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Full Length Research Paper

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

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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 hyperstimmulation (COH). Twelve oocyte donors were used. They underwent two endometrial biopsies 2-7 days after human chronic 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 hyperstimmulation.

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 (E_2) 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-

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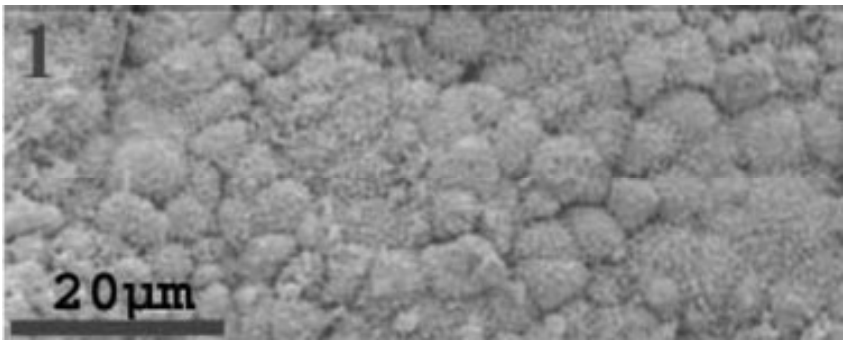

Figure 1. Scanning electron micrograph of early luteal phase endometrium of oocyte donors under COH, showing microvilli on the nonciliated cells are denser on the central portion of the cells. Bar = 20 µm.

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 hyperstimmulation (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 leopold 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. Tow 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 projecttions 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 hyperstimmulation, 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

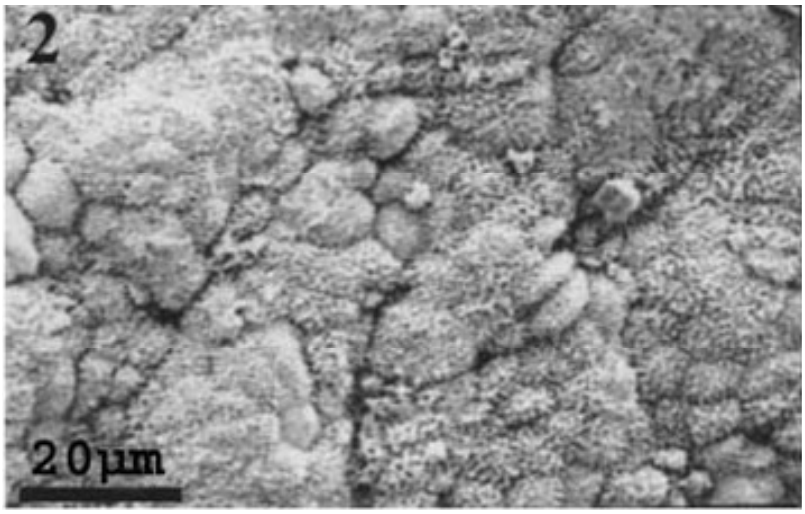

Figure 2. Scanning electron micrograph of early luteal phase endometrium of oocyte donors under COH, showing microvilli are more diffuse over the nonciliated cells. Cells are more protruded into the lumen. Bar = 20 µm.

