Messages from the placentae across multiple species: A 50 years exploration

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A B S T R A C T

This review explores eight aspects of placentation in multiple mammalian.
  1) Specialities of gestational trophoblastic disease.
  2) Clinical significance of single umbilical artery (SUA) syndrome.
  3) Pulmonary trophoblast embolism in pregnant chinchillas and DIC in pregnant giant panda.
  4) Genetics status and placental behaviors during Japanese serow and related antelopes.
  5) Specific living style and placentation of the Sloth and Proboscis monkey.
  6) Similarities of placental structures between human and great apes.
  7) Similarities of placental forms in elephants, manatees and rock hyrax with different living styles.
  8) Specialities of placental pathology in Himalayan mountain people.
Conclusions: It was taught that every mammalian species held on placental forms applied to different environmental life for their infants, even though their gestational lengths were different.

1. Introduction

Last October, *Scientific American* published a special issue about a baby's first organ, the placenta [1]. It is full of surprises and amazing science. A history of placental studies was given by Douglas Haynes in 1983 [2]. The term placenta was first used in English writings in 1677, and a depiction of the placenta was shown on ancient Egyptian royal sculpture, dating from 3400 B.C.

In Japan, an exhibition of prehistoric art from 10,000 years ago, during the Jomon period, has just been held at Tokyo National Museum [3]. Japanese people living in this period continued their independent life style in the Jomon national environment [4]. They carved "Dogu" giving form and beauty for prayers, and they prayed for safe childbirth. After the birth, they carefully handled the delivered placenta, putting it into a pot called "Umegame"; thereafter it was buried under the floor and many people tramped over the "Umegame" to ensure the baby's healthy future. An exploration of placenta morphology and pathology diverse species is presented with emphasis our species rarely dismissed and on pathology of significant to both mother and embryo/fetus.

2. Postwar increase of gestational trophoblastic disease in Japan

After the Second World War, we had many patients suffering from gestational trophoblastic disease, hydatidiform mole and choriocarcinoma, at Tokyo Medical School Hospital. The prognosis of metastatic choriocarcinoma patients was extremely poor, because of lack of effective chemotherapeutic agents. In 1959, I was fortunate to receive an invitation from Prof. Kurt Benirschke at the Boston Lying-in Hospital. Before that, I had written to Prof. Arthur T. Hertig, Chairman of Pathology, Harvard Medical School, asking to study human trophoblast. He passed my letter to Prof. Benirschke. When I first met him at the Lying-in Hospital, he showed me a cut section of umbilical cord and asked me "How many vessels are within the cord?" Surprisingly, there were just two vessels with a single umbilical artery (SUA). My research on SUA started then and has continued until now. I will show you my report on SUA cases later.

While studying human trophoblast in tissue culture at Harvard [5], I received an invitation from Dr. Roy Hertz, NIH in Bethesda, to deliver a lecture on diagnosis and chemotherapy of metastatic choriocarcinoma [6]. After the lecture, I was asked to see patients with trophoblastic disease who received chemotherapy with MTX and was amazed to see patients with good effect. Dr. Roy Hertz kindly provided me some MTX regimens. After this, many Japanese patients with trophoblastic disease were helped.

Gestational trophoblastic diseases (GTD) have a wide spectrum including hydatidiform mole, invasive mole and choriocarcinoma. In addition, GTD-related diseases are classified as placental site trophoblastic tumor (PSTT) and epithelioid trophoblastic tumor (ETT). PSTT occurs most commonly after normal pregnancy or abortion, and is uncommon after hydatidiform mole. The immunohistochemical distribution of hPL and hCG in PSTT shows a close resemblance to intermediate trophoblastic cells.

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Fig. 1. SEM findings of umbilical cord with SUA.

2.1. Placental site trophoblastic tumor (PSTT)

Case: A 43-year-old Japanese woman presented without any pregnancy history and had undergone salpingectomy on the right side due to hydrosalpinx 10 years previously. She complained of severe genital bleeding from a cervical myoma polyp [7].

On partial resection of the polyp, bleeding became more severe. The tumor polyp was diagnosed as PSTT.

Tumor cells consisted of prominent cytotrophoblasts resembling intermediate trophoblast cells with relatively larger nuclei and clear cytoplasm, as well as rare mitoses in the removed polypoid tissue, which stained positively for SP1 as well as hPL and inhibin-α. In addition, cytotrophoblast-like cells resembling intermediate trophoblasts stained well for hPL and cytokeratin.

2.2. Ultrastructural features of epithelioid trophoblastic tumor (ETT)

Case (Hiroshima University) [8]: A 38-year-old woman suffered from complete hydatidiform mole (CHM) at the age of 27 following four full-term deliveries from 24 to 37 years old. In addition, when CHM was diagnosed 11 years ago, she had undergone chemotherapy with MTX. This time, she was diagnosed with thoracoscopy as metastatic pulmonary choriocarcinoma.

Tumor lesions were composed histologically of trophoblastic cell masses. In immuno-histochemistry, tumor lesions were composed of a trophoblastic cell mass which was positive for hPL, CK7, SP1 and inhibin-α. Electron microscopy showed tumor cells consisting of mononuclear cells and binuclear cells with abundant cytoplasm. Tumor cells were polygonal with large nuclei being surrounded by bundles of intermediate-type filaments and desmosomes. As a result, this tumor was diagnosed as ETT in the lung.

3. Placental lesions associated with SUA

The incidence of SUA (Fig. 1) is slightly less than 1%, but SUA frequently is associated with other congenital malformations. SUA syndrome with absence of one cord artery is related to a 10- to 20-fold increase in the incidence of congenital malformations. Since Benirschke and Brown [9] emphasized that SUA was associated with increased incidence of congenital anomalies, many studies concerning the incidence, pathogenesis and clinical significance of SUA have been reported. Findings from pathologic reports of 7057 placentas at Tokyo Medical University Hospital from 1979 show 34 with SUA infants (0.48%) in which perinatal mortality and fetal anomalies occurred in 14 (41.1%) [10]. In addition, Saitama Medical University Hospital from 2009 to 2017 reported 46 infants with SUA (0.87%), of which 11 were intrauterine fetal deaths or abortions (23.9%) and 11 infants had chromosomal anomalies including 18-trisomy (9), 13-trisomy (1) and 21-trisomy (1). Twins included were 6 (13.0%) (Table 1) [11].

Case SP213-222: A 27-year-old woman, para 1, was delivered of a female infant weighing 1675 g suffering from 18-trisomy, aortic stenosis and ventricular septal defect at 37 weeks of gestation (Fig. 2). The cord[A)] was 45 cm long with SUA. The placenta weighed 235 g with an accessory lobe. Histology of the chorionic villi showed marked chorangiosis[B)]. At the ultrastructural level, enlarged intravillous capillaries connected with each other in the villous stroma[C)]. Several lamellar bodies were found within the trophoblast layer[D)]. (Fig. 2)

Case SP216-22: A 33-year-old woman, para 1, was delivered of a female stillborn infant weighing 1782 g at 34 weeks of gestation. The umbilical cord was brownish and 24 cm long with SUA. The placenta weighed 200 g (Fig. 3a). Histologically, multiple intravillous capillaries were observed as chorangiosis (Fig. 3b). By SEM, two cord vessels were filled with clots and intraluminal canals were occupied with a fibrin net as well as confetti-like erythrocytes. TEM study of the chorionic villi showed circular hemosiderin deposits along the subtrophoblast layer (Fig. 3c). These iron deposits were demonstrated using Energy Dispersion X-Ray Spectrometry (EDS).(Fig. 3d)

Case SP217-606: A 40-year old woman, para 0, was artificially aborted of a female fetus weighing 140 g suffering from 18-trisomy. The cord was 17 cm long, short and slender, with SUA. The placenta weighed 90 g. Marginal hemorrhages were distinct. At the ultrastructural level, marked hemosiderin deposit lines were distinct under the trophoblast layer. By use of EDS mapping, the circular deposits lines were shown to be iron.

4. Pulmonary trophoblast embolism in a pregnant chinchilla

During human pregnancy, syncytial sprouts detach from the placenta, migrate via the maternal circulation and get into the pulmonary circulation as trophoblast embolisms (Fig. 4a).

