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HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDIES ON THE LOCALIZATION OF IMMUNOGLOBULINS IN PORCINE PLACENTA

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Porcine placentae from 18 to 114 days of gestation were investigated histologically, histochemically and immunohistochemically.

The placenta was histologically divided into four areas: the chorionic fossa (CF), chorionic ridge (CR), regular areola (RA) and irregular areola (IRA). The IRA was further subdivided into fetal IRA, maternal depression and ruffled area of the maternal depression (RAMD). In histochemical analysis, a reaction product suggestive of acid mucopolysaccharides was detected exclusively in the uterine and chorionic epithelia of the IRA. In immunohistochemical analysis, the localization of IgG and IgM was demonstrated in the endometrial lamina propria mucosae, and the epithelia of the RAMD and fetal IRA. Regional IgG and IgM localization was identical to that of the acid mucopolysaccharides. In the fetal lymphatic tissues, a small number of IgG-containing cells was first noted at 114 days of gestation. In a serological survey, IgG and IgM were demonstrated at a constant level in all fetal sera from days 40 to 114 of gestation, although IgA was negative except for 2 of 23 sera from fetuses. Comparing the relative levels of maternal immunoglobulins (Igs), the fetal Igs were 1/200 in IgG and 1/500 in IgM.

These results suggest that there is a possible diaplacental transition of IgG and IgM from the mother into the fetus occurring via epithelia exclusively at the RAMD and the fetal IRA.

Key words: immunoglobulins, immunohistochemistry, porcine placenta

INTRODUCTION

It is known that passive antibodies of maternal origin play a significant role in the prevention of microbial infections in neonatal animals whose immune systems are immature. The routes of the passive immunity from mother to fetus are different among species of animals. BUTLER (1973) classified animals into groups I to III on the

basis of the modes and the routes of transferable immune substances. Humans and rabbits were classified in group I because the maternal IgG was selectively transferred into fetuses during gestation. Ungulates in which IgG was transferred via the colostrum belong to group III. Group II includes such as rodents and carnivores, in which the transference occurs via routes other than those of groups I and III. KUTTER & RATNER (1923) suggested that the diversity in the efficiency of passive immunity was dependent on the degree of permeability of globulins, and that the permeability was limited by the histological placental barriers. In the single layered-hemochorial placenta in human and rabbits, the maternal antibodies can pass into the fetus through the placenta, while in cows the antibodies remain on the maternal side of the three-layered epitheliochorial placenta.

It was suggested in recent studies that maternal IgG was transmitted into fetuses across the yolk-sack epithelium or the syncytial trophoblasts of the hemochorial placenta via a receptor-linked transporting system^{1,7,15,22}. At the present time, however, it is generally believed that the diaplacental transmission of maternal IgG into fetuses does not occur in ungulates because they are furnished with an epitheliochorial placenta having a more highly obstructive histological structure, although gamma globulins^{3,6} Igs²⁹ and IgG^{26,28} were demonstrated in the sera of colostrum-deprived neonatal piglets. Therefore, it is as yet unclear how the placental structures are involved in the diaplacental transmission of maternal antibodies into the fetuses²¹.

This study was performed to clarify the morphological and immunohistochemical natures of porcine placentae in various gestational periods in view of the ungulate's passive immunity, and discussed the possibility of whether the maternal Igs are transmittable into fetuses via epitheliochorial placentae or not.

MATERIALS AND METHODS

Animals: Forty-five pregnant sows and 26 fetuses in various gestational stages were obtained from a local slaughterhouse. Individual gestational stages were estimated by the crown-rump-length (CRL) of the fetuses¹⁴.

Histology and histochemistry: After an arterial perfusion of periodate-lysine-paraformaldehyde solution (PLP), placentae were refixed in the PLP or 10% formalin, and 4 μ -thick paraffin sections or frozen sections 10 μ in thickness were prepared. They were stained with haematoxylin-eosin (HE), alcian blue, pH 1.0, (AB 1.0), alcian blue, pH 2.5, (AB 2.5), toluidine blue, pH 4.0, (TB), sudan black B, sudan III or Berlin blue. Some serial sections were immersed in Gomori's incubation medium for the detection of acid phosphatase (ACPase) or alkaline phosphatase (ALPase).

