



Universidade de São Paulo

Biblioteca Digital da Produção Intelectual - BDPI

Sem comunidade

WoS

2012

Morphology and Involution of the Yolk Sac during Early Gestation Bovine (*Bos indicus*)

ACTA SCIENTIAE VETERINARIAE, PORTO ALEGRE RS, v. 40, n. 4, supl. 1, Part 1-2, pp. 42-50, SEP, 2012

<http://www.producao.usp.br/handle/BDPI/40903>

Downloaded from: Biblioteca Digital da Produção Intelectual - BDPI, Universidade de São Paulo

Morphology and Involution of the Yolk Sac during Early Gestation Bovine (*Bos indicus*)*

Antônio Chaves de Assis Neto, Franceliusa Delys de Oliveira, Maria Vitória Piemonte Constantino & Maria Angélica Miglino

ABSTRACT

Background: The bovine yolk sac derives from visceral endoderm and its development occurs between days 18-23 of gestation. The study of this membrane is important for comparative data and has already been performed in rodents, sheep and in cattle, especially *Bos taurus*. In species *Bos indicus* the yolk sac has not quite been studied and is believed that there are morphological differences between these species. The yolk sac undergoes a process of involution and degeneration during embryonic development and none vestige of it is found in late gestation. The period in which occurs the involution of the yolk sac coincides with the period of increased pregnancy loss in cattle, and changes in the morphology of this membrane may indicate the reasons for such high loss rates. Thus, considering that the yolk sac is important for embryonic circulation and metabolic transmission, besides participating actively in the process of cattle placentation, this study aimed characterize morphologically the involution of the bovine yolk sac.

Materials, Methods & Results: The early gestational period was determined between days 20 and 70 post-insemination (p.i), according to the exterior characteristics of embryo/fetus. For macroscopic analyzes the uterus was dissected to expose the fetal membranes and subsequently the embryo/fetus was photographed. The samples were fixed for light microscopy and transmission electron microscopy. The yolk sac that emerges from the ventral part of the embryo was prominent and composed by a central part with two thin peripheral projections of different lengths. The bovine yolk sac with about 9 cm on day 25 p. i. of pregnancy permanently decreased its total length during this study. Histologically, the yolk sac is composed of three cell layers: the mesothelium, the mesenchyme and the endoderm. In mesenchyme are found blood islets. In the endoderm are formed cells invaginations toward the mesenchyme originating small canaliculi. The ultrastructure of yolk cells presented many mitochondria, rough endoplasmic reticulum, vesicles, euchromatin and the presence of two nucleoli.

Discussion: The real first blood circulation in the bovine is attached with the development of yolk sac, differently from other membranes, such as the corium, that does not present evidence of vascularization by the age of 20-30 days. The erythroblasts found in the yolk sac are related to vasculogenesis and the process of differentiation of blood cells during the erythropoiesis. It could be observed on the histology of the yolk sac, in embryos of 30-50 days old, the presence of canaliculi and small folds of the epithelium. The canaliculi collapse is associated with the degeneration of the endoderm wall of the yolk sac. The organelles present in the endoderm cells of the yolk sac are associated with the function of protein metabolism and in the exchange of substances between the mesenchyme and the mesothelium. For these findings, could be observed that the yolk sac epithelium is found active until the 50th day of gestation, and thereafter regresses. However, remnants of this membrane may be present until the 70th day. These features may represent a presence of an active chorionvitelline placenta in this period responsible for the maintenance of pregnancy whereas the chorioallantoic placenta is not definitively established.

Keywords: *Bos indicus*, bovine, placenta, embryo, fetus, yolk sac, early gestation.

INTRODUCTION

The yolk sac morphology or function has been previously studied in rodents [6,7,14,16,17]. Moreover, these studies were described in domestic animals such as sheep and cattle [19]. Especially in cattle, studies related to their morphology were reported in *Bos Taurus*; however, it not has been elucidated in *Bos indicus*. The gestation length of *Bos indicus* cows (284 days to more than 300 days) is longer than *Bos taurus* cows (280 to 285 days) [3,4,18]. However, not much has been known regarding the differences between extra-embryonic membranes between these two bovine species.

The bovine yolk sac develops from days 18 to 23 post-insemination (p. i.), when the embryo converts from a trilaminar disc into a cylindrical body [24]. This process occurs during gastrulation, in which the yolk sac derived from visceral endoderm or hypoblast. [5,20,22]. The study of the yolk sac and the other extra embryonic membranes of ruminants become mainly relevant for further comparative data. It is known that embryos from IVF are more effective than cloned embryos. Thus, the morphological changes in the yolk sac may represent a key to elucidate the high rates of embryonic loss, since this membrane is functional in the bovine placentation [10,15].

