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Morphological and cytological differentiation of goat spleen (*Capra hircus*)

Varsha Gupta*, M.M. Farooqui, Ajay Prakash, Archana and Prabhakar Kumar
Department of Anatomy,
College of Veterinary Sciences and Animal Husbandry, (DUVASU), Mathura-281 001, Uttar Pradesh, India.
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ABSTRACT
Development of goat spleen at various stages of gestation was studied. Primordium of spleen was observed on 46th day of gestation in close proximity to the developing rumen. All the parameters *viz.* length, width, thickness and weight were increased with advancement of age. Differentiating capsule and trabeculae were noticed on 69th day and 102th days of gestation, respectively. Reticular, collagen and elastic fibers were noticed at 60, 55 and 124 days of gestation, respectively. Red and white pulp was not fully differentiated till term.

Key words: Anatomy, Histology, Spleen, Goat.

INTRODUCTION

The spleen performs both hematopoietic and immunologic function. The embryonic origins of the spleen are obscure, with most studies describing the earliest rudiment of the spleen as a condensation of mesodermal mesenchyme on the left side of the dorsal mesogastrium (Kristin *et al.,* 2000).

During fetal life, spleen acts as an organ of blood formation but after birth only lymphocytes and monocytes are formed (Copenhaver *et al.*, 1975 and Dellmann and Eurell , 1996). Documentation of normal, embryonic and fetal development is necessary to understand the consequences of harmful influences at various stages of gestation (Evans and Sack, 1973). There are only a few histological studies on the goat fetal spleen in literature, more comprehensive analysis are needed to fully understand the changes occur during the fetal life. On perusal of literature it has been revealed that no sequential study was conducted on spleen during embryonic life in goat. Therefore, the present study was designed to study the gross and histological structure of spleen at various stages of gestation.

MATERIALS AND METHODS

The study was conducted on the developing spleen of goat embryos/foetii of different gestational age. The approximate age of embryos/foetii was estimated by using formula derived by Singh *et al.* (1979) in goat after interpolation of formula given by Hugget and Widdas (1951) in mammals. The abdominal cavity was opened by giving ventro abdominal incision. The developing spleen was exposed by careful dissection. Shape, size, weight and relations of the spleen with adjacent structures were recorded. For histological studies, spleen was collected and fixed in the 10% neutral buffered formalin term. shTissues were processed by routine paraffin embedding technique (Luna, 1968). Thick paraffin sections (06 µ) were stained with haematoxyline and eosin (Luna, 1968) for ddemonstration of general histo-architecture, Wilder's reticular stain (Luna, 1968) for demonstration of reticular fibers, Mallory's triple Stain (Crossman's Modification, 1937) for demonstration of connective tissue fibers and Weigert's method (Luna, 1968) for demonstration of elastic fibers. term. shopDifferent histological observations were recorded in the stained slides.

RESULTS AND DISCUSSION

Gross study: In the present study, the splenic primordium was noticed at 46 days of gestation. Spleen was in differentiating stage at 46 days of gestation and was in close contact with developing rumen. The human spleen developed at 35-40 days of gestation Copenhaver *et al.* (1975). In rat, primordium of spleen was distinguished at 16th day of gestation (Jifei *et al*., 1991). Late appearance of spleen in goat as compared to human and rat might be due to species differences in gestation period.

The spleen was located on left side of the abdominal cavity ventral to the first lumbar vertebra and opposite to the dorsal part of 11-13th ribs (Fig. 1) as reported earlier (Nickel *et al.,* 1973). It was creamish in early stage of development and gradually became reddish in color (Figs.1 and 2). Nickel *et al.* (1973) stated that the color of the sheep spleen after birth was reddish brown while goat had reddish grey color. The spleen was somewhat quadrilateral in shape. Spleen was thickest at cranio-dorsal angle and thins out caudally and ventrally. It has parietal and visceral surfaces, and four borders viz. cranial, caudal, dorsal and ventral borders. Parietal surface was convex and was in contact with developing diaphragm while visceral surface was deeply concave and was related to the parietal surface of dorsal and cranial sac of developing rumen. Cranial border was adherent to the rumen while the ventral border was free. The cranial border was

