Morphological Features of the Secretory Phase Endometrium in Women With Unexplained Infertility

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Morphological features of the secretory phase endometrium in women with unexplained infertility

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ABSTRACT

Background: In this study, we evaluated the structural features of the endometrial tissues, the immunohistochemical expression of MUC-1, which plays an important role in implantation, and the biochemical markers during the implantation window.

Methods: Randomly chosen 18 fertil and 18 infertil women, that have 27-32 days long menstrual cycle, normal hormonal values, normal USG findings of ovary and endometrium were included. Five, six, and seven days after ovulation, endometrial biopsies were taken and prepared in accordance with light and electron microscopy tissue preparation methods. Immunohistochemical methods were used to determine MUC-1 expression in the tissues. Serum hormone levels were determined.

Results: The MUC-1 immunoreactivity, as well as the serum levels of FSH, LH, TSH, estrogen, progesterone, and total testosterone did not differ significantly between the two groups; however, prolactin levels were higher in the infertile group. In the infertile samples intraepithelial lymphocytes were frequently observed, the microvilli of the surface columnar epithelium were widespread, cells with pinopodes as well as vesiculated cells were minimal, pinopode development was insufficient, the development of the endometrial glands was deficient.

Conclusions: Considering all these findings, it was concluded that these structural differences observed in the surface and glandular epithelium of the endometrium in infertile patients may be due to the insufficiency of these cells in responding to steroid hormones; therefore, these changes may affect the implantation of the blastocyst in the endometrium.

KEYWORDS: Unexplained infertility, endometrium, ultrastructure, mucin 1

BACKGROUND

The human endometrium is a tissue affected by the hormone estrogen and progesterone, and its primary function is to provide a suitable environment for embryo implantation. The endometrium demonstrates cyclic changes, known as the menstrual cycle (approximately 28 days long), with maximum receptivity to blastocyst implantation between days 19-21 [1]. At this stage, the endometrial epithelial cells exhibit distinct structural and molecular features [2, 3]. These ultrastructural features are characterized by the development of pinopodes, giant mitochondria, the nucleolar channel system, and increased glycogen storage which are also considered reliable indicators of the implantation window [1, 4, 5]. Pinopodes are apical protrusions of the epithelial cells, that extend towards the uterine lumen and are fine structural markers of the implantation window [6]. It is also accepted that pinopodes are the constructs that provide the relationship between the blastocyst and luminal epithelium during

implantation and are therefore considered biological markers of endometrial receptivity [7]. Studies have shown that well-developed pinopodes, in the endometrial epithelium of fertile women, attach to the embryonic cells, and that defects in the development of these structures may lead to implantation failure [8-10]. It is also reported that mucin-1 (MUC-1), in the glycocalyx of epithelial cells, plays an important role in the implantation of the blastocyte, and that related secretion disorders may cause infertility [11, 12].

In women with an unexplained infertility diagnosis, studies on the structure of the endometrium during the implantation window seem to be inadequate in providing any solution. Therefore, determining the morphological features of the endometrium during this period and the functional evaluation of these features may provide a new perspective on unexplained infertility. For this reason, we aimed to evaluate the morphological features, using light and electron microscopy, and the mucin-1 expression of endometrial tissues between days 19-21 of the menstrual cycle in women diagnosed with unexplained infertility in comparison to fertile women.

MATERIALS AND METHODS

Patient Selection

In this study, 18 patients from the Çukurova University's Faculty of Medicine, Department of Obstetrics and Gynecology, Turkey, who were unable to fall pregnant due to unexplained infertility, were selected as the study group. The control group consisted of 18 fertile women, sourced from the Çukurova University's Faculty of Medicine, Department of Obstetrics and Gynecology, Turkey, who did not have any fertility problems; however suffered from uterine myomas or other gynecological problems unrelated to fertility

The control group consisted of women with a history of a menstrual cycle length of 27-32 days, normal hormonal values, normal ultrasonographic (USG) findings related to ovary and endometrium health, no use of oral contraceptives and intrauterine devices for the last 6 months, at least one spontaneous pregnancy, and gynecological complaints unrelated to fertility, such as myomas. Women in the study group were selected randomly from those diagnosed with unexplained infertility despite having regular menstrual cycles and ovulation criteria, normal thyroid functions, LH, FSH, estradiol, progesterone and prolactin levels, and although engaging regularly in sexual intercourse, were unable to fall pregnant.

In both groups, ovulation was determined by follicular development using USG on days 11-14 of their cycle and endometrial biopsies were taken 5, 6, and 7 days after ovulation. The endometrial tissue biopsies were obtained using pipelle sampling, from the women from the study and control groups, on the 5^{th} day (n = 6), 6^{th} day (n = 6) and 7^{th} day (n = 6) after ovulation and prepared accordingly for light and electron microscopy. Peripheral

blood samples, from all women, were also taken for biochemical analysis at the same time as the endometrial biopsies taken on day 2 or 3 of their menstrual cycle.

Biochemical Sampling Methods

5 mL blood samples were taken from the women in both the fertile and infertile groups on days 2 and 3 of their menstrual cycle as well as 5, 6 and 7 days after ovulation. All blood samples were tested for FSH, LH, TSH, Estrogen, Progesterone, Prolactin, and total testosterone (Beckman Coulter kit, USA) at the Central Laboratory, Biochemistry Department, Faculty of Medicine, Balcali Hospital, University of Çukurova, Turkey using a Beckman Coulter DXI800 (USA, California) automatic device.

Tissue Preparation for Light Microscopy

Some of the tissue samples obtained from endometrial biopsies were fixed in 10% neutral formalin for 48-72 h for light microscopy examination. Thereafter, routine tissue preparation was performed using the Leica TP1020 (Germany) automatic tissue processor. Sections were taken from the paraffin blocks containing the endometrial tissues and then stained with Hematoxylin-Eosin (80). Thereafter, immunohistochemical methods for MUC-1 detection were applied and the tissues were examined using an Olimpus BX51 (Japan) light microscope.

Immunohistochemical Methods

In order to determine the expression of MUC-1, sections 5 μm thick were placed on polylysine-coated slides and deparaffinized at 60°C. The sections were hydrated at room temperature and then immunohistochemical staining was performed using the fully automated IHC slide staining instrument, Ventana BenchMark XT (USA). The primer antibody (Cell Marque MRQ-17, USA) for MUC-1 was applied in 1/100 dilution. Thereafter, the antibody was visualized using a basic AEC detection kit (Basic AEC detection, USA), consisting of indirect biotin, streptavidin, and AEC chromogen, which detects the primary antibodies present. Sections were then mounted using the mounting medium AML030 (Scytek). Tissue slides were examined using the Olympus BX51 (Japan) light microscope. The scoring of MUC-1 expression in the endometrial epithelium was rated either low (+), moderate (++) or high (+++).

Tissue Preparation for Electron Microscopy

A portion of the endometrial biopsies were cut into 1 mm^3 sections for electron microscopy examination and immersed in 5% glutaraldehyde for 4 hours. Thereafter, a second fixation was performed using 1% osmium tetroxide solution for 2 hours at 4°C. After the dehydration process, the tissues immersed in the resin were embedded in polyethylene BEEM capsules (size 00) in order to obtain tissue blocks. Then semi-thin sections were taken from the blocks using the Reichert FCS Ultracut S Cryo Ultramicrotome. Thereafter, the thin sections, with

a thickness of 50 nm, were stained with uranyl acetate, and saturated in 70% ethyl alcohol and Reynold's lead citrate solution. The tissues were examined using the Jeol JEM 1400 (Japanese) transmission electron microscope and micrographs were obtained.

Statistical Analysis

The SPSS 20.0 package program was used for statistical analysis of the data. The Chi Square / Pearson test statistic was used to compare the results of the two groups. The Kruskal-Wallis test was used to test whether the numerical data from the two groups represented a normal distribution. The Mann Whitney U test was used to test the null hypothesis when no assumptions were held regarding the numerical data of the two groups. The statistical significance level was set at $p < 0.05$ for all tests.

