Uttar Pradesh Journal of Zoology

Volume 45, Issue 18, Page 62-70, 2024; Article no.UPJOZ.3995
ISSN: 0256-971X (P)

Comparative Anatomical Studies of Adrenal Glands in Large White Yorkshire Pigs Reared at Low and High Altitudes in Kerala

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i184424

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3995

Original Research Article

Received: 28/06/2024
Accepted: 30/08/2024
Published: 02/09/2024

ABSTRACT

The adrenal gland plays a crucial role in the body's homeostasis and during the stress response. However, less attention was given to the structural variation of the adrenal glands in pigs reared at different altitudes. Hence, the present study was conducted to correlate structural variation in the

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Cite as: *S., Patgiri, Rajani, C.V., Surjith, K.P., Patki, H.S., Leena, C., and Indu V.R. 2024. "Comparative Anatomical Studies of Adrenal Glands in Large White Yorkshire Pigs Reared at Low and High Altitudes in Kerala". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (18):62-70. https://doi.org/10.56557/upjoz/2024/v45i184424.*

adrenal glands in 24 ten-month-old Large White Yorkshire pigs of either sex reared at low-altitude and high-altitude regions of Kerala, India. Microscopically, the adrenal gland presented the three typical concentric cortical zones*:* zona glomerulosa, zona fasciculata, zona reticularis, and a central medulla. The arrangement pattern of cells of the zona glomerulosa resembled the arcs; zona fasciculata, similar to cords separated by intervening trabeculae; and zone reticularis, like irregularly anastomosing cords. Two types of cells were present in the medulla: large, granular, intensely-stained cells towards the periphery and small polyhedral cells towards the centre of the medulla. Chromaffin was identified in the medulla, while undifferentiated cells were identified within the capsule, subcapsular zone, the transitional zone between zona glomerulosa and zona fasciculata, and even medulla. Zona fasciculata formed the largest zone of the adrenal cortex, and its thickness was significantly higher in the low-altitude male and female groups than in the corresponding high-altitude groups, which was correlated to serum cortisol levels. The comparative micrometrical data revealed a larger zona fasciculata in the low-altitude male and female groups than in the corresponding high-altitude groups, indicating variation between the groups.

Keywords: Altitude; histology; pig; undifferentiated cells.

1. INTRODUCTION

The pig industry has emerged as one of India's fastest-growing divisions of the agricultural sector. Production depends on physiological and environmental conditions, and pigs are very sensitive to high ambient temperatures. The adrenal gland comprises an outer cortex subdivided into three zones: zona glomerulosa, zona fasciculata, and zona reticularis. Zona glomerulosa produces mineralocorticoids; zona fasciculata produces glucocorticoids, predominantly cortisol and androgens; and zona reticularis produces adrenal androgens and glucocorticoids. The inner adrenal medulla produces the stress hormones: epinephrine and norepinephrine [1]. The hormones produced by the adrenal glands play a crucial role in the body's homeostasis, thereby adapting to the environment and for production [2]. Although a few studies reported the structure of adrenal glands in pigs [3-5], the information available is limited. Studies reported the occurrence of constant remodelling of the adrenal gland in the postnatal period in humans, rats, and mice [6-9]. Further, earlier literature reported alteration in the adrenal gland function in high-altitude in humans and rats and during thermal stress in goats [10-12].

However, a detailed investigation of the microscopic structure of the adrenal gland and its structural variation between pigs reared at different altitudes is very limited. Similarly, reports on the postnatal remodelling of the gland in pigs are lacking. Hence, the present study was undertaken with the following objectives: whether any structural variation exists in the adrenal glands of Large White Yorkshire pigs reared in low-altitude and high-altitude regions of Kerala and whether any structural alterations occur postnatally in Large White Yorkshire pigs.

2. MATERIALS AND METHODS

The samples were collected from 24 ten-month-old Large White Yorkshire pigs from the slaughterhouses located in the low-altitude (Meat plant, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala elevation 2.83 m) and in the high-altitude (Sulthan Bathery, Wayanad, Kerala elevation 930 m) areas. Animals were divided into four groups, *viz*., low-altitude male group, low-altitude female group, high-altitude male group, and high-altitude female group; each group comprised six animals. Histomorphological features of the adrenal gland and serum cortisol levels in Large White Yorkshire pigs reared at low-altitude and high-altitude regions of Kerala were studied.

