Indian Journal of Animal Sciences **75** (7): 773-775, July 2005

Cytodifferentiation of FSH-and LH-gonadotrophs in pars distalis adenohypophysis cerebri of buffalo foetii

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Received: 10 October 2003; Accepted: 1 April 2005

ABSTRACT

The study was conducted on the hypophysis cerebri of 19 buffalo (*Bubalus bubalis*) foetii ranging from 30mm to 630mm in crown-rump length (CRL). The immunoreactive follicle stimulating hormone producing (FSH) cells were observed first time at 190mm CRL and were distributed throughout the lobe except in the lateral regions where the cells were rare. At 240mm CRL the cells were more in the rostro-dorsal region of the lobe. The cells were relatively more in number and were distributed throughout the lobe at 482mm stage. However, the density of the cells was relatively more near the cleft and cells were also seen along the blood vessels at this stage. At 630mm CRL strongly positive immunoreactive FSH cells were mostly observed singly and rarely in small groups of 2-3 cells. The immunoreactive luteinizing hormone-producing (LH) cells were first time noticed at 190mm CRL and were distributed singly throughout the lobe. The cells were mainly seen in the rostral region at 240 mm CRL. At 482mm CRL the LH cells were quite clear with strong immunoreactivity.

Key words: Adenohypophysis, Buffalo, Cytodifferentiation, Gonadotrophs, Immunohistochemistry, Pars distalis

Earlier, the cytodifferentiation of gonadotrophs in the developing foetal pars distalis had been reported in goat (Singh and Dhingra 1979), sheep (Thomas *et al.* 1993), pig (Danchin and Dubois 1982, Dacheux 1984) and camel (Marai *et al.* 1990). In buffalo, no such report was available and hence, the present study was undertaken to elucidate the same.

MATERIALS AND METHODS

Nineteen buffaloes (*Bubalus bubalis*) foetii ranging from 30mm to 630 mm in crown-rump length (CRL) were collected from the slaughter house. The hypophysis cerebri were fixed in Bouin's Holland sublimate (Humason 1972). The tissues were processed and sectioned at 5µm thickness which were stained with haematoxylin and eosin, periodic acid schiff (PAS) and alcian blue-PAS stainings (Luna 1968), luxol fast blue-erythrocin-orange G-aniline blue, Elftman's paraldehyde fuchsin-PAS orange G, Herlant's alcian blue-PAS-orange G and Masson's trichrome stainings (Girod 1976). The serial sections of 5µm thickness were stained for immunohistochemistry with Avidin Biotin method using rabbit-anti-human FSH serum (working dilution of 1:500 and incubation period of 4 hr at room temperature) and rabbit-anti-human LH serum (working dilution of 1:250, incubation period of 24 hr at 4°C and 4hr at room temperature).

RESULTS AND DISCUSSION

Follicle stimulating hormone producing (FSH) cells

The diffused blue granules in the cytoplasm (indicative of FSH cells) could be observed at 120mm stages in buffalo foetii (Fig. 1) with various stainings. The immunoreactive FSH cells were absent at 85mm CRL and sections were not suitable for results at 120mm CRL so these cells were confirmed first time at 185mm CRL buffalo foeti. These findings were not similar, as they first appeared at CRL 135mm in goat (Singh and Dhingra 1979), by day 100 in sheep (Thomas *et al.* 1993), between 45 and 60 days (Danchin and Dubois 1982) and on day 45 of gestation (Dacheux 1984) in pig, and at 24-28 weeks of gestation in camel (Marai *et al.* 1990).

The FSH cells at 185 and 190mm stage were observed as columnar cells, arranged in follicular forms with dispersed granules in the ventral part of the lobes (Fig. 2). At 240mm CRL the cells were more in the rostro-dorsal region of the lobe. The cells relatively increased in number throughout the lobe at 482mm CRL. The density of the immunoreactive cells was relatively more near the cleft and alongwith the blood vessels (Fig. 3). Occasional cells with a mixed tinge of

