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Smooth muscle differentiation at endometrio-myometrial junction

An ultrastructural study

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Summary. To determine whether myometrial smooth muscle is newly produced at the endometrio-myometrial junction (EMJ) of the adult uterus, we examined the ultrastructure of mesenchymal components at this site during the menstrual cycle and during early pregnancy. Cells having some features of smooth muscle were found among the usual endometrial stromal cells in every specimen examined. In the follicular phase of the menstrual cycle, such cells resembled myofibroblasts, but in the luteal phase and during early pregnancy, they had more distinct cytoplasmic filaments with dense bodies and dense plaques, and other fairly well developed characteristics of smooth muscle. The identification of smooth muscle-like cells at the EMJ in the adult uterus and the finding that their morphology changes into cells having many of the characteristics of smooth muscle cells during the luteal phase and early pregnancy, suggests that smooth muscle differentiation possibly occurs from multi-potential mesenchymal cells in the endometrial stroma.

Key words: Emdometrio-myometrial junction – Myofibroblast – Smooth muscle – Differentiation

Introduction

During the early stages of the development of the human uterus the genital canal is formed by the fusion of the paramesonephric ducts, while the shape of the uterus is the result of proliferation of the surrounding undifferentiated mesenchymal cells. In an ultrastructural study of the human fetal uterus, we recently found that myometrial smooth muscle cells originate from these mesenchymal cells, which first differentiate into immature smooth muscle cells resembling myofibroblasts at about 18 weeks, and then develop into mature smooth muscle cells of the myometrium by 31 weeks of gestation (Konishi et al. 1984). Immature smooth muscle cells can always be identified at the endometrio-myometrial junction (EMJ) both at 26 and 31 weeks of gestation, when bundles of myometrium made of almost mature smooth muscle cells are already formed (Konishi et al. 1984). This view of fetal uterine smooth muscle is consistent with the assumption of Bird and Willis (1965) that myometrial smooth muscle is newly produced by endometrial stroma in certain pathological conditions in the adult. However, the value of their observations is uncertain because of the difficulties in identifying cells by conventional light microscopy (Burnstock 1981).

To see if myometrial smooth muscle may be newly produced by the EMJ of the adult uterus as well as by that of the fetal uterus, we examined the ultrastructure of the mesenchymal component in this region in the adult uterus of different phases of the menstrual cycle and during early pregnancy.

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Materials and methods

Thirteen human uteri were obtained following hysterectomy for cervical carcinoma in situ (ten cases) and termination of pregnancy (three cases). None of the specimens had leiomyomas. The patients, whose ages ranged from 36 to 45 years, all had regular menstrual cycles. Five uteri were obtained during the follicular and five during the luteal phase. Uteri from pregnant women were obtained at the 7th, 9th and 14th weeks of gestation.

Tissues, including endometrium, EMJ, and myometrium obtained from the anterior wall of the uterine corpus and the corpus wall opposite the implantation site at pregnancy were fixed in 4% glutaraldehyde with 0.1M cacodylate buffer for several h and postfixed in 1% osmium tetroxide for 2 h. They

