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Original Article

Ultrastructural features of endometrial-myometrial interface and its alteration in adenomyosis

Ying Zhang[1], Li Zhou[1], Tin C Li[2], Hua Duan[1], Pei Yu[1], Hong Y Wang[3]

[1]Department of Gynecology Minimally Invasive Center, Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Qi He Lou Street 17#, Dongcheng District, Beijing, China, Post code: 100006; [2]Department of Reproductive Medicine and Surgery, The Jessop Wing Sheffield Teaching Hospital, The Jessop Wing Tree Root Walk, Sheffield, United Kingdom, Post code: S10 2SF; [3]Department of Pathology, Fu Wai Hospital Cardiovascular Institute, Chinese Academy of Medical Sciences Peking Union Medical College, Beijing, China, Post code: 100037

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Abstract: The endometrial-myometrial interface (EMI) is a specific functional region of uterus. However, our knowledge on EMI ultrastructure both in normal uterus and adenomyosis is far from enough to understand its pathology. In this study, used the samples of EMI and outer myometrium (OM) from the adenomyosis hysterectomy specimens and the subjects from the control uteri, we prospectively compared the ultrastructure of myocytes from EMI and OM, the ultrastructural changes of EMI between the proliferative and secretory phases, and the ultrastructural difference of EMI between adenomyosis and the control group. In both adenomyosis and control group, there were differences in ultrastructure between myocytes from EMI and OM. Specifically, the myocytes from EMI were rich in organelles. In contrast, the myocytes from OM had abundant contractile structural components. In the proliferative phase, the myocytes from EMI in adenomyosis had significantly smaller cell and nucleus diameter than those from the control group, but in the secretory phase, the difference was not significant. In the control group, the various ultrastructural features of myocytes from EMI including the mean diameter of cell and nuclei and the myofilaments/cytoplasm ratio exhibited cyclical changes, but in adenomyosis, the normal cyclical changes were absent. In conclusions, there are significant ultrastructural differences between the myocytes from EMI and OM. The myocytes in women with adenomyosis were significantly different to the control subjects, primarily because the normal cyclical changes were absent.

Keywords: Adenomyosis, endometrial-myometrial interface, outer myometrium, ultrastructure

Introduction

The endometrial-myometrial interface (EMI), is also known as “junctional zone”, or “the inner myometrium”, first described in 1983 by Hricak et al [1]. EMI is different from the outer myometrium (OM) in respect of the embryological origin, structure, function and pathology. Embryologically, just like the endometrium, EMI is of Müllerian origin, while the OM is of non-Müllerian, mesenchymal origin [2, 3]. Structurally, EMI is the inner one third of myometrium composed of shorter muscle bundles. The OM is the outer two third of myometrium predominantly composed of longer muscle fibers. However, on light microscopic examination, the whole myometrium appears as a homogeneous structure comprised of smooth muscle cells, myocytes [2]. Tetlow et al. [4] used biopsy needle obtained EMI tissue from the uterus directed by ultrasound; morphometric analysis of the specimen demonstrated a greater total nuclear area in EMI than the OM. Functionally, contractile activity in the non-pregnant uterus, a type of peristalsis-like contraction, has been found to be present in EMI. The peristalsis is involved in sperm transport, regulation of implantation and hemostasis during menstruation [6-11]. Therefore, EMI plays a vital role in the various functions of the non-pregnant uterus, while the OM is mainly responsible for parturition. Pathologically, EMI is the most common region to be affected in adenomyosis and is characterized by irregularity and thickening [2, 3, 6, 8, 9, 12, 13]. Altered EMI contraction can be observed in adenomyosis including hyperperistalsis and