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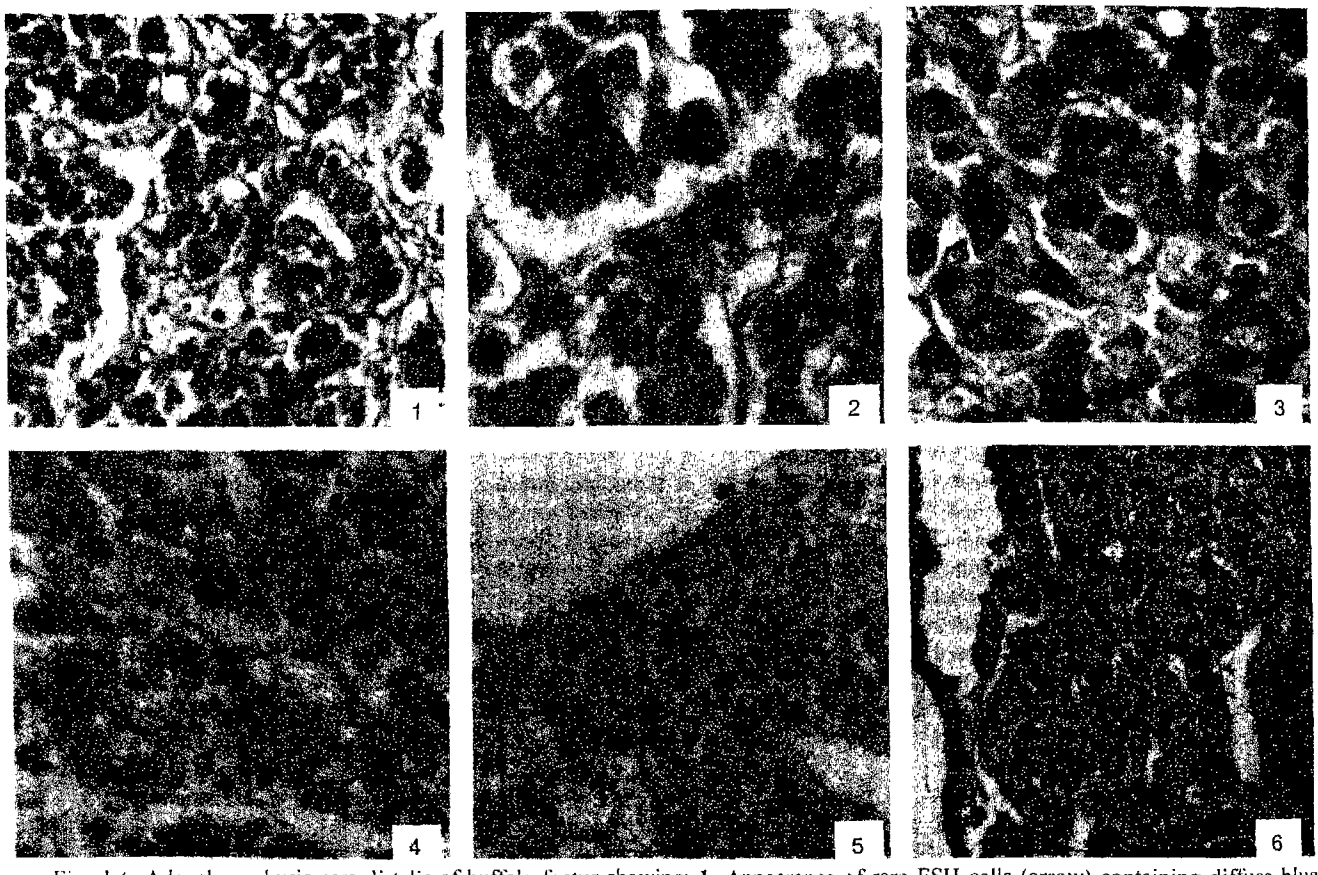


Figs 1-6. Adenohypophysis pars distalis of buffalo foetus showing; **1.** Appearance of rare FSH cells (arrow) containing diffuse blue granules at 120mm CRL, also seen in GH cells (Dark bluish green), Luxol fast blue–erythrocin–orange G–aniline blue stain × 700; **2.** Columnar type of cells arranged in follicular form containing diffuse blue granules (FSH cells) in the most ventral region of the lobe at 190 mm CRL, × 350; **3.** Immunoreactive FSH cells at 482mm CRL, avidin-biotin stain × 175; **4.** Frequent light blue cells in the central region of the lobe at 482 mm CRL, also seen is a cell with mixed granules (blue and erythrophilic), luxol fast blue-erythrocin-orange G-aniline blue stain × 700; **5.** PAS positive gonadotrophs in the central region of the lobe at 600mm CRL, Herlant's alcian blue–PAS-orange G stain × 700; **6.** Light blue FSH cells distributed throughout the gland at 600mm CRL, also seen are dark blue TSH cells and bluish green GH cells, Luxol fast blue–erythrocin–orange G–aniline blue stain × 700.

erythrophilia were identified singly or in small groups particularly in the ventral region and along the cleft (Fig. 4).