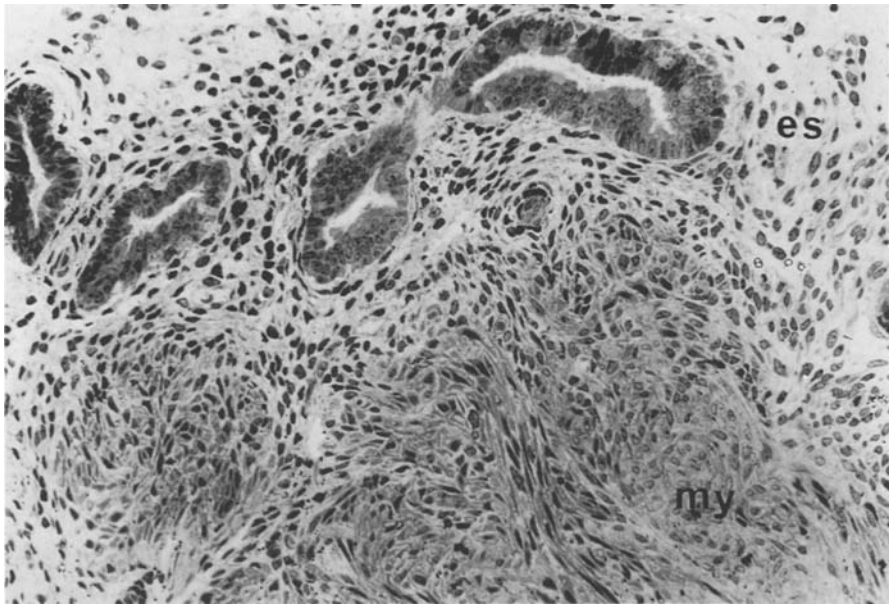

Fig. 1. EMJ in the follicular phase. Myometrial smooth muscle bundles (my) were easily distinguished from endometrial stroma (es) which contains glands. (toluidine blue, ×200)

were dehydrated in an ethanol gradient and propylene oxide, and then embedded in Epon 812. After being trimmed, ultra thin sections were made on a Porter-Blum MT-2 ultramicrotome and stained with uranyl acetate and lead citrate. They were examined under a Hitachi HU-11D electron microscope.

Results

Light and electron microscopic findings

At the EMJ in the follicular phase smooth muscle bundles of the myometrium could easily be distinguished from the endometrial stroma, which contains endometrial glands on light microscopy. Endometrial stroma at the EMJ was composed of round or spindle-shaped cells with scanty cytoplasm (Fig. 1).

Ultrastructurally, the smooth muscle bundles of myometrium showed the typical features of smooth muscle (Fig. 2), including abundant cytoplasmic filaments with dense bodies occupying almost all of the cytoplasm, scant perinuclear organelles, and dense plaques. They were enveloped by an external lamina. Higher magnification also revealed well developed surface vesicles.

Endometrial stromal cells were spindle-shaped and resembled fibroblasts (Fig. 2). Intracytoplasmic organelles were moderately developed and collagen fibrils were easily seen in the matrix. Among these fibroblastic cells, cells more elongated and smaller than myometrial smooth muscle were observed (Fig. 2, arrow). These cells had well developed intracytoplasmic organelles (mitochondria and rough-surfaced endoplasmic reticulum[rER]) (Fig. 3). Filaments were sparse and existed in only some portions of the cytoplasm (Fig. 3). Dense bodies among the filaments and dense plaques along the cell membranes were not distinctly seen. Surface vesicles, identified as invaginations of the cell membrane, were infrequent or absent. These cells were incompletely enveloped by a material like the external lamina in patches.

In the luteal phase with the light microscope, spindle-shaped cells, more elongated than those in the follicular phase, were often encountered (Fig. 4).

Ultrastructurally, the myometrium consisted of bundles of smooth muscle bound together by varying amounts of connective tissue. Smooth muscle cells with typical features (Fig. 5) looked larger than those in the follicular phase. At the EMJ spindle-shaped cells were scattered, usually singly. Among these cells, we often saw cells having focal cytoplasmic filaments with dense bodies and dense plaques along the cell membrane (Fig. 5, inset). These cells contained well developed intracytoplasmic organelles (mitochondria and rER) (Fig. 6). The intracytoplasmic filaments seemed to be more abundant and clearly visible than those in the follicular phase. Spindle-shaped cells with cytoplasmic filaments in some portions of the cytoplasm had fairly well developed surface vesicles and were enveloped by a material like that of the external lamina (Fig. 6).

In early pregnancy light microscopy revealed spindle-shaped cells at the EMJ which were more