The chinchilla (*Chinchilla lanigera*) is a rodent that dwells in the high Andes of South America. This animal has fur that is possibly the thickest, softest and warmest of any animals.
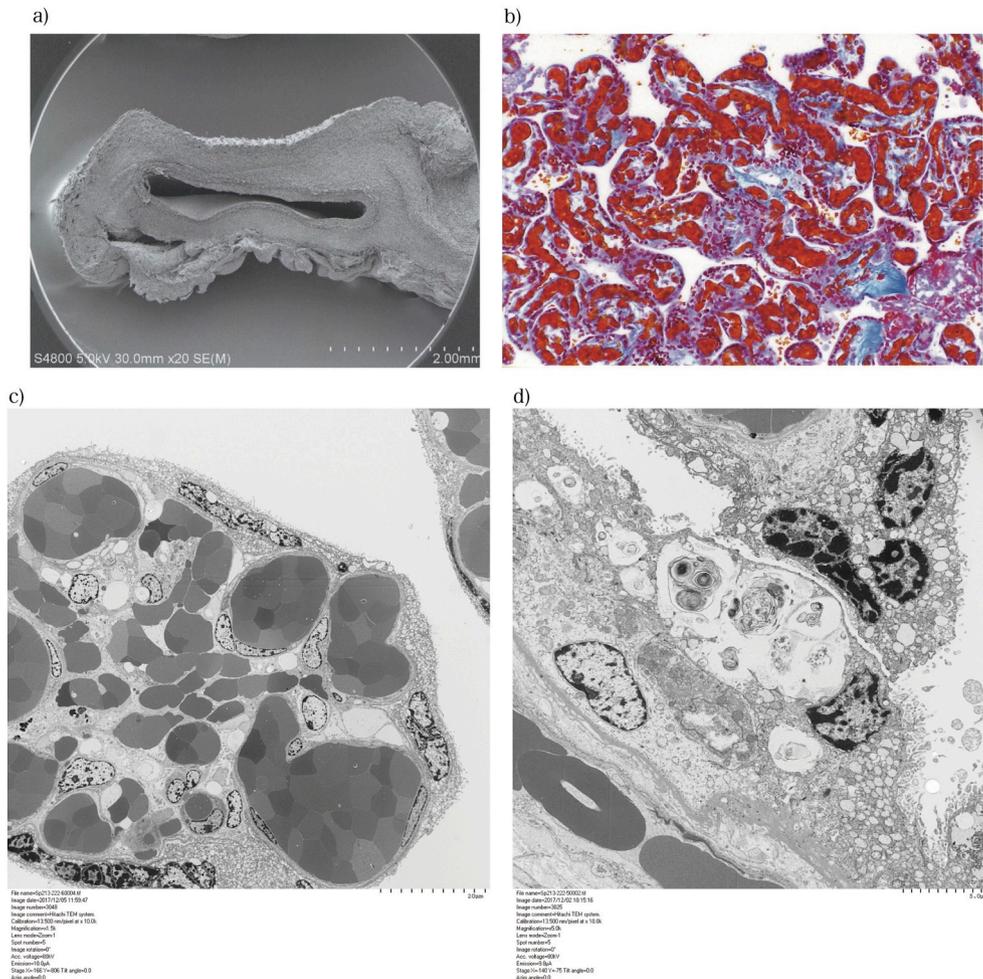
Table 1

Authors	Total number of SUA cases	Incidence of SUA	Perinatal mortality	Anomalies	Remarks
Soma & Yoshida(1979) (Tokyo Med.Univ)	34	34/7057 placentas (0.48%)	14/34 (41.1%)	14/34 (41.1%)	4 twins
Soma et al (2017) (2009–2017) (Saitama Med.Univ)	46	46/5267 placentas (0.87%)	11/46 (23.9%)	Chromsomal anomalies 18-trisomy … 9 13-trisomy … 1 21-trisomy … 1 11/46(23.9%)	6 twins

Fig. 2. Case SP213-222:A 27-year-old woman, para 1, was delivered of a female infant weighing 1675 g suffering from 18- trisomy, aortic stenosis and ventricular septal defect at 37 weeks of gestation. The cord was 45 cm long with SUA (Fig. 2a). The placenta weighed 235 g with an accessory lobe. Histology of the chorionic villi showed marked chorangiosis(b). At the ultrastructural level, enlarged intravillous capillaries connected with each other in the villous stroma(c). Several lamellar bodies were found within the trophoblast layer(d).

Billington and Weir [12] have published a report of deportation of trophoblast in the pregnant *Chinchilla laniger*. The chromosome number of chinchilla is 2n = 64 with a large X-chromosome.(Fig. 4b) The gestational length of chinchilla is about 110 days. She delivers 1-3 sibs. The placenta is of the discoid and hemochorial type (Fig. 4c and d). Giant cell embolism can be seen in the lungs of pregnant chinchilla (Fig. 5a). On placental histology, the utero-placental interface consists of a layer of clear swollen, giant cells and a dense labyrinth layer (Fig. 4d). In ultrastructures, giant cells with large nuclei and well developed rER were observed (Fig. 4e). They look like those of human X cells in the basal plate [13]. In tissue culture of the giant cell layer, swollen secretory cell growth was seen (Fig. 5d). Giant cells were seen in the pulmonary field of pregnant chinchilla. The giant cells of placenta, uterus and lung were measured. The pulmonary giant cells were found to be larger, but the cytogenesis of these giant cells was not clear.

5. Disseminated intravascular coagulation (DIC) in a pregnant giant panda

The gestational period of female giant panda ranged from 122 to 163 days at the Peking Zoo [14]. However, until now, no description of giant panda placenta has been recorded.

A female giant panda (*Ailuropoda melanoleuca*) in Ueno Zoo, Tokyo, named Lan Lan, aged 10 years and weighing 100 kg, had a poor appetite and suddenly collapsed with convulsions. She had bleeding from the mouth and anuria. Although she received many medications, she died after repeated convulsions and vomiting. Main autopsy findings showed acute hemorrhagic gastritis, acute pancreatitis, chronic nephritis with thrombosis and hyaline degeneration. Acute necrosis and thrombosis as well as atherosis were found in the liver. In addition, there was a uterus duplex with hemorrhagic infusion containing a dead infant weighing 42.6 g with a discoid placenta (3.8 × 1.5 × 0.7 cm) (Fig. 6a). Placental lesions (Fig. 6b) and marked hemorrhage and thrombosis (Fig. 6c and d) were observed at the utero-placental junction. In summary, it is suspected that DIC induced by placental damage and inflammation of the stomach and pancreas may have been the main cause of the panda's death, even though coagulation studies were not performed.

