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RESEARCH ARTICLE

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

[1,]*Paramasivan, S. and [2]Geetha Ramesh

[1]Department of Veterinary Anatomy, Veterinary College and Research Institute, Thanjavur – 614 625, India
[2]Professor and Head, Department of Veterinary Anatomy, Madras Veterinary College, (Tamilnadu Veterinary and Animal Sciences University) Chennai – 600 007, India

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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 I acidophils (orangeophils) ranged from 9.30 ± 0.22 µm in dry animals to 9.90 ± 0.28 µm in pubertal animals. The number of these cells was more in pregnant (1866 ± 95.85 cells/mm^2) and lactating (1687 ± 62.41 cells/mm^2) animals when compared to dry (1650 ± 108.92 cells/mm^2) age groups in sheep. 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). The alveolar size was 39.88 ± 1.39 µm in pregnant sheep, 106.05 ± 14.70 µm in lactating sheep but decreased significantly to 32.45 ± 1.64 µm in the mammary glands of dry sheep. The increased number and size of the lobulo-alveolar system in mammary glands of pregnant and lactating sheep may be correlated with the corresponding increase in the number and secretory activity of somatotrophs in adenohypophysis of hypophysis cerebri of Madras red sheep.

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INTRODUCTION

India stands 6th in sheep population in the world. 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 labourers 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 (Bole-Feysot et al., 1998). The prolactin is the primary hormone involved in the initiation of lactation which increase rapidly during or shortly after parturition in sheep.

Corresponding author: Paramasivan, S.
Department of Veterinary Anatomy, Veterinary College and Research Institute, Thanjavur – 614 625, India.

The hormones involved in the co-ordination of the development of the mammary gland with that of the other parts of the reproductive system are the hormones of the adenohypophysis, placenta and the steroid hormones produced in the ovaries, testes, adrenal cortex and placenta. 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 of the histology and histochemistry of the pituitary gland during prepubertal, pubertal, gestation, lactation and dry sheep are scanty. Therefore, the present study is focused to record the age related cytological differentiation of anterior pituitary gland of Madras red sheep.

MATERIALS AND METHODS

A total of 30 Madras red ewes of different age groups were included in the current study. The ewes used 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).

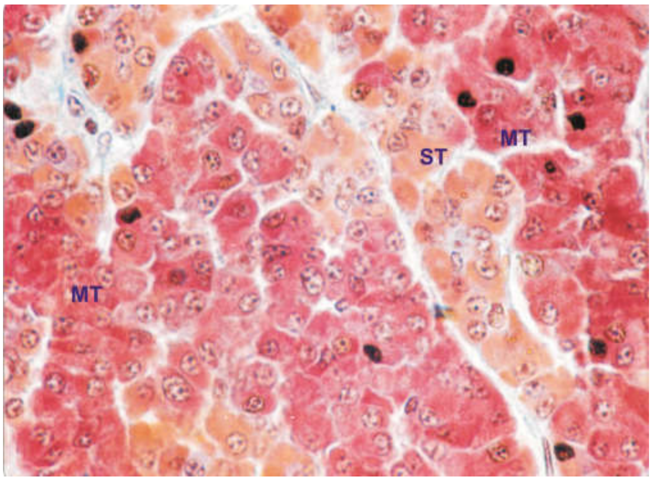

Figure: 1. Photomicrograph of the parenchymal cords of pars distalis adenohypophysis in pregnant sheep showing Type I acidophils / somatotrophs (ST) and Type II acidophils / mammotrophs (MT) in high concentration than other cell types. Mallory's triple stain x 630

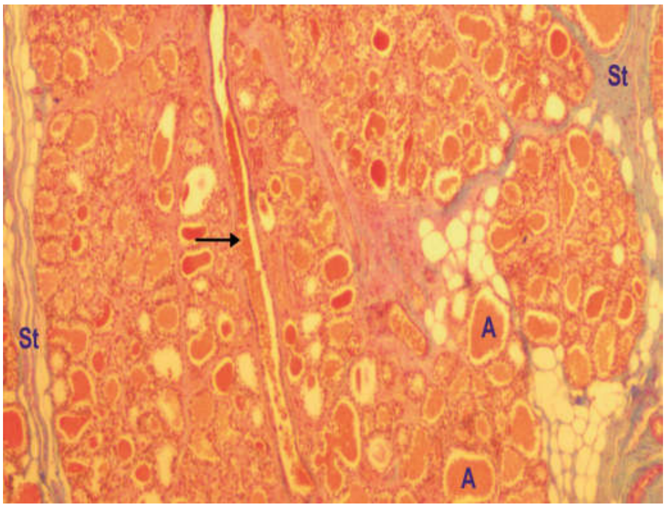

Figure 2. Photomicrograph of the lactating mammary gland showing alveoli filled with secretions in their lumen. A longitudinal section of intralobular duct (arrow) is seen at the centre of the lobule. St – Connective tissue, A – Alveoli. Mallory's triple stain x 630