The involution of bovine yolk sac occur during the period in which the definitive chorioallantoic placenta is formed around the 40 days of pregnancy, and can be associated with embryonic losses on this period of transition [1,2,20].

This is a descriptive study from different stages of bovine embryogenesis describing the structure and ultrastructure of the yolk sac. Our goal was to characterize morphologically the involution of the bovine yolk sac.

MATERIALS AND METHODS

Sample Collection

The samples were collected at a slaughterhouse located in Dracena, São Paulo, Brazil. Immediately after the slaughter, each uterus was removed and the conceptus was taken to examination.

Gestational period determination

The early gestational period between days 20 and 70 p. i. of pregnancy was stipulated according to the exterior characteristics of embryo/fetus greatest length (GL) [23], as well as developmental morphol-

ogy of embryonic/fetal membranes [2]. Based on the established criteria the embryos and fetuses were included in six day-groups of pregnancy: 20 to 25 (n = 26), 25 to 30 (n = 23), 30 to 40 (n = 25), 40 to 50 (n = 24), 50 to 60 (n = 21), 60 to 70 (n = 22)

Macroscopic analysis

The entire process of the macroscopic material for the present study was photographed to analyze the structural characteristics. For this purpose was used a photographic camera¹. The illumination was photoflood light (250 Watts) from up to down and blue counter light (40 Watts) from down to up through a glass container, with the specimen in water immersed therein. The uteri were collected and the uterine horns opened as well as dissected at its dorso-antimesometrial line in order to expose the embryonic/fetal membranes. The GL of embryo and fetus, respectively, was determined and its weight estimated using an analytical scale (0.001 to 200 g). The specimens were fixed in 10 % formaldehyde in order to solidify the tissue, and for histological use.

Processing for light microscopy

The fetal membranes and embryos were reduced and placed in a fixing solution of 4% paraformaldehyde in a saline phosphate solution (PBS). After fixation, the material was dehydrated in a series of ethanol of increasing concentrations (from 70 to 100%) and diaphanized in xylol, followed by inclusion in Paraplast^{®2}. Cuts 5 µm thick were made and stained with hematoxylin-eosin (HE), periodic-Schiff acid (PAS), and toluidine blue. The slides were mounted, and after analyses, photomicrographs were made (light microscope Leica DM2500).

Processing for transmission electron microscopy - TEM

The yolk sac was previously fixed in 2.5% glutaraldehyde³ in a phosphate buffer of 0.1M, pH 7.2. After the membranes were individualized, fragments of the yolk sac were obtained separately. When fixation ended, the material was washed in a sodium phosphate buffer of 0.1 M, pH 7.4, three times during ten minutes and post-fixed in 1% osmium tetroxide² (4% Osmium tetroxide w/w solution in water) for 1 h. After being washed again in a phosphate buffer, the fragments of membrane were dehydrated in ethyl alcohol at 50%, 70%, 90% and 100% and washed in propylene oxide³ (Propylene oxide EM Grade).

For 12 to 16 h, the membrane fragments remained under rotation in propylene oxide and Spurr resin in a ratio of 1:1 (Spurr's kit-Electron Microscopy Sciences, Co. USA). Afterwards this mixture was replaced in pure resin for 4 to 5 h. After this period, they were imbedded with pure resin in molds. Once it was embedded, the membranes remained in an incubator at 69°C for 72 h to consolidate the resin polymerization.

With the purpose of locating and characterizing the areas of interest, the blocks were cut using an ultramicrotome Leica ULTRACUT UCT®. Semi-thin sections (1µm thick) were obtained containing 0.25% of Toluidine blue for observation under light microscope.

The ultrathin sections, about 60 nm thick, were collected on copper grids and contrasted with 2% uranyl acetate in distilled water for 5 min and by lead citrate 0.5% in distilled water during 10 min. The observations and electron micrographs were performed using electronic microscope ZEISS EM-94S2 and JEOL CX-II-100.

RESULTS

The external macroscopic characteristics as well as yolk sac length of embryo/fetus were previously described in published research [2]. From days 20 to 30 p. i., the embryonic membranes, with exception of the amnion including the embryo, were transparent and wrinkled and macroscopically not easy to detect. From days 30 to 50 p. i., the outer chorioallantoic membrane of conceptus was presented slick being pressured out of the uterus. After days 50 to 70 p. i., this “elapse” didn't happen anymore. Due to this phenomenon the conceptuses had to be collect at different periods.

