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Development of smooth muscle in the human fetal uterus: an ultrastructural study

IKUO KONISHI, SHINGO FUJII, HITOSHI OKAMURA AND TAKAHIDE MORI

Department of Obstetrics and Gynecology, Kyoto University School of Medicine, 54 Shogoin-Kawahara-cho, Sakyo-ku, Kyoto, 606, Japan

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INTRODUCTION

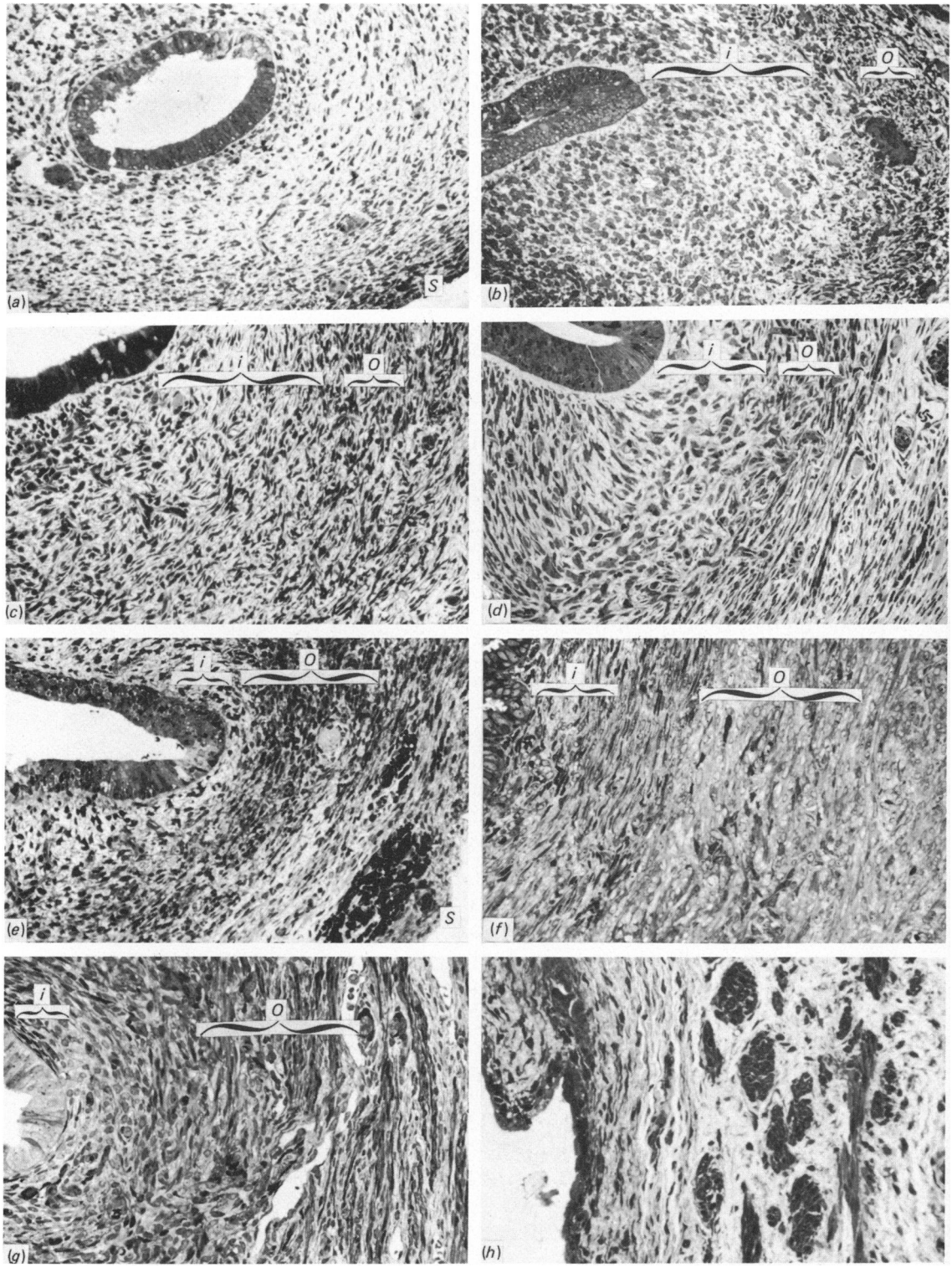
During the early stages of development of the human fetal uterus, the genital canal is initially formed by the fusion of the paramesonephric ducts, while the eventual shape of the uterus is the result of proliferation of the surrounding mesenchymal cells. However, differentiation of the myometrium and the endometrial stroma during the later stages of fetal development has not been clearly defined. Although smooth muscle is reported to exist in the uterus before the middle of gestation (Hunter, 1930; O'Rahilly, 1977; Valdés-Dapena, 1979), differentiation of the smooth muscle cells and other cytological features of this tissue have not been adequately defined at the ultrastructural level. Nor is it known what chemical factors regulate the differentiation of the myometrium and endometrium, although placental oestrogens are generally considered to affect the development of the fetal genital organs in the female (Witschi, 1970).

In the interest of clarifying these areas of uncertainty the ultrastructure of the mesenchymal component has been examined in the human fetal uterus, since it is this embryonic tissue that differentiates into myometrium and endometrial stroma. In addition, the potential role of sex steroid hormones in the development of the uterus has been considered.

MATERIALS AND METHODS

Human fetal uteri were obtained at autopsy from seven aborted fetuses of 12, 14 (two cases), 16, 18 and 20 (two cases) weeks, and three stillborn infants at 26, 31 and 40 weeks of gestation. The gestational ages were calculated from the dates of the last menstrual periods of the mothers. Crown–rump lengths of the fetuses of 14, 16, 18 and 20 weeks were also available, and were 95, 120, 145 and 160 mm respectively.

Specimens of corpora uteri were sliced transversely across the long axis of the uterine canal, and fixed in 4·0% glutaraldehyde with 0·1 M cacodylate buffer, pH 7·4, for several hours. They were rinsed in 0·1 M cacodylate buffer, post-fixed in 1·0% osmium tetroxide, dehydrated in ethanol gradients and propylene oxide and embedded in Epon 812. Transverse sections 1 μm thick were stained with toluidine blue and observed by light microscopy. After trimming, ultrathin sections were made on a Porter–Blum MT-2 ultramicrotome, and stained with uranyl acetate and lead citrate. They were observed using an Hitachi HU-11D electron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microscope. The specimen of 40 weeks of gestation was examined by light microscopy only, because it was not adequate for ultrastructural observation due to postmortem changes.

In order to compare the ultrastructure of uterine smooth muscle with the development of smooth muscle in other organs, two urinary bladders from the 14 weeks fetuses were also examined by electron microscopy.

RESULTS

Light microscopy

The fetal uterine body of each gestational age consisted of the epithelium which lined the uterine cavity, the outermost serosa and the interstitial mesenchyme between these two linings. Since the mesenchymal development of the uterus was the primary objective of this study, the following description is restricted to the interstitial mesenchyme.

