

Review

Morphology and physiology of rat placenta for toxicological evaluation

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Abstract: The placenta plays a pivotal role in fetal growth, and placental dysfunction and injury are associated with embryo/fetal toxicity. Histological examination of the rat placenta for safety evaluation provides valuable clues to the mechanisms of this toxicity. However, the placenta has specific and complex biological features unlike those of other organs, and placental structure dramatically changes depending on the time during the gestation period. Thus, time-dependent histopathological examination of the rat placenta should be performed based on the understanding of normal developmental changes in morphology and function. The placentas of rats and humans are both anatomically classified as discoid and hemochorial types. However, there are differences between rats and humans in terms of placental histological structure, the fetal-maternal interface, and the function of the yolk sac. Therefore, extrapolation of placental toxicity from rats to humans should be done cautiously in the evaluation of risk factors. This review describes the development, morphology, physiology, and toxicological features of the rat placenta and the differences between the rat and human placenta to enable accurate evaluation of reproductive and developmental toxicity in studies. (DOI: 10.1293/tox.2018-0042; *J Toxicol Pathol* 2019; 32: 1–17)

Key words: histopathology, human, placenta, rat, reproduction

Introduction

The placenta plays a pivotal role in fetal growth even though it is a temporary organ during pregnancy. It is the interface between the dam and developing embryo/fetus and is a multifunctional organ that serves as the liver, lung, gut, kidney, and endocrine/exocrine glands. Its functions include anchoring the developing fetus to the uterine wall, mediating maternal immune tolerance, hormone production, nutrient uptake, waste elimination, and gas exchange via the maternal blood supply during embryonic/fetal development¹. Furthermore, the placenta serves as a protective barrier that protects the embryo/fetus against chemical injury. Placental dysfunction and injury have adverse effects on the maintenance of pregnancy and fetal development². Detection of chemically induced placental damage in rats provides a valuable clue to the mechanisms of embryo/fetal

toxicity in safety evaluation. Therefore, the placenta is an important organ for evaluating reproductive and developmental toxicity³.

The large placental surface area comes in contact with a relatively large volume of maternal blood⁴. The placenta, which is rich in protein, may bioconcentrate chemical residues by means of protein binding and release these residues into the placental circulation. Due to these biological features, the placenta is vulnerable to toxicants, and placental toxicity has been reported for many chemicals and other factors (Table 1). However, the placenta has not received proper consideration as a target organ in safety evaluation of risks for dams and embryos/fetuses, because the placenta has the following complex biological features: a) a complicated structure composed of multiple tissues⁵, b) drastic changes in placental structure and function over time due to rapid development, and c) wide variations of placental structure among different animal species⁶. Additionally, the placentas of both rats and humans are anatomically classified as discoid and hemochorial types. However, there are differences between rats and humans in terms of the placental histological structure, the fetal-maternal interface, and the function of the yolk sac (Table 2). Therefore, extrapolation of placental toxicity from rats to humans should be done cautiously in the evaluation of risk factors. This review describes the development, morphology, physiology, and toxicological features of the rat placenta and the species-based

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Table 1. Chemicals Toxic to the Placenta

Part of placenta	Pathological lesion	Chemicals / factors				
Labyrinth zone	Hypoplasia	1-b-D-arabinofuranosylcytosine	5-azacytidine	Azathioprine	Bestatin	Busulfan
		Cisplatin	Chlorpromazine	Dibutyltin	Ethyl nitrosourea	Glucocorticoid
	Necrosis	Hyperphenylalaninemia	Maternal undernutrition	Methyl methane-sulfonate	6-mercaptopurine	Risperidone
		T-2 toxin				
		Cadmium	Glucocorticoid	Interleukin-1	Iron-deficiency	Lipopolysaccharide
Cystic dilatation	Tobacco	Triamcinolone	Tumor necrosis factor	Valproate		
	Epoxiconazole	Estradiol	Ethanol	Hyperphenylalaninemia	Ketoconazole	
Hypertrophy	Methylparathion					
	Alloxan	Epoxiconazole	Ethanol	Ketoconazole	Letrozole	
	Other changes	Adrenomedullin antagonist	Benzyl benzoate	Caffeine	Cyclosporin A	Ethanol
Basal zone	Hypoplasia	Hyperthermia-treatment	Iron-deficiency	PM2.5	Streptozotocin	TCDD
		Carbenoxolone	Chlorpromazine	Chromium	Cisplatin	Ethanol
	Cystic degeneration	Glucocorticoid	Hyperphenylalaninemia	Methotrexate		
		Chlorpromazine	Cisplatin	Dibutyltin	Epoxiconazole	Hyperphenylalaninemia
	Hypertrophy	6-mercaptopurine	PPAR β/δ agonist	Streptozotocin	TCDD	Topiramate
Alloxan		Ethanol	Ketoconazole	Low-sodium diet	Methylhydrazine	
Decidua	Hypoplasia	Methyl parathion	Streptozocin			
		Benomyl	Chromium	Dibutyltin	Doxycycline	Hydroxyurea
	Necrosis	Metyl-benzimidazole carbamate	NG-nitro-L-arginine methyl ester	Streptozotocin	Triphenyltin	
Chlorpromazine		Cocaine	Cold stress	Cyclosporin A	Ethanol	
Metrial gland	Hypotrophy	Hyperthermia	Interleukin-1	Lipopolysaccharide	Methyl parathion	Ovariectomy
		Salmonella dublin	Topiramate	Tumor necrosis factor		
Yolk sac	Degeneration	Chlorpromazine	Cisplatin	Doxycycline	Estrogen	Ethanol
		Lipopolysaccharides		Ovariectomy	Tamoxifen	
Others	Enlarged placenta	Antiserum	Dextran	Dinitrophenol,	Ethanol	Estrogen
		Hemoglobin-based oxygen carriers	Leupeptin	Polyvinylpyrrolidone	Somatomedin inhibitor	Sodium aurothiomalate
	CYP induction	Sucrose	Suramin	Triton WR-1339	Trypan blue	
Others	CYP induction	Carbon monoxide	Hemorrhage	Hypertension	Indomethacin	Tributyltin
		Vessel ligation				
		Diallyl disulfide	Dioxin	β -Naphthoflavone	Phenobarbital	Pregnenolone-16 α -carbonitrile
		Tobacco				

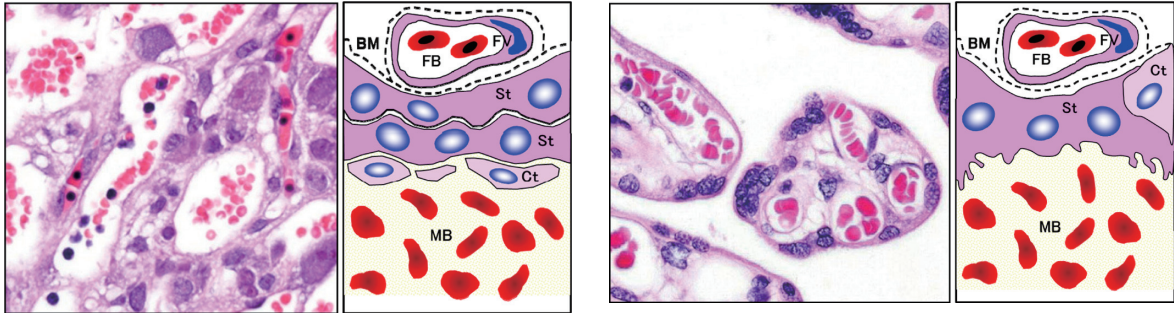
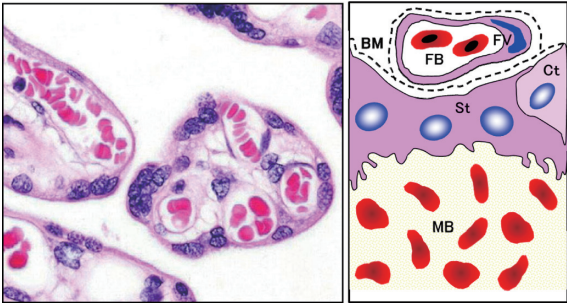
differences between the rat and human placenta to enable accurate evaluation of the effect of reproductive and developmental toxicity in studies.

Normal Development of the Rat Placenta

Table 3 shows the reproductive data (embryonal/fetal weight, placental weight, embryonal/fetal placental weight ratio, etc.) of 171 dams from gestation day (GD) 11 (GD 0 is designated as the day when the presence of a vaginal plug is identified) to GD 21 in control groups from our previous studies using Wistar Hannover rats. The placental weights

gradually increase with pregnancy progression and reach a plateau on GD 19, whereas the fetal weights rapidly increase from GD 17 to GD 21^{7,8}. The placental weight is approximately equal to the fetal weight on GD 15 and declines to one-fourth on GD 17 and one-tenth on GD 21. Figure 1 shows the time-dependent macroscopic changes in placental diameter in CrI:CD (SD) rats. The minor axis and thickness reach a plateau on GD 17, and the major axis gradually increases until GD 21.