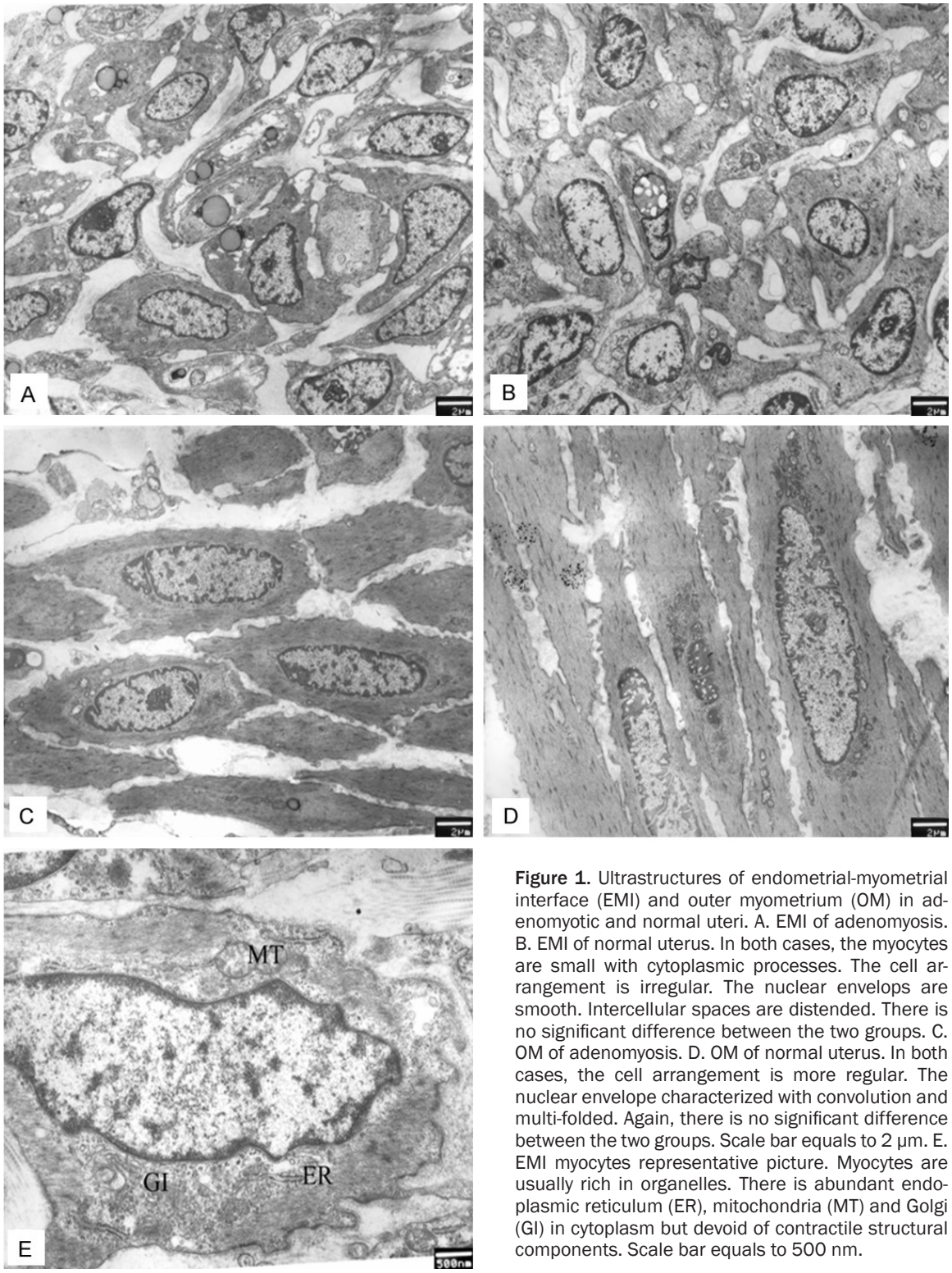

Figure 1. Ultrastructures of endometrial-myometrial interface (EMI) and outer myometrium (OM) in adenomyotic and normal uteri. A. EMI of adenomyosis. B. EMI of normal uterus. In both cases, the myocytes are small with cytoplasmic processes. The cell arrangement is irregular. The nuclear envelops are smooth. Intercellular spaces are distended. There is no significant difference between the two groups. C. OM of adenomyosis. D. OM of normal uterus. In both cases, the cell arrangement is more regular. The nuclear envelope characterized with convolution and multi-folded. Again, there is no significant difference between the two groups. Scale bar equals to 2 μm. E. EMI myocytes representative picture. Myocytes are usually rich in organelles. There is abundant endoplasmic reticulum (ER), mitochondria (MT) and Golgi (GI) in cytoplasm but devoid of contractile structural components. Scale bar equals to 500 nm.

abnormal peristalsis which may be responsible for the associated clinical manifestations such as dysmenorrhea, metrorrhagia and infertility [3, 5, 6, 8, 9, 14, 15].