*Corresponding author's e-mail: drvarshaguptavet@gmail.com

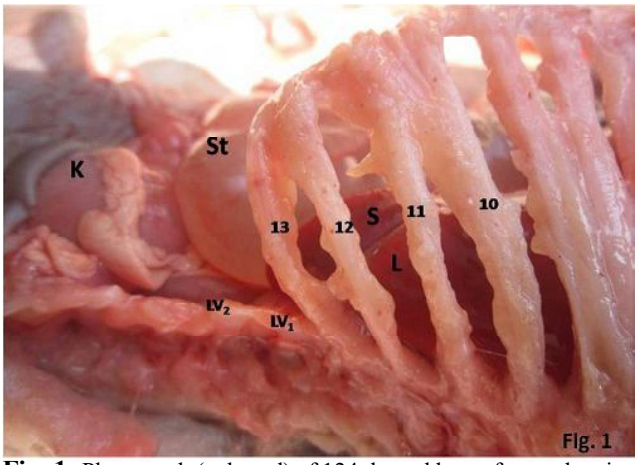

Fig. 1: Photograph (enlarged) of 124 days old goat foetus showing spleen (S), stomach (St), liver (L), kidney (K), ribs (10,11,12and 13) and lumbar vertebrae (LV_1 and LV_2)

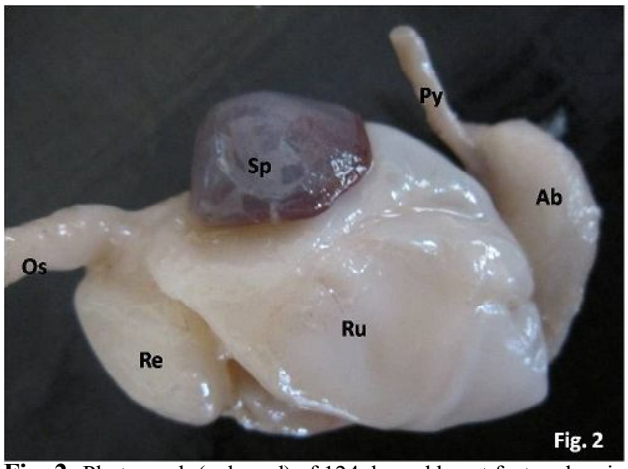

Fig. 2: Photograph (enlarged) of 124 days old goat foetus showing spleen (Sp), rumen (Ru), reticulum (Re), oesophagus (Os), abomasum (Ab) and pylorus (Py)

straight, thick dorsally and thins ventrally. The caudal border was thin and slightly concave. Dorsal border was thick cranially and thinner caudally whereas ventral border was thin and rounded caudally (Fig. 2). Hilus was present at the cranio- dorsal angle. At 69, 82, 87, 92, 102 and 124 days of gestation the length of the spleen was 0.6, 0.9, 1.1, 1.2, 2.6 and 2.7 cm, respectively and width was 0.4, 0.6, 0.8, 1.0, 1.9 and 1.9 cm, respectively. At 69, 82, 87, 92, 102 and 124 days of gestation the thickness of the spleen was 0.12, 0.16, 0.17, 0.21, 0.25and 3.0 cm, respectively and weight was 0.023, 0.107, 0.130, 0.214, 0.793 and 1.0 gm, respectively. Biometrical analysis showed that the dimensions and weight of the prenatal goat spleen increased with the advancement of age. This observation was in full agreement with the earlier report of prenatal human spleen (Ungor *et al.* 2007). They further substantiated that the prenatal human spleen showed positive significant correlation between age and various dimensions. Similar views have been reported by Asha *et al.* (2008) in prenatal goat spleen.

Histogenesis: At 46 days of gestation fetal spleen showed a discontinuous layer of flat cells lining the loosely arranged parenchyma, future mesothelium (Fig. 3). Arey (1954) mentioned that peritoneal mesothelium lay over the mesenchymal cells. At few places there was discontinuation of epithelial cells. The nuclei of these cells were vesicular type. Just below the mesothelium, loosely arranged undifferentiated cells were noticed, precursor of future capsule. These cells were mostly squamous shape whose chromatin was finely granular with scanty cytoplasm. 3-4 cells were in the close proximity of each other and can be spoken as the beginning of formation of blood vessels**.** Jifei *et al.* (1991) also reported that splenic blood vessels appeared just after the appearance of primordia of spleen. Some of the nuclei of the cells were large and ovoid in shape. Towards the center, the cells were running in a parallel row in a cord like fashion and were nearer to each other.

