

BASOPHILS OF PARS DISTALIS ADENOHYPHYSIS IN MADRAS RED SHEEP (*Ovis aries*) – HISTOLOGY WITH REFERENCE TO VARIOUS AGE GROUPS

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Abstract: Histophysiological study of pituitary gland was conducted on Madras red sheep to record the age related changes. The pars distalis adenohypophysis comprised of cells arranged in irregular cords, grouped as acidophils, basophils and chromophobes. The basophils were round, oval and irregularly polygonal cells with distinct cell boundaries observed as 4 variants. The type I basophils were angular or polygonal cells stained intensely with aniline blue both in Mallory's triple stain and Mallory's Azan technique. Type II basophils were oval, polygonal in shape packed with coarse granules and showed strong PAS reaction. Type III basophils were oval or round cells, purple to violet in acid fuchsin and aniline blue preparations. These were aldehyde fuchsin negative and showed a mild PAS reactivity as compared to type II basophils. Type IV basophils were irregularly oval to elongate in shape with their processes extended through the neighbouring cells, moderately PAS positive but strongly positive for lead Haematoxylin. The number of basophils ranged from 862 ± 52.98 cells/mm² in lactating animals upto 1428 ± 83.14 cells/mm² in pubertal animals. The increased basophils during pubertal age groups of sheep indicated that the activity of FSH and LH cell that contributed the hormones required for the estrous cycles.

Keywords: Histology, Pituitary gland, Adenohypophysis, Basophils, Sheep.

Introduction

Sheep and goats contribute greatly to the agrarian economy, especially in areas where crop and dairy farming are not economical. They play an important role in the livelihood of a large proportion of small and marginal farmers and landless labours by providing supplementary employment and an additional source of income (Pulina and Nudda, 2004).

Reports are available on histological and histochemical features of bovine pituitary gland and on buffalo pituitary gland (Dellmann, 1987). However, reports on the correlative studies on the histology and histochemistry of basophils during prepubertal, pubertal, gestation, lactation and dry sheep are limited. Therefore, the present study is focused to record the age

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related cytological differentiation of basophils of anterior pituitary gland of Madras red sheep.

MATERIALS AND METHODS

The current histological study was carried out on pituitary glands collected from 30 Madras red ewes divided into five age groups, viz. prepubertal, (4 to 6 months), pubertal (7 to 18 months), pregnant (1.5 to 2.5 years), lactating (2 to 4 years) and dry (4 to 8 years) animals. The head of each animal collected was flushed with 2% sodium citrate solution through common carotid arteries and fixed with various standard fixatives, viz. 10% neutral buffered formalin, Zenker's fluid, Carnoy's fluid, and Bouin's fluid. The pituitary gland from each head was dissected out and preserved in corresponding fixatives.

All tissues collected were processed by routine Alcohol-Benzene schedule and paraffin blocks were cut at 5-7 μm thickness for histological study. The sections were stained with standard Haematoxylin and Eosin, Masson's trichrome method for collagen and muscle fibres, Verhoeff's method for elastic fibres, Periodic acid Schiff (PAS) technique for mucopolysaccharides, Lead Haematoxylin stain for endocrine cells in pituitary, Crossman's modification of Mallory's triple staining for connective tissue fibres and cytodifferentiation of basophils of pituitary gland, Von Kossa method for calcium, Mallory-Azan (Heidenhain's) method for endocrine cells in adenohypophysis (Bancroft and Gamble, 2003).

Micrometry was done using the Carl Zeiss Videoplan image processing system and Image Pro 5.1 (Olympus) software. Differential cell count was conducted through special (differential stains) histological techniques for basophils in randomly selected fields of each regions of adenohypophysis of all age groups. The average diameter of basophils and their nuclei were measured in the sagittal sections of adenohypophysis of each animal from all groups. The micrometrical observations on lobulo-alveolar numbers and size were made in mammary glands of all age groups. The observations were subjected to statistical analysis and tabulated as per the procedures of Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The pars distalis adenohypophysis comprised of cells arranged in irregular cords, clusters and follicles in various age groups of sheep. In Haematoxylin and eosin staining, the cells were noticed as acidophils, basophils and chromophobes which were observed to be scattered throughout pars distalis. Nagamalleshwari *et al.* (1994), however, identified two types of cells forming cords and follicles in the goat pituitary viz., chromophobes and four types of chromophils. The acidophils had a strong affinity for acidic dyes and the intensity of staining

depended upon the concentration of cytoplasmic granules. These cells were round, oval or polygonal cells with eccentrically placed vesicular nuclei. The basophils were round, oval and irregularly polygonal cells with distinct cell boundaries. Their cytoplasmic granules were basophilic and showed positive reaction to the alcian blue, aldehyde-fuchsin and Schiff's reagent. The nuclei of the basophils were generally round and centrally placed. Four types of basophils were noticed in the present study depending upon the difference in their shape, and the stainability of their cytoplasmic granules.

