

HISTOLOGICAL AND MICROMETRIC ANALYSIS OF MYOID CELLS IN THE THYMUS OF ASEEL BIRD

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ABSTRACT

The present study was conducted to investigate the age-related histomorphology and micrometry of thymic myoid cells in healthy TANUVAS Aseel birds. Birds belonging to five age groups (day-old, 4, 8, 18, and 32 weeks) were obtained from the Poultry Research Station, Madhavaram Milk Colony, Chennai. Thymic tissues were fixed in 10% neutral buffered formalin, processed routinely, sectioned at 5–6 µm, and stained using routine histological and special staining techniques. Myoid cells were observed in both the cortex and medulla of the thymus in all age groups. Two morphological types, namely spindle-shaped and oval-shaped myoid cells, were identified, with both exhibiting cytoplasmic striations and centrally placed round nuclei. In day-old birds the spindle shaped myoid cells were noticed, whereas spindle and oval cell types were observed in older age groups. Spindle-shaped myoid cells predominated in younger birds, while oval-shaped cells were more numerous in adult birds. Masson's trichrome stained myoid cells red, whereas phosphotungstic acid haematoxylin (PTAH) stained them bluish, confirming their identity. Myoid cells were frequently located near Hassall's corpuscles and showed a characteristic circular arrangement in adult birds. Micrometric analysis demonstrated an age-related increase in the size of both oval- and spindle-shaped myoid cells, with the largest cells recorded in 32-week-old birds. The increased number and size of myoid cells with advancing age suggest their possible association with thymic involution and age-dependent functional changes.

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INTRODUCTION

Poultry production is a globally important economic sector experiencing significant growth. In India, the poultry industry plays a substantial role in the GDP

and contributes to poverty reduction through the production of meat and eggs (Prabakaran *et al.*, 2020).

Native chickens are renowned for their adaptability to local agro-climatic conditions, robustness, and the capacity to thrive on locally available feed with minimal care and management. Additionally, native chicken meat is appreciated for their distinctive flavour and taste. In India, native chicken breeds make a substantial

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contribution to the overall poultry population, constituting approximately 33 per cent of the total.

Birds possess an immune system that can be considered intermediate in development between lower vertebrates, such as fish, amphibians, and reptiles, and mammals (Rose, 1979). Like other vertebrates, birds have both innate and adaptive immune responses. Their innate immunity provides the first line of defense through physical barriers, phagocytic cells, inflammation, and antimicrobial substances. The adaptive immune system includes B and T lymphocytes, which provide specific and long-lasting protection against pathogens. A distinctive feature of the avian immune system is the bursa of Fabricius, a specialized lymphoid organ where B cells develop and mature. The thymus is primarily involved in T-cell development, as in mammals. Birds also possess spleen, bone marrow, and other lymphoid tissues that contribute to immune responses. Although avian immunity shares several characteristics with mammalian immunity, there are important structural and functional differences. Thus, the avian immune system represents an important evolutionary stage between the simpler immune systems of lower vertebrates and the more complex immune system of mammals.

In all the age groups, the parenchyma of the thymus was composed of lymphoid cells or thymocytes, reticuloepithelial cells, myoid cells, macrophages and melanocytes as the predominant component of the chicken thymus (Panwar *et al.*, 2020) in kadaknath fowl.

A histological investigation of the thymus in adult *Gallus domesticus* demonstrated the presence of abundant large cells in the thymic medulla. These bodies were identified as myoid cells, members of a cell type peculiar to the thymus of various species, and characterized by the presence in their cytoplasm of striated myofibrils similar to those found in skeletal muscle (Ruth *et al.*, 1974) in domestic fowl.

The multiple studies have shown different functions of the thymic myoid cells. They have been described to have a role in myogenesis during postnatal skeletal muscle regeneration (Cumpanas *et al.* 2018), because they express muscle-specific proteins such as desmin, myosin, and myogenic regulatory factors, indicating myogenic potential. Similar to muscle satellite cells, they may proliferate and differentiate into muscle cells during tissue repair. Their embryonic origin from progenitor populations with myogenic potential may also explain their ability to contribute to muscle formation and regeneration. Additionally, their location within the thymus suggests a possible link between immune regulation and muscle repair. Thus, their myogenic properties support a potential role in skeletal muscle regeneration.