Immunohistochemistry: Placentae, fetuses less than 5 cm in CRL that were cut in half sagittally, and the fetal duodenum, jejunal lymph nodes and spleens of fetuses whose CRLs were over 5 cm in length, were immersed in PLP, and 4 μ paraffin sections

were prepared. Indirect peroxidase-labeled antibody methods were applied to estimate the distribution of IgG, IgM or IgA in the placentae and to determine the initial localization of Ig-containing cells in fetal lymphatic tissues.

Transmission electron microscopy (TEM): Placental regions of the interareola (IA), regular areola (RA) and irregular areola (IRA) were fixed in 3% glutaraldehyde, then postfixed in 1% OsO₄, and embedded in Quetol 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (HITACHI, HU-12A).

Immunoelectron microscopy: The intraplacental localization of IgG was analysed immunoelectron microscopically using frozen sections of PLP-fixed placentae.

Scanning electron microscopy (SEM): Placentae were cut into 5 cm portions and immersed in 0.07 M phosphate-buffered saline with 1 mM ethylenediamine tetraacetic acid (EDTA) and 1% sucrose¹³⁾. After immersion for 5 to 10 min in the medium, the chorioallantois was gradually stripped from the endometrium and fixed in 3% glutaraldehyde. It was then osmificated by a conductive tannin-osmium method²⁴⁾, and dried in a critical point drying apparatus (HITACHI, HCP-1), coated with gold, and observed with a scanning electron microscope (JEOL, T-200).

Serological survey: Maternal and fetal sera were examined to determine the relative concentrations of IgG, IgM and IgA by an enzyme-linked immunosorbent assay (ELISA). The determination was performed by means of a sandwich method for IgG, and an indirect method for IgM and IgA.

The terms used in the present study follow the description of BRAMBEL (1933), and the areas located in the maternal depressions occurring on the endometrium are tentatively termed the ruffled area of the maternal depression (RAMD).

RESULTS

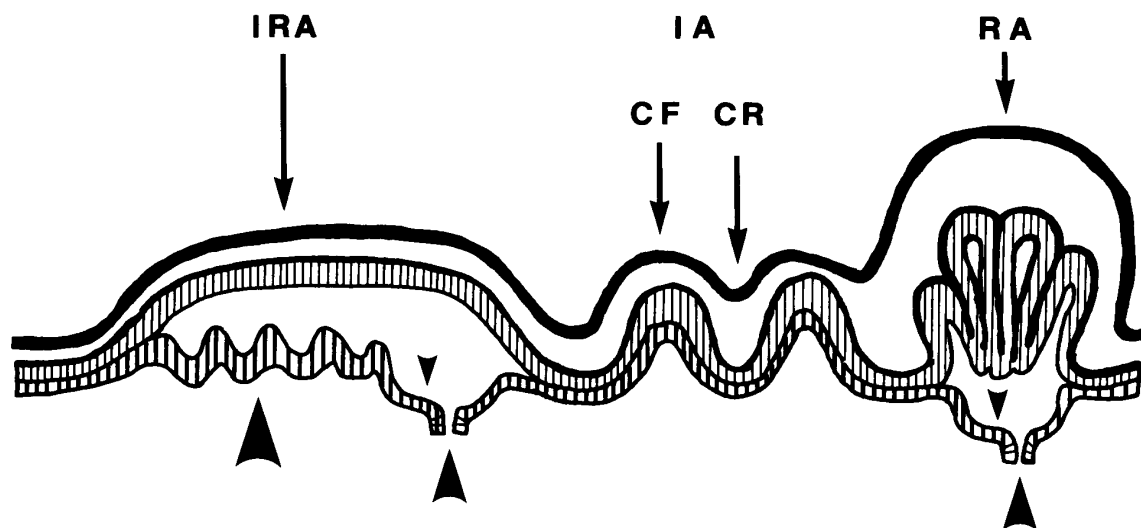
Macroscopic and microscopic architectures of placenta

The placenta was divided into the areola and IA according to the contacts between the maternal and fetal territories (Fig. 1). The IA was further subdivided histologically into the chorionic fossa (CF), which had higher columnar epithelial cells, and the chorionic ridges (CR) furnished with a cuboidal epithelium and adjacent fetal capillaries. The areola area was also subdivided into the RA and IRA on the basis of their morphologies. The IRA consisted of fetal IRA, maternal depressions with uterine gland openings and RAMD (Figs. 1-3). Thus, it was determined that the porcine placenta was constituted of the following four areas; CF, CR, RA and IRA.