2.1 Processing of Tissues for Histological Studies

Representative tissue samples from the cranial, middle, and caudal ends of both right and left adrenal glands were collected and preserved in 10 percent Neutral Buffered Formalin for histological studies. Following proper fixation, tissue samples were processed for paraffin embedding, and paraffin sections of 5 µm thickness were prepared using a rotary microtome. The sections were then stained by Haematoxylin and Eosin (H and E) staining technique for routine histological studies; Gomori's rapid one-step trichrome method for collagen fibres [13] and Schmorl's reactions for Chromaffin cells [14]. The stained sections were examined under a binocular light microscope (Model- Olympus CX21*i*LED), and photomicrographs were taken using an attached camera (Model- MagcamDC10). The micrometric parameters, *viz*., the total thickness of the adrenal cortex and medulla and the thickness of various layers of the adrenal cortex, were recorded using the Magvision software.

2.2 Processing of the Samples for the Scanning Electron Microscopy

Scanning electron microscopic (SEM) studies were conducted on the representative tissue samples of the adrenal glands of males and females at low and high altitudes. Specimens were fixed in 2.5 percent glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 hours at 4 ºC. Later, the samples were dehydrated in ascending grades of ethanol. Subsequently, the samples were dried before the sputter coating process. The dried samples were mounted over the stubs with double-sided carbon conductivity tape, and a thin layer of gold coat was applied by using an automated sputter-coater (Model- QUORUM SC7620) for twenty minutes and scanned under SEM (Model- TESCAN VEGA-3-LMU) at a magnification of ×150 up to × 5000 as per the standard procedures.

2.3 Processing of the Samples for the Hormonal Assay

Prior to slaughter, the blood samples were collected from the ear vein. The serum was separated and stored at -20 ºC for hormonal assay. Serum cortisol was estimated by radioimmunoassay, and the procedure employed 50 µL of each standard, sample, or control in separate antibody-coated tubes. Later, 500 µL of I125-Cortisol (tracer) was added, and the tubes were incubated for one hour at 18-25 ºC with shaking (≥ 400 rpm). The contents of the tubes were aspirated carefully to estimate radioactivity in a gamma counter (Model- WIZARD 2470-0010, S/N: 3130261, SW 2.2). A standard curve was prepared using six serum standards ranging from (0-2350) nmol/L of cortisol. Unknown values were calculated from the standard curve by interpolation.

2.4 Statistical Analysis

The data were analysed statistically using SPSS software (version 24.0). An Independent t-test was performed to determine the correlation between the parameters of adrenal's micrometric and serum cortisol concentration with sex and altitude.

3. RESULTS

3.1 Histoarchitecture of the Adrenal Gland

The transverse and longitudinal sections of the adrenal gland presented an outer cortex or inter-renal tissue and an inner medulla or chromaffin tissue. A dense, distinct, fibrous connective tissue capsule surrounded the gland (Fig. 1A-1C). The outer zone of the capsule enclosed numerous large-sized blood vessels and was more loosely arranged than its inner zone. The inner zone of the capsule presented very thick trabeculae enclosing blood vessels, which extended at right angles from the capsule at regular intervals into the cortex traversing towards the medulla (Fig. 1A-1C). The trabeculae were chiefly composed of collagen fibres and were indistinct beyond the outer region of the zona fasciculata.

The cortex constituted a significant part of the parenchyma of the gland. It revealed three concentric regions *viz.,* zona glomerulosa or zona arcuata, zona fasciculata, and zona reticularis from the periphery towards the centre. Four to five layers of darkly stained cells enclosing irregular nuclei in the subcapsular area constituted the zona arcuata. The zone enclosed numerous sinusoidal capillaries, evident as vacuoles in the scanning electron microscopy (Fig. 1A, 1C). The zone cells were columnar and arranged on the basement membrane and resembled inverted U-shaped arches or curves to the capsule (Fig. 1A, 1B). The cytoplasm of those cells stained less intensely to basic stains. The zona fasciculata consisted of radially arranged cords of cuboidal cells separated by intervening sinusoids (Fig. 1D, 2A). The cords of this zone were usually one cell layer thick, and cells were often binucleate (Fig. 1D). The cells appeared to enclose cytoplasmic vacuoles, as the lipid secretions within the vacuoles were lost during the fixation and processing. Therefore, the cells presented a foamy appearance and were termed spongiocytes. The zona fasciculata cells contained more vesicular and achromatic nuclei than the other zones. Zona reticularis consisted of polyhedral cells arranged in an irregular network of anastomosing cords (Fig. 2B, 2C). Further, the cortical cells were demarcated from the medullary cells at the cortico-medullary junction.

