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Research Article

Immunohistochemical Distribution of Desmin and Vimentin in Bovine Spleen During Fetal Development

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Abstract

As one of the largest secondary lymphoid organs, the spleen, besides having a key role in filtering the blood, supporting the lymphocyte differentiation as well as maintaining immune system control, is the centre of haematopoiesis during the fetal period. Such essential cellular processes as cell maintenance, cell division and communication between cells are regulated by intermediate filaments. Among these filaments, vimentin and desmin play an important role in keeping mechanical strength and giving support to the cellular differentiation processes. Therefore, the presented study aims to find out how immunohistochemical distributions of vimentin and desmin are done in the fetal bovine spleen during gestation periods. In this study, from each gestational period, 10 spleen tissue samples were collected. Before serial sections were prepared and stained immunohistochemically, those spleen tissue samples were processed via basic histological procedures and embedded in paraffin. The study demonstrate that vimentin and desmin exhibited various levels of staining intensity within splenic cell during gestation. As a result, throughout prenatal development, vimentin and desmin proteins have been seen to have different dynamics with regards to the staining intensity and distribution in the spleen. With this study, it was concluded that proteins from both might have a crucial role in giving contribution to the essential biological processes, like cell proliferation and differentiation, the structural organization of the splenic stroma, blood cell formation, and the regulation of immune responses.

Keywords: Bovine fetus, Desmin, Immunohistochemistry, Spleen, Vimentin

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Introduction

Being one of the largest secondary lymphoid organs, the spleen has a number of vital roles; such as contributing to hematopoiesis and regulating the immune system during the fetal period (Srivani and Pillai 2019). The spleen functions as a hematopoietic organ during the fetal period as well as, controlling the production of erythrocytes and granulocytes. Besides, it has such many other roles as controlling immune system mechanisms, blood filtration, removal of apoptotic cells from circulation, and lymphocyte differentiation (Srivani and Pillai 2019, Yatagiri et al 2019, Ungor et al 2007). That these duties be carried out is done by the white and red pulp, which have distinct differences in their vascular networks and cellular merge. Histologically, whilst the white pulp in the spleen consists of the periarteriolar lymphoid sheath (PALS), follicles, and marginal zone, the remainders constitute the red one (Kraus 2003, Eroschenko 2008, Srivani and Pillai 2019). After all, the spleen is covered with a capsule which is made of fibrous connective tissue. There are staggered trabecular extensions containing smooth muscle and fibroelastic tissue that extends from the capsule to the splenic parenchyma. These trabeculae also include vessels, and nerve fibers. Additionally, the lymphatic vessels located here constitute the efferent (outgoing) pathways by which lymphocytes are carried from the spleen to lymph nodes (Holkunde and Sakhare 2018).

Microfilaments (actin), intermediate filaments (IF), and microtubules are three primary components of the cytoskeleton which have critical role in governing fundamental cellular processes, including morphology, division, adhesion, and interactions between cells (Guharoy et al 2013, Dutour-Provenzano and Etienne-

Manneville 2021, Hakibilen et al 2022). As well as maintaining the proper positioning of the nucleus and other organelles inside the cell, vimentin and desmin, part of the intermediate filament family, have contribuiton to the mechanical stability in cells. Vimentin is classical present in mesenchymal cells, while desmin is characteristically found in muscle cells. Furthermore, both vimentin and desmin can be found in the same cell and interfacing with mitochondria, influencing the cell (Dayal et al 2024). Vimentin has also been noted to be demonstrated in endothelial cells, fibroblasts, and smooth muscle cells in postnatal life (Paulin et al 2022). Generally, vimentin plays important roles, including mediating intercellular signaling, acting as a cell-surface cofactor for the sequestration or activation of proteins and nucleic acids, and providing cellular machinery (Hol and Capetanaki 2017, Patteson et al 2021). Desmin, a key constituent of IFs in skeletal and smooth muscle cells, primarily plays an act in ensuring structural and mechanical support to cells. Subsequent research has also shown that desmin plays a role in many physiological processes (Agnetti et al 2021). Based on this information, in fetal spleen development, vimentin regulates the formation and development of the stromal framework as well as hematopoiesis and vascular formation. Desmin, on the other hand, plays a role in the differentiation of muscle-containing structures (capsule, trabeculae, and perisinusoidal cells) in the developing spleen, giving these structures contractile potential and ensuring the mechanical integrity and functional dynamics (blood filtration, storage) of the organ. Thus, it is anticipated that both proteins play critical roles in fetal spleen development and, together, can make significant contributions to spleen development by providing the spleen framework, vascular network, and cellular development (Agnetti et al 2021, Hakibilen et al 2022).

