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Chorioallantoic and yolk sac placentation in the plains viscacha (*Lagostomus maximus*) – A caviomorph rodent with natural polyovulation

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ABSTRACT

Objectives: Reproduction in the plains viscacha is characterized by the polyovulation of hundreds of oocytes, the loss of implantation and the development of 1–3 offspring. Our goal was to determine whether placental development was affected by these specializations.
Study design: Thirteen placentas from early pregnancy to near-term pregnancy were analyzed using histological, immunohistochemical and transmission electron microscopy.
Results: An inverted, villous yolk sac was present. Placentas were formed by the trophospongium, labyrinth and subplacenta. A lobulated structure with a hemomonochorial barrier was established early in pregnancy. Proliferating trophoblast that was clustered at the outer border and inside the labyrinth was responsible for placental growth. Trophoblast invasion resulted from the cellular trophoblast and syncytial streamers derived from the subplacenta. Different from other caviomorphs, numerous giant cells were observed.
Conclusions: The principle processes of placentation in caviomorphs follow an extraordinarily stable pattern that is independent of specializations, such as polyovulation.
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1. Introduction

The plains viscacha, *Lagostomus maximus* Desmarest, 1817, is a guinea pig-related caviomorph rodent with an ample distribution throughout Argentina [1]. It is one of the larger rodents of South America, with females weighing between 2 and 4.5 kg, and males weighing between 5 and 8 kg [1]. This species is characterized by the unique reproductive feature of natural polyovulation. Originating from the suppression of apoptosis, females polyovulate up to 800 oocytes per cycle [1–6] and just 10 to 12 blastocysts are implanted [7,8]. However, usually only two fetuses are maintained to birth after 154 days [1,2]. The surviving fetuses are those implanted near the cervical end of the uterus [4]. With the exception of basic data obtained from delivered tissues [9], placentation in *L. maximus* is unknown. Placentation has been well studied in several caviomorph species [10–25] because they are more attractive animal models for human placentation than other rodents [26,27]. Similarities to humans include the processes of trophoblast invasion and placental growth, a hemomonochorial barrier and a precocial reproductive strategy [13,18,19,21,24–29]. Previous data indicate that placentation in caviomorphs occurs in a stable pattern that is largely independent of body size [17,20,25]. However, no parallel mechanism to the unusual polyovulation in the plains viscacha has been observed in other caviomorphs. Thus, we aimed to substantiate its potential influence on the differentiation of both the chorioallantoic and yolk sac placenta in *L. maximus*.

2. Methods

Samples were obtained from free-living female viscachas from Estación de Cría de Animales Silvestres, Buenos Aires. The procedures followed that of established studies [e.g., [22,25]]. Materials included 15 placentas from early pregnancy to full-term pregnancy (Table 1). They were analyzed using the following techniques: histology (hematoxylin and eosin (HE) and periodic acid-Schiff (PAS) staining), lectinhistochemistry with DBA (*Dolichus biflorus*) lectin for recognize uNK cells, immunohistochemistry for cytokeratin (to mark epithelial/trophoblast cells; mouse monoclonal anti-human primary antibody 1:300; Clone 1A4, DakoCytomation) and vimentin (to identify mesenchymal/endothelial cells; mouse monoclonal anti-human primary antibody 1:200; V9, sc-6260, Santa Cruz Biotechnology), proliferating cell nuclear antigen (mouse monoclonal anti-human primary antibody 1:800; PC10, sc-56, Santa Cruz Biotechnology; negative control using PBS) and transmission electron microscopy (TEM).

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Table 1
Morphometric parameters of fetuses and placentas at different gestation stages.

Pregnancy stage[a]	Age (days)	*N*	CRL (cm)	PD (cm)
Early pregnancy	40–60	6	3.0–5.7	1.1–2.8
Middle-term pregnancy	80–120	5	8.0–10.5	2.2–3.4
Near-term pregnancy	130–150	4	11–13.0	2.5–3.0

N: number of animals studied; CRL: fetal crow-rump length; PD: placental diameter.
[a] Total gestation time = 154 days.

