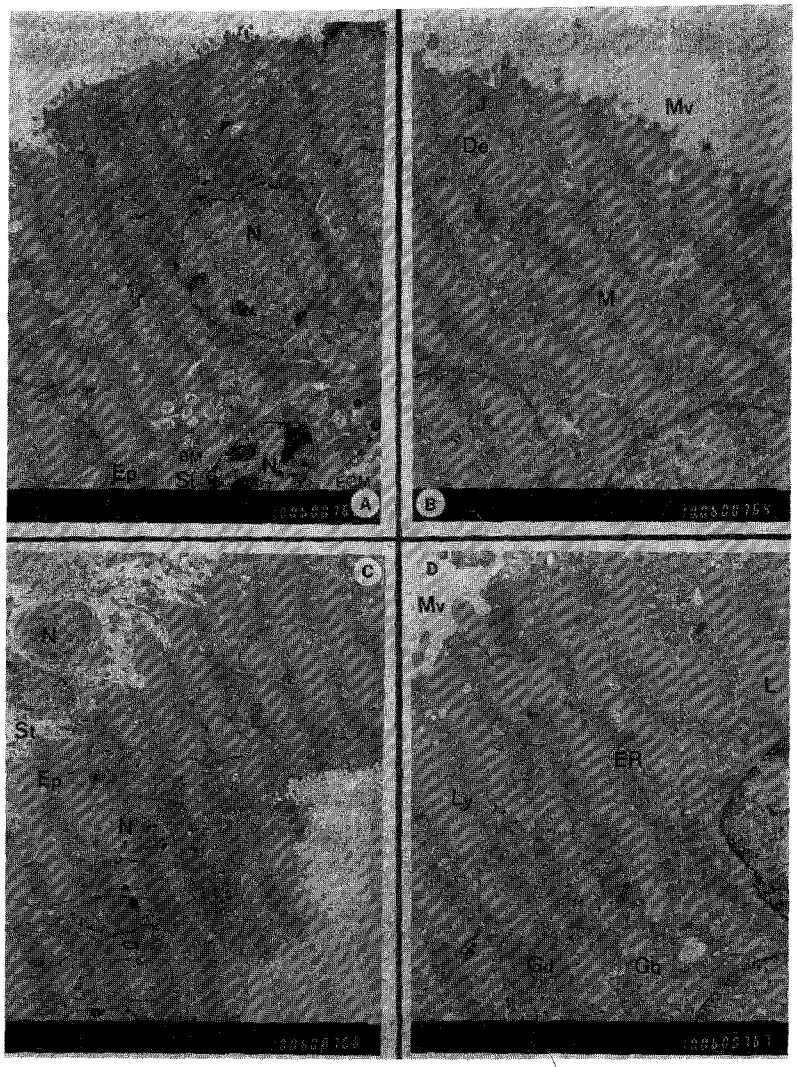

Figure 2. Transmission electron micrographs of the mouse biopsied endometrial cells. The endometrium was consisted of 3-layers, a simple columnar epithelial cells (Ep), basement membrane (BM) and stromal cells (St) filled into the extracellular matrix (ECM). **(A~B)** At D1, the epithelial cells were polarized, with microvilli (Mv) on the surface (A: ×4,000), apical tight junctions (J) and desmosomes (De) between epithelial cells (B: ×10,000). **(C~D)** At D5, in epithelial cells, the distribution of microvilli, endoplasmic reticulum (ER), Golgi body (Gb), lipid (L) an glycogen deposits (Gd), secretory granules and surface areas were increased. C: ×2,000, D: ×10,000.

regions at the luminal surface. And the several number of them was developed the "fully developed" stage, and they were not appeared the same stage in other sites even though positioned at the same sample. That is, the appearance of pinopodes was found at D5, commonly designated implantation time but the stage of pinopode process was different from each other. These differences will be caused to the different implantation time, so that they might be the reason of implantation failure. Also, the shape of pinopode in mouse was different from human. In mouse, the pinopode was a protrusion with rounding up, while in human that was a bulging with shapes resembling mushrooms or flowers.[1] These aspects