The structure and function of EMI has been receiving increasing attention from investigators. Ultrasound and MR imaging are used for localization of EMI and diagnosis of the EMI-

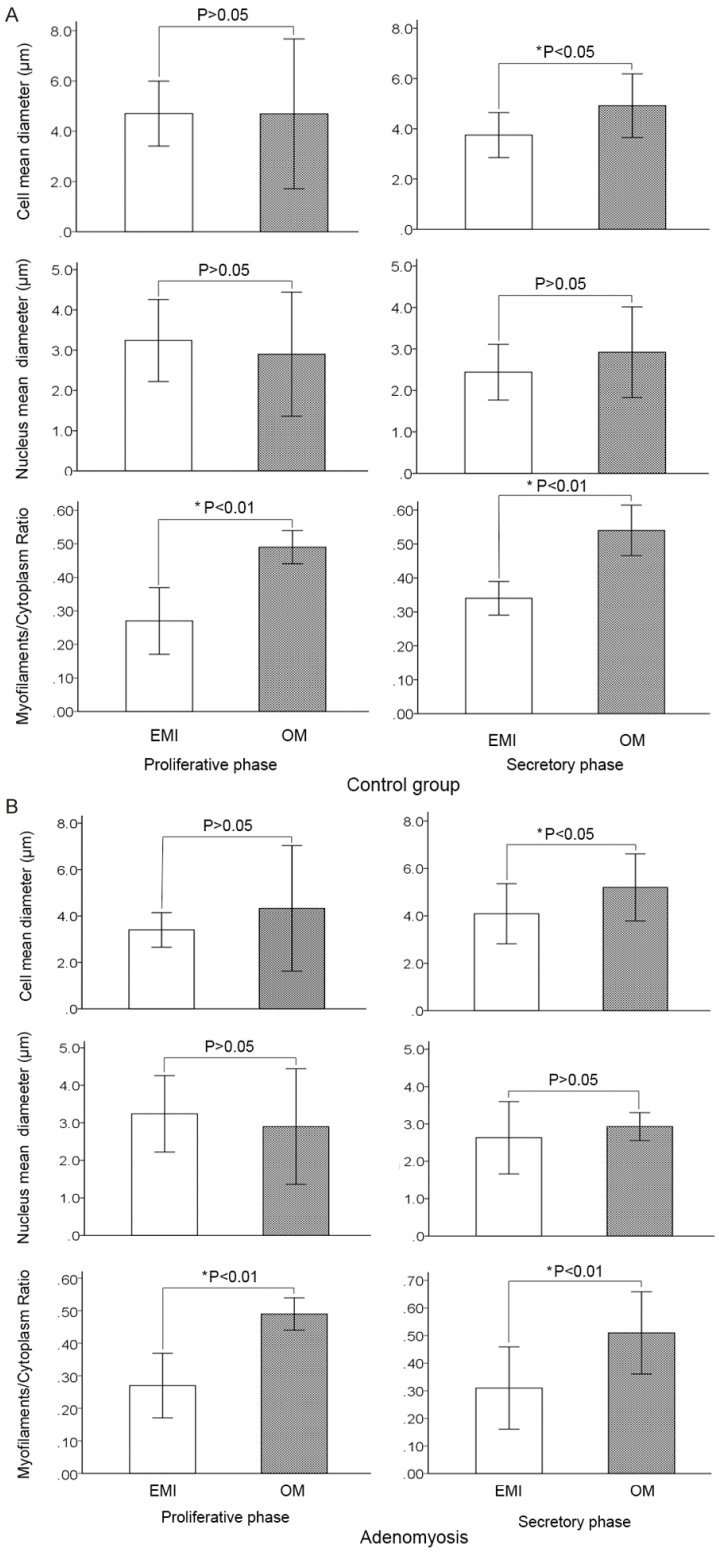

Figure 2. A comparison of myocytes between endometrial-myometrial interface (EMI) and outer myometrium (OM) in the control group (A) and adenomyosis (B). In both groups, myocytes from EMI are significantly (P<0.05) smaller than those from OM in the secretory phase. The myofilaments/cytoplasm ratio of myocytes from EMI is significantly (P<0.01) less than that from OM in both the proliferative and secretory phases.