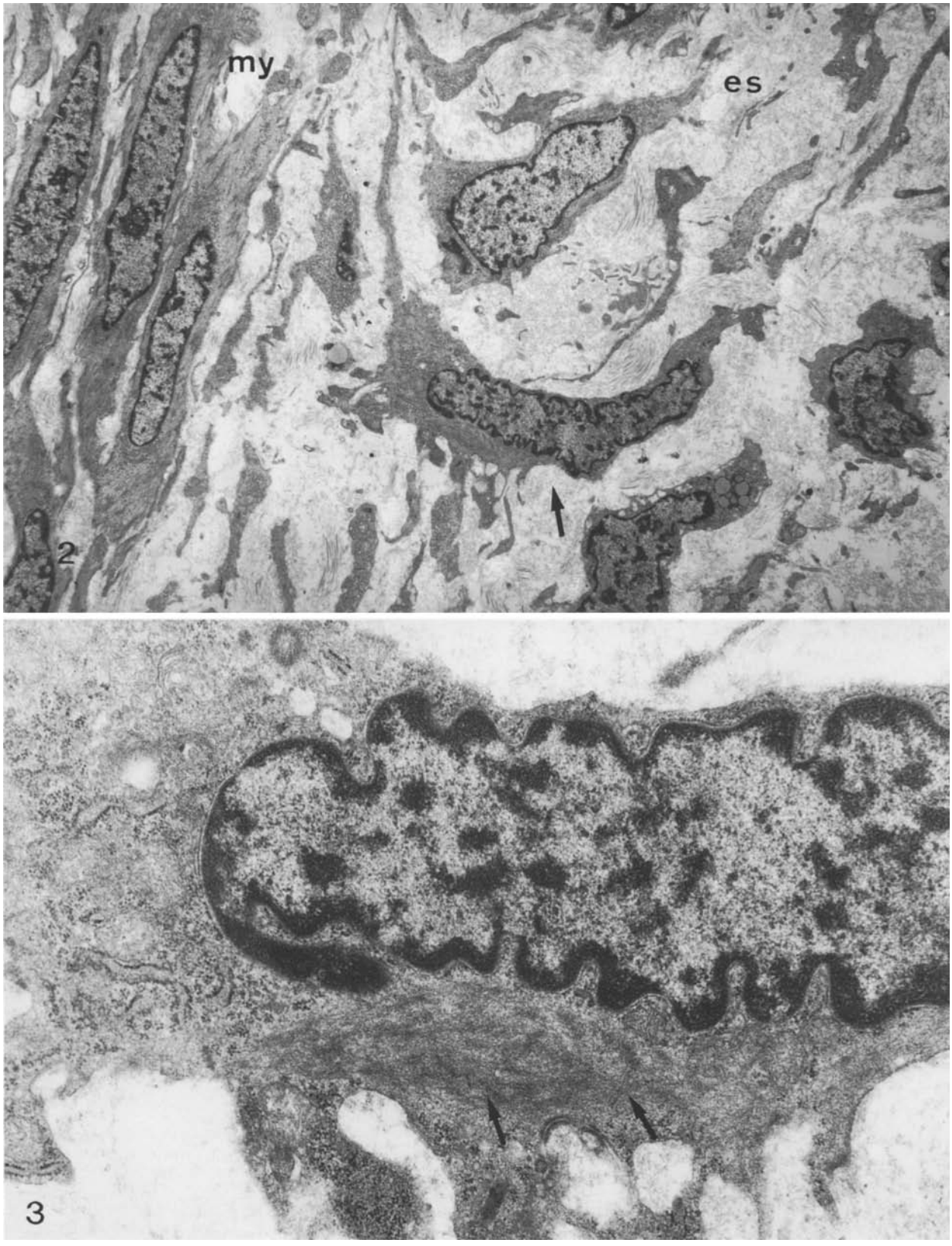

Fig. 2. Ultrastructure of EMJ in the follicular phase. Note smooth muscle of the myometrium (my) and spindle-shaped cells having focal cytoplasmic filaments (*arrow*) among endometrial stromal cells (es). (× 4000)

Fig. 3. Higher magnification of the cell with *arrow* in Fig. 2. Note intracytoplasmic filaments (*arrow*) with indistinct dense bodies. Dense plaques, surface vesicles, and external lamina are unclear. (× 21 600)

