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Light and Electron Microscopic Study of Decidual Cells in the Human Ovary during Pregnancy

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ABSTRACT

Decidual tissue in the human ovary obtained from 53 women by gynecological operation or cesarean section at 8-42 weeks of normal pregnancy was studied by light and electron microscopy.

Histologically, ovarian decidual cells had round nuclei with prominent nucleoli and abundant eosinophilic cytoplasm. Immunoreactive prolactin was detected in the cytoplasm of some decidual cells.

Electron microscopically, nuclei were oval or ellipsoidal in shape with distinct nucleoli and enchromatin. The cytoplasm contained well developed rough and smooth endoplasmic reticulum, Golgi apparatus, abundant rod-shaped mitochondria, intermediate filaments, glycogen particles and lysosomes. Peduncular protrusions containing secretory bodies bounded by a limiting membrane were characteristically observed. Some protrusions showed exocytosis of secretory bodies composed of numerous granular contents. Acid phosphatase (AcPase) activity was demonstrated in the secretory bodies by a cerium-based method in combination with a microslicer. Periodic acid-silver methenamine (PA-silver methenamine) technique also revealed deposits of silver in the secretory bodies.

These findings strongly suggest that ovarian decidual cells secrete granular contents positive for AcPase and PA-silver methenamine reactions from the peduncular protrusions with the mode of exocytosis and some decidual cells contain prolactin in the cytoplasm. These are quite similar to uterine decidual cells.

Since the first description of decidual cells of the human ovary during pregnancy by Kinoshita[11], decidua in other organs during pregnancy has also been reported in such diverse organs as the uterine cervix[24], vagina[28], peritoneum[21], mesentery[10], bowel[10], liver surface[4], appendix[9], diaphragm[3] and pelvic lymphnodes[2]. Recently, Starup and Visfeldt have shown decidual changes in the ovary as early as the ninth week of gestation[23]. However, the function of decidual cells in the human ovary still remains to be elucidated.

This study was undertaken to gather further data on the frequency of ovarian decidua in all trimesters and to compare the ultrastructure of ovarian decidual cells with that of uterine decidual cells.

MATERIALS AND METHODS

Small ovarian biopsies were obtained from 53 women by gynecological operation or cesarean section at 8-42 weeks of normal pregnancy (Table 1). Ovarian decidual tissue was identified by its appearance as slightly elevated, hypervascu-

Table 1. Specimens used in this study

Period of biopsy (weeks)	1st trimester (8—13)	2nd trimester (20—22)	3rd trimester (34—42)
Number of cases	10	3	40

and postfixed for 1 hr with 1% OsO_4 solution adjusted to pH 7.4 with 0.1M cacodylate buffer. These specimens were dehydrated in ethanol and embedded in Poly/Bed 812. Semi-thin sections (1 μm) were stained with 1% toluidine blue solution. Section cut on a Porter-Blum MT-1 ultramicrotome were stained with uranyl acetate

Table 2. Procedure of ABC method for localization of prolactin immunoreactivity

Successive inculations of tissue sections	Time	Temperature (°C)
(1) Deparaffinized sections (4um) in PBS*	30 min	20
(2) 0.03% H_2O_2 solution	10 min	20
(3) PBS	3×5 min	20
(4) Rabbit anti-human prolactin (×200, UCB)	24 hrs	4
(5) PBS	3×5 min	20
(6) Biotinylated sheep anti-rabbit IgG (×500)	30 min	20
(7) PBS	3×5 min	20
(8) ABC**	60 min	20
(9) PBS	3×5 min	20
(10) DAB*** in 0.05M, pH7.6 Tris buffer with 0.01% H_2O_2	30 min	20

*PBS=phosphate buffered (pH7.4) saline, **ABC=avidin-biotin peroxidase complex, ***DAB=3,3' diaminobenzidine tetrahydrochloride

Table 3. Procedure of periodic acid-silver methenamine technique

Successive incubations	Time	Temperature(°C)
(1) Ultrathin sections on 1% aqueous periodic acid solution	20 min	20
(2) Wash and storage in distilled water (D.W)	3×5 min →overnight	20
(3) Silver methenamine reagent*	60 min	60
(4) Wash in D.W	over 60 min	20
(5) 5% sodium methenamine reagent	5 min	20

*silver methenamine reagent
5% silver nitrate solution+3% methenamine solution+2% sodium borate solution

lar and soft foci on the free, convex surface of the ovary.