At 12 weeks of gestation, the mesenchymal cells were distributed sparsely in the uterine mesenchyme. They were mostly round in shape, with high nucleo-cytoplasmic ratios (Fig. 1 *a*). Smooth muscle was not identified at this stage. By 14 weeks, two layers of mesenchymal cells could be delineated: the cells near the serosa (the outer layer) were most abundant and elongate, whereas the cells toward the lumen (the inner layer) were sparse and round in shape (Fig. 1 *b*). Rich vascular networks were observed between the outer layer and the serosa. From 14 to 20 weeks spindle shaped cells of the outer layer gradually increased in number and at 20 weeks it was obvious that this layer was even more thick than the inner layer (Fig. 1 *c–e*). Mitotic figures of the mesenchymal cells were most frequent in the inner layer between 14 and 20 weeks. At 26 weeks the thickness of the outer layer increased markedly (Fig. 1 *f*) and at 31 or 40 weeks bundles of cells like smooth muscle were obvious in this layer (Fig. 1 *g*). These observations implied that the outer mesenchymal layer of the body of the fetal uterus gave rise to the myometrium and that the inner layer corresponded to endometrial stroma of the adult uterus.

By comparison, in the mesenchyme of the urinary bladder, regular bundles of elongated cells which resembled smooth muscle were observed as early as 14 weeks of gestation (Fig. 1 *h*). This suggested that there may have been an organ specific period of smooth muscle differentiation during fetal life.

Electron microscopy

12 *weeks of gestation*

The mesenchymal cells in the body of the uterus were round or stellate, about 10 μm in length, and contained large, round nuclei (Fig. 2). Nuclear chromatin was condensed near the nuclear membrane. Mitochondria, granular endoplasmic reticulum and free ribosomes were scarce. Intracytoplasmic filaments were not observed. There were negligible intercellular junctions between the cells. Collagen fibrils were

Fig. 1 (*a–h*). Transverse sections of human uterine corpora at 12 (*a*), 14 (*b*), 16 (*c*), 18 (*d*), 20 (*e*), 26 (*f*), and 31 (*g*) weeks of gestation. Mesenchymal cells arrange themselves into an outer (*O*) and inner (*i*) layer at 14 weeks. The outer layer, which increases in thickness with advancing age, develops into smooth muscle. For comparison, Fig. 1 (*h*) is a section of fetal urinary bladder at 14 weeks of gestation. *S*, serosa. × 100.

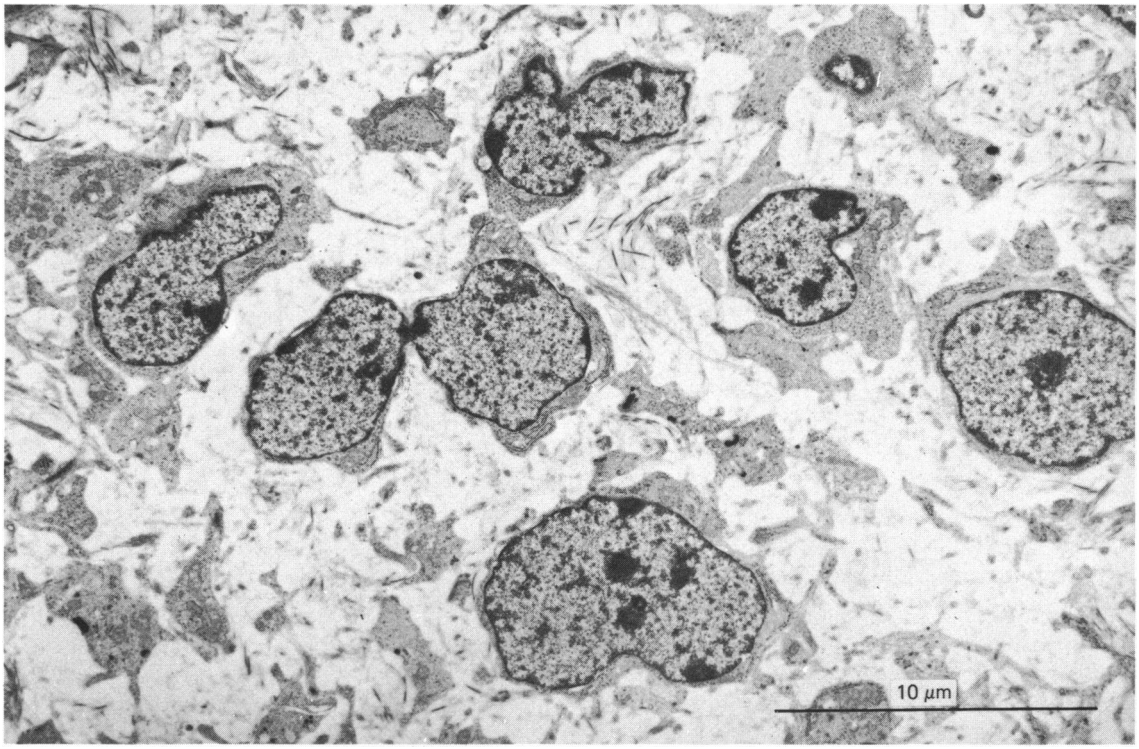

Fig. 2. Electron micrograph of the body of the uterus at 12 weeks of gestation. Mesenchymal cells have large nuclei with marginally condensed chromatin and cytoplasm with poorly developed organelles. × 4300.

