

Full Length Research Paper

Epithelial lining of the endometrium during the luteal phase in patients under controlled ovarian hyperstimulation

Reda Awadallah Saleh¹, Mohamed A. Dkheil², Abdel-Azeem S. Abdel-Baki² and Saleh Al-Quraishy²

¹Department of Anatomy, Faculty of Medicine, Al-Azhar University, Egypt.

²Department of Zoology, College of Science, King Saud University, P.O. Box: 2455, Riyadh- 11451, Saudi Arabia.

Accepted 13 November, 2009

The endometrium is receptive for the embryo and presents an implantation window for a limited time. This study is aimed at highlighting an alternation in pinopod expression and to provide more analysis of the structural characteristics of epithelial lining of the endometrium during luteal phase in patients undergoing controlled ovarian hyperstimulation (COH). Twelve oocyte donors were used. They underwent two endometrial biopsies 2-7 days after human chorionic gonadotrophin (HCG) administration. Endometrial epithelial surface appearance was evaluated by scanning electron microscopy. Microvilli became more uniformly distributed as the luteal phase progressed. Also the central aspects of the cells appeared to protrude more into the endometrial lumen as the cycle progressed. Apical protrusions (pinopods) were noted to develop and regress during the midluteal phase after HCG administration. Pinopods began development in the region of the glandular orifices to become much denser at the glandular orifices than in regions further away from the glands. In conclusion, epithelial lining of endometrium in stimulated cycle during the luteal phase progresses in an orderly manner. This advanced development may result in an alteration of the window of implantation between the developing endometrium and the developing blastocyst and affect pregnancy rates in women undergoing controlled ovarian hyperstimulation.

Key words: Epithelial lining, endometrium, ovarian hyperstimulation.

INTRODUCTION

Endometrium may provide important clues to receptivity because the implanting blastocyst must first come into physical contact with the overlying epithelium (Daayana and Holland, 2009). Ludwig and Metzger (1976) provided the first systematic descriptive analysis of the endometrial surface patterns throughout the menstrual cycle using scanning electron microscopy (SEM). Further attempts to understand how the administration of exogenous hormones affected the ultrastructural characteristics were proven (Nilsson et al., 1980; Novin et al., 2007) and the effects of levonorgestrel and estradiol (E₂) evaluated. Implantation failure is a major factor limiting the success of *in vitro* fertilization (IVF). On the average, up to 90% of

apparently healthy zygotes transferred into the uterus are destined to vanish (Liu et al., 1988), giving no signs of trophoblastic attachment and production of human chorionic gonadotrophin (HCG). The initiation of implantation requires a blastocyst to interact with an endometrium that has gained receptivity. While the importance of embryo quality has been clearly demonstrated (Liu et al., 1988; Rajesh et al., 2007), a further cause for the reduced implantation rates may be an impairment of endometrial receptivity, due to high concentrations of sex steroids resulting from ovarian stimulation used of IVF (Daayana and Holland, 2009). This suggestion is supported by higher implantation rates in hormonal replacement treatment (HRT) cycles after ovum donation than the standard IVF cycles (Edwards et al., 1991; Nikos et al., 2006). At the time of implantation, the apical membranes of the epithelial cells lining the uterine cavity loses their microvilli and develop large and smooth membrane pro-

*Corresponding author. E-mail: mohameddkheil@yahoo.com.
Tel: 00966-14675754.

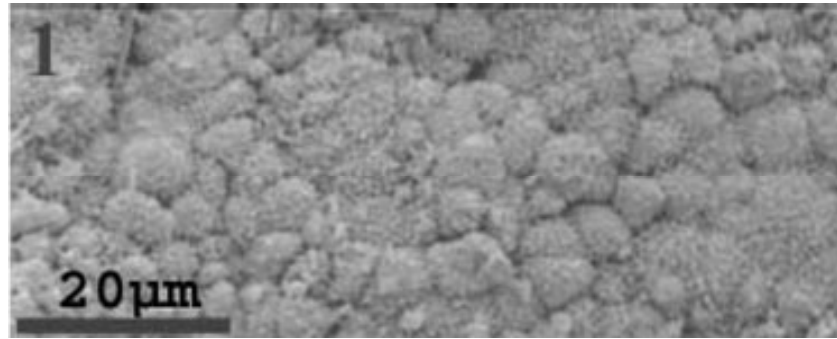


Figure 1. Scanning electron micrograph of early luteal phase endometrium of oocyte donors under COH, showing microvilli on the nonciliated cells are denser on the central portion of the cells. Bar = 20 μm .

jections (Daayana and Holland, 2009; Psychoyos and Mandon, 1971). Due to their pinocytotic function, these projections were named pinopodes (Enders and Nelson, 1973).

