

## MICROSCOPIC ANATOMY OF A DEVELOPING FETAL SPLEEN: A CROSS SECTIONAL STUDY FROM NORTHERN INDIA

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### Abstract

**Background:** Spleen is the largest lymphatic organ present in the upper part of the abdominal cavity. It is a blood forming organ in fetal life and blood destroying organ in postnatal life. It filters the blood from blood borne antigens and microorganisms. The present study was conducted to describe the detailed histological changes of human fetal spleen during its development. The aim is to study the histogenesis of spleen in fetuses of different gestational age. **Materials and Methods:** The present study was a prospective, observational study conducted in the Department of Anatomy along with the Department of Obstetrics and Gynaecology at a tertiary care teaching hospital situated in Uttarakhand state from March 2021 to July 2023. Human fetuses between 13 to 40 weeks of gestational age and without any congenital malformations were collected after routine medical termination of pregnancies, still birth or intra uterine death for study. **Result:** The microscopic anatomy of the spleen was observed at different gestational ages. During first trimester of pregnancy primary vascular system was seen. Formation of red pulp and white pulp was seen clear during second trimester of pregnancy. At the beginning of the third trimester, white pulp shows lymphatic nodule with eccentrically placed arteriole. **Conclusion:** The detail study of development of fetal spleen suggest the functional aspect of spleen in fetal life.

## INTRODUCTION

The spleen is the largest lymphoid organ and appears at about sixth week of gestation,<sup>[1]</sup> simultaneously in several adjoining areas which soon fuse to form a lobulated spleen of dual origin. The spleen is surrounded by a capsule and located in the pathway of the blood stream (splenic artery and vein). The spleen is enclosed by a dense connective tissue capsule from which trabeculae extend into the parenchyma of the organ.<sup>[2]</sup> The connective tissue of the capsule and trabeculae contains myofibroblasts.<sup>[3,4]</sup> These contractile cells also produce extracellular connective tissue fibers. The substance of spleen, the splenic pulp, consists of red pulp and white pulp.<sup>[5]</sup> The red pulp consists of a dense network of reticular fibers that contains numerous erythrocytes, lymphocytes, plasma cells, macrophages and other granulocytes. The main function of the red pulp is to filter the blood. It removes antigens, microorganisms, platelets, and aged or abnormal erythrocytes from the blood.<sup>[6]</sup> The

white pulp is the immune component of the spleen and consists mainly of lymphatic tissue.<sup>[7]</sup> Lymphatic cells that surrounds the central arteries of the white pulp are primarily T cells, whereas the lymphatic nodules contain mainly B cells. The Spleen plays multiple supporting roles in the body. It acts as filter for blood as part of the immune system, which helps fight infectious diseases. It also detects abnormal red blood cells.

The variations in the lymphoid tissue, including spleen are well documented in the adults but few studies have documented the variations in the structure of spleen at different stages of development in the prenatal period. The present study was conducted at a tertiary care center to study the macroscopic and histological development of the human spleen, by observing changes in the gross splenic morphology and histology in fetuses of different age groups during the prenatal period.

## MATERIALS AND METHODS

The present study was a prospective, observational study conducted in the Department of Anatomy at a tertiary care teaching hospital situated in Uttarakhand state from March 2021 to July 2023.

### Inclusion and Exclusion Criteria

All fetuses ranging from the gestational age of 13 to 38 weeks, obtained from the Department of Gynaecology and Obstetrics of the tertiary care teaching hospital as a result of medical termination of pregnancy (MTP), intra-uterine death or still birth were included in the study. Fetuses showing any sign of congenital malformation were excluded from the study.

### Specimen Collection and Preservation

The specimen/ fetus were collected from the Department of Obstetrics and Gynaecology after obtaining due written, informed consent from the parents. These fetus specimen were collected within a window of one to two hours of the delivery. The gestational age was determined by reviewing the medical records available in the hospital and confirmation was done by taking the following measurements: crown-rump length, crown-heel length, length of the foot and the bi-parietal diameter. The weight of the fetus specimen was measured followed by injection of 10% formalin in the body cavities. Each fetus was then transferred and kept in a container filled with 10% formalin solution for seven to ten days.

### Specimen processing

The abdomen of the fetus was opened and the spleen, the location and appearance of the spleen was observed and recorded. The spleen was then carefully dissected out and further processing of the spleen tissue was done as per standard histological techniques.<sup>[2,8,9]</sup> Briefly, the specimen underwent grossing, fixation, dehydration, clearing and impregnation. The spleen tissue was then embedded in paraffin blocks and sectioning was done to obtain slices of around 10-12µm thickness. Finally, staining of the slides was done using Haematoxylin and Eosin stain and the slides were observed under light microscope using 10x (low power) and 40x (high power) objective lenses.

