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HISTOPHYSIOLOGICAL STUDIES ON THE HYPOPHYSIO-MAMMARY AXIS IN SHEEP *(Ovis aries)* - MAMMOTROPHS

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Abstract: The pituitary gland and mammary glands of 30 Madras red ewes of different age groups were utilized for the current study. Two types of acidophils were identified in the pars distalis adenohypophysis. The size of type II acidophils (carminophils) ranged from 9.15 ± 0.26 µm in prepubertal age to 10.05 ± 0.29 µm in lactating sheep. The average number of type II acidophils in prepubertal sheep was 550 ± 34.88 cells/mm^2 which increased gradually upto lactation (2043 ± 107.48 cells/mm^2) but showed marked decrease in dry animals (1269 ± 117.71 cells/mm^2). These cells had the largest electron dense, oval or elliptical shaped secretory granules varied in size from 200 to 700 nm. in pregnant and lactating sheep. Immature forms of granules were smaller, and more variable in size and shape.

Keywords: Sheep, Pituitary gland, Mammotrophs, Histology.

INTRODUCTION

Sheep (*Ovis aries*) 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. In tropical countries, sheep milk is mainly for home consumption and could be an important item of diet. Sheep milk is as rich as the buffalo in fat and even richer in protein (Pulina and Nudda, 2004). The anterior pituitary gland secretes several important hormones including growth hormone and prolactin. The growth hormone plays a key role in epithelial differentiation, milk synthesis and secretion. The prolactin is the primary hormone involved in the initiation of lactation which increase rapidly during or shortly after parturition in sheep. The present study is focused to record the age related cytological differentiation of mammotrophs in pituitary gland in correlation with the development of mammary gland in Madras red sheep.

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MATERIALS AND METHODS

Thirty Madras red ewes used in the current study were divided into five age groups viz. prepubertal, (4 to 6 months), pubertal (7 to 18 months), pregnant (1.5 years to 2.5 years), lactating (2 to 4 years) and dry (4 to 8 years) with 6 animals in each group. The tissue samples collected from pituitary glands of all these animals were fixed in various standard fixatives viz., 10% neutral buffered formalin, Zenker's fluid, Carnoy's fluid, and Bouin's fluid. All tissues collected as above 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 acidophils of pituitary gland, Mallory-Azan (Heidenhain's) method for endocrine cells in adenohypophysis (Bancroft and Gamble, 2003). The micrometrical parameters were recorded for pituitary and mammary glands for correlative study.

RESULTS AND DISCUSSION

The pars distalis comprised of cells arranged in irregular cords, clusters and follicles in various age groups of sheep. The cell cords were intimately related to the sinusoids lined with endothelium and the connective tissue fibres of the stroma. The arrangement of cells into longitudinal cords was common in prepubertal and pubertal age groups of sheep. However, the formation of cell clusters and follicles filled with colloid material was frequently seen in pregnant and lactating animals. However, they were arranged in irregularly oriented cell cords with more number of cysts were observed in the pars distalis of dry sheep.

In Haematoxylin and eosin staining, the cells were noticed as acidophils, basophils and chromophobes which were observed to be scattered throughout pars distalis. The acidophils had a strong affinity for acidic dyes and appeared as round, oval or polygonal cells with eccentrically placed vesicular nuclei. Two types of acidophils were recognized as Type I – Orangeophils / Somatotrophs and Type II – Carminophils / Mammotrophs in the pars distalis based on the staining affinity, in all the age groups of sheep.

The type II acidophils were oval or round cells distributed singly all along the pars distalis adenohypophysis in prepubertal and pubertal sheep and occurred in cords or clusters in pregnant and lactating ewes. They appeared bright red in colour with the cytoplasm packed with coarse secretory granules. These cells were showed positive reaction for acid fuchsin (Fig.1). Most of the cell had centrally placed vesicular nuclei. The increased population (Table. I) of these cells corresponded very high content of the prolactin in sheep hypophysis during these periods. These findings are in agreement with the findings of Bernabe *et al.* (1996) who concluded that PRL cells increased in number and size with increasing age, and were greater in males than in females. Guda *et al.* (2002) identified the lactotrophs and reported that population varied in number through different ages of the buffalo.

In correlation with the number and size of mammotrophs the development of mammary gland also showed the corresponding variations in lobulation and alveolar parameters. The length of the lobules was 467.66 ± 32.42 µm in pubertal age (Fig.4), 741.83 ± 81.01 µm in pregnant sheep and increased significantly to 1333.65 ± 76.80 µm in lactating mammary glands. However, it decreased significantly in dry mammary glands measuring only 686.33 ± 85.41 µm. The breadth of the lobules also showed the same trend among the age groups studied (Table.1).

In the pregnant mammary glands, the development of alveoli was distinct. The mammary parenchyma at this stage of development consisted of proliferating ducts and solid rounded alveolar buds with considerable lumen. The number of alveoli per lobule was 254.77 ± 14.96 in pregnant animals, which reduced significantly to 108.27 ± 15.64 during lactating animals. However, the number of alveoli per lobule was only 27.11 ± 3.11 in the mammary glands of dry animals. In the present study, the reduction in the number of alveoli per lobule during lactation might be due to the greater enlargement of the alveoli and lumen.

