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Histomorphological Study on the Neurohypophysis of Jaffarabadi Buffalo during Different Seasons

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ABSTRACT

The study was conducted on neurohypophysis of 30 adult Jaffarabadi buffaloes to explore the histomorphological changes during different seasons. The tissue samples were collected during the winter, summer and rainy seasons (10 samples during each season) from the slaughterhouse. The samples were processed and stained with various histological stains, by standard procedures. The pars nervosa and infundibulum were two main components of the neurohypophysis, which forms the caudo-dorsal portion of the pituitary gland. Both parts consisted of axon bundles and pituicytes (cells of neurohypophysis). The neurosecretory material was distributed diffusely along with the axonal bundles and blood vessels. In addition to diffuse neurosecretory material, membrane-bound, round or oval-shaped accumulations (Herring body) were also observed between the axonal bundles and close to the blood vessels. During winter the average diameter of the Herring body was smallest which increased gradually during summer and the rainy season. The diameters of Herring bodies were significantly higher during the rainy season. The pituicytes showed a wide variation in the shape and size of cells and nuclei. The highest cell density was observed during the summer season as compared to the winter and rainy seasons.

Keywords: Neurohypophysis; Jaffarabadi buffalo; Seasonal; Histomorphology

INTRODUCTION

The pituitary gland produces several hormones that influence the activities of other endocrine glands and directly or indirectly regulates various physiological functions of the animal body. The pituitary gland is composed of two parts namely, the adenohypophysis and the neurohypophysis. The neurohypophysis formed the dorso-caudal part of the pituitary (Sharma *et.al*, 2019). Neurohypophysis is an integral part of the neuro-humoral system, which involves the storage and release of oxytocin and vasopressin. These hormones produced by the hypothalamus are conveyed to neurohypophysis via specialized axon bundles. The hormones released from neurohypophysis play a crucial role in reproductive functions and fluid balance (Eurell, and Frappier. 2006).

Detailed histomorphological studies on neurohypophysis were mainly reported in buffalo (Pathak and Bansal, 2017), Madras red sheep (Paramasivan and Ramesh, 2016), camel (Alim *et al*, 2012; Ye *et al*, 2018), but there is lack of information on the season related structural changes in neurohypophysis, especially in the buffalo. Hence, the present study has been conducted to find out histomorphological and micrometrical changes in the neurohypophysis of the adult Jaffarabadi buffalo (*Bubalus bubalis*) during different seasons to understand the effect of environmental changes on the structure and function of neurohypophysis.

MATERIALS AND METHODS

The present study was carried out on the pituitary glands of 30 adult Jaffarabadi buffaloes. The pituitary glands were collected from the slaughterhouse, following the opening of the cranial cavity just after the slaughter.

Immediately after collection, the tissue samples were fixed in 10% Neutral buffered formalin. The fixed tissue samples were processed through standard procedures of the preparation of paraffin blocks (Luna, 1968). The sections of the whole pituitary gland were sectioned with the help of a manual rotary microtome. The sections of 5 µm were mounted on glass slides and these sections were subjected to Hematoxylin and Eosin, Masson's trichrome, and Weigert's resorcin fuchsin stain for

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routine histomorphological observations, collagen fibers, and elastic fibers (Luna, 1968). Gridley's method (Sheehan and Hrapchak, 1973) for reticular fibers. Cameron's method of Aldehyde fuchsin staining (Cameron and Steele, 1959) for neurosecretory material. The micrometry was performed with the help of microscopic image analysis software.

RESULTS AND DISCUSSION

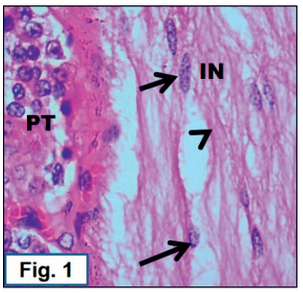
The pituitary gland was covered by a connective tissue capsule of the dura mater and consisted of two parts viz., adenohypophysis and neurohypophysis. The neurohypophysis formed caudo-dorsal part of the pituitary gland and mainly constituted by infundibulum and pars nervosa.

