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Prenatal Morphohistogenesis of Thymus in Kosali Breed of Cattle: A Developmental Study of Histological Changes during Embryonic Development

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ABSTRACT

Background: Kosali cattle are a native breed from Chhattisgarh, India, primarily raised in rural areas on agricultural residues. However, there is limited information about the prenatal development of their thymus. Therefore, current study was conducted to understand the prenatal morphohistogenesis of thymus in Kosali breed of cattle.

Methods: The present investigation involves 24 Kosali foetuses for the prenatal gross morphometric development of the thymus and categorized on the basis of crown-rump length (CRL) into four groups: Group I (CRL <20 cm), Group II (20-40 cm), Group III (40-60 cm) and Group IV (>60 cm). A midventral cervical incision from the larynx to the thoracic inlet was made to expose the thymus for *in situ* examination of its topography and morphology. Thymus samples were collected and processed for Hematoxylin and Eosin, Van Gieson's, Verhoeff's and Gomori's staining for histological changes.

Result: In Group I, the thymus had paired cervical parts and one unpaired thoracic part, with the right limb extending to the middle thyroid cartilage and the left to the cricoid cartilage. By Groups II to IV, the left limb shifted laterally. The thymus color changed from cream to pink and while lobes became more distinct, their number decreased and size increased. The capsule thickened and became more vascular. The stroma contained interlobar and interlobular septa that thinned over groups and the number of lobules per field decreased significantly, indicating involution of the thymus and functional adaptations with age. These observations suggest that the thymus undergoes progressive involution with age. The structural and vascular changes seem to reflect the functional adaptations that occur during development.

Key words: Cattle, Development, Embryonic, Kosali, Morphohistogenesis, Prenatal, Thymus.

INTRODUCTION

The Kosali cattle are the thirty-sixth registered breed in India and the first recognized breed from Chhattisgarh and hold significant importance in rural ecomony. This breed is well-known for its disease resistance and ability to thrive in hot climates. Additionally, it is managed at a low cost, primarily by feeding on agricultural by-products like paddy straw, which is commonly utilized by rural communities (Jain *et al.,* 2017). The thymus is the first lymphoid organ to develop during embryonic development. It serves as the initial site for the development of T cell immunological functions and is crucial for the maturation of other lymphoid organs, such as the spleen, lymph nodes and tonsils. Thus, it plays an essential role in the acquisition of immunological competence (Chaurasia *et al.,* 2021). It is recognized as the lymphopoiesis pacemaker (Nickel and Schummer, 1977). The thymus is larger and operates most effectively during the newborn period (Baishya *et al.,* 2001). Understanding the processes that drive its development, maturation and involution is critical for fully comprehending the function of the entire lymphoid system, as it regulates the activity of other lymphoid organs. Defects can occur, such as failure to develop a proper thymus structure. However, scientific data on the prenatal development of their visceral organs, particularly the thymus are scarce. So, considering the importance of thymus during the phases of its development and involution, the primary goal of present study was undertaken to understand the different stages of prenatal morphohistogenesis of thymus and its

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developmental histological and histochemical changes in Kosali breed of cattle for providing baseline anatomical data that can serve as a valuable reference for further anatomical, physiological and clinical research on this breed.

MATERIALS AND METHODS

Place of research work

The present study was carried out during year 2023-2024 in confinement of Department of Veterinary Anatomy, College of Veterinary Science and A.H. Anjora, Durg (C.G.) and was conducted on twenty-four Kosali foetuses, which were collected immediately after the death of Kosali cows in the nearby districts of Rajnandgaon, Balod and Durg.

Study design

The weight, volume and crown-rump length (CRL) of the foetuses were measured after collection of sample as methodology outlined by Joubert (1956). Based on the CRL measurements, the foetuses were categorized into four groups, with six foetuses in each group: Group I (below 20 cm CRL), Group II (above 20 cm up to 40 cm CRL), Group III (above 40 cm up to 60 cm CRL) and Group IV (above 60 cm CRL).

Gross topography and morphology

A midventral cervical incision was made from the larynx to the thoracic inlet to expose the thymus. The topography and morphology of the thymus were studied *in-situ*. The morphology and topography of the thymus was done after washing sample with normal saline to make it clear and morphometric measurement like weight, volume, length, width and thickness was done by using thread.