3. Results

3.1. The general structure of chorioallantoic placenta and yolk sac

The chorioallantoic placenta had a discoidal shape with a main placenta and a distinct subplacenta (Fig. 1A) and was attached to the uterus by a peduncle. The umbilical cords included a variable number of vessels; most often, there were two arteries and two veins. From early pregnancy onward, the main placenta was lobulated and was characterized by a labyrinth and a trophospongium around the lobes (Fig. 1B,C). The labyrinth was dually vascularized from the maternal and fetal systems, whereas the trophospongium had no fetal capillaries (Fig. 1B,C). The chorioallantoic placenta was covered by the non-villous parietal yolk sac above a well-developed

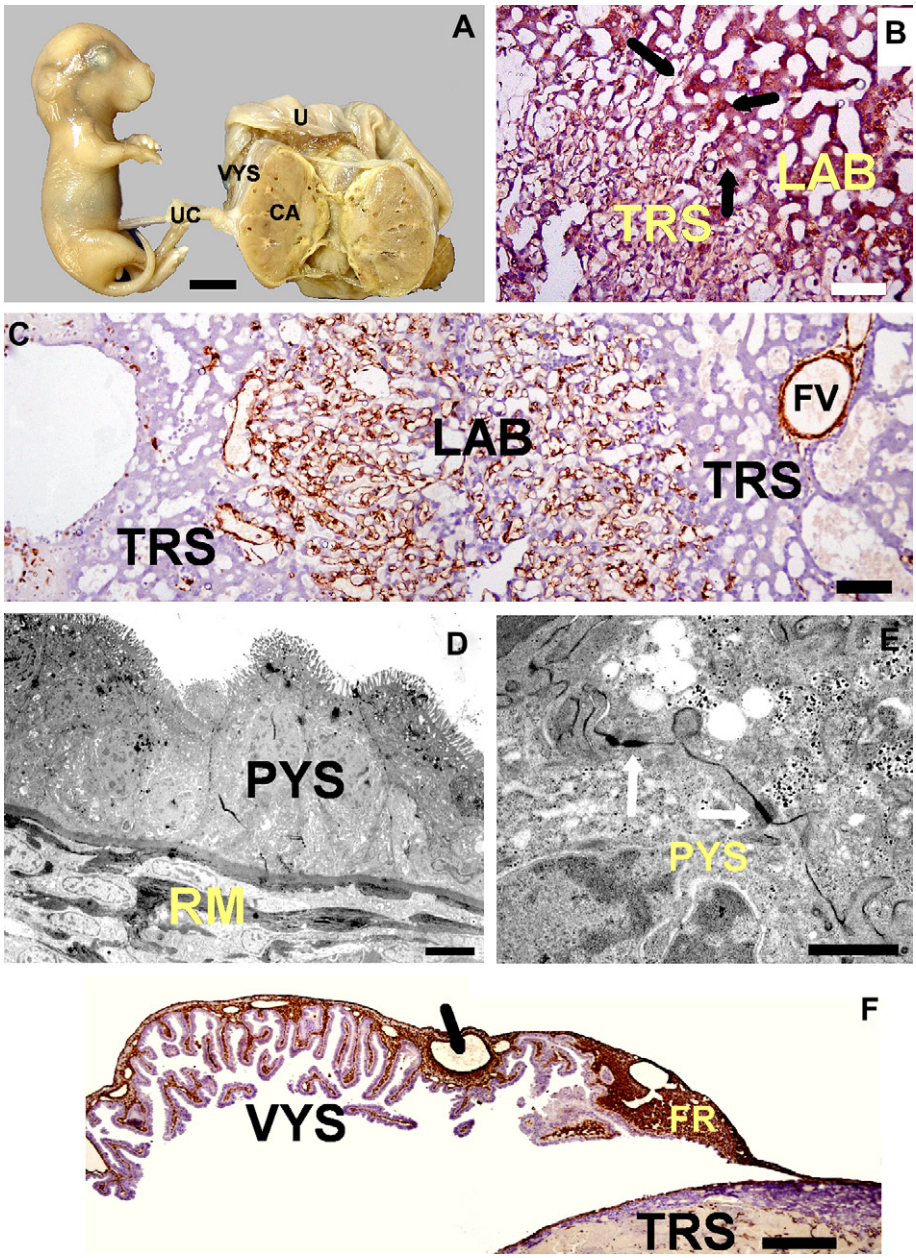

Fig. 1. The general structure of chorioallantoic and yolk sac placenta. (A) Embryo in mid-gestation with umbilical cord (UC), chorioallantoic placenta (CA), visceral yolk sac (VYS) and uterus (U). (B,C) Early pregnancy. (B) Immunostaining for cytokeratin marked trophoblast (arrows) in the trophospongium (TRS) and the labyrinth (LAB). (C) Immunostaining for vimentin. Only the labyrinth showed fetal vessels (FV) with positive endothelial cells. (D,E) Near-term pregnancy. TEM. One-layered parietal yolk sac (PYS) above the Reichert's membrane (RM). The cells had close contact (noted with arrows) at the bottom. (F) Early pregnancy. Vimentin. The visceral yolk sac (VYS) was well vascularized (arrow) and villous. A fibrovascular ring (FR) was present. Bar A: 1 cm. Bars B, C: 100 μm. Bar D: 2 μm. Bar E: 1 μm. Bar F: 500 μm.