RESULTS

Biochemical Findings

With regards to serum FSH, LH and TSH levels measured on days 2 and 3 of the menstrual cycle, as well as 5, 6, and 7 days after ovulation, no significant difference was detected between the infertile and control group.

Blood serum levels of progesterone, estrogen, and total testosterone did not show any significant difference between the infertile and the control group; however, the prolactin level was significantly higher in the infertile group compared to the fertile group ($p = 0.001$), with this increase more prominent on the 7th day ($p = 0.030$). Estrogen levels were lower in the infertile group only on the 7^{th} day after ovulation ($p = 0.027$; Table I and table II).

Light Microscopy Findings

From the samples obtained from both the infertile and control groups, on the 5^{th} day after ovulation, it was observed that the surface epithelium of the endometrium consisted of simple columnar cells with thin, long basophilic stained nuclei. The endometrial glands, in the endometrial stroma, were slightly tortuous and lined with simple columnar epithelium. In the endometrial stroma between the glands, edematous areas were observed (Figure 1A, B).

In the samples obtained from the control group, on the 6^{th} day after ovulation, the endometrial surface was lined with simple columnar epithelium, whereas in the infertile group, the surface epithelium contained intermittent areas of pseudostratified and stratified epithelia. The endometrial glands were more abundant and very tortuous in the fertile group, whereas the infertile group exhibited more sparse and straight tubular endometrial glands. It was noted that the endometrial stroma were structurally more profound and compact in the infertile group (Figure 1C, D).

In the samples obtained on the 7th day after ovulation, it was observed that the fertile group exhibited a simple columnar endometrial epithelium; however, the infertile group demonstrated intermittent patches of pseudostratified and stratified epithelia**.** The endometrial glands in the infertile group were observed to be scattered and less tortuous than those in the fertile group, and vacuoles, in the basal cytoplasm of the cells at the epithelial lining of the gland, were observed. Macrophages and lymphocytes were observed in the endometrial stromal cells in both groups (Figure 1E, F).

Immunohistochemical Findings

High expression of MUC-1 was evidenced at the apical surfaces of the endometrial epithelial cells in the control group; however, there were cases where it was weakly stained (Figure 2A, C, E). In the epithelial cells of the endometrial glands, MUC-1 expression was higher than in the surface epithelial cells. In addition to the strong staining at the cells' apical surfaces, MUC-1 expression was also present in the apical cytoplasm and weakly expressed in basal cytoplasm (Figure 2A, B, C, D, E, F).

When the MUC-1 immunoreactivity was statistically evaluated, in both the surface and glandular epithelial cells of the control and infertile groups, no significant difference was observed between the two groups in terms of percentage staining (Table III).

Electron Microscopy Findings

Ultrastructural examination of the both groups surface and glandular epithelium showed that the simple columnar epithelium was composed of four distinct cell type: ciliated cells, vesiculated cells, cells with pinopodes, and microvilli-rich cells.

Five days after ovulation

The surface epithelium of the endometrium of the fertile women was composed of 4 prominent cell type. Ciliated cells were rarely observed; however, cells with pinopodes were common in the epithelium. Microvilli-rich cells were observed to have a well-developed nucleolar canal system in their nuclei as well as enlarged endoplasmic reticulum cisternae, membrane-surrounded vesicles, mitochondria, giant mitochondria, granular endoplasmic reticulum cisternae, and well-developed Golgi complexes in their cytoplasm. Cells with pinopodes were normal in appearance with dome shape protrusions into the lumen. It was observed that the vesicular cells contained glycogen particles, lipid droplets, and membrane-enclosed secretory vesicles in the cytoplasm (Figure 3A).

Although ciliated and pinopode cells were rarely observed in the surface epithelium of infertile women compared to the control group, it was noted that microvilli-rich cells were quite common. Furthermore, the

presence of lymphocytes between the surface epithelial cells was also noteworthy. In some areas, it was observed that the intercellular distance was enlarged, and that the glycogen particles were in close proximity to the lipid droplets in the apical and basal cytoplasm of the cells (Figure 3B).

In the fertile group, the endometrial glandular epithelium was predominantly composed of microvilli-rich cells and rarely contained cells with pinopodes and cilia. It was observed that the cytoplasmic extensions, of the cells with pinopodes, were diminished and that the microvilli were in excess in comparison to the cells in the surface epithelium. In the infertile group, the glandular epithelium was composed of microvilli-rich cells and intraepithelial lymphocytes were observed between the epithelial cells (Figure 3C, D).

Blood vessels, stromal cells, collagen fibers, and mononuclear phagocytic cells, as well as mild edema, were common in both groups(Figure 3E, F).

Six days after ovulation

In the fertile group, ciliated, microvilli-rich cells, cells with pinopodes and vesiculated cells were found to have normal ultrastructural features within the surface epithelium of the endometrium. Tight junctions were observed between the epithelial cells in the apicolateral region with desmosome-type connections just below this (Figure 4A).

In the infertile group, different types of surface epithelia were observed, with different micrographs evidencing the presence of simple columnar low columnar, pseudostratified, and stratified epithelia. The microvilli-rich cells, with smooth apical surfaces, were widespread, while cells with pinopodes and ciliated cells were rare. Nucleolar channel systems were also well developed. The nucleolar channel system was composed of ovoid-shaped euchromatic nuclei, containing 1-2 nucleoli (Figure 4B).

In the fertile group, the cells lining the endometrial glands were columnar in nature, the number of cells with pinopodes increased and ciliated cells were very rare. In some areas, common autophagic vacuoles were observed in the cytoplasm of microvilli-rich cells as well as cells with pinopodes. These autophagic vacuoles were surrounded by membranes and contained a cytoplasm, endoplasmic reticulum cisterns, mitochondria, electron dense granular structures, and membrane-surrounded vesicles (Figure 4C).

In the infertile group, the epithelium of the endometrial glands consisted of microvilli-rich cells, plenty cells with pinopodes and vesiculated cells, with few ciliated cells. It was observed that the number of microvilli on the apical surfaces of the microvilli-rich cells were normal. It was also noted that the glandular epithelium was pseudostratified in some areas and stratified in others, with evidence of numerous mitotic figures between epithelial cells (Figure 4C, D).

In the fertile and infertile groups, stromal cells, macrophages, and lymphocytes, as well as collagen fibers and blood vessels, were considered normal in terms of what is expected in endometrial stroma (Figure 4E, F).

Seven days after ovulation

In the fertile group, the columnar surface epithelium, including microvilli-rich cells, cells with pinopode, ciliated cells, and vesiculated cells were observed as normal in nature. Intraepithelial lymphocytes were also occasionally observed between epithelial cells (Figure 5A). In some of the cells with microvilli, the presence of giant mitochondria located near glycogen aggregates, associated with the endoplasmic reticulum cisterns located in the basal cytoplasm, was noted (Figure 5A).

While the epithelium of the infertile group was usually simple columnar in nature, stratified epithelial patches were also observed in some areas. The stratified epithelium was found to have a thickness of 5-6 cell layers; the cells on the surface were cuboidal or squamous in nature, with some possessing short, blunt microvilli on their smoothened apical surface, while others also contained cilia with microvilli. While these cells were attached to neighboring cells by means of junctional complexes on their lateral surfaces, it was observed that they were attached to the underlying cells at the basal surfaces by means of desmosome-type connections. In the deeper layers of the surface epithelium, some epithelial cells contained spherical, euchromatic nuclei, widespread free ribosomes, and small mitochondria in their cytoplasm. These cells were observed as clusters in the epithelium (Figure 5B).

In the fertile group, the epithelium covering the endometrial glands was mostly composed of cells with microvilli and pinopodes. These cells were observed as normal in terms of histological structure with mild indented nuclei, widespread mitochondria, GER cisterns, well developed Golgi complex, and free ribosomes in their cytoplasm. However, an increased number of glycogen particles were observed in the cytoplasm, with dense clusters evidenced in the basal and apical cytoplasm as well as in perinuclear areas (Figure 5C). Widespread stromal cells, lymphocytes, macrophages, and blood vessels were also observed in the endometrial stroma of the fertile group.