Histomophology and histochemistry analysis

Identical samples of thymus was collected and fixed in 10% neutral buffered formalin. The fixed tissue samples were processed in alcohol-xylene sequence, in accordance with standard protocol and were embedded and blocked in paraffin wax at 58^{o}-60^{o}C melting point. These sections of the paraffin embedded tissues were cut at 3-5 μ thickness and stained using Haematoxylin and Eosin (H and E) according to the standard protocol (Singh and Sulochana, 1996) for microscopic analysis under a light microscope to obtain details of the histological structure. For elastin, collagen and reticular fibers, sections were stained with Verhoeff's, Van Gieson's and Gomori's stain as per procedure of Singh (2003).

Statistical analysis

The collected data of various parameters were subjected to analysis of variance (ANONA) and Duncan's multiple range test (DMRT) in order to compare the mean values between groups by using the statistical software SPSS v20.0 to analyze all the data and were displayed as mean±Standard error with the level of significance was set at P<0.05.

RESULTS AND DISCUSSION

Gross topography and morphology

The thymus consists of a paired cervical part and an unpaired thoracic part, as observed from groups I to IV Chaurasia *et al.* (2019). The cervical thymus has right and left limbs: the right limb extends cranially to the middle part of the thyroid cartilage, while the left limb reaches the level of the cricoid cartilage across all groups (Fig 1 and 2). These findings are consistent with previous research by Nickel *et al.* (1977) in calves, Dyce *et al.* (1987), Machado *et al.* (2001) in buffalo, Cunningham *et al.* (2001) in unborn lambs and Baishya *et al.* (2001) in pig fetuses, as well as Konig and Leibich (2004). The significant increase in crown-rump length, fetal weight, thymic weight and volume from group I to group IV indicate symmetrical exponential growth of the thymus relative to crown-rump length and fetal size. Similar reports have emerged, such as the one by Baishya *et al.* (2001), which documented that in crossbred pig fetuses, thymic weight displayed ascending ontogeny tendencies with advancing fetal age. Press and Landswork (2006) found that the thymus progressively increases in size up to the age of puberty. Igbowke and Ezenwaka (2017) reported that in indigenous Large White pigs, the absolute weight of the thymus shows significant ($p \leq 0.05$) increases in size with advancing fetal age, though the increase is not significant in the postnatal period.

The weight and volume of the left limb of the cervical thymus increased, showing a maximum ten- and eleven-fold rise respectively from group I to group II, although this change was not statistically significant (Fig 2). In contrast, the minimum increases in weight and volume were recorded from group III to group IV, which were significant.

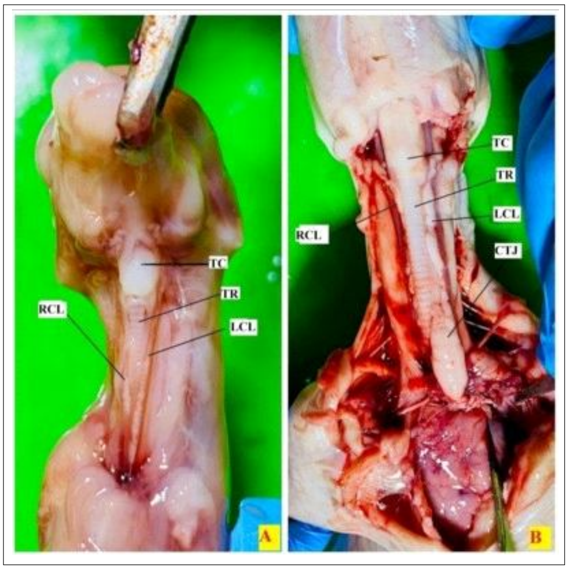

Fig 1: Photographs of thymus in situ of group I (A) and group II (B) showing left cervical limb (LCL), right cervical limb (RCL), cervicothoracic junction (CTJ), trachea (TR) and thyroid cartilage (TC).