The embryo with 0.60 cm GL, 25 p. i., (Figure 1) shows, macroscopically distinct the yolk sac and a central portion, from which two projections are particularly visible by their content of blood vessels. The plan to cuboidal endodermal epithelium became visible by the characteristics cells, which showed isolated from each other, and also as a specifically large binucleated one. It was observed that the blood islet formed in the mesenchyme by the detection of dark cells and a large nucleus. In comparison, the capillary showed a distinct, “mature” endothelium (Figure 2).

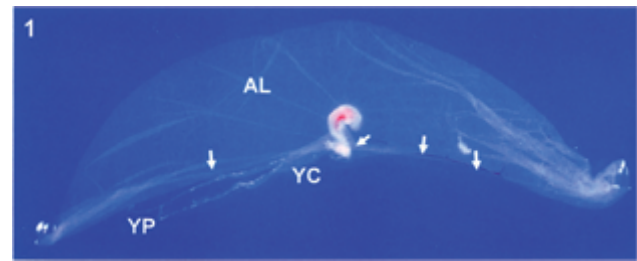


Figure 1. Embryo - 0.60 cm GL, 25 days. Central part (YC) of the yolk sac, two projections extends (YP) and blood islets (clear stipples). The allantois (AL) (arrows). The arrows show blood vessels in the ventral portion of the embryo. The vessels are from the chorion which was removed [Bar: 1 cm].

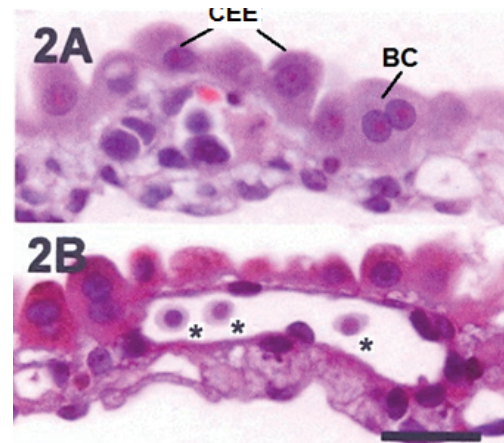


Figure 2. Yolk sac histology - projection part (A) and central part (B) - corresponding to the embryo in Figure 1. A: The cuboidal to compressed endodermal epithelium (CEE), presence of large and binucleated cell (BC). B: The capillary with three erythroblasts (asterisks). HE. [Bar: 20 µm].

On the edge of the embryo yolk sac with 0.71 cm GL, 26 p.i, the endodermal epithelium became cuboidal to columnar by the shape of its cells, therefore the localization of this edge is apically each other or merely have a gap between the yolk sac and the lumen. Erythropoiesis is visible in small vessels and in a niche vessel. Some of the vessels contain small amount of erythroblasts, which do not represent blood islets, but the existence of blood flow. Mesothelial cells are distinct in this embryo (Figure 3).

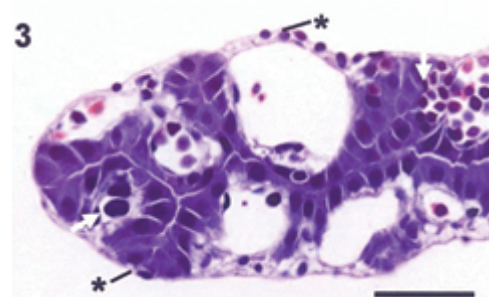


Figure 3. Projection of embryo yolk sac histology - 0.71 cm GL, 26 days. The endodermal epithelium presented cuboidal to columnar cells. The erythropoiesis is visible in a cluster of blood vessels (arrows). Mesothelial cells are distinct (*). [Bar: 40 µm].

The histology of the embryo yolk sac of - 0.95 cm GL, 27 p.i - showed that the epithelium of it is composed of three layers: a single layer of endodermal cells - the endoderm, lining the vitelline cavity; a simple mesothelial layer, aimed at exoceloma - the mesothelium; and a vascular mesenchymal - the mesenchyme, but only one layer is considered cuboidal. In the mesenchyme, the erythropoiesis onset became visible due to a group of primitive haemoblast cells. Still, the yolk sac showed a single layer of goblet and basal cells. Right below, were the basal membrane and a thin layer of highly vascular mesenchymal tissue. The blood vessels were filled with primitive blood cells: primary erythroblasts. This layer was not surrounded by the endothelium, and presenting also, a few red blood cells in the cytoplasm (Figure 4).

In embryos with 1.03 cm GL, 30 p.i, was also observed goblet cells arranged in a single layer which rest on the mesenchyme; and, a duct in the yolk sac, in which the endothelial cells where very variable in form and size. The mesenchyme was replete with blood vessels and primary erythroblasts cells. Endothelium was already formed in two capillaries (one containing

an erythroblast). The goblet cells and, sometimes, in a single layer, were presented in the yolk sac, as the mesenchyme, with erythroblasts, and the mesothelium. The lumen was presented covered by endodermal epithelium. The space between the endoderm and mesothelium was thin and the blood vessels were different in size in the mesenchyme. The blood vessel with a distinct endothelium and pericytes was in advanced development stage being typical in the duct part of yolk sac (Figure 5).