In the infertile group, it was evidenced that the height of the epithelial cells, composed mostly of cells with microvilli, lining the glands was reduced. The apical surface of these cells had no protrusions. Cells with pinopodes, with extensive microvilli in their apical surfaces, were rarely observed in the glandular epithelium (Figure 5D). In the infertile group, the stromal cells were considerably enlarged, and contained euchromatic nuclei, with a glycogen-rich appearance in the cytoplasm. There was an increased number of macrophages and

lymphocytes in the endometrial stroma, as well as an increased presence of edema during this period (Figure 5E, F).

DISCUSSION

Successful implantation involves a series of complex interactions between the endometrium and the embryo [13-15]. The period in which the endometrium receives the blastocyst is called the implantation window [16]. During this period, suitable morphological characteristics of the endometrium and hormonal status are very important factors for successful implantation.

In our study, baseline FSH, LH, TSH, and E2 values were measured using blood samples taken on the second and third day of the menstrual cycle with the aim of determining ovarian reserve. FSH, LH and TSH values from 5, 6, and 7 days after ovulation showed no difference between the two groups, while prolactin levels were significantly higher in the infertile group. It is reported that decidual cells have the capacity to secrete prolactin from the 23rd day of the menstrual cycle and that this secretion is dependent on progesterone [17]. Although prolactin is primarily secreted by the lactotrophic cells of the pituitary gland, its secretion from the uterine decidual cells are important for decidualization maintenance [18]. Increases in serum prolactin levels, even if within physiological limits, have been noted to cause unexplained infertility via the proinflammatory effect [19]. In our study, the significant increase in prolactin serum levels in infertile women on the 5th and 7th day after ovulation, as well as the presence of macrophages and lymphocytes in the endometrial stroma, lead us to believe that the increased prolactin levels caused implantation failure in these patients through the proinflammatory effect. The higher prolactin levels in the infertile group, compared to the fertile group, may be due to the impaired response by the stromal cells to the progesterone hormone, suggesting that infertility may also be due to intensive stress experienced during pre-treatment and treatment. As a matter of fact, Csemiczky et al. [20] reported that prolactin levels were increased in women during stressful situations.

Significant morphological differences were observed between the fertile group and those diagnosed with unexplained infertility, by examining the endometrial biopsies obtained 5, 6 and 7 days after ovulation using light and electron microscopy. These ultrastructural differences in the infertile women, especially in the endometrial surface and glandular epithelial cells, were characterized by changes in the type of luminal epithelium, increased intraepithelial lymphocytes, differentiation of structure and the proportion of pinopode cells. It was observed that the surface of the endometrium was generally lined with simple columnar epithelium; however, the endometrial surface of the infertile group was lined with low columnar, pseudostratified or stratified squamous epithelium in some areas. Epithelial cells revealed columnar or pseudostratified epithelial characteristics at the end of the

proliferative phase, and evidence of mitosis might be observed in some cells [21]. These changes in the endometrial epithelium in the early and mid-secretory phase, which were evaluated in this study, suggests that the observed proliferative phase characteristics of these cells may be indicative of response failure to steroid hormones. Hence, it was evidenced that the endometrial epithelium exhibits premature cyclic characteristics. Studies have reported that changes in endometrial dating are caused by developmental abnormalities and phase lag in these patients. Considering these changes in endometrial dating, it was reported that a delay of one or more days in embryo transfer would be more successful with regards to IVF [22]. On the other hand, the stratified endometrial surface epithelium in infertile women suggests that metaplasia occurs in these areas. It was reported that this metaplasia may be induced by abnormal stimuli, such as changes in pH, hormonal balance, cigarette smoke and alcohol. Although this adaption of the epithelial cells occurs in response to environmental stressors, it is not clear whether this tissue can return to its normal structure [23]. Ultrastructural examinations of the metaplastic areas showed that the epithelial cells were cuboidal shape and formed 5-6 cell layers, and that the cells in the superficial layer contained microvilli and cilia on the apical surfaces. The existence of these epithelial cell surface specializations in the superficial layer differed from the previously described squamous metaplasia [24]. These identified areas may therefore be described as early stage squamous metaplasia. It was also observed that these cells were held together by numerous desmosomes. These changes in the endometrial epithelium, i.e., being multi-layered with an increased number of desmosomes throughout the epithelial types (which are actually expected to decline during this period of the cycle), suggests that the embryo may be adversely affected with regards to the attachment to the endometrial epithelium and subsequent invasion into endometrial stroma.

It was noted that intraepithelial lymphocytes, in the luminal epithelium, were more common in infertile woman in comparison to fertile women. Pace et al. [25] reported that granular intraepithelial lymphocytes were found in the endometrium during the proliferative and early secretory phase, and that their numbers increased during the late secretory and early gestation period, with the number of non-granulated intraepithelial lymphocytes increasing in the early and late secretory period. Given that Gamma delta T lymphocytes are also referred to as intraepithelial lymphocytes and constitute a defense mechanism against microorganisms in epithelial tissues, the intraepithelial lymphocytes observed in the infertile women may have increased for protection purposes.

It was noticed that the microvilli-rich cells in the surface epithelium of the fertile and infertile groups were widespread, and that the pinopode cells in the infertile group were observed less frequently. Bartosch et al. [1] noted that the proportions of microvilli cells, pinopodes, vesiculated, and ciliated cells differ depending on the hormonal status and the phase of the menstrual cycle. They also reported that the number of ciliated cells increased

in the proliferative phase; however, these ciliated cells, as well as cells with microvilli, decreased during the secretory phase due to their subsequent differentiation to cells with vesicles and pinopodes. Our ultrastructural evaluations of infertile women's endometrial tissue suggest that microvilli-rich cells are more prevalent, suggesting that the process whereby cells with microvilli differentiate into cells with pinopodes and vesicular cells may be impaired. In their studies, Novotny et al. [26] have classified pinopodes of the luminal epithelium as either developing, developed or regressing pinopodes during the early and middle secretory phase. However, reports on the ultrastructural characteristics of pinopodes are also inconsistent. Some researchers have described pinopodes as microvilli- and organelle-free, apical cytoplasmic protrusions [1], while some have reported that they include microvilli and membranous organelles [27], whereas others have reported that pinopodes contain microvilli on the surface when they first developed, lose their microvilli when developed, and contain microvilli again during the regressive period [28]. In the present study, we observed pinopodes without organelles and the presence of microvilli only in the luminal epithelium of fertile women on the 5th day after ovulation. Other than on the 5th day after ovulation, we observed pinopodes containing microvilli on their apical surfaces with a cytoplasm containing organelles, such as mitochondria and lysosomal structures, in both groups. However, the structural comparison of the pinopode cells indicate that they were shorter and narrower in the infertile women and did not cover the entire apical surface of the cell. Considering that pinopode development is dependent on progesterone [28] and that a high level of estrogen adversely affects pinopode formation [29], pinopodes are less common in infertile women. The observed structural changes associated with pinopodes could therefore be related to the irregularity of individual steroid hormones or an insufficient response by these cells to steroid hormones and may consequently be one of the causes of implant failure. It was reported that failure in progesterone secretion or functioning causes non-phase endometrial histopathology [22, 30]. Giant mitochondria were observed in the endometrium, basal or apical cytoplasm of microvilli-rich cells and in the surface and glandular epithelium of both fertile and infertile women. Armstrong et al. [31] demonstrated the presence of giant mitochondria in the glandular epithelium in the endometrium between days 13 and 22 of the menstrual cycle, and that these mitochondria respond to steroid hormones, resulting in fusion of normal mitochondria. The giant mitochondria observed in both groups in this study were therefore evaluated as normal structural findings.

