

## Examination of the Histology and Development of the Fetal Spleen at Various Stages of Gestation

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Received: 25-02-2024 / Revised: 23-03-2024 / Accepted: 25-04-2024

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Conflict of interest: Nil

### Abstract:

**Background and Objectives:** The spleen is a largest collection of lymphoid tissue with peculiar anatomical and physiological features. Spleen plays an important role in fetal hematopoiesis and immunomodulation. The aim of the study is to perform detailed histological analysis of human fetal splenic specimens of various gestational ages and compare the findings with earlier studies.

**Material and Methods:** The present study included 40 fetal cadaveric spleen and morphometric features i.e., weight was measured, and the sections of the spleen were stained with Haematoxylin and Eosin stain and were observed under compound light microscope.

**Results:** In the present study the histology of spleens of prenatal group showed the well-defined red pulp, venous sinusoids and diffusely spread lymphocytes at 18 weeks of gestation and the organization of lymphoid follicles was noticed at 24-28 weeks. At 32 weeks well defined white pulp was observed and the microscopic architecture of the spleen was similar to the histology of adult spleen.

**Conclusion:** A detailed knowledge of splenic morphometric dimensions, Histology and Histogenesis is crucial in deciphering the role of spleen in fetal development and fetal wellbeing.

**Keywords:** Spleen, Hematopoietic, Microscopic, Fetal.

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### Introduction

The spleen is a largest secondary lymphoid organ which plays an important role in fetal hematopoiesis and immunomodulation. The spleen arises from a mesenchymal proliferation in dorsal mesentery or mesogastrium during 5th week of gestation [1,2]. The spleen assumes its definitive morphological structure during the 3rd month, the size gradually increases during the fetal period as mentioned by Sir Henry Gray in 1854 in the book "The structure and the use of spleen" [2]. Spleen by tissue nature belongs to the lympho-reticular organs but unlike the 'white' lymph nodes it is not included in the lymph but in the blood circulation [3]. In the antenatal period, the spleen plays an important role in the immunomodulation, acts as a primary haemopoietic centre until late in the fetal period, also plays an important role in apoptotic cell clearance, immune tolerance, efficient removal of blood-borne microorganisms, lymphocyte differentiation and activation [4]. These functions are spleen, the white pulp (including the marginal zone) and the red pulp, which are vastly different in their microscopic architecture, vascular organization, and cellular composition [5,6]. The white pulp is subdivided into periarteriolar lymphoid sheath (PALS), the follicles, and the marginal zone. In the antenatal

period the histogenesis of spleen occurs in three stages: 1. Preliminary stage also called the "primary vascular reticulum," lasts up to the 14th gestational week. The primordium of the spleen appears in the embryos of size 6-7 mm in the 5th gestational week, and the hematopoietic cells were observed in the vascular lumen in 10th week [7]. Numerous erythrocytes, normoblasts and macrophages are seen among a network of mesenchymal cells and argyrophilic fibers.

**Stage of transformation:** The characteristic structure of the organ becomes established during this stage beginning with the 15th gestational week.

Splenic lobules begin to form during the 15th to 17th gestational week.

### Stage of Lymphoid Colonization

The development of the white pulp is correlated with the stage of lymphoid colonization around the central arteriole occurs during the 18-20 weeks. Around the 23rd week the aggregation of primary follicles is discernible at the periphery of the PALS and by 30-33 weeks distinct red and white pulp will be formed [4,7]. However there is limited research regarding the histology and histogenesis of spleen

at different gestational ages which justifies the purpose of this study.

### Material and Methods

The present study is a prospective type of study conducted in the department of Anatomy, at Darbhanga Medical College and Hospital Laheriasarai Darbhanga, Bihar. Medically terminated fetuses of both sexes and relevant obstetric data were collected from Madhubani Medical College Madhubani, which includes 40 dead aborted fetuses of both sexes ranging from 16 weeks of gestation to term. The ethical committee approval and consent of the relatives were obtained.

