Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2015) 45: 706-713
© TÜBİTAK
doi:10.3906/sag-1402-153

Comparison of endometrial biopsies of fertile women and women with repeated implantation failure at the ultrastructural level

Leyla BAHAR[1,*], Semra KAHRAMAN[2], Nazan ERAS[3], Caroline PIRKEVI[2]
[1]Vocational High School of Health Services, Mersin University, Mersin, Turkey
[2]İstanbul Memorial Hospital, ART & Reproductive Genetics Center, İstanbul, Turkey
[3]Department of Medical Biology and Genetics, Faculty of Medicine, Mersin University, Mersin, Turkey

Received: 24.02.2014 • **Accepted/Published Online:** 06.09.2014 • **Printed:** 30.06.2015

Background/aim: The aim of this study was to compare the cellular properties of endometrial tissues from fertile patients and patients having at least 3 previous in vitro fertilization failures, during the implantation window. The ultrastructural evaluation of the endometrium in the implantation window may shed light on the complexity of the implantation failure paradigm.

Materials and methods: The study involved 23 women, 14 infertile with a clinical diagnosis of repeated implantation failure (RIF) and 9 fertile, defined as the control group. Endometrial samples were examined by transmission electron microscopy (TEM).

Results: In the control group, secretory vacuoles and cytoplasmic projections filled with secretory material, called pinopodes, were noted; microvilli were observed on some apical surfaces; and ciliated cells were absent. In the RIF group, the number of pinopodes was remarkably lower, with some of them being immature. Moreover, decidualization of stromal cells was not frequent and fewer epithelial cells with poor secretory vacuoles were discerned.

Conclusion: TEM analyses of endometrial samples from the RIF group revealed dramatic differences at the ultrastructural level compared to the controls, which may well be an underlying cause of their infertility.

Key words: Endometrial receptivity, recurrent implantation failure, transmission electron microscopy

1. Introduction

The implantation of an embryo in the uterus wall, which involves a complex series of interactions and events, is the first requirement for the embryo to develop beyond the blastocyst stage and progress to term (1). The successful establishment of endometrial receptivity and the subsequent differentiation of the endometrium into decidua are the key events in embryo implantation (2,3). Age is considered an important factor in endometrial receptivity during embryo implantation (4). Abnormalities occurring at the physiological, structural, biochemical, and/or molecular levels during this period could be associated with implantation failures and recurrent miscarriages (5,6). Understanding these changes would help in deciphering the still unknown biological mechanism of not only implantation but also of infertility related to implantation failure, which remains a major problem in assisted reproductive techniques (ARTs).

Hallmarks of the endometrium in the implantation window are: (a) the engorgement of the endometrial lumen's surface epithelium with secretory vacuoles, (b) the subsequent formation of pinopodes, and (c) the existence of small tips of microvilli structures, all occurring under the influence of ovarian hormones at the luteal phase (7). Therefore, a detailed scanning electron microscopy (SEM) assessment of an endometrial biopsy could be reliable and useful for the evaluation of uterine receptivity on an individual basis with the aim of obtaining a clinical pregnancy in an ART cycle (8,9). However, as SEM does not give details on cellular morphology, the structural distribution of organelles, and the condensation of chromatin, transmission electron microscopy (TEM) appears to be more appropriate and is a very good option to consider (10).

Morphological changes on the apical surface of the endometrium and structures such as pinopodes should be well defined and functionally characterized by TEM since a controversy still exists regarding their role in the receptivity of the endometrium and thus implantation (11,12). This study was designed to use TEM to compare the endometrial tissues biopsied in the implantation window from infertile patients with at least 3 previous

* Correspondence: laylabahar@gmail.com

ART failures and the endometrial tissues of fertile women, in order to evaluate the differences at the cellular level and to assess their potential role in implantation.

2. Materials and methods

2.1. Study groups and endometrial biopsy

All women participating in this study gave their informed consent and the use of their endometrial tissue for research purposes was approved by the Ethics Committee of Memorial Hospital (İstanbul, Turkey). The study group consisted of 14 infertile patients (mean age: 32 ± 4.3 years) who had 3 or more ART failures despite good-quality embryo and/or blastocyst transfer. They were categorized as having repeated implantation failure (RIF), which is defined as consecutive negative pregnancy tests following transfer of morphologically good embryos and/or blastocysts. All patients in the study group had clarified fallopian tubes opened with hysterosalpingography or laparoscopy. Patients diagnosed with endometriosis were excluded.