Table 2. Morphological Differences Between the Rat and Human Placenta

	Rat	Human
Site of first attachment	Anti-mesometrial	Anti-mesometrial
Placental shape	Discoid placenta	Discoid placenta
Placental structure	Labyrinth zone	Placental villi
	Basal zone (Junctional zone)	Basal plate
	Decidua Metrial gland	Decidua
Fetal-maternal interface	Labyrinth	Villous
	Hemochorial type Hemotrichorial - Three layers	Hemochorial type Hemomonochorial - One layer
		
Yolk sac	Initial bilaminar yolk sac Inverted yolk sac placenta until parturition	Not function as placenta Disappearance by 12 weeks
Pathway of steroid hormone biosynthesis	Absence of aromatase	Absence of CYP17

BM, Basal membrane; Ct, Cytotrophoblast; FB, Fetal blood; FV, Fetal vessel; MB, Maternal blood; St, Syncytiotrophoblast.

Table 3. Reproductive Data of Wistar Han Rats During Pregnancy

Gestation day	No. of dams	No. of live embryo/fetus	Dead embryo ratio (%)	Embryonal/fetal weight (mg) ^a	Placental weight (mg) ^a	Embryonal/fetal-placental weight ratio (mg/mg) ^a
11	12	12.8 ± 2.4	0.0 ± 0.0	ND	ND	ND
12	4	14.3 ± 0.5	1.7 ± 3.3	ND	74.4 ± 8.3	ND
13	25	12.2 ± 2.1	4.2 ± 3.6	70.8 ± 13.1	104.6 ± 22.7	0.7 ± 0.3
14	4	13.3 ± 3.7	1.8 ± 3.6	141.3 ± 7.5	160.7 ± 8.6	0.9 ± 0.1
15	41	12.2 ± 2.2	4.5 ± 6.5	261.8 ± 19.8	213.0 ± 31.3	1.3 ± 0.2
16	14	12.0 ± 2.9	7.9 ± 9.1	521.7 ± 125.6	276.7 ± 40.7	1.9 ± 0.3
17	27	11.3 ± 2.4	3.3 ± 6.4	853.0 ± 220.8	325.4 ± 60.1	2.6 ± 0.4
19	4	11.0 ± 0.6	2.5 ± 2.2	2062.2 ± 80.0	448.7 ± 13.0	4.8 ± 0.1
21	40	11.6 ± 2.7	2.1 ± 7.1	4962.4 ± 374.1	464.0 ± 59.9	11.0 ± 1.0
Mean/total	171	11.9 ± 2.6	3.7 ± 6.5	ND	ND	ND

Mean ± SD. ^aMean of individual litter values. ^bTotal number of dams. ND, no data.

Embryology, Morphology, and Physiology of the Rat Placenta

The rat placenta is histologically divided into a fetal part and a maternal part⁵. The fetal part is composed of the labyrinth zone, basal zone (also referred to as the junctional zone), and yolk sac. The maternal part is composed of the

decidua and metrial gland. Figure 1 and 2 show the respective time-dependent thickness and biological features of each part of the placenta. Developmentally, the trophoblast, which differentiates into the placenta, consists of the mural trophoblast and polar trophoblast. The mural trophoblast surrounding the blastocyst cavity arrests cell division and differentiates into primary trophoblastic

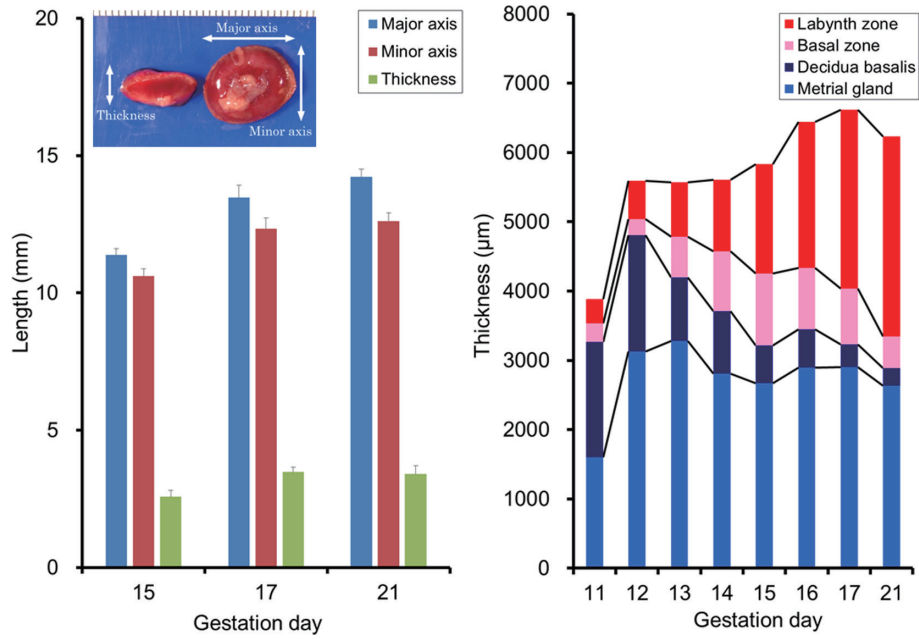


Fig. 1. Time-dependent changes in diameter and thickness of each part of the placenta in rats. Left, placental diameter on GD 15, 17, and 21 in CrI:CD(SD) rats. Error bar represents SD. Right, thickness of each part of the placenta from GD 11 to GD 21 in Wistar Han rats.

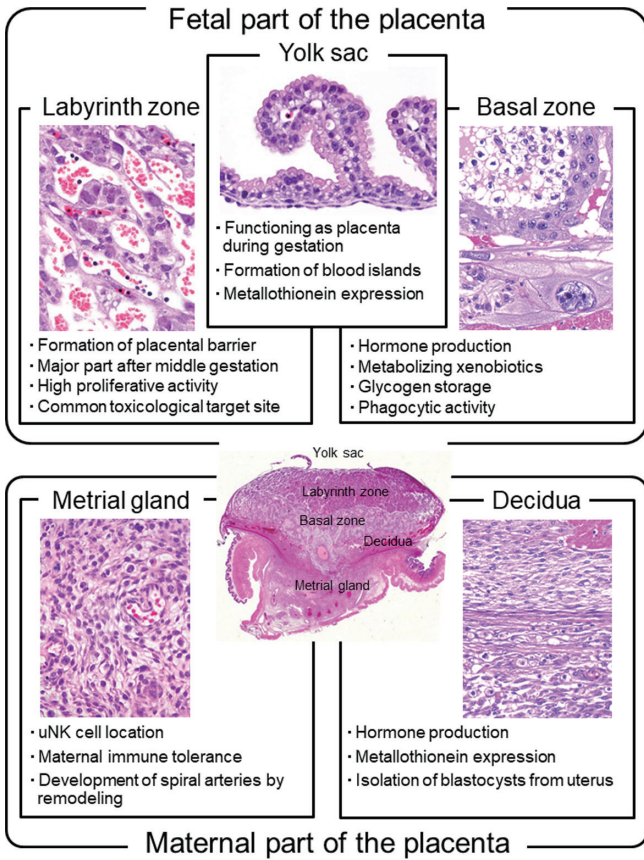


Fig. 2. Biological features of each part of the placenta in rats.

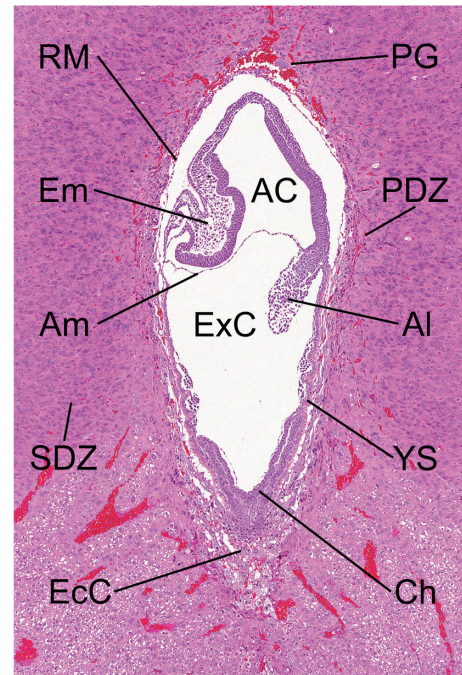


Fig. 3. Morphology of the embryo on GD 9.5. HE stain. AC, amniotic cavity; AI, allantois; Am, amnion; Ch, chorionic plate; Ecc, ectoplacental cone; Em, embryo; ExC, exocoelomic cavity; PDZ, primary decidual zone; PG, primary trophoblastic giant cell; RM, Reichert's membrane; SDZ, secondary decidual zone; YS, yolk sac.