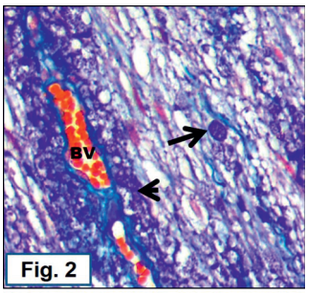
The infundibular stalk was a narrow and elongated, rostro-dorsal extension of neurohypophysis which connected the pars nervosa to the ventral aspect of the hypothalamus at tuber cinerium/median eminence. The rostral part of the infundibulum was surrounded by the pars tuberalis (Fig. 1). The infundibulum mainly consisted of the parallelly arranged axon bundles and these bundles were surrounded by blue-colored diffuse neurosecretory material. A few small Herring bodies were also observed (Fig. 2). The cells of neurohypophysis were neuroglia-like cells called as pituicytes, in infundibulum. They were fewer in number as compared to pars nervosa and interposed between axon bundles. The collagen fibers, reticular fibers, and blood capillaries were also observed between the axon bundles (Fig. 3 & 4). A thick layer of collagen fibers and reticular fibers interposed between the infundibulum and pars intermedia. The elastic fibers were not observed in the infundibulum. There were no distinct histological seasonal variations observed in the infundibulum.

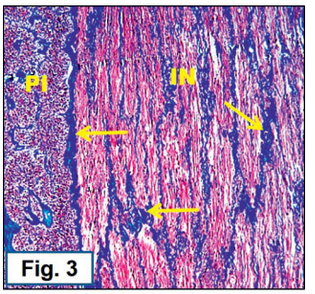
The caudal terminal part of the infundibulum expanded to form the pars nervosa. The pars nervosa was separated from pars distalis by the pars intermedia. The Pars intermedia was interdigitated into the caudal most part of pars nervosa and covers the caudo-ventral part of pars nervosa (Fig. 5 & 6). Pars nervosa showed an irregular arrangement of the non-myelinated axonal fibers. The nucleus of pituicytes exhibited a wide variation in shape and size. These cells were categorized as cells with a large rounded nucleus, cells with a thin elongated nucleus, cells with a large oval nucleus and cells with small round and dark nuclei (Fig. 7). The neuronal cells were not observed in the neurohypophysis. The cellular density of pituicytes in pars nervosa was 1506.00 ± 54.73, 1769.50 ± 76.13 and 1650.17 ± 55.63 cell/mm^2, respectively during winter, summer and rainy seasons. The data showed non-significant increase in number of pituicytes during the summer season. However, Alim *et al.* (2012), observed a significantly higher number of pituicytes in the nervous lobe of camels during the summer in comparison to winter.

The pars nervosa showed the presence of collagen fibers, reticular fibers and blood capillaries (Fig. 8 & 9). The collagen and reticular fibers were primarily observed around the blood capillaries and were less abundant than the infundibulum. Aldehyde-fuchsin stain demonstrated blue-colored neurosecretory material, distributed diffusely along the axonal bundles and blood vessels. In addition to this diffuse neurosecretory material, membrane-bound, round or oval-shaped vesicle-like structures (Herring body) were also observed (Fig. 9). The occurrence of Herring bodies was more common in pars nervosa than in the infundibulum. Present findings were corroborated well with the findings of Pathak and Bansal (2017) in buffalo and Paramasivan and Ramesh (2016) in Madras red sheep, who reported the Herring bodies in both infundibulum and pars nervosa. However, Ye *et al.* (2012) reported the Herring bodies, only in pars nervosa of Bactrian camel. In the present study, the diameter of Herring bodies ranged from 8 µm to 15 µm in different parts of the neurohypophysis. The mean (±SE) diameters of Herring bodies were 9.28 ± 0.40 µm, 10.66 ± 0.27 µm and 12.73 ± 0.73 µm, respectively during winter, summer and rainy seasons. During winter the average diameter of the Herring bodies were the least which increased gradually during summer and the rainy season. However, this increase in the diameters of Herring's body, was statistically significant ($P < 0.05$) only during the rainy season as compared to winter and summer. In an earlier study, Paramasivan and Ramesh (2016) reported the greater diameter of Herring bodies (12 µm to 200 µm) in Madras red sheep and they stated that the increase in number and size of Herring bodies indicated the release of higher concentration of hormones.

In conclusion, the neurohypophysis of Jaffarabadi buffalo was composed of two main parts: the pars nervosa and the infundibulum. Both parts were mainly composed of axon bundles and pituicytes. In







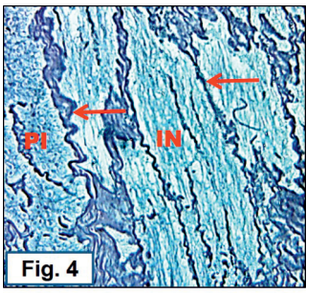
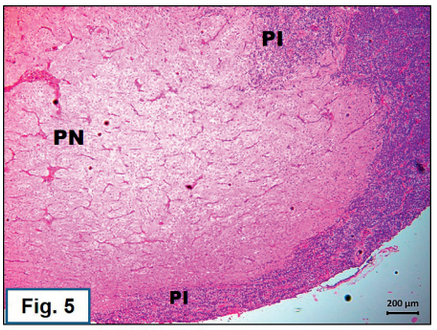
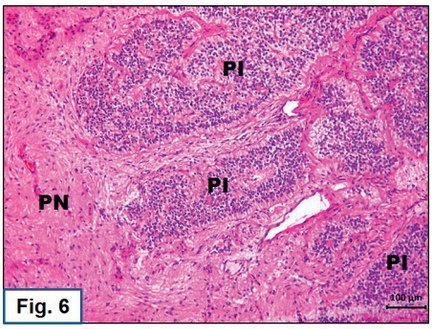


