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Spiral Artery of Placenta: Development and Pathology -Immunohistochemical, Microscopical, and Electron-Microscopic Study

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The spiral artery (=SA) is an important muscular artery, which controls the blood volume to the placenta. Preeclampsia is thought to be induced by the failure of the placenta by dysfunction of SA. To clarify the function of SA, we examined forty-eight placentae and its morphological and biological characteristics: 36 normal placentae and 12 placentae with preeclampsia. Gestational age of normal placentas was between 19 and 40 weeks and placentae with preeclampsia was between 31-36 weeks. The wall of the placental segment of SA by both light and electron microscope, and the wall width of SA and gestation age were compared each other. The wall of SA, with the invasion of trophoblast, was thin, but SA without trophoblasts was thick in width. At normal placenta, the diameter of SA was dilative constantly, but the width of the wall showed a tendency of getting thinning as advances. Ultrastructurally, we found the trophoblast of thin wall of SA with dilated lumen. These ultrastructural alternations were consistence with the light microscopical findings. In preeclampsia, the lumen of SA between normal pregnancy and one with preeclampsia was almost same, but the wall width was thick, compared with normal pregnancy ($P<0.05$). We concluded that trophoblastic invasion control the functions of SA.

Many descriptions about placental circulations have been reported since Freidlander's ⁷⁾ report in eighteenth century. In 1967, the relation of gestation age and morphological changes of SA were published already.³⁾ Some investigators indicated also the relations of alternations of SA and gestational age. The change of SA wall is reported to be associated with the trophoblastic invasion ³⁾. The examination of trophoblasts was done by ultrastructural ^{6,11,17)} and immunohistochemical methods.^{5,15)} Wolf ¹⁶⁾ disclosed the transmission electron microscopical (TEM) features of the trophoblasts in SA. Sheppard ¹²⁾ used the scanning electron microscopy (SEM) in analysis of the surface structure of SA.

On the other hand, the many pathological studies on pregnancy of the complicated by pre-eclampsia have been performed.^{7,9)} Veall and Broune ¹⁾ reported that the pregnancy with pre-eclampsia and hypertension induced the placental failure, and that the blood flowed from maternity decrease. As well known many concepts, morphological studies of pregnancy with preeclampsia have complicated with hypertension and intrauterine growth.^{2,6)} Brosens examined the morphological change of SA during pregnancy process with hypertension.⁴⁾ He proposed that the stenosis of SA by atherosclerosis may reduce blood volume to the placenta in pregnancy with hypertension.

Many investigators analyzed the uteroplacental segments of SA in normal pregnancy and ones with preeclampsia. The morphological studies by TEM are a few,^{4,11,16,17)} and the reports

by use of SEM are very rare. Its morphological studies are important to clarify the pathological background of maternal preeclampsia and others.

MATERIAL AND METHOD

We tried to study the detail features of placental segments of SA and measure these dimension of lumen and wall width of SA and do the observations by the methods of TEM and SEM, to elucidate the mechanism of maternal-fetus circulations and the causes of preeclampsia. Informed consent of all materials of placentae were gotten before pathological examination.

