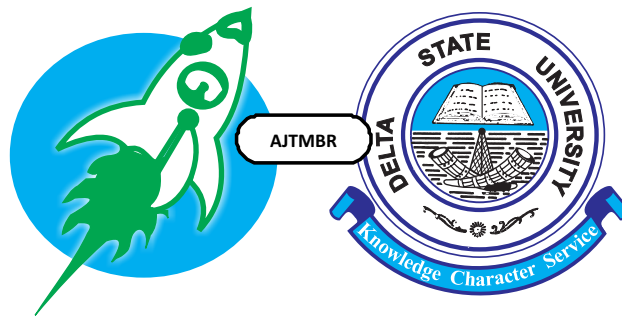


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Microanatomy of Term Placenta: Insights from Human and Wistar Rat Studies

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ABSTRACT

Introduction: The placenta is crucial for the development and survival of the maturing foetus. Wistar rat models are commonly used in biomedical research to study human biological responses. However, there is paucity of literature on the histology of Wistar rat placenta in open-access journals.

Materials and Methods: In this study, young adult male and nulliparous female Wistar rats were obtained, acclimatized, and allowed to mate overnight. On the 20th day of gestation, the pregnant rats were euthanized, and their placentas were collected and fixed in 10% neutral buffered formalin. Human placentas were sampled from patients who had normal vaginal deliveries. All samples were processed using haematoxylin-eosin, Masson trichrome, periodic acid-Schiff (PAS), reticulin.

Results and Conclusion: Both human and Wistar rat placentas were haemochorial, meaning the trophoblasts in both species are in direct contact with maternal blood. Differences were observed at the foetal-maternal interface and in the presence of the yolk sac. The highly vascularized labyrinth of the rat placenta features maternal and embryonic blood spaces separated by a trilaminar trophoblast layer and embryonic endothelial cells. In contrast, human-term placental villi consist of thin-walled capillaries lined by endothelial cells, which are surrounded by a syncytiotrophoblast layer and an inconspicuous cytotrophoblastic layer. While the basal plate and chorion of the human and Wistar rat placentas have similar anatomical positions, their morphologies differ. Additionally, the distribution of PAS positivity, collagen, and reticulin fibres varies between the two species. Therefore, caution is advised when extrapolating findings from toxicological studies in rat placentas to humans.

Keywords: Term Placenta, Microanatomy, Human, Wistar rat

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INTRODUCTION

The placenta serves as an interface between the pregnant mother and the developing foetus, playing a vital role in the development and survival of the maturing foetus. Its functions include anchoring the developing foetus to the uterine wall, producing hormones, and

facilitating nutrient uptake, oxygen and carbon dioxide exchange, and waste elimination. Placental dysfunction and injury can have adverse effects on the maintenance of pregnancy and foetal development. Given its significance, understanding placental structure and function is crucial for studying developmental diseases and

conditions that impact pregnancy outcomes.¹⁻⁸

The rat placenta consists of both foetal and maternal components. The foetal part includes the yolk sac, chorion, labyrinth zone, and basal zone (also known as the junctional zone). The maternal part includes the decidua and metrial gland. Placental cells originate from the trophoctoderm of the embryo and the endometrium of the mother.⁷

The first cell lineages to differentiate in a mammalian embryo are the trophoctoderm cells.^{2,3,4} The trophoctoderm further differentiates so that the portion surrounding the inner cell mass becomes the polar trophoblast, while the part around the blastocoel becomes the mural trophoblast. The polar trophoblast actively proliferates, stimulated by signals from the inner cell mass (ICM). In contrast, mural trophoblast cells are not in close contact with the ICM, and do not benefit from the trophoblast growth factors produced by the embryonic cells. They therefore, exit the mitotic cell cycle, enlarge, and undergo rounds of DNA replication without mitosis, eventually becoming polyploid and forming primary giant cells.^{3,4,10,11,12} After implantation, the mural trophoctoderm differentiates into primary trophoblastic giant cells, which play a crucial role in regulating decidualization. This is the process by which the maternal endometrium is gradually remodelled into the specialized tissue (decidua) needed to support the conceptus. Furthermore, once the polar trophoblast comes into contact with the endometrium, it begins to proliferate and develop into the extra-embryonic ectoderm and the ectoplacental cone. The edge and centre of the ectoplacental cone then differentiate into secondary trophoblastic giant cells and spongiotrophoblasts, respectively, ultimately forming the basal zone. The chorion, which is the embryonic-side membrane of the

ectoplacental cone, fuses with the allantois and eventually differentiates into the labyrinth.^{3,6,9,10,11}

Wistar rat models have become a common choice in biomedical research, particularly in Nigeria, due to their physiological and anatomical similarities to humans.¹³ A key aspect of understanding rat models for human developmental biology is having a solid grasp of the normal anatomy of the placenta. However, free open-access literature regarding the histology of Wistar rat placenta—both normal and abnormal—is relatively sparse. This study aims to fill this gap by providing a detailed comparative analysis of the foetal part of the term placentae in humans and Wistar rats. Such comparisons will enhance our understanding of placental biology and improve the reliability of Wistar rat models in studying human reproductive health.