6. Placentation of the Japanese serow (*Capricornis crispus*)

The Japanese serow, now protected by law as an endangered species, is thought to be a relict species on the island of Japan (Fig. 7a). There are two groups of serows. Sumatran serows are scattered through the Himalayan region and Southeast Asia. The Formosan serow, native to Taiwan, is smaller than the Japanese serow, but the chromosomes of both have a similar diploid number of 2n = 50 (Fig. 7b). The Sumatran serows have a diploid number of 2n = 48 [15,16]. Finally, there are the related gorals (*Nemorhaedus* spp.), widely distributed from the Himalayas in China through Korea to Siberia and in North Korea; The latter
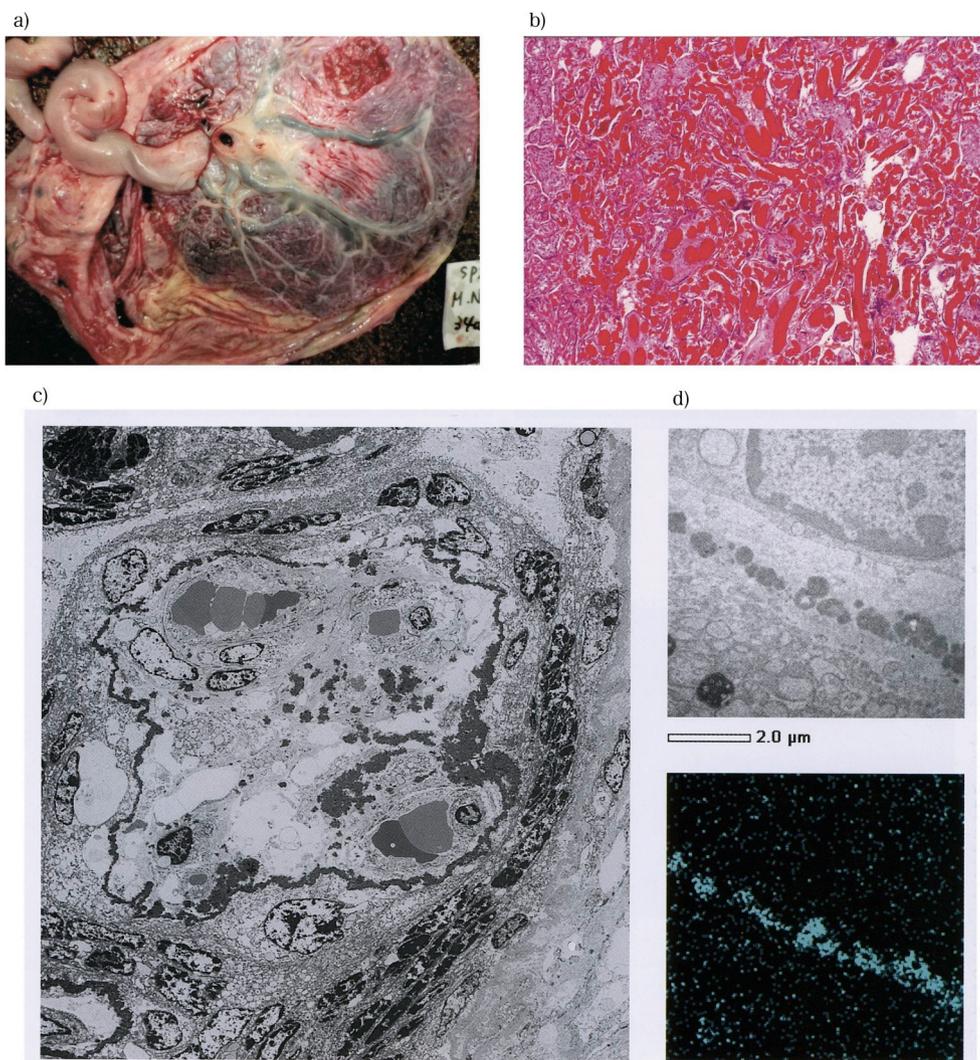
Fig. 3. Case SP216-22: A 33-year-old woman, para 1 was delivered of a female stillborn infant weighing 1782 g at 34weeks of gestation. The umbilical cord was brownish and 24 cm long with SUA. The placenta weighed 200 g(a). Histologically, multiple intravillous capillaries were observed as chorangiosis(b). By SEM, two cord vessels were filled with clots and intraluminal canals were occupied with a fibrin-net as well as confetti-like erythrocytes. TEM study of the chorionic villi showed circular hemosiderin deposits along the subtrophoblast layer (c,d).

(*N. caudatus*) is commonly called a Korean serow. These gorals possessed a diploid number of 2n = 56 which is different from that of the Japanese serow. The gestational length of Japanese serow is estimated at about 212 days and the sib weighing 760 g is situated in the left horn of the uterus. The placental structure is syndesmochorial with about 60–90 cotyledons (Fig. 7c,d,e). There are 4 umbilical cord vessels and around the cord insertion metaplasia is shown. The number of cotyledons in Japanese serow is greater than the 37-50 lobes in chamois and rocky mountain goat. A comparison of placentation between *Capricornis* species and other antelopes was given.

7. Placentation of a sloth

Sloths are the most completely arboreal of all South American forest animals [17](Fig. 8a). They eat, sleep, mate and give birth while hanging upside-down from branches, suspended from powerful hook-like claws on each foot. Sloths, armadillos and anteaters belong to a group of mammals now called Xenarthra. The estimated gestational length in sloths is over 200 days due to delayed implantation. During delivery, the pregnant sloth climbs down to ground to give a birth.

Sloths include the 3-toed sloth with 9 cervical spines, 2-toed sloth with 7 cervical spines and Hoffman's sloth with 6 cervical spines. About 90 years ago, the placental structures of the sloths were reported by Wislocki [18]. We examined a placenta (*Choloepus hoffmanni*) weighing 120 g with a gingko leaf-like form and 9 lobes, 20 × 16 × 1 cm (Fig. 8b). The umbilical cord was 9.5 cm long with 3 vessels. Placental structures consisted of blood spaces surrounded by large clear endothelial cells, and a swollen trophoblast layer (Fig. 8c). At the ultrastructural level, endothelial cells had large nuclei and clear cytoplasm (Fig. 8d). However, release of placental hormone from these cells has not been clarified.

8. Placentation of leaf-eating monkeys

Proboscis monkeys (*Nasalis larvatus*) are found mainly in coastal swamp forests and alongside the rivers of Borneo (Fig. 9a). They belong to the leaf-eating monkey family (Colobinae) and are close relatives of the langurs (e.g. *Presbytis* spp.). Leaf-eating monkeys range from the high altitude of the Himalayas through the dry zone of South India to the rainforests of South Asia. However, the proboscis monkey is a large
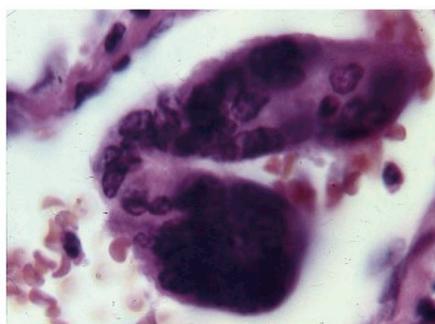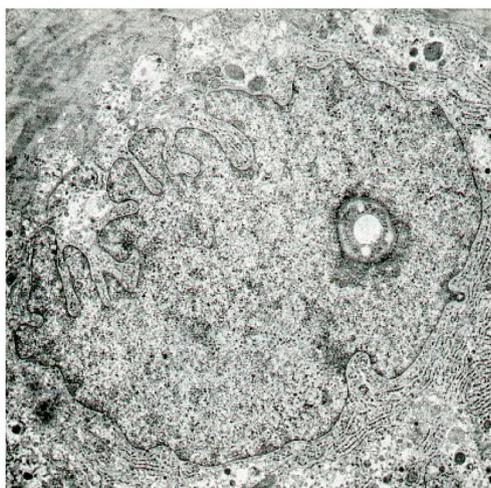
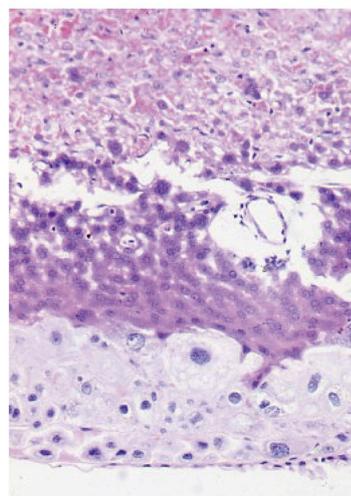a : Trophoblast embolism in the human pregnant lung
(5 months)   (x900)
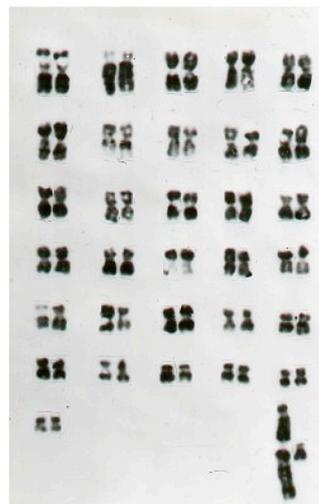
b : Chromosome of Chinchilla 2n=64

c : Chinchilla`s fetus and placenta

e : Giant cells with large nuclei in ultrastructure
(x1000)

d : Placental structural layer includes giant cells   (x200)

Fig. 4. a:Trophoblast embolism in the human pregnant lung (5 months) (x900). b: Chromosome of Chinchilla 2n = 64. c:Chinchilla's fetus and placenta. d: Placental structural layer includes giant cells (x200). e:Giant cells with large nuclei in ultrastructure (x1000).

and remarkable animal and unlikely to be confused with any other leaf-eating monkey. We investigated the karyotype of the proboscis monkey and found a diploid number of 2n = 48 [19], which is dissimilar from that of *Presbytis* (2n = 44) [20](Fig. 9b).

