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RESEARCH ARTICLE

COMPENSATORY MECHANISM OF PITUITARY GLAND DURING VARIOUS PHYSIOLOGICAL STATUS IN SHEEP (*Ovis aries*) – STRUCTURAL ASPECTS

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ABSTRACT

Histology under light and transmission electron microscopy was conducted on the pituitary glands collected from 35 Madras red ewes of different age groups. The chromophobes were round or irregularly oval shaped cells, which occurred in groups in the centre of the cell cords of the pars distalis adenohypophysis of prepubertal, pubertal and dry animals. Their population was more in the rostro-dorsal and rostro-ventral regions of the pars distalis with no affinity towards any of the acidic or basic dyes. These cells are thought to be non-functional reserve cells that become chromogenic when the cells become active. The folliculo-stellate cells were observed mostly around the follicles with a characteristic star-like morphology and long agranular cytoplasmic processes. These cells have supportive role by provided mechanical support to the endocrine population and also by producing many growth factors and cytokines In the current electron microscopic study showed some immature endocrine cells closely associated with chromophobes and folliculo-stellate cells forming an incomplete follicle suggesting that these two types of cells are likely to be involved in the differentiation of new endocrine cells as required by the physiological status of the sheep.

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INTRODUCTION

The application of histology and electron microscopy on study of pituitary cytology has contributed significantly to the understanding of the histophysiology of the adenohypophysis. The hormones produced by the adenohypophysis are secreted by cell types with different ultrastructural characteristics. Correlative studies on morphologic and physiologic characters in laboratory animals have been extensively studied. However, details on the histology of adenohypophysis and its compensatory mechanism during various physiological status are not available for the ruminants. Hence, the present observation on histology and ultrastructure of adenohypophysis specially focused on the occurrence of chromophobes and follicular supporting stellate cells and their role in regulation of cellular subdivisions in the gland.

MATERIALS AND METHODS

A total of 35 Madras red ewes of different age groups were included in the current study.

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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 7 animals in each group. The head of each animal collected was flushed with 2% sodium citrate solution through common carotid arteries of both sides to wash out the blood clots. Subsequently the heads were perfused individually with various standard fixatives viz., 10% neutral buffered formalin, Zenker's fluid, Carnoy's fluid, and Bouin's fluid. The pituitary gland was dissected out from the hypothalamus at infundibular stalk after fixation of head. Hypothalamus was dissected out from the brain by outlining its anterior limit as optic chiasma, posterior limit as caudal to the mammillary body and the lateral limits 2-3 mm on either side of the lateral margin of the mammillary body. The hypothalamus and pituitary with infundibular stalk intact were dissected out from some animals to study the structural relations of hypothalamus with the pituitary gland. All tissues collected as above were processed by routine Alcohol-Benzene schedule and paraffin blocks were made (Luna, 1968). Sections were cut at 5-7 µm thickness for histological study. For histochemistry of lipids and enzymes, frozen sections of 15-20 µm thickness were cut using a cryostat from the tissues fixed in 4°C chilled formol-calcium or 10% neutral buffered

formalin. The sections were stained with the following standard histological and histochemical techniques,

- Standard Haematoxylin and Eosin (H&E) method for the routine histological study (Bancroft and Gamble, 2003).
- Masson's trichrome method for collagen and muscle fibres (Luna, 1968).
- Lead Haematoxylin stain for endocrine cells in pituitary (Bancroft and Stevens, 1996).
- Crossman's modification of Mallory's triple staining for connective tissue fibres and cytodifferentiation of acidophils of pituitary gland (Bancroft and Stevens, 1996).
- Mallory-Azan (Heidenhain's) method for endocrine cells in adenohypophysis (Bancroft and Stevens, 1996).
- Periodic acid Schiff (PAS) technique for mucopolysaccharides (Luna, 1968).