METHODS

Young adult male and nulliparous female Wistar rats were obtained and quarantined for one week. They were allowed to mate overnight. The rats were fed pelletized feed and had access to clean water *ad libitum*. On day 20 of gestation, the pregnant rats were euthanized.^{4,9} The placentas were collected, separated, and fixed in 10% neutral buffered formalin. They were then dehydrated using increasing gradients of alcohol, cleared in xylene, and embedded in paraffin wax. This study received approval from the Ethical Review Committee of the University of Ilorin.

Paraffin blocks of human placentas were donated by Dr. Folaranmi from the Department of Anatomic Pathology at the University of Ilorin Teaching Hospital. The placenta samples were obtained from control group patients involved in an earlier study. Participants were recruited from the labour ward, comprising maternal ages ranging from 18 to 40 years, with term deliveries having normal clinical and laboratory parameters.

Participants provided consent for the study, which was also approved by the Ethical Review Committee of the University of Ilorin Teaching Hospital.

Five-micron thick sections were cut using a rotary microtome. The sections were subsequently stained with Haematoxylin and Eosin, Masson's Trichrome (to demonstrate the presence of collagen and fibrin), and Periodic Acid-Schiff (PAS) to highlight polysaccharides, along with a reticulin stain for reticulin fibres.^{14,15,16}

Slides were examined using a light microscope, and regions of interest were captured with a camera attached to a light microscope using Amscope 3.7 software. White balance correction was performed using Adobe Photoshop Lightroom (v9.50).

RESULTS

The human and Wistar rat placentae are

haemochorial, meaning that trophoblast cells come into direct contact with maternal blood. There are notable differences between rats and humans regarding the histological structure of the placenta which include the foetal-maternal interface and the presence of the yolk sac. The chorionic plate, chorionic villi and basal plate in the human placenta correspond in anatomical position to the chorion, labyrinth, and basal zone in the Wistar rat, respectively.

Haematoxylin-Eosin Staining:

The foetal portion of the Wistar rat placenta comprises the yolk sac, chorion, labyrinth, and basal zone, also known as the junctional zone (Figure 1). The yolk sac has a papillary structure and is attached to the chorion (Figure 1). Its epithelial lining consists of a single layer of cuboidal cells with central, round nuclei, characterized by scalloped edges at the apex. Within the yolk sac are thin-walled, capillary-sized vascular channels (Figure 2).

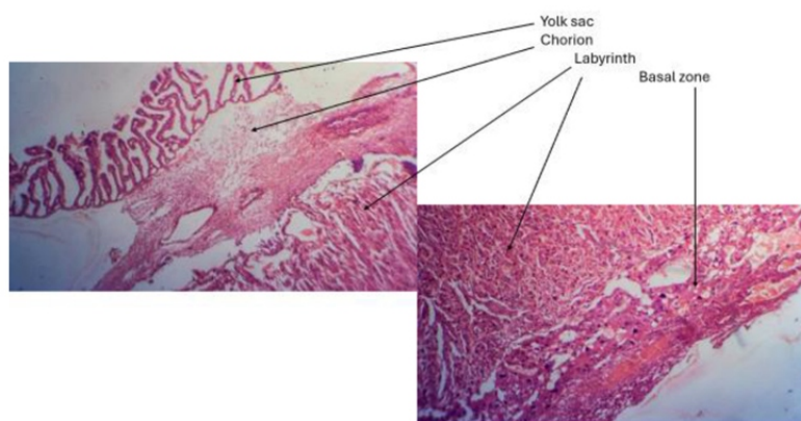


Figure 1. H&E photomicrograph of the layer of the foetal part of Wistar rat placenta. x40

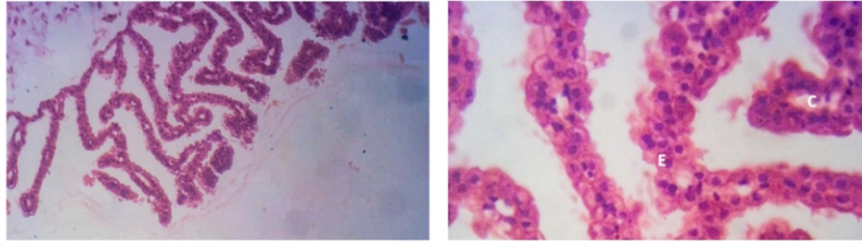


Figure 2. H&E photomicrograph of the yolk sac. Ax100, Bx400.
E: epithelial cell, C: capillary sized vascular channel.