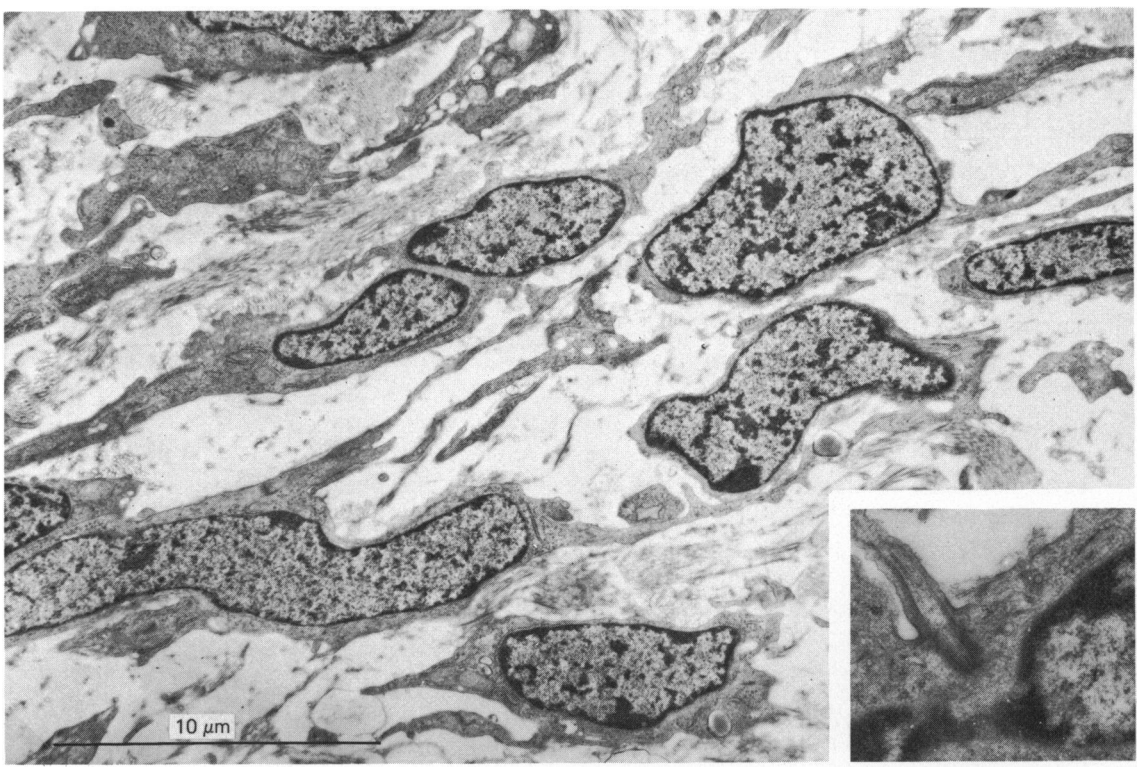

Fig. 3. Outer layer of the body of the uterus at 14 weeks of gestation. Mesenchymal cells are slighuly elongated, but cytoplasmic filaments with dense bodies are not present. × 4500. Inset. A single cilium protrudes into the matrix from some cells. × 16200.

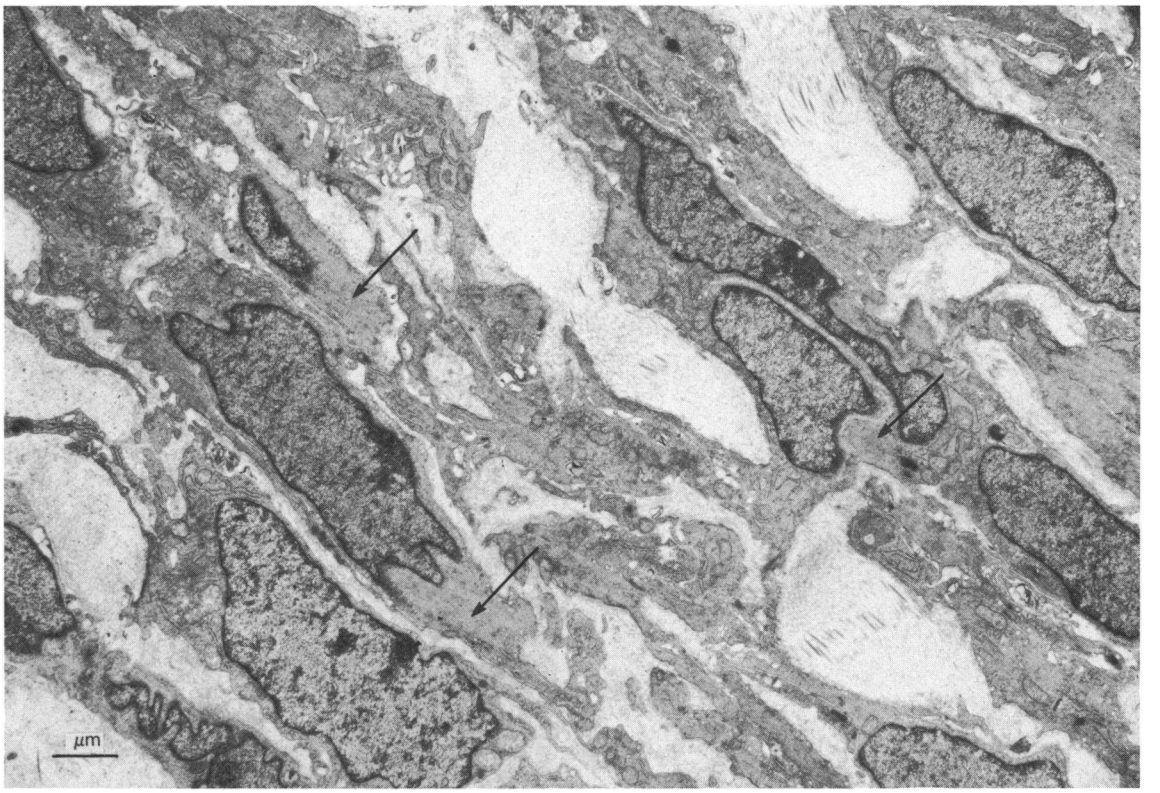

Fig. 4. Mesenchymal cells in the outer layer of the body of the uterus at 20 weeks of gestation contain oval nuclei with dispersed chromatin and a few intracytoplasmic filaments (arrows). × 5800.

sparse in the matrix. Collectively, these ultrastructural features suggested that the mesenchymal cells remained undifferentiated at this stage.

14–16 *weeks of gestation*

At this stage, the outer layer of the uterine wall consisted of elongated cells, about 10–20 μm in length, which had oval nuclei with condensed chromatin along their margin (Fig. 3). Cytoplasmic organelles were not well developed; a few filaments but no dense bodies were present in some of these cells. Such cells had tiny vesicles along the cell membrane, but the vesicles were smaller in number than the surface vesicles of typical smooth muscle cells. Single cilia occasionally protruded into the matrix of these cells (Fig. 3, inset). A few intercellular contacts were observed, but desmosome-like junctions were rare.

On the other hand, the cells in the inner layer were stellate or round in shape, and had features similar to the cells in the 12 weeks fetus.

18–20 *weeks of gestation*

In the outer layer, the cells were even more elongated than those of 14–16 weeks, measuring about 20–40 μm in length, and were greater in number (Fig. 4). The nucleus was oval and the nuclear chromatin was dispersed within the nuclear matrix. The nucleo-cytoplasmic ratio was reduced. Intracytoplasmic organelles such as