The thymic index of the left limb exhibited highly significant differences from group II to group III, peaking at that stage (Table 1). The presence of paired cervical and junctional parts alongside the unpaired thoracic part in group I (corresponding to the apparent first trimester of pregnancy) contradicts previous reports by Baishya *et al.* (2001), who noted that all fetuses had cranial and cervical lobes by days 45 to 46, while the thoracic lobe became evident between days 50 and 52 (second trimester of pregnancy). Similarly, Hyttel *et al.* (2010) found that in horses at 60 days and dogs at 30 days of gestation, the thoracic part forms only after these time frames. According to Press and Landswork (2006), the thymus originates as a solid epithelial outgrowth from the epithelium of the third pharyngeal pouch, developing into an irregular bi-lobed structure located in the cranial mediastinum during early life, with further development occurring until puberty. Ramayya *et al.* (2008) reported that buffalo fetuses possess paired cervical and cranial parts, as well as unpaired thoracic parts within their thymus. Hyttel *et al.* (2010) documented that developed thymus structures consist of a paired cervical part and an unpaired thoracic part in ruminants by 40 days of gestation. The significant reduction in the number of lobes from groups I to IV in this study may be attributed to the fusion of lobes with advancing age and the resultant increase in lobe size. The recorded weight, volume, length and width of the right limb of the thymus were greater than those of the left limb across groups I to IV, which contrasts with findings by Ramayya *et al.* (2008) in buffalo fetuses, where they noted that the left limb was marginally longer than the right.

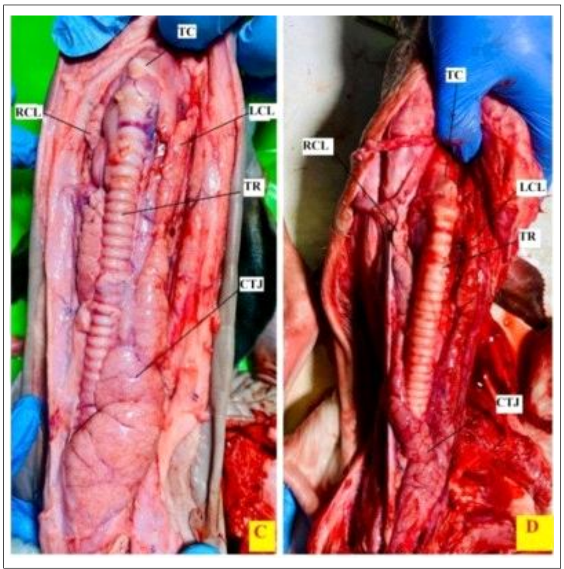

Fig 2: Photographs of thymus in situ of group III (C) and group IV (D) showing leftcervical limb (LCL), right cervical limb (RCL), cervicothoracic junction (CTJ), trachea (TR) and thyroid cartilage (TC).

Histomorphological analysis

In the samples from all four regions of the thymus, specifically the thoracic area, cervical-thoracic junction and right and left cervical limbs, the capsule was primarily composed of loose to slightly dense areolar connective tissue (groups I to IV). Some samples from group I contained sections of various blood vessels. The vascularity of the capsule increased from group I to group IV, with notable aggregations of blood vessels present in multiple locations. This increase in vascularity was particularly evident in tissue samples from the cervicothoracic junction and thoracic regions of the thymus from groups II to IV. The average thickness of the capsule increased significantly from 38.12 ± 5.95 µm in group I to 138.8±3.5 µm in group IV (Table 2). The increase was significant between groups I and IV, II and IV, as well as between groups I, II and III. However, the difference between

Table 1: Gross parameters and percentage increase of foetus and thymus in different groups.