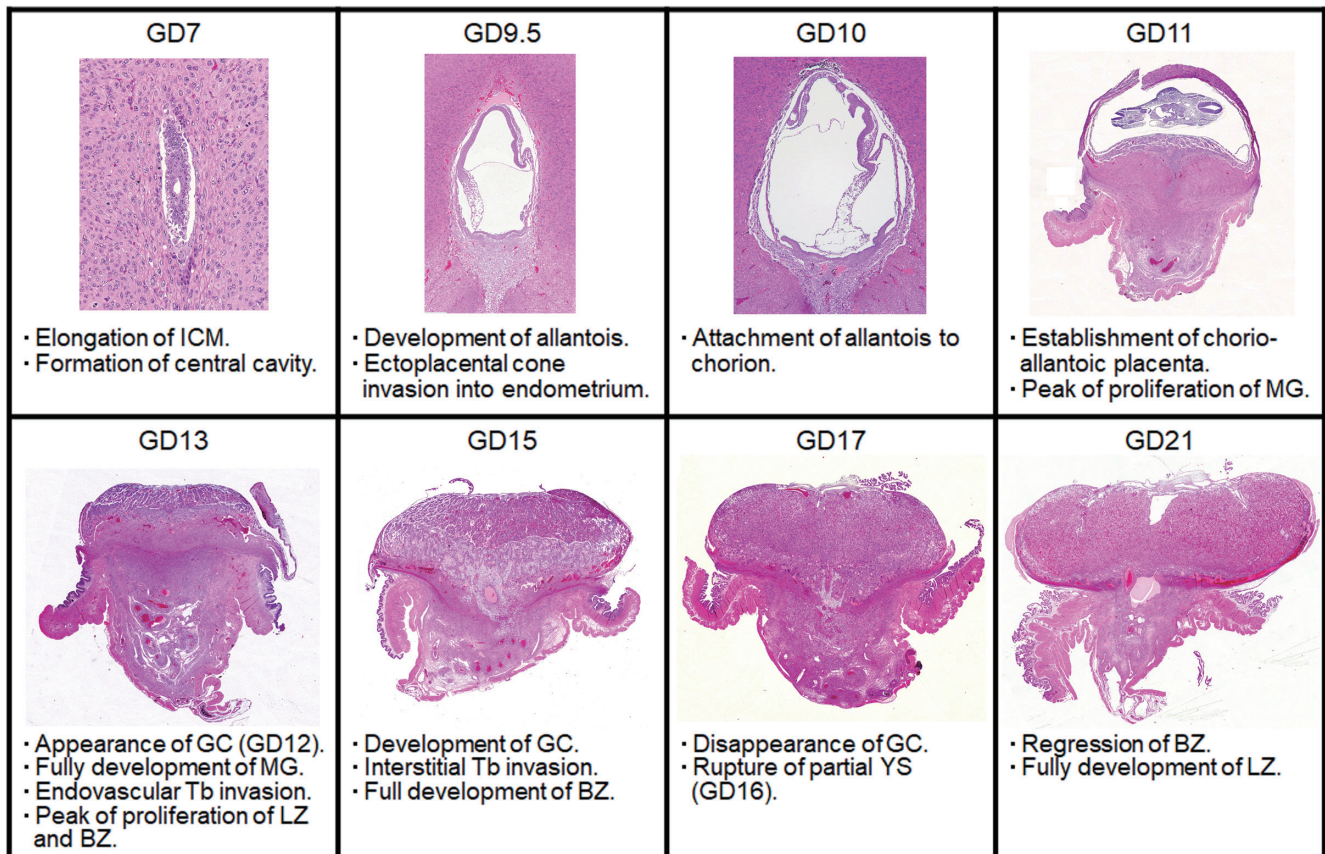


Fig. 4. Morphological development of the rat placenta and histological events at each point. BZ, Basal zone; GC, Glycogen cell; ICM, Inner cell mass; LZ, Labyrinth zone; MG, Metrial gland; Tb, Trophoblast; YS, Yolk sac. HE stain.

giant cells just after implantation. The polar trophoblast forms the ectoplacental cone and invades the decidua. The edge and center of the ectoplacental cone differentiate into the basal zone (Fig. 3)⁹. The chorionic plate, which is the membrane of the ectoplacental cone on the embryonic side, differentiates into the labyrinth zone. In the endometrium, the decidua is derived from endometrial stromal cells by stimulation of decidualization. The decidua rapidly grows and fills the uterus. Primary decidualization takes place on the anti-mesometrial side in response to implantation of the blastocyst. This is followed by secondary decidualization on the mesometrial side. The metrial gland is composed of nodular aggregates of heterogeneous tissue that develop in the mesometrial triangle in the uterine wall⁹. Figure 4 shows the process of overall morphological development of the placenta with the events in each stage from GD 7 to GD 21.

1) Fetal part of the placenta

(1) Labyrinth zone

• Morphology

The labyrinth zone rests atop the flat, broad chorionic plate and is a thick vascular plate that serves as the terminus for the umbilical cord¹⁰. It is composed of the maternal sinusoids, trophoblastic septa, and fetal capillaries. In the trophoblastic septa, the outer trophoblast, which comes

into direct contact with the maternal blood, is referred to as the cytotrophoblast with a microvillous surface. Under this trophoblast, there are two layers of syncytiotrophoblasts (syncytiotrophoblast I and syncytiotrophoblast II from the maternal sinusoid side) (Fig. 5 and 6). The continuity of the syncytiotrophoblast layer provides a placental barrier¹¹. Connexin 26 is localized in the gap junctions connecting the two syncytiotrophoblast layers and allows these layers to act functionally as a single syncytial layer for the transfer of small molecules across the placental barrier¹² (Fig. 5). The maternal sinusoids filled with blood cells are formed amid these cells. Developmentally, the trophoblast layer appears on GD 10 and is occupied by syncytiotrophoblasts and cytotrophoblasts¹³. There is a decrease in the cellular density in the trophoblastic septa and an increase in the size of the cytotrophoblast with pregnancy progression (Fig. 6). The cellular proliferative activity of the trophoblasts is at its peak on GD 13 and then gradually decreases until GD 21¹⁴ (Fig. 7). In response to this, the labyrinth zone develops with advancing pregnancy and forms the majority of the fetal part of the placenta (Fig. 1).

• Physiological function and toxicological features

Syncytiotrophoblasts form a layer that separates the fetal circulation from the maternal circulation. These cells express several efflux and influx transporters that serve to

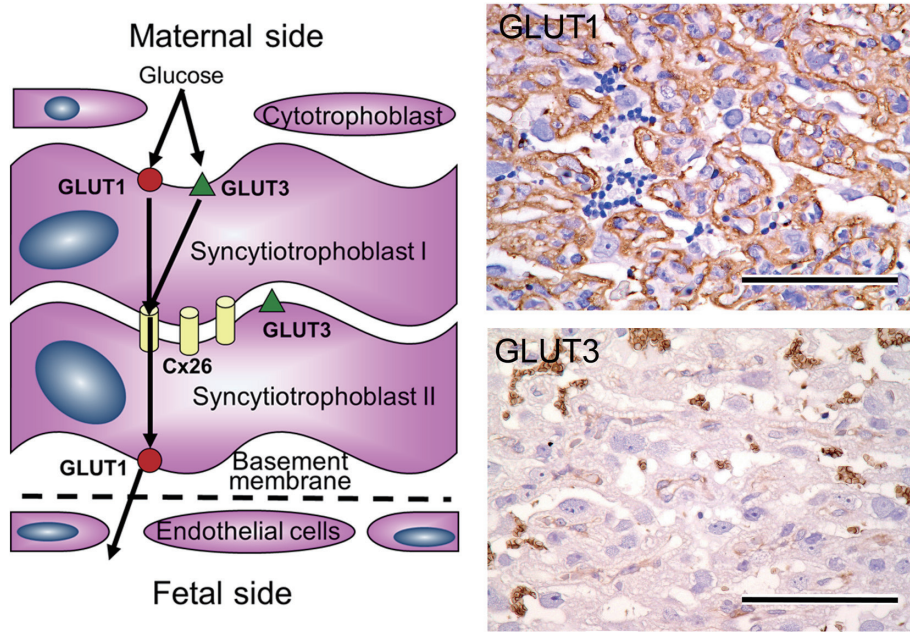


Fig. 5. Expression of GLUT1 and GLUT3 in trophoblastic septa. Left, schema of trophoblastic septa. Pathway of glucose via GLUT1 and GLUT3. Upper right, immunohistochemical expression of GLUT1. Bar=100 µm. Lower right, immunohistochemical expression of GLUT3. Bar=100 µm.