related disease such as adenomyosis [3, 4, 16-19]. Videovaginosonography, hysterosalpingoscintigraphy, High-resolution ultrasound and cine MR imaging can all detect the contraction of EMI [3, 8, 20, 21]. Immunohistochemistry results reveal that EMI is rich in estrogen and progesterone receptors which indicate that ovarian steroid hormones may regulate EMI function [22]. However, the research on ultrastructure of EMI is relatively scarce except for the study of Mehasseb et al. which found that there was no difference between EMI and OM [5]. In this study, we examined EMI and OM tissue in different menstrual phases obtained from women with and without adenomyosis with a view to describing the ultrastructural changes in myocytes in women with adenomyosis.

Materials and methods

Adenomyosis and control group

Ten hysterectomy specimens from pre-menopausal subjects with adenomyosis, 5 from the proliferative phase and 5 from the secretory phase, constituted the study group. The diagnosis of adenomyosis was made before surgery based on the clinical symptoms and transvaginal ultrasonography, and then histologically confirmed postoperatively. The clinical symptoms included dysmenorrhea and/or metrorrhagia. The ultrasonographic diagnostic criteria as described in previously published studies [23, 24] were as follows: an asymmetric or globular uterus; asymmetrical thickening of junctional zone in the anterior or posterior wall; subendometrial linear striations; heterogeneous myometrial echotexture and myometrial cysts. In our study, we selected the cases with the thickened junctional zone in posterior wall. Another ten hysterectomy specimens from pre-meno-

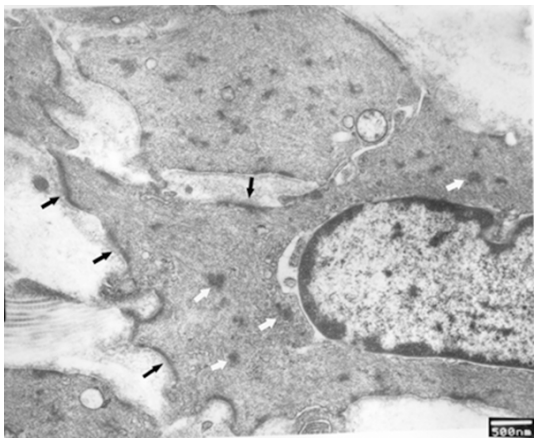

Figure 3. The typical dense bodies (white arrows) and the long dense bands (black arrows) are rich in myocytes from OM, unlike the myocytes from EMI. Myocytes are usually lack in organelles. Scale bar equals to 500 nm.

pausal subjects without adenomyosis but with early cervical neoplasm (CINIII and cervical cancer stage Ia), 5 from the proliferative phase and 5 from the secretory phase, constituted the control group. The mean±SD age of the study and control groups were 45.5±5.5 and 47.6±3.0 years respectively (no significant difference). All patients had regular menstrual cycle (25-35 days), without myoma, previous uterus surgery and hormone use in the preceding three months. Patients with vascular and renal disease were excluded. This study was approved by our institutional ethics review board. Informed consent was obtained from each patient.

EMI specimens

After surgical removal, the uteri were immediately opened with a Y-shaped incision. Multiple 5 mm^3 samples were obtained from the EMI (underneath the endometrium) and the outer third of the myometrium. All samples were obtained from the posterior wall of the uterus near the fundus. Parallel full uterine samples were obtained for traditional histological examination to confirm the presence or absence of adenomyosis and the stage of the menstrual cycle.

Transmission electron microscope assessed

Samples of EMI and OM were fixed in 2.5% buffered glutaraldehyde for 24 hours. The samples were then washed by 0.1 M phosphate buffer, post-fixed in buffered 1% osmium tetroxide for 30 minutes, rewashed and dehydrated in acetone series. They were embedded in Epon812 and then trimmed. One um semi-thin section was cut and stained by toluidine blue and basic fuchsin. Under optical microscope, EMI specimens were re-positioned and selected. Excess tissue was trimmed. Ultrathin 70-nm sections were cut from each sample, collected on 300 copper mesh grids, counter stained with 2% uranyl acetate and Reynolds' lead citrate and examined using a JEM1010 transmission electron microscope (Jeol, Tokyo, Japan).