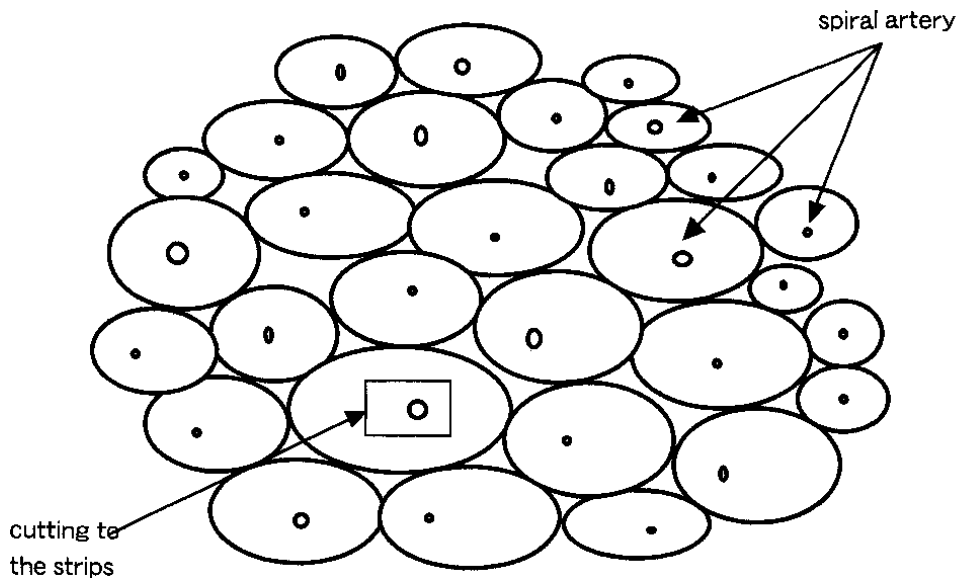


Fig. 1. Cut to the strips; -horizontal with the basal plate.

Patients:

We examined 48 placentae: 36 normal placentae and 12 ones with preeclampsia. Normal placentae were between 19 and 40 weeks of gestational age, and placentae with preeclampsia were between 31-36 weeks. In thirty-six cases of normal placentas, there were no any complications such as cardiovascular and endocrine, autoimmune, renal diseases and so furthers. Twelve placentae with preeclampsia showed maternal hypertension, proteinemia, and systemic edema. Clinical history and informations were summarized in Table I-a, I-b. Placentas were fixed in 10% buffered formalin solution, from 6 blocks about 2 by 3 by 0.5 cm in size were made. Many cotyledons are seen on the maternal surface of placenta. The specimens were cut horizontally to the strips on the basal plate of the placentae. Placental specimen had SA (Fig. 1) in the center of block. They were processed routinely in paraffin embedding, and cut 4 μ m. Histological sections were stained with HE (Hematoxylin Eosin) and EVG (Elastica van Gieson). We calculated media diameter by internal and external media by EVG and then estimated an idealized diameter. Mean $\pm$ SD of the lumen and wall width of SA was calculated finally. A student's test was used to evaluated clinical and morphological differences between normal pregnancy and ones with preeclampsia by Stat View 4.5 package. Statistical significance was considered at the 0.05 level.

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Electron microscopy:

We analyzed fifty samples of placental tissue of 31-36 gestation weeks. For TEM analysis, the tissue was minced into 1-mm cubes following surgical removal, fixed overnight in half-strength Karnovsky's fixative, postfixed in osmium tetroxide, dehydrated in alcohols, and embedded in Epon mixture. Thick sections at 1 μm were cut and stained with toluidine blue to trim adequate thinner sectioning. Proper areas containing SA were selected for thin sectioning. Thinner sections stained doubly with uranyl acetate and lead citrate, and observed under transmission electron microscope. At SEM analysis, the specimen was minced in small blocks and fixed by 2.5% glutaraldehyde for 4 hours. They were rinsed several times in 0.1M phosphate buffer and then immersed in 8 N HCl for 45 minutes at 60°C. They were rinsed again several times in phosphate buffer, treated with a 2% tannic acid solution for one hour, followed by rinsing in buffer. The specimens were then immersed in 0.1% osmium tetroxide for 2 hours at room temperature and transferred into scintillation vials filled with phosphate buffer. The vials were placed in an ultrasonic cleaner bath containing tap water to the level of the buffer in vials where they were subjected to ultrasonic energy at a set frequency of 80 kHz for 2 minutes. The buffer became slightly dark due to release of osmium from the tissue. Tissue blocks were then dried in a series of acetones with graded concentration of acetone and finally in a critical point drier using acetone and CO₂. Dried specimens were mounted on specimen stubs with conductive bridges, sputter-coated with gold for 600 seconds, and examined in a SEM at an accelerating voltage of 20kV and a working distance of 20 mm.

