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RESEARCH ARTICLE

MICROANATOMY OF PARS TUBERALIS AND INTERMEDIA OF PITUITARY GLAND IN MADRAS RED SHEEP (*Ovis aries*)

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ABSTRACT

Microanatomical observations were recorded on the pituitary glands of various age groups of Madras red sheep. The pars tuberalis appeared as a thin sleeve and surrounded the infundibular stalk separated by a thin connective tissue layer. It was located at rostro-ventral part of pituitary stalk, extended downward and backward and mingled with the pars distalis at zona tuberalis without any line of demarcation. The hypothalamic-hypophyseal portal system originated in the primary capillary plexus in the median eminence supply the anterior pituitary by passing through pituitary stalk. The pars tuberalis consisted of longitudinally oriented cells cords and follicles separated by sinusoids. Pars tuberalis consisted mainly of gonadotrophs interspersed with few corticotrophs and thyrotrophs, histologically resembling those of pars distalis. Somatotrophs and lactotrophs were not identified. The pars intermedia was located along the anterior, ventral and lateral walls of the neurohypophysis and formed the caudal wall of the hypophysial cavity. The pars intermedia composed of basophilic cells arranged in clusters or cell cords along with scattered chromophobes. A bee-hive shaped or elliptical projection of the pars intermedia called as cone of Wulzen was noticed in the middle of the hypophysial cavity. The hypophysial cavity was well developed and seen in all age groups of animals in the present study. Cysts of various sizes and shapes in pars distalis and pars intermedia of the adenohypophysis.

INTRODUCTION

The pituitary gland in domestic animals consists of various lobes with distinct cell groups separated by connective tissue septa. The 'hypophysiotropic hormones' or releasing hormones from various nuclei of hypothalamus diffuse into the capillary plexus of the median eminence and are carried by the veins of the pituitary stalk to the sinusoids of the pars tuberalis and pars distal is of pituitary gland. This vascular system has been designated as the hypophysial-portal circulation, by analogy with the hepatic portal system (Sulman, 1970). Literatures are available on histological and histochemical features on anterior and posterior lobes of pituitary glands in various domestic animals (Dellmann, 2005). However, reports on the pars tuberalis and pars intermedia of pituitary gland during various physiological stages in sheep are scanty. Therefore, the present study is focused to record the age related cytological differentiation of pars tuberalis, intermedia and associated structures in pituitary gland of Madras red sheep.

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MATERIALS AND METHODS

The 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 7 animals in each group. The head of each animal was collected and 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. 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 4oC 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). Micrometry was done using the Carl Zeiss Videoplan image processing system and Image Pro 5.1 (Olympus) software.

RESULTS AND DISCUSSION

Pars tuberalis

The pars tuberalis appeared as a thin sleeve and surrounded the initial part of the infundibular stalk (Fig.1) which confirms the report of Dellmann *et al.*, (2005) in domestic animals. It was separated from the infundibular stalk by a thin layer of connective tissue. Rostro-ventral part extended downward and backward and mingled with the pars distalis at zona tuberalis without any line of demarcation.

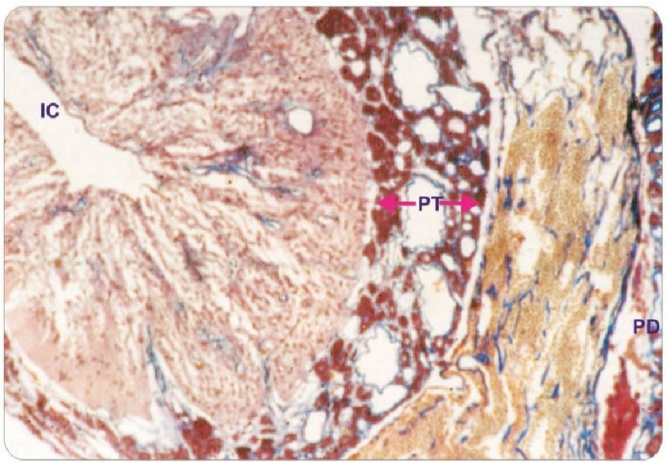

Figure 1. Photomicrograph of the pituitary gland of pubertal sheep showing the infundibular stalk surrounded by pars tuberalis with a large vein. IC – Infundibular cavity, PT – Pars tuberalis, PD – Pars distalis (Mallory's triple stain x 100)