Images analysis

Images were evaluated and the related indices were measured by two independent electron microscopy specialists who were blinded to the information of the specimen. For each specimen examined under electron microscopy, 10 images were selected randomly in low magnification and high magnification, respectively. Using the Scion image measurement software, the mean diameter of cells and nuclei were measured through the nuclear center of the cells' longitudinal axis in the low magnification pictures (2 μm, ×2500). The muscle fiber area and the cytoplasmic area were measured through the nuclear center section in high magnification pictures (500 nm, ×10000). The average myofilaments/cytoplasm ratio was calculated.

Statistical analysis

Statistical analysis was performed using SPSS for Windows 17.0. Data were expressed as the mean and standard errors of the mean. Comparisons of the means were made using Student's t test for non-paired samples. A P value of <0.05 was considered statistically significant.

Results

Comparison of ultrastructural features of myocytes from EMI and OM

The features of EMI and OM were shown in **Figure 1A-D**. At EMI, the myocytes were small and with cytoplasmic processes. The cell arrangement was irregular. At OM, the cytoplasmic processes were less frequently found. Compared to OM, EMI had more distended intercellular spaces and with more prominent collagen bundles; myocytes from EMI were richer in organelles. In myocytes from EMI, there was an abundance of endoplasmic reticulum, mitochondria

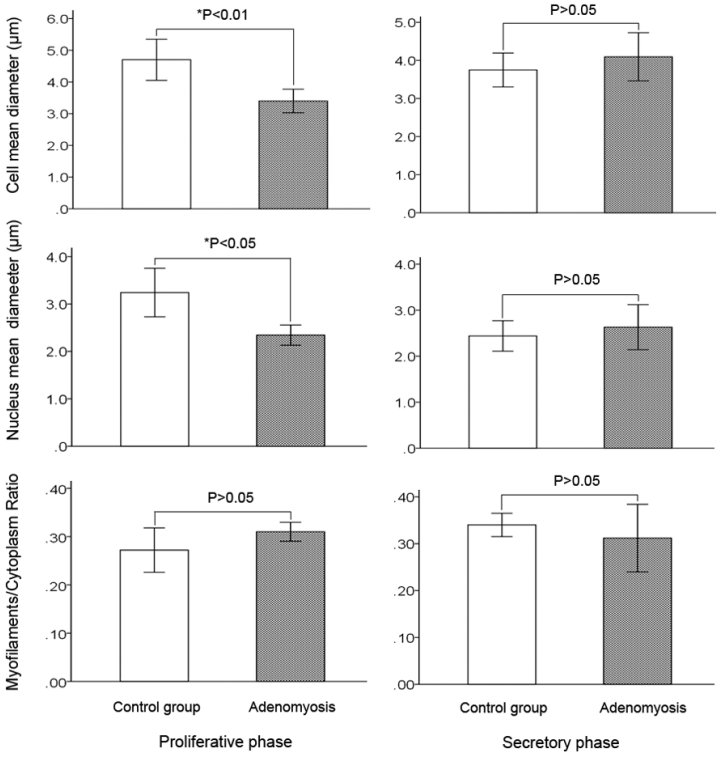

Figure 4. A comparison of endometrial-myometrial interface (EMI) ultrastructure between adenomyosis and the control group. The comparison is made separately for the proliferative and secretory phase. In proliferative phase, the mean diameters of cells and nuclei in adenomyosis are significantly smaller than in control group ($P<0.01$, $P<0.05$, respectively). There is no significantly different in myofilaments/cytoplasm ratio ($P>0.05$). In secretory phase, all parameters are not significantly different between the two groups ($P>0.05$).

and Golgi (**Figure 1E**), the mitochondria exhibited unfolding of the internal cristae, and the rough endoplasmic reticulum and Golgi apparatus were more prominent, denoting active protein synthesis; however, the contraction related structures, such as cytoplasmic myofilaments, dense bodies and dense bands in cell membrane were less abundant, denoting diminished contractive capacity. In addition, myocytes from EMI had smooth nuclear envelope, in contrast to the convoluted nuclear envelope observed in myocytes from OM.