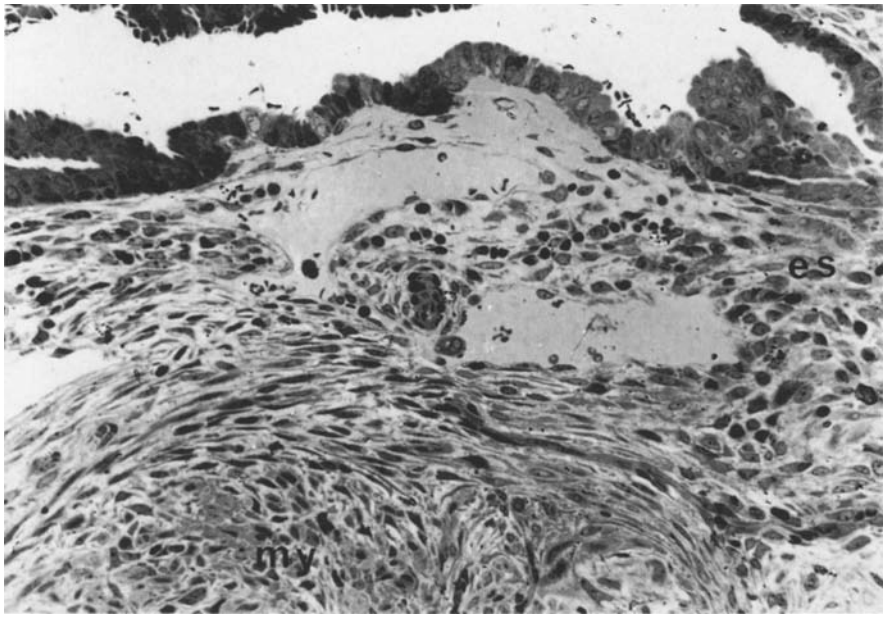

Fig. 4. EMJ in the luteal phase. Note elongated spindle-shaped cells around myometrial bundles (my). (toluidine blue, ×400)

elongated and more numerous than those in the luteal phase (Fig. 7). They had abundant cytoplasm.

Ultrastructurally, these cells were seen to contain relatively abundant cytoplasmic filaments with distinct dense bodies and dense plaques along the cell membrane (Fig. 8). They had fairly well developed surface vesicles and were enveloped by material resembling the external lamina (Fig. 8). Some cells had a paucity of surface vesicles. The cells usually contained abundant cytoplasmic organelles, including dilated rER surrounded by a narrow bundle of cytoplasmic filaments (Fig. 8). Such cells were more numerous than in the luteal phase and were clearly distinguishable from myometrial smooth muscle and endometrial stromal cells, with or without decidual changes.

Discussion

Since the identification of myofibroblasts, cells intermediate between smooth muscle cells and fibroblasts, in granulation tissue (Gabbiani et al. 1971), numerous neoplastic and non-neoplastic lesions once thought to be characterized by the proliferation of either fibroblasts or smooth muscle cells have shown to contain myofibroblasts in varying numbers (Ghahdially 1982). In a variety of disorders of the female genital tract, the participation of smooth muscle cells and myofibroblasts has been a point of intense investigation in recent years (Scully 1981). Non-neoplastic and neoplastic abnormalities of native smooth muscle have been reported in many regions of the female genital tract. Cells of smooth muscle type may be encountered in pathological processes in which they seem to originate from other elements, including endometrial stromal cells (Bird et al. 1965; Song et al. 1970), ovarian stromal cells (Cozzutto 1981), the cells of the subcoelomic mesenchyme (Parmley et al. 1975; Fujii et al. 1980, 1981; Tavassoli and Norris 1982), and possibly other cells as well. Recent investigations (Scully 1981; Fujii et al. 1981; Mazur and Kraus 1984; Konishi et al. 1983) suggest a possible origin for the smooth muscle elements or myofibroblasts of these lesions in multipotential mesenchymal cells that can differentiate into endometrial stromal cells, myofibroblasts, smooth muscle cells, and, perhaps, other elements.

However, the participation of smooth muscle cells and myofibroblasts in the normal female genital tract is not clearly defined. In an ultrastructural study we found that during the development of the human fetal uterus, immature smooth muscle cells resembling myofibroblasts originate from undifferentiated mesenchymal cells that surround paramesonephric ducts; the immature smooth muscle cells differentiate into mature cells to form the myometrium (Konishi et al. 1984).