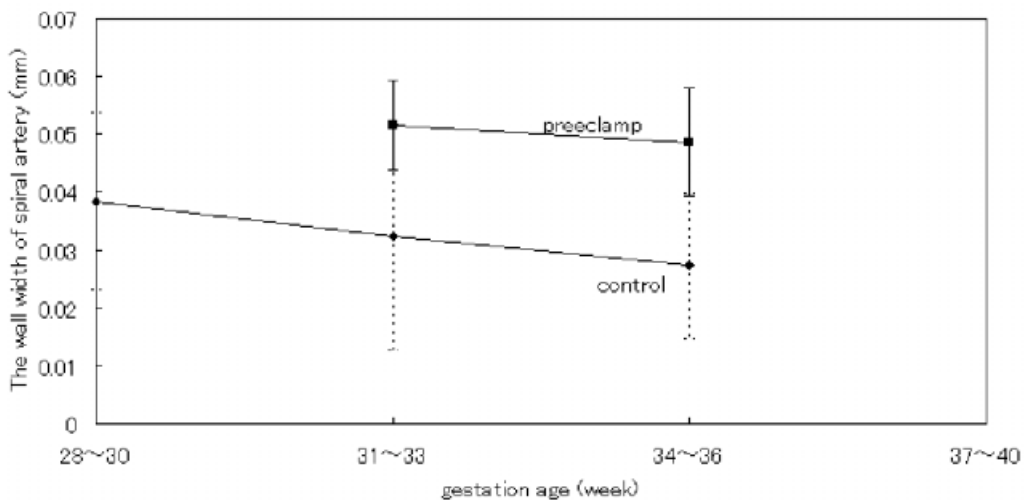


Fig. 2. The Relations of the Wall Width of Spiral Artery (SA) between Normal Pregnancy and Preeclampsia.

Immunohistochemistry:

We tried immunohistochemical studies to distinguish intermediate trophoblasts from decidual cells. Immunohistochemical staining was performed by the avidin-biotin-peroxidase complex method. Monoclonal antibodies used in this study were CAM5.2 diluted 1:80.

Table I-a: Clinical data of pregnancy without preeclampsia.

Case	Age (year)	Gestation week (week)	Placental size (cm)	Placental weight(g)	Number of cotyledon	Clinical diagnosis other than preeclampsia
<u>19-21 gestational weeks</u>						
1	26	19	11X 9	172	6	Placenta previa, PROM
2	39	20.5	13X11	230	4	Placental previa, CAM
3	23	22.1	15X12	250	16	NC
4	35	21.6	15X12	260	22	EROM, Abortion
<u>22-24 gestational weeks</u>						
5	27	22.5	16X13	330	17	NC
6	29	23	10X 9	170	6	Cervical incompetency
7	28	23.1	14X13	270	9	EROM, Myoma
8	32	23.4	13X10	270	17	NC
9	22	24.3	19X14	450	25	NC
<u>25-27 gestational weeks</u>						
10	36	25.1	16X13	350	19	Placenta previa
11	22	25.4	14X12	360	15	Cervical incompetency
12	23	25.6	15X14	230	22	Placental previa
13	35	26.6	17X15	380	13	Chorioamnionitis
14	33	27.3	16X16	445	16	Chorioamnionitis
<u>28-30 gestational weeks</u>						
15	23	29.3	13X12	330	15	Cervical incompetency
16	24	30	14X 8	410	17	NC
17	34	30	14X11	380	13	NC
18	30	30	26X16	850	3	Placenta previa
19	29	30.1	18X14	390	12	NC
20	36	30.2	18X17	470	23	EROM
<u>31-33 gestational weeks</u>						
21	21	31.5	20X15	455	18	NC
22	30	31.6	15X14	300	18	PROM, CAM
23	31	32	19X17	500	31	EROM
24	33	29	19X17	390	18	NC
25	30	33.1	16X16	425	14	NC
26	34	33.2	21X16	485	23	NC
<u>34-36 gestational weeks</u>						
27	30	34	15X15	480	18	EROM
28	31	34	16X15	540	10	Placenta previa
29	28	34.2	18X14	630	16	EROM
30	35	34.4	14X14	360	13	NC
31	26	34.5	28X21	1100	25	NC
32	34	34.5	18X14	520	13	NC
<u>37-40 gestational weeks</u>						
33	30	37	16X12	520	23	Placenta previa
34	33	37.3	21X13	545	16	NC
35	28	37.4	21X17	620	40	NC
36	26	38.1	21X16	560	39	NC

PROM=premature rupture of the membrane, EROM=early rupture of the membrane,
NC=no complication, CAM=chorioamnionitis

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Table I-b: Clinical data of pregnancy with preeclampsia.