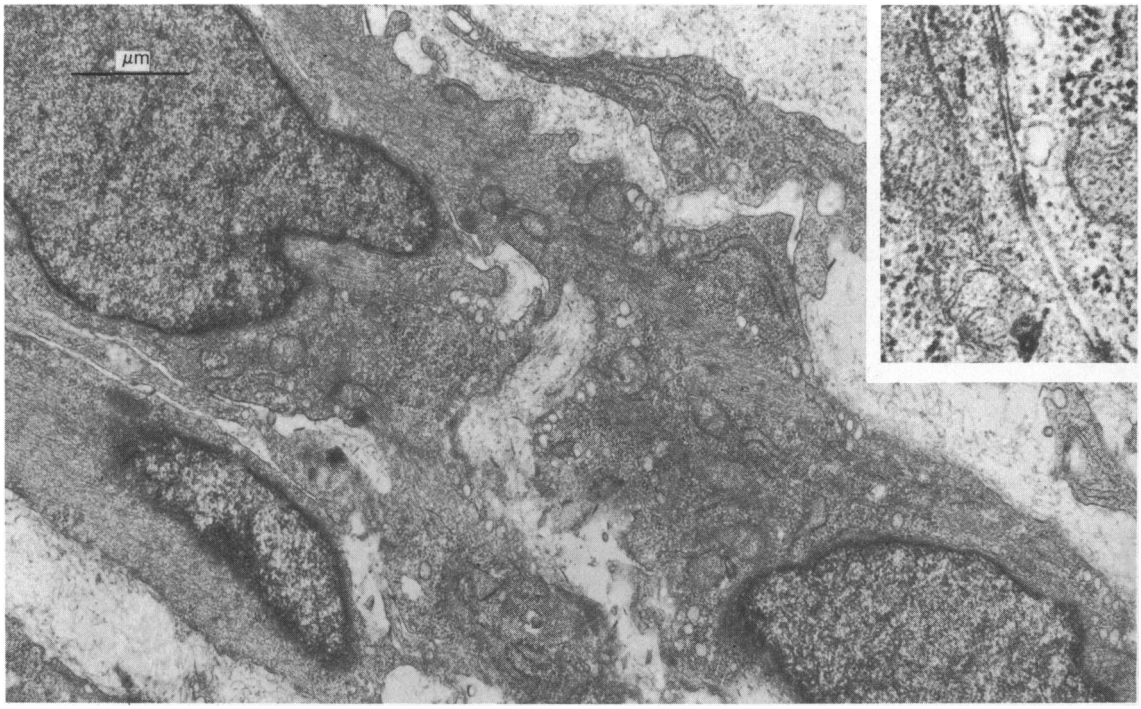

Fig. 5. Higher magnification of Fig. 4, demonstrating well developed cytoplasmic organelles, such as free ribosomes, granular endoplasmic reticulum, mitochondria, and the Golgi apparatus. Note that there are a few filaments with dense bodies and many surface vesicles of the cell membrane (immature smooth muscle cells). × 15600. Inset. Intercellular contacts with desmosome-like junctions. × 49500.

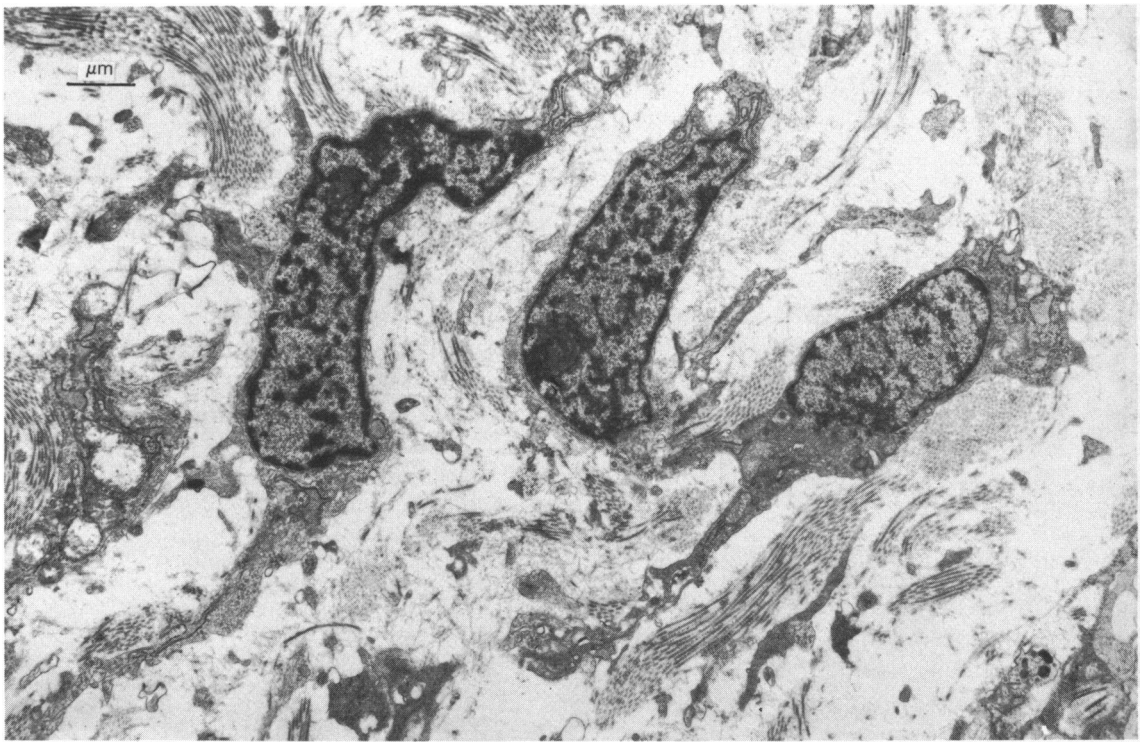

Fig. 6. Inner layer of the body of the uterus at 20 weeks of gestation. Spindle shaped mesenchymal cells are surrounded by numerous collagen fibrils. × 6400.

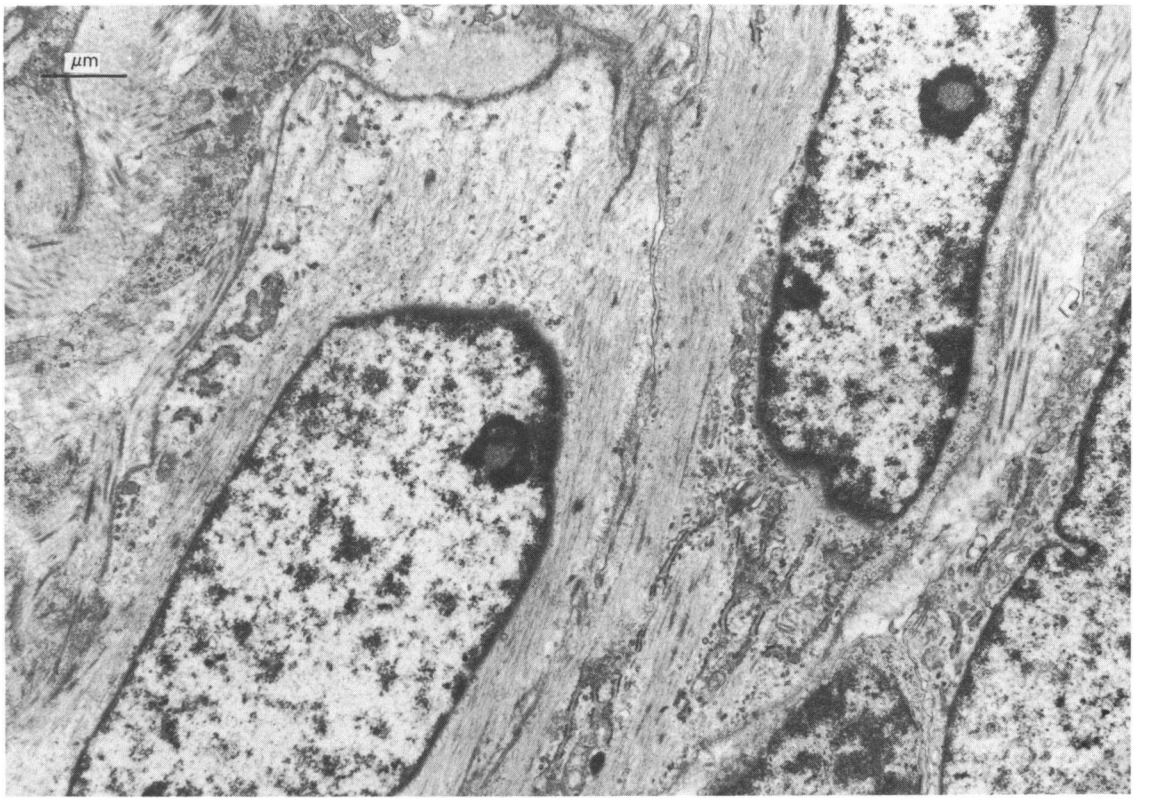

Fig. 7. Mesenchymal cells in the outer layer of the body of the uterus at 26 weeks of gestation. The cytoplasm contains many filaments with dense bodies, and intercellular contacts are conspicuous. × 9700.