The medulla was subdivided into two distinct portions: outer and inner zones (Fig. 2D, 3A). Large granular, intensely stained cells with highly basophilic cytoplasm in follicular arrangement constituted the outer zone. However, the cells in the inner zone were small polyhedral cells, less granular, and were compactly arranged (Fig. 2D). The medulla was identified distinctly from its fluffy appearance of cells in scanning electron micrographs (Fig. 3A). Groups of nerve bundles, along with the nerve fibres, was also observed in the medulla. The centre of the medulla presented a large central vein, and its endothelial cells were surrounded by a few bundles of connective tissue fibres (Fig. 2D, 3A) and sinusoidal vessels of variable sizes. (Fig. 3B). Schmorl's technique identified chromaffin as blue-coloured pigments in the cytoplasm of the cells (Fig. 3B).

The study also revealed a few trabeculae, which enclosed blood vessels extending interior even up to the medulla. Adjacent to the invading trabeculae presented the less differentiated zona glomerulosa and its transition to the other cortical zones in a radiating manner in the same order (Fig. 1B). All the three cortical zones mentioned above were depicted as concentric zones encircling the cross sections of trabeculae in the inner cortex (Fig. 3C). The boundary between the deeper part of the capsule and the zona glomerulosa layer revealed a group of relatively undifferentiated 'capsular' cells (Fig. 3D). The subcapsular area presented a cluster of undifferentiated parenchymal cells that resembled the cells of zona fasciculata and the cells of the inner medulla (Fig. 3D). Further, a cluster of lightly stained undifferentiated cells enclosing some secretions was observed at the transitional or intermediary zone, junction of zona glomerulosa and zona fasciculata (Fig. 4A). In addition, the medulla presented undifferentiated or less differentiated cells that resembled the cortical zones (Fig. 4B).

The comparative micrometrical data of both adrenal glands in the low-altitude male and female groups to the corresponding high-altitude male and female groups were summarised (Fig. 5A, 5B).