The features of myocytes from EMI and OM in adenomyosis and control group were compared in **Figure 2A**, **2B**. In proliferative phase, the mean cell diameter and the mean nucleus diameter of myocytes from EMI were not significantly different from those of OM both in the adenomyosis and the control groups. In the secretory phase, the mean cell diameter of myocytes from EMI was significantly ($P<0.05$) smaller than those from OM both in the adenomyosis and the control groups; the mean nucleus diameter was not significantly different. The myocytes from OM were rich in contractile structural components. The cytoplasm contained an abundance of myofilaments, with their associated dense bodies. The length of dense bands in cell membrane was longer and more distinct which were shown in **Figure 3**. In adenomyosis, the mean±SE myofilaments/cytoplasm ratio of myocytes from OM in proliferative phase (0.54±0.03) was significantly ($P<0.01$) higher than that from EMI (0.31±0.02); the mean±SE myofilaments/cytoplasm ratio of myocytes from OM in secretory phase (0.51±0.06) was also significantly ($P<0.01$) higher than that from EMI (0.31±0.06). In the control group, the mean±SE myofilaments/cytoplasm ratio of myocytes from OM in proliferative phase (0.49±0.02) was significantly ($P<0.01$) higher than that from EMI (0.27±0.04); the mean±SE myofilaments/cytoplasm ratio of myocytes from OM in secretory phase (0.54±0.03) was also significantly ($P<0.01$) higher than that from EMI (0.34±0.02) (**Figure 2A**, **2B**).

Comparison of EMI ultrastructure between adenomyosis and control groups

Except for the cells and the nuclei size in the proliferative phase, other ultrastructural features of myocytes from EMI of adenomyosis and control uteri were not significantly different from each other. The comparisons are shown in **Figure 4**.

In the proliferative phase, the mean±SE cell diameter of myocytes in adenomyosis (3.40±0.30 μm) was significantly ($P<0.01$) smaller than that in the control group (4.70±0.52 μm). The mean±SE nuclei diameter of myocytes in adenomyosis (2.34±0.17 μm) was also significantly ($P<0.05$) smaller than that in the control group (3.24±0.41 μm). The mean±SE myofilaments/cytoplasm ratio in adenomyosis

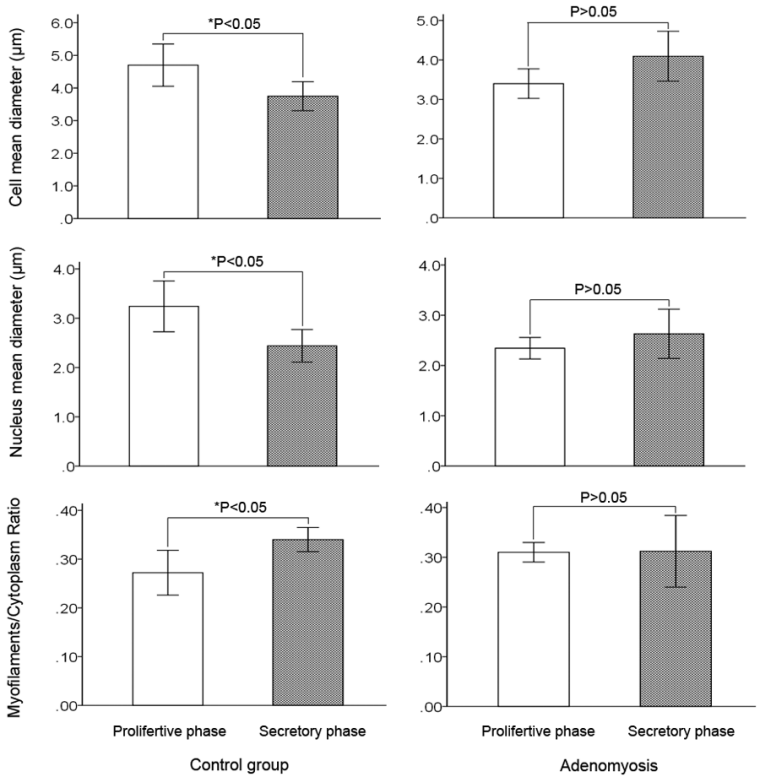

Figure 5. A comparison of endometrial-myometrial interface (EMI) ultrastructure between the proliferative phase and the secretory phase. The comparison is made separately for adenomyosis and the control group. In control uteri, cells and nuclei mean diameters and the myofilaments/cytoplasm ratio exhibit the cyclical changes ($P<0.05$). In contrast, in adenomyosis, there are no significant differences between cycle phases in the measure criteria.