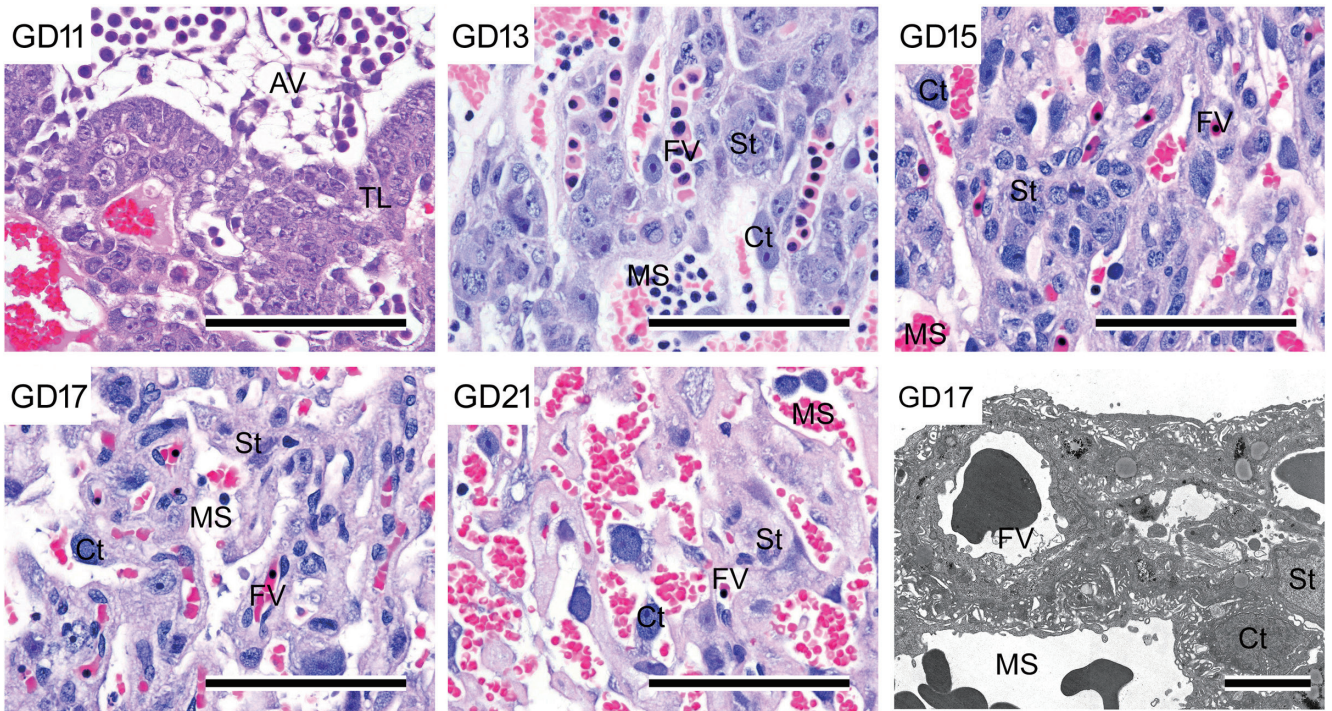


Fig. 6. Morphological development and ultrastructure of trophoblastic septa in the labyrinth zone. Decrease in cellular density in trophoblastic septa and increase in size of cytotrophoblasts with pregnancy progression; HE stain. Bar=100 µm. Lower right, ultrastructure of trophoblastic septa. Bar=15 µm. AV, allantoic vessel; Ct, cytotrophoblast; FV, fetal vessel; MS, maternal sinusoid; St, syncytiotrophoblast; TL, trophoblast layers.

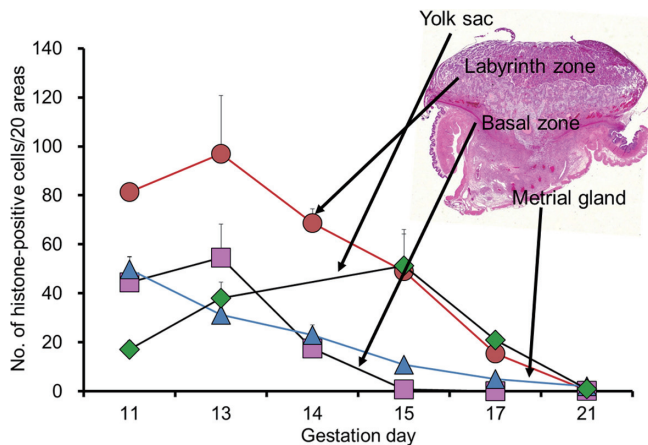


Fig. 7. Cellular proliferative activity of each placental layer during pregnancy. Error bar represents SD.

control transfer of chemicals between the fetus and dam. In order to regulate the transfer process of chemicals, the plasma membranes are equipped with various transporters between the maternal and fetal side: multidrug resistance protein (Mdr)1a, Mdr1b, multidrug resistance-associated protein (Mrp)1, Mrp5, organic cation transporter (Oct)3, organic cation transporter novel (Octn)1, organic anion-transporting polypeptide (Oatp)3, Oatp12, four metal transporters (ZnT1, divalent metal transporter 1, Menkes, and Wilson), a prostaglandin, ATP-binding cassette sub-family G member (AbcG)8, equilibrative nucleoside transporter (ENT)1, and ENT2¹². In addition, glucose is the main fuel for growth and energy metabolism of fetoplacental tissues, and the transplacental movement of glucose is a stereospecific saturable and carrier-mediated process of facilitated diffusion¹⁵. The expression of two maternal-to-fetal glucose transporter isoforms has been demonstrated in rats, namely GLUT1 and GLUT3 on the trophoblastic septa^{16, 17}. GLUT 1 is localized in the membrane of syncytiotrophoblast layer I facing the maternal blood side and is present in the membrane of syncytiotrophoblast II facing the fetal capillaries (Fig. 5). In contrast, GLUT3 is localized in the membrane of syncytiotrophoblast layer I facing the maternal blood side and is present in between syncytiotrophoblast layers I and II. The asymmetric distribution of GLUT3 across the placental barrier may suggest asymmetric transfer of glucose, which would be beneficial to provision of a stable milieu for fetal development while also preventing the loss of glucose at times of maternal hypoglycemia. The glucose transport capacity develops primarily due to an increase in the total number of glucose transporters found on the membranes of the trophoblasts. In addition, the expression of glucose transporters has been found to be increased as an adaptive change in small placentas¹⁸. Cytotrophoblasts and fetal endocapillary cells are nonfunctional for a placental barrier, since they are fenestrated. The cytotrophoblasts function by slowing the maternal blood flow and forming local regions of blood stasis behind the fenestrations that facilitate fet-

maternal transport¹⁹.

The labyrinth zone plays a role in O₂/CO₂ exchange, providing nutrients for the fetus and removing waste products. Labyrinth zone damage has a high correlation with intrauterine growth retardation (IUGR)¹⁴. The labyrinth zone is vulnerable to the target site in placental toxicity because of high blood flow, high cellular proliferative activity, and long proliferation period as compared with other parts of the placenta. It has been reported that antiproliferative or apoptotic agents result in a small placenta and IUGR due to labyrinth zone hypoplasia^{20–22} (Table 1). Conversely, estrogen is an inhibitor of placental growth, and estrogen deficiency induces placental hypertrophy^{23, 24}. The labyrinth zone is a hormone-dependent tissue, and aromatase inhibitors induce labyrinth zone hypertrophy, which is associated with an increase in mitosis of trophoblasts and the cystic dilatation of maternal sinuses due to the antiestrogenic effect^{25, 26}.

(2) Basal zone

• Morphology

The basal zone forms just below the labyrinth zone and is composed of spongiotrophoblasts, glycogen cells, and (secondary) trophoblastic giant cells²⁷. Developmentally, there are two types of trophoblastic giant cells. The primary trophoblastic giant cells derived from the mural trophoblasts are present outside Reichert's membrane²⁸ (Fig. 3). These cells are of paramount importance for successful implantation but are not components of the basal zone. The secondary trophoblastic giant cells (hereinafter referred to as trophoblastic giant cells) are formed from the edge of the trophoblastic ectoplacental cone²⁹. These cells are large polyploid cells with a characteristically enormous, deeply divided nucleus. They provide the first surface layer for the ectoplacental cone on GD 10 and are located between the spongiotrophoblast layer and decidua basalis. The spongiotrophoblasts are derived from the outer layers of the ectoplacental cone on GD 10 and are located immediately above the trophoblastic giant cell layer. They are a main structural component of the basal zone. The glycogen cells, which accumulate glycogen-rich granules, transiently appear on GD 12 and form small clusters on GD 14. They develop into glycogen cell islands and comprise a large part of the basal zone on GD 15 and 16. They exhibit pyknosis after GD 17 with basal zone regression, and then almost all glycogen cells disappear on GD 21 (Fig. 8). The glycogen cells are reportedly derived from the spongiotrophoblasts. However, the same evidence suggests that glycogen cells are distinct from spongiotrophoblasts³⁰. The cellular proliferative activity in the basal zone is at its peak on GD 13 and then nearly disappears until GD 15 (Fig. 7). In response to this, the basal zone fully develops on GD 15, and this gradually leads to regression before parturition (Fig. 8).