In the department of anatomy the collected fetuses were observed for congenital anomalies and they were preserved in 10% formalin. After one week of preservation, the abdomen was opened by using routine dissection method and the morphological observations were made Insitu. Later spleen were removed by using routine dissection method and outer surface of fetal spleens were dried with blotting paper and then weighed by using digital weighing balance (SHIMADZU-ATY224-UNibloc). For microscopic observation, the antenatal specimens were broadly categorized into 5

groups. Representative samples from each group were subjected to routine histological processing for H & E and reticulin stains. The sections were observed under 10x and 40x objective piece of microscope and representative fields were photographed by using photomicrographic equipment and the results were analyzed. Haematoxylin and Eosin staining. Reticulin stain to demonstrate reticular fibres.

The collected data was subjected to statistical analysis by computing the mean of each parameter with respect to the gestational age-wise groups by using SPSS 20 version.

### Results

The Crown-rump length of all the fetuses were initially measured and fetal gestational age is calculated in weeks and based on the age in weeks the fetal specimens were categorized into 5 groups i.e., 16-20 weeks, 21- 24 weeks, 25-28 weeks, 29-32 weeks and 33 weeks to Term. The largest group was fetuses with gestational age 16-20 weeks with 10 specimens. The gender-wise distribution was 57.5% and 42.5 % for male and female groups respectively.

**Table 1 : Gestational age/ Gender wise distribution of prenatal group**

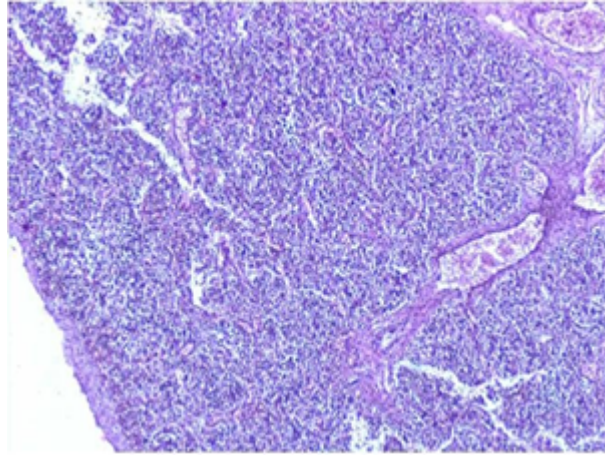
| Group | Gestational age (weeks) | Male (%)   | Female (%) | Total (%) |
|-------|-------------------------|------------|------------|-----------|
| A     | 16-20 Weeks             | 6 (26)     | 4 (23.5)   | 10(25)    |
| B     | 21-24 Weeks             | 3(13)      | 2(11.7)    | 5(12.5)   |
| C     | 25-28 Weeks             | 3(13)      | 6(35.7)    | 9(22.5)   |
| D     | 29-32 Weeks             | 6 (26)     | 2(11.7)    | 8(20)     |
| E     | 33 – Term               | 5 (22)     | 3(17.6)    | 8(20)     |
|       | Total                   | 23 (100 %) | 17(100 %)  | 40 (100)  |

The splenic weight is maximal in 29-32 weeks group with mean  $3.33 \pm 1.01$  gm. The weight of the spleen ranged from 0.1 gm to 4.9 gm. The average weight of the fetal spleen is  $1.98 \pm 1.22$  gm. In the present study the earliest specimen observed was that of fetal spleen of 16 weeks gestational age. The observations made in prenatal group were as follows. Fetal spleen sections showed thickened capsule and dilated sinusoids filled with blood cellular elements, diffuse aggregations of lymphoblasts.