The control group consisted of 9 fertile volunteers (mean age: 31 ± 3.2 years) who were normo-ovulatory with regular cycles and had not been on steroid hormone medications within 3 months of the endometrial sampling. The fertile women presented with the following various diseases: uterine prolapse (n = 3), uterine leiomyoma (n = 1), ovarian cyst (n = 3), and pelvic pain (n = 2).

Each subject's luteinizing hormone (LH) surge was estimated by the patient herself based on the first day of her menstruation, and each had her uterus evaluated by transvaginal ultrasonography. Human endometrial samples were obtained in the receptive (LH + 5) and (LH + 7) phases during the same cycle of all 23 women (13). Serum progesterone levels were measured on the day of the midluteal phase (days 19–21, estimated to be representative of the receptive endometrium). Endometrial samples were examined according to the endometrial biopsy dating method by Van Vaerenbergh et al. (14). Endometrial biopsies were taken using Pipelle catheters (Endocell, France) under sterile conditions, and samples were rinsed with phosphate buffered saline. After fixation, the routine histological process was applied to the endometrial samples. Additionally, TEM was used to study the morphology of cells and their organelles.

2.2. Preparation for transmission electron microscopy

Endometrial tissue samples were fixed in 2.5% glutaraldehyde and excess fixative was removed by washing with 0.1 M Dulbecco's phosphate buffered saline (DPBS), pH 7.4. After the biopsy materials were rinsed in DPBS, specimens were fixed in 1% OsO_4 and washed again thoroughly with DPBS (3 × 15 min) before the final application of a series of graded ethanol. The dehydration was performed in ethyl alcohol. The samples were then exposed to propylene oxide and embedded in an araldite resin (Araldite 212, Agar, Italy) for 1 h at room temperature. Polyethylene capsules were filled with araldite as an embedding material, and the plastic blocks were placed at 60 °C for 36 h. Afterwards, the blocks were cut into thin sections (70–100 nm) and contrasted with uranyl acetate and lead citrate for a final observation by TEM (JEOL JEM-1011, Japan).

2.3. Statistical analysis

Endometrial samples of 14 women from the RIF group and 9 from the control group were analyzed under TEM and 10 micrographs were randomly taken at different locations in each sample to check for reproducibility. The statistical analysis was done using an independent samples test and the Kolmogorov–Smirnov test with SPSS 11.5.

3. Results

3.1. TEM examination of the endometrial tissue

The mean clinicopathologic characteristics of the study and control groups are shown in Table 1. In the control group, some regions of the endometrial epithelium facing the lumen were irregular in appearance. Some of the endometrial epithelial cells had microvilli on their apex (Figure 1a) and some others had cytoplasmic projections called pinopodes, which contain secretory vacuoles. Some pinopodes and their secretory material devoid of their communication with the cells were also

Table 1. Mean clinicopathologic characteristics of the study and control groups.

	Women with repeated implantation failure (n = 14)	Fertile women (n = 9)	P-value
Mean age (years)	32 ± 4.3	31 ± 3.2	P > 0.05
Duration of infertility	3.2 ± 2.9	–	*
Previous failed cycles	3.8 ± 1.6	–	*
Live births	–	1 ± 1.3	*
Good-quality embryos transferred	3.2 ± 1.4	–	*
Hormonal treatment (tailoring the stimulation protocols)	4.1 ± 1.7	Last 3 months untreated with steroids	*

* P < 0.05.

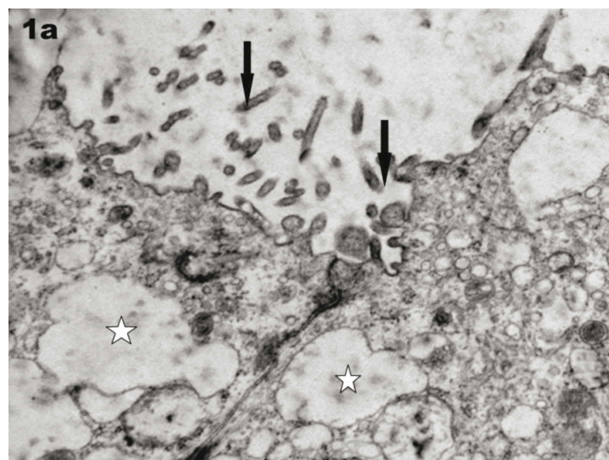

Figure 1a. Microvilli on the surface epithelium of the control group. Black arrows = microvilli, stars = vacuoles, and scale = 1.1 µm.