(0.31±0.02) was not significantly ($P=0.13$) different from that in the control group (0.27±0.04).

In the secretory phase, all parameters including mean cell diameter, mean nuclei diameter and the myofilaments/cytoplasm ratio in adenomyosis were not significantly different from those in the control group. The amount and the morphology of the perinuclear cell organelles, such as endoplasmic reticulum, mitochondria and Golgi, were not different between the two groups in both the proliferative and secretory phases.

Comparison of EMI ultrastructure between proliferative and secretory phases

During the menstrual cycle, EMI ultrastructure in normal uteri exhibited characteristically cyclical changes. In contrast, the normal cyclical changes were absent in adenomyosis. The comparisons are shown in **Figure 5**.

In the control group, the mean±SE cell diameter of myocytes in the proliferative phase (4.70±0.52 μm) was significantly ($P=0.03$) larger than that in the secretory phase (3.75±0.36 μm). The mean±SE nuclei diameter of myocytes in the proliferative phase (3.24±0.41 μm) was significantly ($P=0.02$) larger than that in the secretory phase (2.44±0.27 μm). The mean±SE myofilaments/cytoplasm ratio of myocytes in the proliferative phase (0.27±0.04) was significantly ($P=0.03$) less than that in the secretory phase (0.34±0.02). Therefore, the cell and nuclei size and the myofilaments/cytoplasm ratio all demonstrated significantly different between the proliferative phase and secretory phase.

In adenomyosis, the mean±SE cell diameter of myocytes in the proliferative phase (3.40±0.30 μm) was not significantly ($P=0.06$) different from that in the secretory phase (4.09±0.51 μm). The mean±SE nuclei diameter of myocytes in the proliferative phase (2.34±0.17 μm) was not significantly ($P=0.23$) different from that in the secretory phase (2.63±0.39 μm). The mean±SE myofilaments/cytoplasm ratio of myocytes in the proliferative phase (0.31±0.02) was not significantly ($P=0.96$) different from that in the secretory phase (0.31±0.06). Therefore, the cell and nuclei size and the myofilaments/cytoplasm ratio had no significant difference between the proliferative phase and secretory phase.

Discussion

In this study, we have examined the ultrastructural features of myocytes from EMI and OM, and the impact of the stage of the cycle and the presence of adenomyosis on the ultrastructure. The novel findings of this study are that the myocytes of EMI is the contractile phenotype of

smooth muscle cells; whilst myocytes from EMI belonging to the synthetic phenotype.

Myocyte ultrastructure of EMI and OM

In comparing the ultrastructure of myocytes from EMI and OM, we found a number of significant differences. Myocytes from OM had all the characteristics of cells involved in contractile function, including a higher content of myofilaments in the cytoplasm, more prominent dense bodies and dense bands, and the convolutional nuclear membrane. The convoluted nuclear membrane is thought to be due to forces exerted on the internal structure of the myocytes by the contractile elements, which is one of the features of the contractile smooth muscle cells [25]. On the other hand, myocytes from EMI appear to have a different set of ultrastructure usually associated with protein synthesis, namely an increased number of organelles, the larger nucleus/cytoplasm ratio and the less contractile components.

Previous studies on the vascular muscles showed that there are two spectrums of smooth muscle cells, contractile phenotype and synthetic phenotype. At the contractile extreme are smooth muscle cells with a fully functional contractile apparatus. The cells are characterized by densely packed elements of the contractile machinery, (myofilaments, dense bodies, dense bands), but with minimal rough endoplasmic reticulum, Golgi, and free ribosome [26, 27]. This type of cells is responsible for producing muscle force. At the synthetic phenotype extreme, the ultrastructure shows a cytoplasm devoid of contractile bundles with extensive rough endoplasmic reticulum, Golgi, and ribosome [28]. A synthetic phenotype is correlated with smooth muscle cells proliferation and is reported to play an important role in regulating local microenvironment [29, 30].