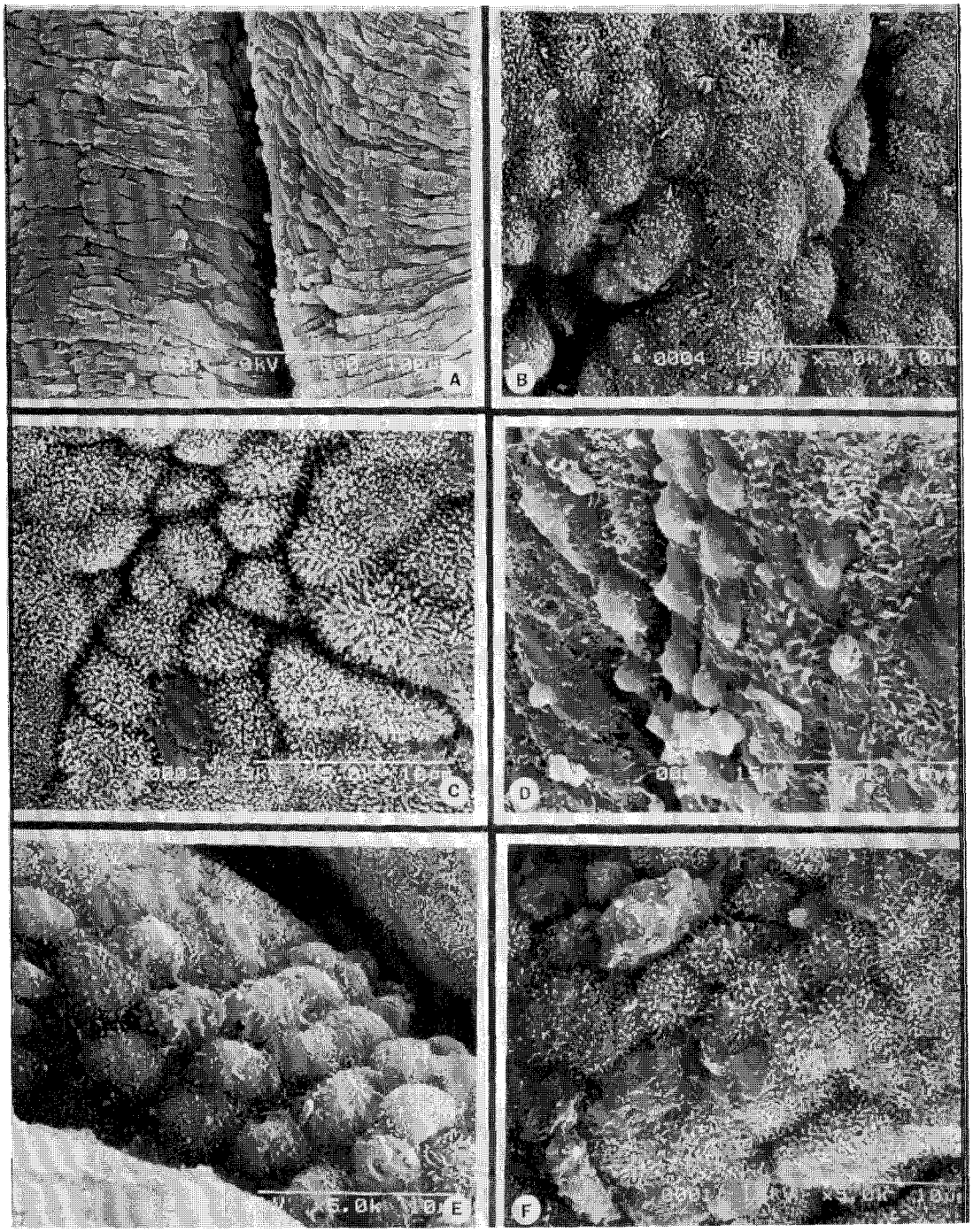

Figure 3. Scanning electron micrographs of the mouse uterine luminal apical surface. **(A)** At D1, there were many graves and foldings. ×500. **(B)** At D1, the cells were bulging and covered with well-developed microvilli. These patterns were positioned at folding areas. ×5,000. **(C)** At D5, the cell bulging was increased and the microvilli appeared more longer. Wide areas were covered by these shapes in same sample. ×5,000. **(D)** At D5, the microvilli were virtually absent in perfect protrusions so that they were seemed the cells with smooth naked membrane. These patterns were distributed at narrow sites some folding areas. ×5,000. **(E~F)** At D5, cell membrane was wrinkled, with few small tips of microvilli. ×5,000.

about irregularity of distribution (spotty) and stages (different shapes) of mouse uterine pinopodes resemble in case of the rat.[12] Anyway, the pinopode as the indicator of implantation window, plays a role in producing apposition of the hatched blastocysts.[12] In addition, the successful implantation could be achieved resulting in ovo-endometrial synchrony.[1] Therefore, to speculate the implantation mechanisms *in vivo*, it is required the establishment of culture system *in vitro* with uterine receptivity.

These results indicated that the mouse endometrium was appeared the different shape of pinopode with human but the similar processes of dramatic morphological changes during peri-implantation period, so that these aspects demonstrated the species-specific. The further study needs to be established *in vitro* 3-dimensional culture system on the basis of results of the present study.