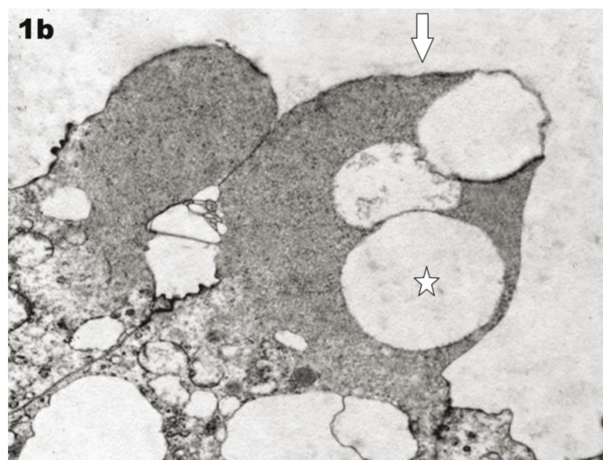

Figure 1b. Epithelium of the control group. White arrow = pinopode, star = vacuole, and scale = 1.1 µm.

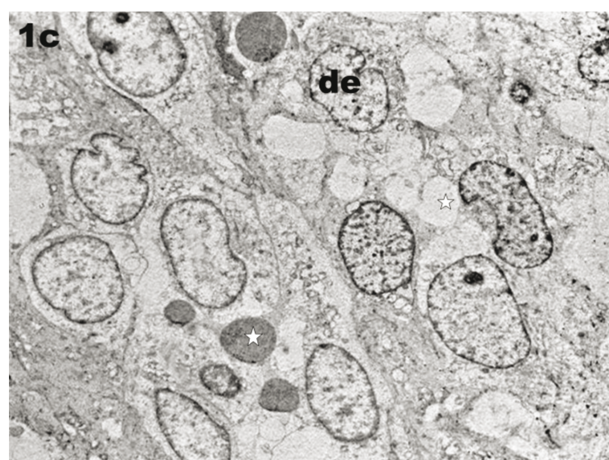

Figure 1c. Endometrial stroma of the control group. de = decidual cell's nuclei, stars = lipid and other secretory vacuoles, and scale = 4 µm.

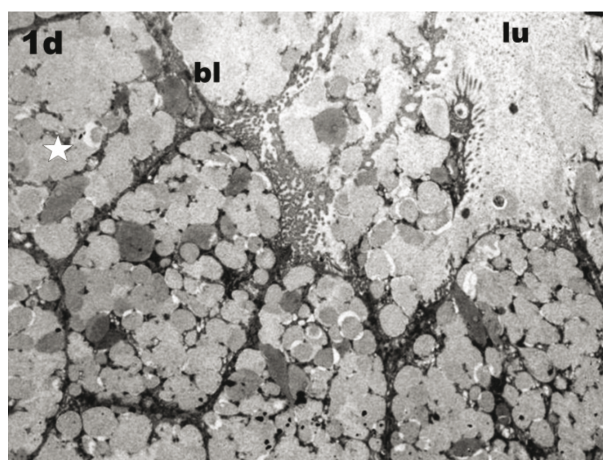

Figure 1d. Epithelial cells of the secretory gland in the control group. bl = basal lamina, stars = secretory vacuoles, lu = lumen, and scale = 1.7 µm.

observed in the lumen. Secretory vacuoles were on the basal and supranuclear regions of the cells. The vacuoles in the supranuclear regions and pinopodes were larger and merged with each other to create giant vacuoles (Figure 1b). Ciliated cells were almost completely absent and nonciliated cells displayed microvilli, which were dense, of average height, and limited in number, and covered most of the cell apical membrane. Secretory vacuoles and lipid droplets were also seen in the cytoplasm of endometrial epithelial cells. Most cells had secretory-like vacuoles (Figure 1a). The extracellular matrix was dense because of decidualization. The stroma, localized beneath the basal lamina, was cell-rich and euchromatic decidualized cells were frequent. Moreover, the stroma contained gland cells with many randomly distributed cytoplasmic secretory vacuoles. Therefore, the structure and organization of the extracellular matrix in the endometrial tissues, as well as the decidual cells, had an exceptional appearance, attributed to the receptive endometrium (Figure 1c). Occasionally, a special extracellular matrix surrounding each secretory gland cell was also observed (Figure 1d).