Reichert's membrane (Fig. 1D). The cells contained apical microvilli (Fig. 1D), and they were interconnected by tight junctions at the base (Fig. 1E). A visceral yolk sac was present throughout gestation and was completely inverted, highly villous and well vascularized (Fig. 1F). A fibrovascular ring occurred where the yolk sac was attached to the placenta and contained a network of capillaries that positively immunostained for vimentin (Fig. 1F).

3.2. Internal placental structure

In early pregnancy, the trophospongium was the dominant structure. Toward the decidua, trophoblastic giant cells that were bi- or poly-nucleated and contained evident chromatin were present (Fig. 2A). The trophoblast was located on internally directed lamellae of the fetal mesenchyme (Fig. 2B). The trophoblast lined the tubular channels that formed the inner side of the trophospongium (Fig. 2C). The syncytial trophoblast lined the maternal blood spaces (Fig. 2C). Clusters of cellular trophoblast appeared between the syncytial layer and the mesenchyme (Fig. 2D). Placental growth resulted primarily from cells that were actively proliferating during early and mid-gestation (Fig. 2E,F). In the labyrinth, maternal blood spaces and fetal capillaries were closely intermingled (Fig. 3A), but only the latter had an intact endothelium (Fig. 3A). The barrier between the maternal blood spaces and fetal capillaries consisted of cellular and syncytial trophoblasts (Fig. 3B), with the latter appearing as the dominant trophoblast form from mid-gestation onwards. The barrier eventually decreased to a thin syncytial layer (Fig. 3C). Both the cellular and syncytial trophoblast showed electron dense inclusions. The cellular trophoblast proved to be actively proliferating (Fig. 3D), indicating that the labyrinth is an additional region of proliferation.

3.3. Subplacenta and junctional zone

The subplacenta was highly folded (Fig. 4A) and characterized by layers of cellular and syncytial trophoblast. This organ developed