Case	Age (year)	Gestational Week (week)	Placental Size (cm)	Placental Weight(g)	Number of Cotyledon	Clinical Daignosis Other than Toxicosis
31-33 gestational weeks						
1	32	31	15X10	390	7	-
2	30	31.6	18X14	400	32	-
3	32	31.2	13X10	380	19	-
4	30	33	23X12	460	20	-
5	35	33.5	17X13	245	16	-
34-36 gestational weeks						
6	37	34.2	14X14	370	8	-
7	34	34.2	18X16	480	32	Uterin rapture
8	22	34.3	16X13	440	19	SFD
9	30	34.3	17X13	580	6	-
10	40	34.3	17X12	430	13	-
11	25	36.5	17X14	410	16	-
12	37	36.6	13X10	250	14	IUGR

SFD=small for dates, IUGR=intrauterine growth retardation

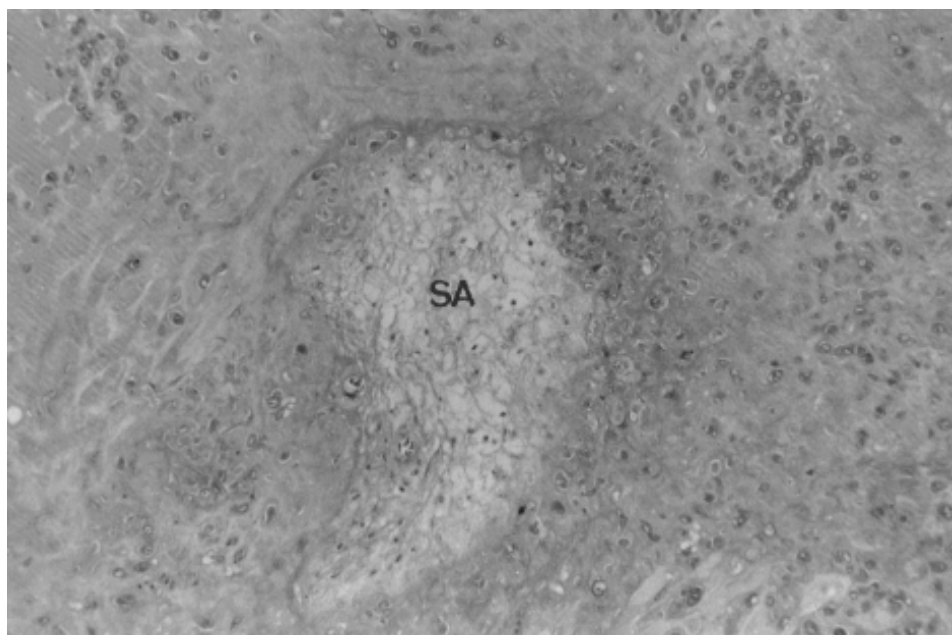


Fig. 3a. The invasion of trophoblasts, showing thinner wall of SA, 38-gestation weeks, normal pregnancy, HE×200.