The spleen, with its rich cellular diversity, dynamic immune functions, role in pathological processes, and complex stromal architecture, has influenced the design of our study. This study aims to examine the expression distribution of desmin and vimentin according to gestational periods and to reveal their possible roles in fetal spleen development physiology.

Material and Methods

Obtaining tissue samples and preparing tissues

The study used 30 bovine fetuses from different periods of gestation. The ages of the retrieved fetuses were calculated after testing the crown-rump length (CRL) according to the notation suggested by Harris et al (1983). Age-determined fetuses were divided into three groups to cover all stages of gestation: the first period (69-89 days, n=10), the second period (99-178 days, n=10), and the third period (190-269 days, n=10). Subsequently, tissue samples were obtained through dissection from fetuses at each stage of gestation. The samples were fixed in 10% neutral buffered formalin dissolution for 18 hours. Afterwards, the tissues were taken into routine tissue follow-up protocol; they were dehydrated with alcohol series with increasing concentrations from 70% to 100%, made transparent with xylene and then turned into paraffin blocks. Sections 5 µ thick were taken from the prepared blocks. Some sections were mounted on standard slides for Masson Trichrome staining, while the others were mounted on adhesive-coated slides for immunohistochemical (IHC) staining.

Immunohistochemical staining

Sections mounted on adhesive-coated slides were stained with the indirect streptavidin-biotin complex technique of immunohistochemical staining (Karakoc et al 2019, Ertuğrul and Ceylan 2022). After deparaffinization, sections were incubated in xylol for 10 minutes each, then rehydrated in 100%, 96%, 80%, and 70% ethanol series for 3 minutes each, and then cleaned with distilled water. Following this, the sections were put in a methanol solution containing 3% H_2O_2 for twenty minutes to block endogenous peroxidase activity. The sections were then washed three times in PBS, each lasting five minutes. For antigen retrieval, 0.01 M, pH 6.0 citrate buffer was prepared; the sections were heated in this buffer at 95°C for twenty minutes and then left to cool at 24 °C. After cooling, the sections were washed again with PBS. To prevent nonspecific binding, sections were exposed to protein blocking solution for 15 minutes at 24 °C. Without washing, the study sections were subjected to 18 hour at +4°C with primary antibodies to Vimentin (Mouse monoclonal, catalog no: MS-129-R7, Thermo Scientific) and Desmin (Mouse monoclonal, catalog no: MS-376-S1, Thermo Scientific) diluted 1/200. Following incubation, the preparations were washed three times (5 minutes each) with PBS, and then the sections were exposed to biotinylated secondary antibody for 20 minutes at 24 °C. After this step, the sections were washed three times with PBS and then subjected to a streptavidin-peroxidase complex for 20 minutes. After the reaction, the sections were washed three times with PBS and exposed to 3,3'-diaminobenzidine hydrochloride for 10-20 minutes, depending on the reaction rate, to visualize the antigen-antibody complex. The sections were then rinsed with distilled water to finish the DAB treatment. For background staining, the sections were stained with Mayer's hematoxylin for 2-3 minutes and then cleaned in tap water for approximately 4 minutes. In the final step, the sections were placed in distilled water, passed through an alcohol (80%, 96%, 100%, 100%) and xylene (I and II) series, and then covered with entellan. Immunoreactivity