For transmission electron microscopy, tissue pieces from pituitary gland were cut into 1-2 millimeter thickness and fixed in 2.5% Glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 24 hours at 4°C. The tissues were washed in sodium cacodylate buffer for 30 minutes and post fixed in 1% aqueous Osmium tetroxide for 2 hours at 4°C. The samples were washed in sodium cacodylate buffers two times for 15 minutes each. After the post fixation, the samples were dehydrated in a series of graded acetone, infiltrated and embedded in Spurr's resin. Semi thin sections of 200-300 nm thickness and ultra thin sections, 50-70 nm thick were cut with a glass knife on a Leica Ultracut UCT-GA-D/E-1/00 ultra microtome. The semi thin sections were stained with toluidine blue. The ultra thin sections were mounted on grids and stained with saturated aqueous uranyl acetate and counter stained with 4% lead citrate (Bozzola and Russell, 1998). The specimen preparation, staining and the observations at various magnifications under the transmission electron microscope (Model: Hitachi, H-7500) were done at Christian Medical College Hospital, Vellore. Micrometry was done using the Carl Zeiss Videoplan image processing system and Image Pro 5.1 (Olympus) software.

RESULTS AND DISCUSSION

Chromophobes

These cells were round or irregularly oval shaped cells, which occurred in groups in the centre of the cell cords of the pars distalis adenohypophysis of prepubertal, pubertal and dry animals. However, they were distributed singly in the cords of pregnant and lactating sheep. Their cell boundaries were not very clearly discernible. Their nuclei were surrounded by a thin rim of lightly stained cytoplasm (Fig.2 & 3). Roy (1970) reported that the chromophobes in pituitary were small irregularly oval cells, contained agranular lean cytoplasm. The author further stated that their percentage population varied from 24.5 to 30.7 per cent in Indian buffalo. Their population was more in the rostro-dorsal and rostro-ventral regions of the pars distalis in all the age groups of sheep. The cytoplasmic granules showed no affinity towards any of the acidic or basic dyes. This is in conformity with the findings of Singh (1973) who also described the chromophobes, in goat pituitary as oval or round cells with faintly granular cytoplasm, which did not react to acidic or basic stains, including PAS. Their number appeared more in the rostro-median and ventro-caudal basophilic areas of the pars distalis adenohypophysis in goat. The nuclei of chromophobes were small, rounded or oval in shape with diffuse chromatin material and centrally placed nucleoli. Chromophobes are thought to be non-functional reserve cells that become chromogenic when the cells become active. Some chromophobes may be supporting cells with long branching processes forming a network within the parenchyma of the organ. Other chromophobe cells are not numerous and may be either transitionally degranulated cells (Fig.2) or may be considered as reserve cells. The total number of chromophobes in the adenohypophysis was 2045 ± 86.73 cells/mm^2 in prepubertal animals and decreased significantly in pregnant (981 ± 44.36 cells/mm^2) and lactating animals (857 ± 58.84 cells/mm^2). Their number was 1410 ± 90.53 cells/mm^2 and 1381 ± 82.79 cells/mm^2 in pubertal and dry animals, respectively. These findings coincided with the finding of Khan (1995) who noticed that the chromophobes decreased and the acidophils as well as the basophils increased with the advancement of age. Also with the cyclic changes and changes during pregnancy there was an increase in the proportion of acidophils and basophils with a decrease in the proportion of chromophobes.

Follicular cells or stellate cells

The folliculo-stellate cells were observed mostly around the follicles in the pars distalis adenohypophysis bordering the sinusoids (Fig.3) of sheep of all age groups studied. These cells have a characteristic star-like morphology with the population or 3-6 per cent of total count in adenohypophysis. The cytoplasm was thin with no staining affinity in the adenohypophysis of all the age groups studied. The long agranular cytoplasmic processes of these cells extended to intermingle in a fashion that produced three dimensional anatomical network enmeshing hormone secreting cells. These cells have been considered to give a supportive function for adenohypophysial cells cords as also reported by Jimenez *et al.*, (1986).