Hormonal treatments have been shown to advance or retard the timing of pinopode formation. During ovarian stimulation with clomiphene citrate followed by human menopausal gonadotrophin (HMG)/HCG, pinopodes form earlier, on days 17 or 18 (Martel et al., 1987). In contrast, HRT pinopodes form later, around the 22 day (Psychoyos and Rider, 1994). Yet these days represent only mean values derived from a group of patients. When sequential (every 2-3 days) midluteal samples were taken from natural or HRT cycles, the timing of pinopode appearance was found to vary up to 5 days between women. Fully developed pinopodes were always confined to one sample, showing a life span of less than 48 h. Finally the number of pinopodes was different between patients and there was a strong correlation between pinopode numbers and implantation after embryo transfer (Nikas et al., 1996, 1997; Nikas and Psychoyos, 1997).

The aim of this study is to highlight alternations in pinopod expression and to provide more analysis of the structural characteristics of epithelial lining of the endometrium during luteal phase in patients undergoing controlled ovarian hyperstimulation (COH).

MATERIALS AND METHODS

Twelve oocyte donations were used in this study. All patients used gave written consent before the start of the experiment. Donors underwent controlled ovarian hyperstimulation using standard IVF methodology. The stimulation protocol has been described previously (Sauer et al., 1989). Briefly, the patients initially underwent pituitary down-regulation with the Gonadotropin-releasing hormone (GnRH) agonist leupold acetate, which was continued until HCG administration. Follicle stimulation was achieved with HMG and ovulation was triggered with 10000 IU of HCG. A total of 24 biopsies were performed using a pipelle. Two biopsies were performed during each cycle, ranging from days 2-7 after HCG administration. The samples were preserved immediately in 3% glutaraldehyde solution. Specimens then were processed for SEM

by dehydration in graded series of ethanol, followed by a graded series of ethanol- hexamethyldisilazone solution to minimize structural damage and shrinkage (Adams et al., 1987). Specimens were mounted on an aluminum stub with colloidal graphite and coated with gold.

RESULTS

The epithelium covering the human endometrial surface is composed of two cell types, the nonciliated cells, which bear microvilli and the ciliated cells. The ciliated cells appeared to be distributed randomly over the endometrial surface. Ciliated cells were isolated from one another by the nonciliated cells. This study was focused on the nonciliated cells. The ratio between the ciliated and nonciliated cells ranged from 1:20 to 1:30 and remained constant between individual patients as the cycle progressed.

The nonciliated cells underwent a number of changes. In the early luteal phase (2 days after HCG administration), the microvilli were located more centrally and were organized into tufts (Figure 1). As the cycle progressed, the microvilli become more diffuse over the cell surface. Furthermore, the nonciliated protruded into the lumen as the cycle progressed (Figure 2).

Uterine pinopods, which are believed to mark the window of implantation, were noted to develop in an orderly manner. The pinopods initially appeared as small projections or droplets on the nonciliated cells (Figure 3). Their appearance was noted to occur 4-7 days after HCG administration, which correlates with cycle days 17-20. In addition, the pinopods were noted to be much denser at glandular orifices than in regions further away from the glands. After 7 days of hyperstimulation, pinopods were noted to be regressed at the glandular orifices (Figure 5) in comparison to Figure 4.

DISCUSSION

In the present study, the endometrium tended to develop in an orderly synchronous manner. However, as marked

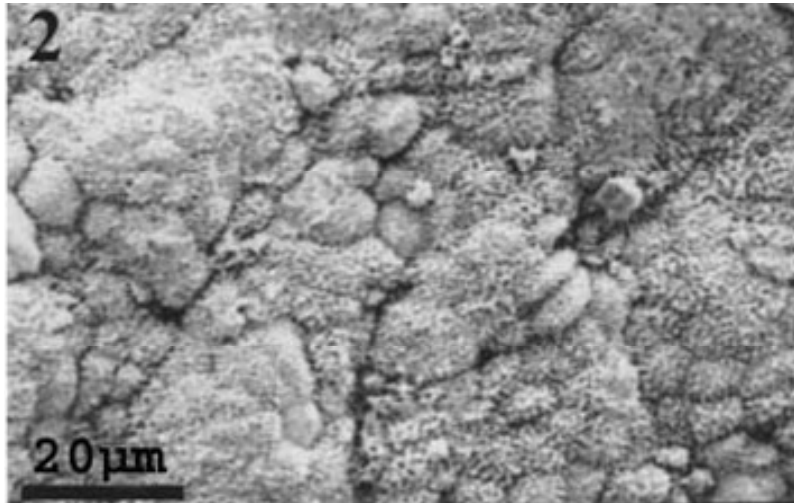


Figure 2. Scanning electron micrograph of early luteal phase endometrium of oocyte donors under COH, showing microvilli are more diffuse over the nonciliated cells. Cells are more protruded into the lumen. Bar = 20 μm.