• Physiological function and toxicological features

The basal zone is a site of production of steroids and peptide hormones, which play an important role in the maintenance of pregnancy³¹. The peak placental progesterone production is on GD 11, and testosterone production reaches its maximum level on GD 17³². Synthesis of progesterone

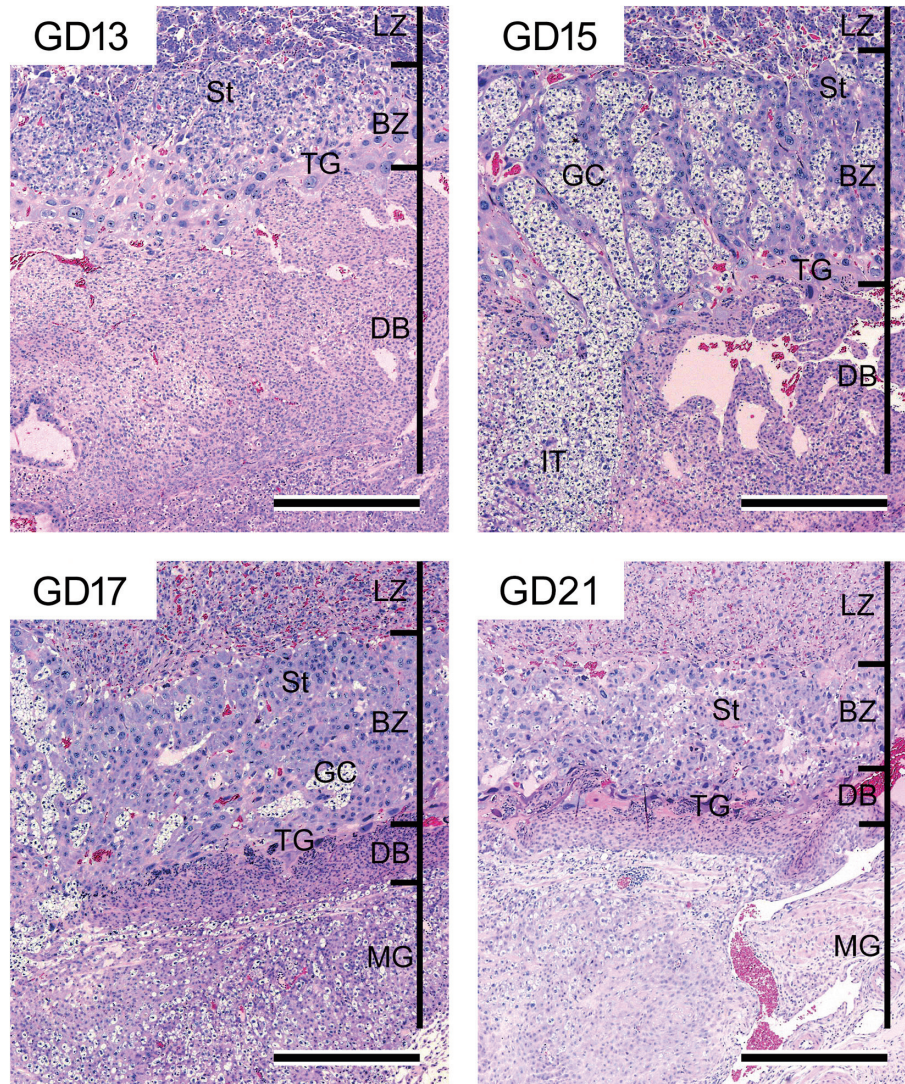


Fig. 8. Morphology of the basal zone and decidua basalis from GD 13 to GD 21. Regression of the decidua basalis from GD 13. Full development of the basal zone with glycogen islet formation on GD 15 and then gradual regression. Penetration of interstitial trophoblasts through the decidua basalis into the metrial gland from GD 15. HE stain. Bar=500 μ m. BZ, basal zone; DB, decidua basalis; GC, glycogen cell; IT, interstitial trophoblast; LZ, labyrinth zone; MG, metrial gland; St, spongiorophoblast; TG, trophoblastic giant cell.

and testosterone falls precipitously from GD 17 onward, and this change reflects the reduced functional importance of the basal zone in association with the higher level of apoptosis in late pregnancy³³. In ovariectomized rats treated with estrogen and progesterone, overgrowth of the basal zone is induced by a response to the hormonal imbalance³⁴. The trophoblastic giant cells, spongiorophoblasts, and invasive trophoblasts are capable of expressing components of the prolactin family^{35, 36}. The spongiorophoblasts, in particular, are a major source of them.

The basal zone plays an important functional role in metabolism of chemicals³⁷. CYP3A1, a major component of the CYP system during pregnancy, is mainly located in trophoblastic giant cells. The expression of CYP3A1 is induced by diallyl disulfide³⁸, pregnenolone 16- α -carbonitrile³⁹, and phenobarbital⁴⁰. In addition, CYP17 (17- α -hydrox-

ylase/C17,20-lyase) is detected during the second half of pregnancy⁴¹, and is located in the spongiorophoblasts and trophoblastic giant cells in the basal zone and in trophoblasts in the labyrinth zone.

The glycogen cells are the storage site for glycogen that is produced from maternal glucose⁴². This glycogen is suggested to be an important nutrient for the fetus. It is known that glycogen cells are increased in the basal zone with the expression of hypoxia in rats given a low-sodium diet⁴³. The disappearance of glycogen cell islands is thought to meet the increased demand for glycogen as an energy substrate for the final period of fetal growth^{44, 45}. Cystic degeneration describes abnormal retention of extensive cytoplasmic vacuolation within glycogen cells. It is induced by remaining glycogen cell islands that should regress and disappear at the end of pregnancy, as a result of placental developmental

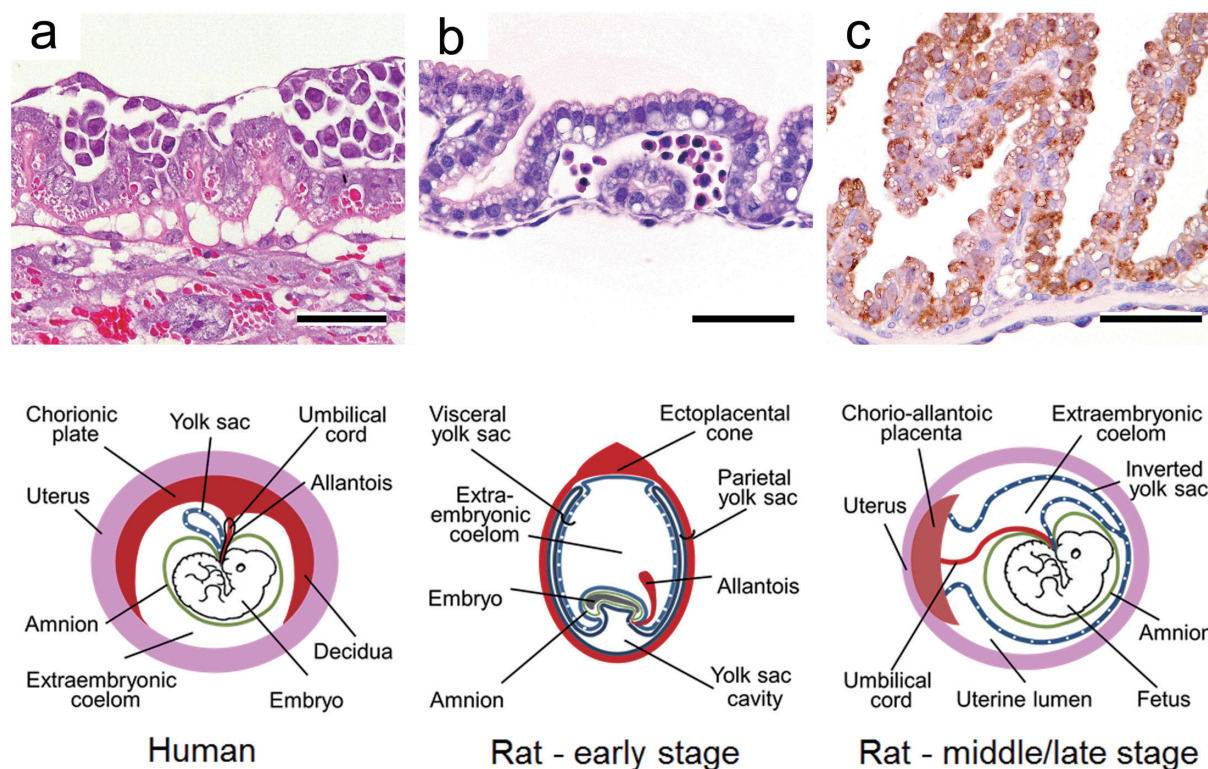


Fig. 9. Morphology of the parietal and visceral yolk sac in rats and morphological differences in the yolk sac between rats and humans. a. Parietal yolk sac on GD 9.5 in rats. HE stain. Bar = 50 μ m. b. Visceral yolk sac on GD 9.5 in rats. HE stain. Bar = 50 μ m. c. Metallothionein expression in the visceral yolk sac on GD 17 in rats. Bar = 50 μ m. Lower schema. Morphological differences of yolk sac between humans and rats (early stage and middle/late stage).

delay.