Between the hypothalamus and the anterior pituitary, there is a system of blood vessels known as the hypothalamichypophyseal portal system which originates in the primary capillary plexus - a series of capillary loops in the median eminence. Most of the arteries that supply the anterior pituitary do not form a capillary network amongst the epithelial cells; rather, they course upwards into the pituitary stalk where they empty into a network of capillary sinusoids. The blood leaving this primary capillary plexus then flows in parallel veins, the long portal vessels, down the pituitary stalk to the anterior lobe. Here the portal veins break up into sinusoids which form the main blood supply of the anterior pituitary. In the present study, pars tuberalis consisted of longitudinally oriented cells cords and follicles separated by sinusoids which is also true in mammals (Gross and Page, 1984). The cells of the pars tuberalis mostly comprised of faintly stained chromophils and indistinctly outlined chromophobes which had small amount of cytoplasm and round nuclei as in buffalo (Roy, 1970). However, Skinnner and Robinson (1996) identified gonadotrophs in antero-ventral region of pars tuberalis of ewe. In the current study Type I basophils were also identified as aldehyde fuchsin positive cells in the pars tuberalis. Type IV basophils were found scattered in between the chromophobes and they were strongly reactive with lead Haematoxylin in all the age groups studied. This is in agreement with Prasad and Sinha (1983) noticed that the glandular cells of the pars tuberalis of Indian buffalo were basophilic and argyrophilic. Pars tuberalis consisted mainly of gonadotrophs interspersed with few corticotrophs and thyrotrophs, histologically resembling those of pars distalis. Somatotrophs and lactotrophs were not identified.

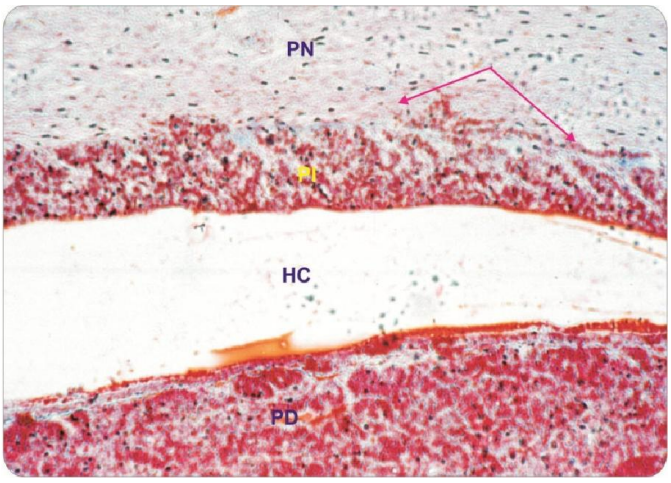

Figure 2. Photomicrograph of pituitary gland of pregnant sheep showing a thin glial connective tissue (arrow) separating the pars intermedia from pars nervosa. PN – Pars nervosa, PI – Pars intermedia, HC – Hypophysial cavity, PD – Pars distalis (Mallory's triple stain x 100)