MATERIALS AND METHODS

The current research was done on healthy TANUVAS Aseel birds. Birds of different age groups irrespective of sex including day-old chicks and birds aged 4, 8, 18, and 32 weeks, were used for the study. Six birds were chosen from each

age group. All birds were obtained from the Poultry Research Station, Madhavaram Milk Colony, Chennai-51, and maintained under standard management conditions and found healthy.

For light microscopic study, the tissues were collected in 10 per cent neutral buffered formalin. For histological examination the collected tissue samples were subjected to routine histological processing. The fixed tissues were initially washed appropriately and dehydrated through a series of ascending grades of alcohol to remove water from the tissue. Following dehydration, the tissues were cleared in xylene to replace the alcohol and facilitate penetration of the embedding medium. The cleared tissues were then infiltrated and embedded in molten paraffin wax to obtain tissue blocks. After solidification, the paraffin-embedded tissue blocks were trimmed and mounted onto the microtome. Serial sections of approximately 4–5 μm thickness were carefully obtained using a rotary microtome. The sections were subsequently floated on a warm water bath to remove wrinkles and were collected on clean glass slides. The prepared sections were dried appropriately and used for further histological staining methods (Bancroft and Stevens, 1996).

Microscopic images were captured using the Leica microscope (CH9345 Heerbrugg). Micrometry was done using the Leica application suite (LAS V4.4). The microscopic features of Myoid cells size were measured by using Leica micrometer.

The recorded data were analysed by repeated measures of ANOVA (SPSS23) with Arithmetic Mean and Standard Deviation for the data were calculated as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

In all the age groups studied, myoid cells were observed in both the cortex and medulla whereas Hashimoto and Sugimura (1976) in White Pekin ducks reported that the myoid cells were frequently found in the medulla, corticomedullary region and occasionally found in the cortex. Two types of myoid cells were identified based on their morphology: oval and spindle-shaped. In both types, the myoid cells appeared elongated and spindle-shaped with noticeable striations in the cytoplasm. The nucleus was round and centrally placed. This observation aligns with Gilmore and Bridges (1974) and Kendall (1980) in chicken, Tamilselvan *et al.* (2017) in guinea fowl, and Geetha and Vijayaragavan (1997) in buffalo calves. Whereas, Robert *et al.* (1978) described a different observation in human thymus, noting that myoid cells were rare. This suggests that the presence and distribution of myoid cells in the thymus can vary across species, emphasizing the importance of considering species-specific differences in thymic structure and composition.

In day-old birds, only the spindle-shaped myoid cells were observed, while in other age groups under this study revealed the presence of both oval-shaped and spindle-shaped myoid cells.

In younger age groups except day-old, the proportion of spindle-shaped myoid cells were more prevalent, while in adult age groups such as 18 weeks and 32 weeks, oval-shaped myoid cells were more prominent (Fig.1).

The presence of myoid cells was further confirmed using special staining techniques, such as Masson's trichrome and PTAH method. In Masson's trichrome, myoid cells stained red (Fig.2), whereas in the PTAH method, these cells appeared bluish (Fig.3). With Masson's trichrome staining, the myoid cells appeared red, which is characteristic of the staining of muscle fibres and cytoplasmic proteins. In contrast, with the phosphotungstic acid-haematoxylin (PTAH) method, the myoid cells appeared bluish, indicating the presence of muscle-associated structures, particularly myofibrillar components.

Within the medullary region, myoid cells were found close to the Hassall's corpuscles. In the adult age groups myoid cells were arranged in a circular fashion around the Hassall's corpuscles.

The overall size of myoid cells showed an increase with advancing age (Table1). This observation prompts the hypothesis that these myoid cells, either directly or indirectly, may be linked to the organ's involution, aligning with the functional synchronization with the levels of sex hormones in the bloodstream

(Vijayaragavan, 1988). While Bockman (1968) hypothesized that myoid cells played a role in the mechanism of tolerance to muscle self-antigen in humans. Mandel (1968a and b) expressed the opinion that thymic striated muscles might serve as a local source of antigen for the self-recognition of skeletal muscle in guinea pigs. The myoid cells were believed to aid in the movement of lymphocytes through their spasmodic contractions (Raviola and Raviola, 1967).