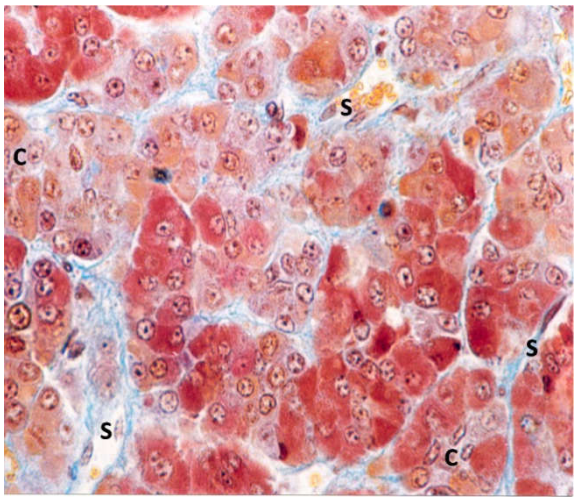

Fig.1. Photomicrograph of the parenchymal cords of pars distalis adenohypophysis in pregnant sheep showing chromophils, chromophobes (C) and folliculo-stellate cells (S). Mallory's triple stain x 630

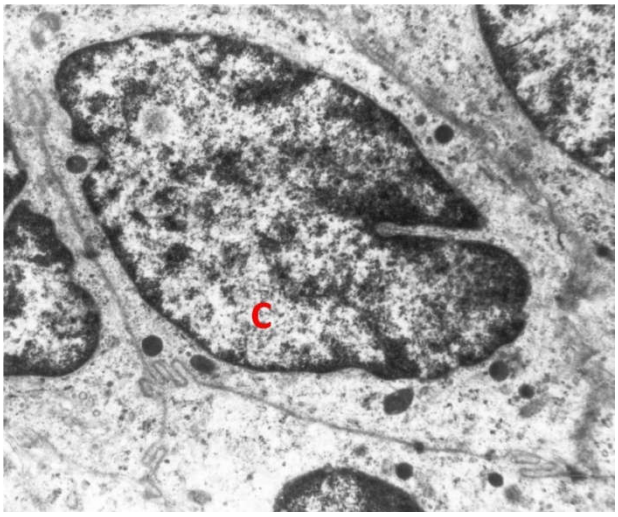

Fig. 2. The electron photomicrograph of adenohypophysis showing chromophobes (C) with very few secretory granules located in thin cytoplasm TEM X 10000

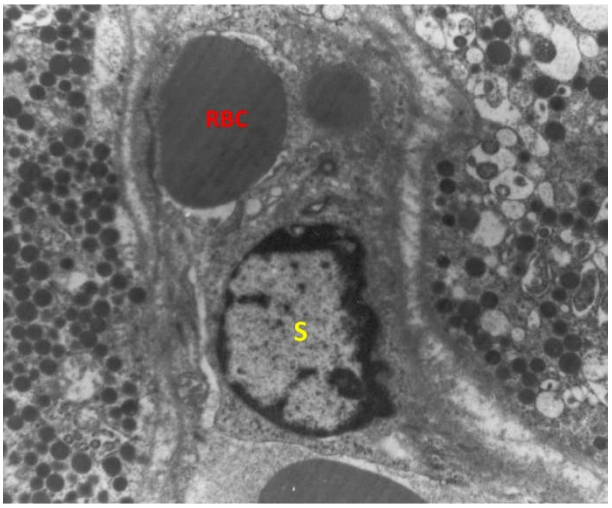

Fig.3. The electron photomicrograph of adenohypophysis showing the folliculo-stellate cell (S) projecting into the lumen of a sinusoid filled with RBCs and surrounded by endocrine cells filled with secretory granules TEM X 7000

The nucleus was usually oval to elongate in shape which contained coarse granular chromatin as clumps. The presence of non-hormonal folliculo-stellate cells with hormone-secreting cells in the anterior pituitary gland indicated that it has supportive role by provided mechanical support to the endocrine population and also by producing many growth factors and cytokines (Soji *et al*., 1997).

The endocrine cells are encased in the cytoplasmic processes of these folliculo-stellate cells and thereby a close contact is established. The interdigitation of these cells between endocrine cells evidences a role for intercellular communication between the two cell types. In the current electron microscopic study showed some immature endocrine cells closely associated with chromophobes and folliculo-stellate cells forming an incomplete follicle suggesting that these two types of cells are likely to be involved in the differentiation of new endrocrine cells as required by the physiological status of the sheep.

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