Ultrastructural investigation of the EMJ of the adult uterus has shown there are cells with some of the fine features of both smooth muscle cells and fibroblasts among endometrial stromal cells, as in the same region of the fetal uterus. In addition, they were morphologically somewhat different during the follicular and luteal phases of the

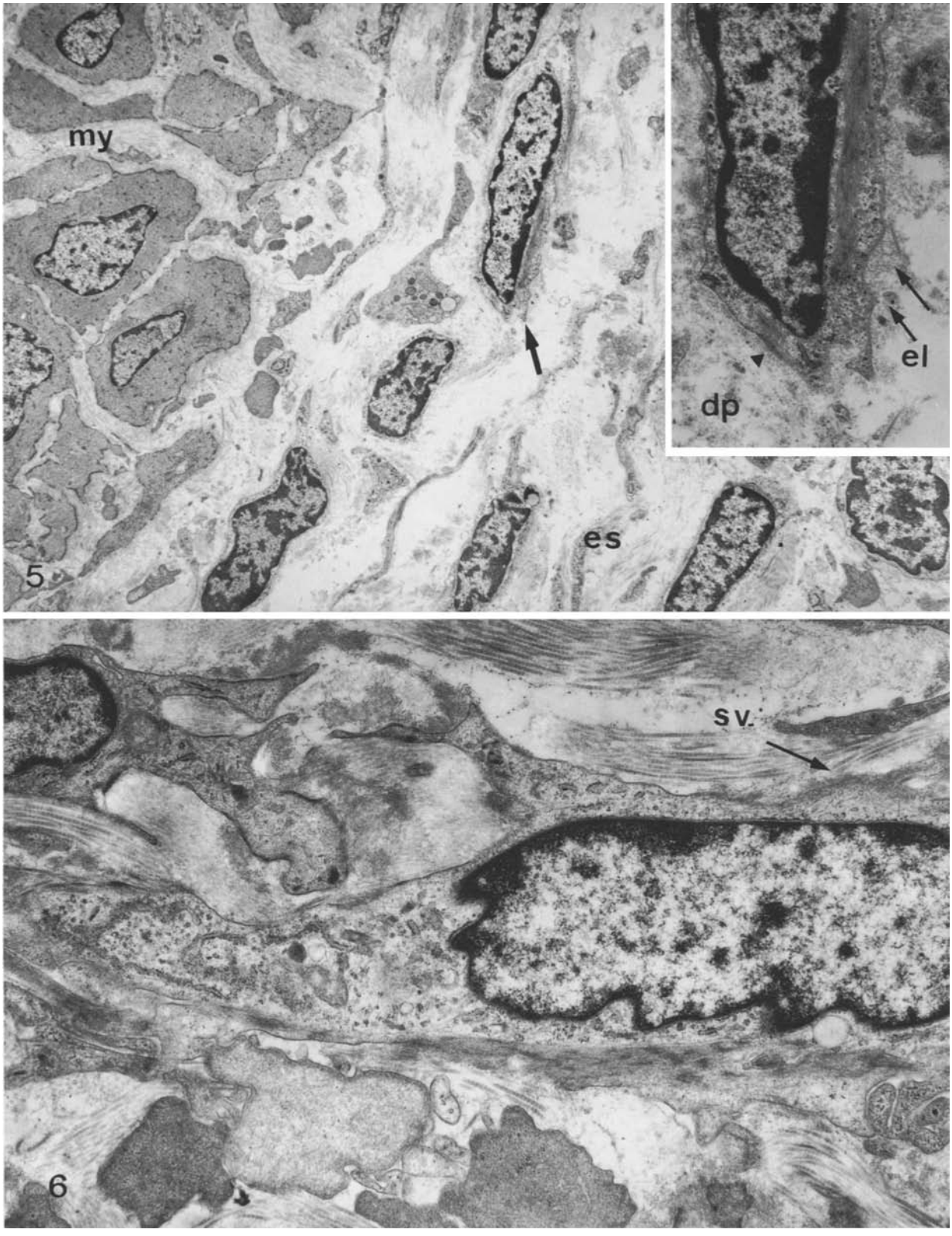

Fig. 5. Ultrastructure of EMJ in the luteal phase. Note smooth muscle of the myometrium (my) and spindle-shaped cells having focal cytoplasmic filaments (*arrow*). (×4000) *Inset:* Higher magnification of the cell shows cytoplasmic filaments with dense bodies and a dense plaque (dp: *arrow head*) along cell membrane. Note fairly well-developed external lamina-like material (el: *arrow*). Surface vesicles are unclear. (×12000)

Fig. 6. Another cell in the luteal phase. The ultrastructural features are almost the same as in Fig. 5. Surface vesicles (sv: *arrow*) were fairly well developed (×12000)

