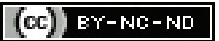


Histogenesis of White Pulp of the Human Foetal Spleen at Different Gestational Age Groups: A Cross-sectional Study

S MADHUMALAR¹, A THENMOZHI², N MYTHILY³, M ARUL SRUTHI⁴, K ANAND⁵

ABSTRACT

Introduction: The spleen is a highly vascular, ductless and the largest lymphoid organ in our body. Its functions include phagocytosis and immune responses. In the foetus, haematopoiesis is the primary function of the spleen, which regresses after birth; however, the production of lymphocytes continues after birth.

Aim: To investigate the histological changes in the immune component, specifically the White Pulp (WP), of the human foetal spleen at various gestational age groups.

Materials and Methods: The present cross-sectional histological study was conducted in the Department of Anatomy, Government KAPV Medical College, Trichy, Tamil Nadu, India, from July 2019 to December 2021. Study was carried out on 50 samples of aborted human foetuses between 10 weeks and 40 weeks of gestational age, without any obvious congenital abnormalities, in a South Indian population. The samples were collected from the Department of Obstetrics and Gynaecology at Government Mahatma Gandhi Memorial Hospital, Trichy, Tamil Nadu, India, after obtaining institutional ethical clearance and consent from the mothers. After processing the splenic tissues, slides were

prepared and stained with Haematoxylin and Eosin (H&E). The slides were observed under a compound light microscope using 4x, 10x, 40x and 100x magnifications. The appearance of lymphocytes, their developmental growth, and changes in WP were observed and interpreted. Statistical analysis was performed by computing the mean number of lymphoid follicles across gestational age groups using Statistical Package for the Social Sciences (SPSS) software version 21.0.

Results: Among the 50 foetuses, the capsule appeared between the 10th and 12th weeks, and trabeculae appeared at the 18th week. Lymphocytes were first observed at the 14th week, and lymphoid aggregation around the arteriole began at 22-24 weeks. The Periarteriolar Lymphatic Sheath (PALS) was observed at the 24th week, with definite WP noted at 36 weeks. Vascular loops appeared at the 12th week, and the vascularity of the WP increased with the age of gestation.

Conclusion: The present study provides detailed knowledge of the histogenesis of the WP of the spleen, which is valuable for anatomists and pathologists. The foetal spleen exhibits high vascularity, suggesting its potential use for transplantation in cases of splenectomy in the future.

Keywords: Central arteriole, Eccentric, Follicle, Lymphocyte, Nodules

INTRODUCTION

The spleen is a secondary lymphoid organ that plays a role in foetal haematopoiesis and immunomodulation. Haematopoietic function of the spleen continues throughout the foetal period and regresses after birth; however, the production of lymphocytes continues in postnatal life [1]. The spleen arises from the mesenchymal proliferation of the mesogastrium during the fifth week of gestation and reaches its definitive morphological structure during the third month [2]. The spleen is termed the graveyard of Red Blood Cells (RBCs), as it destroys aged RBCs; splenic macrophages engulf any blood-borne antigens. Foetuses exposed to antigen-related diseases undergo morphological changes in lymphoid organs, presumably as a consequence of the primary foetal immune reaction. These changes are characterised by an increase in the number of lymphoblasts and partly of macrophages in the spleen and lymph nodes. Exposure of foetuses to antigen-related diseases appears to cause marked changes in the normal development of lymphoid organs [3].

The splenic parenchyma is divided into two components: White Pulp (WP) and red pulp. The WP occupies 5% to 20% of splenic tissue and mainly contains lymphocytes. The WP is subdivided into the PALS, follicles, and the marginal zone. Through the hilum, the splenic artery enters, branches, and then passes into the splenic parenchyma via the trabeculae of the spleen. When it enters the WP, it is called the central artery [4].

The PALS are lymphocyte aggregates around the central artery. PALS appear expanded in places due to the aggregation of B lymphocytes, resembling lymphatic follicles. They are visible as white, semiopaque dots to the naked eye on a freshly cut spleen and are usually situated near the terminal arterioles. These nodules displace the central artery to an eccentric position, which differentiates the spleen from the lymph node [5].