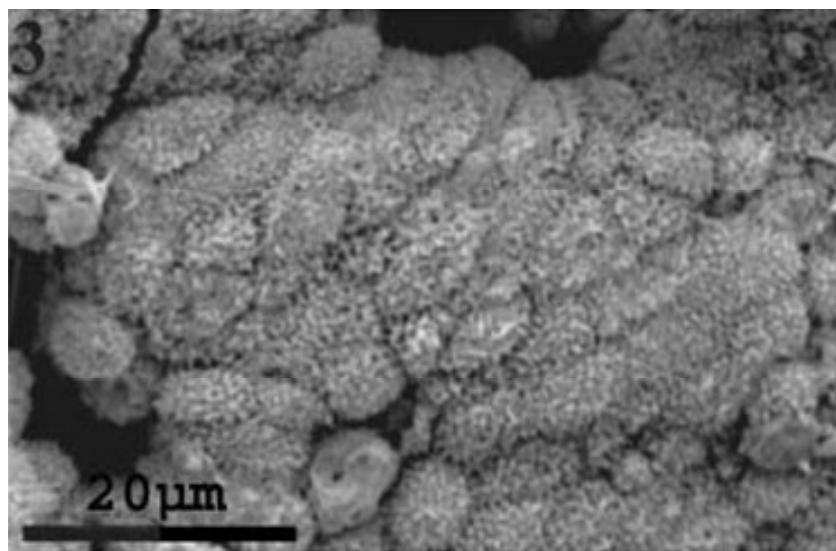


Figure 3. Scanning electron micrograph of early luteal phase endometrium of oocyte donors under COH, showing: formation of pinopods as small projections from the nonciliated cells. Bar = 20 μm.

by the premature appearance of pinopods formation, the progression of the luteal phase appeared to be advanced by approximately 24 - 48 h. Ciliated cells remained relatively constant throughout the luteal phase as regard to their distribution, density and ultrastructural characteristics. This may reflect the ability of these cells to withstand the effect of variable hormonal stimulation. However, nonciliated cells underwent a series of changes as the luteal phase progressed. In the early luteal phase, the microvilli initially developed more centrally in tufts and then became diffuse over the cell surface as the cycle progressed. In addition, the cells began to protrude into

the lumen as the cycle advanced and took on a cobblestone appearance. This protrusion may be secondary to uptake of fluids from the endometrial cavity as the uterus prepares for arrival of the blastocyst into the cavity.

In nonciliated cells, fine projections from their apical parts were believed to coincide with the expected period of blastocyst implantation. These projections have been demonstrated to be involved actively in the pinocytosis of uterine fluids and macromolecules (Enders and Nelson, 1973; Comillie and Lauweryns, 1984). Their appearance is thought to be related closely to uterine receptivity to im-

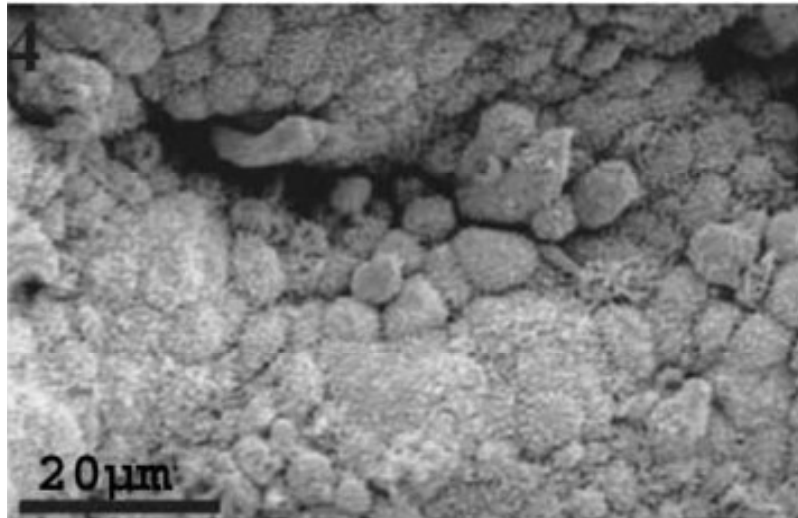


Figure 4. Scanning electron micrograph of early luteal phase endometrium of oocyte donors under COH, showing: well developing and maturation of uterine pinopods as a projection of non ciliated cells around the glandular orifices. Bar = 20 μm .