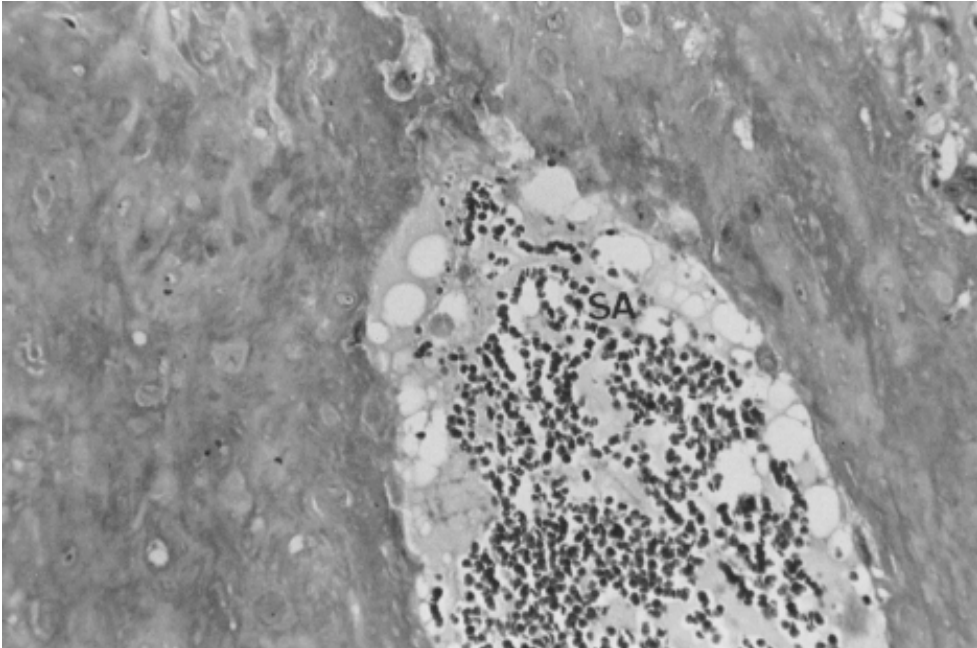


Fig. 3b. No trophoblasts invasion, showing thick wall of SA, 32-gestation weeks, normal pregnancy, HE $\times 200$.

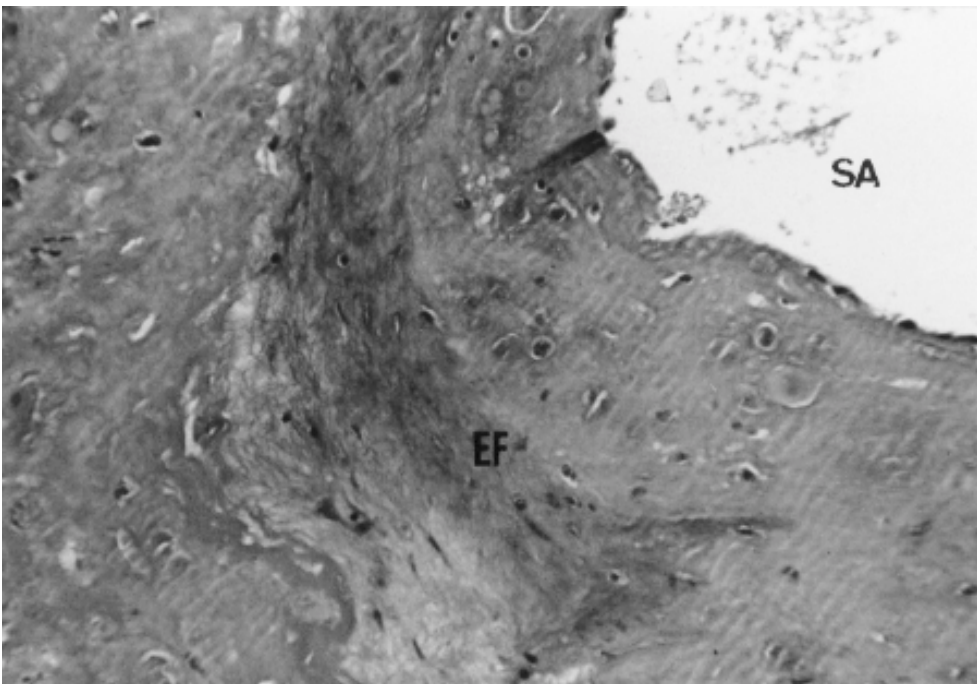


Fig. 4. In the wall of SA, there are remnants of elastic fibers with a few trophoblastic invasion. Preeclampsia, 31-gestation weeks, EF:elastic fiber Elastica van Gieson's stain, $\times 200$.

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RESULTS

Summary of statistical comparative study of SA was summarized in Table II. At the normal pregnancy, the wall of SA showed a tendency of getting thinning as pregnancy proceeding. Statistically, the wall of SA was thicker in preeclampsia than normal pregnancy ($P < 0.05$) (Fig. 2). In normal pregnancy, the wall of SA, without the invasion of trophoblasts, was thick (Fig. 3a) at 31 gestational weeks, and a SA wall with the invasion was thin at 35 gestational weeks (Fig. 3b). At normal pregnancy, elastomuscular of the SA wall were hardly seen, but SA had remnants of elastic fiber in 31 gestational weeks of preeclampsia (Fig. 4).