The PALS and follicles are centres for lymphoid aggregation and proliferation. After antigenic stimulation, there is intense B cell proliferation and the development of germinal centres similar to those found in lymph nodes. Antigen-presenting dendritic cells present in the follicles are responsible for this germinal centre formation. Within 24 hours after antigenic stimulus, these germinal centres become very large and are visible to the naked eye. They are called splenic nodules or Malpighian corpuscles. When antigenic stimulation abates, the germinal centre regresses [6].

The marginal zone, or perifollicular zone, is located at the periphery of PALS, in between the red pulp and WP. It is considered a separate compartment and not part of the WP. From this site, many lymphocytes leave the circulation and migrate into the appropriate B and T lymphocyte aggregations. Here, lymphocytes are loosely arranged between a dense network of reticular fibres and reticular cells [7]. The role of the marginal zone is to identify blood-borne antigens and pathogens present in systemic circulation, which activate antigenic reactions [8].

According to Vellguth S et al., the development of the foetal spleen is described in the following three stages [1]: In the preliminary stage, erythroblasts, normoblasts and macrophages are observed. In the transformation stage (15th-18th week), splenic lobules begin to form, consisting of a central artery surrounded by sheaths of lightly stained stationary cells like myofibroblasts. In the stage of lymphoid colonisation (18-24 weeks), WP started to develop at the 18th week of gestation. Accumulation of lymphocytes around the central arterioles (PALS) is seen around the 19th-20th week. Primary follicles assemble at the periphery of PALS. In the present study, the authors aimed to find out the histological changes in the WP of the human foetal spleen across various gestational age groups. The present study is compared with national and international studies based on the appearance of lymphoid follicles, the diameter of the lymphoid follicles, the development of the central arteriole, PALS and the marginal zone.

MATERIALS AND METHODS

The present cross-sectional histological study was conducted in the Department of Anatomy, Government KAPV Medical College, Trichy, Tamil Nadu, India, from July 2019 to December 2021. Ethical committee approval was obtained from the Institutional Ethical Committee (IEC No. 16/2020).

Inclusion criteria: Human fetuses aborted or stillborn, with a gestational age between 10 weeks and 40 weeks, were included in the study.

Exclusion criteria: Foetuses less than 10 weeks old and those with major congenital anomalies, such as omphalocele, were excluded from the study.

Study Procedure

Fifty samples of human fetuses [Table/Fig-1] were collected from the Department of Obstetrics and Gynaecology at Mahatma Gandhi Memorial Hospital, Trichy, Tamil Nadu, India, after obtaining prior consent from the mothers. The study samples were arbitrarily divided into five groups according to gestational age:

Group I: 10-15 weeks;

Group II: 15-20 weeks;

Group III: 21-25 weeks;

Group IV: 26-32 weeks;

Group V: 33-40 weeks.



[Table/Fig-1]: Samples of 50 fetuses.

In each group, 10 fetuses were studied. The gestational age of the fetuses was determined based on the last menstrual period and ultrasonogram reports from antenatal cases in the medical records during the collection. The age was confirmed by Crown Rump Length (CRL) calculation. The collected fetuses were preserved in 10% formalin for 15-20 days. Dissection of the fetuses was performed following the standard protocol described by Enid Gilbert Barness for foetal autopsy.

After dissection, the spleen was kept in 10% neutral buffered formalin for 2-4 days. The spleen was processed using the following technique:

1) tissue fixation; 2) tissue cutting; 3) dehydration; 4) clearing; 5) embedding; 6) microtomy and sectioning of the tissue block. Splenic tissues were processed for H&E staining. All sections were examined under a binocular light microscope, and photographs were taken using a USB camera. All the photographs were studied and analysed.