The first successful captive breeding of a proboscis monkey was reported from San Diego Zoo with an estimated gestation of about 166 days. A term placenta of proboscis monkey obtained from the Japan Monkey Center was bidiscoid in form and weighed 145 g [21](Fig. 9c). The umbilical cord was 19 cm long with 3 vessels. Fraternal twins of proboscis monkey were born at the Japan Monkey Center (Table 2). One twin survived, but the other twin died 2 days after birth. The bidiscoid placenta showed dichorionic-diamniotic fusion and weighed 130 g. Marginal infarcts were seen. Although the chorionic plate of *Nasalis* was thick, the subchorionic state was poor. Crowded terminal
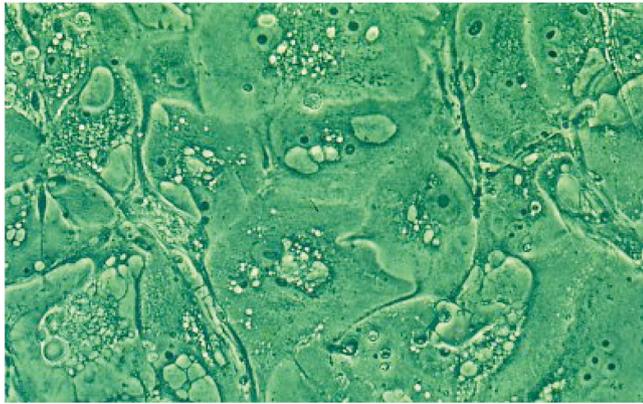
a: In tissue culture of giant cell, swollen secretory cell growth was seen. (x2000)
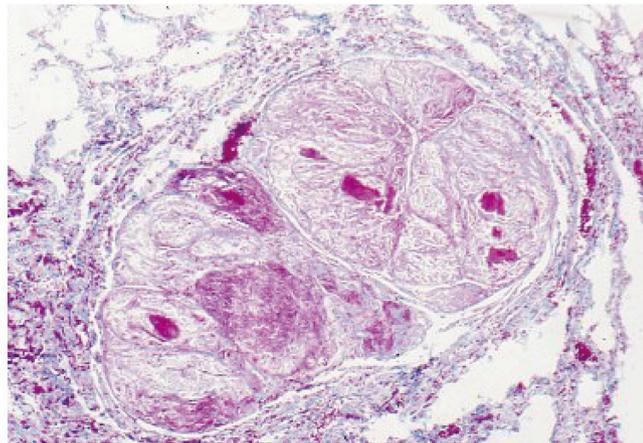
b: Giant cells in the Chinchilla`s lung （Mallory stain x100）

Fig. 5. a: In tissue culture of giant cell, swollen secretory cell growth was seen. (x2000). b: Giant cells in the Chinchilla's lung (Mallory stain x100).

villi were observed (Fig. 9d). Syncytial bridges and fusion as well as fibrinoid necrosis of the villi were seen.

The placenta of Hanuman langur (*Semnopithecus entellus*) obtained at San Diego Zoo was bidiscoid in form and weighed 171 g [21]. In the subchorionic plate of the Hanuman langur placenta, infarction and laminated intervillous thrombosis were evident. A female dusky leaf monkey (*Trachypithecus obscurus*) died at Yokohama Zoo. A dead fetus weighing 165 g was found in the uterus. The gestational length of dusky leaf monkey is thought to be approximately 160 days. The placenta was bidiscoid and weighed 45 g. The umbilical cord was 17 cm long. The secondary disc presented circumvallation and marked intervillous thrombosis (Table 2).

Nuclear DNA content of trophoblast of the placenta of proboscis monkey and *Presbytis* monkeys was measured using a spectrophotometer. The nuclear DNA content of trophoblast of both leaf-eating monkeys had a model number of 3n-4n, being similar in that respect, but the mode of nuclear DNA content in proboscis monkey is different from that of Japanese monkey, patas monkey, spider monkey and great apes.

9. Hydatidifom mole in a pregnant chimpanzee

According to published reports on the placental structure of the great apes, the gorilla, the chimpanzee and the orangutan, anthropoid placenta is similar to human placenta (Fig. 10). Therefore, placental examination of the great apes might provide clear-cut answers for investigation of causes of fetal death, prematurity and infection [22–24]. The average birthweight of 14 chimpanzee neonates and the mean weight of 26 chimpanzee placentas (excluding two twin placentas) were 1748 ± 306 g and 290.3 ± 60.8 g, respectively, while the average birthweights of small-for-date (SFD) (N = 171) and premature infants (N = 306) were 2179 ± 349 g and 1973 ± 908 g, respectively. Average placental weights for SFD and premature infants were 367 ± 103 g and 336 ± 158 g respectively. Thus, the average placental/fetal weight ratio in chimpanzee of 0.165 more closely approximates that of SFD and premature human infant groups than that of
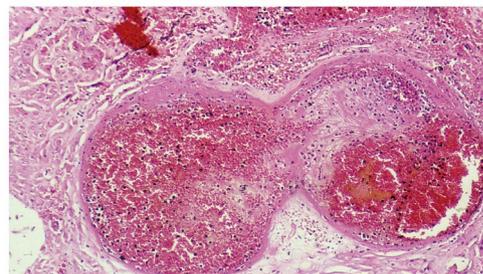

a: Stillborn fetus of Giant panda escaped away in uterine wall

b: Diffuse placental infarct displayed cut section of the uterus

c : Large hematoma and inflammation from cut section of utero-placental junction　(HE stain x100)

d : Large hematoma and infiltration in the placenral basal plate (HE stain x 100)

Fig. 6. a: Stillborn fetus of Giant panda escaped away in uterine wall. b: Diffuse placental infarct displayed cut section of the uterus. c: Large hematoma and inflammation from cut section of utero-placental junction (HE stain x100). d: Large hematoma and infiltration in the placental basal plate (HE stain x100).
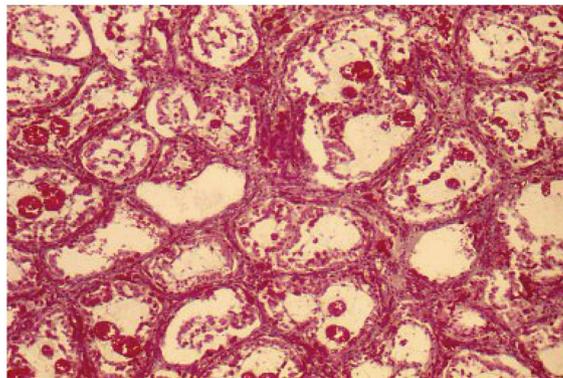
a : Japanese female serows and sibling

b : Chromosome karyotypes of Japanese serows
2n=50   above male below female

c : Placentation of Japanese serow(fetal side)

d : Placentation of Japanese serow(Maternal side)

e : Cotyledonary villous structures of Japanese serow placenta
show up diploid nuclei (Mallory stain x200)

Fig. 7. a:Japanese female serows and sibling. b:Chromosome karyotypes of Japanese serows 2n = 50 above male below female. c: Placentation of Japanese serow (fetal side). d: Placentation of Japanese serow (Maternal side). e: Cotyledonary villous structures of Japanese serow placenta show up diploid nuclei (Mallory stain x200).

combined or severe toxemic groups. The ultrastructure of chorionic terminal villi between orang-utan and chimpanzee placentas seems to be similar (Fig. 10).

Hydatidiform moles occur not infrequently in pregnant women. Although placental structures of the great apes are very similar to those of the human placenta, the occurrence of hydatidiform mole in non-human primates has not been documented.

Hydatidiform moles occur as complete or partial entities, which differ in morphology and cytogenetics. Complete hydatidiform moles (CHM) present rapidly progressing villous hydropic swelling affecting the placenta as a whole, with trophoblastic hyperplasia and absence of an embryo/fetus. Partial moles (PHM), however, exhibit only focal villous swelling with fetal trophoblastic hyperplasia with the presence of a fetus. In humans, the karyotype of CHM consists of 46 XX

chromosomes, all of parental origin (androgenesis). The karyotype of PHM can show different patterns.