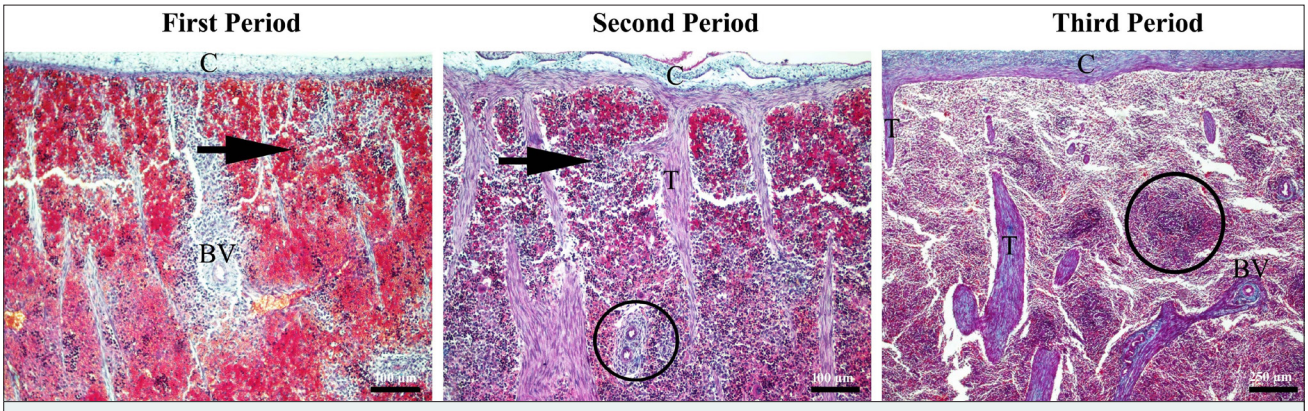

Figure 1. Demonstration of fetal bovine spleen at the first period, second period, and third period of gestation with Masson trichrome staining. Capsule (C), Blood Vessel (BV), Lymphocyte (arrow), Trabeculae (T), Lymph Follicle (circle). Scale Bar: 100, 100, 250 µm and magnification: 10X, 10X, 4X, respectively.

assessment was performed utilizing a Nikon Eclipse E400 model microscope.

Histological scoring was performed for staining results plying the H-scoring method (Aşır et al 2024a). Twenty fields were determined for each tissue sample, and vimentin and desmin expression was analyzed in these areas. These analyses were performed by two experts blinded to each other, and the scores were recorded. Expression scoring was as follows: negative (0); weak (1); middle (2); and strong (3). Negative control sections were subjected to PBS instead of the primary antibody. Fetal heart tissue was used as a positive control to demonstrate the accuracy of the immunohistochemical method.

Semi-quantitative histological scoring

Antibody staining intensity measurements were performed using Image J software (version 1.53, http://imagej.nih.gov/ij) (Aşır et al 2024a). Signal intensity in tissues was determined according to the method used by Crowe and Yue (2019), and five fields were selected from each tissue sample for quantitative scoring, and the results were recorded. In the tissue samples used in the study, brown staining indicated a positive antibody reaction, while blue indicated a negative one. The signal intensity in the area designated for quantification was calculated by dividing the antibody intensity of interest by the entire area of the tissue sample. For each tissue sample, five areas were considered, and the value was calculated as the staining area/entire area. As a result, an average value was tested for the antibody in each tissue sample, thus performing a semi-quantitative immunohistochemistry scoring. H-score values used for statistical analysis were calculated based on quantitative staining intensity and positive area measurements obtained using ImageJ software. The preparations were imaged and recorded utilizing a Nikon Eclipse E400 research microscope endowed with a digital camera. The images were then processed and quantified using Image J software.

Statistical analysis

GraphPad Prism version 9 software was used for statistical analysis of the study. The While the Shapiro-Wilk test was applied for the normal distribution of the data, the Kruskal Wallis analysis test was applied for the comparison between data groups that did not show normal distribution. The Dunn test was applied as a post-hoc test, and $p<0.05$ was considered statistically significant.

Results

Histological results

In the first period (69-89 days); the fetal spleen was surrounded by a thin capsule. While significantly enlarged sinusoidal spaces were observed, these spaces were found to be filled with blood cell elements. However, clusters of lymphoblasts were observed, but these were scattered (Figure 1).

In the second period (99-178 days); the capsule became more prominent, and thickened trabeculae, which divided the parenchyma into incomplete lobules, were observed. Red and white pulp features began to appear. Lymphocyte clusters and lymph follicles with central arterioles were observed. The sinusoids were more developed, and increased vascularization was observed (Figure 1).

In the third period (190-269 days); a well-developed capsule was observed. The red and white pulp were clearly defined. Numerous sinusoids and blood vessels were present, and trabecular vessels also appeared. However, lymph follicles were observed along with eccentrically positioned arterioles (Figure 1).

Immunohistochemical results

The immunohistochemical distribution of vimentin observed in the fetal spleen during gestation is shown in Figure 2. The immunoreactivity observed in the first period of gestation was found to be strong in some smooth muscle cells in the capsule surrounding the