The chorion is made up of plump to spindle-shaped cells with oval to spindle nuclei and abundant eosinophilic cytoplasm (Figure 3A).

Its stroma is loose, collagenous and includes thick-walled vascular channels.

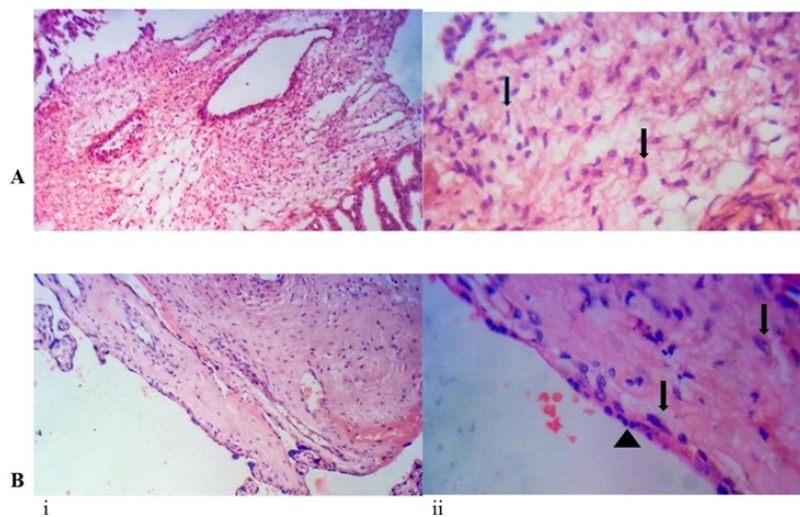


Figure 3. H&E photomicrograph of the A: Chorion in Wistar rat, B: Chorionic plate in human placenta. i: x40, ii: x400. Arrow: Spindle cells; Arrow head: Amnion.

In the highly vascularized labyrinth (Figure 4A), maternal and foetal blood spaces are separated by a trilaminar trophoblast, which includes a single layer of sinusoidal trophoblast lining the maternal sinusoids and two layers of trophoblast cells. The sinusoidal trophoblast cells are relatively large, featuring large basophilic nuclei and ample cytoplasm. The

lining of the maternal sinusoids by the trophoblastic cells is not continuous. In contrast, other trophoblastic cells in the trilaminar wall are smaller, with round nuclei, and some are multinucleated. The non-sinusoidal trophoblasts form a continuous layer. Anucleated red blood cells are present within both the maternal sinusoids and foetal capillaries.

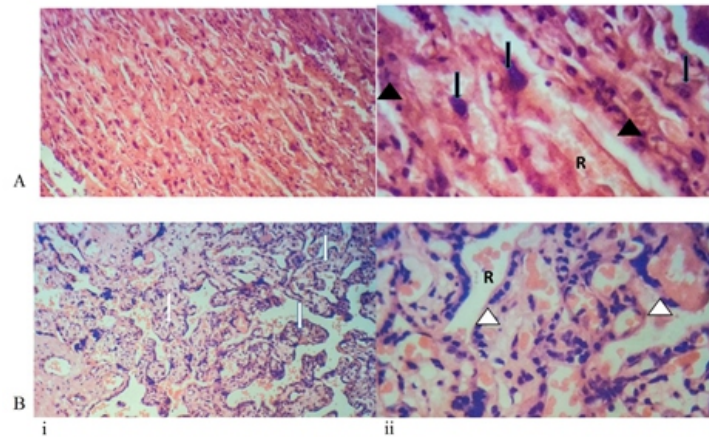


Figure 4. H&E photomicrograph of the A: labyrinth, B: villi. i: x40, ii: x400

Black arrow: sinusoidal trophoblast, black arrow head: trillaminar wall, white arrow: villi, white arrowhead: syncytiotrophoblast. R red blood cells

Beneath the labyrinth lies the basal zone, which contains spongiotrophoblasts and giant trophoblastic cells. Maternal sinuses, lined by giant trophoblastic cells, can also be found within the basal zone (Figure 5A). The giant trophoblasts are large, fairly pleomorphic, and much larger than the sinusoidal trophoblasts, featuring huge basophilic nuclei and abundant eosinophilic cytoplasm. Sheets of

spongiotrophoblast cells are found beneath the giant trophoblasts; these spongiotrophoblasts are monomorphic, consisting of plump cells with oval basophilic nuclei and eosinophilic cytoplasm. Numerous capillary-sized blood vessels lined by flattened endothelial cells are observed within the sheets of spongiotrophoblast, along with dilated congested blood vessels.