a). Basophil – I / Thyrotroph

The type I basophils were angular or polygonal cells with distinct cell boundaries. They were concentrated more in the rostro-dorsal and mid-dorsal regions of the pars distalis adenohypophysis of all the age groups of sheep. However, they were scattered among the other basophils throughout the adenohypophysis of pregnant animals. The fine granules in the cytoplasm of these cells stained intensely with aniline blue both in Mallory's triple stain and Mallory's Azan technique (Figure.1). These cells stained mildly with the Schiff's reagent but selectively with Gomori's aldehyde fuchsin technique. The vesicular nucleus was seen centrally in the cytoplasm. Gomez *et al.* (1989) identified the TSH-cells located in the anterior area in sagittal sections and in caudodorsal band and transversely in the ventral and medial region in adenohypophyseal pars distalis of a kid.

b). Basophil – II / FSH gonadotroph

The type II basophils were oval, polygonal in shape. They often appeared to be scattered in the periphery of cell cords. They were distributed throughout the pars distalis with a higher concentration at periphery of the mid-dorsal and mid-ventral regions in all age groups of sheep. The cytoplasm of these cells was packed with coarse granules and showed strong PAS reaction (Figure.2). The nuclei of the type II basophils were large and round and eccentric in position in the cytoplasm. Khan (1995) described that the FSH cells were only 5 to 15 % of the total number of cells in the adenohypophysis of buffalo.

c). Basophil – III / LH gonadotroph

The type III basophils were oval or round cells. These cells were relatively larger in size when compared to the type II basophils. Their cytoplasmic granules were fine and stained pale purple to violet in acid fuchsin and aniline blue preparations. These were aldehyde fuchsin negative and showed a mild PAS reactivity as compared to type II basophils (Figure.2). Singh and Dhingra (1991) identified these cells as delta-I and stated that these cells were oval and stained light pink with AB-PAS-OG method of staining, which were

responsible for the secretion of luteinizing hormone in pregnant ewes. The nuclei of the type III basophils were generally spherical and placed centrally or sometimes eccentrically. One or two nucleoli could also be identified in the sparsely granulated chromatin network. These cells were larger in size and increased in number in the pars distalis of pregnant sheep.

d). Basophil – IV / Corticotroph

The type IV basophils were irregularly oval to elongate in shape with their processes extended through the neighbouring cells. They were scattered singly or in occasional groups in the rostro-ventral region of the pars distalis adenohypophysis of all the age groups of sheep. The cytoplasmic granules were moderately PAS positive but strongly positive for lead Haematoxylin (Figure.3). The granules were fine and distributed homogeneously in the cytoplasm. The vesicular nuclei of these cells were mostly eccentric in position. These cells showed no changes in the number and size between different age groups in sheep.

The cyclic changes during pubertal age and developmental changes during pregnancy involved in an increase in the proportion of acidophils and basophils with a decrease in the proportion of chromophobes. The number of basophils ranged from 862 ± 52.98 cells/mm² in lactating animals upto 1428 ± 83.14 cells/mm² in pubertal animals (Table 1). The increased basophilic during pubertal age groups of sheep indicated that the activity of FSH and LH cell that contributed the corresponding hormones required for the estrous cycles. The pregnant sheep showed increased values for acidophils which secreting the hormones that may be required for the development and growth of body of the foetus and mammary gland.

Table 1. Cell population of pars distalis adenohypophysis in different age groups of Madras red sheep

Parameters (cells / mm²)	Prepubertal	Pubertal	Pregnant	Lactating	Dry
Acidophils	2297 ^a ± 144.69	2909 ^b ± 132.50	3687 ^c ± 126.88	3775 ^c ± 154.40	2933 ^b ± 113.13
Basophils	1169 ^b ± 63.61	1428 ^c ± 83.14	1008 ^{ab} ± 73.54	862 ^a ± 52.98	1136 ^b ± 69.10
Chromophobes	2045 ^c ± 86.73	1410 ^b ± 90.53	981 ^a ± 44.36	857 ^a ± 58.84	1381 ^b ± 82.79