At 55 days, spleen had similar cytological character as on 46 day of gestation. However, nuclei of few epithelial

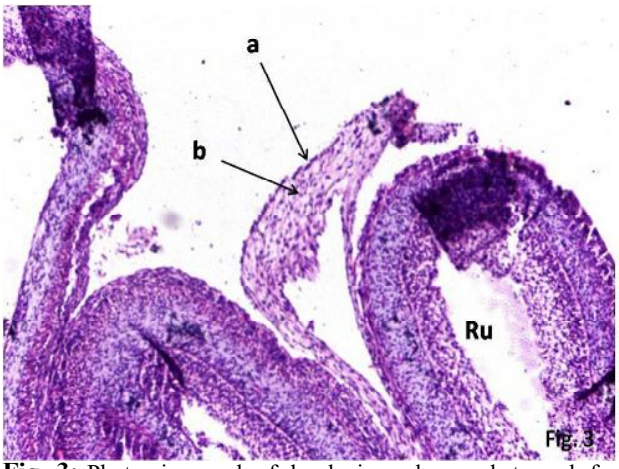

Fig. 3: Photomicrograph of developing spleen and stomach from a 46 day old goat foetus showing mesothelium (a), parenchyma (b) and rumen (Ru). H&E X 10

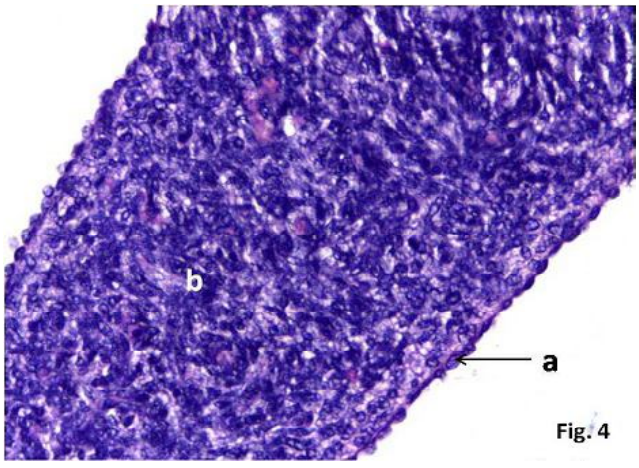

Fig. 4: Photomicrograph of 55 days old goat foetal developing spleen showing Future capsule (a) and parenchyma (b). H&E X 40

cells were bulged out from the surface (Fig. 4). Fine reticular fibrils were observed around the differentiating mesenchymal cells i. e. future capsule. The parenchyma was not differentiated into white and red pulp. However, at several places congested blood vessels were observed. The lining cells of blood vessels had large, spherical or oval vesicular nuclei. The parenchyma appeared to be concentrically arranged in intermeshing manner around developing blood vessels. The nuclear chromatin of most of the cells of parenchyma was adhering at the nucleolema gave a bright basophilic appearance and central part of the nucleus was mostly empty. The parenchyma was formed by irregularly dispersed cells which formed a continuous network. The parenchyma was more pronounced at the central region as compared to periphery. Abundant fine reticular fibers were noticed around the mesenchymal cells.