In lactating animals, the structure of the alveoli was generally the same as observed in pregnant mammary glands except that the alveolar lumina were considerably wider (Fig.2). In addition, the alveoli were mostly rounded in shape in pregnant animals, whereas they were round, oval or elliptical in shape mostly filled acidophilic secretions in lactating animals. The increased cell population of acidophils could be well correlated with the increased need for growth hormone and prolactin hormone for the development of the mammary glandular epithelium during pregnancy and for synthesis of milk during lactation. Hence it may be concluded that the hypophysio-mammary axis is distinctly functional as evidenced by the histomorphological parameters of hypophysis cerebri and mammary gland in Madras Red sheep during different age groups investigated.

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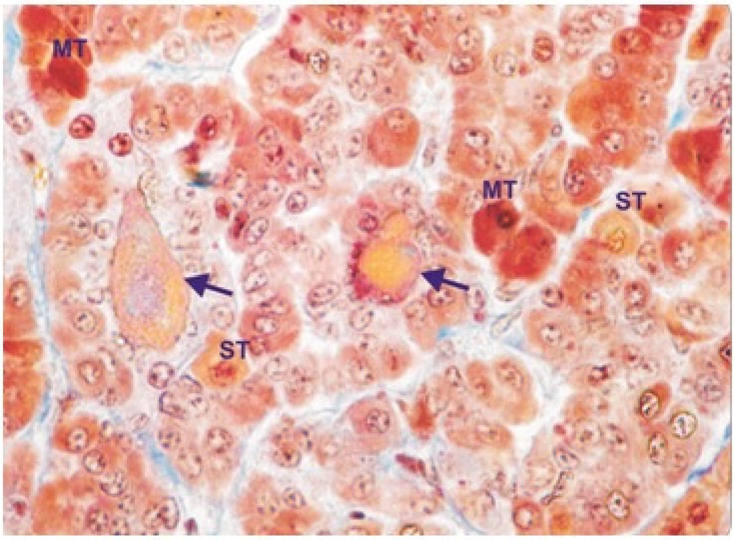

Figure 1: Pars distalis adenohypophysis of pregnant sheep showing the follicular lumen filled with colloid material showing acidophilic secretion (arrow). ST– Type I acidophils / Somatotrophs, MT – Type II acidophils / Mammotrophs. **(Mallory's triple stain x 630)**

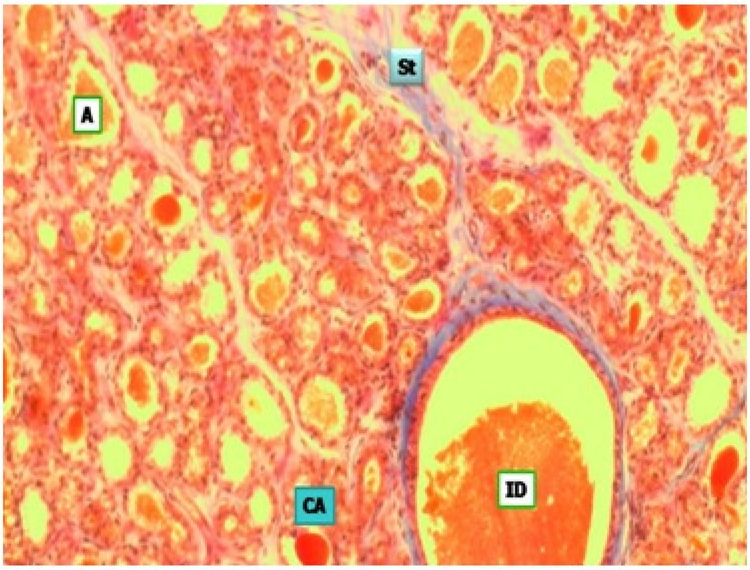

Figure 2: Photomicrograph of the lactating mammary gland showing alveoli (A) filled with secretions in their lumen, Interlobular duct (ID), St – Connective tissue, CA – Corpora amylacea. **(Mallory's triple stain x 100)**

Table: 1. Age related micrometrical parameters of Hypophysis cerebri and Mammary glands of Madras red ewes

Parameters (µm)	PREPUBERTAL	PUBERTAL	PREGNANT	LACTATING	DRY
MAMMOTROPHS IN ADENOHYPOPOHYSIS					
Total count of Mammotrophs	$550^{a} \pm 34.88$	$751^{a} \pm 41.08$	$1853^{c} \pm 97.53$	$2043^{c} \pm 107.48$	$1269^{b} \pm 117.71$
Diameter of mammotrophs	$9.15^{a} \pm 0.26$	$9.40^{ab} \pm 0.23$	$9.57^{ab} \pm 0.16$	$10.05^{b} \pm 0.29$	$9.30^{a} \pm 0.25$
Diameter of Nucleus	$4.52^{ab} \pm 0.11$	$4.27^{a} \pm 0.10$	$4.65^{b} \pm 0.12$	$4.40^{ab} \pm 0.12$	$4.37^{ab} \pm 0.11$
MAMMARY GLAND					
Length of lobule	-----	$467.66^{a} \pm 32.42$	$741.83^{b} \pm 81.01$	$1339.65^{c} \pm 76.80$	$686.33^{b} \pm 85.41$
Breadth of lobule	-----	$226.78^{a} \pm 19.41$	$448.56^{b} \pm 44.51$	$613.39^{c} \pm 44.82$	$274.72^{a} \pm 29.28$
Number of alveoli per lobule	-----	-----	$254.77^{c} \pm 14.96$	$108.27^{b} \pm 15.64$	$27.11^{a} \pm 3.11$
Alveolar Size	-----	-----	$39.88^{a} \pm 1.39$	$106.05^{b} \pm 14.70$	$32.45^{a} \pm 1.64$