In the present histological examination, areolae were observed first in the fetuses at 18 days of gestation, and classified as RA or IRA at 28 days, after which structural diversity was maintained throughout the preparturitional period.

The morphology of the endometrial epithelium of the RAMD varied with the various stages of development. In ultrastructural analysis, protrusions on the surface

Fig. 1 Schematic presentation of the porcine placenta. The placenta was divided into IRA, RA and IA. IA was composed of CF and CR. The IRA consisted of fetal IRA, the maternal depressions (small arrow heads) with uterine gland openings (medium arrow heads), and RAMD (large arrow head).



RA : regular areola, IRA : irregular areola
 IA : interareola
 CF : chorionic fossa, CR : chorionic ridge

of the epithelium were seen frequently and it was revealed suggestive structures on an active transportation of metabolic substances when they were investigated by SEM. The epithelium of the RAMD consisted of cells whose vesicles were of high electron density (Fig. 4). The ultrastructures of the vesicles changed as maturation proceeded. At the immature stage, they appeared as smaller vesicles of high electron density occurring around the Golgi apparatus (Fig. 5a), and then changed to a lower density and were clouded by fibrous materials as they increased in size. The vesicles next fused together to form larger vacuoles adjacent to the nucleus (Fig. 5b) or periphery of the cytoplasm (Fig. 5c). The same materials found in the vesicles were also demonstrated in the uterine cavity, exclusively facing the fetal IRA (Fig. 5d).

Histochemistry of placenta

The histochemical results are summarized and presented in Table 1.

Polysaccharides: It was demonstrated that there were PAS-reactive materials at all regions of the chorionic epithelium of the placenta. The areas positive for AB were identical with those metachromatic for TB, and they were the epithelium of the fetal

TABLE 1. Histochemical natures of the chorionic and uterine epithelia in porcine placentae

STAINING	RA	IRA	IA	
			CF	CR
PAS	+	+	+	+
AB 1.0	-	+	-	-
AB 2.5	-	+	-	-
TB	-	+	-	-
Sudan black B	+	+	+	+
Sudan III	+	+	+	+
Berlin blue	+	+	+	+
ACPase	+	+	+	+
ALPase	+	+	+	+

+ : present, - : not detected

IRA, the RAMD and, slightly, the intraluminal materials of the IRA. In duplicated staining with PAS and AB (pH 1.0 or 2.5), a tendency for the epithelium of the RAMD in the IRA to have a greater stainability with AB for acid mucopolysaccharides was noted (Fig. 6). These findings on the intraplacental localization of acid mucopolysaccharides corresponded well with the locations of the vacuoles in the present ultrastructural analysis.

Lipids: Sudan black B was more sensitive to lipids than was Sudan III, and lipids were common to all the placental epithelia.

Iron: Localization of iron was confirmed in small granules of the epithelia of both maternal and fetal placentae by Berlin blue staining.

ACPase and ALPase: ACPase and ALPase were commonly demonstrated in all the epithelia, and ALPase was extensively localized in the connective tissues around the uterine glands.

Immunohistochemistry of placenta

Intraplacental distribution of Igs: IgG was demonstrated in the epithelia of the RAMD, the endometrial lamina propria mucosae (Fig. 7a, b), and, slightly, in the vacuoles of the chorionic epithelia of the IRA (Fig. 8). This regional localization of placental IgG was first found at 28 days of gestation and continued throughout the gestational

period. These immunohistochemical results corresponded to those of the localization of acid mucopolysaccharides demonstrated histochemically.