Micrometric analysis revealed that the overall size of oval-shaped myoid cells (mean $\pm$ standard deviation) were $1813.81 \pm 965.78 \mu\text{m}$, $2382.92 \pm 708.37 \mu\text{m}$, $1585.79 \pm 742.76 \mu\text{m}$ and $2493.78 \pm 971.11 \mu\text{m}$ in four, eight, eighteen and thirty two-week aged birds respectively. Similarly, the overall size of spindle-shaped myoid cells (mean $\pm$ standard deviation) as found to be $816.26 \pm 690.97 \mu\text{m}$, $1633.45 \pm 776.44 \mu\text{m}$, $1507.52 \pm 581.56 \mu\text{m}$, $1911.67 \pm 647.88 \mu\text{m}$, and $3766.23 \pm 1551.99 \mu\text{m}$ in day-old, four, eight, eighteen and thirty two-week aged birds respectively (Table-1). Thus micrometric observations revealed that there was an increase in overall size of both oval and spindle-shaped myoid cells in 32-week-old birds when compared to younger age groups which is in accordance with the findings of Gilmore and Bridges (1974) in chicken.

Table-1: Mean $\pm$ S.D of various parameters viz., size of the Myoid cells (oval and spindle shaped), in TANUVAS Aseel chicken at different age groups (Values in μm) (Twenty numbers in each age groups))

S. No.	Parameters		Age in Weeks					F value	Wilks' Lambda
			Day-old	4 weeks	8 weeks	18 weeks	32 weeks		
1	Thymic myoid cell								
	i	Ovalshaped myoid cell							
		a Length	--	44.85 $\pm$ 10.83	53.82 $\pm$ 10.92	42.08 $\pm$ 9.86	53.13 $\pm$ 11.18	108.878	0.11
		b Width	--	38.23 $\pm$ 10.30	43.64 $\pm$ 5.31	35.92 $\pm$ 9.06	45.01 $\pm$ 10.90	96.557	0.007
		c Overall size	--	1813.81 $\pm$ 965.78	2382.92 $\pm$ 708.37	1585.79 $\pm$ 742.76	2493.78 $\pm$ 971.11	32.444	0.026
	ii	Spindle-shaped myoid cell							
		a Length	58.34 $\pm$ 31.25	92.12 $\pm$ 37.98	108.31 $\pm$ 21.60	107.84 $\pm$ 36.96	139.87 $\pm$ 39.23	13.914	0.225
		b Width	12.76 $\pm$ 3.03	17.86 $\pm$ 5.79	13.91 $\pm$ 4.26	18.24 $\pm$ 5.22	27.46 $\pm$ 9.88	18.737	0.149
		c Overall size	816.26 $\pm$ 690.97	1633.45 $\pm$ 776.44	1507.52 $\pm$ 581.56	1911.67 $\pm$ 647.88	3766.23 $\pm$ 1551.99	27.572	0.194