Parameter	Group 1	Group 2	% Inc (Gr 1-2)	Group 3	% Inc (Gr 2-3)	Group 4	% Inc (Gr 3-4)
CRL (cm)	14.01±1.59^{d}	29.83+2.49^{c}	113%	47.33±2.34^{b}	59%	66.16±1.64^{a}	40%
Wt of foeti (gm)	118.25±21.00^{d}	1283.33±335.42^{c}	985%	3650.33±576.05^{b}	184%	9411.66±1035.21^{a}	158%
Wt of thymus (cm)	0.62±0.65^{d}	8.49±0.51^{c}	1269%	30.12±0.59^{b}	254%	102.95±0.98^{a}	241%
Re % wt of thymus	0.9±0.3^{a}	0.8±0.15^{a}	-11%	0.7±0.08^{a}	-13%	1.15±0.13^{a}	64%
Wt of CT (gm)	0.38±0.03^{c}	7.51±0.31^{bc}	1876%	18.41±5.18^{b}	145%	67.30±6.24^{a}	266%
Re % Wt of CT	0.43±0.16^{a}	0.41±0.07^{a}	-5%	0.30±0.05^{a}	-27%	0.37±0.03^{a}	23%
Wt of TT (gm)	0.24±0.02^{c}	0.97±0.20^{c}	304%	11.70±3.49^{b}	1106%	35.65±3.72^{a}	205%
Re % Wt of TT	11.01±1.60^{c}	38.05±0.93^{c}	246%	52.79±1.6^{b}	39%	63.6±7.9^{a}	21%
Vol of foeti (ml)	117.33±14.86^{c}	1205.67±299.56^{c}	928%	3536.67±574.85^{b}	193%	9038.33±908.60^{a}	156%
Vol of thymus (ml)	0.54±0.06^{d}	7.84±0.31^{c}	146%	20.41±0.84^{b}	161%	98.33±3.56^{a}	390%
% Vol of CT	0.28±0.01^{a}	0.41±0.07^{a}	46%	0.29±0.04^{a}	-29%	0.35±0.02^{a}	21%
% Vol of TT	0.19±0.01^{c}	0.05±0.01^{b}	-74%	0.29±0.04^{ab}	480%	0.36±0.06^{a}	24%
No of lobes	54.5±0.96^{a}	41.5±0.6^{b}	-24%	36.12±0.02^{c}	-13%	25.8±2.2^{d}	- 30%
Length of thymus (cm)	4.61±0.64^{b}	5.55±0.15^{b}	20%	13.56±1.98^{a}	144%	13.59±1.05^{a}	0%

a, b, c, d:- Superscript in each row, means with different superscripts are significantly different ($P<0.05$).

groups III and IV was not significant. Furthermore, the stroma of the thymus consisted of interlobar and interlobular septa made of loose to slightly dense irregular connective tissue (groups I to IV). The interlobar septa extended from the capsule, while the interlobular septa originated either from the capsule or the interlobar septa.

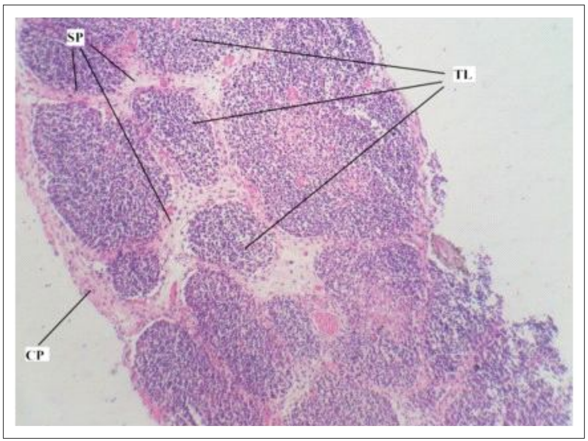

Fig 3: Photomicrograph of cervicothoracic junction of thymus of group I showing capsule (CP), interlobar septa (SP) and thymic lobes (TL) (H and E, 100X).

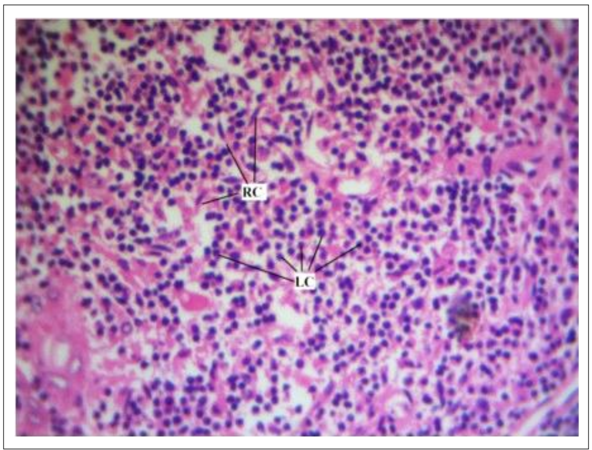

Fig 4: Photomicrograph of thoracic thymus of group III showing reticular cell (RC) and lymphocytes (LC) in medulla (H and E, 1000X).