The intraplacental localization of IgM was commonly identical to that of the IgG, although it had a lesser reactivity in the endometrial lamina propria mucosae as compared with that of the IgG. No IgA localization was confirmed.

Distribution of cells containing Igs: A small number of large lymphocytes and plasma cells were scattered in regions of the endometrial lamina propria mucosae, although no transplacental cellular migration was confirmed. They were mainly IgG- or IgM-containing cells.

Immunohistochemistry of fetal lymphatic tissues

Although no immunoreactive cells were demonstrated in any fetal lymphatic tissues until day 40 of gestation, on day 70, IgG- or IgM-immunoreactive medium- or large-sized lymphocytes were observed in all the lymphatic tissues examined. In the spleen, a few IgG-immunoreactive cells first appeared at 114 days of gestation (Fig. 9).

Serological analysis

IgG and IgM were commonly detected in all the sera from fetuses aged from 40 days until parturition, although no IgA was contained in almost sera except for 2 of 23 (Fig. 10). The ELISA end point titers of IgG and IgM in the fetal sera varied from 10^2 to 10^4 (Fig. 10). The relative proportions of the end point titers of the fetal sera were 1 : 200 in IgG and 1 : 500 in IgM when they were compared with those of the maternal sows.

DISCUSSION

It is known that passive immunity in the pig is completed due to the active transference of immunoglobulins via the colostrum from mothers to neonates^{6,25}. However, it was reported that macroglobulins,^{3,6} Igs²⁹ and IgG^{26,28} were present in the sera of colostrum-deprived neonatal piglets.

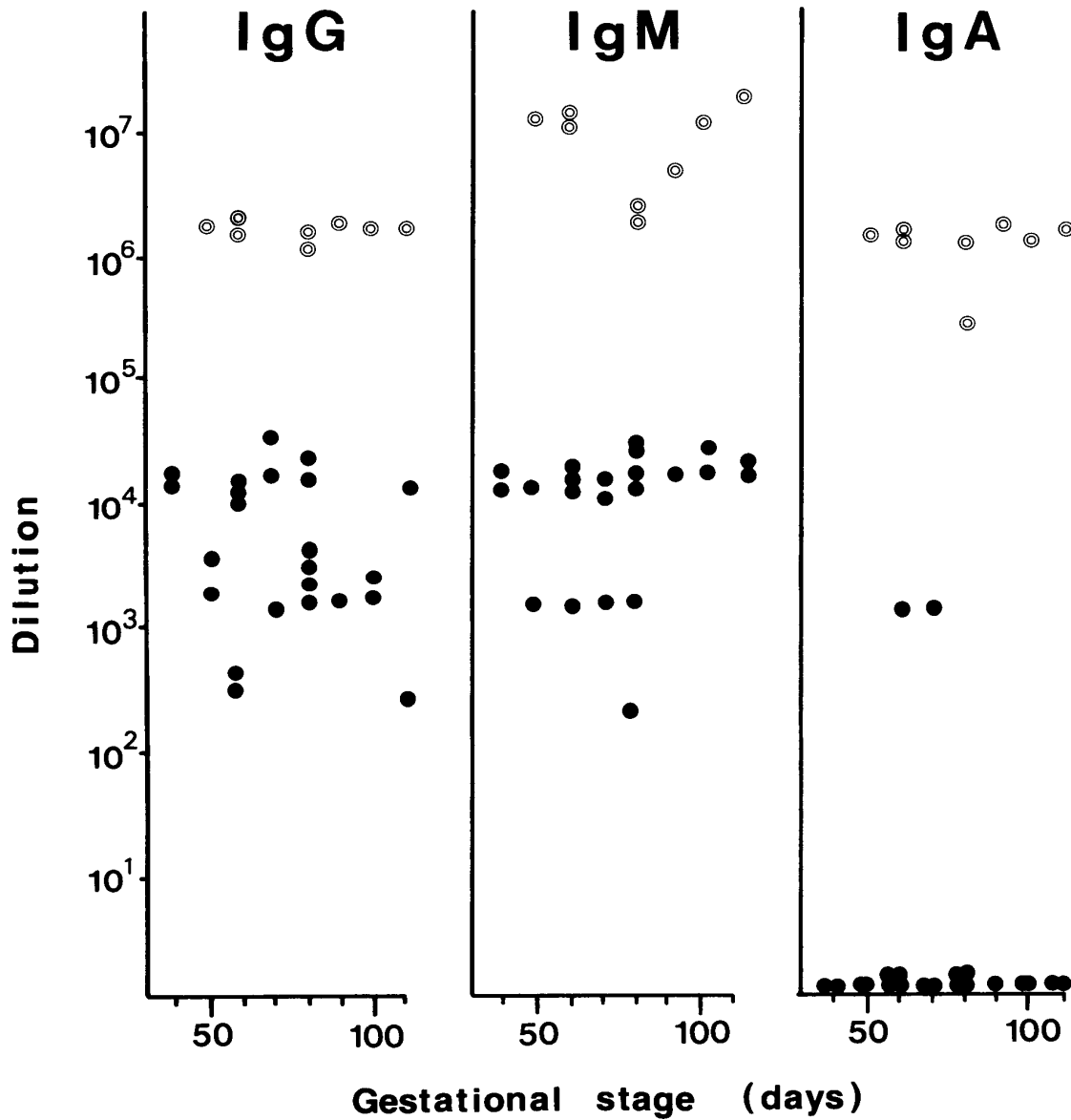
In the present serological survey, IgG and IgM were detected in the fetal sera from fetuses aged 40 to 114 days of gestation. This confirmed the view that Igs were not only present in the sera of colostrum-deprived neonatal piglets but also in the fetal sera, even in the early stages of pregnancy²⁹. In addition, an antibody for tetanus toxoid was also detectable in the sera of neonatal piglets whose maternal sows were previously immunized with the toxoid²³. In contrast, KIM et al. (1966) were of the opinion that there was no IgG in the serum of new-born piglets. Therefore, whether there is a diaplacental contribution of maternal immunity to fetuses in pigs has been a matter of dispute. However, ELISA provides an effective method for the determination of relative quantities of immunoglobulins at the pg/ml level, thus making more exact analysis possible⁴.