Preparation for light microscopy

The specimens were fixed in Zamboni's fixative or 10% formaldehyde, embedded in paraffin and stained with hematoxylin-eosin (H-E) or periodic acid-Schiff (PAS) reaction. Serial sections were used for the demonstration of immunoreactive prolactin in ovarian decidual cells by avidin-biotin peroxidase complex (ABC) method (Table 2). Primary antibody used in this study was rabbit anti-human prolactin (UCB, ×200). A control incubation was made by replacing the primary antibody by non-immune rabbit serum.

Preparation for electron microscopy

The tissues were fixed for 2 hr with 1.5% glutaraldehyde solution adjusted to pH 7.4 with 0.1M cacodylate buffer containing 5% sucrose, and Raynolds' lead solution, coated with carbon, and examined with a Hitachi H-300 type electron microscope.

Periodic acid-silver methenamine technique (Table 3)[16]

Some specimens were used for periodic acid-silver methenamine stain. Ultrathin sections were transferred to 1% periodic acid solution for 20 min at room temperature, washed 3 times in distilled water and stored overnight in the bath of distilled water. This procedure was necessary to wash off periodic acid, which is an essential step to avoid precipitation. In a dark room, 5 ml of silver nitrate solution are measured in a 10 ml-cylinder and added to 45 ml methenamine solution. The final solution was obtained by adding 5 ml sodium borate solution. The specimens were transferred to the final solution poured into a Petri dish and allowed to float for 60 min

Table 4. Procedure of acid phosphatase reaction by the cerium-based method

Successive incubations	Time	Temperature(°C)
(1) Fixation in 1.5% glutaraldehyde solution in buffer*	30 min	4
(2) Immersion in buffer	overnight	4
(3) Microslicer sections (30—40 μm)		
(4) Preincubation without substrate from incubation medium**	60 min	37
(5) Incubation with substrate	30 min	37
(6) Immersion in buffer	overnight	4
(7) Postfixation in 1% OsO_4	60 min	20
(8) Dehydration in ethanol and embedded in Poly/Bed 812		
(9) Observation		

*buffer = 0.1M cacodylate buffer containing 5% sucrose, pH 7.4
**incubation medium: 0.1M acetate buffer (pH5.0)
1 mM β-glycerophosphate (disodium salt, Sigma)
2 mM $CeCl_3$ as capturing agent

at 60°C. They were floated for a few minutes on distilled water, transferred for 5 min to 5% sodium thiosulfate solution, finally rinsed in the bath of distilled water, and then mounted on Formvar-coated nickel grids by simply touching the tissue sections.

Acid phosphatase activity

Ultracytochemical localization of acid phosphatase (AcPase) activity was investigated in decidual cells in the ovary according to the method of Robinson and Karnovsky[19]. Incubation medium used is shown in Table 4.

A control incubation was made by omitting the substrate from the incubation medium.

RESULTS

Macroscopic findings of ovarian decidual foci

Decidualized areas on the ovarian cortex in the second and third trimester of normal pregnancy appeared as slightly elevated, red in color and edematous foci. Upon closer observation, these foci were hypervascular and gentle pressure easily caused them to rupture and bleed (Figs. 1a, b and c). On the Other hand, typical foci described above could not always be identified in the first trimester of pregnancy, but somewhat reddish patches under the ovarian surface could be found.

Light microscopic findings

At the 13th week of gestation or later, the presence of ovarian decidual cells was histologically confirmed (Figs. 2a, b and c). Decidual areas were classified as surface or subsurface in their distribution. In the former case, they were covered only with one layer of the peritoneal epithelium (Figs. 2a and 2c) and in the latter case, several cellular layers of connective tissue were interposed between the peritoneal epithelium and decidual cells (Fig. 2b). There were many vessels or capillaries in the decidual foci and decidual cells were arranged around the vessels. Typical ovarian decidual cells were polygonal, ellipsoidal, fusiform or elongated in shape. They had round nuclei with prominent nucleoli and abundant eosinophilic cytoplasm. Their cell borders were strongly stained with PAS (Fig. 3) and showed metachromasia by toluidine blue stain. Immunoreactive prolactin was detected in the cytoplasm of some decidual cells and the intensity of staining was varied by each cell (Figs. 4a and 4b).