Capsule: At 60 days, the surface epithelium was continuous and had flattened squamous shaped cells. Number of bulging nuclei greatly reduced than previous age group. Reticular fibers became coarser in capsule region. At 64 days of gestation, reticular and mesenchymal cells were present in the mesenchyma. At 69 day of gestation the capsule was composed of mesenchymal tissue having fine reticular and collagen fibers which surrounded the parenchyma of the spleen (Fig. 6). It was covered by a continuous layer of squamous cells, the mesothelium. Few of the reticular fibers were wavy and arranged parallel to the mesothelium. The mesenchymal cells were spherical to fusiform in shape. They had spheroid or oval, vesicular nuclei. The cytoplasm of these cells was eosinophilic. At 82 day of gestation capsule became thick and had three to four cell layer. The capsule contained numerous, parallely arranged differentiated fibroblasts with dark stained fusiform to ovoid nuclei and had eosinophilic cytoplasm. From 87th day of gestation few cells of mesothelium became cuboidal in shape and had rounded nuclei. At this stage the capsule was relatively thicker, fibrous and wavy. It had two types of cells in addition to the mesenchymal cells. One of them was fusiform in shape and had darkly stained nuclei while other cells were rounded contained vesicular nuclei. The reticular fibers were relatively coarser and were abundant at this stage. The fibers were intermingled with each other. The collagen fibers became coarser and were arranged in loose bundles. At 102 days of gestation spleenic capsule showed similar cytological characters like 87 days and there was increase in thickness of the capsule (Fig. 7).

At 124 day of age the capsule became more fibrous (Fig. 8). The reticular and collagen fibers were abundant and the collagen fibers were arranged in the compact bundles and the reticular fibers showed branching.

Appearance of reticular and collagen fibers in developing spleen capsule were noticed at 55 and 69 days of gestation, respectively. Arey (1954) in mammals described that thin collagen fibrils appeared at third month of gestation. Collagen fibrils at later stage of gestation assumed parallel position and aggregated into wavy bundles. Asha *et al.* (2008) in prenatal goat spleen reported that the thickness of the capsule increased with maturity of fetal age and contain fibroblasts, smooth muscle fibers, blood vessels and nerves. Khalil *et al*. (2009) reported that the spleen of desi chicken (*Gallus domesticus*) was surrounded by thin capsule in prenatal life, which gradually became thicker in postnatal life. Human fetal spleenic capsule was thin in early gestation and with became slightly thicker with advanced age. It was composed of longitudinally arranged collagen and elastic fibers (Linda *et al*., 2011)

Thin reticular fibrils with few mesenchymal cells and isolated collagen fibers at various places invaginated from the capsule into the underlying parenchyma designing to become the future trabaculae at 69 day of gestation. Arey (1954) and Mc Geady *et al*. (2009) in mammals and bovines, respectively mentioned that principle structure of foetal spleen composed of capsule, trabaculae, white and red pulp and

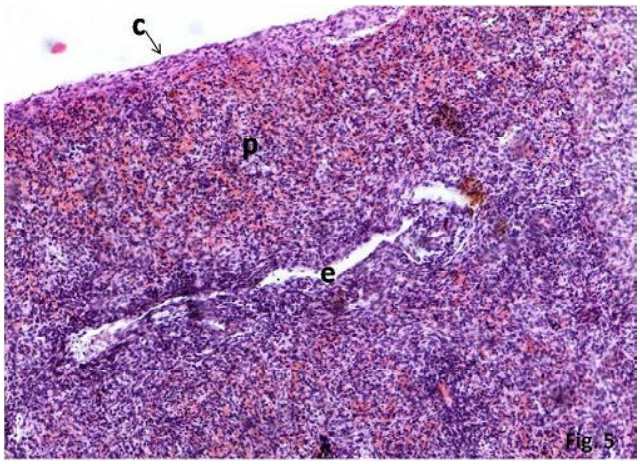

Fig. 5: Photomicrograph of 69 days old goat foetus showing capsule (c), parenchyma (p) and empty space (e). H&E X 20

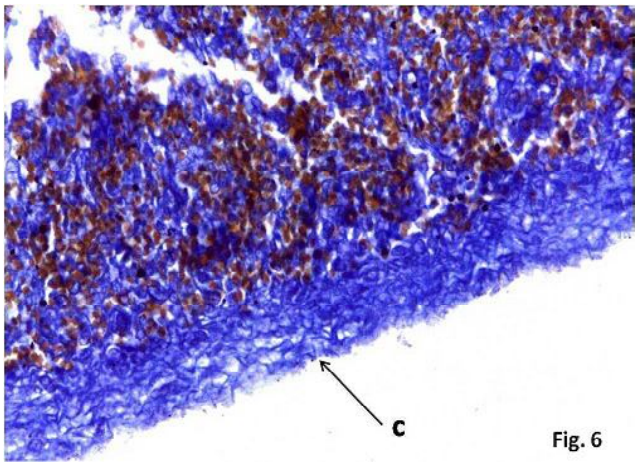

Fig. 6: Photomicrograph of 69 days old goat foetus showing collagen fibers in capsule (a). Mallory's Triple Stain X 40