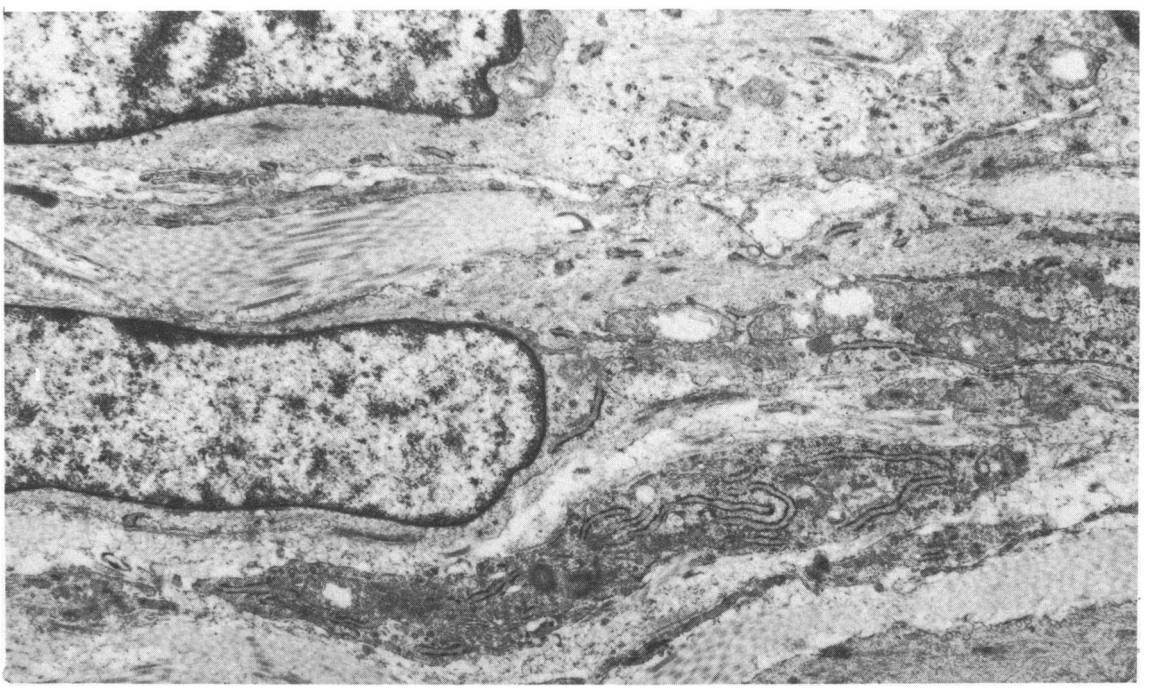

Fig. 8. Between the outer and inner layers of the body of the uterus at 26 weeks of gestation, the cells contain a few filaments and well developed organelles. These features are similar to immature smooth muscle cells at 18–20 weeks of gestation. × 11200.

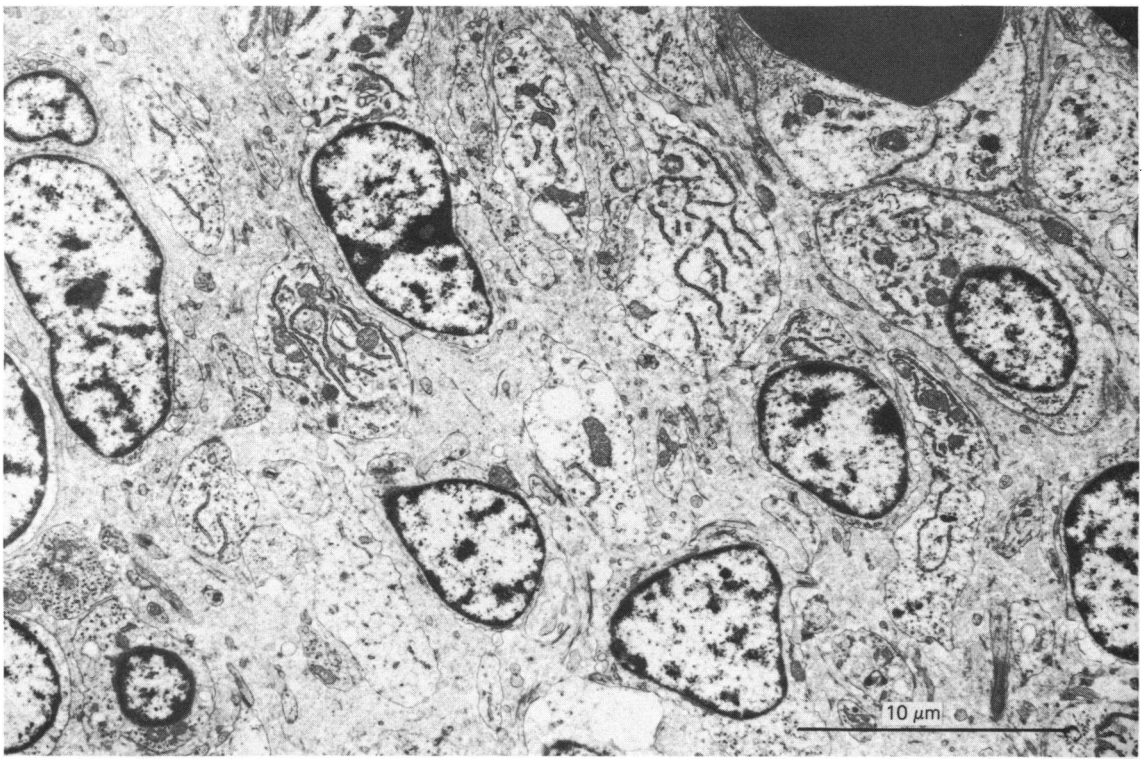

Fig. 9. In the inner layer of the body of the uterus at 26 weeks of gestation, mesenchymal cells around blood vessels have abundant cytoplasm with well developed granular endoplasmic reticulum (predecidual-like cells). ×4200.

mitochondria, granular endoplasmic reticulum, and Golgi apparatus were well developed. Free ribosomes were especially numerous. Intracytoplasmic filaments with a few dense bodies were observed in some portions of the cytoplasm (Fig. 5). These filaments were about 6–8 nm in diameter. Surface vesicles along the cell membrane increased in number by 20 weeks. However, no external lamina or dense plaques could be identified along the cell membrane. There were more intercellular contacts and a few desmosome-like junctions were observed (Fig. 5, inset). These ultrastructural features were characteristic of immature smooth muscle cells, especially since a few myofilaments were present. These immature smooth muscle cells first appeared at 18 weeks and increased by 20 weeks. Filaments were more abundant in cells located near the subserosal vascular networks than in cells near the inner layer, at 20 weeks of gestation.

At 18–20 weeks, the mesenchymal cells in the inner layer of the uterine wall were spindle shaped, 10–20 μm in length, and resembled fibroblasts. Intracytoplasmic organelles were moderately developed and collagen fibrils were more obvious in the matrix (Fig. 6), as compared to the tissue as seen at 14–16 weeks.