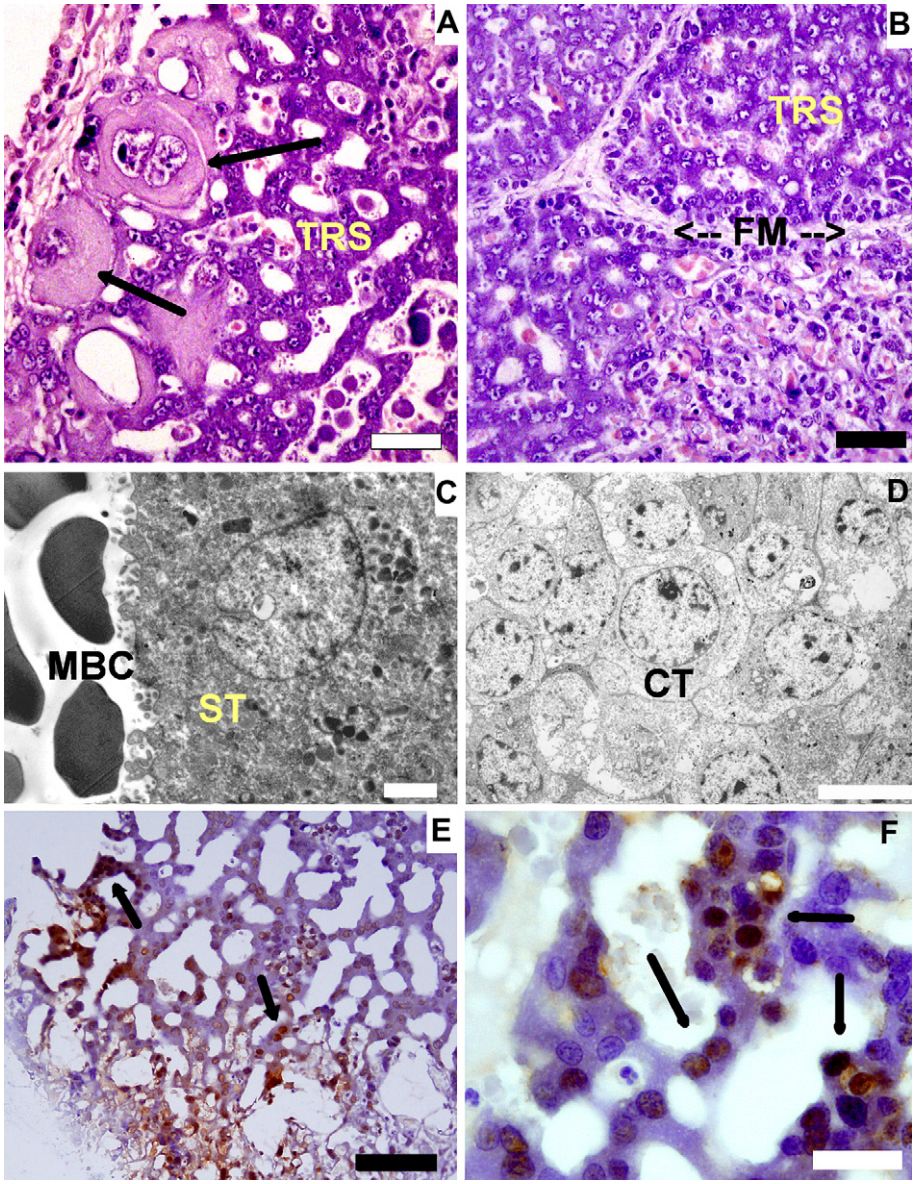

Fig. 2. Trophospongium. (A–D) Near-term pregnancy. (A) HE. The outer area of the trophospongium (TRS) with giant cells (noted with arrows). (B) HE. A fetal mesenchyme (FM) divided this area. (C) TEM. Syncytial trophoblast (ST) between the maternal blood channels (MBC). (D) TEM. Clustered cellular trophoblast (CT). (E,F) Early pregnancy. PCNA showed proliferation activity of these trophoblast cells (shown by arrows). Bar A, B, F: 50 μm Bar C: 2 μm. Bar D: 10 μm. Bar E: 200 μm.