The central part of yolk sac (Figure 6) showed up replete with blood vessels and primary erythroblasts cells. Erythroblasts cells are found in groups, forming blood islets, with different vessels sizes. Some endodermal cells tented to round up.

The transmission electron microscopy (TEM) showed the cytoplasm of vitelline cells, which exhibited mitochondria, mostly located between the nucleus and the luminal extremity. A rough endoplasmic reticulum (ER) is sparsely distributed in large quantities. Small, discrete vesicles were observed throughout cytoplasmic region. Most of the nuclei were spherical, euchromatin with one or two nucleoli (Figure 7).

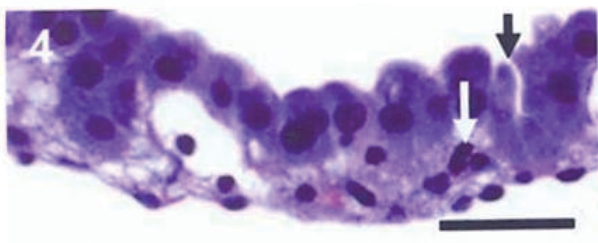


Figure 4. Yolk sac histology of embryo - 0.95 cm GL, 27 days. There are three layers, but only one layer is considered cuboidal (black arrow). Layer of mesenchymal cell (white arrow) located between the endoderm and mesothelium. HE. [Bar: 20 µm].

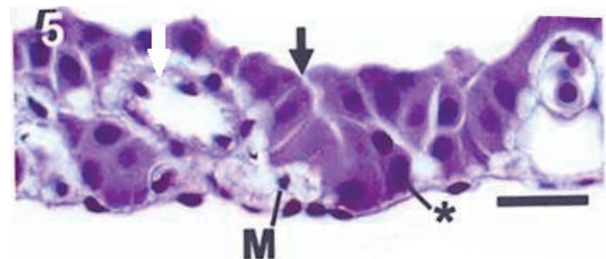


Figure 5. Yolk sac duct of embryo 1.03 cm GL, 30 days. There is a chamber from the luminal side (black arrow). This epithelium almost reaches the mesothelium. Cells with oval nuclei and distinct cytoplasm (*) are present in these structure. The blood vessel with distinct endothelium and pericytes (white arrow). M = mesenchymal cell. HE. [Bar: 20 µm].

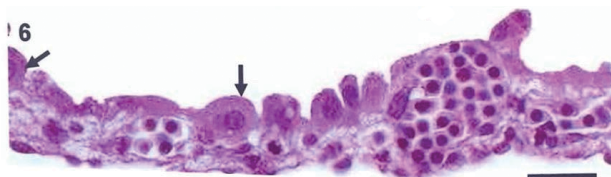


Figure 6. Yolk sac central part corresponding to the embryo of Figure 5. Three fully developed blood islets. Some endodermal cells show tendency to round up (arrows). HE. [Bar: 20 µm].

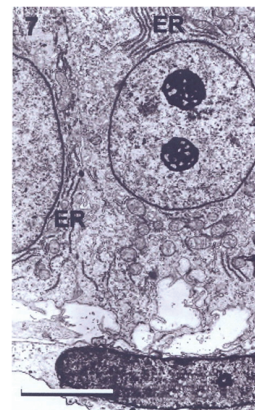


Figure 7. TEM of Two endodermal cells located in front of the luminal indentation corresponding to figure 5. Rough endoplasmic reticulum (ER), mitochondria (M). [Bar: 6 µm].

Still, in TEM, intercellular spaces were evident between the epithelial cells of endodermal yolk sac, and a possible opening area of the endothelial lining. Primitive erythroblasts were found inside the vessel (Figure 8).

The embryo with 1.74 cm GL, 36 p.i, presented macroscopically the yolk sac distinctly in three parts of vitelline duct, the central part and two projections. The central part of the yolk sac showed up in red color and in this particular region, is when the erythropoiesis may be happening (Figure 9).

It was observed that the lumen of the yolk sac is surrounded by two walls which are noted not as blood islets anymore (Figure 10), but many small capillary sections which contain few erythroblasts. The endoder-

mic epithelium was presented irregularly and columnar showing dark and clear cells. The mesothelium, on the outside of yolk sac, was distinct. The mesothelial cells showed to be shaped flattened and sometimes ovoid.