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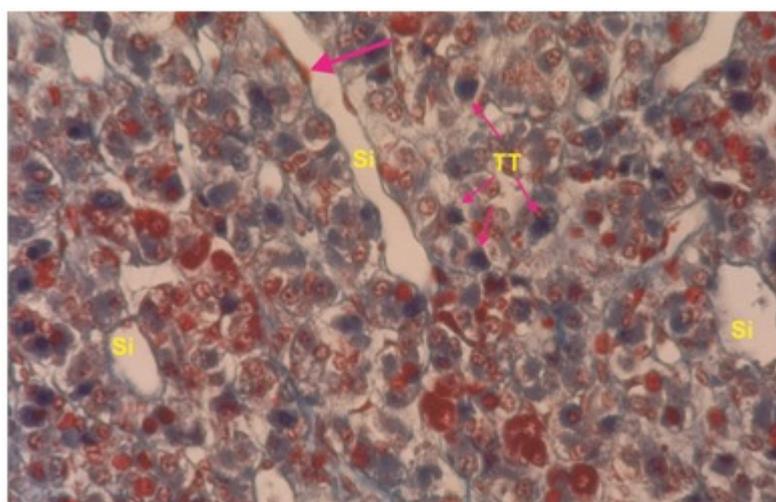


Figure 1. Basophilic cell cords showing scattered thyrotrophs (TT) in pars distalis adenohypophysis of pubertal sheep. Sinusoids (Si) lined by endothelial cells (arrow).
Mallory Azan x 400

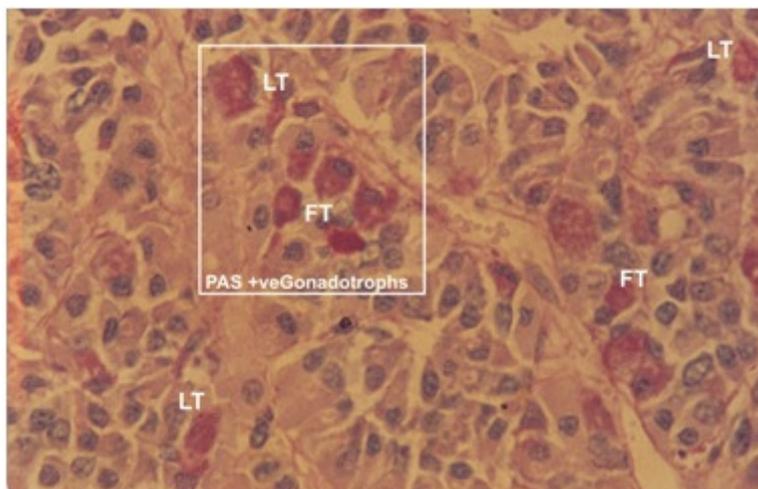


Figure 2. Pituitary gland showing strongly PAS reactive FSH gonadotrophs (FT) and LH Gonadotrophs (LT) in pubertal sheep. **PAS x 630**

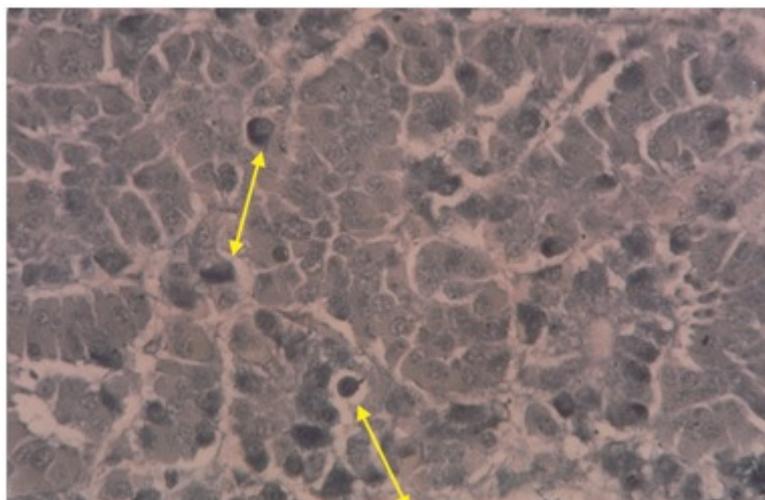


Figure 3. Photomicrograph of pars distalis adenohypophysis of lactating sheep showing the scattered Type IV basophils / corticotrophs (arrow). **Lead haematoxylin x 400**