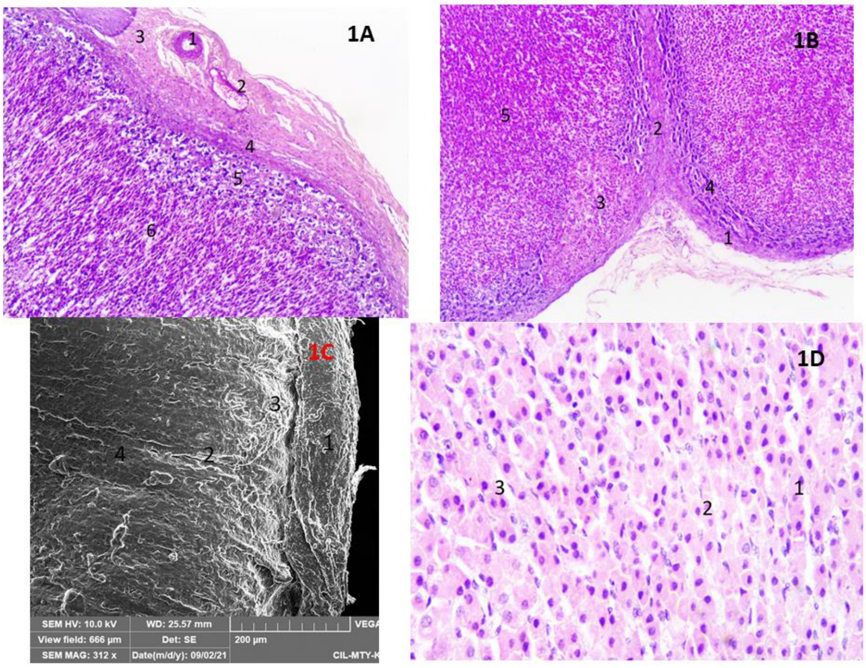

Fig. 1. 1A. Photomicrograph of transverse section of adrenal, male at high altitude: 1-Blood vessel (Cross section), 2-Blood vessel (Longitudinal section), 3- Outer capsule, 4-Inner capsule, 5-Zona glomerulosa, 6-Zona fasciculata H & E × 40. 1B. Photomicrograph of transverse section of adrenal, female at low altitude: 1-capsule, 2- Trabecula, 3- Cluster of undifferentiated cells, 4- Zona glomerulosa, 5- Zona fasciculata H & E × 40. 1C. SEM of Adrenal gland: 1- Capsule, 2- Trabeculae, 3- Zona glomerulosa, 4- Zona fasciculata × 312. 1D. Photomicrograph of zona fasciculata layer of the adrenal cortex: 1- One cell thick cord, 2- Cuboidal cells, 3-Binucleate cells H & E × 100

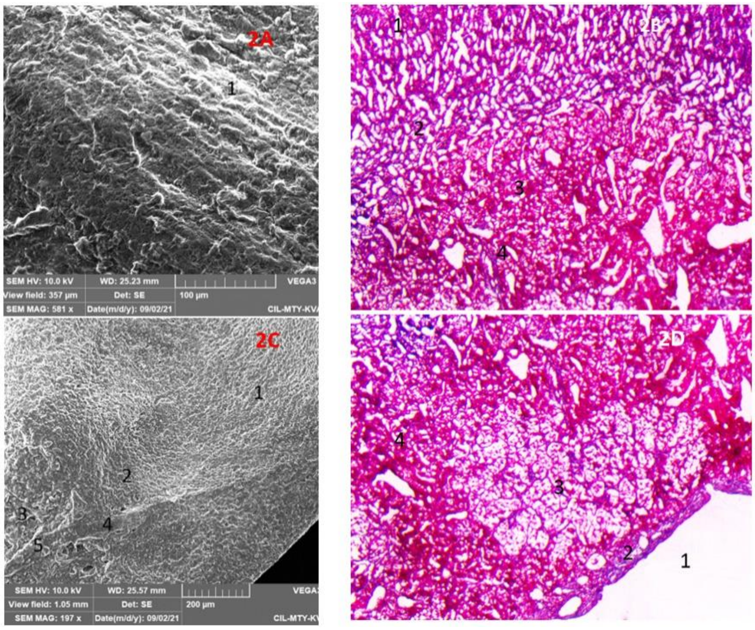

Fig. 2. 2A. SEM of Adrenal gland: 1- Cord-like arrangement of Zona fasciculata × 581. 2B. Photomicrograph of adrenal showing cortico-medullary junction: 1- Zona fasciculata, 2- Zona reticularis, 3- Outer medulla, 4- Blood capillary Gomori's one step Trichrome method × 100. 2C. SEM of Adrenal gland: 1- Zona fasciculata, 2- Zona reticularis, Trabeculae, 3- Medulla, 4- Large blood vessel, 5- Branch of blood vessel × 197. 2D. Photomicrograph of adrenal showing medulla and central vein: 1- Central vein, 2- Endothelial lining, 3-Inner medulla, 4- Outer medulla Gomori's one step Trichrome method × 100