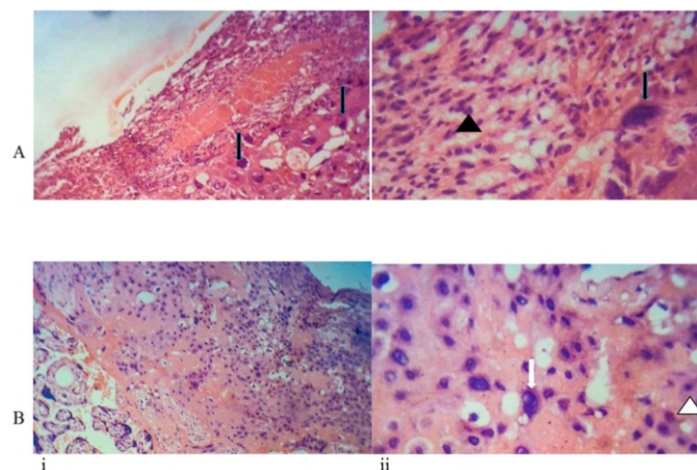


Figure 5. H&E photomicrograph of the A: Basal zone in Wistar rat, B: Basal plate in human. i: x40, ii: x400.

Black arrow: giant trophoblast, black arrow head: spongiotrophoblast, white arrow: extravillous trophoblast, white arrowhead: decidual cell,

The human term placenta consists of the chorionic plate, villous parenchyma, and basal plate (Figure 6). The chorionic plate is formed by the amnion and chorion (Figure 3B). The

amnion is a single layer of flattened epithelial cells, while the chorion is fibrocollagenous and contains medium-sized vascular channels.

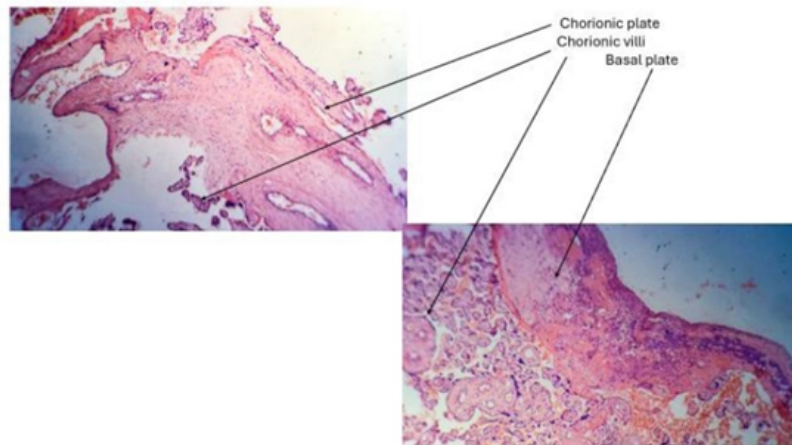


Figure 6. H&E photomicrograph of the human placenta. x40

The chorionic villi, which vary in size and are oval to irregular in shape, are filled with capillaries and lined by a single layer of syncytiotrophoblast (Figure 4B). The villi can be categorized as stem, intermediate, and terminal, with stem and intermediate villi being larger than the smaller terminal villi. The core of the villi is fibrocollagenous and contains spindle cells (fibroblasts) with tapered nuclei and thin-walled capillaries. In terminal villi, there is close opposition between the capillaries in the core and the lining syncytiotrophoblast. Syncytial knots are primarily observed in the terminal villi. Red blood cells are found in the surrounding spaces (maternal sinusoids) adjacent to the villi.

The basal plate (maternal surface) includes extravillous trophoblast cells, decidual cells, fibrin, and vascular channels (Figure 5B). Extravillous trophoblast cells are large, featuring oval basophilic nuclei and scant cytoplasm. In

contrast, decidual cells are much smaller, characterized by central oval nuclei and abundant eosinophilic cytoplasm. The fibrin appears as light eosinophilic deposits (Figure 5B).