In the RIF group, the surface epithelium of the endometrium was composed of low cylindrical cells. There were plenty of unevenly distributed microvilli on the apical surface of most of the cells. Although they had secretory vacuoles near the lumen, they did not have intact pinopode structures (Figure 2a). However, little pinopode-like structures were spread in the lumen. Ciliated cells were observed in some places. The connections between the cells near the apical regions were pronounced (Figure 2b). Nevertheless, neighboring cells, especially those near the basal and the middle parts, were extremely enlarged

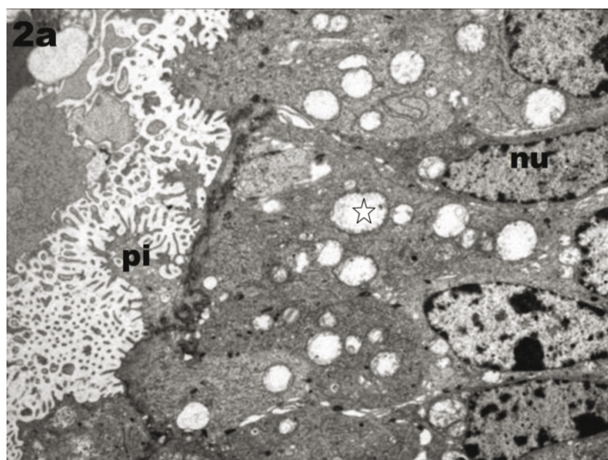

Figure 2a. Surface epithelium and pinopode-like structures in the RIF group. pi = pinopode-like structures, star = secretory vacuoles, nu = nucleus, and scale = 2.2 µm.

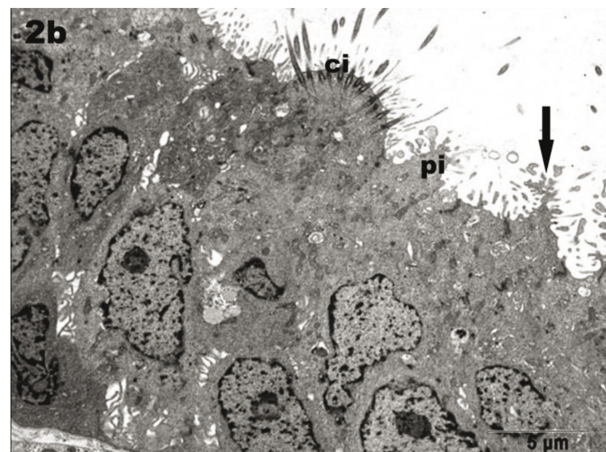

Figure 2b. Cilia and pinopode-like structures on the apical side of the RIF group's surface epithelium. ci = cilium, pi = pinopode, nu = nucleus, white arrow = interdigitations, black arrow = microvilli, and scale = 2.5 µm.

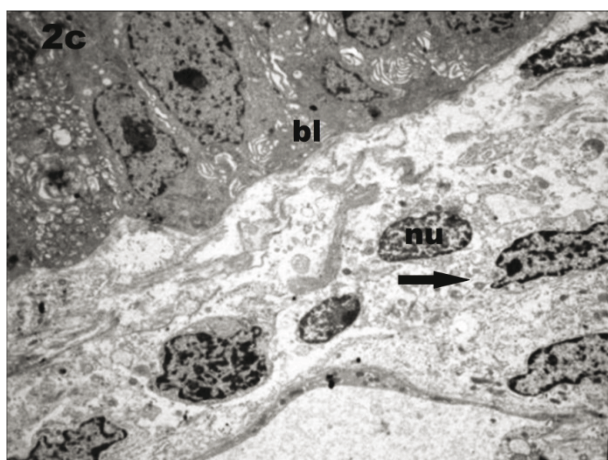

Figure 2c. Stroma below the basal lamina of the endometrial epithelium in the RIF group. bl = basal lamina, nu = nucleus, black arrow = stromal cells, white arrow = interdigitations, and scale = 3.2 µm.