Case: A 30-year-old female chimpanzee, para 10, stillbirth 1, died at the Amsterdam Zoo and was presented for necropsy [25]. The chimpanzee was believed to be pregnant because of 4 months' amenorrhea. From the end of November, she had lost appetite, and a swollen belly was noted (Fig. 11). Two months later, she had massive genital bleeding and aborted. Within two days she died. A dead female fetus, weighing 68 g, was found in the uterus (Fig. 11a). The fetus was connected to the placenta by a 20 cm long umbilical cord. The placenta attached to the uterine wall included molar vesicles containing yellowish clear fluid (Fig. 11b). By histological examination of placental tissue, villi with hydropic swelling and cistern formation were noted (Fig. 11c). In addition to the hydropic villi attached to the uterus, chorionic villi
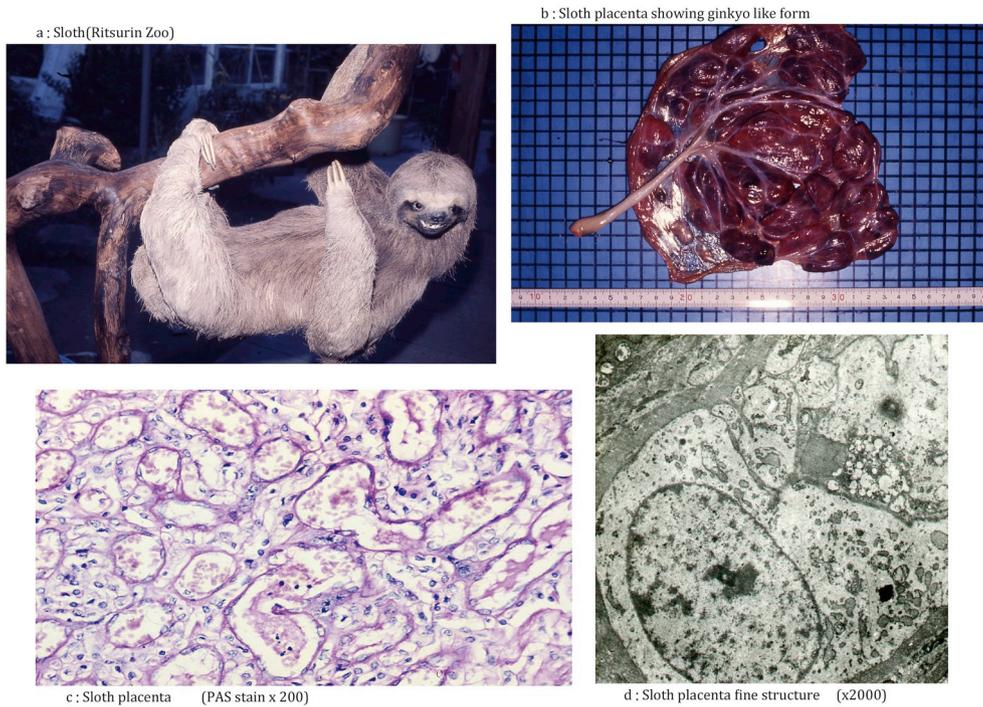
a : Sloth(Ritsurin Zoo)

b : Sloth placenta showing ginkyo like form

c : Sloth placenta   (PAS stain x 200)

d : Sloth placenta fine structure   (x2000)

Fig. 8. a: Sloth (Ritsurin Zoo). b: Sloth placenta showing ginkyo like form. c: Sloth placenta (PAS stain x 200). d: Sloth placenta fine structure (x2000).

a : Proboscis monkey and infant(San Diego Zoo)

b : Chromosome of proboscis monkey
2n=48

c : Bilobed placental structures of proboscis monkey

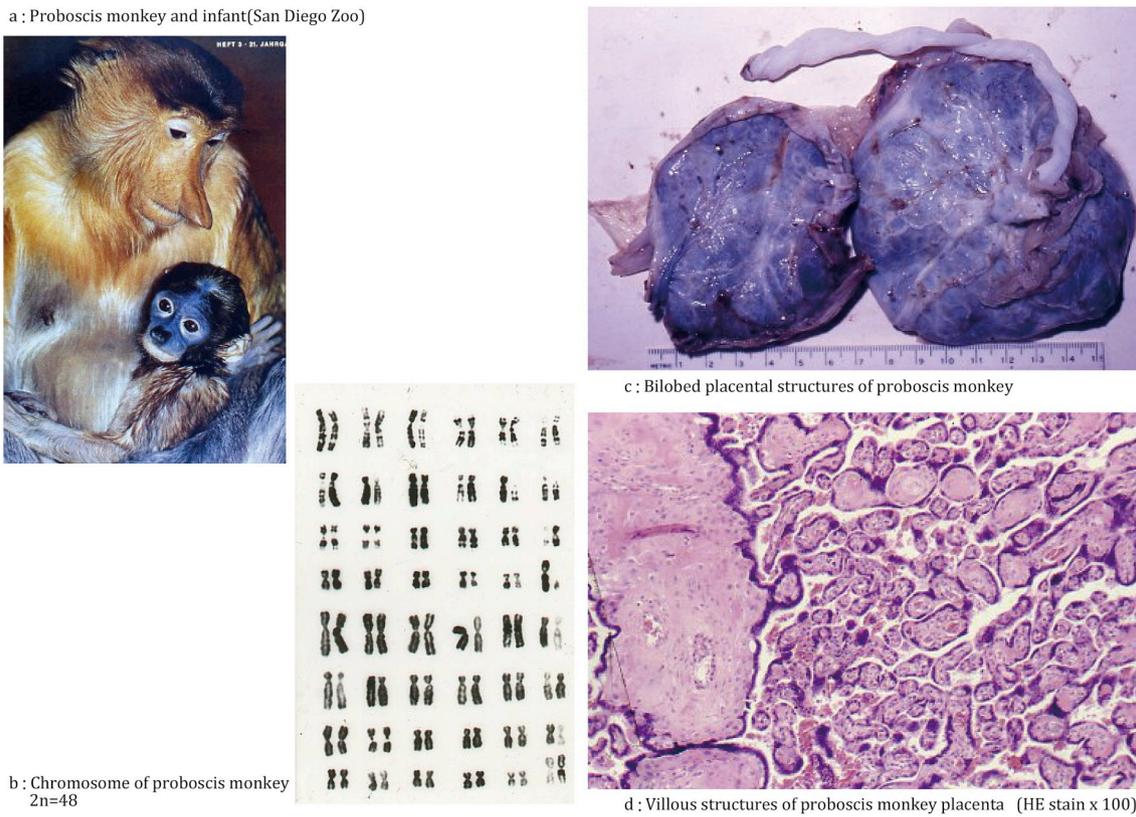d : Villous structures of proboscis monkey placenta   (HE stain x 100)

Fig. 9. a: Proboscis monkey and infant (San Diego Zoo). b: Chromosome of proboscis monkey 2n = 48. c: Bilobed placental structures of proboscis monkey. d:Villous structures of proboscis monkey placenta(HE stain x 100).

Table 2
A Comparison Between Placentas of Proboscis monkey and Presbytis monkey.

Species Zoo	Date obtained samples Chromosome	Fetus Sex B.W.	Cord Length Short Distance No. of vessels	Membrane Connection between P.&S. (distance)	Weight Form	Placenta Primary lobe Dimension	Secondary lobe Dimension
Proboscis monkey (*Nasalis larvatus*) JMC	Nov.18,1978 2n = 48 (Soma, 1974)	Male unknown Living	19cm4cm(3)	Incomplete 4 (2 cm)	145g bidiscoid	Decidual necrosis 10x9x1cm 90g	Decidual necrosis 7x7.5 × 1 cm 55g
Proboscis monkey JMC	Oct.2,1980	unknown	Unknown 2.5 cm (3)	Complete 4 (7 cm)	35g bidiscoid	Lobulated (8) 8 × 8 × 0.7 cm (25 g) Marginal infarcts 1.4 × 0.7 cm Maternal floor infarcts 2 × 2cm 1 × 0.6 × 1cm	Lobulated (6) Thrombosis 7.5 × 5.5 × 0.7 cm (10 g) infarct 1x0.4 × 0.7 cm
Proboscis monkey JMC	April 2, 1982 6.21.1977 born) fixed in formalin solution	Fraternal twin one: living other: died 2 days after birth	Unknown A:3 cm B:2.5 cm	Incomplete DiDi fused	130 g bidiscoid	Lobulated (9) 7 × 10.5 × 1 cm Infarcts: 1.5 × 0.4 × 0.5 cm 1 × 0.7x0.5 cm 0.4 × 0.2 × 0.5 cm	5x9x1.3 cm Marginal infarcts 1x0.7x0.5 cm 0.5x0.5x0.5 cm
Dusky Luton (*Trachypithecus obscurus*) Yokohama Zoo	Jan.21,1980 Maternal death 2n = 44 (Chiarelli, 1963)	Male 165 g CH 14.5 cm Death in utero	17 cm 2 cm (3)	Complete 4 (7 cm)	45 g bidiscoid	Thrombosis 0.4 × 0.4 cm 5.5x5.5 × 1cm	Circumvallate Thrombosis 0.2x0.2 cm 5.0x5.0 × 1cm
Hanuman langur (*Semnopithecus entellus*) San Diego Zoo	March 29, 1977 2n = 44 (Chiarelli, 1963)	Male Living	25 cm 2.0 cm (3)	Complete 2 (10 cm)	171 g bidiscoid	Lobulated (5) Thrombosis 1.4x1x0.7 cm laminated 9.5x9.5x1.5cm 90g	Lobulated (4) Subchorionic fibrin 9.5x7.5x1.4 cm 81g

invaded the myometrium. The case was diagnosed as an invasive mole comparable with invasive mole in the human uterus. By immunohistochemical studies, elevated hCG level was 1350 mIU/ml and elevated SP1 level was 1000 ng/ml. Both proteins were localized in the syncytiotrophoblast (Fig. 11d). Flow cytometric DNA analysis of isolated nuclei showed an aneuploid pattern.

