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Seasonal histomorphology of Acidophils in the Hypophysis cerebri of Gaddi goats: A comparative study between the year 2000 and the present in the context of climate change

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ABSTRACT

The present study was conducted to investigate the seasonal histomorphological variations in acidophil cells of the pituitary gland in Gaddi goats, with reference to changing climatic conditions between (2000) and now. A total of 35 heads from adult, pluriparous, non-pregnant Gaddi does were procured from local slaughterhouses in the Palampur region of Himachal Pradesh during different seasons of the year. The pituitary gland, appeared as a light grey, oval or pyriform structure in the interpeduncular fossa. Histological analysis of the pars distalis adenohypophysis revealed significant seasonal differences ($P<0.05$) in the relative proportions of acidophils The acidophils were maximum in percentage during the monsoon season. These were further classified into somatotrophs (orangeophils) and lactotrophs (carminophils) using Crossman's modification of Mallory's trichome stain**,** both showed minimal to nil PAS reactivity. The average diameter of somatotrophs was largest in monsoon (11.4±0.20 µm) and smallest in winter (10.01±0.33 µm). Similarly, lactotrophs showed maximum diameter during monsoon (10.6±0.14 µm) and minimum in spring season (8.41±0.22 µm). Comparative evaluation with previous seasonal histomorphological studies revealed discernible alterations in the cellular dimensions of acidophils over time. These changes may reflect the influence of rising temperature and humidity over the past two decades, potentially affecting the spatial distribution and functional dynamics of pituitary acidophils in Gaddi goats, with implications on reproductive performance and productivity.

Keywords: Acidophils, Climate change, Gaddi goat, Histomorphology, Pituitary gland, Reproduction, Seasonal variation.

The hypophysis cerebri is a pivotal endocrine organ regulating essential physiological functions, including growth, metabolism, and reproduction (Ganong 2005). In goats, acidophil cells within the pars distalis predominantly secrete prolactin and growth hormone, and are known to be influenced by environmental factors such as temperature, humidity, and photoperiod (Sejian *et al*. 2010). These hormones exhibit seasonal secretion patterns, particularly in seasonally breeding animals like goats (Singh *et al.* 1991). The Gaddi goat, indigenous to Himachal Pradesh, is traditionally reared by the nomadic Gaddi community, and contributes significantly to their livelihood through milk, meat, fiber, and manure production (Pathak 2006). These goats undergo transhumance, migrating to alpine pastures in summer and returning to lower altitudes in winter. However, regional climatic shifts over the past two decades (2001 to 2023), characterized by rising temperatures and increased humidity, have increasingly disrupted this migration pattern (IPCC 2021). Reduced annual rainfall and declining snowfall are negatively affecting forage availability and increasing heat stress on the animals (Sejian *et al*. 2018). Environmental stressors can impair the hypothalamic-pituitary axis, resulting in hormonal imbalances and compromise reproductive performance (Sejian *et al.* 2013). Earlier histological studies confirm that the pituitary gland undergoes marked seasonal morphological adaptations,

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notably in the distribution of acidophils, basophils, and chromophobes (Prasad and Singh., 1980). Despite their critical importance, detailed seasonal histomorphological assessments of the hypophysis cerebri in context to climate change in Gaddi goats remain scarce. This study aims to address this gap by offering a detailed evaluation of the seasonal histomorphological and histochemical alterations in acidophil cells of the pituitary gland in Gaddi goats.