The initiation of interlobular septa formation was observed in the thoracic and cervicothoracic junction samples of group I, but from groups II to IV, these septa distinctly divided the lobes into incomplete lobules across all thymic regions corroborating the findings of Kadam *et al.* (2023). They reported that the thin connective tissue capsule of thymic lobes gives rise to septa to divide lobes in to lobules. In the samples of group I, the lobes were primarily oval in shape. In contrast, from groups II to IV, the lobules were typically oval, elongated oval, or polygonal. The average maximum diameter of the lobule increased from 498.83±27.16 µm in group I to 755.31±3.41 µm in group IV. Similarly, the average minimum diameter rose from 291.67±27.16 µm in group I to 426.67±6.16 µm in group IV. The increase in maximum diameter was significant between groups II and III, but not between groups III and IV. Conversely, the increase in minimum diameter was significant across groups II, III and IV. Additionally, the average number of thymic lobules per field (10X) decreased from 13.00±6.16 in group II to 5.33±0.21 in group IV. The decrease in the number of lobules per field was significant between groups II to IV. Lobules were not clearly distinguishable in group I. The septa contained sections of blood vessels, with their vascularity increasing from group I to group IV, particularly in the cervicothoracic junction and thoracic tissue samples from groups II to IV. The thickness of the septa gradually decreased from group I to group IV, while their vascularity increased during the same progression.

The stroma of the thymus consisted of interlobar and interlobular septa made of loose to slightly dense irregular connective tissue (across groups I to IV). The interlobar septa penetrated the parenchyma from the capsule, while the interlobular septa descended from either the capsule or interlobar septa (Fig 3). These septa contained segments of blood vessels, with both the density and size of these vessels increasing from groups I to IV. The loose connective tissue progressively transformed into dense connective tissue with fetal age. The thickness of the septa decreased from groups II to IV, corroborating the findings of Lieshchova and Gavrilin (2020), Ahmed *et al.* (2025), who recorded an increase in connective tissue in septa up to two months, followed by a decrease in *Bos taurus*

Table 2: Histomorphological parameters and percentage increase of thymus in different groups.

Parameter	Group 1	Group 2	% Inc (Gp1-2)	Group 3	% Inc (Gp 2-3)	Group 4	% Inc (Gp3-4)
Thickness of capsule	38.12±5.95^{c}	89.93±3.25^{b}	136%	127.15±1.60^{a}	41%	138.8±3.5^{a}	9%
Thickness of septa	73.33±3.93^{a}	46.50±2.57^{b}	-37%	25.33±2.6^{c}	-46%	12.33±1.28^{d}	-51%
Thickness of cortex	-	388.33±41.74^{c}	-	560.00±15.27^{b}	44%	637.33±5.72^{a}	14%
Diameter of medulla	-	410.00±59.16^{b}	-	720.00±17.32^{a}	76%	776.67±2.10^{a}	8%
Maximum diameter of lobule	-	498.83±27.16^{b}	-	691.67±15.14^{a}	39%	755±3.41^{a}	9%
Minimum diameter lobule	-	291.67±11.37^{c}	-	373.33±6.67^{b}	28%	426.67±6.16^{a}	14%
Number of lobules per field	-	13.00±.57^{a}	-	7.83±0.47^{bc}	-40%	5.33±0.21^{d}	-32%
Diameter of Hassall's corpuscles	-	144.33±3.24^{a}	-	38.33±3.83^{b}	-73%	13.00±0.68^{c}	-66.65%

a, b, c, d:- Superscript in each row, means with different superscripts are significantly different (P<0.05).

fetuses. Conversely, Mainde *et al.* (2017) reported that the thickness of septa increased consistently until 142 days of gestation in goat fetuses, nearly reaching the end of the gestation period. The parenchyma of the lobes and lobules comprised different stages of lymphocytes, reticular cells, macrophages, mast cells, mesenchymal cells and other free connective tissue cells, along with fine connective tissue fibers (Fig 4). The initiation of differentiation of thymic lobular parenchyma into peripheral cortex and central medulla began in group I, though the boundary between these two areas was not distinct. A clear division between the cortex and medulla was observed starting from group II. The medulla served as a common entity for all lobules within a lobe, with interlobular septa dividing the lobes into incomplete lobules. Similar differentiation into cortex and medulla was noted at mid-gestation by Chandra and Parmar (2004) and at 50 days of gestation by Mainde *et al.* (2017) in goat fetuses and at 41 to 55 days by Baishya *et al.* (2001) and at 60 to 75 days by Igbowke and Ezenwaka (2017) in pig fetuses.