Central part of the embryo yolk sac is showed in Figure 10. The vessels were presented with primary erythroblasts, located inside and outside the vessels. The yolk epithelium along with the connective tissue formed a fold that projects itself into de light of the yolk sac resulting in canalicular structures. These folds were bordered by a cuboidal endodermal epithelium. The mesenchyme only enclosed a moderate amount of capillaries with small content of erythroblasts. Nearby the mesothelium the mesenchyme specifically showed numerous fibroblasts (Figure 11).

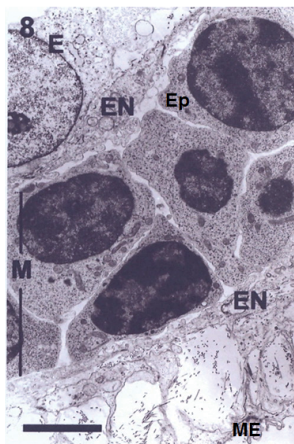


Figure 8. TEM - section through the central part of yolk sac showing a blood islet corresponding to figure 6. E = endodermal cell; M = mesenchymal layer, EN= Endothelium without nuclei; ME = thin mesothel, Ep = Primitive erythroblasts. [Bar: 6 µm].

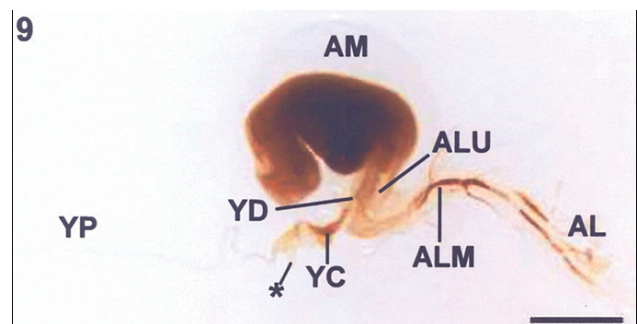


Figure 9. Embryo - 1.74 cm GL, 36 days. The yolk sac macroscopically distinctly shows the three parts of vitelline duct (VD), the central part (YC) and the two projections (YP, one of them was cut [*] for TEM-processing). Note the central part distinctly red-stained as signifying erythropoiesis to be particularly in this part of yolk sac. AL= allantois with umbilical (ALU) and blood vessels (ALM). AM = amnion. [Bar: 1µm].

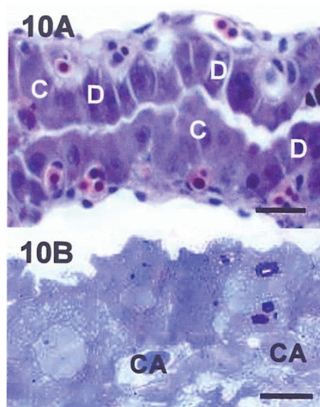


Figure 10. A: Yolk sac histological view of the transition of the central to the projecting parts corresponding to the embryo of figure 9. The yolk sac lumen (YL) is flanked by the two walls which are conspicuous not of blood islets anymore (compare figure 6). The simple endodermic epithelium is irregularly columnar showing dark (D) and clear (C) cells. HE. [Bar: 20 µm]. **B:** The semifine section specifically shows dark and clear cells. CA = capillaries. HE. [Bar: 10 µm].



Figure 11. Yolk sac central part of embryo of Figure 10. Nearby the mesothelium (M) the mesenchyme shows specifically numerous fibroblasts (black arrow). HE. [Bar: 20 µm].

In the embryo with 3 cm GL, 45 p.i the yolk sac was macroscopically visible. It was observed that the extremities become thinner and more transparent. The central region of the yolk sac resembled a small flattened grain in contact with the amnion. An abrupt decrease in the lengths of the extremities and the total length of the yolk sac was seen clearly (Figure 12).

The folds at 45 p.i old were very distinct (Figure 13). The walls of the yolk sac presented folds which formed small channels. In the mesenchyme, no erythroblasts were visualized. Also, the yolk sac was bordered by a cuboidal endodermal epithelium. The

capillaries in part are extended and numerous, and contain some erythrocytes.

It was observed ultra-structurally in embryos with 2.47 cm GL, 43 p.i, goblet cells rough endoplasmic reticulum and numerous mitochondria in the apical pole. The nucleus presented defined spherical shape by the nuclear membrane with evident nucleoli. On the surface was observed short luminal microvilli of different sizes (Figure 14).

In the fetus with 5.6 cm GL, 56 p.i, was observed macroscopically the central part of the yolk sac (compare the pin-head nearby). The involution of the yolk sac was found to be established; however, signs could be seen in some fetuses (Figure 15).