The relationship between the histological architecture of the porcine placenta and

Fig. 10 Relative values of Igs in fetal and maternal sera analysed by an ELISA method.

IgG and IgM were detected in all fetal sera from 40 days of gestation, but IgA was in only 2 sera of 23 tested. Their end point titers in fetal sera were from 10^2 to 10^4 for IgG and IgM.

○: sow's sera, ●: fetal sera



the mode of transmission of Igs is still in dispute. CROMBIE (1972), and YABIKI & NAMIOKA (1976) demonstrated the fetal sanguiferous localization of IgG by immunofluorescence methods, and reported that no fluorescence appeared in the fetal placenta or the endometrial epithelial cells, although IgG was confirmed in the endometrial lamina propria mucosae. It was demonstrated in the present study that IgG and IgM in the maternal animals were exclusively present in the epithelium of the RAMD, and that they were, to a slight degree, also present in the fetal chorionic epithelia in the IRA.

The IRA is a type of areola. BRAMBEL (1933) and CROMBIE (1972) described its structures and allantochorionic differentiation in detail with light microscopy, and they divided the components into RA, IRA, IA, cysts, hippomanes and petrification. Present histochemical results on the RAMD corresponded well with the findings described by CROMBIE (1972) that acid mucopolysaccharides were detected in the RAMD, but were not observed in any other endometrial areas. CHRISTIE (1968) also suggested that only a limited number of epithelial cells could contain acid mucopolysaccharides. In the present study, acid mucopolysaccharides were common in the epithelium of the RAMD in PLP-fixed specimens. PLP is known to be one of the best fixatives for glycoprotein preservation. This may suggest that the preservation of the materials depends on the fixative applied. In ultrastructural analysis, the granular materials of low electron density in the endometrial epithelial cells of the various areas corresponded to the AB-positive ones in which IgG coexisted, as revealed by immunohistochemistry. This result was supported by reports that acid mucopolysaccharides existed in the yolk-sac visceral endodermal layers of guinea pigs¹⁹⁾ and rats¹⁷⁾, that horseradish peroxidase (HRP)-labeled IgG was preferentially taken up from the layer and that IgG might be taken up with the acid mucopolysaccharides²⁰⁾.