MATERIALS AND METHODS

Pituitary glands from 35 heads (seven heads per season) of apparently healthy, adult (around 2.5-4.0 years of age) cyclic, non-pregnant, pleuriparous Gaddi goats were collected from the slaughter houses around the Palampur area. Meteorological data such as relative temperature and humidity were collected from department of Agronomy, CSKHPKV, Palampur from years 2001-2023 to assess climatic trends and changes. The age, regular body structure, and state of health of the animal were noted before the slaughter of animal. The age was calculated using the Noden and de Lahunta (1985) established dental pattern. The common carotid arteries on both sides of each head were flushed with a 25% sodium citrate solution to remove any remaining blood clots. Following that, each head was individually perfused with fixative 10% neutral buffered formalin for histological and histochemical examination. Tissues were processed using standard paraffin embedding technique (Luna, 1968). Serial sections of 5 µm thickness were prepared and stained with routine Harris' hematoxylin and eosin (Luna, 1968) to examine the general histoarchitecture and identify cell types in the pars distalis adenohypophysis. Additionally, Crossman's modified Mallory's trichrome stain was employed to highlight connective tissue and distinguish acidophils into somatotrophs (orangeophils) and lactotrophs (carminophils) (Crossman, 1937). Periodic Acid-Schiff (PAS) stain was used to assess carbohydrate content and reactivity (Mallory, 1961). Samples were serially sectioned from end to end for the percentage of various cell types, as well as their relative distribution. For quantitative cytoanalysis, the sagittal sections of the adenohypophysis were divided into six regions: rostro-dorsal, rostro-ventral, mid-dorsal, mid-ventral, caudo-dorsal, and caudo-ventral portions. .The transverse sections were divided as dorsoperipheral, mid-peripheral, ventro-peripheral, dorso-medial, mid-medial, and ventro-medial. The somatotrophs and lactotrophs were counted in sagittal and transverse section of hypophysis cerebri using haematoxylin and eosin stained sections. Maximum cellular and nuclear diameters of each cellular subtype were recorded season-wise using a compound microscope with an ocular micrometer. In each sample, 12 cells of each subtype were used to calculate the average diameter. Seasonal values were compared statistically using ANOVA followed by the Tukey-Krammer multiple comparison test (using the INSTAT-Graph PAD software) and results with $P < 0.05$ were considered significant. Results were comparatively analysed with previous seasonal histomorphpological studies and correlated with the relative increase in temperature and humidity (from years 2001-2023) to identify the possible effects of climate change on reproduction potential and overall productivity of does.

RESULTS AND DISCUSSION

Histomorphological assessment of the pituitary gland in Gaddi goats revealed polygonal, oval, or round acidophil cells, with eccentrically placed vesicular nuclei distributed either singly or in clusters along the periphery of parenchymal cords (Figure 1). These cells exhibited strong affinity for acid dyes (Figures 1 & 2) and were PAS negative (Figure 5). The amount of granules in cytoplasm greatly influenced their staining intensity. These findings are in agreement with previous observations in sheep and goats reported by Singh and Dhingra (1991), Paramsivan (2000) and Pathak (2001). Acidophils constituted 42–48% of the anterior pituitary population, with the highest mean percentage observed in the monsoon (48.4 ± 1.97%) and the lowest in spring (42.0 ± 1.79%), (Table-1). These seasonal fluctuations in the percentage and distribution of acidophils were statistically significant and reflect the findings of Paramsivan (2000) and Pathak (2001), although their seasonal peaks were reported in summer and autumn, respectively. The altered peak in the monsoon in the present study may indicate a shift in endocrine responsiveness due to climate-induced alterations in environmental factors such as average annual rainfall pattern, day length, and forage

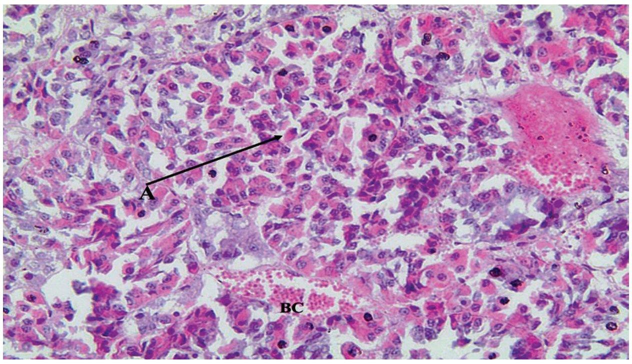

Fig. 1. Pars distalis adenohypophysis showing acidophils (A) and blood capillaries (BC). Pituitary H & E × 200.