Ocular and stage micrometer were used to measure the diameter of the lymphoid follicles. The shape of the lymphoid follicles in the spleen is either oval or spherical, so two measurements were taken: one for the vertical diameter and another for the transverse diameter. The diameter of the lymphoid follicle was calculated as follows:

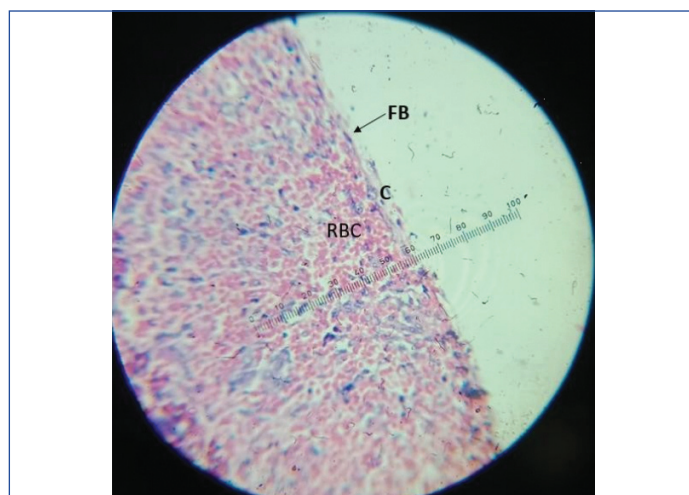
Diameter of lymphoid follicle=(maximum transverse diameter+ maximum vertical diameter)÷2.

The average diameter values were calculated in millimeters by converting measurements from the ocular micrometer to the stage micrometer.

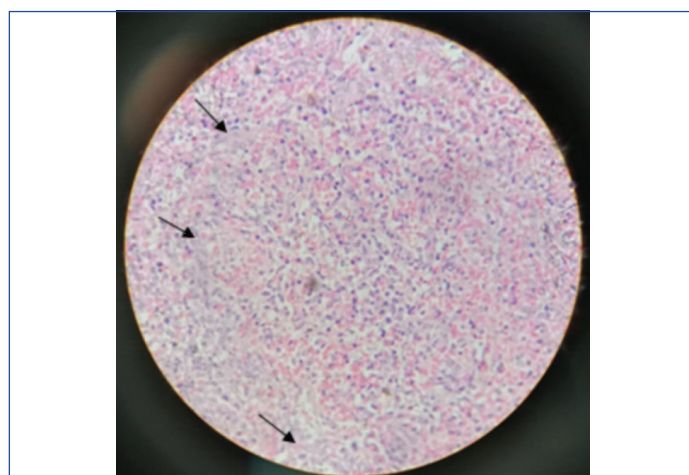
RESULTS

In 50 fetuses, the observations were categorised into five groups based on gestational age.

Group I (10-15 weeks): Lymphocytes were diffusely present in the parenchyma of the spleen. Definite WP not formed in this group [Table/Fig-2]. In the 14th week, lymphocytes appeared in a scattered manner [Table/Fig-3].



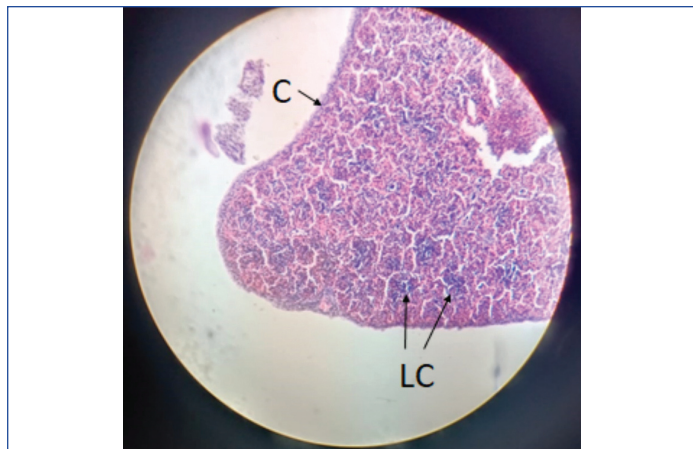
[Table/Fig-2]: A 10th week fetus reveals a thin capsule (C) with flat Fibroblast (FB) cells (H&E, 40x).