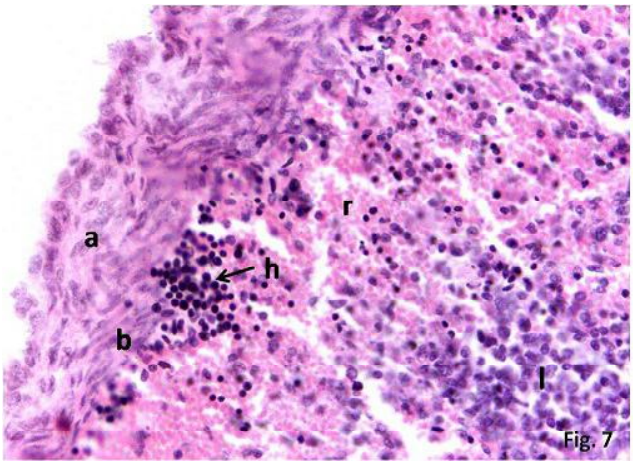

Fig. 7. Photomicrograph of 102 days old goat foetus showing round cells (a), fusiform cells (b) developing red pulp (r), aggregation of hemopoetic cells (h) and developing lymphatic nodule (l) H&E X 40

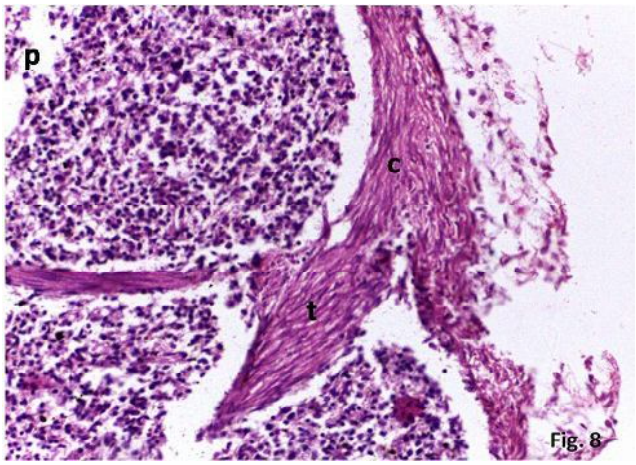

Fig. 8: Photomicrograph of 124 days old goat foetus showing capsule (c), trabaculae (t), developing red pulp and white pulp (p). H&E X 20

blood vessels. Asha *et al.* (2008) reported that the goat fetal spleen had a thin capsule by 3rd month of gestation but trabaculae did not extend into the parenchyma. At 82 day of gestation these trabaculae had few mesenchymal cells, differentiating fibroblasts and occasionally few hemopoetic cells. At 87 day of fetal age, the developing trabaculae were composed of relatively coarser reticular and collagen fibers along with the structures as observed on 82 days. The reticular fibers were abundant and became more coarser in trabaculae. At 102 days of gestation well formed trabaculae were observed which had small arterioles, fibroblasts and few differentiating smooth muscle cells. Fibroblast cells were compactly arranged. Moreover, at this stage the trabaculae showed branching and anastomosing within the parenchyma (Fig. 9). The thickness of the trabaculae also varied at various places in the parenchyma. It was thin at origin and became thick in the center o parenchyma. Asha *et al.* (2008) observed well formed trabaculae by 4th month of gestation which were made up of fibroblasts, smooth muscle fibers, blood vessels and nerves. Similar content of trabaculae was also described by Arey (1954) and Mc Geady *et al.* (2009) in mammals and bovines, respectively. In 124 days old fetus the trabaculae became more fibrous and thicker. Wavy and radially arranged reticular fibers were observed with branching and anastomosing pattern. Collagen fibers were abundant and were arranged in compact bundles.