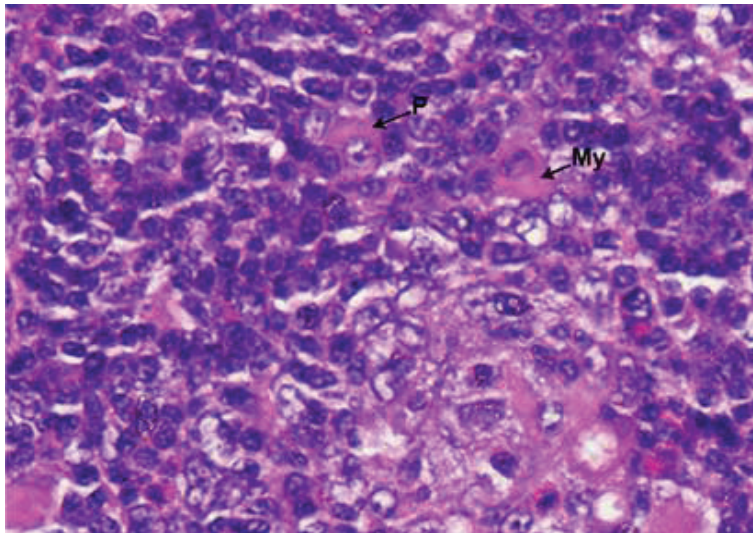


Fig.1. Photomicrograph of the thymus of thirty two week-old Aseel chicken showing the MY - oval myoid cell and P - Plasma cell H&E x 1000

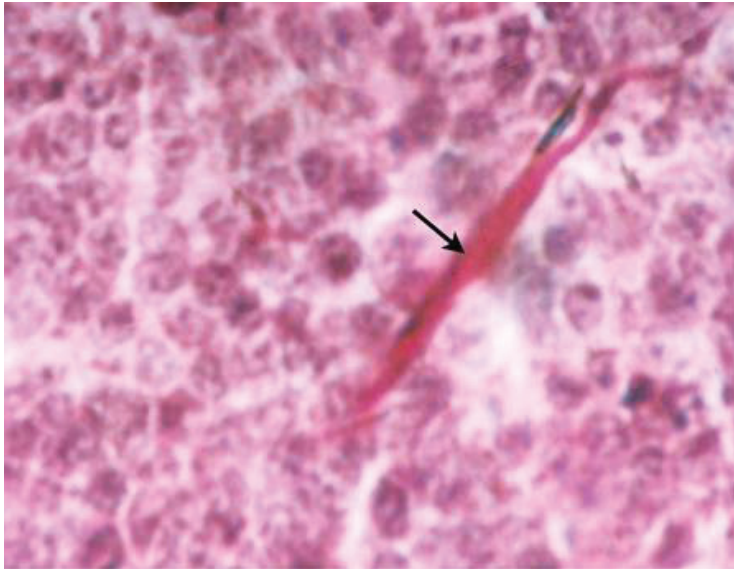


Fig.2. Photomicrograph of the thymus of day-old Aseel bird showing the myoid cell (in arrow) MTC x 1000

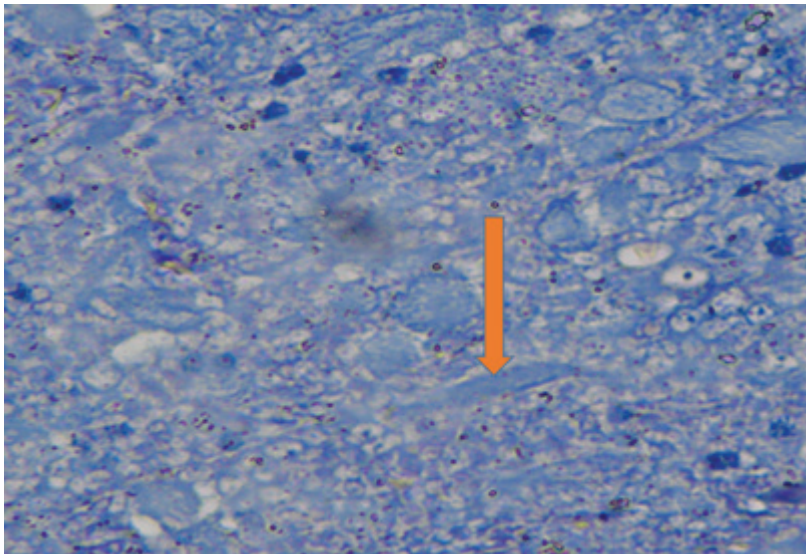


Fig.3. Photomicrograph of the thymus of eight week-old Aseel bird showing the myoid cell (arrow) PTAH x 1000

CONCLUSION

Myoid cells were closely associated with Hassall's corpuscles and showed a characteristic circular arrangement in adult birds, particularly at 32 weeks of age. These age-related changes in their morphology and distribution suggest that myoid cells may contribute to thymic maturation, involution, and the functional organization of the thymus.

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