[Table/Fig-3]: In the 14th week fetus showing the appearance of lymphocytes (arrows) (H&E, 40x).

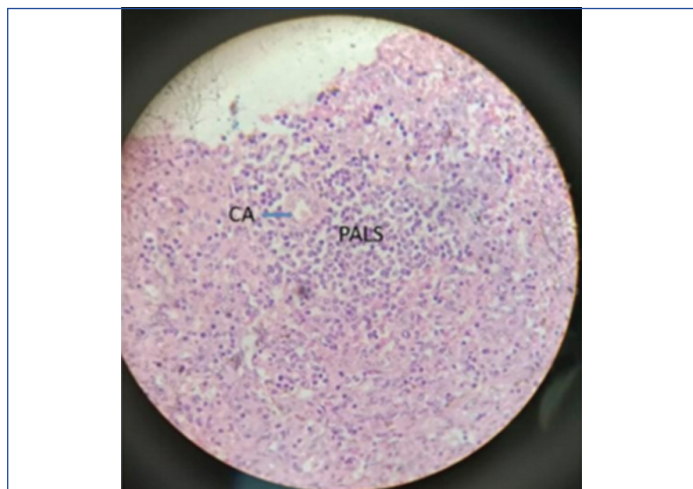
Group II (16-20 weeks): A few lymphocytes appeared in small groups and had no central arterioles [Table/Fig-4]. There were no dense, well-demarcated lymphoid aggregations, so definite WP did not develop in this group. There was no clear demarcation between the red pulp and WP. PALS began to form, and lymphocyte differentiation started to appear around the arterioles.

Group III (21-25 weeks): Lymphoid aggregations differentiated around the central arterioles to form PALS [Table/Fig-5]. The number of lymphoid aggregations increased, but they were not compactly arranged. There was no clear demarcation with the surrounding tissues. Centrally placed arterioles with lymphoid follicles were recognised. The WP region consisted of a greater number of reticular fibres than the red pulp.

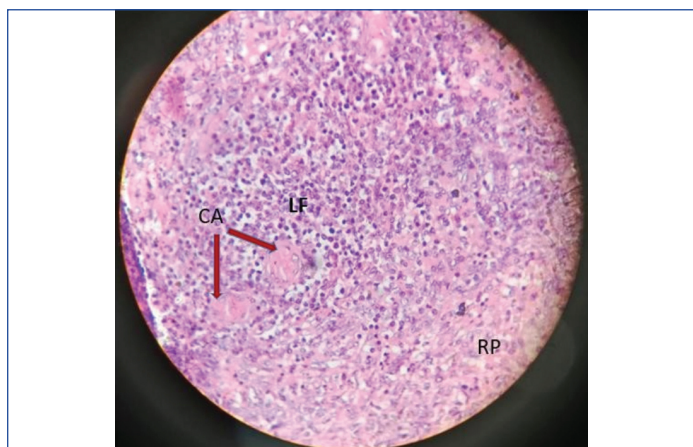


[Table/Fig-4]: A 16th week foetus showing capsule (C) with no trabeculae. Lymphocytes (LC) are arranged in small groups and are scattered without Central Arterioles (CA) (H&E, 10x).

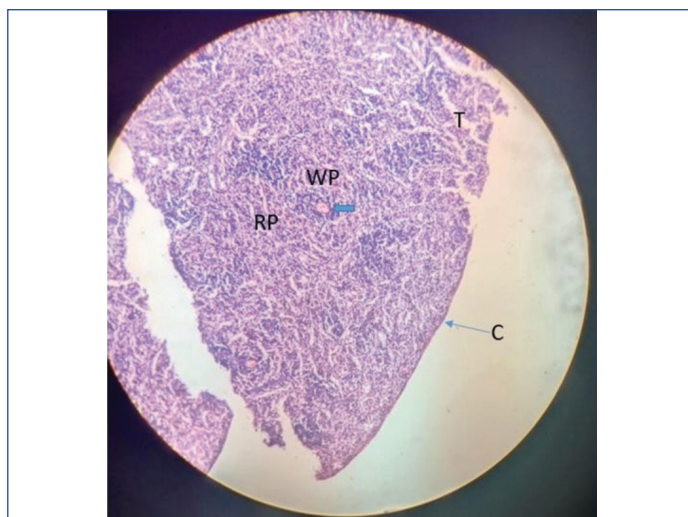
Group IV (26-32 weeks): Definite WP formed at the 28th week. Lymphocytes were compactly arranged to form lymphatic nodules. At the 30th week, a marginal zone appeared between the red pulp and WP. A few follicles showed double central arterioles [Table/Fig-6]. Well-established lymphatic follicles with central arterioles were observed [Table/Fig-7].