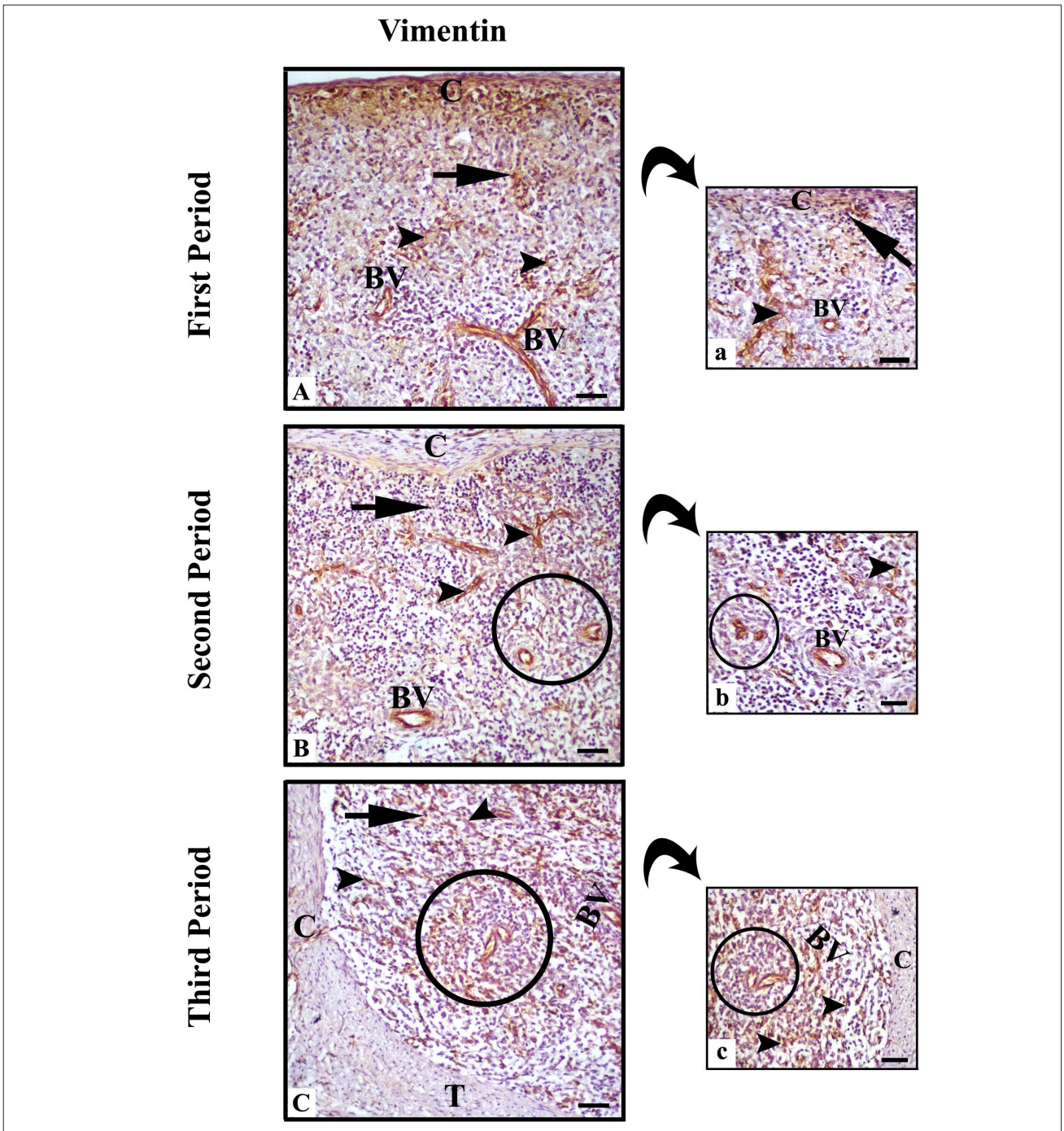

Figure 2. Immunolocalization of Vimentin in fetal bovine spleen at the first period (75 days) (A, a), second period (150 days) (B, b), and third period (232 days) (C, c) of gestation. Capsule (C), Blood Vessel (BV), Lymphocyte (arrow), Reticular Fibers (arrow head), Trabeculae (T), Lymph Follicle (circle). Scale Bar: 50µm, (A, B, C), 25 µm, (a, b, c) and magnification: 20X (A, B, C), magnification:40X (a, b, c).

spleen, lymphocytes, reticular fibers, and endothelial cells of blood vessels (Figure 2 A, a). While immunoreactivity in some smooth muscle cells in the capsule decreased in the second and third periods, immunoreactivity in lymphocytes, reticular fibers, and endothelial cells of blood vessels to be strong in the second and third periods, as in the first period. A positive vimentin reaction was observed in the endothelial cells of the central arterioles within the lymphocyte clusters in the white pulp area, which begin to form during the second period and become more pronounced during the third period (Figure 2 B, C, b, c).