Masson Trichrome (MT) Staining:

Masson trichrome staining highlights collagen fibres, which can be used to assess and stage fibrosis. In this staining method, collagen appears blue, fresh fibrin stains orange-yellow, mature fibrin is red, and old fibrin is blue. The human chorion is densely collagenized when compared to the chorion in Wistar rats. The labyrinth zone contains very few collagen fibres. The cores of terminal villi show little to no collagen, in contrast to the intermediate and stem villi. In the basal plate of the human placenta, old fibrin appears blue with the Masson trichrome stain, while mature fibrin appears red. In the basal zone of Wistar rat placentas, there is an absence of collagen fibres and fibrin deposition (Figure 7)

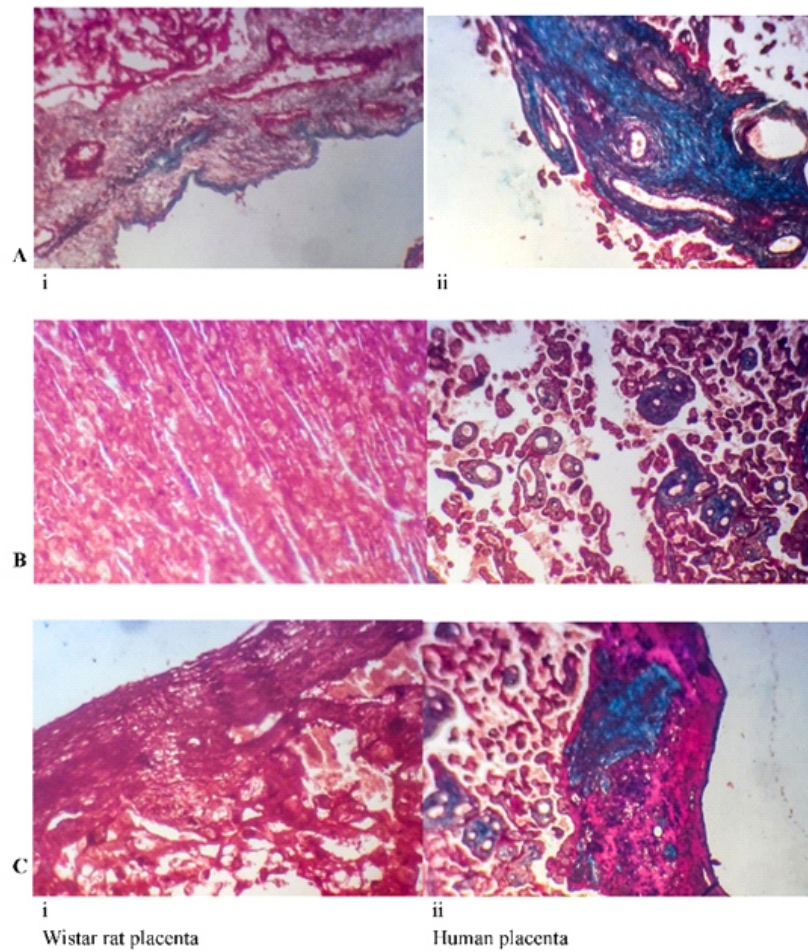


Figure 7. Masson Trichome

A: i. Chorion, ii. Chorionic plate.

B: i. Labyrinth, ii. Villi.

C: i. Basal zone, ii. Basal plate.

Reticulin Staining:

Reticulin staining stains reticular fibres black. Reticular fibres are thin type III collagen fibres. The distribution of reticulin fibres closely mirrors that of collagen fibres. The human chorion demonstrated a dense distribution of reticulin fibres compared to the chorion in Wistar rats. The labyrinth zone has no strands of

reticulin fibres located within the trophoblastic septae. Villi exhibit varying amounts of reticulin, with the most abundance found in the stem and intermediate villi. There are no reticulin fibres present in the basal zone of Wistar rat placentas, while the basal plate of the human placenta shows perivascular reticulin fibres (Figure 8).