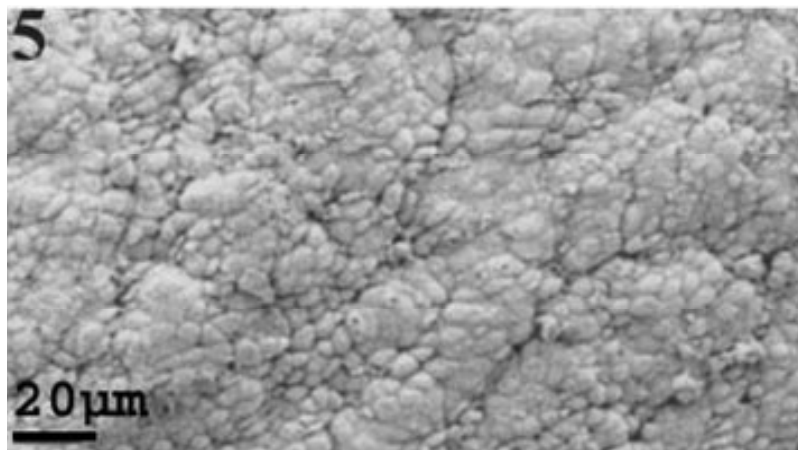


Figure 5. Scanning electron micrograph of early luteal phase endometrium of oocyte donors after 7 days of hyperstimulation showing regression of the small projection (pinopods) from the non-ciliated cells. Bar = 20 μm .

plantation. In rat, Psychos and Mandon (1971) suggested that the presence of fully formed pinopods is associated with a short period of uterine receptivity for implantation of the ovum. Lifespan of pinopods lasts for only 48 h and the fully developed pinopods exists for 24 h only (Psychoyos and Rider, 1994; Nikas et al., 1996; Usadi et al., 2003).

In the present study, pinopods expression was present 4-7 days after HCG corresponding to cycle days 17-20. In contrast, in the natural cycle it was days 19 to 21 (Martel et al., 1987).

In natural cycles, there is an inherent synchrony between the maturing uterus and the developing embryo,

ensuring that both partners meet at the right stage. It is therefore reasonable to postulate that reduced implantation in IVF cycles is due to an ovo-endometrial asynchrony. In IVF, embryonic development is probably delayed while the uterus is advanced, resulting in an early closure of the nidation window, before the zygote eventually reaches a stage capable of initiating implantation. Additionally, implantation failure may be explained by the disparity in maturation between the stroma and the epithelium observed in histology (Toner et al., 1993). Since a paracrine communication between epithelium and stroma may be important at the beginning of implantation, this disparity could compromise uterine receptivity

or early trophoblastic invasion.

Ovarian stimulation using gonadotrophins alone appears more physiological than using a combination of clomiphene citrate and gonadotrophins, since in the latter the surface morphology was greatly advanced, with pinopodes already regressed by day 20, in 85% of cycles studied (Martel et al., 1987). This is in agreement with the fact that ovarian stimulation using gonadotrophins only leads to higher pregnancy rates (Menchaca and Rubianes, 2007). However the findings of this study coincided with those of Kolb et al. (1997) who observed pinopodes as soon as 4 days after HCG. These investigators might have possibly included as pinopodes small apical projections which appear occasionally at the uterine folds during early luteal phase. In conclusion, epithelial lining of the endometrium in stimulated cycle during the luteal phase progresses in an orderly manner. Pinopods expression was noted at an earlier phase of endometrial maturation. This advanced development may result in an alteration of the window of implantation between the developing endometrium and the developing blastocyst and affect pregnancy rates in women undergoing COH.

ACKNOWLEDGMENTS

This research was supported by King Saud University through the Nobel Laureate collaboration project; number (NLCP-1/2009). We, therefore, thank the Nobel laureate, Prof. Günter Blobel (Laboratory of Cell Biology, Howard Hughes Medical Institute, The Rockefeller University, New York).