The immunohistochemical distribution of desmin protein in the fetal spleen during gestation is shown in Figure 3. The immunoreactivity observed in the first period of gestation was moderate in some smooth muscle cells in

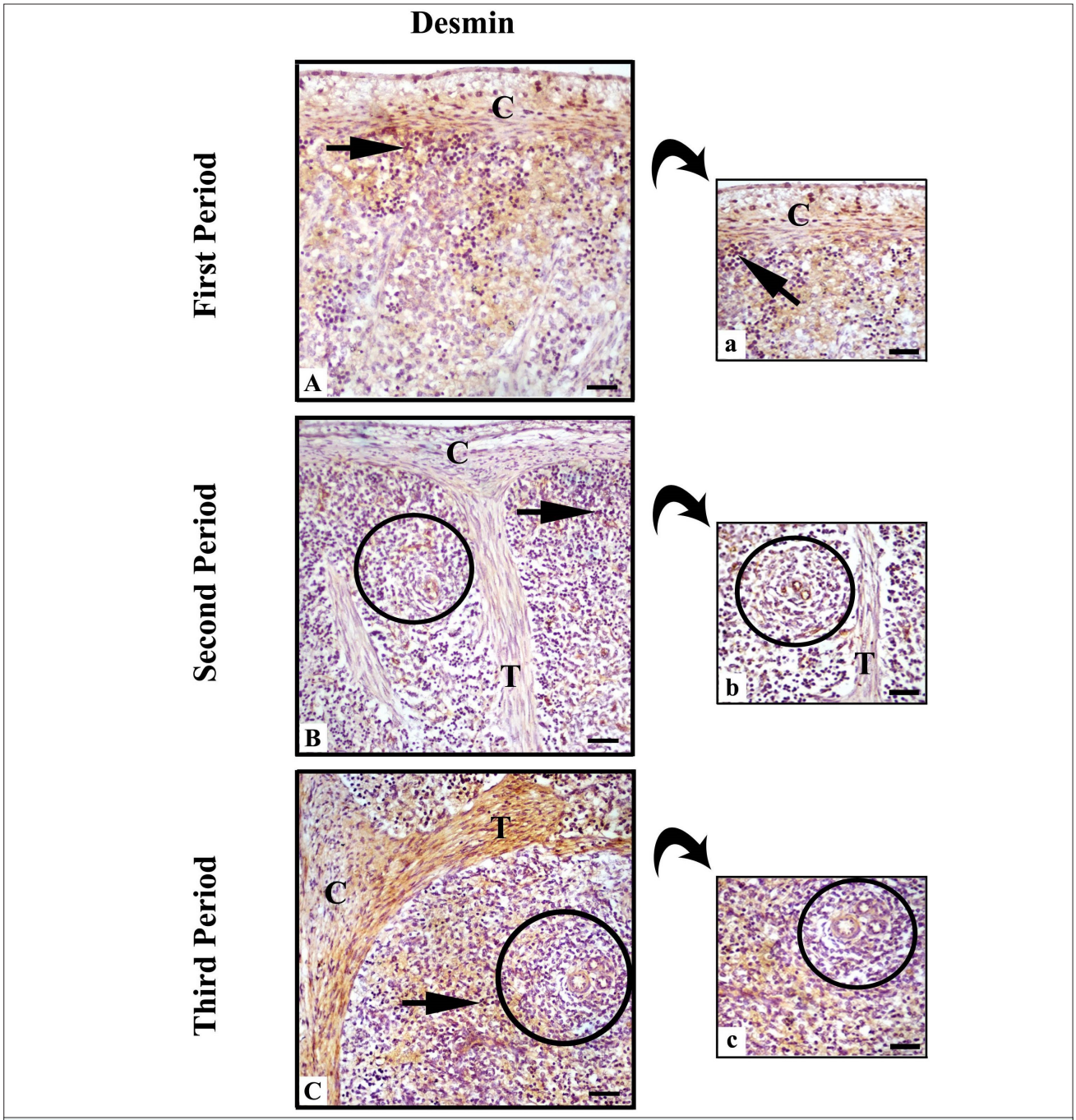

Figure 3. Immunolocalization of Desmin in fetal bovine spleen at the first period (78 days) (A, a), second period (136 days) (B, b), and third period (232 days) (C, c) of gestation. Capsule (C), Blood Vessel (BV), Lymphocyte (arrow), Trabeculae (T), Lymph Follicle (circle). Scale Bar: 50µm, (A, B, C), 25 µm, (a, b, c) and magnification: 20X (A, B, C), magnification:40X (a, b, c).

the capsule and some lymphocytes in stroma (Figure 3 A, a). It was determined that the immunoreactivity in the second period of gestation was weak in the smooth muscle cells of the capsule, while it was close to moderate intensity in some lymphocytes in the stroma (Figure 3 B, b). The intensity of the immunoreactivity in the third period of gestation was found to increase again in the smooth muscle cells of the capsule compared to the second period and to be moderate in some lymphocytes in the stroma and in some vascular endothelial cells (Figure 3 C, c). A positive desmin reaction was observed in the endothelial cells of the central arterioles within the lymphocyte clusters in the white pulp area, which began to form in the second period and became more prominent in the third period (Figure 3 B, C, b, c).