Our observation also suggests that there are two subtypes in the uterus. Myocytes from OM are the contractile phenotype of smooth muscle cells; whilst myocytes from EMI belonging to the synthetic phenotype. The characteristics of myocytes from EMI and OM are consistent with their differential functions. The force during labor exerted by myocytes from OM is more powerful than the peristaltic waves produced by myocytes from EMI. Synthetic phenotype of myocytes from EMI is supposed to secret functional proteins to regulate EMI morphogenesis and to participate in non-pregnant uterine function, such as interacting with endometrium to regulate trophoblastic invasion in early pregnancy [31-33].

Cyclical changes in myocyte ultrastructure

In comparing the ultrastructure of myocytes between adenomyosis and normal uteri, we found evidence of cyclical changes in the myocytes in control subjects but the cyclical changes appeared to have disappeared in subjects with adenomyosis. In the control group, the myocyte size, nuclear size, and the myofilaments/cytoplasm ratio in the proliferative phase are all significantly different from those in the secretory phase. However, all the parameters in adenomyosis group are not significantly different between the proliferative phase and secretory phase, which may provide an explanation for the loss of cyclical changes of peristalsis observed in the EMI.

In contrast to our observation that there was a cyclical change in the ultrastructural features of the myocytes from EMI, Mehasseb et al. [5] were unable to find any significant difference in the ultrastructure of myocytes between different phases of the menstrual cycle. The most likely explanation for the difference in the observations is in the sample size. The number of cases in Mehasseb's study was rather small, with a total of only four adenomyosis cases and 6 control cases, and with only one adenomyosis case in the secretory phase. The small sample size could have limited the power of the study to detect a significant cyclical difference. In contrast, our study involved a larger sample size, and the samples were carefully matched for the different phases of the menstrual cycle.

Noe et al. [22] found that myocytes in EMI is rich in estrogen and progesterone receptors which parallel those of the endometrium and exhibit a cyclic pattern, suggesting that the sex steroid hormones play a role in regulating the structure and function of the myocytes of EMI. It has been found that uterine contractions emanating from EMI vary throughout the menstrual cycle [3, 6, 7, 10], which in turn produce the cyclical peristaltic waves in normal subjects. Alternation in ER expression in the endometrium and inner myometrium [34] may account for the abnormal peristalsis observed in adenomyosis [9, 15, 35].

In this study, we have detected subtle ultrastructural changes in the myocytes of women with adenomyosis in the proliferative phase, but there does not appear to be any obvious changes in the myofilaments. It seems that the contractile elements in myocytes of adenomyosis are not significantly deranged. The previous observation [6, 15, 35] that there are recognized hyperperistalsis in adenomyosis cannot be explained by changes in the ultrastructure of the myocytes. An earlier study by Mehasseb et al. [5] suggested that myocytes from EMI of adenomyosis displayed features of hypertrophy which in turn account for the thickening of the EMI observed in women with adenomyosis [3, 12, 13]. However, our finding that myocytes of EMI are smaller in adenomyosis (in proliferative phase) suggests that increase cell number is a more likely explanation for the thickening of the EMI. Morphometric study is required to confirm if it is indeed the case.

Future studies

To the best of knowledge, the observations in our study that (a) there are significant differences in ultrastructure between myocytes from EMI and OM, and (b) the normal cyclical changes in ultrastructure observed in myocytes appear to have been lost in adenomyosis, are original. The latter observation identified the link between structural changes in myocytes of EMI and adenomyosis. However, in this study, we have not examined the functional aspects of myocytes from the EMI. Further studies are required, almost certainly involving expression of protein and mRNA from biopsy specimens obtained from the EMI. A specially designed hysteroscopically directed myometrial biopsy instrument, called spirotome, has been developed by Prof Stephan Gordts (personal communication).

Conclusion

To conclude, the myocytes from EMI and OM have distinctive ultrastructural features adapted for different functions. We observed cyclical ultrastructural changes of EMI in control subject but the changes appear to be lost in women with adenomyosis. The observations are consistent with functional disruption in the EMI of women with adenomyosis.

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Disclosure of conflict of interest

None of the authors have a conflict of interest.

Address correspondence to: Dr. Hua Duan, Department of Gynecology Minimally Invasive Center, Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Qi He Lou Street 17#, Dongcheng District, Beijing, China, Post code: 100006. Tel: 086-13501019066; Fax: 086-01065235524; E-mail: duanhua888@163.com

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