HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE SPLEEN OF GOAT FOETUS IN EARLY STAGES OF GESTATION

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ABSTRACT

Histomorphological and histochemical studies were conducted on the spleen of 8 healthy and normal goat embryos/foeti of either sex between 22 to 50 days of gestation. The splenic primordium formed by the densely packed mesenchymal cells in the dorsal mesogastrium was first observed at 32 days gestation. Up to 36 days gestation, the dorsal (parietal) surface was covered by a layer of simple squamous epithelium, the mesothelium but at 41 days of gestation, parietal as well as the major part of the visceral surface was covered by a layer of flattened cells. At 46 days gestation, the fibroblasts began to appear. Fine but short reticular fibers were observed for the first time in the spleen of 46 days old foetus. From 41 days onwards, the parenchyma had mesenchymal cells and aggregated hemopoietic cells along with isolated nucleated and anucleated erythrocytes. The red and white pulps were not observed until 50 days of foetal life. The mesenchymal cells of splenic parenchymaand mesothelial cells that covered the spleen showed mild reaction for PAS positive substances. In the early prenatal period, the nuclei of mesenchymal cells showed moderate to intense Feulgen's reaction. It was concluded that up to 50 days gestation, the foetal goat spleen was engaged in the erythropoiesis.

Keywords: Foetal, Goat, Histogenesis, Histochemistry, Spleen

Goat (Capra hircus) is considered as a mini cow as it plays a significant role in the economy and nutrition of landless, small and marginal farmers in the country. They are disseminated all over the world because of their great adaptability to varying environmental conditions and the different nutritional regimes under which they were evolved and subsequently maintained. The spleen is demanding constant attention from an anatomical, immunological and clinical point of view (Alim et al., 2012). It is a complex lymphoid organ which assists in the defence mechanism of the body against the pathogenic organisms by producing antibodies. In mammals, this organ is classified as defensive type (man, rabbit), storage type (horse, dog, cat) and intermediate type (ruminant, swine). In most of the mammals, during fetal life, spleen acts as an organ of blood formation but after birth, only lymphocytes and monocytes are formed (Dellmann and Eurell, 1996). The literature on foetal goat spleen is meager, hence, the present investigation was planned to observe the histogenesis along with certain histochemical reactions of spleen at early stages of gestation.

MATERIALS AND METHODS

The present study was conducted on the developing spleen from 8 healthy embryos/ foeti (22 to 50 days of gestation) of either sex of non- descript goat (*Capra hircus*). Prior to the commencement of the study, approval was obtained from the animal ethics committee of DUVASU, Mathura (U.P.). Each embryo/foetus was

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weighed in grams with the help of analytical balance. The age of embryos/foeti was ascertained by using formula derived by Singh *et al.* (1979) for goat foetus, W1/3 =0.096 (t-30), where W = body weight of foetus in gram and t = age of foetus in days. The entire embryo was fixed in 10% neutral buffered formalin. Upto 41 days of gestation, serial sections of the embryo were obtained. In the foetuses after 41 days of foetal life, the spleen was exposed by giving ventral midline abdominal incision, collected and fixed in the neutral buffered formalin. Tissues from each embryo were processed by routine paraffin embedding technique (Luna, 1968). Thick paraffin sections (6μ) were stained with haematoxylin and eosin (Luna, 1968) for demonstration of general histo-architecture, Gordon and Sweet's method (Luna, 1968) for reticular fibers, Mallory's triple Stain (Crossman, 1937) for connective tissue fibers and Weigert's method (Luna, 1968) for elastic fibers, term. Periodic Acid Schiff's (Luna, 1968) for polysaccharides (PAS), Muller's Colloidal (hydrous) ferric oxide (Luna, 1968) for acid mucopolysaccharides (AMPS), Feulgen's reaction (Bancroft et al., 2013) for DNA and Sudan Black B method (Pearse, 1968) for lipids.

RESULTS AND DISCUSSION

In serial sections of goat foetuses, the first evidence of the primordium of spleen was observed at 32 days of gestation. At this stage and in 36 days old foetus, the primordium was present on the dorsal part of the mesogastrium close to the developing rumen (Fig.1). At 39 days of gestation, the primordium was relatively more developed and projected dorsally from the mesogastrium, and it was near to with the developing kidney (Fig. 2). In 41 days old foetus, the primordium became elongated and was separated from mesogastrium, except its cranial part where it was in contact with dorsal part of the developing rumen (Fig. 3). The dorsal (parietal) surface of the primordium was convex and was towards the kidney, whereas, the ventral (visceral) surface was partially concave to straight and was toward the developing rumen. In human foetus, splenic primordium was observed at the fifth week of gestation (Radhika et al., 2012) and 6th week of gestation by Mukhia et al. (2016). Jifei et al. (1991) in rat and Gupta et al. (2017) in goat observed in the splenic primordia at 16 and 46 days of gestation, respectively. According to Alex et al. (2015), the primordium of human foetal spleen was dual in origin as it originated from the thickening in the coelomic epithelium as well as from the underlying an iogenic mesenchyme of dorsal mesogastrium.