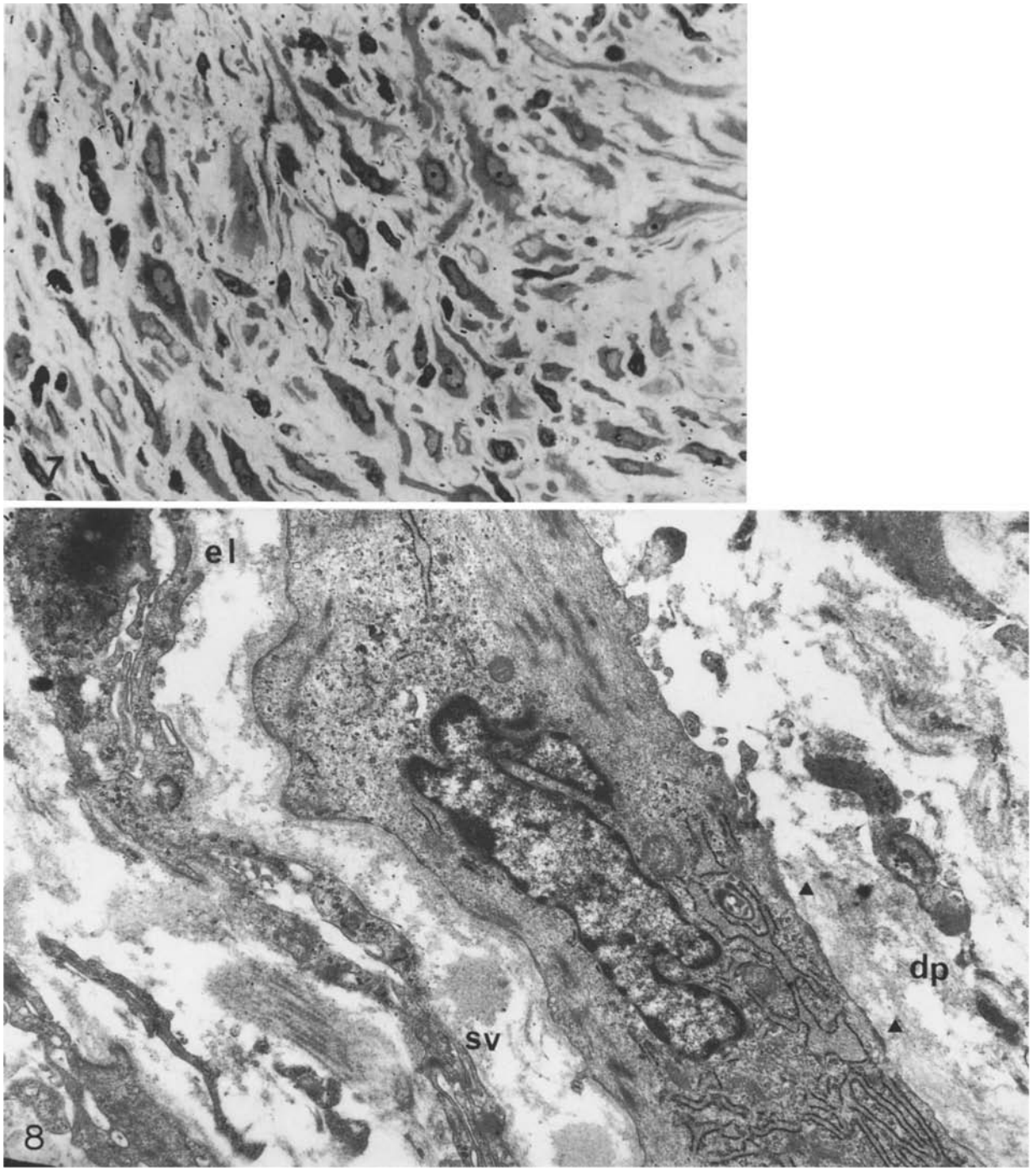

Fig. 7. EMJ in early pregnancy. Spindle-shaped cells containing abundant cytoplasm can be seen. The myometrium is not shown. (toluidine blue, ×400)

Fig. 8. Ultrastructure of EMJ in early pregnancy. Note fairly well-developed cytoplasmic filaments with dense bodies, surface vesicles (sv: *arrow*), dense plaques (dp: *arrow head*), external lamina (el: *arrow*) and abundant cytoplasmic organelles, particularly rER. (×12600)

menstrual cycle and during early pregnancy. Cells in the follicular phase were ultrastructurally very similar to the myofibroblasts described in granulation tissue and avascular fibrous tissue (Gabbiani et al. 1971; Ghahdially 1982) since they had filaments with indistinct focal densities in only some portions of the cytoplasm, and well developed organelles such as mitochondria and rER. However, dense plaques and surface vesicles were few or absent along the cell membrane, and the external lamina occured only in patches.