Electron-microscopy:

At the normal gestation, TEM of SA showed the large lumen with irregular endothelial cells and fibroblasts in the wall (Fig. 5). SA in preeclampsia showed thick wall and narrow lumen by SEM analysis (Fig. 6).

Immunohistochemistry:

Invasion by intermediate trophoblasts to SA was clearly stained by CAM5.2, at normal pregnancy (Fig. 7).

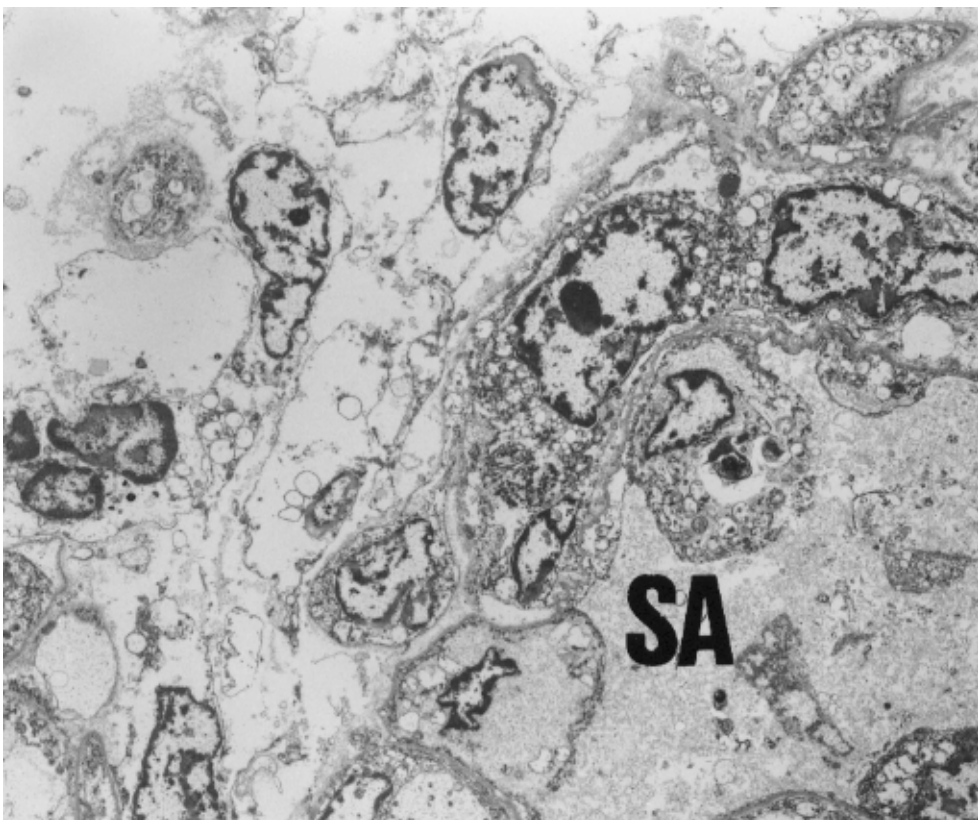


Fig. 5. Transmission electron microscopic figure of SA shows edematous wall with trophoblastic cells, without elastic fibers in normal pregnancy, 34-gestation weeks, Uranium acetate and lead nitrate stains (double stains) $\times 2000$.