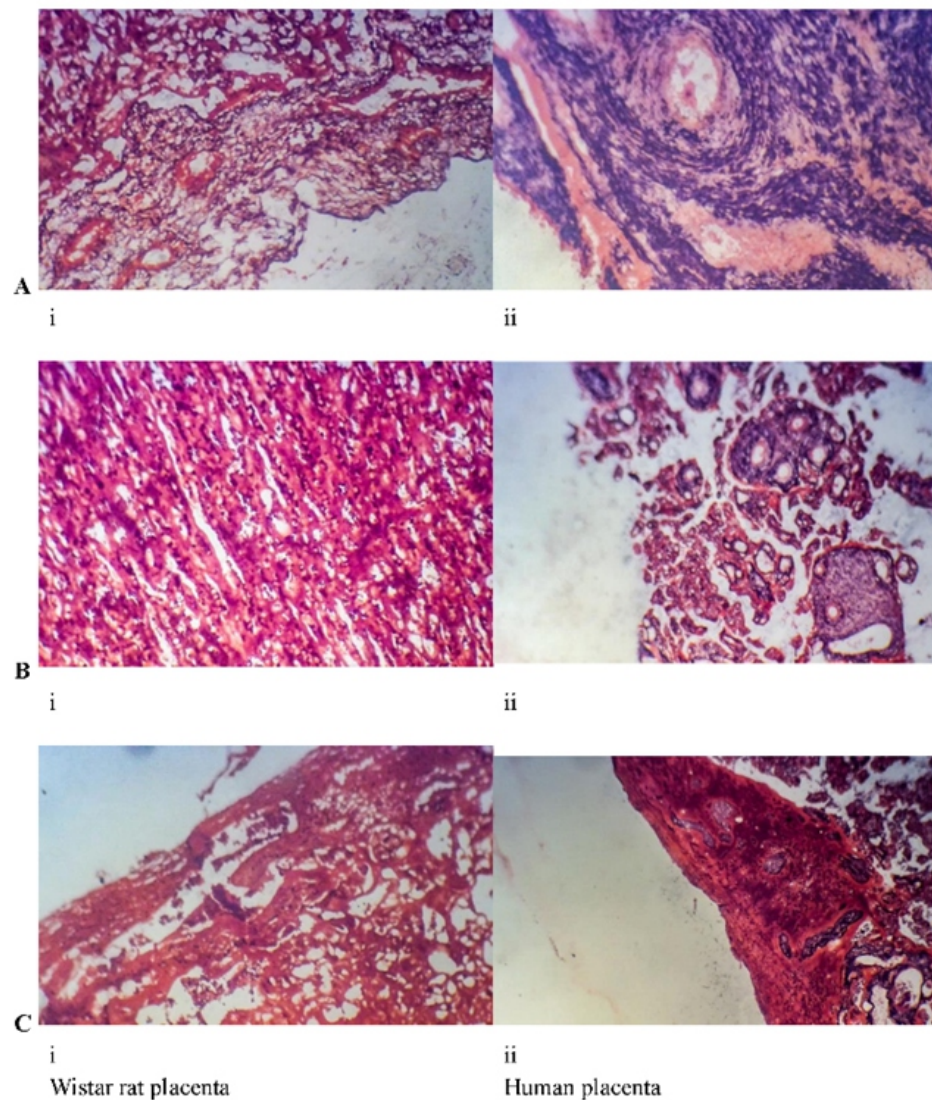


Figure 8. Reticulin stain A: i. Chorion, ii. Chorionic plate.
 B: i. Labyrinth, ii. Villi.
 C: i. Basal zone, ii. Basal plate.

Periodic Acid-Schiff (PAS) Staining:

PAS stains basement membrane matrix, glycogen, and mucopolysaccharides magenta. The chorion in Wistar rat and chorionic plate demonstrated loose and dense PAS positive areas respectively (Figure 9). The foetal capillary

endothelial basement membrane are the only areas showing positive PAS stain in the labyrinth (Figure 9, Figure 10). The cores of stem, intermediate, and terminal villi demonstrated positive reactions to PAS staining. Foci of intracellular and extracellular positive PAS were

seen in the basal zone of Wistar rat. The extracellular PAS positivity were noticed around the vascular channels (Figure 10). Extensive

regions of positive PAS staining were found in the basal plate of the human placenta (Figure 9).