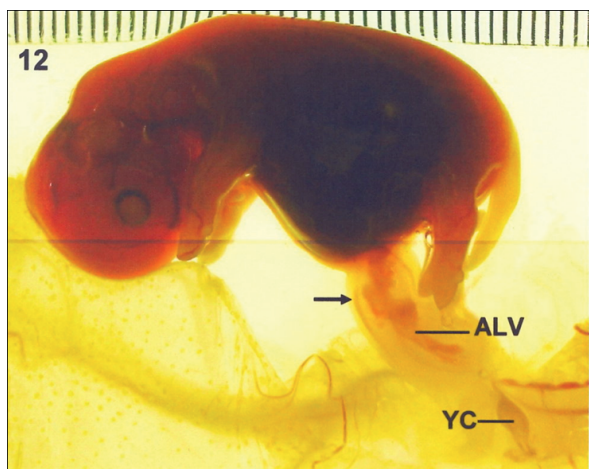


Figure 12. Embryo - 3 cm GL, 45 days. The yolk sac is macroscopically visible in part by its duct at cranial location of umbilicus (arrow) and the central part (YC). ALV = allantoic blood vasculature.

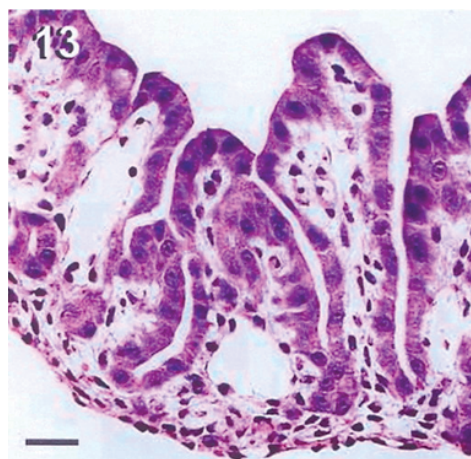


Figure 13. Yolk sac central part histology of corresponding embryo in Figure 12. HE. [Bar: 20 µm].

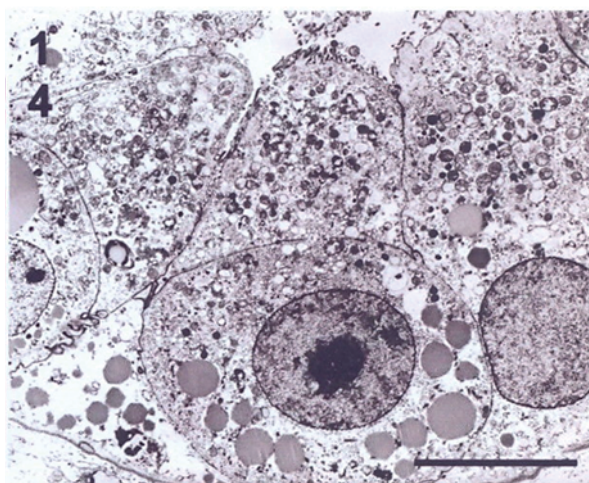


Figure 14. TEM of yolk sac endodermal epithelium of embryo - 2.47 GL, 43 days. ACC = apoptotic characteristic cell. [Bar: 9 µm].

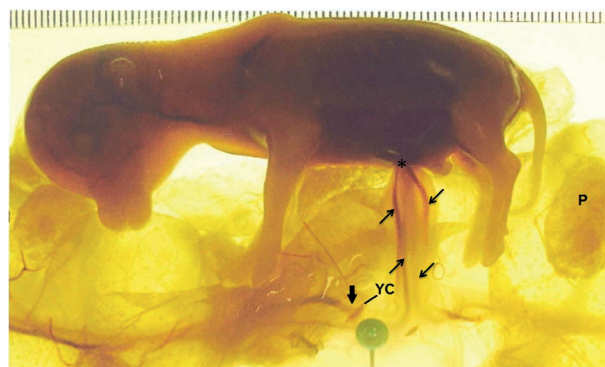


Figure 15. Fetus - 5.6 cm GL, 56 days. Yolk sac central part is visible (YC, compare the pin-head nearby). Note the umbilicus containing the allantoic vessels: the two umbilical arteries and the two umbilical veins (black arrows) which converge to a single one nearby the body of fetus. P = placentomes through the amnion and allantochorion having been prepared and partly removed to show the umbilical region.

DISCUSSION

Macroscopically the yolk sac is well developed in the first trimester of pregnancy, still remaining up to 70 days, when it is reduced in size. This findings suggests that in this period this structure plays an important role for the maintenance of pregnancy, whether the mechanisms of maternal-embryonic exchange or as na organ of nutrition.

The real first blood circulation in the bovine is attached with the development of yolk sac. The blood vessels of the yolk sac are not macroscopically visible, but can be visualized by the red color of blood or erythrocytes therein. The intensity of this coloration corroborates with the activity of erythropoiesis that probably ends between days 25 to 30 p. i. This fact, however, must be refined histologically. The blood vessels present fenestrations which suggests that the proteins are transferred to the vascular portion.