SUMMARY

This study was designed to identify the ultrastructural changes of mouse endometrium during peri-implantation period and obtain the fundamental information for the establishment of 3-dimensional culture system of mouse endometrial cells *in vitro*. The used female ICR mice (6~8 wks) were conducted on pregnant. The biopsies were obtained from whole uterus at cycle day 1 (D1) and day 5 (D5) after hCG injection and mating. The biopsied materials were fixed 2.5% glutaraldehyde and 1% osmium tetroxide. Subsequently, for observation using light and transmission electron microscopy (LM and TEM), they were dehydrated and embedded in Epon and the embedded biopsies were sectioned and stained. For scanning electron microscopy (SEM), the fixed specimens were dehydrated, dried and coated with gold. 1) For LM, the biopsied materials at D5 (late secretory phase) were appeared the extended stromal layer by increased connective tissues and the fully developed endometrial glands and vessels compared with D1 (early secretory phase). 2) For TEM, the mouse endometrium was consisted of 3-layers, a simple polarized columnar epithelial cells, basement membrane and stromal cells. At D5, the distribution of microvilli, endoplasmic reticulum, Golgi body, lipid and glycogen deposits, secretory granules and surface area of basement membrane were increased. 3) For SEM, the degree of folding and microvilli of surface of mouse epithelial cells was became more and more according to the process of secretory phase, and at D5, implantation time of mouse, the appearance of pinopodes as a specific marker of uterine receptivity was found. The uterine pinopodes of mouse were found in narrow sites at the luminal surface, irregularity and appeared the different stages in the same sample. Therefore, these results indicated that the mouse endometrium was experienced dramatic morphological changes during peri-implantation period.

REFERENCES

1. Nikas G, Osman HD, James PT, Howard WJ. Endometrial pinopodes indicate a shift in the window of receptivity in IVF cycles. Hum Reprod 1999; 14: 787-92.
2. Bentin-Ley U, Pedersen B, Lindenberg S, Falck Larsen J, Hamberger L, Horn T. Isolation and culture of human endometrial cells in a three-dimensional culture system. J Reprod Fertil 1994; 101: 327-32.
3. Park DW, Yang HW, Kwon HC, Chang KH, Kim SK, Cho DJ, et al. 3-Dimensional culture system of endometrial cells for studying the human implantation mechanism. Kor J Fertil Steril 1999; 26: 1-8.
4. Van Blerkom J, Chavez D. Morphodynamics of outgrowths of mouse trophoblast in the presence and absence of a monolayer of uterine epitheilium. Am J Anat 1981; 162: 143-55.
5. Lindenberg S, Hyttel P, Sjøgren A, Greve T. A comparative study of attachment of human, bovine and mouse blastocysts to uterine epithelial

monolayer. Hum Reprod 1989; 4: 446-56.
6. Taniguchi F, Harada T, Yoshida S, Iwabe T, Onohara Y, Tanikawa M, et al. Paracrine effects of bFGF and KGF on the process of mouse blastocyst implantation. Mol Reprod Dev 1998; 50: 54-62.
7. Nilsson O. Ultrastructure of mouse uterine surface epithelium under different estrogenic influences 2. Early effect of estrogen administered to sprayed animals. J Ultrastruct Res 1958; 2: 73-95.
8. Bentin-Ley U, Lindenberg S, Horn T, Falck Larsen J. Ultrastructure of endometrial epithelial cells in a three-dimensional cell culture system for human implantation studies. J Assist Reprod Genet 1995; 12: 632-8.
9. Nikas G, Psychoyos A. Uterine pinopodes in peri-implantation human endometrium. Ann N Y Acad Sci 1997; 816: 129-42.
10. Segalen J, Lescoat D, Chambon Y. Ultrastrucural aspects of uterine secretion during the establishment of pregnacy in the rabbit: role of the egg. J Anat 1982; 135: 281-9.
11. Loke YW, Ashley K. Decidualisation. In: Human implantation: cell biology and immunology. Cambridge Univ Press 1995: 18-31.
12. Enders AC, Nelson DM. Pinocytotic activity of the uterus of the rat. Am J Anat 1973; 138: 277-300.
13. Hogan B, Beddington R, Costantini F, Lacy E. Manipulating the mouse embryo. A laboratory manual 2nd ed. Cold Spring Harbor Laboratory Press; 1994. p51-54.
14. Psychoyos A, Mandon P. Scanning electron microscopy of the surface of the rat uterine epithelium during delayed implantation. J Reprod Fertil 1971; 26: 137-8.