[Table/Fig-5]: A 24th week foetus showing the Periarterial Lymphatic Sheath (PALS) with Central Arterioles (CA) (H&E, 10x).

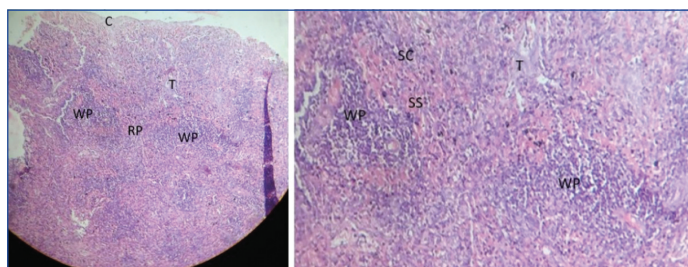


[Table/Fig-6]: A 30th week foetus showing a Lymphoid Follicle (LF) with two Central Arterioles (CA) and Red Pulp (RP) (H&E, 10x).



[Table/Fig-7]: A 32nd week foetus showing a thick capsule (C) and White Pulp (WP) with Central Arterioles (CA) (H&E, 10x).

Group V (33-40 weeks): At the 34th week, well-defined WP with clear differentiation from the surrounding area was observed. Both the size and the number of WPs increased. The number of lymphocytes in the red pulp area decreased. The WP exhibited matured lymphoid follicles with eccentrically placed arterioles [Table/Fig-8]. Well-demarcated Malpighian corpuscles were observed. At the 38th week, well-demarcated multiple WP components were observed. Lymphoid follicles were compactly arranged with densely aggregated lymphocytes and peripheral arterioles. Germinal centre not yet formed by term. Between the 38th and 40th weeks, the splenic structures resembled those of an adult spleen, without germinal centres. Changes in WP were observed and tabulated by group [Table/Fig-9]. Definite lymphoid follicles began to form from 30 weeks onward. [Table/Fig-10] shows the dimensions of lymphoid follicles in Groups IV and V.



[Table/Fig-8]: In a 38th week foetus showing White Pulp (WP) shows eccentrically placed arterioles (SS: Splenic sinusoids; SC: Splenic cords) (H&E, 40x).

Groups	Gestational age (weeks)	White Pulp (WP) changes
I	10-15	Lymphocytes appeared in parenchyma
II	15-20	Small groups of lymphocyte appeared and arranged in Scattered manner
III	21-25	Lymphocytes aggregation begins around the arteriole and form PALS
IV	26-32	Definite Lymphoid follicles started to form
V	33-40	Well-defined WP and clear differentiation from surrounding area observed

[Table/Fig-9]: White Pulp (WP) changes in different groups.

Gestational age (weeks)	Diameter of lymphoid follicle (in mm) (40x)
30	0.22
32	0.26
34	0.30
36	0.33
38	0.28

[Table/Fig-10]: Diameter of the lymphoid follicles in 30-38 weeks of gestational ages.

DISCUSSION

According to Pal M et al., lymphocytes are observed in the parenchyma of the spleen at the 16th week; by the 17th week, lymphoid aggregations around the arterioles had started, but they were not well-defined [9]. Between the 26th and 30th weeks, the density of lymphocytic aggregation increased, but there was no clear line of demarcation from the surrounding area. Lymphoid follicles were well-defined between the 31st and 35th weeks of gestation. Between the 36th and 40th weeks, a greater number of well-defined WPs were observed, with clear demarcation from the surrounding tissue. This study closely correlates with the present study; lymphocytes appeared at the 14th week, lymphoid aggregation started at the 18th week, and well-defined WP formed at the 34th week.