Electron microscopic findings

Nuclei of ovarian decidual cells were oval or ellipsoidal in shape with distinct nucleoli and euchromatin (Fig. 5). The cytoplasm contained well developed rough and smooth endoplasmic reticulum, Golgi apparatus, numerous rod-shaped mitochondria, intermediate filaments, glycogen particles and lysosomes (Figs. 6, 7 and 8). The whorl formation of smooth endoplasmic reticulum was characteristically recognized (Figs. 11 and 12). An external lamina 50 nm in thickness was found around the outer aspect of the plasmalemma of most decidual cells (Fig. 6). In the connective tissue space among these decidual cells, numerous collagen fibers running irregularly and amorphous materials were seen (Fig. 5). Peduncular protrusions containing secretory bodies, measuring 0.3 to 0.7 μm in diameter, bounded by a limiting membrane and enveloped by a thin rim of cytoplasm appeared to protrude through the external lamina and the attachment

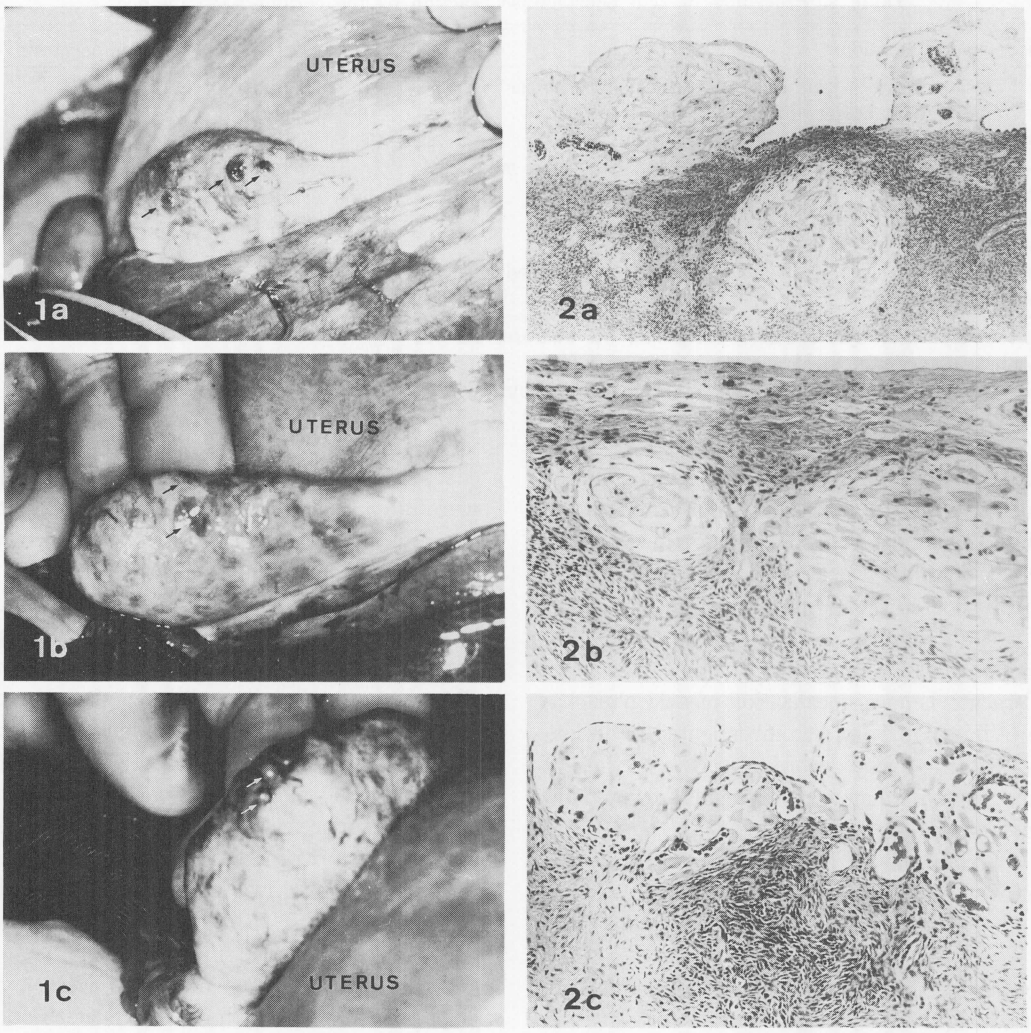

Fig. 1a, b and c. The human ovary at cesarean section at late pregnancy. Macroscopically, decidualized areas (arrows) appear as slightly elevated, hypervascular and edematous foci (1a and 1c) and patches (1b).
Fig. 2a, b and c. Photomicrographs of ovarian decidual tissues. Figures 2a, b and c are the histological features of Figures 1a, b and c, respectively. H-E stain, × 100.

of these structure to the cell by a thin cytoplasmic stalk was recognized (Figs. 6 and 7). Granular contents, measuring 30-50 nm in diameter, were often observed in the secretory bodies and secreted with the mode of exocytosis (Figs. 9 and 10). A single secretory body was usually observed in the peduncular protrusion, but some protrusions contained 2 secretory bodies (Fig. 6). Collagen fibers were scant around the secretory granules (Fig. 5).

PA-silver methenamine technique revealed deposits of silver in the secretory body in the peduncular protrusion (Fig. 13). In addition, the acid phosphatase activity was clearly demonstrated in the secretory bodies by a cerium-based method in combination with a microslicer (Fig. 14). No reaction products were seen in the control incubation.

DISCUSSION

It is well known that endometrial stromal cells are transformed into characteristic decidual cells during pregnancy in the human uterus (Lawn et al[12], Stark and Kaufmann[22], Liebig and Stegner[13]) as well as in the rat uterus (Abrahamsohn[1]). Decidualization of stromal