10. Comparison of placental structures of Sirenia, Proboscidea and Hyracoidea

According to current phylogenetics, the Sirenia (manatee and dugong) and Proboscidea (elephants) as well as Hyracoidea (hyraxes) share a common ancestor dating back to 45 million years ago, even though they live in quite different environments (sea, plain and rock) and have quite different body forms.

The African elephant (*Loxodonta africana*) usually gives birth to a single young after a long gestation period of 22 months. At Gunma Safari World Zoo, Japan, a female African elephant gave birth to a male baby weighing 120 kg after prolonged labor [26]. One year later, another female African elephant at the same zoo gave birth to a female baby weighing 80 kg. Their gestational ages were about 2 years and 629 days, respectively. Their placentas weighed 13 kg and 10.5 kg (Fig. 12a and b). The umbilical cords with marginal insertion measured 170 cm and 65 cm long and had 3 blood vessels. On the fetal surface of the placenta, multiple allantoic pustules and pendulous polyps were observed. Histology of both placentas showed a labyrinth of the endotheliochorial type similar to that of the manatee (Fig. 12c). Ultrastructurally, electron-dense bodies were visualized beneath the trophoblast layer. (Fig. 12d) There are thought to be hemosiderin crystals or iron deposits arising from extravasation in the marginal hemophagous regions.

Two manatee placentas (*Trichecus manatus*) were obtained from the Okinawa Churaumi Aquarium [27]. A stillborn manatee placenta weighed 460 g and one born at 16 months gestation weighed 4.43 kg. These placentas had allantoic verrucoids on the surface of the chorionic plate. Histologically, the labyrinth was endotheliochorial (Fig. 13a and b). There were arcades of columnar trophoblast beneath the chorionic plate, enclosing spaces into which maternal blood escaped. In the basal part of the labyrinth, a syncytial trophoblast layer formed a stratified cavity facing the uterine endometrium.

The rock hyrax (*Procavia capensis*) frequents rocky regions in Africa and the Near East (Fig. 14a). Though resembling a rodent superficially, its taxonomic position is close to the Proboscidea. The gestational period of the hyrax is estimated to be 7 months. The hyrax placenta has a zonary form with resemblance to that of the elephant [28] (Fig. 14b). The histology of the placenta presents columnar labyrinth as seen in the manatee placenta. In addition, the chromosome number of the rock hyrax was found to be 2n = 54 [29] (Fig. 14c and d).

11. Pathological lesions of Himalayan placentas and ultrastructural findings of Nepalese placental villi

The placenta is an important functional unit for gas transfer between mother and fetus. The placental membrane, consisting of trophoblast interposed between maternal and fetal blood, plays an active role for respiration, although no morphological evidence has been documented. Nepal is located in the highest mountains of the world, the Himalayas. The vast majority of Nepal's population lives in small rural settlements, where a chronic shortage of essential health care and low socio-economic status, in addition to rough highways, result in a lack of prenatal care for pregnant women, thereby causing high perinatal mortality and maternal mortality rates. Previously, it has been reported that fetal growth retardation and increased fetal mortality rate often can be seen at high altitude. Thus, it has been argued that newborn infants at high altitude weigh significantly less than those born at sea level. According to a number of comparative studies in the Andes and at
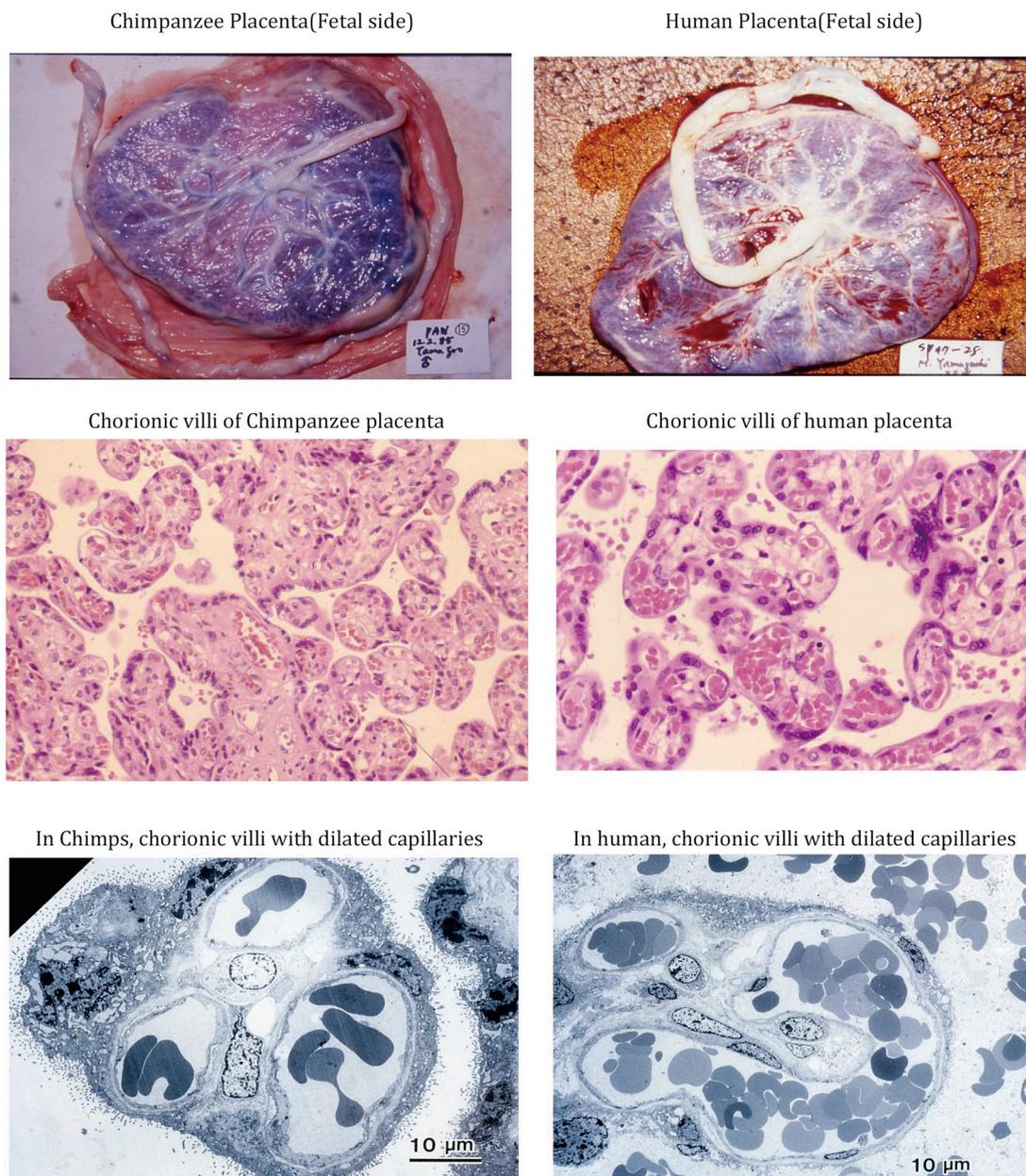
Fig. 10. Chimpanzee Placenta (Fetal side) Human Placenta (Fetal side)
Chorionic villi of Chimpanzee placenta Chorionic villi of human placentaIn
Chimps, chorionic villi with dilated capillaries In human, chorionic villi with dilated capillaries.

sea level [30], the placenta at high altitudes weighed more than the placenta at sea level, even though birth weights at high altitudes are lower than those at sea level. These placental changes can be explained as an adaptation for maintaining oxygen delivery at high altitudes. In an attempt to clarify such morphological changes of the placentas in the Himalayas, we have investigated Nepalese placentas and Tibetan placentas pathologically since 1977 [31] and the results were compared with those of a Japanese group. We found that both mean neonatal birthweight and placental weight in Nepal were less than those of a Japanese group.