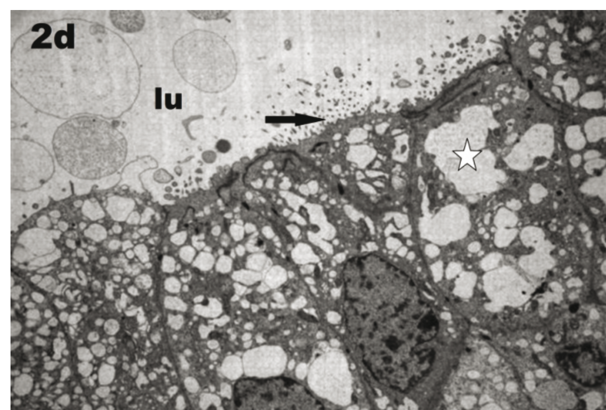

Figure 2d. Epithelial glandular cells in the RIF group. lu = lumen, star = vacuole, black arrow = microvilli, and scale = 3.2 µm.

(Figures 2b and 2c). Stroma cells were observed to be poor. In addition, interdigitations were observed between endometrial epithelial cells in the RIF group (Figure 2c). The secretory epithelial glands usually had cylindrical or low cylindrical cells and varying amounts of secretory materials were found in their lumens. In the fertile group, the glands were quite convoluted, covered a large part of the stroma, and were high in number, while the TEM evaluations of the RIF group revealed only a few flat and slightly convoluted glands (Figure 2d).

In the statistical analysis, a normal distribution was established for each category studied (pinopodes, cilia, and microvilli) and for each group (control and RIF) ($P > 0.05$) (Table 2). Therefore, the independent-samples test and Kolmogorov–Smirnov test were used for statistical analysis. As a result, the numbers of pinopodes, cilia, and microvilli in the RIF group were significantly different than those in the control group ($P < 0.001$). The confidence intervals between the control and RIF group were as follows: pinopodes, 95% CI: 2.918 to 3.968; cilia, 95% CI: −2.361 to −1.479; microvilli, 95% CI: −3.441 to −2.768 (Figure 3).

4. Discussion

Although numerous molecular studies have been conducted on the endometrium at the implantation stage, only a few have performed a morphological evaluation and have established a relationship between endometrial insufficiency and its morphology in RIF patients (15–17). Therefore, this study was designed to examine

Table 2. The normal distribution established for the RIF and control groups for each subcategory.

	Group	n	Mean	±SD	95% confidence interval for mean
Pinopode	Control	9	4.8222	0.70139	4.2831–5.3614
	RIF	14	1.3786	0.51168	1.0831–1.6740
Cilium	Control	9	0.5222	0.47900	0.1540–0.8904
	RIF	14	2.4429	0.50644	2.1504–2.7353
Microvillus	Control	9	2.0667	0.25000	1.8745–2.2588
	RIF	14	5.1714	0.43928	4.9178–5.4251

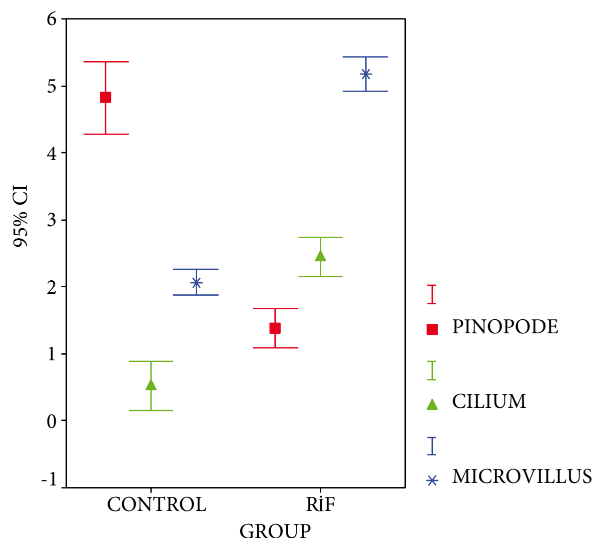

Figure 3. An independent-samples test was applied for the comparison of 2 independent groups.

the morphological features of the endometrium at the implantation stage by TEM in RIF patients compared to fertile controls. Microscopic evaluations of their endometrial samples revealed significant differences in their morphological structures. The most notable morphological finding was domed structures called pinopodes, which appeared during the endometrial receptive period (18) and were found in most of the surface epithelial cells in the fertile group's luteal phase endometrium (Figure 1b). It is well established that without pinopodes, implantation cannot be achieved (7). The pinopodes were well developed in the fertile group, whereas they were few in number in the RIF group due to insufficient epithelial growth (Figures 2a and 2b).