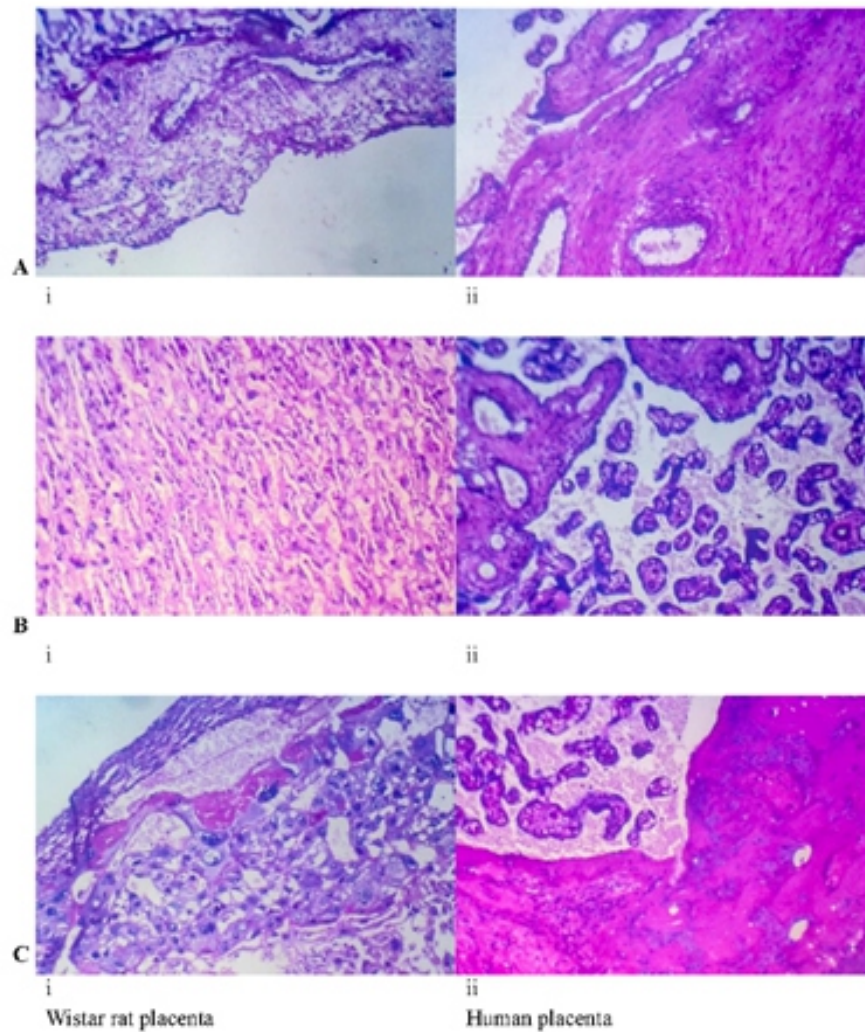


Figure 9. PAS. A: i. Chorion, ii. Chorionic plate.
B: i. Labyrinth, ii. Villi.
C: i. Basal zone, ii. Basal plate.

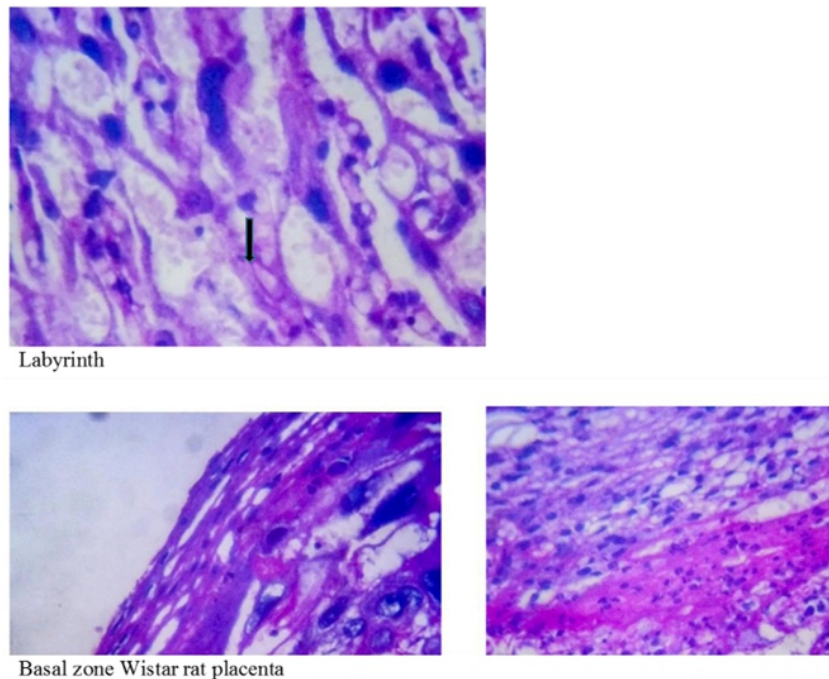


Figure 10. PAS x400.
Black arrow PAS positive endothelial basement membrane.

DISCUSSION

This overview of microscopic placental anatomy serves as a valuable reference for researchers identifying and describing structural changes when working with Wistar rat models of placenta toxicity or diseases.

In mammals, two types of placentas are present during gestation: the yolk sac placenta (or choriovitelline placenta) and the chorioallantoic placenta.^{1,4,6,7} The yolk sac placenta serves as a temporary structure during the early stages of pregnancy before the chorioallantoic circulation is established. The yolk sac actively absorbs nutrients from the chorion and the chorionic cavity, transporting them to the embryo through the yolk sac circulation. In humans, as well as in most mammals, the yolk sac placenta becomes vestigial after the first trimester. It remains as a

small stalk, floats within the exocoelomic cavity and never directly contacts the chorionic plate. In contrast, in rodents, the yolk sac placenta persists and continues to provide nutrients to the foetus until term. The chorioallantoic placenta is the primary placenta in mammals during the middle to late stages of gestation. It is formed from the endometrium of the mother and the trophoblast of the embryo.^{1,4,6,7}

The chorionic plate serves as the attachment site for the umbilical cord vessels [17]. The umbilical vessels enter and exit the placenta at the chorion. Microscopically, the chorionic plate is composed of dense, collagenized connective tissue that surrounds thick-walled arteries and veins. The human chorionic plate exhibits a high degree of collagenisation, as demonstrated by Masson's trichrome (MT) staining. In contrast, the chorion

of the Wistar rat shows minimal collagen compared to that of humans. The distribution of reticulin fibres is similar to that of the collagen fibres.