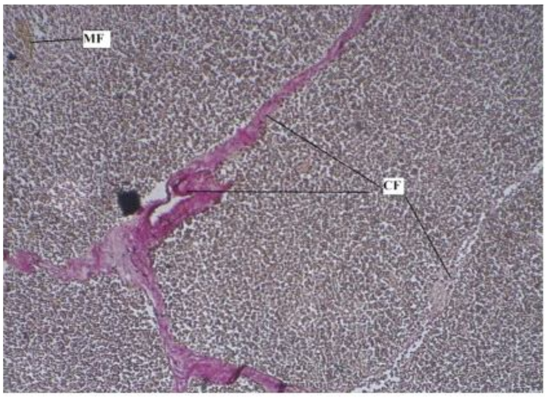

Fig 5: Photomicrograph of cervicothoracic junction of thymus of group IV showing collagen fibers (CF) and muscle fibers (MF) in (Van Gieson's, 100X).

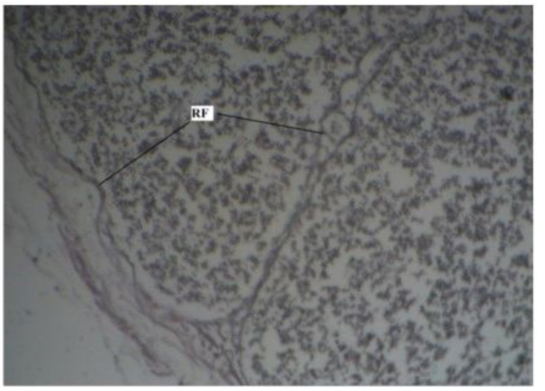

Fig 6: Photomicrograph of left limb of cervical thymus of group IV showing reticular fibers (RF) in interlobar septa (Gomori's, 400X).

Histochemical analysis

Distinct cortex and medullary areas have been reported during fetal development. Well-developed collagen fiber bundles with a wavy course were observed in the capsule and interlobar septa of the cervical limb of the thymus in samples from group IV (Fig 5). This supports the findings of Sharma *et al.* (2004), who noted that collagen fibers increase in number in piglets as they age. In groups I to III, collagen fibers appeared during the maturation phase, indicated by a gradual increase in staining intensity. The density of collagen fibers decreased from the capsule to the deeper parts of the interlobular septa. Additionally, Baishya *et al.* (2001) reported the presence of collagen fibers in the capsule and trabecula of the thymus in pig foeti, noting that smooth muscle and collagen fibers together outnumber reticular fibers. Sparse, very thin, unbranched elastic fibers were recorded in the septa of group I. These fibers developed in both structure and density from group II to IV, with the most developed elastic fibers observed in the interlobular septa of group IV, as well as in the capsule and walls of blood vessels. This observation aligns with findings by Uppal *et al.* (2007), who reported a limited number of elastic fibers in the thymic capsule of neonatal buffalo calves. The density of reticular fibers was greatest in group II, found in the capsule (Fig 6), septa and parenchyma of the lobule. However, their density decreased from groups II to IV, indicating that as lymphoid cells increased in number, connective tissue decreased with the advancing age of the foetus. This finding supports the observations of Lieshchova and Gavrilin (2020) in *Bos taurus* foeti. Similar distributions of reticular fibers in the thymus have also been documented by Baishya *et al.* (2001) in pig foeti, Chandra and Parmar (2004) and Mainde *et al.* (2017) in goat foeti, Uppal *et al.* (2007) in neonatal buffalo calves and Ramayya *et al.* (2008) in buffalo foeti.

CONCLUSION

It could be concluded that the thymus consists of cervical, junctional and thoracic regions, with the highest relative weight found when CRL above 60 cm. The thymus had showed exponential and allometric growth patterns. As the animals aged, the thymic capsule thickened while septal thickness decreased. Additionally, cortical-medullary differentiation and Hassall's corpuscles start appearing when CRL20-40 cm, indicating progressive maturation of the thymic architecture as reticular fiber density decreased with fetal age.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures and handling techniques for experiments were approved by the Institutional Animal.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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