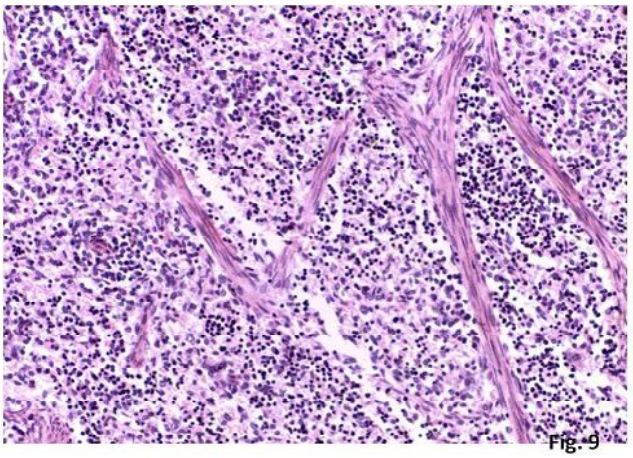

Fig. 9: Photomicrograph of 102 days old goat foetus showing branching and anastomosing pattern of trabaculae. H&E X 20

Parenchyma: At 60 days of gestation at few places group of differentiating hemopoetic cells were first encountered in the parenchyma. These cells were present either singly or in groups at few places. Networking of the parenchymal cells was reduced as compared to 55 days. Number of developing muscular artery increased as compared to 55 days of gestation and was surrounded by differentiating smooth muscle cells. The nuclei of smooth muscle cells were spindle shaped. The parenchyma showed tree like branching pattern of reticular fibers. These fibers became denser and were in the form of rete. Fibers were also observed around blood vessels. Hemopoetic cells like hemocytoblast, hemolymphoblasts and numerous free erythrocytes were observed in the parenchyma of 64 days old fetal spleen. Similarly, Copenhaver *et al.* (1975) described that in early stages of development of human fetal spleen the mesenchymal cells differentiated into reticulum and primitive free cells resembling lymphocytes. The parenchyma of 69 days old prenatal goat spleen was composed of mesenchymal cells, erythrocytes, lymphoblasts, fibroblasts and scattered hemopoetic cells (Fig. 5). In later stages the tissue became myeloid containing all stages of developing erythrocytes, granulocytes and megakaryocytes. At some places particularly beneath the capsule and occasionally in the parenchyma of spleen, the hemopoetic cells were arranged in small groups. The hemopoetic cells were of varying size and had darkly stained basophilic nuclei. At this age several empty spaces were observed within the parenchyma. At few places the empty spaces were

longitudinally arranged which had an interrupted epithelial lining of flattened cells having darkly stained flattened nuclei (Fig. 5). Fine collagen fibers were noticed in the wall of empty spaces. Few isolated fine reticular fibers forming network were seen in the parenchyma and around blood vessels. The reticular fibrils were also noticed around the reticular cells. Onyeanusi (2006) observed that the guinea fowl spleen had a thin but well-defined capsule and internal to this complete network of sinusoids filled with erythrocytes, lymphocytes and granulocytes was observed by day 18 of incubation. Linda *et al*. (2011) described that fetal spleen showed increasing amount of reticulin within the red pulp as spleen matures with advancing gestation. Asha *et al.* (2008) observed the mesenchymal primordia of goat spleen presented irregular vascular network at 2 month of gestation and composed of hemopoetic cells and reticular cells.

Vascularization was pronounced at 82 days of gestation. Arey (1954) also described that the mass of spleenic mesenchyme was well vascularized in advanced human foetus. He further focused that the capsule, trabaculae and pulp cord had been differentiated in later stage of gestation. The number of erythrocytes along with numerous irregularly distributed hemopoetic cells increased. In between these erythrocytes lymphoid tissues of different generations were present either in the form of circular or oval nodules or in the form of elongated cords. The hemopoetic cells showed different developing stages along with normoblast, macrophages etc. The normoblast cell had small nuclei with dense chromatin material and basophilic cytoplasm. The macrophages were irregular with more rounded and somewhat smaller, darkly stained nucleus as compared to that of fibroblast cells. Irregular shape, large sized megakryocyte cells were also present nearer to the trabaculae. These cells had lobulated nucleus and less basophilic granular cytoplasm. Small and large blood vessels were observed in the parenchyma which were usually encircled by concentrically arranged layer of fibroblast and mesenchymal cells. In addition to this few smooth muscle cells and 2-3 layers parellely arranged fibroblast cells were noticed within the parenchyma at many places. Vellguth *et al*. (1985) reported the "primary vascular reticulum," stage lasted up to the 14th gestational week in human. Numerous erythrocytes, normoblasts and macrophages were seen among a network of mesenchymal cells and argyrophilic fibers. Ishikawa (1985) in prenatal human spleen at the 8th week after ovulation observed that reticular cells formed a three-dimensional meshwork. Macrophages appeared at the 8th week of gestation and increased in number with the development of the fetus.