The autophagosomes in the apical and basal cytoplasm of the cells, as well as the microvilli and pinopode cells in the endometrial gland in the fertile women were widespread. It is thought that the mitochondria, that lost their function, are surrounded by the endoplasmic reticulum cisternae and then digested by lysosomal enzymes [32], thereby removing them from the environment by means of autophagosome formation during

autophagocytosis. Choi et al. [33] investigated the role of autophagy in the human endometrium and reported that the expression of the autophagy-associated protein in the endometrial glands was increased throughout the secretory phase. It was therefore concluded that the presence of autophagosomes in the cytoplasm of the epithelial cells in certain areas may be considered normal and resultant of the high functional activity of epithelial cells during the secretory stage. Although it was reported that autophagy is beneficial for decidualization, impaired autophagy, such as in the endometrial cells of obese women or endometriosis patients, may be related to infertility [34].

In our study, well-developed nucleolar canal systems were observed in the nuclei of microvilli, pinopode and vesicular cells in the glandular and surface epithelium in both infertile and fertile women. Zapantis et al. [35] reported that women who underwent controlled ovarian hyperthermia developed the nucleolar canal system prematurely, while Dockery et al. [36] reported the delayed development of the nucleolar canal system in the epithelial cells in women with unexplained infertility. However, we observed that the nucleolar canal system is well developed 5, 6 and 7 days after ovulation in the both the infertile and fertile group.

Diffuse glycogen aggregates in the apical and basal cytoplasm of the surface and glandular epithelial cells was observed in both groups. On the 17th day of the menstrual cycle, it was reported that glycogen clusters were found only in the subnuclear area in the endometrial gland cells, while on the 18th day, they were found in the subnuclear and supranuclear areas, whereas on the 19th day, they were found only in the supranuclear region [37]. However, in our study, aggregates of glycogen particles are observed in the apical, perinuclear, or basal cytoplasm 5, 6, and 7 days after ovulation.

MUC-1 immunoreactivity in the surface and glandular epithelium of the endometrium was quantitatively assessed, and although the infertile group demonstrated a MUC-1 immunoreactivity that was weaker in the surface epithelium and stronger in the glandular epithelium, statistical analysis revealed no significant difference between the fertile and infertile groups. It has been suggested that implantation could not occur because MUC-1 was removed from the environment by paracrine signals between maternal cells and healthy embryo cells during implantation, and that poor quality embryo cells implanted in this area, could not remove MUC-1 from the medium [11, 38]. Weak or malformed MUC-1 has been reported to cause recurrent pregnancy loss as it allows implantation of abnormal embryos [11]. In the present study, MUC-1 immunoreactivity did not show any statistically significant difference between the two groups.

Light microscopy evaluation of tissue samples from infertile women found that endometrial glands were sparse and had a straight tubular structure. In addition, on the fifth day after ovulation in the infertile group, electron

microscope images of the endometrial glands revealed common mitotic figures in certain areas between glandular epithelial cells. Bergeron [39] reported that the secretory phase starts after ovulation (between days 14-28 of a normal menstrual cycle), and that the first changes in the glands occurred in response to progesterone, decreased before DNA synthesis and mitosis, and then stopped completely. Therefore, continued mitotic division in glandular epithelial cells suggests that these cells possess proliferative phase properties.

When the endometrial stroma of the fertile and infertile women were compared, it was observed that stromal cells gained predecidual cell characteristics in both groups; however, macrophages and lymphocytes were increased in the stroma of infertile women. Indeed, Li et al. [40] reported that the endometrial stroma of normal women was no different than that of women with unexplained infertility. Further immunohistochemical studies are needed to improve understanding of these lymphocytes, such as NK cells that originate from normal secretory phase processes, or other types of lymphocytes that take part in the increased inflammatory processes observed in unexplained infertility.

CONCLUSION

In conclusion, when biochemical and morphological results are evaluated from samples taken 5, 6 and 7 days after ovulation in women with unexplained infertility, the following observations are pertinent: the presence of pseudostratified and stratified epithelial patches in surface epithelium; the abundance of cells with well-developed microvilli, sparse cells with pinopodes, and vesiculated cells; structural differences of pinopodes; diffuse intraepithelial lymphocytes; increased prolactin level; and the number of macrophages and lymphocytes in endometrial stroma revealed that endometrial epithelium and the stroma didn't represent the properties of the same cyclic period. The endometrial epithelium indicated ultrastructural characteristics of proliferative phase and metaplasia. Metaplasia, and the related structural differences, may cause infertility. As a result, further studies are needed to explain the reasons behind these structural differences.

DECLARATİONS

Ethics approval and consent to participate

Before commencement, information regarding the purpose of the study was shared with each individual and their written informed consent was obtained. This study was conducted in line with the standards of, and subsequently approved by, the C.U.T.F. Balcalı Hospital Clinical Research Ethics Committee (Approval number: 13/6). All procedures were followed in accordance with ethical standards of the institutional ethical committee and with the World Medical Association Declaration of Helsinki.

Authors' contributions

ÖK and ÖT designed the research. All authors collected the data. All authors analyzed and interpreted the data. ÖK and ÖT wrote the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing Interest

The authors declare no competing interests

Consent for publication

Not applicable.

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REFERENCES

1. Bartosch C, Lopes JM, Beires J, Sousa M. Human endometrium ultrastructure during the implantation window: a new perspective of the epithelium cell types. Reprod Sci. 2011;18:525–9.

2. Aghajanova L, Hamilton AE, Giudice LC. Uterine receptivity to human embryonic implantation: histology, biomarkers and transcriptomics. Semin Cell Dev Biol. 2008;19:204–11.

3. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Am J Obstet Gynecol. 1975;122:262–3.

4. Kabir-Salmani M, Hosseini A, Valojerdi MR. Endometrial receptivity to implantation in humans: biochemical and molecular aspects. Yahkhteh 2008;10:1–24.

5. Kittur N, Zapantis G, Aubuchon M, Santoro N, Bazett-Jones DP, Meier UT. The nucleolar channel system of human endometrium is related to endoplasmic reticulum and R-rings. Mol Biol Cell 2007;18:2296–304.

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

7. Xu B, Sun X, Li L, Wu L, Zhang A, Feng Y. Pinopodes, leukemia inhibitory factor, integrin-β3, and mucin-1 expression in the peri-implantation endometrium of women with unexplained recurrent pregnancy loss. Fertil Steril. 2012;98:389–95.

8. Makrigiannakis A, Minas V. Mechanisms of implantation. Reprod Biomed Online 2007;14:75–82.

9. Rashid NA, Lalitkumar S, Lalitkumar PG, Gemzell-Danielsson K. Endometrial receptivity and human embryo implantation. Am J Reprod Immunol. 2011;66:23–30.

10. Guidice LC. Potential biochemical markers of uterine receptivity. Hum Reprod. 1999;14:3–16.

11. Meseguer M, Pellicer A, Simón C. MUC1 and endometrial receptivity. Mol Hum Reprod. 1998;4:1089–98.

12. Aplin JD, Meseguer M, Simon C, Ortiz ME, Croxatto H, Jones CJ. MUC-1, glycans and the cell-surface barrier to embryo implantation. Biochem Soc Trans. 2001;29:153–6.

13. Tabibzadeh S, Babaknia A. The signals and molecular pathways involved in implantation, a symbiotic interaction between blastocyst and endometrium involving adhesion and tissue invasion. Hum Reprod. 1995;10:1579–602.

14. Paria BC, Reese J, Das SK, Dey SK. Deciphering the cross-talk of implantation: advances and challenges. Science 2002;296:2185–8.

15. Carson DD, Bagchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA, et al. Embryo implantation. Dev Biol. 2000;223:217–37.

16. Cavagna M, Mantese JC. Biomarkers of endometrial receptivity--a review. Placenta 2003;24:S39–47.

17. Handwerger S, Richards RG, Markoff E. The physiology of decidual prolactin and other decidual protein hormones. Trends Endocrin Met. 1992;3:91–5.

18. Kükner S, Danışman N, Yeşilyurt H, Ergin T, Gökmen O. Preeklamptik Gebelerde Kan ve Amniyotik sıvı prolaktin düzeyleri. Perinatoloji Dergisi 1993;1:201–4.