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 acidophils (Type I and II) in randomly selected fields of each regions of adenohypophysis of all age groups. The average diameter of acidophils (Type I and II) 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. 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. Two types of acidophils were recognized viz., somatotrophs and lactotrophs in the pars distalis adenohypophysis based on the staining affinity, in all the age groups of sheep. Type I acidophils or somatotrophs stained with eosin and orange-G but failed to stain with acid fuchsin, aldehyde fuchsin and Schiff's reagent. The type I acidophils were large round, oval or polygonal cells, found scattered singly or in clusters in all the regions of the pars distalis adenohypophysis as observed by Nishimura *et al.* (1998) in goat. These cells were usually situated along the sinusoids and were the most abundant cell type observed in the pars distalis adenohypophysis. The type I cells were found to have granular cytoplasm with eccentric nucleus. The number of type I acidophils was 1442 ± 67.57 cells/mm^2 in prepubertal age which increased significantly to 2658 ± 101.82 cells/mm^2 in adenohypophysis of pubertal sheep. The increased cell count during pubertal sheep could be correlated with the general body growth of the sheep during pubertal periods (Table.1). Their number was more in pregnant (1866 ± 95.85 cells/mm^2) and lactating (1687 ± 62.41 cells/mm^2) animals when compared to dry (1650 ± 108.92 cells/mm^2) age groups in sheep. The current results clearly revealed that the growth hormone released from these cells greatly influenced the growth of mammary gland during pregnancy as reported by Singh and Dhingra (1991) in sheep. The authors found that the orangeophils increased during initial and later stages of pregnancy. The size of somatotrophs ranged from 9.30 ± 0.22 µm in dry animals to 9.90 ± 0.28 µm in pubertal animals.

However, Gomez *et al.* (1989) found that the GH cells in the adenohypophysis of kids measured 12.37 µm in size, which was larger than in sheep as recorded in the current study. The various micrometrical parameters viz. length and breadth of lobule, number of alveoli per lobule, alveolar diameter and luminal diameter all showed increased values during pregnant and lactating age groups of sheep but reduced during dry age groups. Singh and Dhingra (1991) hypothesized that epithelial components in mammary glands of pregnant sheep proliferated at a faster rate and became permeable for various milk recursors under the influence of high levels of circulating lactogenic hormones. In the pregnant sheep mammary glands,

Table 1. Micrometrical parameters of pituitary and mammary glands of Madras red sheep

Parameters (µm)	PREPUBERTAL	PUBERTAL	PREGNANT	LACTATING	DRY
Total count of Acidophils	$2297^{a} \pm 144.69$	$2909^{b} \pm 132.50$	$3687^{c} \pm 126.88$	$3775^{c} \pm 154.40$	$2933^{b} \pm 113.13$
GH-CellsDiameter of cells	9.85 ± 0.16	9.90 ± 0.28	9.70 ± 0.21	9.57 ± 0.28	9.30 ± 0.22
GH-Cells Diameter of Nucleus	$4.74^{b} \pm 0.11$	$4.37^{a} \pm 0.13$	$4.30^{a} \pm 0.10$	$4.45^{ab} \pm 0.08$	$4.27^{a} \pm 0.10$
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	-----	-----	$39.88^{a} \pm 1.39$	$106.05^{b} \pm 14.70$	$32.45^{a} \pm 1.64$

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. The length of the lobules was 467.66 ± 32.42 µm in pubertal age, 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. Kausar *et al.* (2001) also found that the number and size of alveoli per lobule decreased and the parenchyma was replaced by loose connective tissue during non-lactating phase in dromedaries. 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. 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 alveolar size was 39.88 ± 1.39 µm in pregnant sheep, 106.05 ± 14.70 µm in lactating sheep but decreased significantly to 32.45 ± 1.64 µm in the mammary glands of dry sheep. The increased size of the alveoli in lactating mammary gland may be correlated with its active section that fills the lumen and distension of the alveoli when compared to other age group of animals. Similarly, the somatotrophs were highest in population showed increased trend in hypophysis cerebri of pregnant and lactating age groups of sheep. Therefore, the increased cell population of somatotrophs could be well correlated with the increased need for growth hormone for the development of the mammary glandular epithelium during pregnancy and for synthesis and secretion of milk during lactation.

Hence it may be concluded that the hypophysio-mammary axis is distinctly functional as evidenced by the histological parameters of hypophysis cerebri and mammary gland in Madras Red sheep during different age groups investigated.

Conclusion

The current histological data on the pituitary and mammary gland suggested that the increase in somatotrophs during prepubertal stage could be correlated for the general body growth of the sheep in young age. The relative increase of these cells during the pregnant and lactating sheep may be due to the requirement of high concentration of growth hormone for the development of mammary gland and also for synthesis and secretion of milk. The results are strongly suggestive of the existence of hypophysio-mammary axis in sheep.

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