26 weeks of gestation

At this stage of development, the number of elongated cells in the outer layer of the wall of the uterus increased markedly. The cytoplasm of these cells was occupied mostly by filaments with dense bodies. Cytoplasmic organelles were located mainly

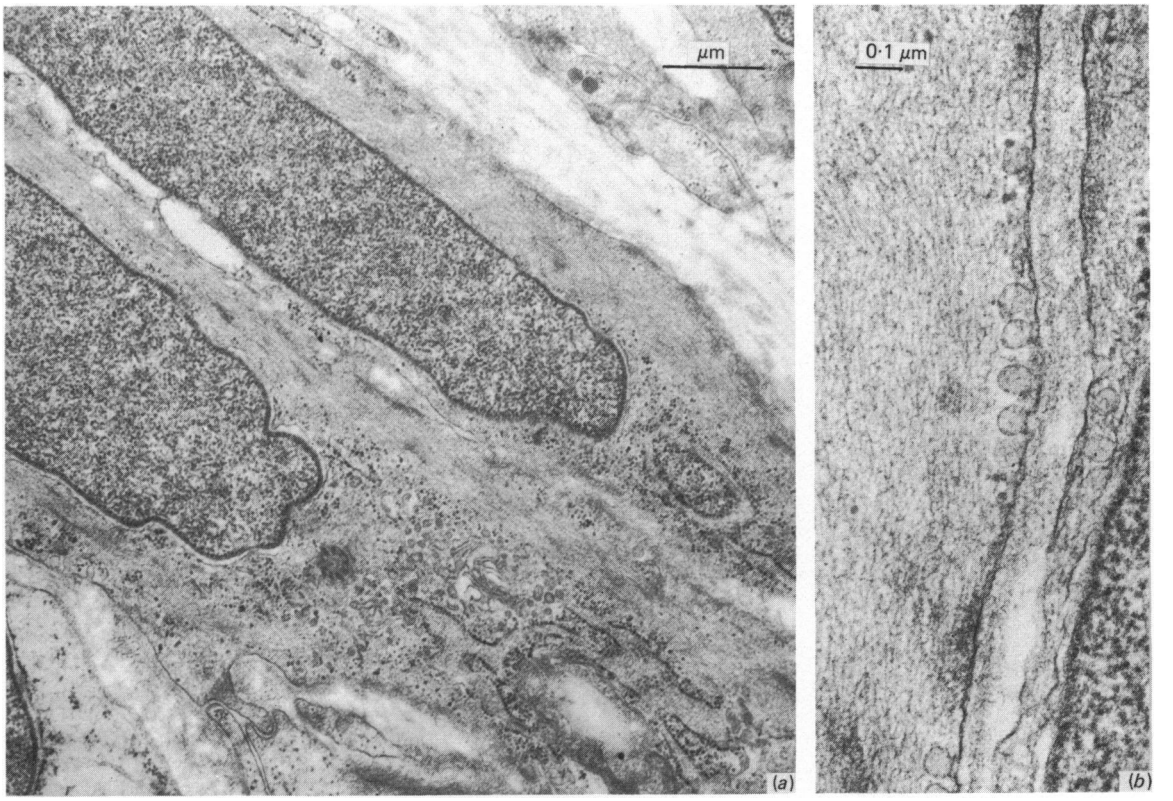

Fig. 10(*a–b*). The cells in the outer layer of the body of the uterus at 31 weeks of gestation have numerous filaments with dense bodies (*a*). × 12500. Dense plaques, surface vesicles, and an external lamina are well developed (*b*). × 55200.

in the perinuclear region (Fig. 7). Surface vesicles were well developed, and there were some dense plaques along the cell membrane. Intercellular contacts with several desmosome-like junctions were prominent; however, development of the external lamina was incomplete. Nuclear indentations were not observed. Therefore, these cells were considered to exhibit the ultrastructural characteristics of smooth muscle cells apart from the appearance of the external lamina and nuclear indentation.

In the region between the outer and inner layers, which corresponded to the junction between the myometrium and the endometrial stroma in the adult, there were a number of spindle shaped cells that contained a few intracytoplasmic filaments and well developed organelles (Fig. 8). Since these cells were ultrastructurally similar to the cells in the outer layer at 18–20 weeks, at this stage this region presumably contained immature smooth muscle cells.

On the other hand, in the inner layer, two morphologically distinct mesenchymal cells were identified. One type was spindle shaped, resembling the fibroblasts observed in this layer at 18–20 weeks. The other type was round, with abundant cytoplasm and was observed in the vicinity of blood vessels. It contained well developed granular endoplasmic reticulum and Golgi apparatus in the cytoplasm, surface vesicles and small cytoplasmic processes projecting toward the matrix. External lamina-like materials partially enveloped these cells (Fig. 9).

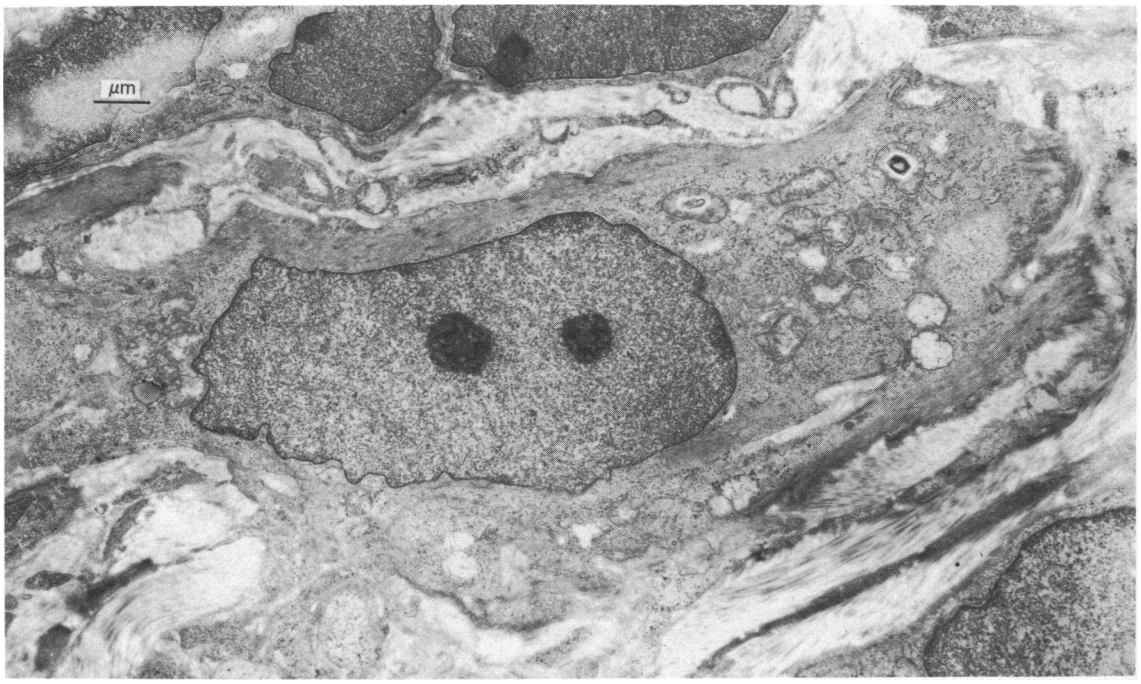

Fig. 11. Immature smooth muscle cells between the outer and inner layers of the body of the uterus at 31 weeks of gestation. × 5800.