19. Kebapçılar A, İlhan TT, Uçar MG, İpekçi SH, Çelik Ç. Relationship between prolactin levels and neutrophil/lymphocyte ratio in patients with unexplained infertility. Med J Aegean Clin. 2015;53:140–5.

20. Csemiczky G, Landgren BM, Collins A. The influence of stress and state anxiety on the outcome of IVF-treatment: psychological and endocrinological assessment of Swedish women entering IVF-treatment. Acta Obstet Gynecol Scand. 2000;79:113–8.

21. Aplin JD. Embryo implantation: the molecular mechanism remains elusive. Reprod Biomed Online 2006;13: 833–9.

22. Kliman HJ, Frankfurter D. Clinical approach to recurrent implantation failure: evidence-based evaluation of the endometrium. Fertil Steril 2019;111:618–28.

23. Giroux V, Rustgi AK. Metaplasia: tissue injury adaptation and a precursor to the dysplasia-cancer sequence. Nat Rev Cancer 2017;17:594–604.

24. Stringfellow HF. Endometrial metaplasia. Diagn Histopathol. 2009;15:564–70.

25. Pace D, Longfellow M, Bulmer JN. Characterization of intraepithelial lymphocytes in human endometrium. J Reprod Fert. 1991;91:165–74.

26. 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.

27. Murphey CR. Understanding the apical surface markers of uterine receptivity: pinopods-or uterodomes? Hum Reprod. 2000;15:2451–4.

28. Quinn CE, Casper RF. Pinopodes: a questionable role in endometrial receptivity. Hum Reprod Update 2009;15:229–36.

29. Martel D, Monier MN, Roche D, Psychoyos A. Hormonal dependence of pinopode formation at the uterine luminal surface. Hum Reprod. 1991;6:597–603.

30. Aunapuu M, Kibur P, Järveots T, Arend A. Changes in morphology and presence of pinopodes in endometrial cells during the luteal phase in women with infertility problems: a pilot study. Medicina (Kaunas) 2018;54:E69.

31. Armstrong EM, More IA, McSeveney D, Carty M. The giant mitochondrion-endoplasmic reticulum unit of the human endometrial glandular cell. J Anat. 1973;116:375–83.

32. Ross MH, Kaye GI, Pawlina W. Histology: a text and atlas: with cell and molecular biology. 4th ed. Philadelphia, PA: Lippincot Williams Wilkins; 2003

33. Choi J, Jo M, Lee E, Oh YK, Choi D. The role of autophagy in human endometrium. Biology Rep. 2012;86:1–10.

34. Yang S, Wang H, Li D, Li M. Role of endometrial autophagy in physiological and pathophysiological processes. J Cancer 2019;10:3459–71.

35. Zapantis G, Szmyga MJ, Rybak EA, Meier UT. Premature formation of nucleolar channel systems indicates advanced endometrial maturation following controlled ovarian hyperstimulation. Hum Reprod. 2013;28:3292–300.

36. Dockery P, Pritchard K, Warren MA, Li TC, Cooke ID. Changes in nuclear morphology in the human endometrial glandular epithelium in women with unexplained infertility. Hum Reprod. 1996;11:2251–6.

37. Ferenczy A, Bergeron C. Histology of the human endometrium: from birth to senescence. Ann N Y Acad Sci. 1991;622:6–27.

38. Aplin JD, Seif MW, Graham RA, Hey NA, Behzad F, Campbell S. The endometrial cell surface and implantation. Expression of the polymorphic mucin MUC-1 and adhesion molecules during the endometrial cycle. Ann N Y Acad Sci. 1994;734:103–21.

39. Bergeron C. Morphological changes and protein secretion induced by progesterone in the endometrium during the luteal phase in preparation for nidation. Hum Reprod. 2000;15:119–28.

40. Li TC, Dockery P, Cooke ID. Endometrial development in the luteal phase of women with various types of infertility: comparison with women of normal fertility. Hum Reprod. 1991;6:325–30.

Table I: Serum Progesteron, Estrodiol (E2), Prolactin and Total testesterone levels in the groups on the biopsy day. *Mann-Whitney U Test *p<0.05

	Progesteron (ng/ml)	**E2 (pg/ml)**	**Prolactın (ng/ml)**	**Total Testosterone (ng/ml)**
	Mean±SD Median (Min-max)			
Fertil Control (n=18)	8,5±6,0 8,7(1,4-23,9)	106.6±46.8 91(62-230.0)	10,6±5 9,6(6,3-26,1)	0,5±0,2 0,5(0,2-0,9)
İnfertil Patients (n=18)	8,1±4,3 8,2(0,7-16,9)	96,3±35,1 94.5(44-158)	18,1±7,4 19(4,5-29)	0,5±0,3 0,5(0,2-1,2)
p	0,938	0,767	0,001*	0,767

Table II. Serum progesteron, estrodiol (E2), prolactin and total testesterone levels in the groups 5, 6, and 7 days after ovulation. * Kruskal Wallis test, *p <0,05

		Mean±SD Median (Min-max)			
		5.day (n=6)	**6.day (n=6)**	**7.day (n=6)**	P**
Progesteron (Ng/Ml)	**Fertile Control**	8.9±6.3 8.3(1,4-23.9)	9,0±4,7 8,9(2,5-16,6)	5,2±3,8 3,5(2-11,1)	0,630
	İnfertile Patients	7,5±3 7,6(3-12,1)	8.3±4,9 4.9(1.3-16,9)	7,6±4,6 8,5(2,0-15,3)	0,854
	P*	0,423	0,631	0,262	
E2 (pg/ml)	**Fertile Control**	129.8±73,0 129.0(71-230)	78,0±8,7 75,0(68-89)	103,0±53,0 83,0(62-202)	**0,027**
	İnfertile Patients	121,0±37 133,0(71-158)	86,1±26,1 84,5(50-125)	81,9±32 85,5(44-115)	0,126
	P*	0,936	0,470	0,522	
Prolactin (ng/ml)	**Fertile Control**	10,3±5,1 9,4(6,3-20,1)	9,0±1,6 9,0(7-11)	12,6±7,0 10,0(7,2-26,1)	0,532
	İnfertile Patients	18,2±6,7 7,4(10-29)	12,3±5,7 10,8(4,5-19,2)	23,7±6,0 24,0(13,5-29)	**0,030***
	P*	**0,050***	0,150	**0,037***	
Total Testosterone (ng/ml)	**Fertile Control**	0,6±0,2 0,7(0,2-0,8)	0,5±0,2 0,5(0,3-0,9)	0,4±0,1 0,4(0,2-0,5)	0,113
	İnfertile Patients	0,6±0,4 0,5(0,2-1,2)	0,6±0,2 0,6(0,3-(0,8)	0,5±0,2 0,4(0,2-0,8)	0,653
	P*	0,423	0,748	0,631	

P* Comparison of the groups (fertil and infetil) for each day

P** Comparison of each group within a day (5., 6. and 7. Day)

Table III. MUC-1 immunorectivities of fertil and infertil group endometriums 5, 6, and 7 days after ovulation.

		Groups n (%)		
		Fertil Control (n=18)	**İnfertil Patients (n=18)**	p
Surface Epithelium	Low (1)	6 (33.3)	7 (38.9)	0.778
	Moderate (2)	5 (27.8)	6 (33.3)	
	High (3)	7 (38.9)	5 (27.8)	
Gland Epithelium	Low (1)	4(22,2)	3(16,7)	0.605
	Moderate (2)	7(38,9)	5(27,8)	
	High (3)	7(38,9)	10(55,6)	

Fig. 1 Light microscopic appearance of endometrium in fertil woman (A), (C) and (E) and infertil patients (B), (D), (F) at 5th, 6th and 7th days after ovulation respectively. The surface columnar epithelium (Ep) and underlying edematous stroma (S) with tortous endometrial gland are normally observed in the endometrium of fertil women. Metaplastic endometrial epithelium (white arrow) in some area, glands with narrow lumina and straight tubules (black arrow) and compact endometrial stroma (S), are seen in endometrium of the infertile woman. (H&E). Bars are 100 μm in A, C, D and 200 μm in B, E, F.