As notable pathological signs in the Himalayan placentas, marked subchorionic fibrin deposits, increased chorionic cysts, and chorangiosis as well as chorangioma were often observed [26,31,32,33] (Fig. 15a). The placental barrier between maternal and fetal circulations consists of the trophoblast layer, basement membrane, and fetal capillaries in chorionic villi. Oxygen transfer between maternal blood in the intervillous space and fetal capillaries in chorionic villi is mainly undertaken by syncytial trophoblast covered with microvilli.

Case NP23-1: A 26-year-old Nepalese woman, para 3, was delivered of a male infant weighing 3300 g at 40 weeks. The cord was 30 cm and there were 3 vessels. The placenta weighed 445 g with dimensions of $19 \times 18 \times 2$ cm. An increase in the numbers of villous capillaries and capillary dilatation were histologically observed. In ultrastructure, the basement membrane of the trophoblast layer was thickened and separated forming lacunae. Thus, villous capillaries were distended and stretched over the trophoblast layer, thereby forming the vasculo-syncytial membrane (VSM), while in organelles of the syncytium, apoptotis was seen.
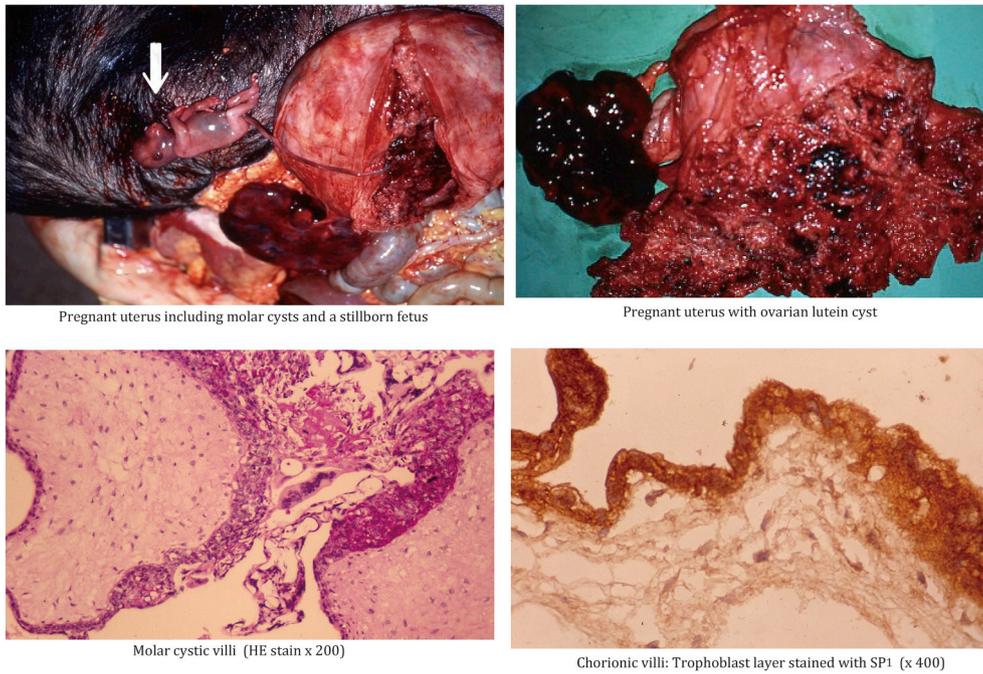
Pregnant uterus including molar cysts and a stillborn fetus

Pregnant uterus with ovarian lutein cyst

Molar cystic villi (HE stain x 200)

Chorionic villi: Trophoblast layer stained with SP1 (x 400)

Fig. 11. a: Pregnant uterus including molar cysts and a stillborn fetus. b: Pregnant uterus with ovarian lutein cyst. c: Molar cystic villi (HE stain x200). d: Chorionic villi: Trophoblast layer stained with SP1 (x400).
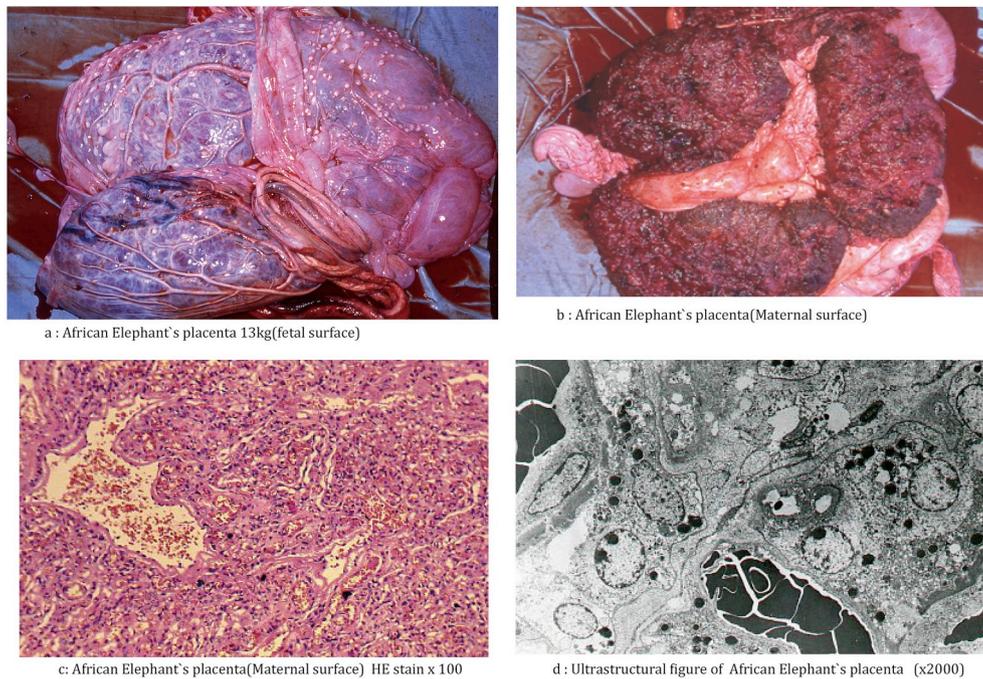

a : African Elephant`s placenta 13kg(fetal surface)

b : African Elephant`s placenta(Maternal surface)

c: African Elephant`s placenta(Maternal surface) HE stain x 100

d : Ultrastructural figure of African Elephant`s placenta (x2000)

Fig. 12. a: African Elephant's placenta 13 kg (fetal surface). b: African Elephant's placenta (Maternal surface). c: African Elephant's placenta (Maternal surface) HE stain x 100. d: Ultrastructural figure of African Elephant's placenta (x2000).

Case NP24-7: A 26-year-old Nepalese woman, para 2, had a male infant weighing 3250 g at 37 weeks of gestation. The umbilical cord was 56 cm long with 3 vessels. The placenta weighed 360 g with dimensions of 18 × 16 × 1.9 cm. Marked subchorionic fibrin and a marginal infarct (4 × 1.2 × 1 cm) were noted. In particular, chorionic villi showed foci of chorangiosis, as was clearly demonstrated by TEM(Fig. 15b).

The syncytium comes into direct contact with the basement membrane; thereby, fetal capillaries are dilated and closely approximate the overlying trophoblast in mature placental villi, forming the vasculo-syncytial membrane (VSM). The VSM is an adaptation to facilitate gas transfer across the placenta. There is a range of compensatory morphological changes occurring in chorionic villi, including increased
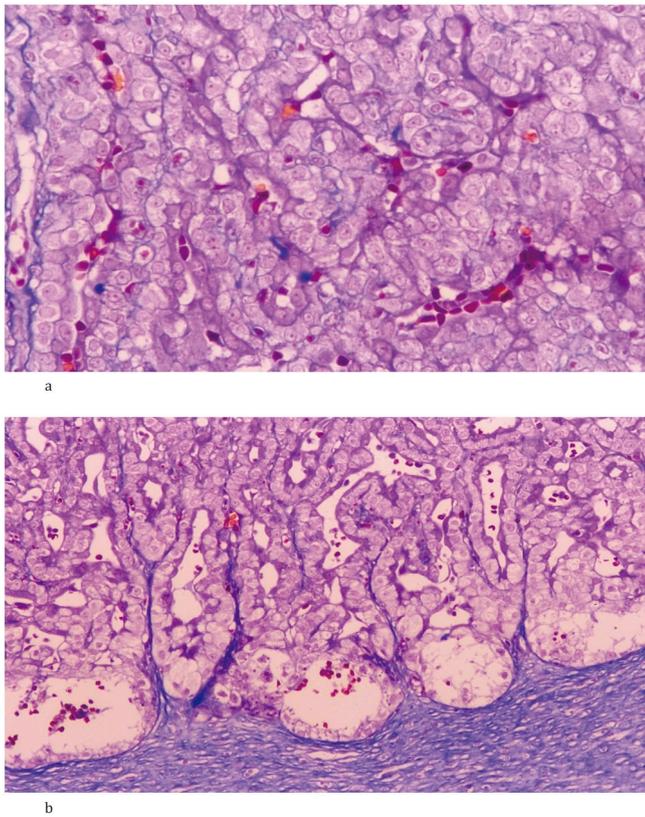
Fig. 13. a,b: Placental villi of manatee showing endothelial form (Mallory stain x100).