As in previous studies, micrographs of the fertile group showed that the pinopodes were located in the apical side of the endometrial cells during the preparatory stage of implantation (7,19) The TEM analyses demonstrated that the pinopodes were filled with secretory material that was extending towards the lumen and with content that could provide the blastocyst with nutrients and even allow it to get close and attach to the endometrium (Figure 1b). Nikas et al. reported that the best observation of pinopodes in the human endometrium could be done on the 20th day of the menstrual cycle when the blastocyst can have contact with the endometrium (20), and the present study revealed that pinopodes in the endometrial samples biopsied on days 19–21 were indeed dense and notable. In this TEM study, we have considered pinopodes as useful markers of receptivity, because although different views about pinopodes still exist, recent evidence suggests that pinopodes are hallmarks of the period with the highest endometrial receptivity (11).

Another structure observed in this study were the microvilli, whose numbers and structures varied with the density of pinopodes, similar to many other studies (6). However, the TEM analyses of endometrial thin sections of the fertile group from days 19–21 showed that microvilli decreased in number and size. The apical parts of the cells were filled with mucoid material, which fed the blastocyst and consisted of numerous vacuoles, as a result of which the microvilli were shortened and occasionally obliterated, allowing the development of the pinopodes (Figures 1a and 1b).

Although the luminal epithelial cells were expected to have giant vacuoles enlarging from the basal to the apical region and obliterated microvilli, when compared with the fertile group, the luminal epithelial cells of the RIF group had insufficient secretory production, which was supported by the presence of poor secretion in the endometrial lumen. A comparison of protein abundances between the fertile and infertile women revealed that several proteins were present at altered levels depending on cycle stage or fertility status (21). Nevertheless, most of the microvilli remained comparable in terms of size and number in both groups despite an irregular appearance in the RIF group (Figure 2a).

Conflicting evidence also exists about the presence of cilia in the human endometrium. The endometrium was reported to be lined with secretory cells without cilia and a simple cylindrical epithelium with cilia (22), but another study also emphasized that the cylindrical epithelium of the uterus has cilia before menarche, but that most of the cells do not have cilia after menarche (23). Moreover, cilia with a rhythmic beat were reported to decrease during the luteal phase in fertile individuals from a 1/20 ratio to a 1/50 ratio (24). In the present study, the absence of cilia in the ultrastructural examination of the fertile group's epithelium was considered positive in terms of implantation. In fact, the presence of cilia and their rhythmic movements during the receptive stage of the endometrium may not allow the blastocyst to attach to the endometrium and thus may render implantation difficult. As opposed to the fertile group, TEM analyses of the RIF group showed that cilia were observed (Figure 2b), which may explain the implantation failures.

Briefly, the TEM analyses showed that the structure of the endometrial epithelium of the RIF patients was completely different from the normal endometrial epithelium. The morphological assessment described above is supported by the statistical analysis, as the ultrastructures of the endometrial epithelium that were studied, namely the pinopodes, microvilli, and cilia, of the controls and the RIF patients were significantly different (Table 2).

Decidualization of the endometrial stroma is initiated in women during the receptive phase (25). Stromal cells with a decidual reaction enable the invasion and the nutrition of the embryo (26). Furthermore, uterine secretions from the endometrial glandular epithelium provide optimal conditions for early embryonic development (27). In recent years, several studies reported that the removal of endometrial secretions immediately prior to embryo transfer provides sufficient material for analysis of receptivity markers without disrupting embryo implantation (28). In our study, few stromal cells were observed in the fertile group, and these were active, meaning that they were turned into decidual cells in most of the areas, providing the morphological appearance of a receptive endometrium (Figure 1c). In contrast, in the RIF group, the stroma was not rich in cells or extracellular matrix (Figure 2c). Deficient glandular activity, usually described as a 'secretory phase defect', has been hypothesized to be an underlying cause of early pregnancy failure in humans (29). Insufficient conversion of stromal cells into decidual cells may also be one of the reasons for implantation failure.

In our study, the glands covered a large part of the stroma in the fertile group, while the RIF group had only a few flat and slightly convoluted glands (Figure 2d). These secretory glandular cells formed few secretory vacuoles and produced little secretion, which could also be considered as a deficiency in terms of the physiology of implantation. Lastly, the endometrial secretion of glandular cells plays a critical role in the implantation and maintenance of pregnancy (30). Parmar et al. maintained that uterine secretions might play an important role in endometrial functions and dysfunctions (31,32). A recent study in China reported that the removal of endometrial secretions immediately prior to embryo transfer provides sufficient material for analysis of receptivity markers without disrupting embryo implantation (28). Recently, Schmitz et al. reported for the first time on a novel endometrial protein, milk fat globule-epidermal growth factor (MFG-E8). They showed that the MFG-E8 protein is mostly expressed in the endometrial epithelial cells and during the window of implantation in normative ovulatory women, both in luminal and glandular epithelial cells, and with dense staining at both the apical and basal cellular parts (33,34).