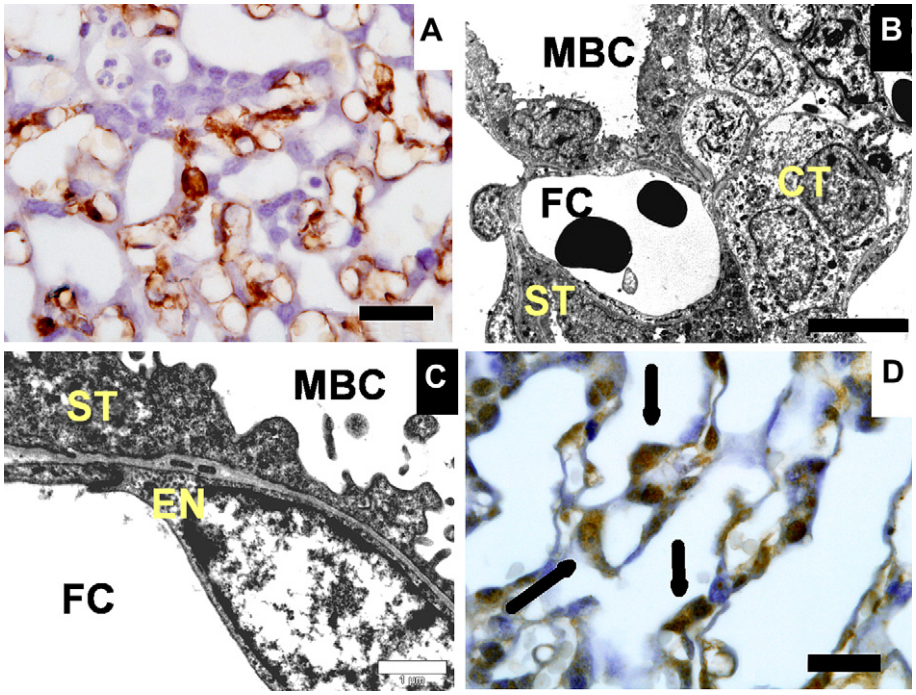

Fig. 3. Labyrinth. Near-term pregnancy. (A) The labyrinth contained vimentin-marked fetal vessels endothelium and trophoblast (vimentin-negative). (B,C) TEM. Syncytial (ST) and cellular (CT) trophoblast and fetal mesenchyme (FM) were observed in the barrier. Partly, there was only a thin syncytial (i.e., hemomonochorial) layer in between the maternal blood channels (MBC) and the fetal capillaries (FC). (D) PCNA. Proliferation activity of the cellular trophoblast (designated with arrows). Bar A, D: 50 μm. Bar B: 10 μm. Bar C: 1 μm.

during early gestation and was in contact with the maternal blood system with the syncytiotrophoblast facing toward the blood spaces. Later, it was supplied by fetal vessels with an intact endothelium (Fig. 4B), which comprised the only blood supply in advanced stages of pregnancy. However, there was some overlap between both systems (Fig. 4B). Neighboring the fetal vessels were clusters of trophoblast cells that were actively proliferating (Fig. 4C). From mid-gestation on, signs of degeneration were frequently observed in the trophoblast. Originating from the subplacenta, the extraplacenta trophoblast cells and syncytial streamers were oriented toward the maternal blood channels (Fig. 4D) and were widespread early on. However, they were rarer during advanced pregnancy. During early pregnancy, the maternal spiral arteries in the decidua were associated with PAS, DBA lectin and vimentin positive cells (Fig. 4E–G). These cells likely represented uterine Natural Killer (uNK) cells. In the more advanced stages, only a few of these PAS and DBA positive cells were observed.

4. Discussion

In the plains viscacha (*L. maximus*), placentation was structurally similar to the guinea pig and related rodents. This process is independent of the uncommon condition characterized by the polyovulation of hundreds of oocytes and the death of most implanted embryos. The lobulated structure-associated growing processes and hemomonochorial types of the barrier were identical in caviomorphs, including the viscacha [10–12,14,17,18,21–23,25,29]. As another characteristic of the group, the visceral yolk sac was inverted, villous and associated with a fibrovascular capillary network, and the parietal yolk sac covered the placenta [10,14,15,17,21–23,25]. Further similarities included the early, invasive and deep process of trophoblast invasion [17,21,24–28]. A subplacenta, serving as the source of origin for the trophoblast invasion and representing a derived condition for Rodentia, was temporarily supplied by both maternal and fetal blood systems, as is the case in other caviomorphs [17,20,25]. The invasion process appeared to be modulated by PAS-positive cells, identified as maternal uNK cells, as has been reported for other rodents, such as mice [30–32]. In addition to PAS, these cells positively immunostained for vimentin, which distinguished them from vimentin-negative, and often cytokeratin-positive, trophoblast cells. This staining supports the maternal origin of these cells, as was recently proposed for other rodents [33,34]. Another marker for uNK cells is DBA-lectin [35]; we found DBA-lectin positive marcation in these cells, similar to what has been described in mice.

In *Lagostomus*, a remarkable population of trophoblast giant cells was observed, which is not common for caviomorph rodents. Giant cells were identified in mice and other rodents as being an important fetal cell lineage for invasion processes and associated with the expression of particular genes and growth factors [e.g., [36–39],]. Because the invasion process in all caviomorphs is induced by the extraplacental trophoblast derived from the subplacenta, the functional meaning of the giant cell population in the plains viscacha is unclear. Further studies are needed to determine whether these cells resemble those of other rodents and to identify their function. In conclusion, our data support the following hypotheses: (1) the principle processes of placentation in caviomorph rodents follow an extraordinarily stable pattern; (2) their placentation is largely independent of body size and specialized modes of reproduction, such as polyovulation in the plains viscacha; and (3) further studies will investigate the processes of implantation and the fetomaternal control of embryonic survival and resorption processes. Understanding the phenomenon of natural polyovulation may also lead to a better understanding of hormone-stimulated superovulation in assisted reproductive techniques in humans, which has indeed caused malfunctions in trophectoderm-derived tissues and in the function of the placenta [40].