The erythroblast islet functions are poorly known; however, Lee *et al.* [12] related those functions to the process of blood cells differentiation during vasculogenesis and erythropoiesis in dogs. At embryos day 30 to 50 p.i., observe the presence of canaliculus and small folds of the epithelium. The canaliculi collapse is associated with the degeneration of the endoderm wall of the yolk sac [19].

The presence of rough endoplasmic reticulum (RER) and mitochondria, visualized by transmission electron microscopy, in vitelline epithelium, is associated with the function of the protein metabolism. Similarly, vesicles are present in the cells cytoplasm of the yolk sac endoderm, which may be involved in the exchange of substances between the mesenchyme and mesothelium [12]. In fact, the endoplasmatic reticulum has the role of storage for subsequent release of proteins, but, the functions of lipid metabolism and the formation of glycogen from glucose is also assigned to the endoplasmatic reticulum [21].

Generally in all stages, the yolk sac presents columnar or sometimes globous cells with large nuclei. Among the most evident organelles are mitochondria, secretory vesicles, the rough and smooth endoplasmatic reticulum and microvilli. Those structures are responsible for the main function of the yolk sac which

are nutrition through the exchange of substances, the production and secretion of proteins and the lipid and sugar metabolism. In the mesenchyme stands out the blood vessels with the presence of primary erythroblasts either internal and the outer portion in the later stages. The presence of such structures is an indicative of na active vitelline circulation that contributes to the functions of this membrane.

Through the performed analyzes, it was observed that the vitelline epithelium is active until day 50 of gestation, although it was present until the day 70 of gestation. Also, this structure presents evidence of morphological characteristics of absorptive epithelium and protein synthesis. This activity may represent a presence of an active chorionvitelline placenta in this period responsible for the maintenance of pregnancy whereas the chorioallantoic placenta is not definitively established.

CONCLUSION

The bovine yolk sac decreases in total length permanently during this study. In most cases, the yolk sac disappears completely from days 50 to 70 p.i.; however, vestiges of the central part of this sac can be observed during this time.

SOURCES AND MANUFACTURERS

¹Sony Cyber-Shot 12.1 mp, Sony-Brasil, São Paulo, SP, Brazil.

²Sigma-Aldrich Corporation, St. Louis, MO, USA.

³Polyciences Inc., Warrington, PA, USA.

Acknowledgments. Special thanks to Professor Rudolf Leiser for his intellectual contribution during the experimentation and preparation of this work. This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and also by the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Process number 2008/58811-6.

Ethical approval. An Experimental protocol (number 631/2005) was authorized by the Bioethics Committee of Veterinary Medicine School, University of Sao Paulo (USP), Brazil.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