Yatagiri SV et al., says that, at 17th week, lymphocytes were seen around central arterioles. By 20th week, PALS was noted. Between 24-30th week, central arteriole becomes eccentrically placed. During 36-40 weeks, well-marked white pulp was seen [10]. In the present study, the splenic lobule appeared at the 18th week. WP begins to develop into the lymphoid colonisation stage between the 18th and 24th weeks, and PALS forms between the 19th and 29th weeks. The assembly of primary follicles around PALS starts at the 23rd week, and the B lymphocytic region consists of specific stationary cells. In the present study, PALS started to form at the 22nd week. This finding closely agrees with the current study.

Alex L et al., state that at the 24th week of gestation, a few scattered lymphocytes appeared in mesenchymal tissues, which is the earliest feature of WP formation [11]. Eccentrically placed arterioles were observed at this stage, and more than one arteriole was seen in most of the follicles. This finding closely correlates with the present study, where two central arterioles [Table/Fig-6] were observed in Group IV from the 28th to 30th weeks.

Mukhia R et al., report that lymphocytic aggregation starts at the 17th week and that PALS are observed at the 20th week; lymphoid follicles were seen between the 21st and 25th weeks [12]. Lymphoid follicles are well-defined at the 34th week, and eccentrically placed arterioles are noted at the 36th week. These study findings are similar to those of the present study, where lymphoid aggregation starts at the 18th week, PALS are observed at the 22nd week, and lymphoid follicles are recognised in Group III between the 21st and 25th weeks. Well-defined and eccentrically placed arterioles are observed at the 36th week.

Holkunde A and Sakhare S states that lymphoblasts are arranged in scattered groups between the 14th and 18th weeks. In the present study, scattered groups were seen in the 16th week, and PALS were noticed around the 20th week; in the present study, PALS were observed at the 22nd week [13]. Between the 22nd and 38th weeks, lymphocytes were compactly arranged, and the arterioles were eccentrically placed. In the current study, from the 28th week onward, lymphocytes were compactly arranged to form lymphatic nodules, and eccentrically placed arterioles were noted at the 36th week, which closely agrees with findings from the Holkunde A and Sakhare S study [13].

Halder A et al., state that up to the 14th week, the formative stage of WP will not be visible, which is the same as in the current study [14]. At the 16th week, scattered lymphocytes are observed, and a few formative stages of WP are noted; in the current study, at the 16th week, a few lymphocytes appear in small groups, and these groups are scattered. There is no definite WP seen between the 18th and 22nd weeks; in the present study, up to Group II (21-25 weeks), definite WP was not observed. WP is more prominent at the 26th week, and definite WP was observed at the 36th week. Between the 32nd and 36th weeks, prominent WP and definite lymphatic nodules with eccentrically placed arterioles were noticed. Definite WP and eccentrically placed arterioles were observed at

the 36th week. All findings of this study closely relate to the present study.

Srivani D and Pillai JJ state that during the 16th to 20th weeks of gestation, diffuse lymphoid aggregation was noted, but well-defined lymphoid follicles, central arterioles, and definite WP were not observed at this stage. Accumulation of lymphocytes around the arterioles to form lymphoid follicles was noted [15]. In the present study, diffuse lymphoid aggregation occurs at the 18th week. During the 25th to 32nd weeks, well-defined lymphoid follicles are seen, and PALS were observed. In the present study, PALS start to form at the 22nd week and are well-developed by the 24th week. From the 33rd week to term, lymphoid aggregation with eccentrically located arterioles was seen, and an increase in the size of the WP region was noted. In the present study, lymphoid aggregation with an eccentrically located central arteriole was observed at the 36th week. This study nearly correlates with the present study.