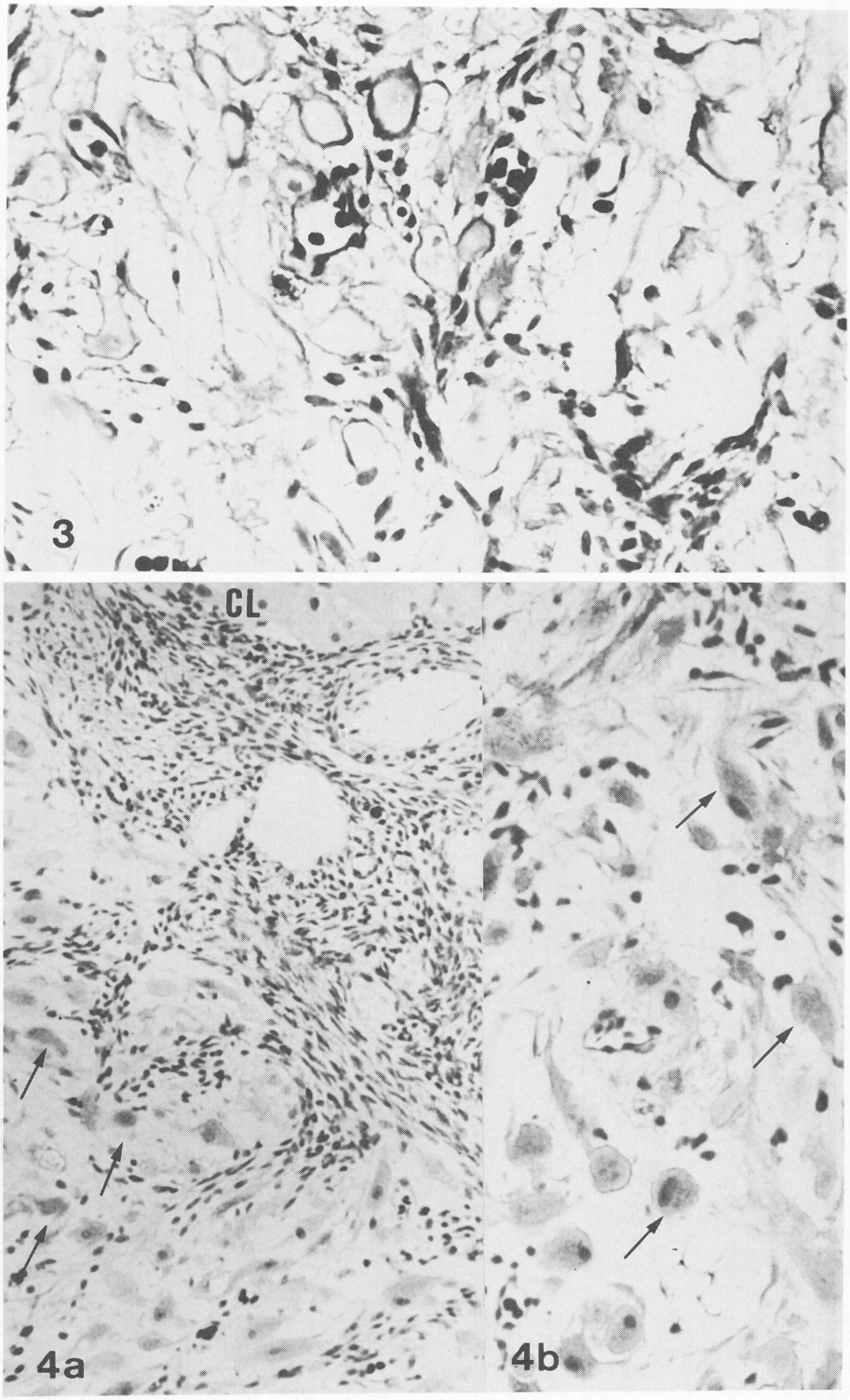

Fig. 3. Photomicrograph of ovarian decidual tissue stained with PAS. Cell borders are strongly positive for PAS. × 100.

Fig. 4a and b. Photomicrographs of ovarian decidual tissue immunoreacted for prolactin with ABC method. Positive immunoreactivity is shown in the cytoplasm of decidual cells (arrows). CL: corpus luteum, counterstain with Mayer's hematoxylin, × 100, × 400.