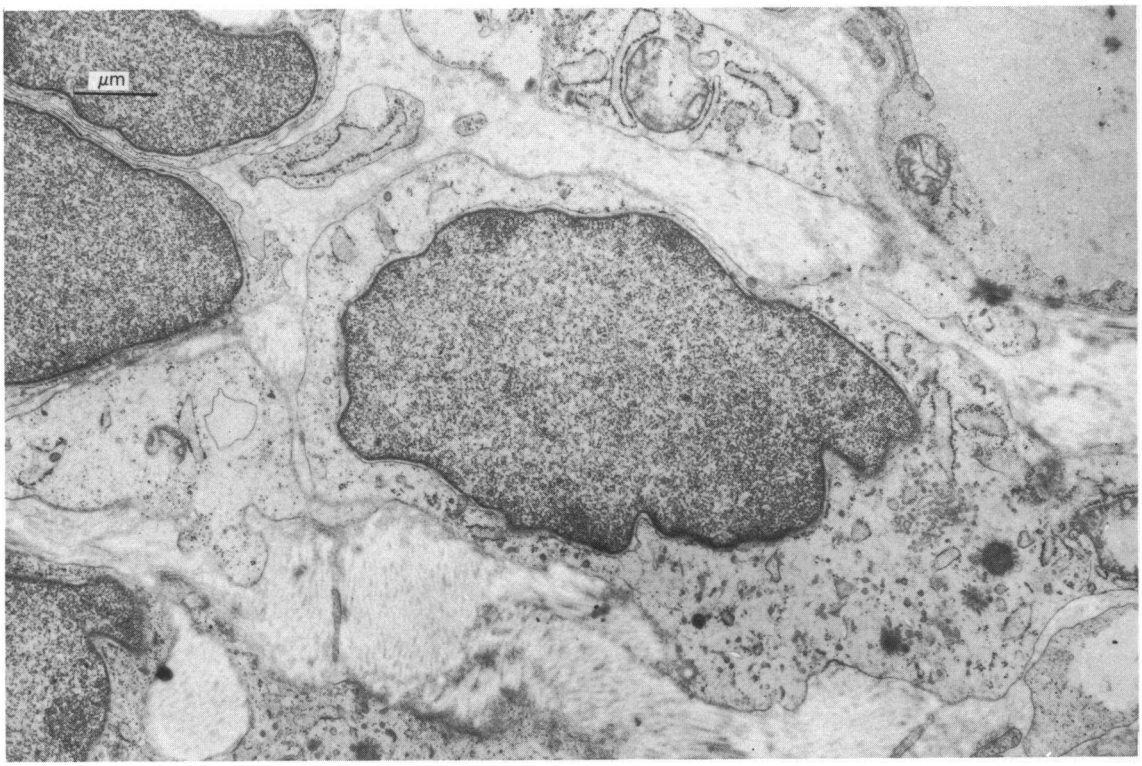

Fig. 12. Predecidual-like cells in the inner layer of the body of the uterus at 31 weeks of gestation. × 10700.

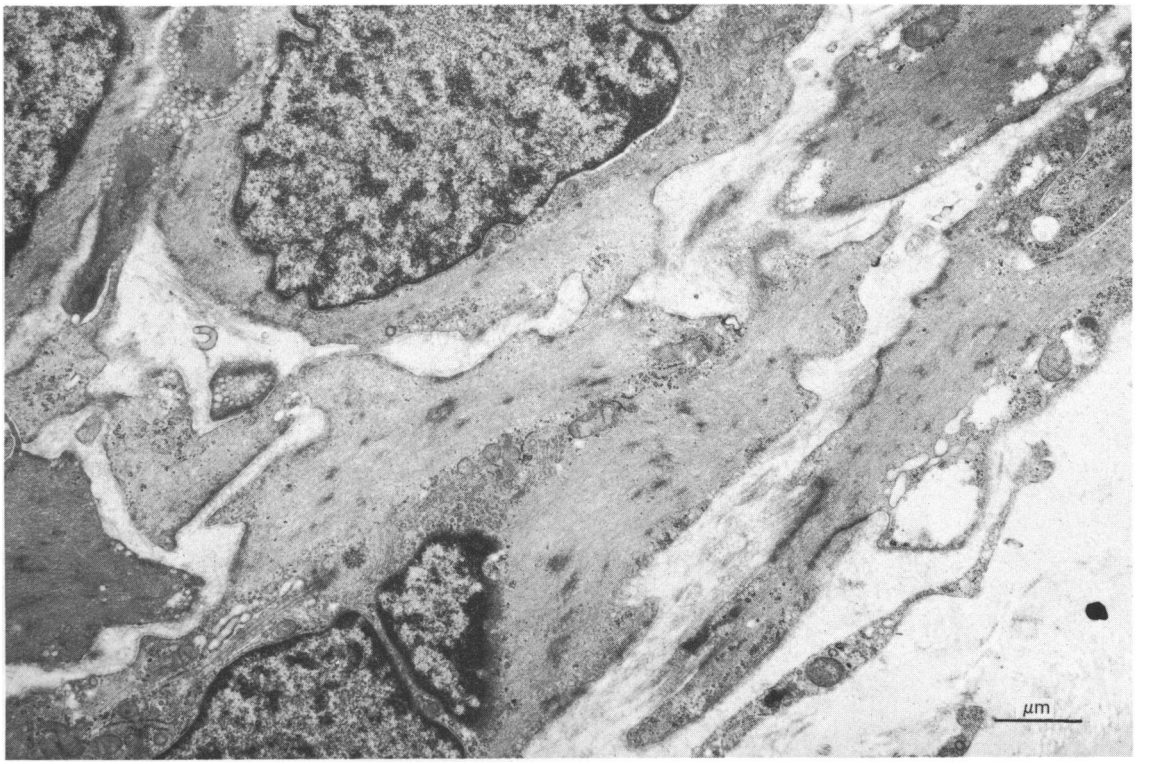

Fig. 13. Smooth muscle cells in the urinary bladder at 14 weeks of gestation, with features characteristic of functionally contractile cells. × 10400.

31 *weeks of gestation*

By this time the cells of the outer layer wall of the uterus were even more elongated, being 50 μm or more in length, and formed bundles. These cells had all the ultrastructural characteristics of smooth muscle cells, including abundant filaments with dense bodies, dense plaques along the cell membrane, numerous surface vesicles and an external lamina. Intracytoplasmic organelles were limited to the perinuclear region (Fig. 10). Intercellular contacts were not as prominent as at 26 weeks. However, the oval nuclei in these cells were not indented and nuclear chromatin was evenly dispersed throughout the nuclear matrix.

The area between the outer and inner layers was several cells thick. These cells had a few filaments with dense bodies, characteristic of immature smooth muscle cells (Fig. 11).