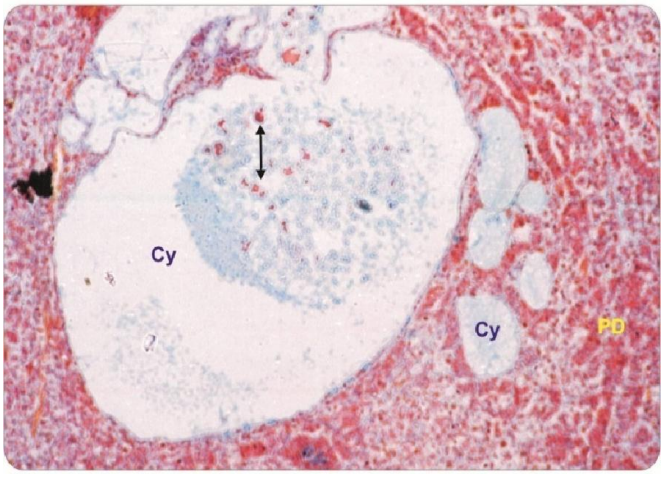

Figure 3: Photomicrograph showing the formation of larger cysts by coalescence of smaller cysts in the pars distalis adenohypophysis of dry animals. The lumen of the cysts show granular basophilic material with few cells (arrow). Cy – Cyst, PD- Pars distalis adenohypophysis. (Mallory's triple stain x 200)

Pars intermedia

The pars intermedia was located along the anterior, ventral and lateral walls of the neurohypophysis and formed the caudal wall of the hypophysial cavity. The latter separated the pars

intermedia and pars distalis adenohypophysis (Fig.2) as in mammals (Dellmann, 1987). However, pars intermedia of buffalo covered the neurohypophysis from all sides (Prasad and Singh, 1980). A thin layer of collage nous glial connective tissue separated the pars intermedia clearly from the neurohypophysis. The pars intermedia composed of basophilic cells arranged in clusters or cell cords. Numerous cells contained the basophilic cytoplasm which stained with PAS or aniline blue. This is in conformity with Roy (1970) in buffalo and Singh and Dhingra (1984) in sheep, where the authors also reported basophilic cells viz., MSH cells and ACTH cells, in the pars intermedia. Along with these cells, chromophobes with large nuclei were noticed. The nuclei of these cells were spherical or oval in shape. A bee-hive shaped or elliptical projection of the pars intermedia called as cone of Wulzen was noticed in the middle of the hypophysial cavity. It was located in the caudal portion of the pars intermedia. In contrary to the current findings, Singh and Dhingra (1984) stated that the Wulzen's lobe was not seen as a distinct projection into the cavum hypophysis of sheep. Wahba *et al.* (1988) considered the origin of Wulzen's cone from the pars distalis in Egyptian buffalo.

The hypophysial cavity was well developed and seen in all age groups of animals in the present study. However, Khatra and Nanda (1983) observed the cavum as S-shaped cavity in the midsagittal section and the depth of the cavum increased with the advancement in age in goats. The hypophysial cavity was filled with PAS positive colloid material (Fig.2) in all the age groups of sheep as described by Roy (1970) in buffalo. The epithelium lining the hypophysial cavity towards the pars intermedia was simple squamous to stratified squamous in type, whereas the same towards the pars distalis adenohypophysis was stratified to pseudo stratified columnar in type. This is in agreement with the findings of Roy (1970) in buffalo where the epithelium lining the cavity was simple squamous on the pars intermedia side, but it was simple cuboidal to pseudostratified ciliated columnar with few goblet cells on pars distalis side.

Cysts

Cysts of various sizes and shapes in pars distalis (Fig.3) and pars intermedia of the adenohypophysis were observed as also reported by Singh and Dhingra (1975) in goats. They were round to irregular in shape and contained a fine granular or homogeneous colloid mass. Some cysts, which were seen in the pars distalis adenohypophysis had PAS positive coarse granular material in their lumina. The cysts were circumscribed by a delicate connective tissue covering and were commonly lined with a simple squamous to cuboidal epithelium. The cysts in various stages of development were clearly seen in the pars distalis. The coalescence of small cysts forming the larger cysts was frequently noticed in the adenohypophysis of dry animals. The cysts were noticed more caudally near the hypophysial cavity. This indicated that these cysts could probably be resulted due to the epithelial infolding from the hypophysial cavity. This is in conformity with the findings of Singh and Dhingra (1984) in sheep.

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