The accuracy of staining was determined using a positive control (heart tissue)(Figure 4).

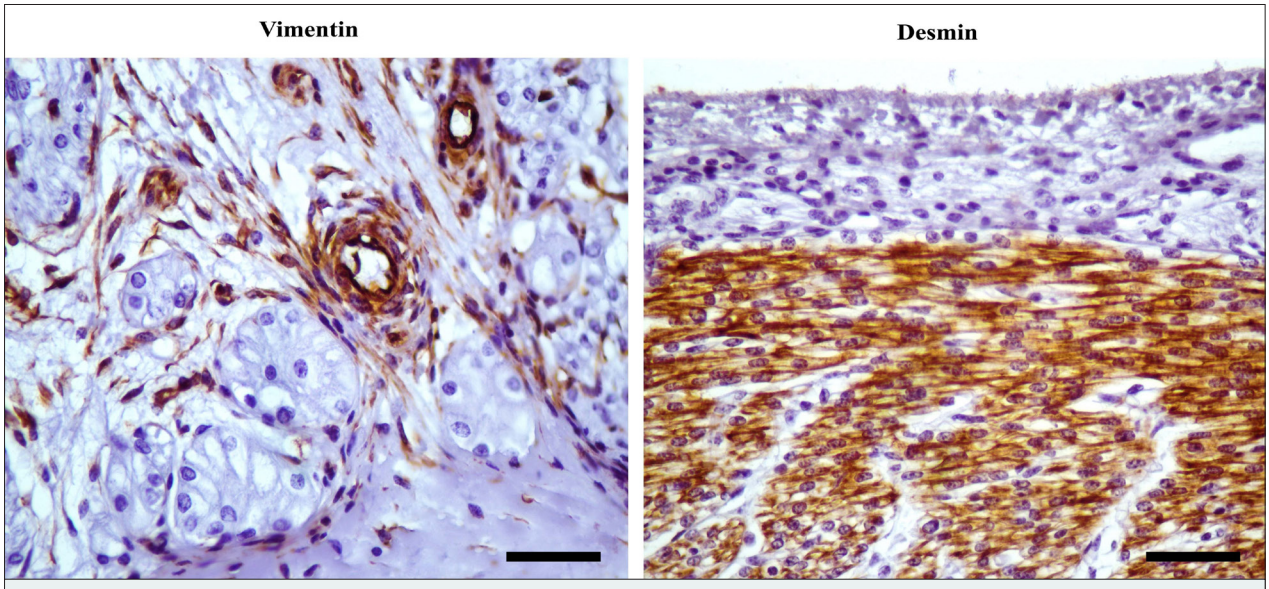

Figure 4. Positive expression of desmin (muscle cells) and vimentin (blood vessels) in fetal heart tissue. Scale Bar: 25µm, magnification:40X.

Statistical results

Vimentin expression intensity was not found to be statistically significant between gestational periods. Desmin expression intensity in the first period of gestation was significantly different from that in both the second and third periodss ($p<0.05$). Furthermore, no statistically significant alter was detected between the second and third periods (Figure 5).

Discussion

Intermediate filaments (IFs), which are cytoskeletal proteins, play critical roles in maintaining cell shape and mechanical entirety, as well as in fundamental processes such as adhesion, motility, and cell division. Desmin and vimentin, two important members of IFs that form transcellular networks via desmosomes and hemidesmosomes, share functional similarities but differ in terms of expression and distribution of functions. Desmin ensures the mechanical entirety of myofibrils and the transmission of contraction force in muscle tissue, while vimentin supports the regulation of processes such as cell migration, division, and signal transduction (Paulin and Li 2004, Capetanaki et al 2007, Eriksson et al 2009, Satelli and Li 2011, Hakibilen et al 2022). This study aims to investigate the distribution of desmin and vimentin expression in the developing bovine spleen and their potential physiological effects in the fetal spleen.