In the inner layer, in addition to fibroblast-like cells, cells with abundant cytoplasm and well developed organelles were usually located around blood vessels, as observed at 26 weeks (Fig. 12).

Urinary bladder at 14 *weeks of gestation*

In the mesenchyme subjacent to the transitional epithelium of the bladder, there were spindle shaped cells which had indented nuclei with marginally condensed chromatin, abundant filaments with dense bodies, dense plaques, surface vesicles and an external lamina (Fig. 13). These ultrastructural features were similar to those of mature smooth muscle cells with contractile function.

DISCUSSION

The current investigation reveals the ultrastructural changes of the uterine mesenchyme during its differentiation into smooth muscle in the human fetus. Until 16 weeks of gestation, cytoplasmic filaments with dense bodies characteristic of smooth muscle cells are not identified in the mesenchymal cells of the uterus. Spindle shaped cells, containing a few myofilaments scattered in the cytoplasm and with well developed organelles such as mitochondria, free ribosomes, granular endoplasmic reticulum and Golgi membranes, first appear in the outer layer of the uterus at 18 weeks when the fetus has a crown–rump length of 145 mm. These cells are interpreted as immature smooth muscle cells, since they have a form intermediate between undifferentiated mesenchymal cells and mature smooth muscle cells. Similar features of developing smooth muscle cells have been reported in studies on the perinatal development of rabbit myometrium (Yamamoto, 1961), rat ureter (Leeson & Leeson, 1965), and mouse ductus deferens (Yamauchi & Burnstock, 1969). Other ultrastructural characteristics of smooth muscle cells develop later; surface vesicles of the cell membrane begin to increase at about 20 weeks, dense plaques along the cell membrane appear by 26 weeks and an external lamina is almost complete by 31 weeks. Although nuclear indentation is not observed, the cells in the outer layer at 31 weeks exhibit all other features of mature smooth muscle cells. Therefore, in the human fetal uterus, it is considered that smooth muscle differentiation begins at 18 weeks of gestation, and that by 31 weeks the myometrium is formed in the outer layer of the wall of the uterus, and is clearly distinguishable from the inner layer corresponding to the endometrial stroma.

The present study demonstrates that uterine smooth muscle cells originate from undifferentiated mesenchymal cells, as observed at 12 weeks of gestation. Although the myometrial layer increases in thickness with advancing age, mitotic figures in mesenchymal cells are detected mainly in the inner layer from 14 weeks. Moreover, immature smooth muscle cells are identified in the region between the myometrial and endometrial stromal layers both at 26 and 31 weeks, when bundles of almost mature smooth muscle cells are already formed. These observations imply that undifferentiated mesenchymal cells which develop into smooth muscle cells may exist in the inner layer of the fetal uterus and that smooth muscle differentiation may occur in the junctional area between the myometrial and endometrial stromal layers. This view of the histogenesis of fetal uterine smooth muscle is consistent with the assumption that uterine smooth muscle may be newly produced by endometrial stroma in certain pathological conditions in the adult (Bird & Willis, 1965). On the other hand, fibroblast-like cells of the endometrial stromal layer are also considered to be derived from undifferentiated mesenchymal cells in the same layer. Ultrastructurally, the undifferentiated cells that develop into endometrial stromal cells cannot be distinguished from the progenitors of smooth muscle cells. Previous experiments by the authors on leiomyomatosis peritonealis disseminata have shown that subperitoneal mesenchymal cells, which are considered to be embryologically identical with the undifferentiated mesenchymal cells that surround the paramesonephric ducts, proliferate and differentiate into cells both of the smooth muscle and decidual types in response to oestrogen and progesterone (Fujii *et al.* 1981). This experimental result and the present observations of fetal uterine mesenchyme suggest that the undifferentiated mesenchymal cells around paramesonephric ducts

have the potential to develop into both smooth muscle cells and endometrial stromal cells.

The mechanism which regulates smooth muscle differentiation in the human fetal uterus is still uncertain. However, the present observation that mature smooth muscle cells occur in the fetal urinary bladder as early as 14 weeks clearly demonstrates a difference between the two organs in the period of smooth muscle development. The slower differentiation of fetal uterine smooth muscle suggests that its development may be controlled by factors other than those of the bladder. Although the differentiation of male genital ducts is controlled by testicular androgens (Jost, 1961), the female ducts are said to differentiate autonomously without the need for any external regulatory factors from the gonads or elsewhere (Pelliniemi & Dym, 1980). However, it is well known that oestrogens and progesterone increase in the maternal and fetal plasma during gestation (Tulchinsky, Hobel, Yeager & Marshall, 1972; Tulchinsky, 1973), and the reduction in size of the uterus after parturition (Hunter, 1930) or after ovariectomy (Jost, 1953) suggests that placental oestrogens or fetal ovarian steroids may have some effect on the development of female genitalia. Moreover, present observations of plump cells, whose cytoplasm contains well developed granular endoplasmic reticulum and Golgi apparatus, which lie around blood vessels in the inner layer of the fetal uterus from 26 weeks, and which resemble predecidual cells (Wienke, Cavazos, Hall & Lucas, 1968; Wynn, 1974) on the basis of ultrastructural criteria implicate the influence of sex steroids on the human fetal uterus. Although decidual cells have been reported in neonatal endometrium by light microscopy (Ober & Bernstein, 1955), the present observation is considered to be the first description of the ultrastructural features of predecidual-like cells in the fetal uterus. Recently, Pasqualini *et al.* (1983) have reported that oestrogen and progesterone receptors become established in the fetal uterus of guinea-pigs from 34 and 50 days of gestation respectively, and that the uptake of tritiated progesterone occurs mainly in the stroma and myometrium, rather than in the luminal epithelium. Therefore, it is likely that the fetal uterus, especially its mesenchymal components of smooth muscle and endometrial stroma, is under the influence of oestrogen and progesterone during pregnancy. However, further research is necessary to verify the exact relationship between the sex steroids and the development of the human fetal uterus.

SUMMARY

Prenatal development of uterine smooth muscle was studied by light and electron microscopy in specimens obtained from ten human fetuses between 12 and 40 weeks gestation. Light microscopical observation of transverse sections of the body of the uterus revealed that the outer, subserosal layer of elongated cells was distinguishable from 14 weeks. This layer was more cellular than the inner layer and increased its thickness with advancing age. Ultrastructurally, the mesenchymal cells of the uterus did not contain myofilaments until 16 weeks. At 18 weeks, however, spindle shaped cells in the outer layer had a few filaments with dense bodies and well developed organelles and were identified as immature smooth muscle cells. By 31 weeks, the cells in this layer developed into almost mature smooth muscle cells, which contained abundant cytoplasmic filaments, dense plaques and surface vesicles along the cell membrane, and an external lamina. Therefore, in the human fetal uterus, smooth muscle differentiation begins at about 18 weeks and myometrium is formed in the outer layer of the wall by 31 weeks gestation. In the inner layer of the uterus that

corresponds to the endometrial stroma, in addition to fibroblast-like cells, there were cells with plump cytoplasm which contained well developed granular endoplasmic reticulum and Golgi apparatus and which were identified around blood vessels by 26 weeks. These features resembled those of predecidual cells, and suggested the influence of sex steroids on the human fetal uterus.

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