The trophoblastic giant cells have phagocytic activity, which mediates the implantation and invasion of the conceptus into the endometrial stroma²⁸. This expression tends to decrease with advancing pregnancy, and the phagocytosis in the final stages of pregnancy reportedly is quite reduced⁴⁶. The trophoblastic giant cells also secrete several factors that control placental growth and maternal reactions to pregnancy. In addition, they are immunologically specialized, and express none of the major histocompatibility complex determinants on their plasma membranes. It is thought that the trophoblastic giant cells play the role of an immunologically neutral buffer zone at the interface between fetal and maternal tissues. As described above, the basal zone consists of 3 different kinds of tissues that develop different functions and regress at different times. Thus, chemically induced lesions in the basal zone largely tend to differ depending on the chemical administration period as compared with other parts of the placenta.

(3) Yolk sac

• Morphological structure

The yolk sac is an extraembryonic membrane surrounding the embryo and is composed of epithelial and mesodermal cells. Developmentally, the yolk sac is derived from embryonic endoderm and mesoderm. It becomes enlarged and surrounds the entire embryo. It is divided into

the visceral yolk sac surrounding the embryo with the amnion and the parietal yolk sac attached to the rim of the chorionic plate (Fig. 3, 9). The parietal yolk sac is lined with Reichert's membrane between the endoderm and trophoblasts⁴⁷. Reichert's membrane is a rodent-specific and acellular thin membrane.

In early development, the blood islands are formed on the surface of the visceral yolk sac and are the first site of embryonic hematopoiesis⁴⁸. They subsequently develop into the yolk sac circulation on GD 10. The inside of the visceral yolk sac becomes exposed to the intrauterine cavity and becomes an inverted yolk sac placenta when the parietal yolk sac ruptures on GD 16⁴⁹ (Fig. 9). The cellular proliferative activity of the yolk sac is at its peak on GD 15 and then gradually decreases until GD 21 (Fig. 7).

• Physiological function and toxicological features

During an early stage, the visceral and parietal yolk sacs play a role as a transient placenta that performs endocytosis of nutrient materials⁵⁰. This yolk sac placenta is also involved with endocrine, metabolic, immunologic, secretory, excretory, and hematopoietic functions⁵¹. It is the only route for major transport between dam and embryo until establishment of the chorioallantoic placenta on GD 11.5⁴⁹. Furthermore, the visceral yolk sac even maintains placental functions, as the inverted yolk sac placenta, until just before parturition⁵. The inverted yolk sac placenta is

a specific biological structure in rodents and rabbits. Thus, impaired structural and functional development of the yolk sac contributes to embryo/fetal toxicity and teratogenicity in rats^{52, 53}. In addition, the presence of metallothionein in the yolk sac protects the fetus from heavy metals. Expression of metallothionein is detected in epithelial cytoplasm from GD 9. It gradually increases until GD 19 and then slightly decreases on GD 21⁵⁴ (Fig. 9).

2) Maternal part of the placenta

(1) Decidua

• Morphology

The decidua is derived from the endometrial stroma and is divided into the decidua capsularis (anti-mesometrial side), decidua parietalis (lateral side), and decidua basalis (mesometrial side) (Fig. 10). Developmentally, the decidual cells surrounding the blastocyst initially form the primary decidual zone. Subsequently, the more loosely packed decidual cells around the primary decidual zone form the secondary decidual zone⁵⁵ (Fig. 3). The primary decidual zone degenerates progressively, and placental and embryonic growth slowly replaces the secondary decidual zone, which develops into a thin layer comprised of the decidua capsularis and decidua parietalis. The decidua capsularis consistently regresses from GD 8 onward and completely ruptures by GD 17-18. Rupture of the decidua capsularis exposes the inverted yolk sac placenta to the reformed uterine epithelium, which is completely restored by GD 15⁵⁶. The decidua basalis is located at the base of the placenta and is an important site for maternal angiogenesis. Most of the development of the decidua basalis occurs during early pregnancy, and the decidua basalis undergoes regression from GD 11 onward. Therefore, the sensitivity of the decidua to chemicals in the organogenesis period is generally lower than that of other parts of the placenta. It is known that the regression of the decidua is mediated by apoptosis⁵⁷. As hemorrhage and edema are sometimes observed in the regression process of the decidua, it is necessary to distinguish them from toxicological lesions. Histologically, the decidual cells are of 2 types: large cells with a large round nucleus and smaller cells with a small nucleus and periodic acid-Schiff-positive vacuolated cytoplasm. The large cells disappear on GD 14, but the small cells remain until the end of pregnancy⁵⁸.

• Physiological function and toxicological features

The decidua has a variety of functions: it acts as a physical barrier to isolate the implanting blastocyst from maternal tissue, as a nutrient source as a result of accumulation of glycogen and/or lipid, and as a producer of hormones, such as prolactin, prostaglandin, steroid, relaxin, etc.⁴⁷. The decidua is a hormone-dependent tissue. Estradiol plays an important role in the growth and differentiation of endometrial stromal cells into decidual cells. In addition, progesterone and prolactin are considered essential for decidual development. Excess estrogen administration in pseudopregnant rats has an inhibitory effect on decidual growth⁵⁹. A reduction in serum progesterone levels also suppresses the decidual cell response in pseudopregnant rats⁶⁰. Estro-

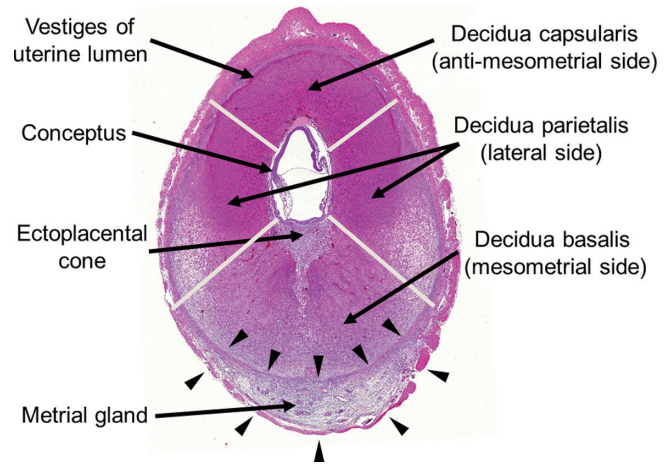


Fig. 10. Transverse sections of the decidua and metrial gland on GD 9.5. Division of the decidua into the decidua capsularis, decidua parietalis, and decidua basalis. The metrial gland in the mesometrial triangle with borders on two smooth muscle layers (arrows). HE stain.

gen receptors (ER) α and ER β are expressed in the decidua and are differentially regulated by prolactin and steroids⁶¹.

The decidua is an important tissue in protection against heavy metals. Metallothionein is present in the decidua from GD 9 onward, increases until GD 15 in tissue surrounding the embryo/fetus⁵³, and then decreases with progression of placental development.

(2) Metrial gland

• Morphology

The metrial gland occupies a space in the mesometrial triangle and borders on two smooth muscle layers in the early gestation period. These muscle layers are disrupted as the metrial gland develops⁶² (Fig. 10, 11). The metrial gland is composed of uterine natural killer (uNK) cells, decidualized endometrial stromal cells, invasive trophoblasts, blood vessels (including spiral arteries), and fibroblasts⁶³. Developmentally, the cellular proliferative activity in the metrial gland is at its peak on GD 11 or 12 and then gradually decreases toward late gestation (Fig. 7). In response, the metrial gland fully develops on GD 13 and comprises the majority of the maternal part of the placenta until parturition^{8, 64} (Fig. 1).

Two types of trophoblasts from the basal zone are present in the metrial gland⁶⁵ (Fig. 11). Interstitial trophoblasts penetrate through the decidua basalis and myometrium on GD 15 and are often situated in perivascular locations of the metrial gland^{11, 44}. They are one of the main cell types constituting the metrial gland after invasion. Endovascular trophoblasts invade into the spiral arteries, where they replace endothelial cells from GD 13 or 14 onward⁶⁶.