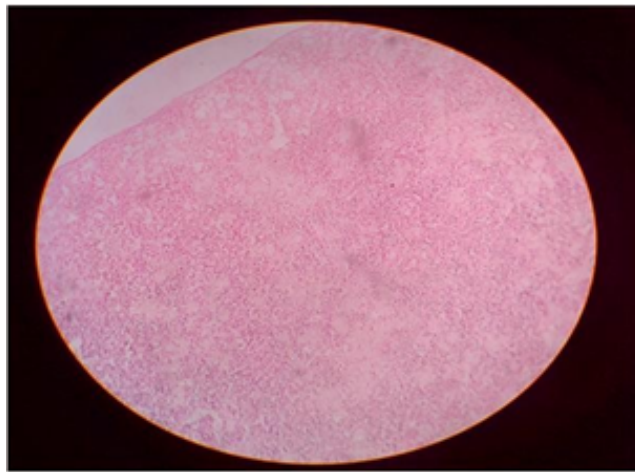
Well defined lymphoid follicles central arterioles and white pulp were conspicuously absent. Megakaryocytes seen with evidence of extramedullary hematopoiesis. The spleen sections showed distinct fibrous capsule, accumulation of lymphoid follicles around arteriole. aggregation of lymphocytes to form lymphoid follicles was noticed. Sinusoids, capillaries with thin endothelial lining were observed. Reticular network, Sinusoids and blood vessels increased in number. Extramedullary hematopoiesis was noticed. Clear

demarcation between red pulp and white pulp was absent. The spleen sections showed white pulp formation in the form of well-defined lymphoid follicles and trabeculae were present. There was a well-defined evolution of Red pulp with RBC. Periarterial lymphoid aggregation was noticed. Well-developed lymphoid follicles with central arteriole were seen.

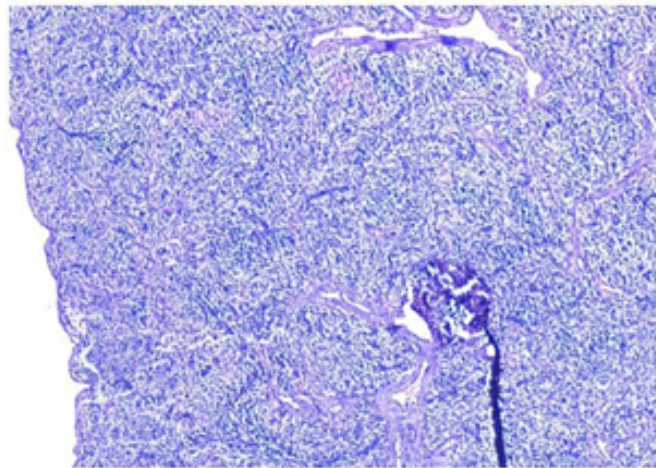
The spleen sections showed well fibrous capsule, numerous sinusoids, aggregation of the lymphoid follicles with an eccentric arteriole. Increased size of the white pulp was noticed. Well differentiated Malpighian corpuscles were present. In this group at term the splenic tissue showed well defined white pulp with dense aggregation of lymphocytes with clear demarcation from the surrounding tissue. White pulp was sparsely associated with collagen fibers. The red pulp showed collagen network of fibers in continuance with trabeculae. It also showed sinusoids filled with RBC, occasional fibroblasts and the microscopic structure at term resembled that of adult spleen architecture.



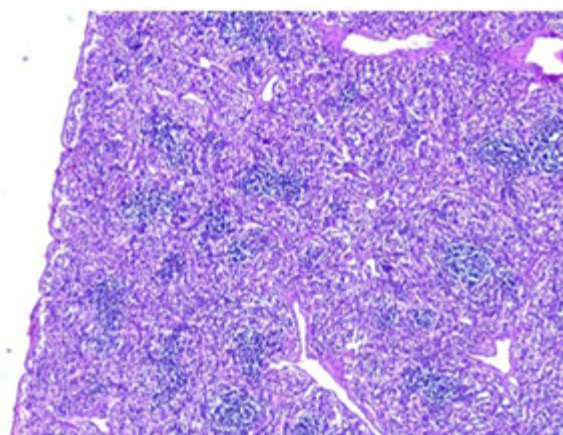
**Figure 1: 18 weeks human fetal spleen section showing thickened capsule and dilated sinusoids filled with blood cellular elements, aggregations of lymphoblasts are seen H&E stain, 40X.**



**Figure 2: 20 weeks human fetal spleen section showing fibrous capsule and dilated sinusoids filled with blood cellular elements. White pulp and malpighian bodies are absent H&E stain, 40X.**