In the luteal phase and in early pregnancy, such cells exhibited more cytoplasmic filaments with dense bodies and dense plaques. In addition, the external lamina and surface vesicles were fairly well developed. Although some cells had a paucity of surface vesicles, these fine features resemble those of the immature smooth muscle cells we found in the fetal uterus (Konishi et al. 1984) more than they resemble those of myofibroblasts.

The finding of smooth muscle-like cells at the EMJ in the adult uterus as well as in the fetal uterus supports the hypothesis that myometrial smooth muscle cells may be newly produced by metaplasia of endometrial stromal cells (Bird and Willis 1965). Bird and Willis suggest this for certain pathological conditions, such as the hyperestrogenic state, and for early pregnancy. In our study, myofibroblast-like cells were observed in the follicular phase, but cells at the same site seemed to have more of the ultrastructural characteristics of smooth muscle in the luteal phase and in early pregnancy.

The influence of sex steroids on the ultrastructural features of uterine smooth muscle have been studied. It has been suggested that cytoplasmic organelles of uterine smooth muscle cells increase after administration of estrogen (Ross and Klebanoff 1967), whereas myofilaments increase with progesterone (Bo et al. 1968). Our previous ultrastructural study (Kawaguchi et al. 1985) of smooth muscle cells cultured from uterine leiomyomas and myometrium under the influence of sex steroids showed that cells cultured in a medium to which estrogen and progesterone have been added have more abundant filaments and dense bodies at every stage of culture examined than cells in either a control medium or in a medium to which only estrogen has been added. This suggests that progesterone, with the assistance of estrogen, may affect differentiation of smooth muscle cells in vitro (Kawaguchi et al. 1985).

The smooth muscle-like cells seen here at the EMJ which exhibited the morphological changes associated with a changing hormonal milieu are almost the same as reported elsewhere (Rossand Klebanoff 1967, Bo et al. 1968; Kawaguchi et al. 1985). Our previous experiments on leiomyomatosis peritonealis disseminata (Fujii et al. 1981) showed that subperitoneal mesenchymal cells, embryologically identical to the undifferentiated mesenchymal cells that surround the paramesonephric ducts, proliferate and differentiate into both smooth muscle and decidual-type cells in response to estrogen and progesterone. This suggests that undifferentiated mesenchymal cells around the paramesonephric ducts have the potential to develop into both smooth muscle cells and endometrial stromal cells under the influence of sex steroids. The present observations that there are smooth muscle-like cells at the non-neoplastic EMJ and that these exhibit morphological changes into the cells having more characteristics of smooth muscle in the luteal phase and in early pregnancy is consistent with previous experimental results and further implies the existence of multi-potential mesenchymal cells that may develop into smooth muscle-like cells at the EMJ.

However, at present, biological behavior of smooth muscle-like cells at the EMJ is unclear. Endometrial cells regenerate continuously to replace cells that are lost during menstruation, but myometrial cells usually do not shed during menstruation. Therefore, it is unlikely to explain that smooth muscle-like cells at the EMJ contribute regeneration of myometrial cells during menstruation. Consequently, we favor to conjecture that smooth muscle-like cells at the EMJ in the luteal phase revert to a myofibroblast stage in the follicular phase, oscillating back and forth during the menstrual cycle. However, further studies are necessary to clarify the biological behavior of smooth muscle-like cells at the EMJ. It has been reported that a monoclonal antibody against alpha-smooth muscle actin is a powerful probe in the study of smooth muscle differentiation in normal and pathological conditions (Skalli et al. 1986). Therefore, a study using a monoclonal antibody against alpha-smooth muscle actin may provide further information on smooth muscle differentiation at the EMJ.

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