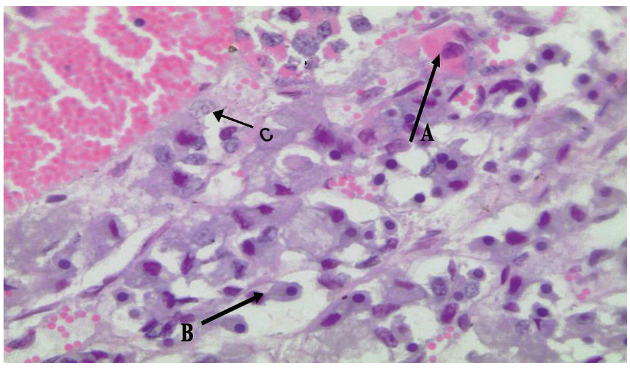

Fig. 2. Acidophils (A), Basophils (B) and Chromophobes (C) in ventral basophillic zone of the pars distalis adenohypophysis. Pituitary. H&E X 400.

growth cycles. These findings are particularly relevant in the context of climate sensitivity, impairing hormonal balance, reproduction and adaptability in livestock. Seasonal variations also affect their distinctive granulation pattern and staining intensity. In the monsoon, acidophils had larger, coarser granules packed more tightly, but in the spring season, they only possessed finer granulation pattern. Jubb and McEntee (1955) explained this variation as a result of the cells' varying cellular activities. Differential staining using Crossman's modification of Mallory's trichome method revealed two distinct acidophil subtypes: somatotrophs (orangeophils) and lactotrophs (carminophils). This histochemical classification is consistent with the descriptions provided by Pathak (2001) in Gaddi doe and Paramasivan and Sharma (2002) in Gaddi sheep and enables further functional correlation with hormonal secretion patterns modulated by seasonal and climatic factors.

Somatotrophs/growth hormone secreting cells: Somatotrophs, which secrete growth hormone (GH), were mainly seen at the periphery of parenchymal cords as large, oval or polygonal cells with darkly stained cytoplasm. With Crossman's modification of Mallory's trichome, they appeared as orangeophils with orange granules, light chromatin and an eccentrically placed round nucleus with a clear nucleolus (Figure 3). These findings were in agreement with Paramsivan (2000) in Gaddi sheep and Pathak (2001) in Gaddi does whereas in contrast to the present findings Nishimura *et al.* (1998) observed these cells as medium- or small-sized, oval, cylindrical, or polygonal in shape in Tokara goat adenohypophysis. In the current study, somatotrophs constituted between 30% and 36% of the acidophil population, with their maximum proportion during spring (36.85 ± 0.51%) and lowest in winter season (30.1 ± 0.41%), (Table 1). In contrast to the present study, Paramsivan (2000) in Gaddi sheep reported lowest population of these cells during winter season and maximum during summer season while Pathak (2001) in Gaddi doe documented it to be lowest during summer season and maximum during autumn season. This divergence suggests a temporal shift in somatotropic activity, likely driven by cumulative climatic changes over the past two decades. This peak in somatotrophs during spring season may be attributed to early onset of forage regeneration and improved nutritional conditions, promoting GH synthesis to support growth and metabolic demands. When compared to other seasons of the year, the difference in mean cell percentage was statistically significant (Table 1). The average diameter of somatotrophs peaked in the monsoon (11.4 ± 0.20 μm) and was lowest in winter (10.01 ± 0.33 μm), indicating seasonal variation in secretory activity. This enlargement during monsoon likely reflects increased growth hormone production in response to better nutrition and favorable environmental conditions. Similar seasonal hypertrophy in buffalo and Daddi sheep somatotrophs was reported by Khan *et al.* (1998) and (Paramsivan 2000) supporting the idea that endocrine cell size reflects functional demand. However, Pathak (2001) in Gaddi doe reported the average cell diameter of somatotrophs was highest in autumn season and lowest in summer and monsoon season. The seasonal difference in cell diameter was significant statistically (Table 1). The nuclear diameter of somatotrophs also demonstrated seasonal modulation, increasing from (3.84 ± 0.13 μm) in winter to (4.92 ± 0.07 μm) in summer (Table 1). This shift contrasts with the previous study of Pathak (2001) who reported nuclear diameter to be smallest in summer and largest in spring in Gaddi doe. This difference may suggest that the animals are now better adapted to summer heat, possibly due to gradual thermal acclimatization or altered heat stress thresholds.