In 32 and 36 days old goat fetuses, the dorsal surface of splenic primordium was covered by a layer of simple squamous epithelium, the cells of which were wider and rounded in the middle but tapering towards the ends. These surface cells were in continuation with the similar cells of the mesogastrium and had eosinophilic cytoplasm with spheroid and oval vesicular nuclei. The part of the ventral surface, which was towards the developing rumen did not reveal any separate covering and was formed by the same mesenchymal cells which were observed in the interior of the primordium. (Fig. 4). In 39 days old foetus, the covering of the splenic primordium was almost same as the previous stage. However, the margins between the adjacent surface cells were indistinct (Fig. 5). At 41 days gestation, the parietal surface of splenic primordium, as well as the major part of the visceral surface, was covered by a layer of flattened cells or squamous cells. Only a few cells showed bulging of the nucleus on their central part (Fig. 6). The epithelial cells covering the dorsal surface were still in continuation with similar cells of mesogastrium. Banks (1993) in domestic animals stated that a thin layer of mesothelium covered the spleen. The mesothelium covering the splenic parenchyma was also reported by Dellmann and Eurell (1996) and Nicander et al. (1993) in domestic animals, Bajpai (1992) in goat and Panchal et al. (1998) in Marwari sheep. At 46 days gestation, the primordium of foetal goat spleen was covered by two layers of cells. The outer layer was formed by distantly arranged flattened cells. Whereas, the inner layer had relatively smaller, oval and lightly stained irregular cells having vesicular nuclei which were fibroblast cells observed for the first time (Fig. 7).

splenic primordium appeared as the condensation of mesenchymal cells in the dorsal mesogastrium. These cells were irregularly arranged and were densely packed. Linda et al. (2011) mentioned that eight millimeters sized human embryo exhibited a swelling at the left side of the mesogastrium which was resulted due to the accumulation of mesenchymal cells beneath the surface epithelium i.e. the mesothelium. On the basis of the shape, the mesenchymal cells of goat foetal primordium were two types. Several of them were lightly stained, elliptical cells having vesicular nuclei while few other cells were relatively darkly stained, more towards oval shape with condensed nuclei. At this stage, there were few blood island with nucleated erythrocytes and were surrounded by densely packed mesenchymal cells. There was no distinct cell lining demarcation around the blood island and surrounding mesenchymal cells (Fig. 1). At 36 days of gestation, a few large and elongated sinuses with nucleated erythrocytes entrapped inside were observed, however, the islands of blood were relatively less in occurrence and smaller in size (Fig. 4). In 39 days old goat foetus, the splenic primordium consisted of the aggregation of haemopoietic cells having anucleated erythrocytes among the condensed mass of mesenchymal cells. The occurrence of haemopoietic aggregations was relatively more toward the periphery of parenchyma than towards the center of parenchyma (Fig. 5). Both lighter and darker cells were observed in the parenchyma before islands and sinuses. The other structural detail of the parenchyma almost resembled with parenchyma of 36 days old foetus, however, at this stage the occurrence of darkly stained cells in the parenchyma was relatively more than the previous stage.

In 32 days old goat foetus, the parenchyma of the

At 41, 46 and 50 days gestation, the parenchyma of the spleen in goat foetus had relatively loosely arranged mesenchymal cells and aggregation of haemopoietic cells along with the isolated nucleated and anucleated erythrocytes distributed throughout the parenchyma (Fig. 6). At 50 days gestation, the occurrence of the anucleated erythrocytes was relatively more in the parenchyma than to the nucleated erythrocytes. The blood sinuses were relatively smaller in size. At 46 days of gestation, the reticular fibers were also observed for the first time which were short, fine and wavy in nature. These fibers were distributed in the parenchyma, towards the periphery of organ and around the small sinuses. In parenchyma, these fibers were present randomly but below the mesothelium lining and around the sinuses, these were relatively dense and more regularly arranged (Fig. 8). Gupta et al. (2017) in goat foetus observed the reticular fibers for the first time at the age of 60 days gestation in capsule and trabeculae of



the spleen, however, differentiation of capsule and trabeculae was not observed in the present study. Collagen and elastic fibers were not observed during this study as reported earlier by Gupta *et al.* (2017) in foetal goat spleen. These authors observed collagen and elastic fibers at 55 and 124 days of gestation, respectively.

In the early prenatal period at 46 and 50 days gestation, the mesenchymal cells, mesothelial cells, and erythrocytes in the parenchyma of goat foetal spleen were mildly positive for PAS (Fig. 9). The reaction for acid mucopolysaccharides in the spleen of these goat foetuses was almost negative in the early prenatal period, however, Gautam and Mishra (2015) in prenatal goat observed a strong PAS-alcian blue reaction in all the splenic components at every gestational age. In the goat spleen of the early prenatal period, the bound lipids in mesenchymal and mesothelial cells were moderately found (Fig. 10). In erythrocytes, these lipids showed an intense reaction at the margins of the cells but the cytoplasm had a mild reaction. In the present study, the nuclei of mesenchymal and mesothelial cells exhibited a moderate amount of DNA whereas, the nuclei of erythrocytes were mildly positive for Fuelgen's reaction.

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