Fig. 1: Photomicrograph showing infundibulum (IN) and pars truberalis (PT) at the rostral part of the pituitary, pituicytes (arrow), and axonal bundles (arrowhead). Summer, H & E X 400; **Fig. 2:** Photomicrograph of infundibulum, showing blood vessel (BV), diffuse neurosecretory material (arrowhead), Herring bodies (arrow). Winter, Cameron's method, X 400; **Fig. 3:** Showing collagen fibers (arrow) in infundibulum (IN) and pars intermedia (PI). Winter. Masson's trichrome X 40; **Fig. 4:** Showing reticular fibers (arrow) in pars nervosa (PN) & pars intermedia (PI). Winter. Gridley's method X 100.





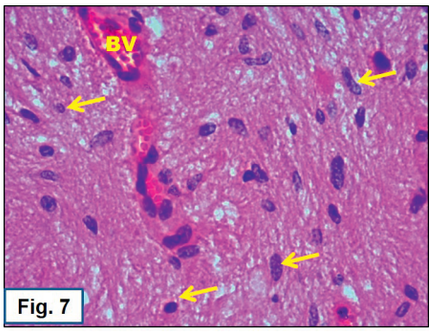
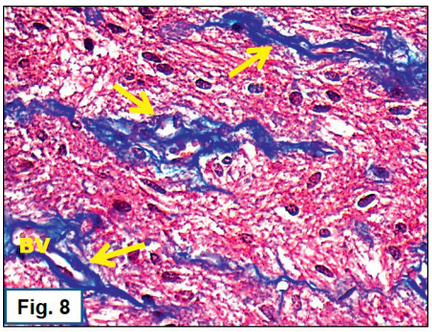
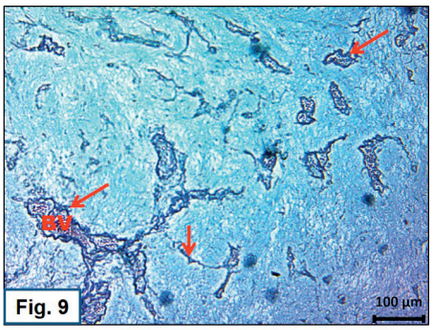


Fig. 5: Photomicrograph of pars nervosa (PN), pars intermedia (PI) covers the caudo-ventral aspect of pars nervosa. Winter. H & E X 40; **Fig. 6:** Pars intermedia (PI), interdigitate into pars nervosa (PN). Winter. H & E X 40; **Fig. 7:** Photomicrograph of pars nervosa showing blood vessels (BV) & different types of pituicytes (arrow). Rainy, H & E X 400





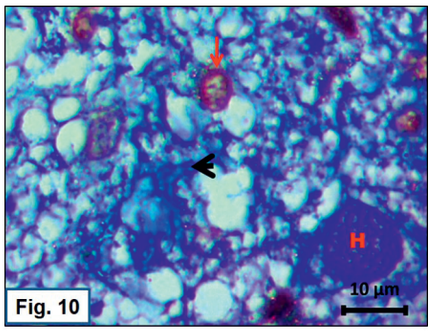


Fig. 8: Pars nervosa showing collagen fibers (arrow) mainly surrounding the blood capillaries (BV). Summer. Masson's trichrome X 400; **Fig. 9:** Pars nervosa showing reticular fibers (arrow) mainly surrounding the blood capillaries (BV). Winter. Gridley's X 400; **Fig. 10:** Pars nervosa showing Herring body (H), diffuse neurosecretory material (arrowhead) and nuclei of pituicytes (arrow). Winter. Cameron's method X 1000

addition to diffuse neurosecretory material along the axonal bundles, membrane-bound accumulations, the Herring bodies, were also observed. There were no distinct variations in the histoarchitecture of the neurohypophysis during different seasons, except in size of Herring bodies and cell (pituicytes) density. Herring bodies were smallest during winter and largest during the rainy season. The highest cell density was observed during the summer season as compared to the winter and rainy seasons. The seasonal changes in Herring body size and cellular density revealed, variation in the secretory activity of the neurohypophysis, which correlated with the functional activity of this part. These seasonal changes might be helpful in the adaptation of animals to variations in environmental conditions during different seasons.

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