It is known in swine that the fetal lymphocytes are responsible for the production of IgG against antigens introduced through the maternal side⁴⁾, and that they differentiate to become surface IgM-bearing cells as a result of treatment with B cell activators¹⁶⁾. PROKEŠOVÁ et al. (1981) demonstrated the embryonic appearance of Ig-forming cells in an autoradiographical study using specific antibodies to gamma, mu and alpha chains labeled with I¹²⁵. They reported that the Ig-forming lymphocytes were first detected in 38-day old fetuses with a markedly increased number of the cells after 60 days of gestation. In the present study, IgG- and IgM-positive lymphocytes occurred in the lymphatic tissues of 70-day-old fetuses. In spleens, a few IgG-containing cells were observed first at 114 days of gestation, although there was no sign of diaplacental migration of such cells of maternal origin. Thus, the cells containing Ig¹⁰⁾ may be surface Ig-bearing lymphocytes. In the lymphatic tissues of pig neonates Ig-producing cells were reported to have appeared on the 6th day in duodenum, and on the 8th day in the spleen and mesenteric lymph node, and it was suggested that a marked increase in the number of such cells occurred at 3 to 4

weeks of postnatal life^{2,8)}.

The results in the present study, therefore, suggested the possibility that maternal IgG and IgM are secreted into the uterine lumen together with acid mucopolysaccharides which exist exclusively in the epithelium of the RAMD, and are then be absorbed by the chorionic epithelium of the fetal IRA and introduced into the fetal circulatory system.

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EXPLANATION OF FIGURES

PLATE I

- Fig. 2 A low-powered view of the porcine placenta.
The placenta consisted of IA, RA and IRA. 75 days of gestation, HE, $\times 28$
- Fig. 3 A scanning electron micrograph showing the epithelium of maternal IRA.
Maternal IRA consisted of MD and RAMD with a more rugged surface. 82 days of gestation, $\times 100$
- Fig. 4 Transmission electron micrograph showing epithelial cells at the RAMD.
The cells contained many large vesicles apically. 114 days of gestation, $\times 3,000$