REFERENCES

- Adams JL, Battjes CJ, Buthala DA (1987). Biological specimen preparation for SEM by a method other than critical point drying. In: Bailey JW, editor. Proceeding of the 45th annual meeting of the Electron Microscopy Society of America. San Francisco: San Francisco Press Inc. pp. 451-452.
- Daayana S, Holland CM (2009). Hormone replacement therapy and the endometrium. *Menopause Int.* 15: 134-138.
- Edwards RG, Marcos S, Macnamee M, Balmaceda JP, Walters DE, Asch R (1991). High fecundity in amenorrhoeic women in embryo-transfer programmes. *Lancet*, 338: 292-294.
- Enders AD, Nelson DM (1973). Pinocytotic activity of the uterus of the rat. *Am. J. Anat.* 138: 277-300.
- Kolb, AB, Najmabadi S, Paulson RJ (1997). Ultrastructural characteristics of the luteal phase endometrium in donors undergoing controlled ovarian hyperstimulation. *Fertil. Steril.* 67: 625-630.
- Lauweryns FJ, Lauweryns JM (1984). Fluid and protein clearance in the rat endometrium. Part II: Ultrastructural evidence for the presence of alternative, non-lymphatic clearance mechanisms in the rat endometrium. *Experientia*, 40: 1264-1266.
- Liu HC, Jones GS, Jones Jr. HW, Rosenwaks Z (1988). Mechanisms and factors of early pregnancy wastage in in-vitro fertilization-embryo transfer patients. *Fertil. Steril* 50: 95-101.
- Ludwig H, Metzger H (1976). The human female reproductive tract: A scanning electron microscopic atlas. Berlin: Springer-Verlag.
- Martel D, Frydman R, Glissant M, Maqqioni C, Roche D, Psychoyos A (1987). Scanning electron microscopy of postovulatory human endometrium in spontaneous cycles and cycles stimulated by hormone treatment. *J. Endocrinol.* 114: 319-324.
- Menchaca A, Rubianes E (2007). Pregnancy Rate Obtained with Short-term Protocol for Timed Artificial Insemination in Goats. *Reprod. Domest. Anim.* 42: 590-593.
- Nikas G, Garcia-Velasco J, Pellicer A, Simon C (1997). Assessment of uterine receptivity and timing of embryo transfer using the detection of pinopodes. *Hum. Reprod.* 12: 60-69.
- Nikas G, Psychoyos A (1997). Uterine pinopodes in peri-implantation human endometrium: Clinical relevance. *Ann. N.Y. Acad. Sci.* 816: 129-142.
- Nikas G, Reddy N, Winston RML (1996). Implantation correlates highly with the expression of uterine pinopodes in ovum recipients under HRT: A preliminary study. Abstr. (FR21) at the IX World Congress in Human Reproduction, Philadelphia PA May 29-June.
- Nikos F, Vlahos Christopher W, Lipari Brandon B, Tsung-Hsuan L, Jeremy AK, Ie-Ming S, Konstantine F, Zhao Y (2006). Effect of Luteal-Phase Support on Endometrial L-Selectin Ligand Expression after Recombinant Follicle Stimulating Hormone and Ganirelix Acetate for *in Vitro* Fertilization. *J. Clin. Endocrinol. Metabolism*, 9: 4043-4049.
- Nilsson O, Englund D, Weiner E, Victor A (1980). Endometrial effects of levonorgestrel and estradiol: a scanning electron microscopy study of the luminal epithelium. *Contraception*, 22: 71-83.
- Novin MG, Bazy P, Rad JS, Sarani SA, Farzadi L, Ghasemzadeh A (2007). Morphometric study of GnRH analog/HMG/HCG effects on ultrastructure of human endometrial epithelium in early and mid-luteal phase. *J. Obstet. Gynaecol. Res.* 33: 681-687.
- Psychoyos A, Mandon P (1971). Study of the surface of the uterine epithelium by scanning electron microscope. Observations in the rat at the 4th and 5th day of pregnancy. *C. R. Acad. Sci. Hebd. Seances. Acad. Sci. D.* 272: 2723-2725.
- Psychoyos A, Rider V (1994). Inhibition of progesterone receptor function results in loss of basic fibroblast growth factor expression and stromal cell proliferation during uterine remodelling in the pregnant rat. *J. Endocrinol.* 140: 239-349.
- Rajesh H, Yong YY, Zhu M, Chia D, Yu SL (2007). Growth hormone deficiency and supplementation at *in vitro* fertilisation. *S. M. J.* 48: 514-518.
- Sauer MV, Paulson RJ, Macaso TM, Francis-Hernandez M, Lobo RA (1989). Establishment of a nonanonymous donor oocyte program: preliminary experience at the University of Southern Carolina. *Fertil. Steril.* 52: 433-436.
- Toner JP, Veeck LL, Muasher SJ (1993). Basal follicle-stimulating hormone level and age affect the chance for and outcome of preembryo cryopreservation. *Fertil. Steril.* 59: 664-667.
- 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.