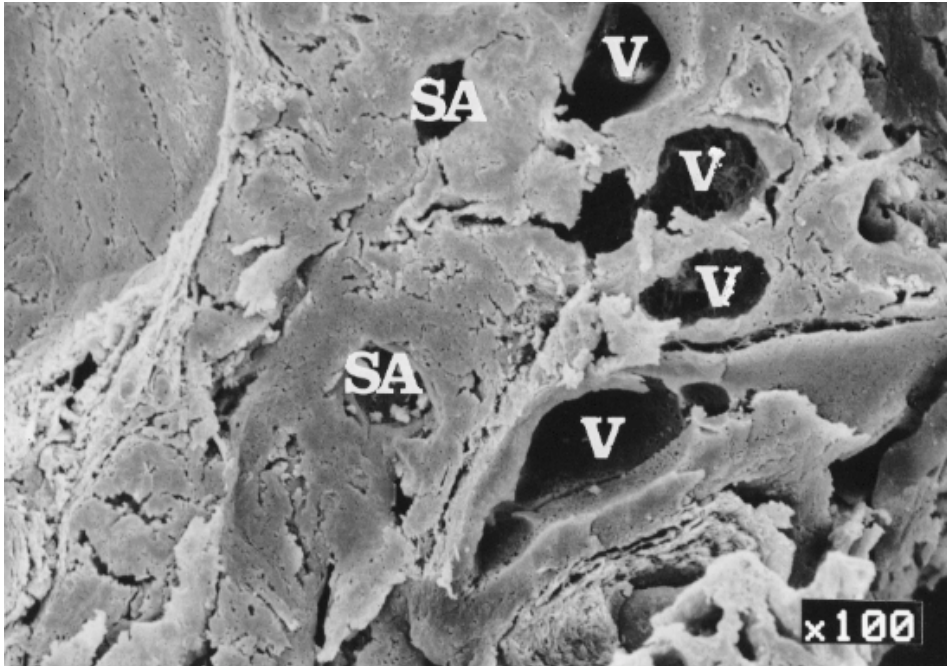


Fig. 6. Scanning electron microscopic figure reveals thick wall of SA, 34-gestation weeks, preeclampsia, V: vein, Gold ion coating, $\times 100$.

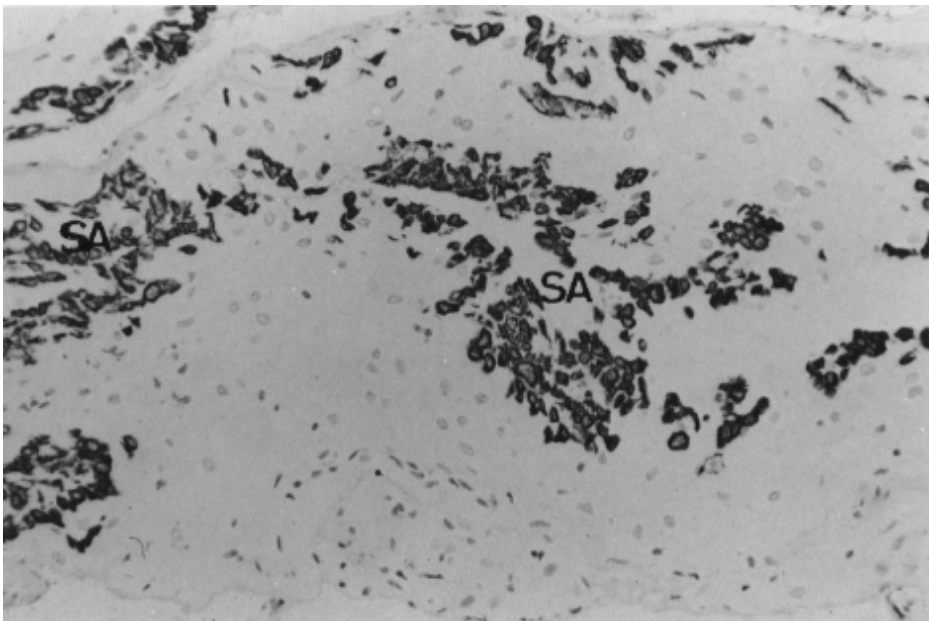


Fig. 7. Trophoblastic invasion to SA in 28-gestation weeks, normal pregnancy. CAM 5.2 stain, $\times 100$.