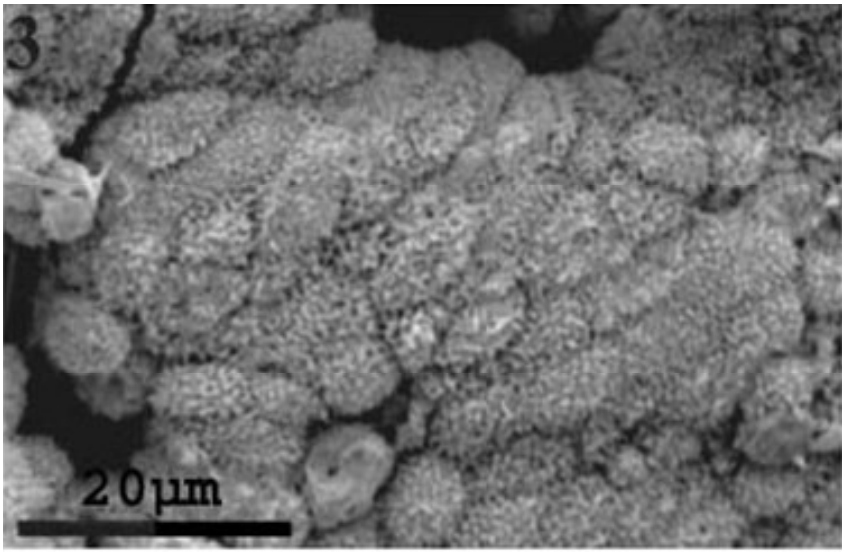

Figure 3. Scanning electron micrograph of early luteal phase endometrium of oocyte donors under COH, showing: formation of pinopods as small projections from the nonciliated cells. Bar = 20 µm.

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-

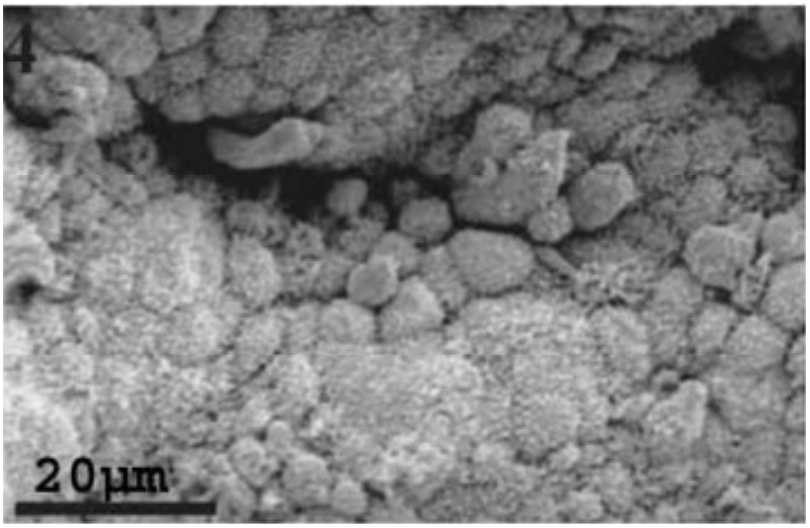

Figure 4. Scanning electron micrograph of early luteal phase endometrium of oocyte donors under COH, showing: well developing and maturation of uterine pinopods as a projection of non ciliated cells around the glandular orifices. Bar = 20 µm.

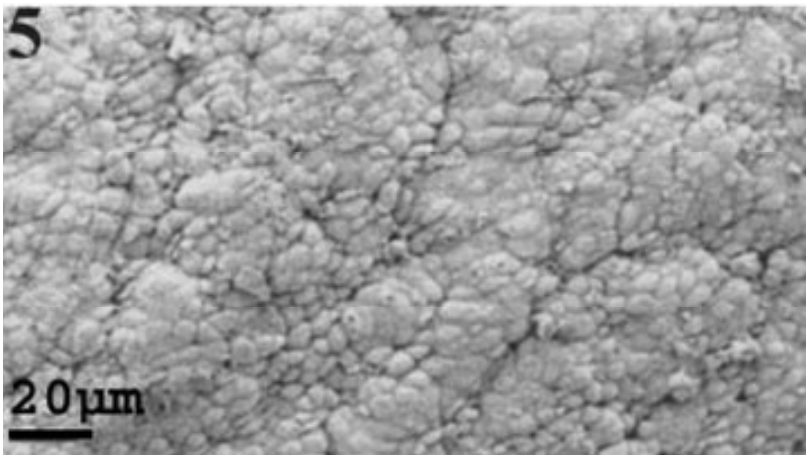

Figure 5. Scanning electron micrograph of early luteal phase endometrium of oocyte donors after 7 days of hyperstimulation showing regression of the small projection (pinopods) from the non-ciliated cells. Bar = 20 µm.

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.

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