Fig. 2 MUC-1 immunoreactivities in the endometrium surface epithelium (Ep), and endometrial glands (arrows) at 5th (A, B), 6th (C, D) and 7th (E, F) days after ovulation are seen in fertile women (A, C, E) and infertile paitens (B, D, F). Bars are 100 μm in A, C, F and 200 μm in B, D, E.

Fig. 3 Endometrial surface epithelium, glandular epithelium and the stroma at the 5th days after ovulation in fertile (A, C, E) and infertile (B, D, F) groups. A) Surface epithelium is seen in normal apperance. Pinopod cell (PC), microvilli (Mv), nucleus (N). Bar= 1 μm. B) Surface epithelium without apical protrusions is seen. Nucleus (N), microvilli (Mv), glycogen particles (gl) and nucleolar channel system (ncs) are indicated. Bar= 0,2 μm. C) In the glandular epithelium giant mitokondria (gm), nucleolar channel system (ncs) and glycogen particles (gl) are observed in the microvilli rich cell (MC) cytoplasm. Bar= 1 μm. D) Intraepithelial lymphocytes (arrows) in the glandular epithelium are striking. Bar= 0,2 μm. E, F) Stromal cells (SC), macrophage (MP) and a capillary (cap) are observed in endometrial stroma. Bar= 2 μm. Bar = 0,5 μm.

Fig. 4 Endometrial surface epithelium, glandular epithelium and the stroma at the 6th days after ovulation in fertile (A, C, E) and infertile (B, D, F) groups. A) Normal ultrastructural apperance of surface epithelium is seen. Bar = 1 μm. B) Infertile group reveals pseudostratified areas in the columnar surface epithelium. Microvilli rich cell (MC), pinopod cell (PC), nucleus (N) and mikrovilli (mv) are indicated. Bar= 2 μm. C) Increased autophagic vacuoles (asterix) are remarkable in the endometrial glandular epithelium. Bar= 0,5 μm. D) Mitosis (arrow) in the glandular epithelium of infertile patients that became stratified is noticeable. Bar=0,2 μm. E, F) Normal stroma of fertile women and infertile patients are seen. Bars= 2 μm and 1 μm respectively.

Fig. 5 Endometrial surface epithelium, glandular epithelium and the stroma at the 7th days after ovulation in fertile (A, C, E) and infertile (B, D, F) groups. A) Columnar surface epithelium is seen normally. In the surface epithelium nucleus (N), mitochondria (m), lipid droplets (L), nucleolar channel system (ncs) and the basal lamina (BL) are observed. Intraepithelial lymphocytes (arrows) are remarkable. Bar= 1 μm. B) The cytoplasm of surface epithelial cells became more electron-dense (eDC), and in some areas more elecron lusent (eLC) compared to the other surface epithelial cells. Bar = 2 μm. C) Glandular epithelium is seen in normal

ultrastructural apperance with pinopod cell (PC), nucleus (N), nucleolar channel system (ncs) and mitocondria (m). Bar= 0,2 μm. D) Glandular epithelium of infertile group reveals pinopod cells (PC) with thinner protrusions and increased microvilli. Bar= 1 μm. E, F) Endometrial stroma of he fertil and infertil women with stromal cells (SC), macrophages (MP) and edematous area (arrow) are seen. Bar = 1 μm.

Figures

Figure 1.

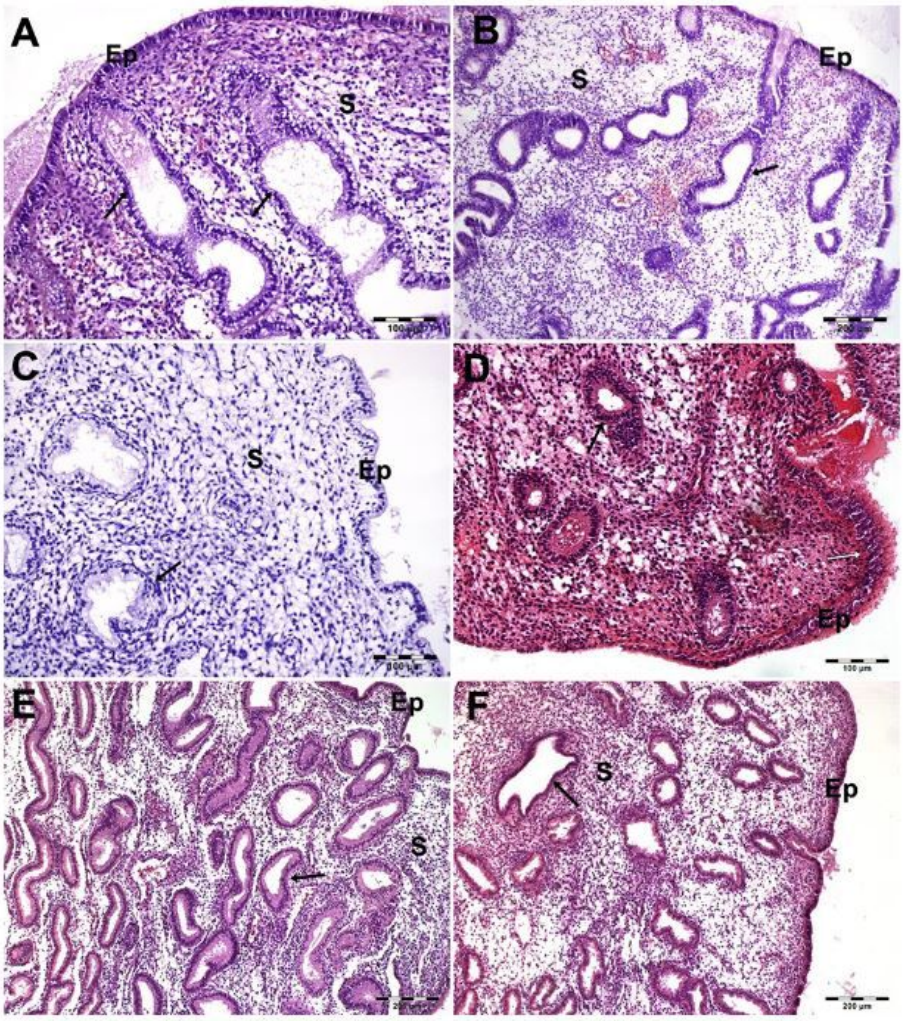

Figure 1

Light microscopic appearance of endometrium in fertil woman (A), (C) and (E) and infertil patients (B), (D), (F) at 5th, 6th and 7th days after ovulation respectively. The surface columnar epithelium (Ep) and underlying edematous stroma (S) with tortous endometrial gland are normally observed in the endometrium of fertil women. Metaplastic endometrial epithelium (white arrow) in some area, glands with narrow lumina and straight tubules (black arrow) and compact endometrial stroma (S), are seen in endometrium of the infertile woman. (H&E). Bars are 100 µm in A, C, D and 200 µm in B, E, F.

Figure 2.