• Physiological function and toxicological features

The metrial gland is a unique decidual tissue characterized by the appearance of uNK cells and is rodent specific. Development of the metrial gland is inhibited in connection with the reduced proliferative activity of uNK cells caused

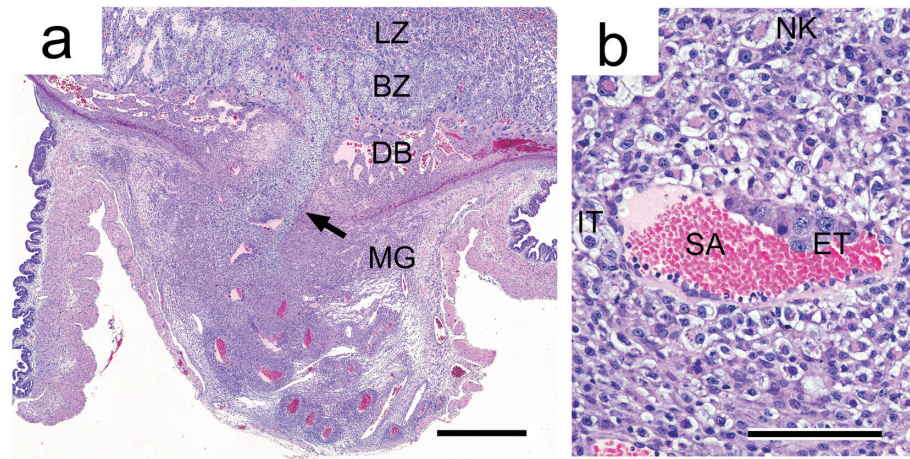


Fig. 11. Morphology of the metrial gland. a. Invasion of interstitial trophoblasts (arrow) into the metrial gland on GD 15. HE stain. Bar=900 μ m. b. Invasion of endovascular trophoblasts into spiral arteries and localization of interstitial trophoblasts around spiral arteries in stroma. HE stain. Bar=100 μ m. BZ, basal zone; DB, decidua basalis; ET, endovascular trophoblast; IT, interstitial trophoblasts; LZ, labyrinth zone; MG, metrial gland; SA, spiral artery; NK, uNK cell.

by tamoxifen⁶⁷, estrogen⁶⁸, and ovariectomy⁶⁹. In uNK gene knockout mice (TgE26 mice), the mesometrial triangle area does not develop into the metrial gland, and the absence of uNK cells results in a small placenta and poor reproductive performance⁷⁰. The importance of progesterone in uNK cell differentiation and proliferation has been shown in studies on the effects of ovariectomy with replacement progesterone treatment^{62, 71}. On the other hand, impaired basal zone development leads to metrial gland hypoplasia^{14, 22, 72}, since invasion of interstitial trophoblasts derived from the basal zone is inhibited⁷³. Thus, it is thought that the metrial gland is a hormone-dependent tissue⁷⁴, and its development is also affected by uNK cell proliferation and basal zone development.

Uterine natural killer cells are the main component of the metrial gland and are also known as granulated metrial gland (GMG) cells in rodents. Decidualized endometrial stromal cells produce signals that trigger recruitment and guide uNK cell differentiation to express a different phenotype from that of circulating NK cells⁷⁵. The uNK cells play critical roles in maternal immune tolerance at the maternal-fetal interface by facilitating the pairing of fetal leukocyte antigen and maternal killer inhibitory receptors⁷⁶. In addition, they produce protease that destroys the spiral arterial basement membranes and produce vascular endothelial growth factor that stimulates endothelial cell proliferation in order to promote vascular remodeling and angiogenesis^{77, 78}. Insufficient uNK cell activation contributes to poor spiral arterial remodeling⁷⁹. Failure of spiral arterial remodeling increases maternal blood pressure⁸⁰ and reduces subsequent placental perfusion. Therefore, alterations of uNK cell function and inadequate remodeling of spiral arteries in the metrial gland are linked to pregnancy-associated diseases such as preeclampsia, IUGR, and premature pregnancy termination^{81–83}.

Comparison with Human Placenta

1) Fetal part of the placenta

The placental villi that show many tree-like projections in humans are functionally analogous to the labyrinth zone in rats. The placentas of humans and rats are categorized as the hemochorial type, as the trophoblasts of both are directly bathed in maternal blood⁸⁴ (Fig. 12). The fetal-maternal interface in humans is comprised of a single syncytiotrophoblast layer and single cytotrophoblast layer and is categorized as hemomonochorial. In contrast, the interface in rats is comprised of three layers and is therefore hemotrichorial (Table 2). This difference between rats and humans might affect fetal-maternal exchange processes in the placental barrier⁸⁵.

The basal plate is the bottom of the intervillous space and represents part of the maternal-fetal junction. It is composed of an admixture of extravillous trophoblasts, various endometrial stromal cells, decidual cells, blood vessels (including spiral arteries), and endometrial glands with fibrinoid overlying the myometrium⁸⁶ (Fig. 12). Multinucleated trophoblastic giant cells are present in the deep basal plate at the myometrial border. The basal plate is roughly analogous to the basal zone in rats, based on to its anatomical location⁸⁵, but the morphology is not necessarily similar. Therefore, caution is required in extrapolation of toxicological effects on the basal zone from the rat placenta to the human placenta.

The yolk sac in humans does not enlarge and remains as a stalk only. The yolk sac floats like a balloon within the exocoelomic cavity and never becomes directly apposed to the chorionic plate, maternal uterine wall, or uterine lumen⁸⁷ (Fig. 9). It is thought that the human yolk sac does not function as a placenta in the sense of being able to transfer nutrients between maternal and embryonic circulations^{86, 88, 89}.

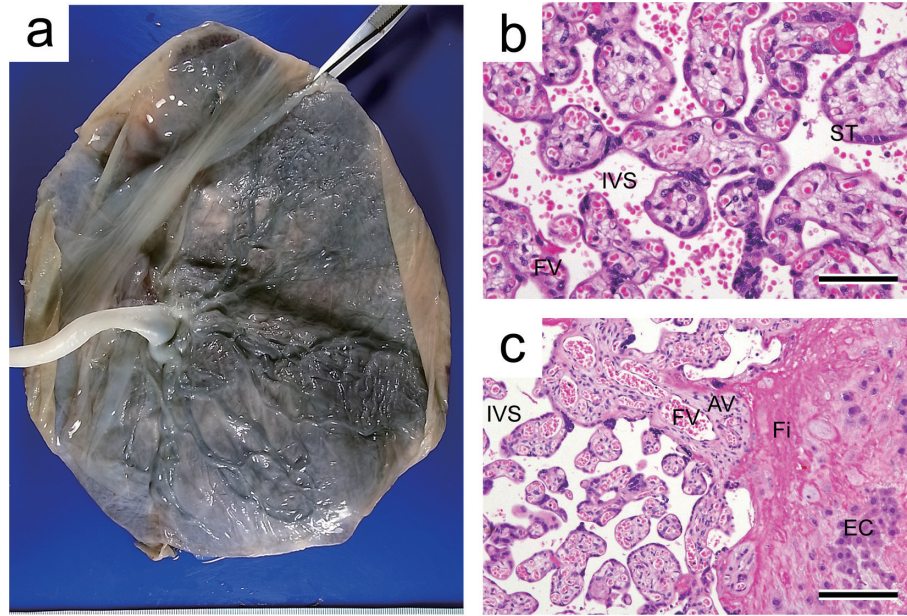


Fig. 12. Human placenta. a. Macrograph of a placenta at term pregnancy. b. Placental villi. HE stain. Bar=75 µm. c. Surface of the basal plate with extravillous cytotrophoblast invasion and fibrinoid deposition. HE stain. Bar=150 µm. AV, anchoring villus; EC, extravillous cytotrophoblast; Fi, fibrinoid; FV, fetal vessel; IVS, intervillous space; St, syncytiotrophoblast.

Therefore, embryo toxicity induced by yolk sac dysfunction during the period before chorioallantoic placenta formation in rats cannot necessarily be extrapolated to humans. Toxicologically, this is one of the important species differences between rat and human placentas⁹⁰. Caution is therefore also required in extrapolation of embryo toxicity from rats to humans during the period before chorioallantoic placenta formation⁹¹. In addition, there is no inverted yolk sac placenta in humans. Thus, an animal model that does not rely upon an inverted yolk sac for embryonic nutrition should be used to elucidate any potential risk of developmental toxicity caused by chemicals in humans.⁸⁹

2) Maternal part of the placenta

In humans, the decidua, which underlies the basal plate, is the site of implantation. The spiral arteries that connect the maternal uterine arteries to the intervillous space penetrate through the decidua. The decidua in humans is analogous to the decidua and metrial gland in rats. There are three types of decidual cells in humans: small predecidual swollen fibroblasts, large undifferentiated decidual cells, and large differentiated decidual cells. These decidual cells are isolated from each other by an investment of fibrinoid and are linked by fewer gap junctions than are found in rats⁴⁷. The decidua contains many uNK cells, which are also known as large granular lymphocytes in humans. Many of these are aggregated around the spiral arteries⁹² and play a role in the control of trophoblast invasion and vascular remodeling⁹³. Therefore, the uNK cells in humans have approximately the same function as those in rats⁸². In humans,

preeclampsia is believed to result in part from inadequate maternal blood flow to the implantation site associated with angiogenesis inhibition and inadequate remodeling of spiral arteries^{94, 95}. The histological feature of preeclampsia is restricted invasion of spiral arteries into the placental bed, with endovascular trophoblast invasion limited to the decidual vessels⁹⁶. Tamoxifen induces the angiogenesis inhibition and inadequate remodeling of spiral arteries in the metrial glands in rats⁶⁷.