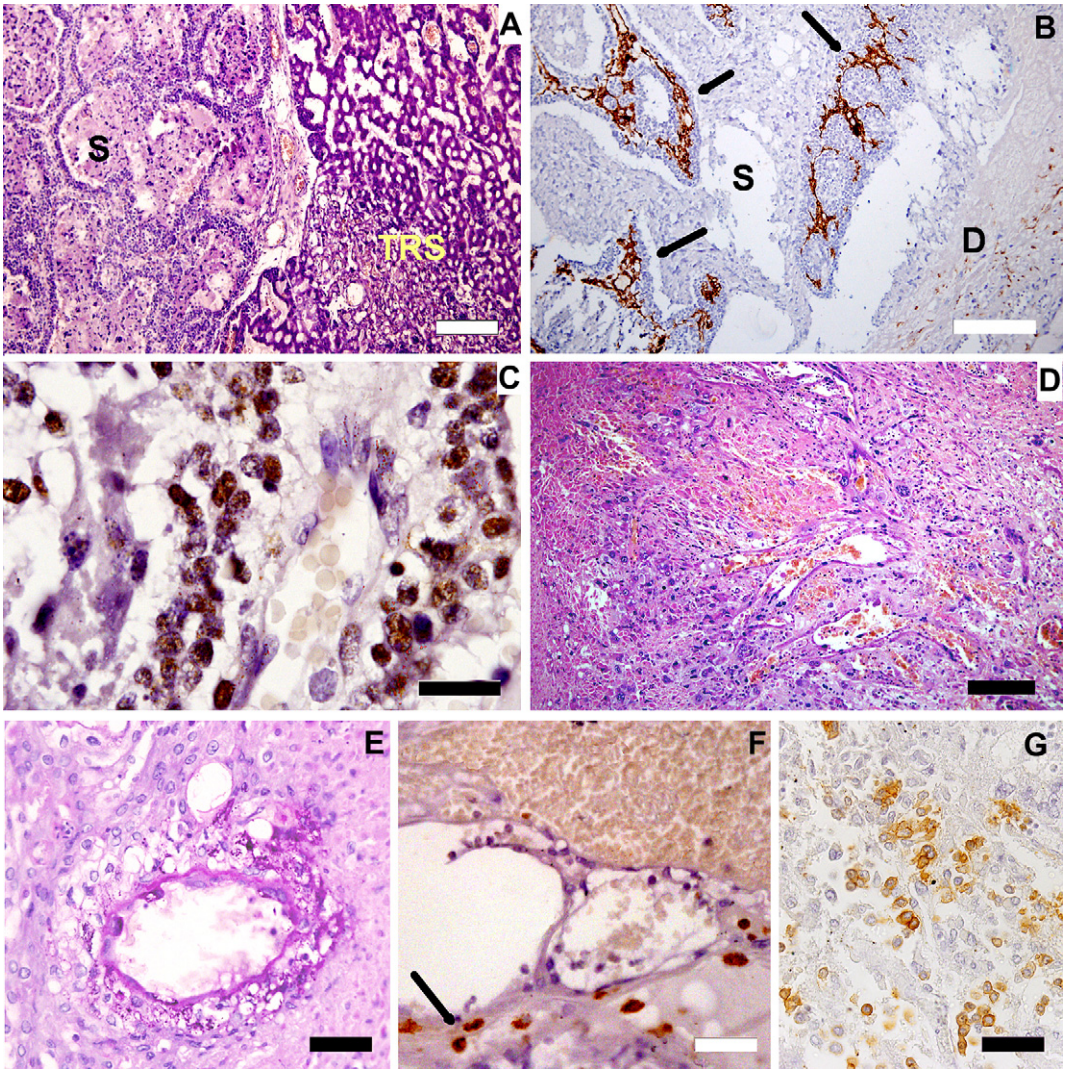

Fig. 4. Subplacenta and junctional zone. (A) Mid-gestation. HE. Folded subplacenta (S) near the trophospongium (TRS). (B). Early pregnancy, vimentin. Positive immunoreactivity (arrows) indicated supply by fetal vessels, in addition to the maternal blood spaces near the decidua (D). (C) Mid-gestation. PCNA. Proliferative trophoblast cells (arrows). (D) Early pregnancy. The trophoblast has invaded the decidua. (E,F,G) PAS, Vimentin and DBA lectin. Positive immunoreactivity of cells associated with the spiral arteries and in other decidual regions, identified as uNK cells. Bar A, D: 100 μm. Bar B: 200 μm. Bar C: 30 μm. Bars E, F, G: 50 μm.

Acknowledgments

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