Yatagiri SV et al., state that at the 17th week, lymphocytes are seen around central arterioles. By the 20th week, PALS are noted [16]. Between the 24th and 30th weeks, the central arteriole becomes eccentrically placed along with ring fibre. During the 36th-40th weeks, well-marked WP is seen. In the present study, only a minimal number of lymphocytes are observed at the 18th week, PALS are noticed at the 22nd week, and definite WP is noted at the 36th week. These study findings are in greater agreement with the present study.

Thomas S et al., state that at the 20th week of gestation, lymphoid aggregation starts around arterioles, and PALS form at the 24th week, similar to the present study [17]. Two central arterioles were observed in a single lymphoid follicle at around the 28th week. During the 32nd week, a greater number of arterioles were surrounded by lymphoid aggregation; in our study, two central arterioles were observed between the 28th and 30th weeks. These primary lymphoid follicles were well differentiated from the neighboring tissues at the 37th to 40th weeks; in the present study, well demarcation was observed from neighboring tissues at the 36th week, which also agrees with the present study.

Souza AD et al., state that during the 10th week, lymphocytes were scattered throughout; in the present study, lymphocytes appeared scattered by the 14th week [18]. No central arterioles were noted until the 12th week; in our study, central arterioles were not observed until the 17th week. During the second trimester, lymphoid aggregation began. PALS were formed at the 20th week, and WP with lymphoid follicles were seen around the 23rd week. Well-developed follicles with central arterioles were observed at the 30th week; by the 36th week, central arterioles moved to an eccentric position in the lymphoid follicles. These findings closely agree with the present study.

Radhika D et al., state that lymphoid aggregation begins at the 11th week; in the present study, diffuse lymphoid aggregation begins at the 18th week [19]. The lymphoid follicles start to develop by the 20th week of gestation. A well-demarcated follicle with a centrally placed arteriole was observed at the 32nd week; in the present study, a well-defined lymphoid follicle with a central arteriole was observed at the 30th week. The matured lymphoid follicle with eccentrically placed arterioles was noted at the 36th week. This study is nearly related to the present study.

Potter EL and Craig JM state that during the first and second trimesters, the foetal spleen consists of few lymphocytes, but perivascular lymphoid aggregation was not seen [20]. In the third trimester, WP consists of lymphoid follicles with a central arteriole. In the present study, lymphocytes, perivascular lymphoid aggregation, and lymphoid follicles appeared in the second trimester by the 14th week. These study findings do not correlate with the current study. WP findings of the present are compared with the past literature [Table/Fig-11] [12,13,15-19].

Name of the study	Year	Sample size	Lymphoid aggregation starts at	PALS observed at	Definite White Pulp (WP) noted at
Radhika D et al., [19]	2012	50	11 th wk	22 wk	36 wk
Souza AD et al., [18]	2015	15	13-24 wk	20 wk	36 wk
Mukhia R et al., [12]	2016	50	17 wk	20 wk	38 wk
Kunde A [13]	2018	30	20 wk	22 wk	38 wk
Srivani D and Pillai JJ [15]	2019	40	16-20 wk	25-32 wk	33-40 wk
Yatagiri SV et al., [16]	2019	50	17 wk	20 wk	36-40 wk
Thomas S et al., [17]	2019	34	20 wk	24 wk	37-40 wk
Present study	2024	50	22-24 wk	24 wk	36 wk

[Table/Fig-11]: A comparison of studies on White Pulp (WP) [12,13,15-19].

Limitation(s)

The limitation of the present study is, it is not focussed on the gross structures of the spleen.

CONCLUSION(S)

The present study was conducted to observe the histogenesis in the WP of the human foetal spleen. Detailed knowledge about the histogenesis of the spleen is essential for anatomists and pathologists studying individual variations. In the gestational age group of 36-40 weeks, the WP reaches full development and attains high vascularity. Therefore, the authors may consider using the foetal spleen for transplantation after 36 weeks, as the chances of graft rejection will be reduced. Further research should be directed at the molecular level to elucidate the role of the spleen in immunity and autoimmunity.

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