**Figure 3: 25 weeks human fetal spleen section showing fibrous capsule, accumulation of lymphoid follicles around arteriole, increased white pulp 40 x H&E stain**



**Figure 4: 28 weeks human fetal spleen showing whitepulp in the form of follicles, with trabeculae, periarteriolar lymphoid aggregation is also seen H&E stain, 40X.**

| Showing comparison of fetal body weight (gms) of present study with literature |               |                            |                      |                           |                           |
|--|---------------|----------------------------|----------------------|---------------------------|---------------------------|
| Gestational age (wks.)   | Present study | Parulekar et al [8] [1995] | Gruenwald [9] [1960] | Schultz et al [10] [1962] | Ajit holkundee et al [11] |
| 16 weeks   | 310           | 200                        |                      |                           | 200                       |
| 20 weeks   | 545           | 460                        |                      | 316                       | 420                       |
| 24 weeks   | 870           | 820                        | 630                  | 748                       | 802                       |
| 28 weeks   | 1270          | 1300                       | 1020                 | 1127                      | 1290                      |
| 32 weeks   | 1809.5        | 2100                       | 1488                 | 1748                      | 1924                      |
| 36 weeks   | 2446          | 2900                       | 2165                 | 2448                      | 2910                      |

## Discussion

In the present study the average weight of the pre-natal spleen was 1.98 gm. The splenic weight ranged from 0.1 gm to 4.9 gm which gradually increased with gestational age significantly till 38 weeks of gestation.

The findings regarding the weight of the spleen in relation to gestational age were in agreement with the observations of Gruenwald P & Minh HN [9] (1960) and with the descriptions of Sir Henry Gray [2] (1854) till 32 weeks of gestation and not in agreement afterwards. On comparison of findings of present study with existent literature, the body weight in relation to gestational age was in agreement with findings of Parulekar et al [1995] [8] and was more when compared to the findings of Gruenwald *et al* (1960) [9], Schulz *et al* (1962) [10].

Vellguth *et al* (1985) [12] proposed three stages of spleen development from 14th-24th week of gestation namely- preliminary stage of splenic primordium [from 14th week], Transformation stage of development of splenic architecture [ 15-18 weeks], followed by Lymphoid colonization & development of white pulp [18-24 weeks] According to Vellguth *et al* [12], at 14 weeks connective tissue, fibroblasts, vascular endothelium are formed and there was increase in blood vessels and mesenchyme. Venous sinuses are not formed. Splenic lobules begin to form in 15 th week, and fully differentiated by 17<sup>th</sup> week. The lobules consisted a

central artery, with red pulp forming at the periphery. The differentiation of the red pulp is closely correlated with the development of the venous system. An accumulation of lymphocytes around the central arteries (PALS) can be recognized during the 19th and 20th week of gestation which marked the formation of white pulp. Around 23rd week the primary follicles were arranged at the periphery of the PALS. In the present study earliest fetal spleen was of 16 weeks which showed dilated sinusoids, aggregation of lymphoblasts with evidence of extra medullary hematopoiesis. At 20-24 weeks, formation of lymphoid follicles was observed. At 30 weeks, white pulp formation in the form of well-defined lymphoid follicles and trabeculae was noticed. At 33 weeks well differentiated white pulp, red pulp and well formed According to Radhika *et al* [13] lymphocytic aggregation started by 11 th week. 20th week marked the development of lymphoid follicles and by 32 weeks well developed lymphoid follicles with central arteriole was observed. Mature lymphoid Malpighian corpuscles were present. follicles with peripherally placed arteriole were noticed at 36 weeks of gestation. These findings are almost in agreement with the present study. According to Rajeev Mukhia *et al* [2016] [14] vasculature, connective tissue were formed and sinusoids were seen at 16-20 weeks. At 20th Gestational week, the spleen shows clear-cut capsule surrounding the spleen with bundles of collagen fibres & fibroblasts. The lymphocyte aggregations started differentiating around the central

arteriole forming the periarteriolar lymphatic sheath (PALS) was noticed by 20 weeks. Distinct white pulp showing lymphatic nodules with peripherally placed central arteriole was observed at 26 - 30 weeks. At 23rd Gestational weeks, the structure of spleen resembles to the adult spleen. Above findings were in concordance with the observations of present study.