### Ethical considerations

The study was conducted as per the ethical guidelines for biomedical research on human subjects as given by the Central Ethics Committee on Human Research (CECHR) of the Indian Council of Medical Research (ICMR) and the 'Declaration of Helsinki' revision of 2013 after obtaining due ethical clearance from the Institute ethics committee. Informed consent was taken from all cases.

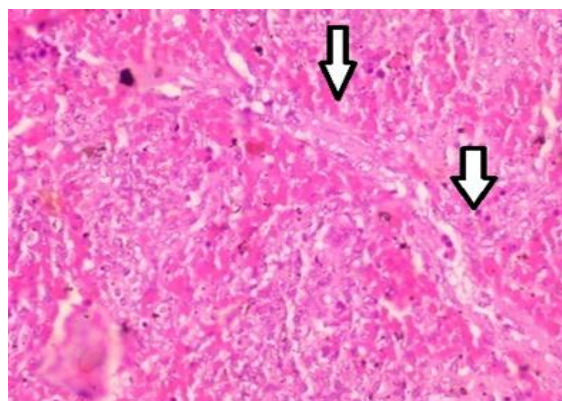
## RESULTS

A total of 60 fetuses were received in the Department of Anatomy during the study period. So, a total of 60 fetal spleen were included in the study. Thirty four

spleen belonged to fetus between 13 to 20 weeks of gestation, 17 had a gestational age of 21 to 30 weeks while nine had a gestational age of 31 to 40 weeks. The following microscopic features at different gestational ages were observed in these fetuses.

### Group 1: 13-20 weeks of gestation

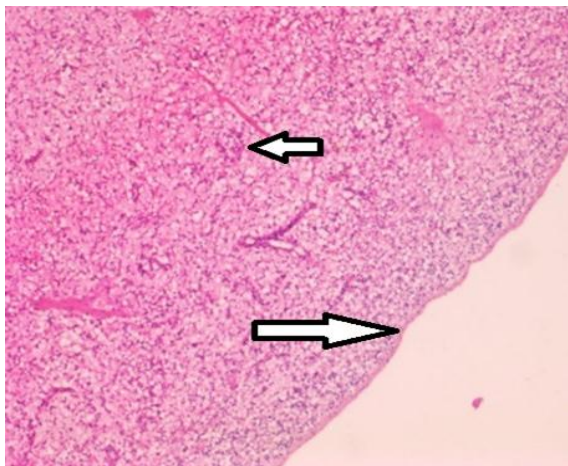
At 13 weeks, Spleen was covered by thin capsule made up of connective tissue. Fibroblast were observed in the capsule. Splenic interstitial tissue was dominated by collagen fibers. More reticular fibers, sinusoidal spaces, and blood vessels were appeared. Development of trabeculae begins within the capsule. Number of haemopoietic cells were increased. Numbers of lymphocytes arranged in groups are seen but they were scattered. At 18 weeks, lymphoid aggregation with centrally placed arteriole was seen. At the margins of these groups, sinusoids and large number of RBCs were observed. At 20 weeks, division of Red and white pulp appeared. [Figure 1] is a histological image of a spleen at 14 weeks showing lymphoblasts and reticular system.



**Figure 1: Photomicrograph of spleen at 14 weeks showing lymphoblasts and reticular system**

### Group 2: 21-30 weeks of gestation

Capsule and trabeculae were seen easily and they become thick. Red pulp and white pulp were seen clearly. Large number of blood vessels were noted. Red pulp containing RBCs and sinusoids were seen. At 30 weeks, white pulp showing lymphatic nodules with eccentrically placed arteriole were seen. Fig. 2 is a histological image of a spleen at 20 weeks showing well-developed capsule along with trabecular vessels.

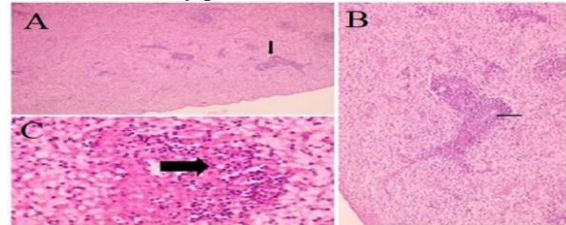


**Figure 2: Photomicrograph of spleen at 20 weeks showing well-developed capsule along with trabecular vessels**

**Group 3: 31-40 weeks of gestation**

At 31-35 week numerous sinusoids were appeared in red pulp, denser lymphocytic aggregation in

lymphoid follicles were seen. Well defined white pulp was seen. Presence of primary lymphoid follicles with germinal centers. At 37 weeks, hemopoietic activity is completely absent. At 38-40 weeks, fetal spleen resembled that of an adult spleen. [Figure 3] is a histological image of a spleen at 38 weeks showing well-developed red and white pulp with eccentrically placed arterioles.