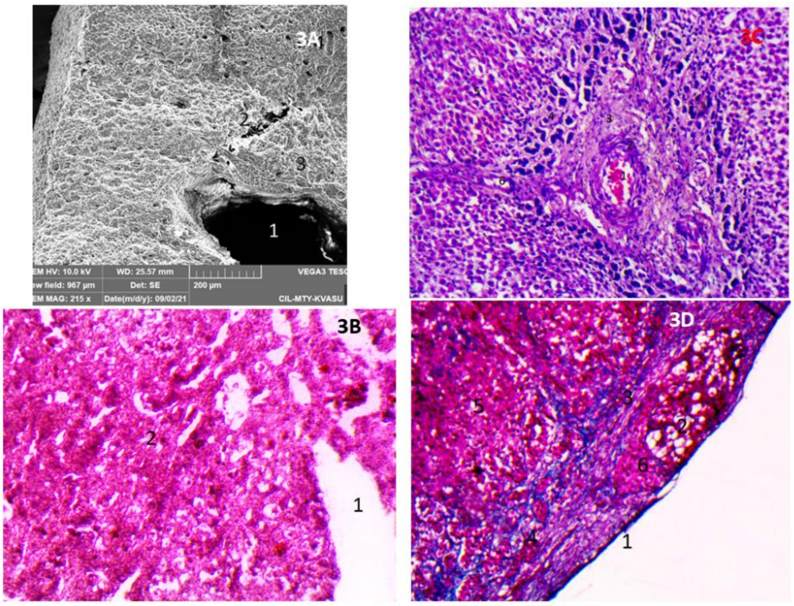

Fig. 3. 3A. SEM of Adrenal gland: 1- Central vein, 2- Blood capillary, 3- Inner medulla, 4- Outer medulla × 215. 3B. Photomicrograph of adrenal medulla: 1- Central vein, 2- Chromaffin Schmorl's reaction for chromaffin × 100. 3C. Photomicrograph of zona fasciculata: 1- Blood vessel, 2- Connective tissue, 3- Zona glomerulosa, 4- Zona fasciculata, 5- Blood vessel (Tangential section) H & E × 100. 3D. Photomicrograph adrenal cortex: 1- Capsule, 2- Cluster of Undifferentiated cells, 3- Sub-capsular connective tissue, 4- Zona glomerulosa, 5- Zona fasciculata, 6- Cells resembling zona fasciculata cells H & E × 100

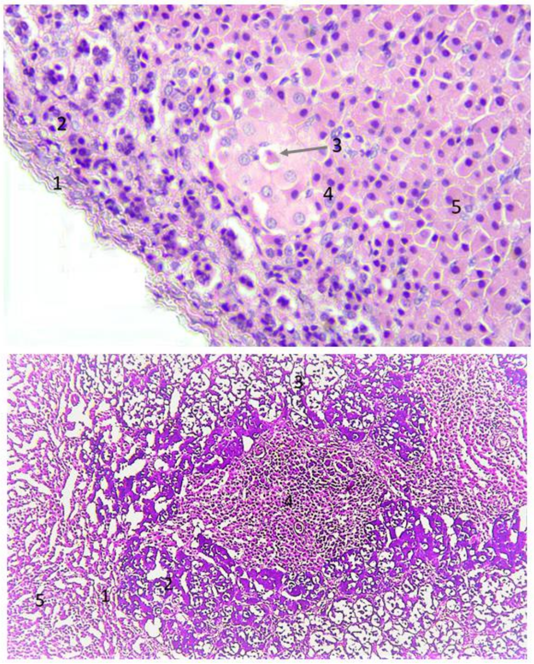

Fig. 4. 4A. Photomicrograph of adrenal cortex: 1- Capsule, 2- Zona glomerulosa, 3- Secretion, 4- Cells of intermediate zone, 5- Zona fasciculata H & E × 100. 4B. Photomicrograph of adrenal medulla: 1- Zona reticularis, 2- Outer medulla, 3- Inner medulla, 4- less differentiated cells resembling adrenal cortex, 5- Zona fasciculata H & E × 40

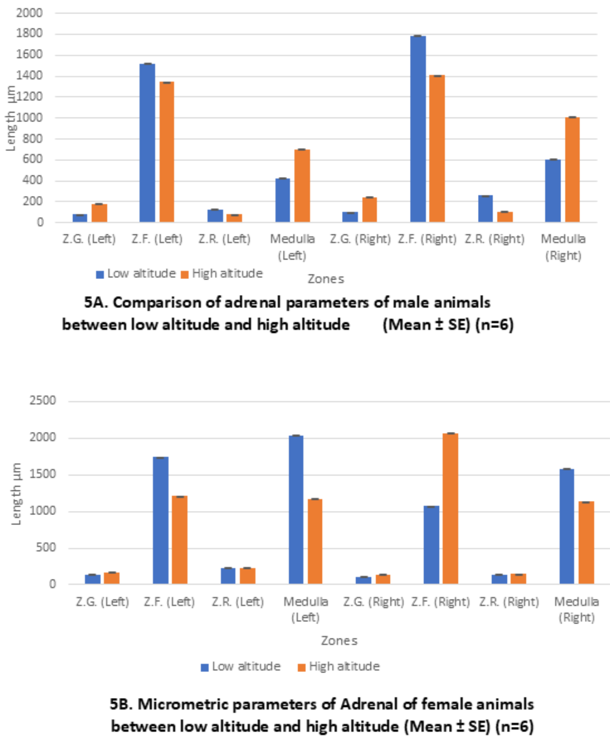

Fig. 5. Comparison of adrenal parameters: 5A. Male animals between low altitude and high altitude; 5B. Female animals between low altitude and high altitude