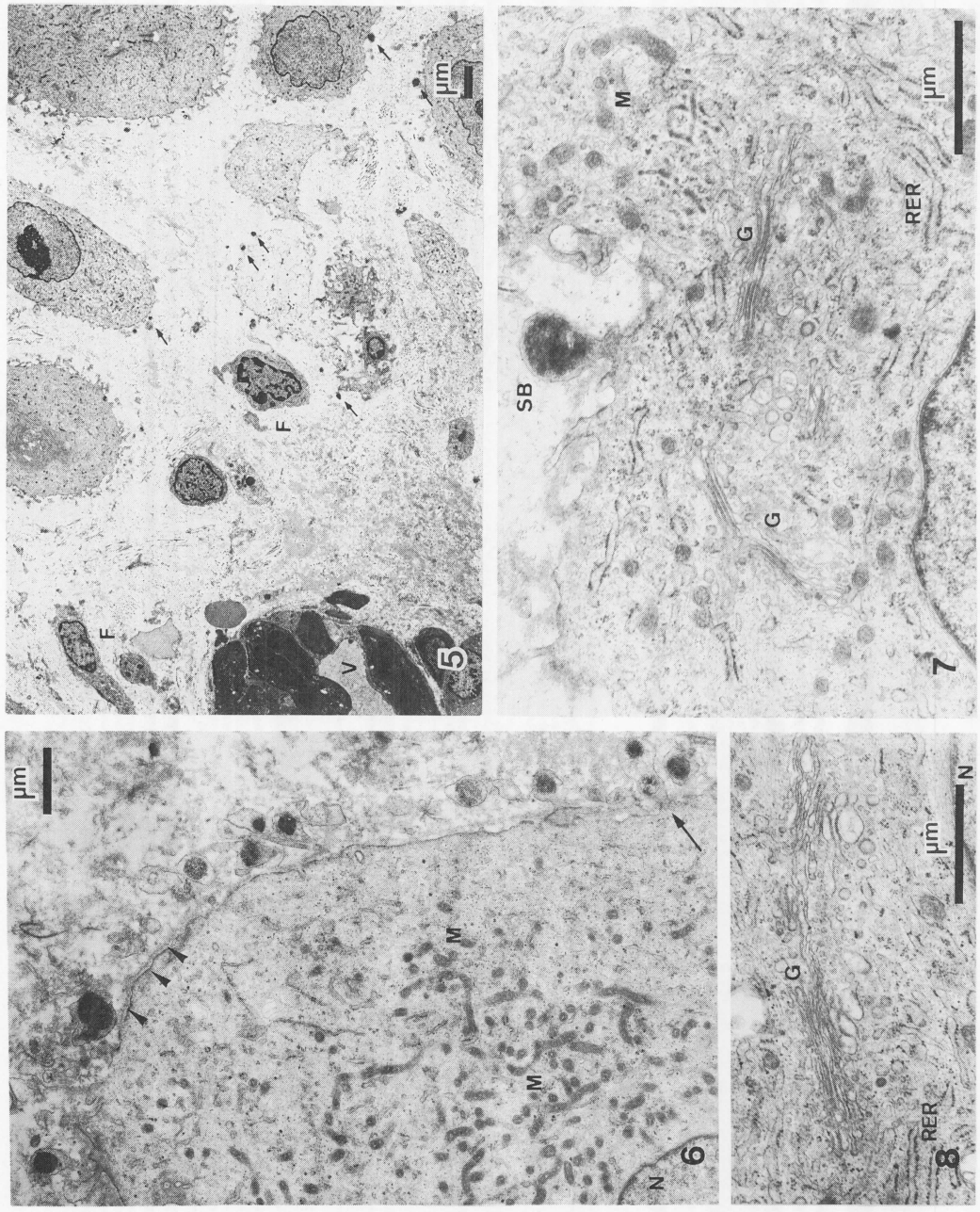

Fig. 5. Decidual cells show oval or ellipsoidal nuclei with prominent nucleoli and euchromatin, and secretory bodies protruded through the external lamina. F: fibroblast-like cell, V: vessel, bar = 1 μm.

Fig. 6. A part of an ovarian decidual cell. Secretory bodies bounded by a limiting membrane are found in the peduncular protrusions. Two secretory bodies within a single peduncle are connected by a thin stalk. (arrow). M: mitochondria, N: nucleus. Arrowheads indicate an external lamina. bar = 1 μm.

Fig. 7. A part of ovarian decidual cell. A secretory body (SB) is noted at the cell margin. Well developed rough endoplasmic reticulum (RER), Golgi apparatus (G) and rod-shaped mitochondria (M) are also present. N: nucleus. bar = 1 μm.

Fig. 8. A part of Golgi apparatus (G). Many Golgi vesicles are seen. RER: rough endoplasmic reticulum. bar = 1 μm.