3) Steroid hormone biosynthesis

There are species differences in the steroid hormone biosynthetic pathway in the placenta that result from differences in enzyme expression associated with this pathway (Fig. 13). In humans, the synthesis of pregnenolone and progesterone from cholesterol is carried out in the placenta. However, pregnenolone is converted to dehydroepiandrosterone in the adrenal gland of fetuses, as CYP17 is absent from the human placenta⁹⁷. Dehydroepiandrosterone is transported into the placenta and is then converted to estrogen⁹⁸. In contrast, pregnenolone synthesized from cholesterol is converted to androgen in the rat, as CYP17 is present in the rat placenta⁹⁹. However, as aromatase is not present in the rat placenta, estrogen is not synthesized in the placentas but is instead synthesized in ovary. The androgen in the rat placentas might contribute to estrogen synthesis in the ovaries. Therefore, caution is also required in extrapolation of toxicological effects on the steroidogenic pathway from rat to human placentas.

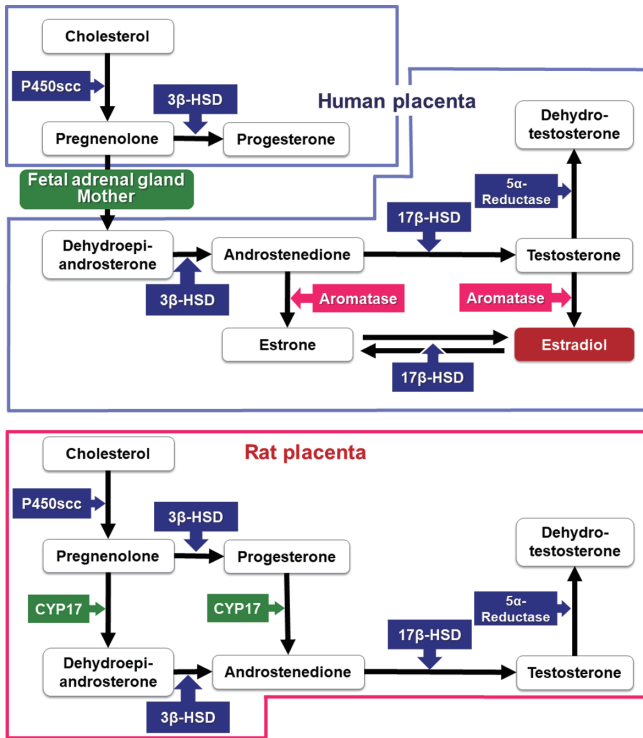


Fig. 13. Pathway of steroid hormone biosynthesis in rats and humans.

Toxicological Significance of Placental Weight Change

Placental weight shows a high correlation with placental transport and metabolic mechanisms that qualitatively and quantitatively affect placental-fetal nutrient exchange¹⁰⁰. In humans, a strong relationship has been reported between fetal and placental weight¹⁰¹. In rats, this relationship at GD 21 is the basis of our previous placental toxicity studies using Wistar Hannover rats, as shown in Fig. 14. The correlation is present except when there is a significant increase in placental weight. Conversely, placental weight is strongly affected by placental histopathological lesions (Fig. 15). In particular, placental weight reflects changes in the labyrinth zone in middle and late gestation, since the labyrinth zone becomes a major part of the placenta with advancing pregnancy. An increase in placental weight is induced by the adaptive response to a circulatory disturbance in the labyrinth zone¹⁰², hypertrophy of the labyrinth zone²⁶ and basal zone²⁴, or a reduction in the number of fetuses to less than 6¹⁰³. A decrease in placental weight is mainly induced by hypoplasia of the labyrinth zone, resulting from apoptosis and/or necrosis of trophoblasts, and usually leads to IUGR. However, despite the fact that placental weight was reduced to about 70% of that of the control group in a previous study, it was found that a compensatory response by the placenta might prevent IUGR from being induced¹⁸. Moreover, it is difficult to detect hypoplasia of the metrial gland from placental weight changes despite induction of IUGR, because

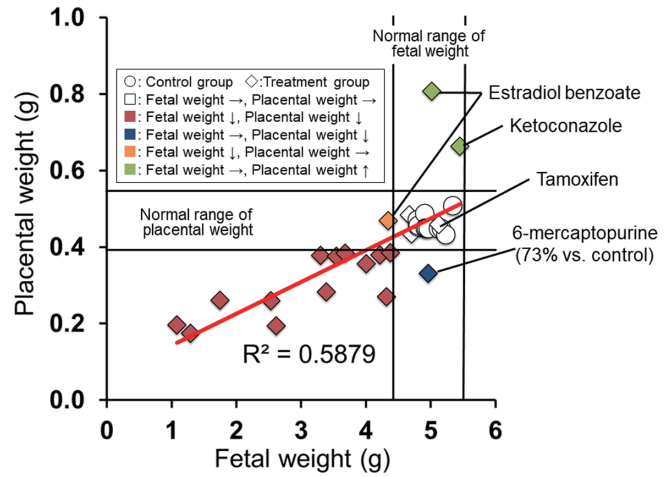


Fig. 14. Relationship between fetal and placental weight. Normal range of weight, mean \pm 3 SD.

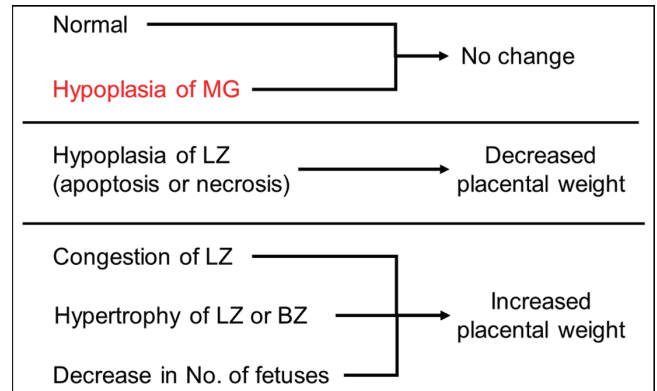


Fig. 15. Pathogenesis of placental weight changes. BZ, basal zone; LZ, labyrinth zone; MG, metrial gland.

the placentas are separated at the basal zone and decidual basalis and are removed from the uterine wall for placental weight measurement. Therefore, although histopathological examination of the placenta is not generally performed in reproductive and developmental toxicity studies, not only placental weight but also placental histopathology should be considered in order to accurately evaluate the secondary effects of placental damage on embryo/fetal toxicity.

Conclusion

The placenta plays a pivotal role in fetal growth, and placental dysfunction and injury are associated with embryo/fetal toxicity. Although the placental weight change is one of the most important indexes of placental toxicity in reproductive and developmental toxicity studies, the placental weight assessment alone is not always enough to evaluate placental toxicity. In order to accurately evaluate reproductive and developmental toxicity studies, it is important to

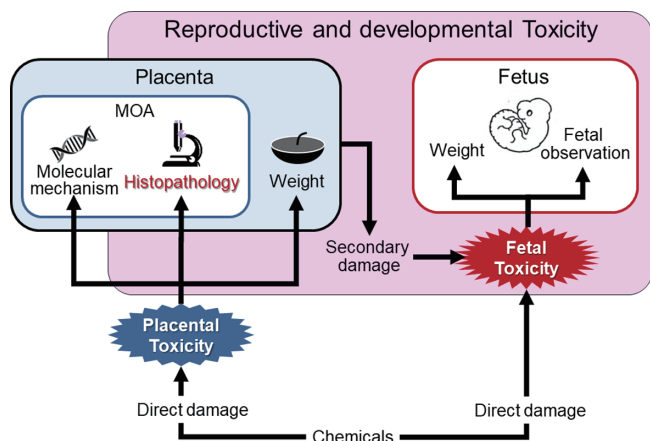


Fig. 16. Significance of placental histopathology in reproductive and developmental toxicity studies.

identify the histopathological changes in each part of the placenta and to elucidate the mechanism of placental toxicity through molecular investigation (Fig. 16). The placenta is toxicologically susceptible to various chemicals because of its high blood flow, rapid growth, and sex-hormone dependence. The placenta has these complex toxicological features, which are unlike those of other organs, and placental toxicity depends on the timing of pregnancy. Thus, the time-dependent histopathological examination of the rat placenta should be based on understanding of normal developmental changes in morphology and function. In addition, extrapolation of placental toxicity from rats to humans should be done cautiously in evaluation of risk factors because of species differences.

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