In conclusion, our TEM study showed significant differences in the morphological features of the endometrial tissue of fertile and RIF patients. One of the leading causes of RIF was found to be an insufficiency of the endometrial tissue; pinopodes and decidualization of the stromal cells were deficient. There is no doubt that understanding endometrial changes will be a key step forward in the elucidation of the implantation mechanism. Thus, perhaps new systems can be developed to characterize new proteins or factors that have been hypothesized to improve fertility by improving blastocyst attachment to the endometrium. New immunohistochemical studies may also help in the delineation of implantation by identifying secretory materials and the signaling mechanisms involved. However, further studies are needed to confirm the morphological features of the endometrium of the RIF patients described in this study in a larger cohort.

References

1. Guzeloglu-Kayisli O, Basar M, Arici A. Basic aspects of implantation. Reprod Biomed Online 2007; 15: 728–739.
2. Nikas G, Aghajanova L. Endometrial pinopodes: some more understanding on human implantation? RBM Online 2002; 4: 18–23.
3. Fan W, Li SW, Li WH, Wang Y, Gong Y, Ma QH, Luo S. FOXO 1 expression and regulation in endometrial tissue during the menstrual cycle and in early pregnancy decidua. Gynecol Obstet Invest 2012; 74: 56–63.

4. Yılmaz N, Kılıç S, Madendağ Y, Madendağ I, Özgün A, Özakşit G, Batıoğlu S. Endometrial parameters in IVF and IUI administration on elderly women. Turk J Med Sci 2010; 40: 343–348.

5. Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, Wang H. Molecular cues to implantation. Endocr Rev 2004; 25: 341–373.

6. Lédée N, Munaut C, Aubert J, Sérazin V, Rahmati M, Chaouat G, Sandra O, Foidart JM. Specific and extensive endometrial deregulation is present before conception in IVF / ICSI repeated implantation failures (IF) or recurrent miscarriages. J Pathol 2011; 225: 554–564.

7. Nardo LG, Sabatini L, Rai R, Nardo F. Pinopode expression during human implantation. Eur J Obstet Gynecol Reprod Biol 2002; 101: 104–108.

8. Murphy CR. Understanding the apical surface markers of uterine receptivity: pinopods or uterodomes? Human Reprod 2000; 15: 2451–2454.

9. Adams SM, Murphy CR. A successful pregnancy following SEM fine tuning of hormonal priming. BMC Pregnancy Childbirth 2001; 1: 3.

10. Salehnia M. Progesterone shifts the pinopodes expression of mouse endometrium to pre-implantation time after ovarian hyperstimulation. Iranian J Reprod Med 2003; 1: 20–23.

11. Quinn CE, Casper RF. Pinopodes: a questionable role in endometrial receptivity. Hum Reprod Update 2009; 15: 229–236.

12. Bahar L, Candan ZN, Kahraman S, Baykal T. Comparatively evaluation of infertile (RIF) and fertile women endometrial biopsies at the ultrastructural level by TEM. In: Dubuisson JB, Gomel V, editors. Reproductive Medicine and Surgery from the Proceedings of the 15th World Congress on In Vitro Fertilization and 4th World Congress on In Vitro Maturation; 19–22 April 2009. Geneva, Switzerland: Medimond Publications; 2009. pp. 174–175.

13. Haouzi D, Assou S, Mahmoud K, Tondeur S, Rème T, Hedon B, De Vos J, Hamamah S. Gene expression profile of human endometrial receptivity: comparison between natural and stimulated cycles for the same patients. Hum Reprod 2009; 24: 1436–1445.

14. Van Vaerenbergh I, McIntire R, Van Lommel L, Devroey P, Giudice L, Bourgaın C. Gene expression during successful implantation in a natural cycle. Fertil Steril 2010; 93: 15–18.

15. Sandra O, Mansouri-Attia N, Lea RG. Novel aspects of endometrial function: a biological sensor of embryo quality and driver of pregnancy success. Reprod Fertil Dev 2011; 24: 68–79.