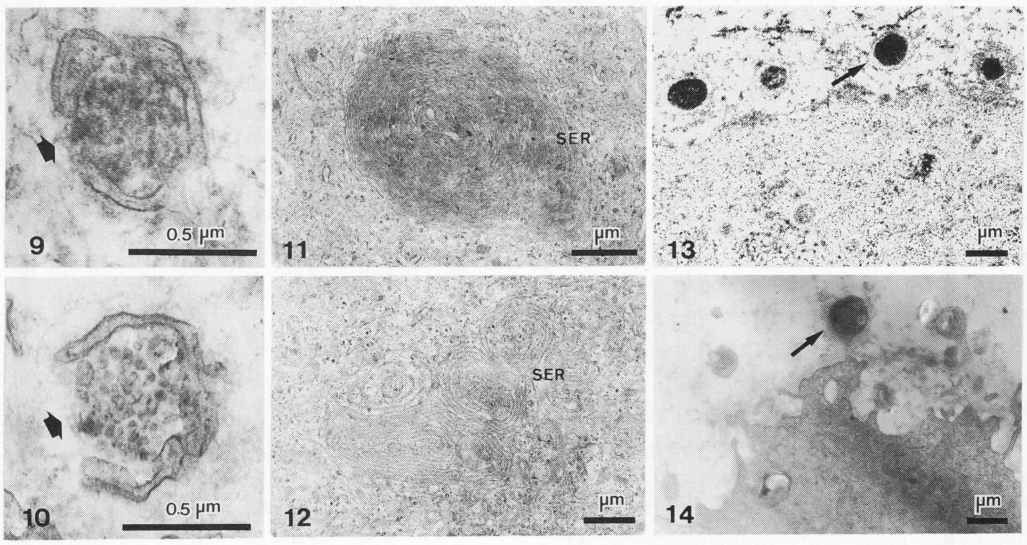

Fig. 9 and 10. The secretory body is composed of numerous granular contents (Fig. 10). The contents are secreted with the mode of exocytosis (arrows). bar = 0.5 μm.
Fig. 11 and 12. Whorl formation of smooth endoplasmic reticulum (SER) is noted. bar = 1 μm.
Fig. 13. Deposits of silver are localized in the secretory bodies (arrow). PA-silver methenamine technique. bar = 1 μm.
Fig. 14. AcPase reaction products are present in the secretory body (arrow). Cerium-based method. bar = 1 μm.

cells seems to play an important role in the background of implantation and development of the fertilized egg. Although ectopic decidual cell reactions have been reported in many organs below the diaphragm during intrauterine pregnancy[2,4,9,10,24], the etiology and function of ectopic decidual cells have not been fully elucidated.

With regard to the stage of pregnancy when ovarian decidua is noted, Starup and Visfeldt reported the decidual changes in the human ovary at the ninth week of gestation[23] and also Herr et al reported the appearance of ovarian decidual cells in the third month of pregnancy[7,8]. According to Herr et al[7,8], ovarian decidua is noted in 100% of the ovaries at term. In the present study, ovarian decidual cells were firstly noted at the 13th week of gestation and recognized in all the specimens from the 13th to 42nd weeks gestation. These data indicate that ovarian decidualization might occur at least within 3-4 months of pregnancy.

The ultrastructure of the human uterine decidual cells has been investigated by several authors (Lawn et al[12], Wynn[25], Okudaira[15] and Yoshida[26]). Ultrastructural features of uterine decidual cells described in these previous reports are euchromatic nuclei with prominent nucleoli, well developed rough and smooth endoplasmic reticulum, whorl formation of smooth endoplasmic reticulum, abundant Golgi apparatus, numerous rod-shaped mitochondria, and secretory bodies bounded by a limiting membrane in the peduncular protrusions. These features of uterine decidual cells are quite similar to those of ovarian decidual cells in the present study.

With regard to secretory bodies, Lawn et al[12] first reported that these electron-dense, membrane-bounded bodies at the periphery of uterine decidual cells could represent either a secretory product or digestive residue of intracellular digestion. Yoshida et al also described the acid phosphatase activity of the secretory granules originated from Golgi apparatus[27]. In the present study, electron-dense secretory bodies composed of 30-50 nm granular contents were recognized at the periphery of the cell and protrusions, and might be released with the mode of exocytosis. Acid phosphatase activity was demonstrated in the secretory bodies and their granular contents by a cerium-based method in combination with a microslicer, and PA-silver methenamine technique revealed the

deposits of silver in the secretory bodies. these findings are in accordance with the earlier observation by Yoshida et al[27] on uterine decidual cells. The positive stain for AcPase and PA-silver methenamine and the scant collagen fibers around the secretory bodies suggest that the secretory body contains an enzyme such as collagenase and helps the implantation of the fertilized egg.

More recently, several authors[6,14,18,20] have shown that prolactin is synthesized and released in the uterine decidual cells, and decidual cells have been considered to be a main source of high concentration of prolactin in amniotic fluid. The decidual prolactin appears to be identical to pituitary prolactin based on chemical, immunological and biological criteria (Golander et al[5], Riddik et al[17]). By the immunostain by ABC method in the present study, immunoreactive prolactin was demonstrated in the cytoplasm of some decidual cells, but all the decidual cells could not be stained and the intensity of staining differed from cell to cell. The reason of this difference could not be elucidated in the present study.

These data indicate that decidualized cell might be capable of producing prolactin in both uterus and ovary.

In conclusion, ovarian decidual cells share many fine structure and function with uterine decidual cells, especially on the release of secretory bodies with the mode of exocytosis.

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