VSM formation in the hypoxic state at high altitude. On the other hand, placental villi often show evidence of continued branching angiogenesis at high altitude. In this study, we attempted to observe ultrastructural changes of chorionic villi of Nepalese placentas at high altitude (Fig. 15c).

Case NP21-21: A 20-year-old Nepalese woman, para 1, was delivered of a female infant weighing 3000 g at 39 weeks. The placenta weighed 340 g with an accessory lobe and marginal infarct. Histology of villi: fibrotic and SK increased with fibrinoid necrosis. In ultrastructure of the trophoblast layer, multivesicular bodies and cisternae with rough endoplasmic reticulum (rER) were observed and mitochondria were increased in number (Fig. 15d).

As characteristic ultrastructural features of chorionic villi of Nepalese placentas, an increase of mitochondria and cystic formation of rER, in addition to appearance of lamellar bodies similar to alveolar epithelial type II cells were observed in the syncytium. These ultrastructural changes of the placental villous capillaries may be ascribed to hypervascularization caused by the chronic hypoxic state. It is, therefore, presumed that trophoblast cells may play an important role for gas transfer under such a hypoxic state at high altitude.

12. Discussions & conclusions

Recently, two memorial reports about late Prof. Kurt Benirschke have been introduced; One report was announced in PLACENTA Oct., 2018 by Dr. M.M.Parast, San Diego [34] and the other was reported in Zoonooz, San Diego Zoo, Jan., 2019 [35]. According to Dr.Parast, Dr.K.Benirschke reminded principal unknown aspects of human placentation as 6 items. He has expected these results later. On the other hand, Zoonooz introduced that Prof.K.Benirschke pioneered the Frozen Zoo to cytogenically preserve reproductive tissues and cell lines form endangered species. I have seen his Frozen Zoo since Dartmouth period.
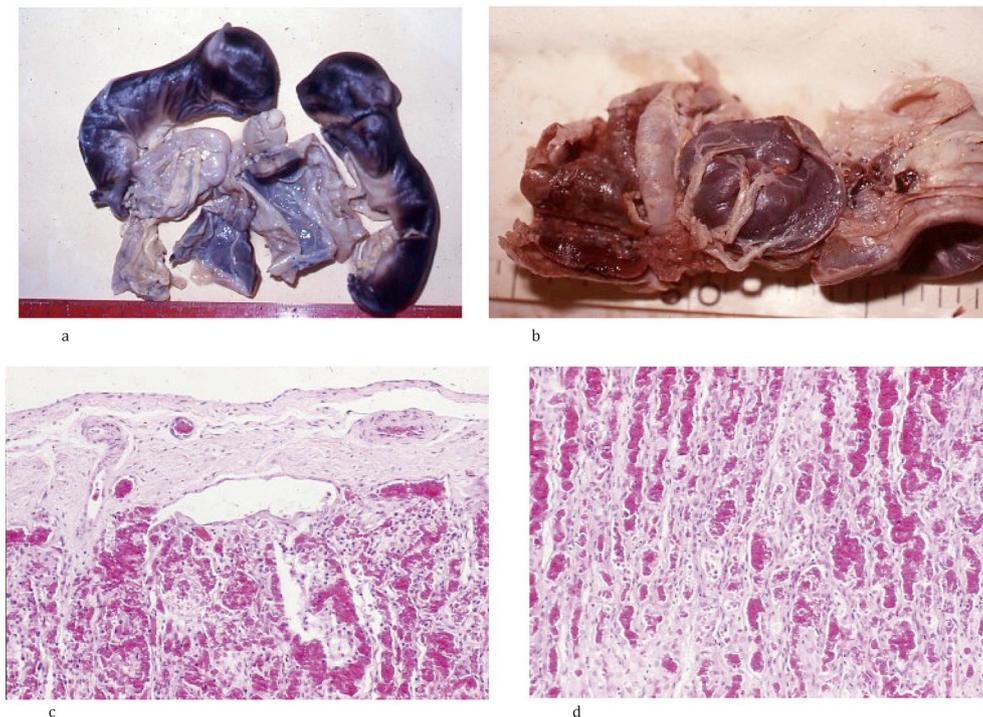

Fig. 14. a,b: Zonary placentation of Rock Hyrax. c,d: Placental microstructures of rock hyrax seeming the manatee's placental structures (HE stain x100).

a : Chorangiosis, Nepalese placenta (HE stain x100)

c : Increased number of intravillous capillaries (x250)

b : Chorangiosis appeared in Nepalese placenta villi (x 1000)

d : An increase of mitochondria and cystic formation of rER in addition to lamullar bodies in the Nepalese placental villi (x2500)
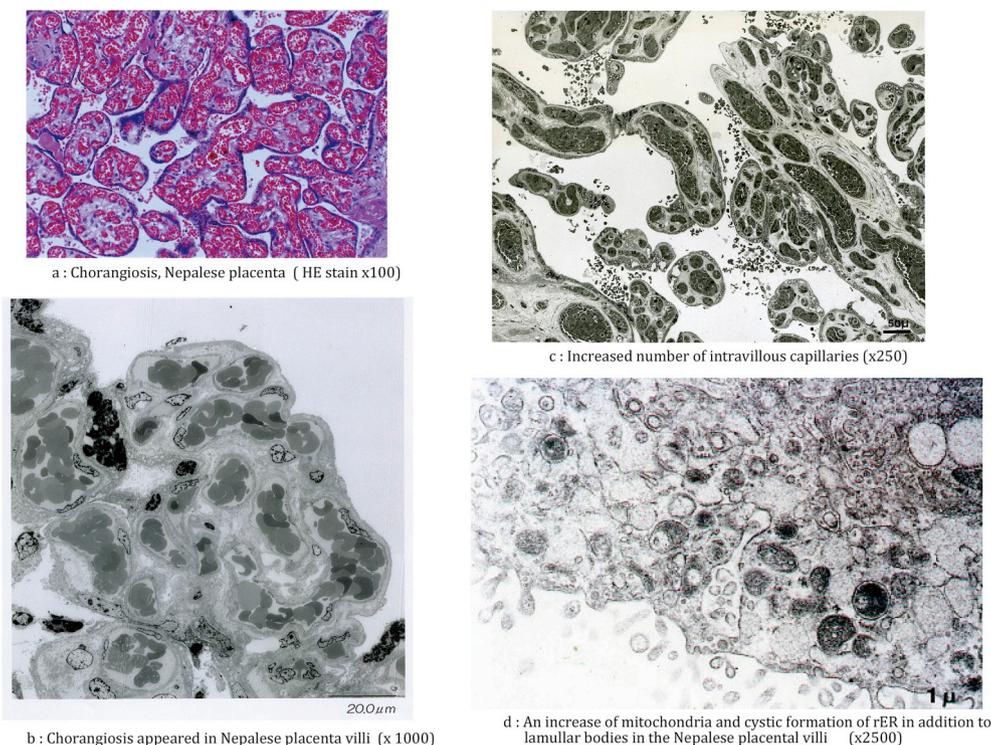
Fig. 15. a: Chorangiosis, Nepalese placenta (HE stain x100). b: Chorangiosis appeared in Nepalese placenta villi (x 1000). c: Increased number of intravillous capillaries (x250). d: An increase of mitochondria and cystic formation of rER in addition to lamullar bodies in the Nepalese placental villi (x2500).

Thereby, I participated to put some species into the Frozen Zoo. This lecture might be given some answers for these question, so that the author would like to devote many thanks for him.

New York Times on December 5, 2018 firstly has appeared an important article called "Why studying Placenta is crucial". Many unresolved problems on its ephemerial organ have been clearly demonstrated by placental researchers in the world. In particular, the missing link between pregnancy complications and development of fetal brain might be influenced by irregular placental development. The author hopes that this memorial lecture will be a role for clarifying unresolved mammalian placental function.

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