**Figure 3: Photomicrograph of spleen at 38 weeks showing well-developed red and white pulp with eccentrically placed arterioles under 4x objective (A), 10x objective (B) and 40x objective (C)**

**Table 1: Previous studies reporting histogenesis of spleen in the literature**

Author	n	Capsule	Lymphocyte aggregation	Lymphoid follicle	Central arteriole	Resemblance with adult spleen
Souza, <sup>[14]</sup>	15	T1	T2	20-23 wk	30 wk	36 wk
Mukhia, <sup>[10]</sup>	50	12-14 wk	-	18 wk	22 wk	38 wk
Thomas, <sup>[11]</sup>	34	10-15 wk	20 wk	21-30 wk	31-40 wk	-
Holkunde, <sup>[12]</sup>	30	13-14 wk	-	20 wk	22 wk	38 wk
Yatagiri, <sup>[13]</sup>	100	12 wk	-	18-24 wk	24-30 wk	38-40 wk
Halder, <sup>[2]</sup>	9	12 wk	T2	-	-	36 wk

T1 = First trimester; T2 = Second trimester; wk = week

**DISCUSSION**

In a study by Mukhia et al,<sup>[10]</sup> 10-15 gestational week the spleen was covered by thin capsule made up of connective tissue. Splenic tissue dominated by collagen fibers. Fibroblast cells observed inside the capsule. Blood cells were RBC'S only. At 14 weeks, capsule along with developing trabeculae were seen in interstitial tissue. Reticular cells forming network were present but they were smaller in size, lymphocytes were present in scattered groups. At 16-20 weeks, spleen shows mixed population of cellular connective tissue components. Blood vessels and splenic sinusoids were more in number lymphocytes aggregation become prominent and could be detected at periphery of arteriole. At 20 weeks, the lymphocyte aggregations started differentiating around the central arteriole forming the peri-arteriolar lymphatic sheath. At 22 weeks, lymphoid aggregation with centrally placed arteriole was seen. At 31 gestational weeks, aggregation of lymphocytes in lymphoid follicle was denser and white pulp was defined.

In the present study, at 13 week, the spleen was covered by thin capsule consist of connective tissue, fibroblast and fibrocyte is present, development of trabeculae begins, hemopoietic cells increases. Thomas et al,<sup>[11]</sup> used hematoxylin and eosin stain to describe microscopic structure of spleen in fetus at 15 weeks, and observed that the trabeculae arose from

the capsule by this time. Arterioles present with no aggregation of lymphocytes. At 16 weeks, arteries were seen within trabeculae at 21 weeks lymphocytic differentiation starts around arteriole.

In the present study from 21 weeks onwards capsule within trabeculae seen prominently. Red and white pulp was distinct. Lymphoid aggregation were present around arterioles forming a peri-arteriolar lymphatic sheath. In a study by Holkunde et al,<sup>[12]</sup> by the end of 13 weeks of gestation, a thin capsule was developed, reticular cells and lymphoblast were seen. By 20 weeks, red and white pulp were seen prominently and lymphocytes were compactly arranged surrounding arterioles. At 36 weeks, onwards pattern of trabeculization was seen, division of red and white pulp were seen prominent. Lymphocytes were compactly arranged and arterioles were eccentric in position. The above findings are very similar to the present study.

Sandhya et al,<sup>[13]</sup> studied the Histogenesis of Human Fetal Spleen at different gestational ages in their study the spleen is lined by capsule at 12 weeks. At 17 weeks, central arteriole starts appearing, venous sinuses were present with scattered hematopoietic cells. Lymphocytes were seen around central arteriole. Around 18-24 weeks, capsule was thicker, trabeculae were seen, and central arteriole were surrounded by few lymphocytes. At 20 weeks, red and white pulp was seen at 24 weeks reticular fibers were seen around white pulp. At 24-30 weeks

germinal center was seen at 28th weeks eccentrically placed central arteriole was seen. At 30 -36 weeks, at 31 weeks capsule was thick, white pulp was well defined with germinal center. 36-40 weeks, the fetal spleen resembled that of an adult spleen, the hemopoietic activity is completely stopped. A few studies reporting the histogenesis of spleen at various gestational age are summarized in [Table 1].<sup>[14]</sup>

## CONCLUSION

The present study shows the histological and developmental changes in the human spleen at various gestational age of fetus. Histogenesis of fetal spleen is a multistep process depending upon the gestational age. Any deviation from normalcy may lead to structural and functional abnormality later in life.

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