16. Puri CP, Katkam RR, Sachdeva G, Patil V, Manjramkar DD, Kholkute SD. Endometrial contraception: modulation of molecular determinants of uterine receptivity. Steroids 2000; 65: 783–794.

17. Imakawa K, Chang KT, Christenson RK. Pre-implantation conceptus and maternal uterine communications: molecular events leading to successful implantation. J Reprod Dev 2004; 50: 155–169.

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

19. Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. Hum Reprod Update. 2006; 12: 731–746.

20. Nikas G, Aghajanova L. Endometrial pinopodes: some more understanding on human implantation? RBM Online 2002; 4: 18–23.

21. Hannan NJ, Stephens AN, Rainczuk A, Hincks C, Rombauts LJF, Salamonsen LA. 2D-DiGE analysis of the human endometrial secretome reveals differences between receptive and nonreceptive states in fertile and infertile women. J Proteome Res 2010; 9: 6256–6264.

22. Demir R, Kayisli UA, Celik-Ozenci C, Korgun ET, Demir-Weusten AY, Arici A. Structural differentiation of human uterine luminal and glandular epithelium during early pregnancy: an ultrastructural and immunohistochemical study. Placenta 2002; 23: 672–684.

23. Singh I. Textbook of Human Histology with Colour Atlas. 6th ed. New Delhi, India: Jaypee; 2011.

24. Kurita T, Nakamura H. Mullerian duct, epithelium, mezenchyme. In: Aplin JD, Fazleabas AT, Glasser SR, Giudice LC, Editors. The Endometrium. 2nd ed. New York, USA: Taylor & Francis; 2008. pp. 1–19.

25. Salamonsen LA, Nie G, Hannan NJ, Dimitriadis E. Society for Reproductive Biology Founders' Lecture. Preparing fertile soil: the importance of endometrial receptivity. Reprod Fertil Dev 2009; 21: 923–934.

26. Gellersen B, Reimann K, Samalecos A, Aupers S, Bamberger AM. Invasiveness of human endometrial stromal cells is promoted by decidualization and by trophoblast derived signals. Hum Reprod 2010; 25: 862–873.

27. Ulbrich SE, Gross K, Schmidt S, Blum H, Rottmayer R, Hiendleder S, Fröhlich T, Arnold GJ, Wolf E, Meyer HHD et al. Does estradiol-17 β cause cycle-dependent modulations of uterine milk protein in bovine endometrium? Reprod Fertil Dev 2006; 19: 202–203.

28. Li MQ, Jin LP. Ovarian stimulation for in vitro fertilization alters the protein profile expression in endometrial secretion Int J Clin Exp Pathol 2013; 6: 1964–1971.

29. Burton GJ, Jauniaux E, Charnock-Jones DS. Human early placental development: potential roles of the endometrial glands. Placenta 2007; 28: 64–69.

30. Tawfeek MA, Eid MA, Hasan AM, Mostafa M, El-Serogy HA. Assessment of leukemia inhibitory factor and glycoprotein 130 expression in endometrium and uterine flushing: a possible diagnostic tool for impaired fertility. BMC Womens Health 2012; 12: 10.

31. Parmar T, Sachdeva G, Savardekar L, Katkam RR, Nimbkar-Joshi S, Gadkar-Sable S, Salvi V, Manjramkar DD, Meherji P, Puri CP. Protein repertoire of human uterine fluid during the mid-secretory phase of the menstrual cycle. Hum Reprod 2008; 23: 379–386.

32. Turgut A, Goruk NY, Tunç SY, Agaçayak E, Alabalik U, Yalinkaya A, Gül T. Expression of extracellular matrix metalloproteinase inducer (EMMPRIN) in the endometrium of patients with repeated implantation failure after in vitro fertilization. Eur Rev Med Pharmacol Sci 2014; 18: 275–280.

33. Schmitz C, Yu L, Bocca S, Anderson S, Cunha-Filho JS, Rhavi BS, Oehninger S. Role for the endometrial epithelial protein MFG-E8 and its receptor integrin avb3 in human implantation: results of an in vitro trophoblast attachment study using established human cell lines. Fertil Steril 2014; 101: 874–883.

34. Franchi A, Bocca S, Anderson S, Riggs R, Oehninger S. Expression of milk fat globule EGF-factor 8 (MFG-E8) mRNA and protein in the human endometrium and its regulation by prolactin. Mol Hum Reprod 2011; 17: 360–371.