Studies have indicated that vimentin is highly concentrated

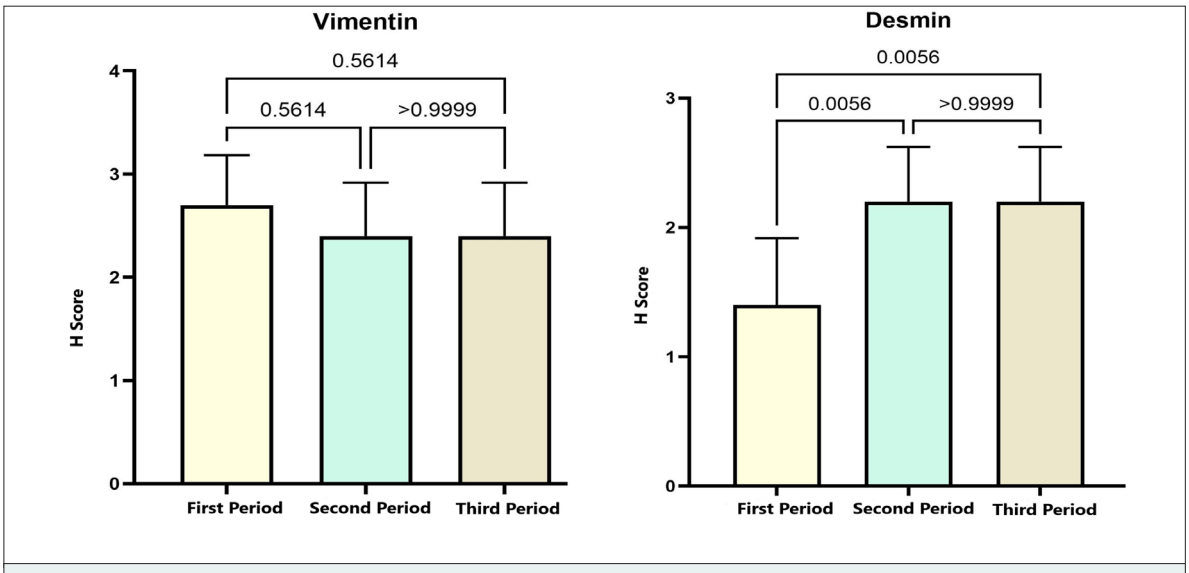

Figure 5. Semi-quantitative signal intensity of Vimentin and Desmin in fetal bovine spleens according to gestational periods.

within cells and plays a critical role in maintaining intracellular structure balance. Furthermore, it has been suggested that vimentin can be released outside the cell, onto the plasma membrane surface, or under diverse physiological and pathological states (Paulin et al 2022, Aşır et al 2024b). Studies on the placenta have reported that vimentin is expressed in placental cells and may affect placental physiology and pathology (Korgun et al 2007, Aşır et al 2024b). Research has shown that vimentin plays critical roles in inflammatory conditions, injuries, wound healing, and tumor metastasis. Furthermore, the observation of vimentin in cataracts, Crohn's disease, rheumatoid arthritis, HIV, and proliferative and undifferentiated cells has indicated that vimentin is a protein associated with many diverse of pathophysiological states (Danielsson et al 2018, Ratnayake et al 2021, Coelho-Rato et al 2024). Studies on vimentin initially demonstrated that the protein is present in bovine lenses. In chickens and mice, it has been shown that the vimentin protein is mainly expressed in connective tissue and central nervous system cells, as well as in erythroid and muscle cells, and that expression is particularly high in proliferating and undifferentiated cells (Tapscott et al 1981, Sax et al 1989, Danielsson et al 2018). In humans, vimentin has been reported to be expressed in numerous tissues (brain, lung, liver, kidney, testis, immune and gastrointestinal systems, skin, pancreas, ovary, and uterus). Expression is particularly high in skin, lung, kidney, bone marrow, and lymph node tissues. Furthermore, vimentin has been associated with numerous pathological conditions, including diseases (neoplasms, eye, endocrine system, fibrosis-related, cardiovascular, reproductive system, infectious, skin, skeletal, diabetes, and inflammatory) (Danielsson et al 2018). The expression of vimentin protein in both decidual and non-decidual cells throughout all days of pregnancy in mice suggests that vimentin is a marker of mesenchymal cells. It has also been suggested that vimentin may be associated with cellular stability and implantation mechanisms, as well as uterine receptivity (Korgun et al 2007). Literature review revealed that studies on vimentin have focused primarily on its presence and role in disease and tumor formation. However, the fact that vimentin which has been reported to be expressed in many organs and tissues in a limited number of research (Danielsson et al 2018) is being studied in fetal bovine spleen for the first times is significant in terms of potantial to serve as a source for new research. Our study revealed strong vimentin immunoreactivity in the spleen during gestation, particularly in the capsule, lymphocytes, reticular fibers, and blood vessels. It has been suggested that vimentin may play a role in cellular organization and functional activities by participating in the structure of connective tissue and stromal cells, as well as in the functional maturation process of lymphocytes for immune response and shape change. Thus, it is thought that vimentin may play important roles directly in splenic development, growth, and physiological functions.