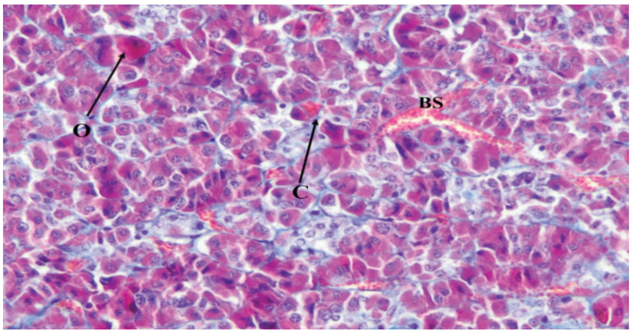

Fig. 3. Pars distalis adenohypophysis showing carminophils (C) as Lactotrophs, orangeophils (O) as Somatotrophs and blood sinusoids. Pars distalis adenohypophysis Crossman's modification of Mallory's trichome X 200.

Table 1. Seasonal variation in differential acidophil cell count, cellular diameter, and nuclear diameter of STH-ocytes and LTH -ocytes in the pars distalis adenohypophysis of Gaddi goats (mean ± SE, n = 7).

Seasons	Somatotrophs (%)	lactotrophs (%)	Somatotrophs Diameter (μm)	lactotrophs Diameter (μm)	Somatotrophs Nuclear Diameter (μm)	lactotrophs Nuclear Diameter (μm)
Winter	$30.1±0.41^{a}$	$69.1±1.06^{c}$	$10.01±0.33^{a}$	$8.54±0.28^{ab}$	$3.84±0.13^{a}$	$4.77±0.09^{c}$
Spring	$36.85±0.51^{c}$	$63.2±0.61^{a}$	$10.6±0.21^{ab}$	$8.41±0.25^{a}$	$4.18±0.09^{ab}$	$4.11±0.09^{a}$
Summer	$30.9±0.34^{a}$	$69.9±0.73^{c}$	$10.3±0.32^{a}$	$9.41±0.17^{c}$	$4.92±0.07^{d}$	$4.67±0.09^{bc}$
Monsoon	$33.9±0.43^{b}$	$65.1±0.75^{ab}$	$11.4±0.20^{b}$	$10.6±0.14^{d}$	$4.84±0.05^{cd}$	$4.92±0.06^{c}$
Autumn	$33.7±0.62^{b}$	$66.3±0.83^{b}$	$10.5±0.12^{ab}$	$9.25±0.11^{bc}$	$4.52±0.07^{bc}$	$4.47±0.06^{ab}$

Note: *Values are presented as mean ± SE (n = 7). Superscripts within a column (^{a}, ^{b}, ^{c}, ^{d}) indicate statistically significant differences between seasonal means at $P < 0.05$. Values sharing at least one common superscript do not differ significantly.*