Goat fetuses of 87 days of age showed aggregation of lymphocytes in the periphery as well as in the center at many places and aggregations of hemopoetic cells in circular or oval nodular form were noticed in between the erythrocytes can be spoken as beginning of spleeniccorpuscles formation. Arey (1954) stated that the spleenic corpuscles appeared after sixth month of gestation in human foeti. The earlier appearance of lymphoid tissue might be due to species variation. Prior to significant lymphoid colonization in human spleen the central arterioles were surrounded by delicate reticulin fibers (Linda *et al*., 2011).

At 102 days of age the parenchyma was in the process of division into red and white pulp and several developing lymphoid nodules (Fig. 7). At few places these nodules were composed of central artery surrounded by mesenchymal, fibroblast and lympholast cells, however at most of the places the artery was not in the center of nodule. The red pulp had numerous erythrocytes along with different generation of scattered lymphocytes, monocytes, plasma and hemopoetic cells, macrophage and megakaryocytes. Large well developed muscular arteries were distinct in the parenchyma. Vellguth *et al*. (1985) observed lobulization of spleen between 15-17 week of gestation in human. The lobule consisted of a central artery, surrounded by a sheath of lightly stained stationary cells and at the periphery of these lobules the red pulp was noticed. These authors referred this stage as transformation stage. Ungör *et al*. (2006) also reported this stage between 13th -16th week of gestation. Copenhaver *et al*. (1975) described that following the development of characteristic distribution of vessels, lymphocytes which were compactly arranged around the arteries to initiate the beginning of white pulp formation. However, the spleenic corpuscles were not formed until the end of human fetal development. Aggregations of hemopoetic cells were pronounced in subcapsular space and decreases towards the center. Large sinuses interconnected to each other and were filled with blood at the center than subcapsular space. Similar views had been explained by Arey (1954) in human foetal spleen. Few megakaryocytes were noticed near the trabaculae and adjoining the sinuses similar to bone marrow. In bone marrow as megakaryocytes mature, they migrate towards the venous sinusus (Weiss and Wardrop, 2010). Abundant reticular fibers with branching and anastomosing pattern and fine collagen fibers were noticed around the wall of blood vessels. Number and dimensions of sinuses were increased at 124 days of gestation. In the wall of blood vessels discontinuous fine elastic fibers were observed. Ungör *et al*. (2006) reported that the period which is related with the development of the white pulp referred as the stage of lymphoid colonization and lasted up to the 22nd week of gestation. Characteristic red and white pulps did not form upto 124 days of gestational age in present study (Fig. 8). Present findings are in line with the earlier report of Copenhaver *et al*. (1975). These authors reported that the characteristic adult structure of red pulp was not attained until after birth in human. Asha *et al*. (2008) reported that the goat splenic parenchyma at four month of gestation presented a form resembling to mature animal with distinct red and white

pulp. Mc Geady *et al.* (2009) stated that the principal structure of bovine fetal spleen viz. capsule, trabaculae, red pulp, white pulp and blood vessels could be distinguished properly by the 3rd month of gestation. Khalil *et al*. (2009) in deshi chicken (*Gallus domesticus*) reported that the splenic pulps were not differentiated into white and red pulp on 15th day of embryonic life (ED15) but they were gradually differentiated into white and red pulp in the late prenatal (ED18) and postnatal period. Onyeanusi (2006) observed in the spleen of guinea fowl dark and light staining zones at 19th day of incubation and referred them as red and white pulps. Granulocytes contained many granules in their cytoplasm at 20 dayofincubation.

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