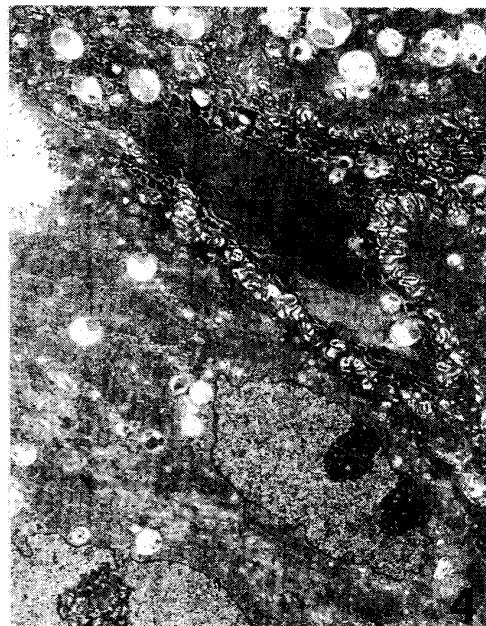
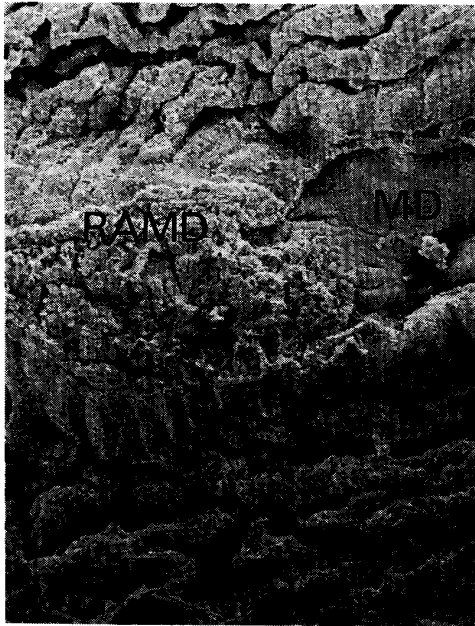
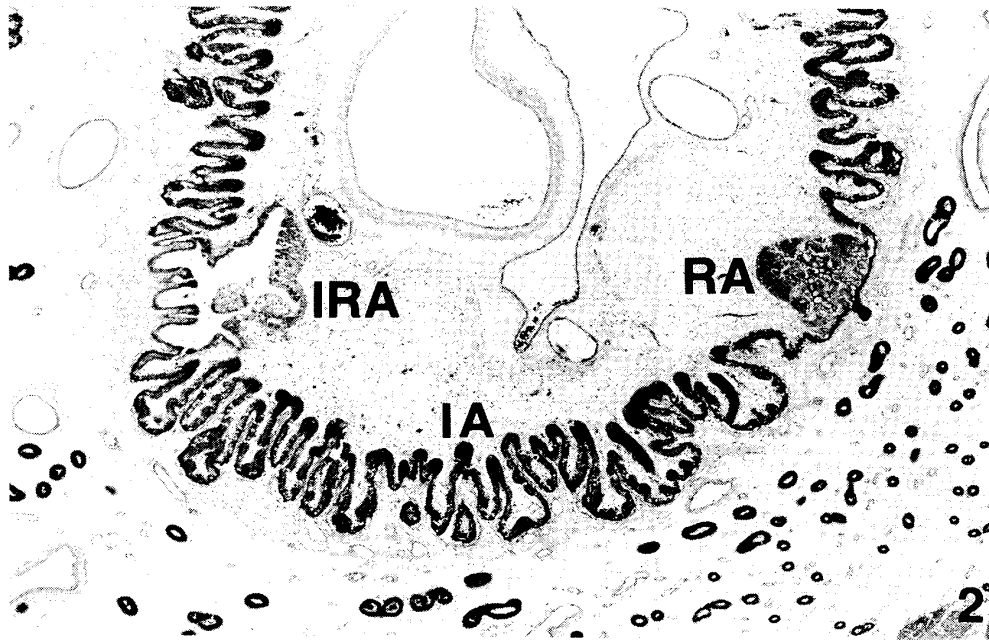


PLATE II

Fig. 5 Vesicles in the epithelial cells of IRA.

Small vesicles, in which intravesicular materials of high electron density are surrounded by a halo, are seen (5a).

The electron density of intravesicular materials decreased and the vesicles fused together to form vacuoles that increased in sizes (double arrow) (5b). Large vacuoles of low electron density (Vac) were located at the periphery of the cytoplasm. Lu: Uterine lumen (5c). Low density materials are also observed in the lumen (Lu) (5d). 114 days of gestation, $\times 12,000$

Fig. 6 Localization of polysaccharides in the placental epithelial cell.

The epithelia of IA and RA are positively stained by PAS reaction. However, that of IRA and some luminal materials reacted positively to alcian blue (arrow). 92 days of gestation, AB (pH 1.0) and PAS, $\times 42$