DISCUSSION

Placentae play an important role in the point of circulation between mothers and fetus of metabolic change, of the hormonal and of immunological ones. These mechanism have been uncertain. After implantation, syntio-trophoblastic villi are formed and invaded at 2 gestational weeks, and decidua is formed from maternal tissues. The fundamental structure of placenta is completed in the 16th week of gestation.¹⁰⁾ After 16 weeks, cyto-trophoblasts show degeneration and diminish.¹⁰⁾ Whereas, SAs are divided from the uterine artery, and are distributed in the endometrium and myometrium at the time of the non-pregnancy.³⁾ At the pregnancy, SAs, which distributed in the decidua basalis and myometrium, were vessels of 100-300 micrometer in diameter, and controls the blood circulation of placenta. We prospected that the role of trophoblast invasions and SA is important during the pregnancy and that it is necessary to examine the ultrastructural and immunohistological appearance to disclose the mechanisms of pregnancy and the cause of preeclampsia.

It is well known that SA is important vessels to maintain pregnancy, and that structures of SA are degenerated with pregnancy advanced.¹⁾ In our microscopic analysis, the wall of SA of normal pregnancy was thin and the one of preeclampsia was thick. Ultrastructural observations demonstrated that thin walls contains trophoblasts and that thick walls did not trophoblasts. This suggested that the invasion of trophoblasts were related with thinning of the wall and decrease of elasticity of SA.

Invasions of intermediate trophoblast to the decidua and SA are seen since the early pregnancy.¹⁵⁾ The discrimination of decidual cell and intermediate trophoblast is not difficult by the methods of ultrastructural observations and immunohistochemical staining of CAM 5.2 and molecular adhesion factor.^{5,8,16)} Wolf¹⁶⁾ performed the ultrastructural examinations of the decidual tissue and trophoblasts. He stated that the trophoblast retain sufficient characteristic features, which were large, irregular in outline, and have one or more nuclei and that decidual cell were round in outline and have one nuclei. The trophoblastic cells of the immunohistochemistry are positive for CAM 5.2.^{5,15)} Our ultrastructural and immunohistochemical examinations confirmed the invasion of trophoblasts of the thin wall of SA.

Table II. Statistical Comparative study of SA.

	Normal pregnancy	preeclampsia	P-value
Overall clinical data			
Age (year)			
Mean $\pm$ SD	29.6 $\pm$ 4.7	32 $\pm$ 5.1	0.141
Range	21-39	22-40	NS
Placental weight(g)			
Mean $\pm$ SD	428.5 $\pm$ 182.1	402.9 $\pm$ 91.9	0.64
Range	170-1100	245-580	NS
Width of SA			
31-33 week			
Mean $\pm$ SD	0.032 $\pm$ 0.0085	0.0052 $\pm$ 0.0077	<0.01
34-36 week			
Mean $\pm$ SD	0.027 $\pm$ 0.0071	0.048 $\pm$ 0.0093	<0.001

NS=not significant, SD=standard deviation

Brozens³⁾ described that remodeling of the SA structures and disruptions of internal elastic lamina and muscular media by fibrinoid material during the normal pregnancy, and he called these alternations 'physiological changes'. Whereas, many pathological studies of SA^{1,2,4,6,7,10)} of the complicated pregnancy have been performed, and SA of preeclampsia¹⁰⁾ indicated absence of physiological change of SA in utero-placental segment. We examined the SA in the placental segment, which did not show complete physiological changes. Most SA of placental segment were dilated and the little remnant of elastomuscular fiber existed. The remnant of elastic fiber increase resistance of SA. This discrepancy could be explained by the site. At the normal pregnancy, the dilations of SA of placental segment occur at early stage.^{8,10)} Preeclampsia occurs at late stage. We suspected that the physiological changes could not effect on the vessels of the placental segment, which had already been dilated at early stage. However, there were some SA showing thickened wall in the placental segment, compared with one of normal pregnancy. We assumed that the absence of physiological changes may occur in the placental segment.

The mechanism of preeclampsia^{3,7)} has been controversy. At preeclampsia, the blood pressure to fetus would decrease, and the narrowing of the lumen by absence of physiological changes induces sufficient blood supply as a compensatory mechanism. We considered trophoblast invasions to the vessels play an important role of controls of blood flow, and the entity of preeclampsia.

The controls of the trophoblast invasion to the wall of SA may contribute to the therapy and prophylaxis of preeclampsia. In additions, trophoblast invasions are related to various cytokine and migration factors, and the further investigations of these factors would be necessary.

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