Both Wistar rat and human placentas depend on maternal blood from the uterus, which enters the placenta through the decidua and then filters through the labyrinth (in rats) or chorionic villi (in humans).^{17,18,19} The labyrinth and villi are responsible for the exchange of oxygen, carbon dioxide, metabolites, and hormones between the foetus and the mother. These structures receive dual blood supply from both the foetal and maternal circulations. Throughout the labyrinth and villi, maternal sinusoids are situated close to foetal vessels. Despite their proximity, the embryonic and maternal circulations never mix at any point during gestation. Although both the labyrinth and villi serve the same function, they differ structurally.^{1,4,5,17,18}

The labyrinth contains maternal sinusoids and trophoblastic septa, which are composed of a trilaminar trophoblastic epithelium and foetal capillaries. As pregnancy progresses, there is a decrease in the cellular density within the trophoblastic septa and an increase in the size of the sinusoidal trophoblasts.^{4,8,9,20} The labyrinth lacks collagen fibres, but reticulin staining reveals a few reticulin fibres in the trilaminar septa. Additionally, there is a scarcity of extracellular matrix in the trilaminar septa, as shown by periodic acid-Schiff (PAS) staining.

Villous trees consist of approximately 40 major primary stem villi, which protrude perpendicularly downward from the chorionic plate. Each villus contains vascular channels and fibrocollagenous stroma. The primary villi divide into 4 to 8 secondary stem villi (also known as intermediate villi) that run parallel to the chorionic plate. These secondary villi further

subdivide into tertiary stem villi (terminal villi), which form the placental lobules and attach to the basal plate. The terminal villi represent the main area for nutrient and gas exchange in the placenta.^{17,18,19,20} They are made up of capillaries and perivascular fibroblasts but have few associated collagen and extracellular matrix as demonstrated with MT and PAS. In contrast, the stem and intermediate villi contain a collagenized core, as demonstrated by MT staining, and do not function as exchange areas within the placenta. PAS stain demonstrated decrease in extracellular matrix from stem to intermediate villi to the terminal villi. Surrounding these villi is a trophoblast bilayer that consists of an outer syncytiotrophoblast layer and an inner cytotrophoblast layer. By midterm to term, the cytotrophoblast becomes less noticeable.

The basal plate is the maternal surface of the placenta. In humans, the basal plate consists of fibrin, extravillous trophoblast cells, decidua basalis, and maternal vascular channels. Endometrial spiral arteries undergo physiological transformation characterized by lumen dilation, invasion of the endothelial lining by extravillous trophoblasts, and the fibrinoid replacement of the muscular and elastic tissue in the arterial wall. As a result, maternal spiral arteries change from high resistance, low capacitance vessels to low resistance, high capacitance vessels.^{5,20,21} Fibrinoid deposition was demonstrated by blue and red MT stains in the basal plate of the human placenta. In contrast, no fibrinoid changes were observed in the basal zone of the Wistar rat placenta. The basal zone in Wistar rat placenta is composed of spongiotrophoblasts, glycogen cells, and trophoblastic giant cells.^{4,6,7} Glycogen cells, which accumulate glycogen-rich granules, form small clusters and develop into glycogen cell islands, comprising a significant portion of the basal zone on gestational days (GD) 15 and 16. They exhibit nuclear pyknosis after GD 17, leading to basal

zone regression, and by GD 21, almost all glycogen cells disappear while spongiotrophoblasts and trophoblastic giant cells become the main structural components of the basal zone.^{4,5} The periodic acid-Schiff (PAS) stain demonstrated few remaining glycogen cells in the basal zone. There is an abundance of extracellular matrix in the basal plate of human placenta as shown by large areas of magenta colour of PAS stain.

In conclusion, this study highlight both the similarities and differences in placental structure between humans and Wistar rats. The haemochorial nature of the placenta in both species indicates functional similarities; however, the significant morphological differences highlight the limitations of directly applying findings from rat toxicology studies to humans.

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Author Contributions: Conceptualization: KMI, OOF Data acquisition: KMI, OOF, MOB, SOR. Interpretation: KMI, OOF. Drafting of the manuscript: KMI, SOR, AIA, AA. Critical revision of the manuscript: OOF. Approval of the final version of the manuscript: all authors.

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