- 1 **Assis Neto A.C., Pereira F.T.V., Santos T.C., Ambrósio C.E., Leiser R. & Miglino M.A. 2010.** Morpho-physical recording of bovine conceptus (*Bos indicus*) and placenta from days 20 to 70 of pregnancy. *Reproduction in Domestic Animals*. 45(5): 760-772.
- 2 **Assis Neto A.C., Santos E.C.C., Pereira F.T.V. & Miglino M.A. 2009.** Initial Development of Bovine (*Bos indicus*) Placentation from the Point of View of the Allantois and Amnion. *Anatomia, Histologia and Embryologia*. 38(5): 341-347.
- 3 **Cavalcante F.A., Martins Filho R., Campello C.C., Lobo R.N.B. & Martins G.A. 2001.** Período de gestação em rebanho Nelore na Amazônia Oriental. *Revista Brasileira de Zootecnia*. 30(5): 1451-1455.
- 4 **Chenoweth P.J. 1994.** Aspects of reproduction in female *Bos indicus* cattle: a review. *Australian Veterinary Journal*. 71(12): 422-426.
- 5 **De-Miguel M.P., Arnalich-Montiel F., Lopez-Iglesias P., Blazquez-Martinez A. & Nistal M. 2009.** Epiblast-derived stem cells in embryonic and adults tissues. *The International Journal of Developmental Biology*. 53(8-10): 1529-1540.
- 6 **Favaron P.O., Carter A.M., Ambrósio C.E., Morini A.C., Mess A.M., Oliveira M.F. & Miglino M.A. 2011.** Placentation in Sigmodontinae: a rodent taxon native to South America. *Reproductive Biology and Endocrinology*. 9:55-68.
- 7 **Flamini M.A., Portiansky E.L., Favaron P.O., Martins D.S., Ambrósio C.E., Mess A.M., Miglino M.A. & Barbeito C.G. 2011.** Chorioallantoic and yolk sac placentation in the plains viscacha (*Lagostomus maximus*) - A caviomorph rodent with natural polyovulation. *Placenta*. 32(12): 963-968.
- 8 **Fléchon J.E. 1978.** Morphological aspects of embryonic disc at the time of its appearance in the blastocyst of farm animals. *Scanning Electron Microscopy*. 2: 541-548.
- 9 **King G.J., Atkinson B.A. & Robertson H.A. 1982.** Implantation and early placentation in domestics ungulates. *Journal of Reproduction and Fertility*. 59(31): 17-30.
- 10 **Kohan-Ghadr H.R., Lefebvre R.C., Fecteau G., Smith L.C., Murphy B.D., Suzuki Junior J., Girard C. & Hélie P. 2008.** Ultrasonographic and histological characterization of the placenta of somatic nuclear transfer-derived pregnancies in dairy cattle. *Theriogenology*. 69(2): 218-230.
- 11 **Latshaw W.K. 1987.** *Veterinary developmental anatomy: a clinical oriented approach*. Toronto: Decker, pp.49-74.
- 12 **Lee S.Y. 1983.** Ultrastructure of the placenta and fetal membranes of the dog: the yolk sac. *The American Journal of Anatomy*. 166: 313-327.
- 13 **Maddox-Hyttel P., Alexopoulos N.I., Vajta G., Lewis I., Rogers P., Cann L., Callesen A., Tveden-Nyborg P. & Trounson A. 2003.** Immunohistochemical and ultrastructural characterization of the initial post-hatching development of bovine embryos. *Reproduction*. 125: 607-623.
- 14 **Miglino M.A., Francioli A.L.R., Oliveira M.F., Ambrósio C.E., Bonatelli M., Machado M.R.F. & Mess A.M. 2008.** Development of the inverted visceral yolk sac in three species of caviids (Rodentia, Caviomorpha, Caviidae). *Placenta*. 29(8): 748-752.
- 15 **Miglino M.A., Pereira F.T.V., Visintin, J.A., Garcia J.M., Meirelles F.V., Rumpf R., Ambrósio C.E., Papa P.C., Santos T.C., Carvalho A.F., Leiser R. & Carter A.M. 2007.** Placentation in cloned cattle: structure and microvascular architecture. *Theriogenology*. 68(4): 604-617.
- 16 **Oliveira M.F., Favaron P.O., Ambrósio C.E., Miglino M.A. & Mess A.M. 2012.** Chorioallantoic and yolk sac placentation in *Thrichomys laurentinus* (*Echimyidae*) and the evolution of Hystricognath rodents. *Journal of Experimental Zoology*. 318(1):13-25.
- 17 **Oliveira M.F., Mess A.M., Ambrósio C.E., Dantas C.A.G., Favaron P.O. & Miglino M.A. 2008.** Chorioallantoic placentation in *Galea spixii* (Rodentia, Caviomorpha, Caviidae). *Reproductive Biology and Endocrinology*. 6: 39-46.
- 18 **Paschal J.C., Sanders J.O. & Kerr J.L. 1991.** Calving and weaning characteristics of Angus-, Gray Brahman-, Gir-, Indu Brazil-, Nellore-, and Red Brahman-sired F1 calves. *Journal of Animal Science*. 69(6): 2395-2402.
- 19 **Ruesse I., Sinowatz F., Richter L., Lehmann M. & Schallenberger E. 1992.** Die Entwicklung des Dottersackes beim Wiederkäuer (Schaf und Rind). *Anatomia, Histologia, Embryologia*. 21(4): 324-347.
- 20 **Talbot N.C., Caperna T.J., Powell A.M., Ealy A.D., Blomberg L.A. & Garrett W.M. 2005.** Isolation and characterization of a bovine visceral endoderm cell line derived from a parthenogenetic blastocyst. *In Vitro Cellular & Developmental Biology - Animal*. 41(5-6): 130-141.

- 21 Tiedemann K. 1976.** On the yolk sac of the cat. Endoderm and mesothelium. *Cell and Tissue Research*. 173: 109-127.
- 22 Wells J.M. & Melton D.A. 1999.** Vertebrate endoderm development. *Cell and Developmental Biology*. 15: 393-410.
- 23 Winters L.M., Green W.W. & Comstock R.E. 1942.** Prenatal development of the bovine. *University of Minnesota Agricultural Experiment Station - Technical Bulletin*. 151: 3-50.
- 24 Wrobel K.H. & Suess F. 1998.** Identification and temporospatial distribution of bovine primordial germ cells prior to gonadal sexual differentiation. *Anatomy and Embryology*. 197(6): 451-467.