Desmin an essential intermediate filament expressed in cardiac, skeletal, and smooth muscle tissues, has a role in regulating cell architecture, force transmission, and mitochondrial functions (Paulin and Li 2004). However, studies show that desmin is expressed in the uterus and in decidual cells of the placenta, and also induced with estradiol or decidualization (Glasser et al 1987). In a study conducted on rats, consistent with the findings of Glasser et al (1987) that desmin was expressed was in the myometrium of the uterus and in the epithelium of the cervix was found out (Takamatu 2023). As a result of the findings of Glasser et al (1987) and Takamatu (2023), desmin may play a role in the differentiation of the cells in which it is expressed. Additionally, desmin has been shown to exist in glomerular epithelial cells of the kidney, in fibroblastic and stromal cells, in Ito cells of the liver, in stromal cells of the intestinal submucosa, and in the testicular interstitium. Bringing all these findings together, desmin has been stated not to be limited to muscle cells but to be able to have roles in such processes as proliferation and cell division in the cells where it is expressed (Yaoita et al 1990). Moreover, defects in skeletal, cardiac, and smooth muscles may occur as a result of deficiencies or mutations in desmin. These have been informed to lead to the disruption of the intermediate filament cytoskeletal network, the loss of normal function and ultimately cell death (Capetanaki et al 2015). Desmin has been demostrated to be connected with angiogenic microvascular pericytes in the tumor stroma of colorectal cancer and expressed in soft tissue tumors. Furthermore, it has been credited with the role in the development of cardiomyopathy and its potential direct association with myocardial deaths. Thus, that desmin might serve as a biomarker for functional variations which may be of help in reducing mortality from cancer and cardiovascular diseases has been put forward (Piesanen et al 2022, Warmke et al 2024). The literature review has found no studies addressing the presence or role of desmin in the spleen. In the current study, it has indicated that desmin exhibits positive immunoreactivity in the spleen throughout fetal development. It has been observed that the formed immunoreaction is intense in the capsule, lymphocytes, and vessel surrounding the forming lymph follicles. The intensity of the observed immunoreactivity was found to be significantly higher in the level in the first period of gestation compared to those of the second and third periods ($p<0.05$). Along with this, immunoreactivity in the capsule has been indicated to be stronger in the first and third periods as opposed to the second period. This

suggests that desmin's association with the mitotic and metabolic activities of smooth muscle cells in the capsule, particularly during the first and third periods may exist. It has also been suggested that desmin, which is generally present in muscle cells, may contribute to the physiological functions of the spleen by providing the capsule with both a dynamic structure and a contraction property. Besides, the positive immunoreactivity of desmin in lymphocytes and vessels throughout gestation aligns with prior studies implying that it could directly influence spleen development and physiology by affecting cell division, proliferation, and differentiation in these structures (Glasser et al 1987, Yaoita et al 1990, Takamatu 2023).

Conclusion

In conclusion, throughout prenatal development, vimentin and desmin have been seen to exhibit different dynamics with regards to the staining intensity and distribution in the spleen. Consequent of the study, it has been determined that the intensity of vimentin immunoreaction has remained similar during the gestation period. However, the desmin immunoreactivity has been statistically more meaningful in the first term than the other two terms. With these findings, the role of both proteins has been observed in such critical processes as cell proliferation and differentiation in the spleen, the organization of the stromal structure of the organ as well as in haematopoiesis, and immune responses. Concurrently, that more updated research is needed for a full elucidation of the functional roles and regulatory mechanism of vimentin and desmin in the spleen development has reached a conclusion.

Declarations

Competing Interests

Authors declares that there are no conflicts of interest related to the publication of this.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author.

Ethical Statement

Dicle University, Animal Experiments Local Ethics Committee on 25/09/2025, 15/10 Number Ethics Committee Decision.

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Author Contributions

Motivation/Concept: UT; Control/Supervision: UT, MEA; Data Collection and Processing: UT, AST, ADC; Analysis and Interpretation: MEA, UT, ADC; Literature Review: UT, AST; Writing the Article: UT, MEA; Critical Review: UT, MEA, ADC

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