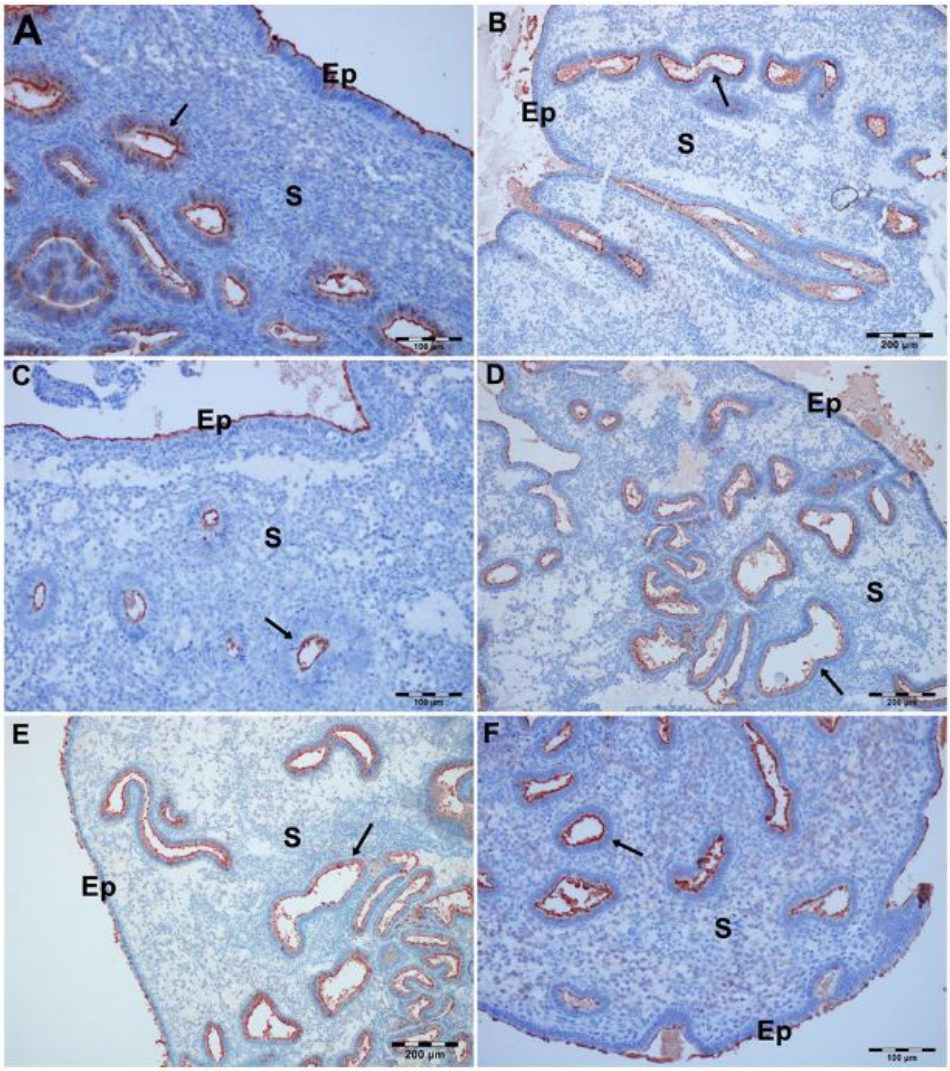

Figure 2

MUC-1 immunoreactivities in the endometrium surface epithelium (Ep), and endometrial glands (arrows) at 5th (A, B), 6th (C, D) and 7th (E, F) days after ovulation are seen in fertile women (A, C, E) and infertile paitens (B, D, F). Bars are 100 μm in A, C, F and 200 μm in B, D, E.

Figure 3.

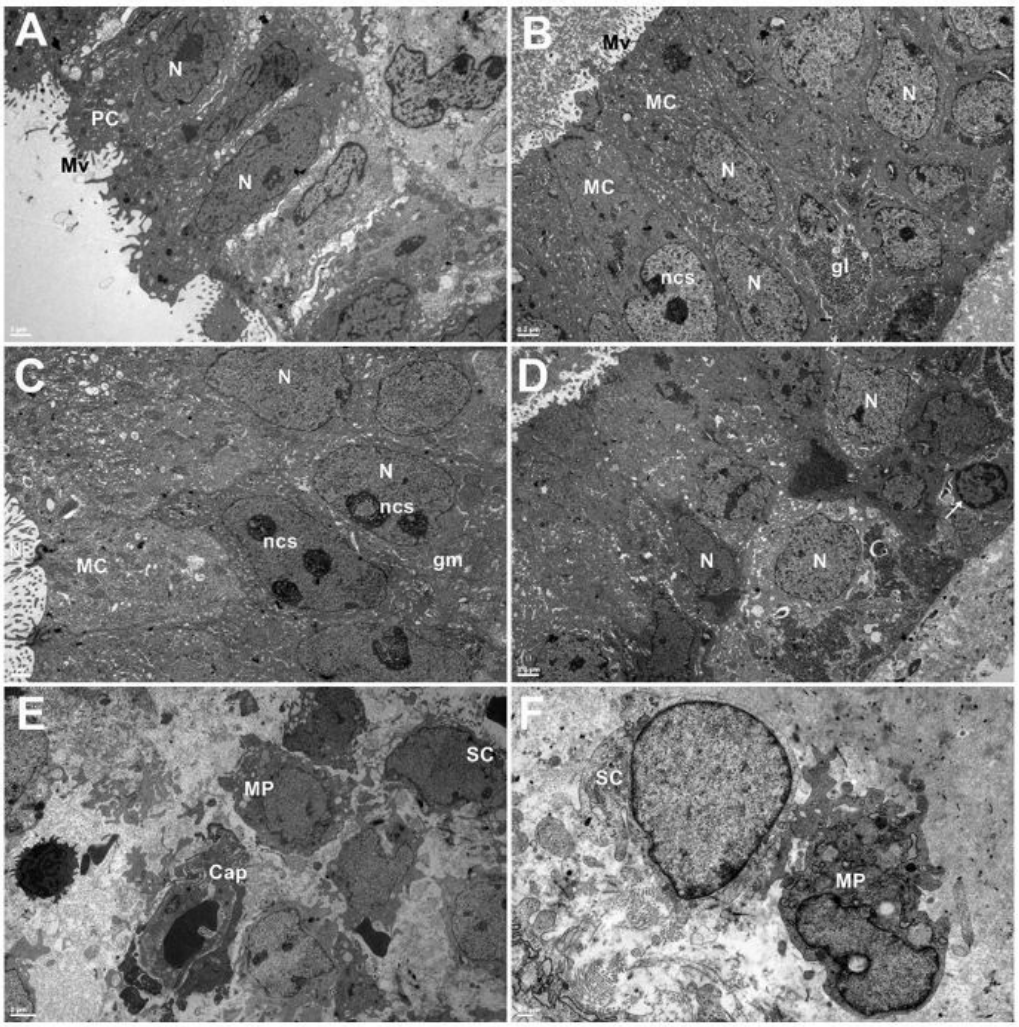

Figure 3

Endometrial surface epithelium, glandular epithelium and the stroma at the 5th days after ovulation in fertile (A, C, E) and infertile (B, D, F) groups. A) Surface epithelium is seen in normal apperance. Pinopod cell (PC), microvilli (Mv), nucleus (N). Bar= 1 µm. B) Surface epithelium without apical protrusions is seen. Nucleus (N), microvilli (Mv), glycogen particles (gl) and nucleolar channel system (ncs) are indicated. Bar= 0,2 µm. C) In the glandular epithelium giant mitokondria (gm), nucleolar channel system (ncs) and glycogen particles (gl) are observed in the microvilli rich cell (MC) cytoplasm. Bar= 1 µm. D) Intraepithelial lymphocytes (arrows) in the glandular epithelium are striking. Bar= 0,2 µm. E, F) Stromal cells (SC), macrophage (MP) and a capillary (cap) are observed in endometrial stroma. Bar= 2 µm. Bar = 0,5 µm.

Figure 4.