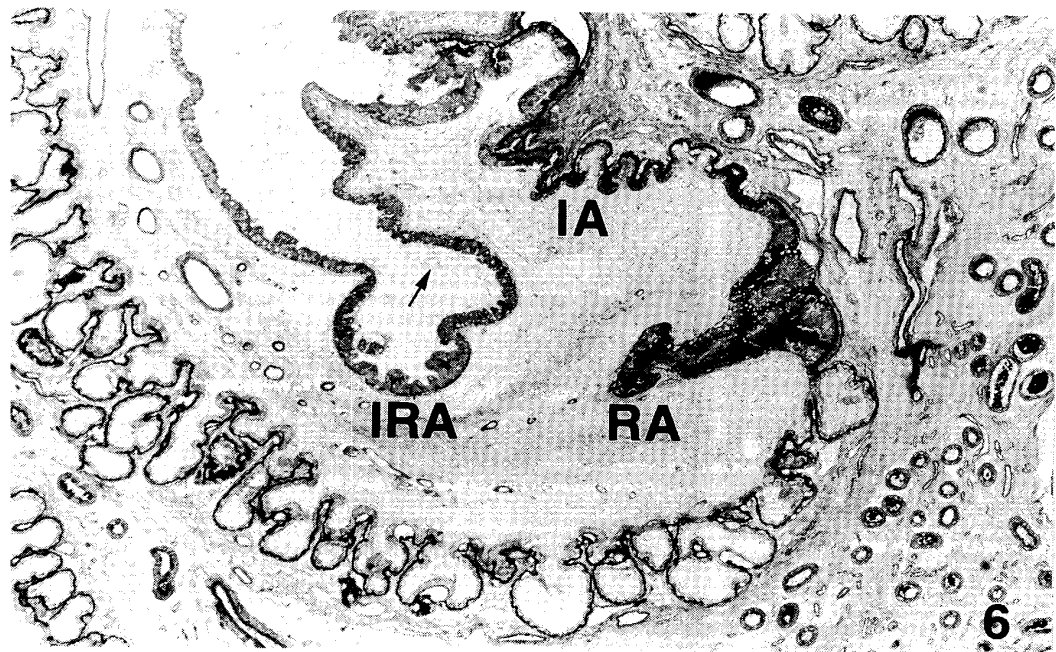
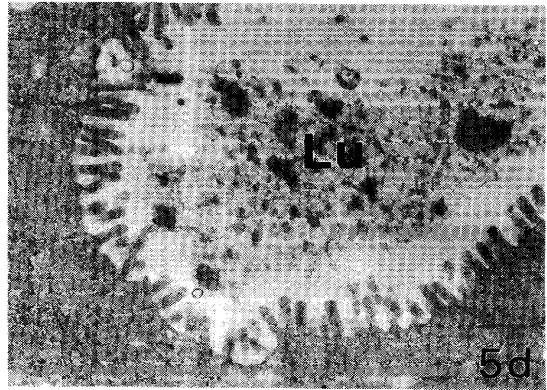
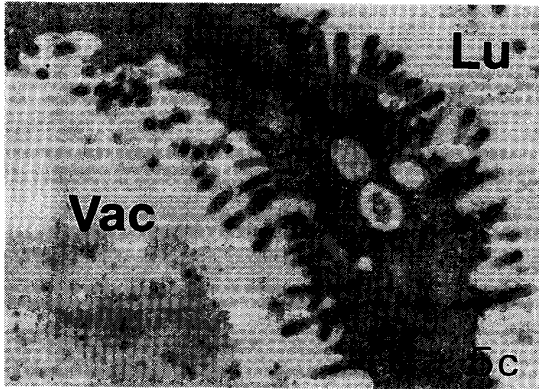
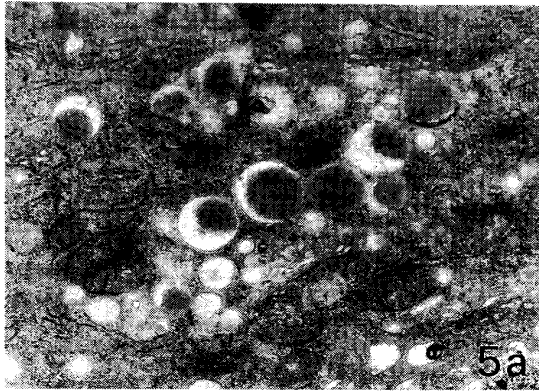


PLATE III

Fig. 7 Localization of IgG in the IRA.

The maternal IgG localization is demonstrated in the intercellular spaces of the epithelium of RAMD, and also in endometrial lamina propria mucosae (7a), $\times 360$. Reaction products are located immunoelectron microscopically in the intercellular spaces of the epithelium (7b). $\times 7,200$. 114 days of gestation.

Fig. 8 Localization of IgG in the IRA.

The reaction products in the fetal areas are sparse in the chorionic epithelium of IRA (arrow). $\times 7,200$. 114 days of gestation. $\times 360$

Fig. 9 IgG-containing cells in a fetal spleen.

A few IgG-containing cells (arrow) are first demonstrated in a fetal spleen at 114 days . $\times 330$