1. Thickened capsule, dilated sinusoids, lymphoblasts and few blood vessels were seen.
2. Evidence of extramedullary hematopoiesis was noted.

**At 20-24 weeks:** Aggregation of lymphocytes to form lymphoid follicles around an arteriole was noticed.

**At 25-32 weeks:** Well defined white pulp, Red pulp, periarteriolar lymphoid sheath [PALS], well developed lymphoid follicles with central arteriole were seen.

**After 33 weeks:** Well differentiated Malpighian corpuscles were present, structure resembled that of adult spleen. These findings were in agreement with the observations of Mrinmoy pal et al [15], Merida- Velasco JA [16].

### Conclusion

The current study was conducted to analyze the histological development of spleen at various gestational ages. We hope a detailed knowledge of Histology and Histogenesis may provide an insight in understanding the role of spleen in fetal development and fetal wellbeing. However further research directed at molecular level and immunobiology is required in elucidating the actual role of spleen in human beings in immunity & autoimmunity.

### References

1. Mebius RE, Kraal G. Structure and function of the spleen. *Nat Rev Immunol*. 2005 Aug; 5(8):606-16.
2. Gray, H.: On the Structure and Use of the Spleen. London, John W. Parker and Son, 1854: 1-53
3. Louis Gross. Studies on the Gross and Minute Anatomy of the Spleen in Health and Disease. *J Med Res*. Jan 1919; 39(3): 311-338.
4. Ungor B, Malas MA, Sulak O, Albay S. Development of spleen during the fetal period. *Surg Radiol Anat*. 2007;29(7):543-550
5. Victor P. Eroschenko. Di fiore's Atlas of histology with functional correlations, 11th edition. Lippincott Williams & Wilkins. 2008;206-208.
6. Madeleine D. Kraus, MD. Splenic Histology and Histopathology: An Update. *Semin Diagn Pathol*. 2003 May; 20(2):84-93.
7. Satoh, T. Sakurai, E. Tada, H. Masuda, T. Ontogeny of reticular framework of white pulp and marginal zone in human spleen: immunohistochemical studies of fetal spleens from the 17th to 40th week of gestation. *Cell Tissue Res*. 2009; 336:287-97.
8. Parulekar SV. Criteria for determination of fertilization age during fetal period. *Practical anatomy*, 1995, 1st ed, p.361.
9. Gruenwald P, Minh HN. Evaluation of body and organ weights in perinatal pathology I. Normal standards derived from autopsies. *Am J Clin Pathol* 1960; 34: 247-253.
10. Schulz DM, Giordano DA, Schulz DH. Weight of organs of fetuses and infants. *Arch Pathol Lab Med* 1962; 74:244-50.
11. Ajit Holkunde et al. The histological study of human fetal spleen. *Indian Journal of Clinical Anatomy and Physiology*, April-June, 2018 ;5(2);259-264
12. Vellguth S, Gaudecker BV, Muller-Hermelink HK. The development of the human spleen. Ultrastructural studies in fetuses from the 14th to the 24th week of gestation. *Cell Tissue Res*. 1985;242(3):579e592.
13. Radhika D, Saila Rekha N. Prenatal Histogenesis of Human Spleen. *Indian Journal of Public Health Research & Development*; Jan-Mar 2012; 3 (1):129.
14. Rajeev Mukhia. Histogenesis of human fetal spleen: *International Journal of Anatomy and Research*, Int J Anat Res. 2016; 4(1):2119-24.
15. Mrinmoy Pal T.H. Naranbabu Singh, C.H. Rajendra Singh. Histogenesis of spleen in human fetuses. *Journal of the Anatomical Society of India*. Dec 2013;62(2): 139-145.
16. Merida-Velasco JA. Histogenesis of the spleen in the human embryo in O'Rahilly's stages 17 to 23. *Arch Anat Histol Embryol*. 1989; 72:97-104.