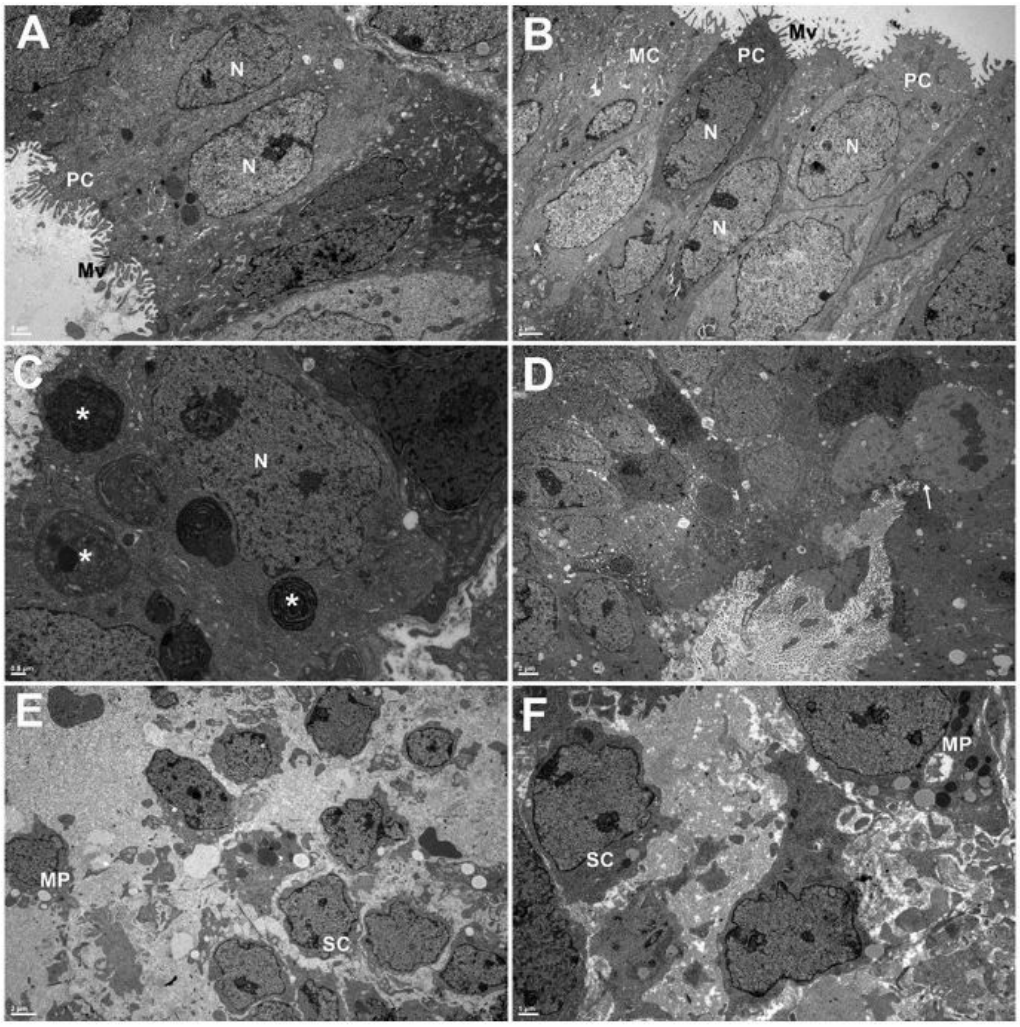

Figure 4

Endometrial surface epithelium, glandular epithelium and the stroma at the 6th days after ovulation in fertile (A, C, E) and infertile (B, D, F) groups. A) Normal ultrastructural apperance of surface epithelium is seen. Bar = 1 µm. B) Infertile group reveals pseudostratified areas in the columnar surface epithelium. Microvilli rich cell (MC), pinopod cell (PC), nucleus (N) and mikrovilli (mv) are indicated. Bar= 2 µm. C) Increased autophagic vacuoles (asterix) are remarkable in the endometrial glandular epithelium. Bar= 0,5 µm. D) Mitosis (arrow) in the glandular epithelium of infertile patients that became stratified is noticeable. Bar=0,2 µm. E, F) Normal stroma of fertile women and infertile patients are seen. Bars= 2 µm and 1 µm respectively.

Figure 5.

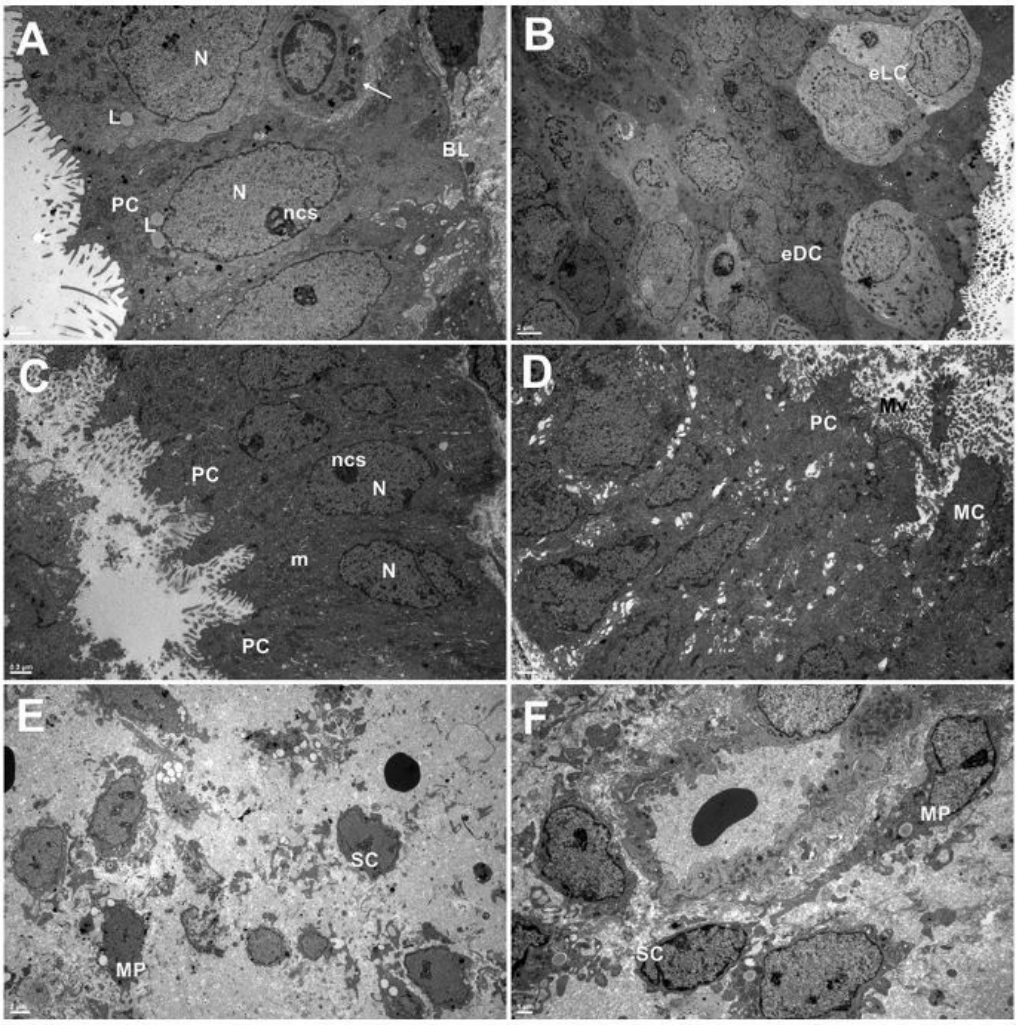

Figure 5

Endometrial surface epithelium, glandular epithelium and the stroma at the 7th days after ovulation in fertile (A, C, E) and infertile (B, D, F) groups. A) Columnar surface epithelium is seen normally. In the surface epithelium nucleus (N), mitochondria (m), lipid droplets (L), nucleolar channel system (ncs) and the basal lamina (BL) are observed. Intraepithelial lymphocytes (arrows) are remarkable. Bar= 1 µm. B) The cytoplasm of surface epithelial cells became more electron-dense (eDC), and in some areas more elecron lusent (eLC) compared to the other surface epithelial cells. Bar = 2 µm. C) Glandular epithelium is seen in normal ultrastructural apperance with pinopod cell (PC), nucleus (N), nucleolar channel system (ncs) and mitocondria (m). Bar= 0,2 µm. D) Glandular epithelium of infertile group reveals pinopod cells (PC) with thinner protrusions and increased microvilli. Bar= 1 µm. E, F) Endometrial stroma of he fertil and infertil women with stromal cells (SC), macrophages (MP) and edematous area (arrow) are seen. Bar = 1 µm.