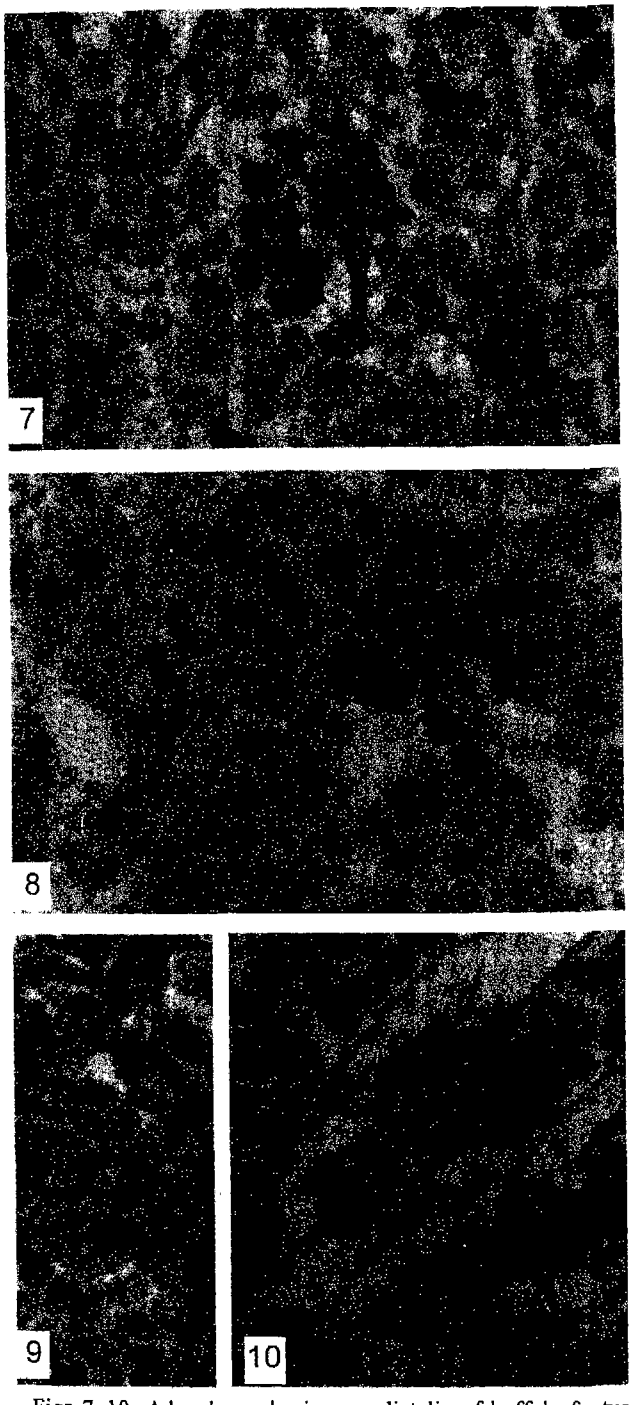
The PAS positive gonadotrophs appeared at 600mm stage in the zona tuberalis which were lacking in the pars tuberalis. The cells predominated dorsally nearer to the cleft (Fig. 5) and were relatively fewer in the central region. The PAS positive cells appeared in higher concentrations in the dorsal region than in the ventral towards caudally. At 600 and 630m stages with luxol fast blue-erythrocin-orange G method, cells with light blue dispersed granules (FSH cells) could be identified throughout the pars distalis (Fig. 6). At 630mm CRL strongly positive immunoreactive FSH cells were mostly observed singly and rarely in small groups of 2-3 cells.

Luteinizing hormone-producing (LH) cells

The immunoreactive LH cells were first time distributed in the lobe at 190mm CRL. However, with the differential staining techniques the LH cells were first identified as groups of 2-3 cells in a section at 482mm CR length in buffalo foetii (Fig. 7), which were not similar as reported at CRL 165mm in goats (Singh and Dhingra 1979), by day 70 in sheep (Thomas *et al.* 1993), between 45 and 60 days (Danchin and Dubois 1982) and day 40 of gestation in camel (Marai *et al.* 1990).

The LH cells were quite clear with strong immunoreactivity at 482 mm CRL (Fig. 8). The most characteristic features of this stage were the occurrence of 2-3 groups of cells that stained violent with luxol fast blue-erythrocin-orange G-aniline blue method representing the LH cells (Fig. 7). At 600mm stages, the violet coloured (LH) cells were seen distributed throughout in the section in countable numbers (about 13) of groups-viz. 1 in central region, 1 in ventral to the mid central, 1 in rostral to mid central, 4 in rostro-dorsal, 2 in the mid dorsal, 2 just caudal to the mid central, 4 in rostro-dorsal, 2 in the mid dorsal, 2 just caudal to the mid central region and 2 in the caudal regions (Fig. 9).

At 630mm stage, yellowish red cells (LH) dispersed individually or in groups in varying numbers were found in



Figs 7–10. Adenohypophysis pars distalis of buffalo foetus showing; **7.** A group of violet stained LH cells in the central region of the lobe at 482mm CRL, Luxol fast blue–erythrocin–orange G–aniline blue stain × 700; **8.** Immunoreactive FSH cells at 482 mm CRL, avidin-biotin stain × 350; **9.** A group of violet LH/ICSH cells seen in the central region at 600mm CRL, Luxol fast blue–erythrocin–orange G–aniline blue stain × 700; **10.** LH cells stained yellowish red dispersed individually and in groups at 630mm CRL, Aldehyde fuchsin–PAS Orange G stain × 700.

different regions of the pars distalis with aldehyde fuchsin-PAS-Orange G method (Fig. 10). The LH cells were found uniformly. However, this finding was not accordance in sheep as reported by Thomas *et al.* 1993.

ACKNOWLEDGEMENTS

We are grateful to Dr K S Roy, Professor cum Head of the Department, for providing the facilities and to Dr Jaswant Singh for his help in the immunohistochemical study. The awarding of antisera for immunohistochemical work, by Dr Terry Taylor, Director, NIDDK, USA in duly acknowledged.

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