3.2 Hormonal Assay

Serum cortisol hormone levels of the low-altitude male, low-altitude female, high-altitude male, and high-altitude female groups were 391.45 ± 7.24 nmol/L, 240.16 ± 6.72 nmol/L, 331.79 ± 7.26 nmol/L, and 214.42 ± 8.47 nmol/L respectively. When a comparison of the data was made concerning sex, both male and female groups revealed significantly higher average serum cortisol levels in the low-altitude group than in the high-altitude group (< 0.001).

4. DISCUSSION

The study established a detailed account of the histoarchitecture of the cortical and medullary zones of the adrenal gland in Large White Yorkshire pigs. The results agreed with the earlier reports on sheep [15], European bison [16], adult Bakerwali goats [17], buffaloes [18-20], and pigs [4]. The cellular morphology of the cells in the outer and inner zones of the medulla indicated them as adrenalin and noradrenalin-secreting cells, respectively, based on comparable cellular features described in buffaloes [18]. Further, chromaffin was identified in the cells of the medulla, suggesting its secretion of catecholamines: adrenaline and nor-adrenaline. The distinct cholesterol band at the junction between the zona fasciculata and zona reticularis reported in pigs [21] was lacking in this study.

The study revealed a transition of the radiating cortical zones adjacent to or encircling the invading trabeculae, and such a feature has not been recorded in the postnatal pig adrenal gland based on the previous literature scanned by the authors. A two-year-old elephant calf has reported a similar finding [22]. But it was limited to the outer cortex and its frequency was less.

Additionally, the work identified undifferentiated or less differentiated cells indicating the existence of stem cells in pigs as recorded earlier in African elephants [23]. The presence of undifferentiated cells in the adrenal in the present study indicates cell proliferation occurs in the adrenal gland, and later, those cells transform to the various adult zones in pigs indicating that regeneration of the adrenal gland occurs postnatally. Further, detailed investigations are required to analyse the factors contributing to stem cell proliferation and its zonal expansion in pigs.

Our recorded data are comparable to the reported adrenal values in goats [24,25] in sheep [26], and in humans [27] that the zona fasciculata comprised the largest zone in all those species and contributed around 80% of the adrenal cortex. The average thickness of zona fasciculata of both the right and the left adrenals was significantly higher in the low-altitude male group to the high-altitude male group as well as in the low-altitude female group in comparison with the high-altitude female group ($p < 0.001$). The significantly higher serum cortisol values ($p < 0.001$) in the low-altitude male and low-altitude female groups than their high-altitude counterparts confirm a larger zona fasciculata layer. The increased cortisol level in the low-altitude group suggests more stress, as cortisol plays a central role in mobilizing reserved energy sources of fat, protein, and carbohydrates during stress [27]. A detailed study incorporating a large sample size will be beneficial for understanding the interplay of hormones, the effect of altitude, and environmental factors.

5. CONCLUSION

The present study recorded the three typical cortical zones and medulla within the adrenal gland in Large White Yorkshire pigs. The comparative micrometrical data revealed a larger zona fasciculata, which produces mainly cortisol, in the low-altitude male and female groups than in the corresponding high-altitude groups. Thus, pigs' adrenal glands reared at low and high altitudes revealed structural variation supported by the serum cortisol. Further, the study identified undifferentiated cells suggesting postnatal regeneration and remodelling in ten-month-old Large white Yorkshire pigs. As the gland plays a vital role in an animal’s well-being and stress responses, its structural variation under various physiological and pathological conditions may be further explored to gain a comprehensive knowledge of the complex interplay of the endocrine glands.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ACKNOWLEDGEMENTS

The authors thank the Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, and

Central Instrumentation Laboratory, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, for providing facilities. Funding by State Plan Fund 21-22 (Government of Kerala), Indian Council of Agricultural Research (ICAR), and Kerala Veterinary and Animal Sciences University are acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3995