Lactotrophs/LTHs/Prolactin (PRL) secreting cells: Lactotrophs were oval and asymmetrically polygonal with round or oval nuclei eccentrically positioned within the cytoplasm (Figures 3 and 4). These cells were infrequently seen in cords or clusters and were dispersed singly over the pars distalis adenohypophysis. This was in agreement with the findings of Paramsivan (2000) in Gaddi sheep and Raja *et al*. (2021) in buffaloes. Crossman's modification of Mallory's trichome stain rendered lactotrophs as red-colored cells (carminophils). While Paramasivan (2000) in Gaddi sheep and Pathak (2001) in Gaddi doe reported PAS-negative staining of lactotrophs. Quantitative analysis revealed significant seasonal variation in distribution and morphology of lactotrophs. The highest mean was observed in summer (69.9±0.73%), and the lowest in spring (63.2±0.61%). This aligns with the increase in plasma prolactin levels during long photoperiods, as described by Misztal et al. (1997) in ewes. Paramsivan (2000) in Gaddi sheep and Pathak (2001) in Gaddi doe also observed increased summer lactotrophs populations correlated with increased daylight and metabolic demand for lactogenesis, but reported lowest values during winter unlike the present study which reported minimum during spring season. This shift may reflect adaptive responses of pituitary acidophils to seasonal changes in forage availability, photoperiod, and temperature under local environmental conditions. Seasonal variations in the proportion of lactotrophs were statistically significant (Table-1). Morphometric analyses indicated that the cellular diameter of lactotrophs varied significantly across seasons (Table 1), ranging from 8.41 ± 0.22 µm in spring to 10.6 ± 0.14 µm in monsoon. Nuclear diameters also exhibited significant seasonal variation, (Table 1), with values ranging lowest (4.11 ± 0.09 µm) in spring to maximum (4.92 ± 0.06 µm) in monsoon. In contrast to the present study, Paramsivan (2000) in Gaddi sheep and Pathak (2001) in Gaddi doe recorded largest diameter of lactotrophs in summer season and smallest in winter and autumn season, respectively. Gebbie *et al.* (1999) also reported that the prolactin drops with cooler temperatures, even when days are long. These results also imply that cooler conditions suppress prolactin secretion even when day length is long.

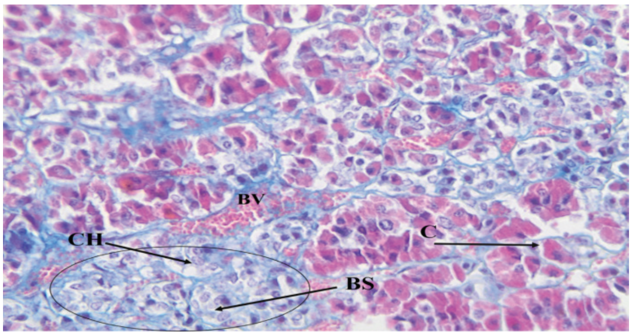

Fig. 4. A basophilic zone showing carminophils (C) as Lactotrophs, chromophobes (CH), basophils (BS) and blood vessels (BV) in pars distalis adenohypopnysis. Pars distalis adenohypophysis Crossman's modification of Mallory's trichome X 200.

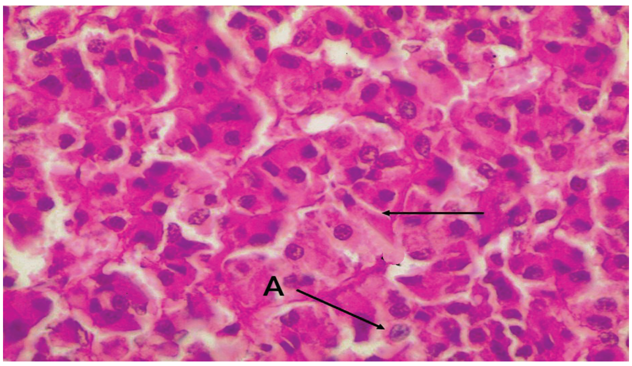

Fig. 5. Pars distalis adenohypophysis showing PAS positive colloid of basophils (Arrow) in the acini and PAS negative acidophil (A). Pars distalis adenohypophysis. PAS X 400

The present study demonstrates significant seasonal variation in the histomorphology and histochemistry of acidophil cells in the anterior pituitary of Gaddi goats, particularly in somatotrophs and lactotrophs. Observed shifts in their distribution and cellular dimensions suggest altered endocrine activity in response to changing environmental factors like relative increase in temperature and humidity from past 23 years. As compared to earlier quantitative studies related to present study the seasonal peaks of both cell types have shifted, indicating possible climate-induced modulation of pituitary functions over the past two decades.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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