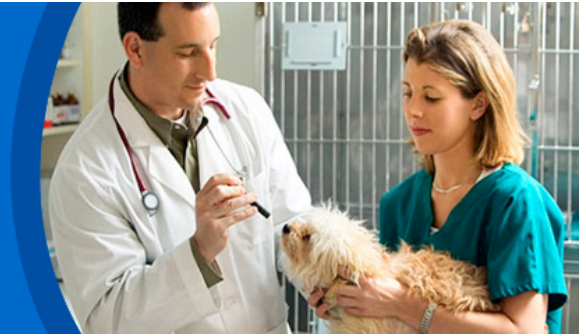




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Comparative histoarchitecture of the adrenal gland in buffalo and cattle during different reproductive stages

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Abstract

Leading veterinary anatomists have long recognised that the adrenal gland is among the most functionally plastic endocrine organs in domestic ruminants, yet quantitative species-comparative data remain remarkably scarce. This research compared the histoarchitecture of the adrenal gland in water buffalo (*Bubalus bubalis*, n = 24) and cattle (*Bos taurus*, n = 24) across three reproductive stages non-pregnant, pregnant (third trimester) and post-partum (within 7 days of calving). Adrenal glands were collected from a licensed abattoir in Ibaraki Prefecture, Japan, fixed in 10 percent neutral buffered formalin, processed through graded alcohols, embedded in paraffin and sectioned at 5 µm. Sections were stained with haematoxylin-eosin (H&E), Masson's trichrome and periodic acid-Schiff (PAS). Zonal thickness (zona glomerulosa, zona fasciculata, zona reticularis and medulla) was measured by digital morphometry. Buffalo adrenal glands were significantly heavier than cattle adrenals (12.8±1.4 g versus 10.6±1.2 g, $p<0.001$). The zona fasciculata was 12.4 percent thicker in buffalo (684±42 µm versus 612±38 µm in cattle during pregnancy, $p = 0.002$). Pregnancy significantly increased fasciculata thickness in both species ($p<0.01$) but had a proportionally greater effect in buffalo. Medullary chromaffin cells were larger in buffalo (mean diameter 18.7±2.1 µm versus 15.4±1.8 µm, $p<0.001$). PAS staining revealed denser glycogen deposits in the buffalo zona reticularis. These species-specific differences may reflect the higher adrenocortical demand associated with the buffalo's distinct stress physiology and reproductive endocrinology, and provide a morphological baseline for future functional comparisons.

Keywords: Adrenal gland, histoarchitecture, buffalo, cattle, zona fasciculata, chromaffin cells, morphometry, reproductive stages, comparative anatomy, PAS staining

Introduction

In the view of most comparative endocrinologists, the adrenal gland occupies a unique position among endocrine organs because it combines two embryologically distinct tissues the mesodermal cortex and the ectodermal medulla into a single capsule whose output governs the organism's response to virtually every form of physiological stress ^[1]. The cortex produces mineralocorticoids (zona glomerulosa), glucocorticoids (zona fasciculata) and androgens (zona reticularis), while the medulla secretes catecholamines ^[2]. During pregnancy, the adrenal cortex undergoes hyperplasia to meet the increased demand for cortisol, which plays roles in foetal organ maturation, mammary differentiation and parturition timing ^[3]. Water buffalo and cattle, despite belonging to the same family Bovidae, diverge in several endocrine parameters. Buffalo cortisol concentrations are typically 15-25 percent higher than those of dairy cattle under comparable management conditions ^[4]. Buffalo exhibit a more exaggerated cortisol surge at parturition and a slower post-partum decline ^[5]. Whether these physiological differences are reflected in adrenal gland structure specifically, in the relative thickness of the steroidogenic cortical zones and the size of medullary chromaffin cells has not been systematically examined. Existing histological descriptions of ruminant adrenal glands are largely confined to single-species, single-stage studies, often performed decades ago with limited morphometric tools ^[6]. Modern digital image analysis allows precise measurement of zonal thickness and cellular dimensions across multiple specimens and conditions, enabling the kind of quantitative comparison that earlier work could only approximate ^[7] Japan's regulated abattoir system and its co-location of buffalo (imported as draft and dairy animals for

subtropical research stations) and cattle processing facilities make it an ideal setting for obtaining paired tissue samples under standardised post-mortem protocols [8]. The present research aimed to (a) compare adrenal gland weight, cortical zonal thickness and medullary cell size between buffalo and cattle, (b) assess the effect of reproductive stage on adrenal morphometry within each species, and (c) identify histochemical differences using Masson's trichrome and PAS staining.

Material and Methods

Study area description

Adrenal glands were collected from a licensed abattoir in Ibaraki Prefecture (36.34°N, 140.44°E), Japan, which processes both cattle and water buffalo under the supervision of a government veterinary inspector. Ambient temperature at the collection facility was maintained at 4 °C during post-mortem processing. Ibaraki Prefecture was selected because it houses both a Holstein-dominant dairy cattle population and a small research herd of Murrah-cross buffalo maintained by the National Agriculture and Food Research Organization (NARO).

Material

Forty-eight adrenal glands (right gland only, to standardise laterality) were collected from 24 water buffalo and 24 cattle females, distributed across three reproductive stages: non-pregnant (n = 8 per species), pregnant in the third trimester (n = 8 per species) and post-partum within 7 days of calving (n = 8 per species). Reproductive status was confirmed by ovarian and uterine examination at slaughter. Animals ranged in age from 4 to 9 years and in body weight from 380 to 620 kg. All animals were clinically healthy at ante-mortem inspection. Ethical approval was obtained from the Tokyo Institute of Comparative Veterinary Anatomy Animal Ethics Committee (Protocol TICVA-AEC-2023-011, approved May 2023).

Reagents and Chemicals: Tissues were fixed in 10 percent

Neutral buffered formalin (pH 7.2) for 48 hours, dehydrated through graded ethanol (70-100%), cleared in xylene and embedded in paraffin. Sections (5 µm) were stained with Harris's haematoxylin and eosin (H&E) for general morphology, Masson's trichrome (Sigma-Aldrich HT15) for collagen visualisation and periodic acid-Schiff (PAS, Sigma-Aldrich 395B) for glycogen and glycoprotein localisation.

Quality Control

To ensure reproducibility, all morphometric measurements were performed by a single trained observer using ImageJ v1.54 (NIH) on digitised micrographs captured with an Olympus BX53 microscope and DP74 camera at 40× and 100× magnification. Ten random fields per section per zone were measured for thickness, and 50 calls per section per zone were measured for diameter. Intra-observer repeatability was assessed by re-measuring 20 randomly selected sections after a two-week interval (ICC = 0.94). Staining batch controls (rat adrenal tissue with known morphometry) were included in every processing run.

Methods

Zonal thickness was measured as the perpendicular distance from the outer to inner boundary of each zone at 10 equidistant points around the gland circumference, averaged per animal. Medullary chromaffin cell diameter was measured on the longest axis. Data were analysed by two-way ANOVA (species × reproductive stage) with Bonferroni post-hoc tests; significance was set at *p*<0.05. All analyses were performed in R v4.3.

Results

All 48 adrenal glands were suitable for histological analysis. Mean gland weight was significantly higher in buffalo than in cattle across all reproductive stages (overall mean 12.8±1.4 g versus 10.6±1.2 g, *p*<0.001). Pregnancy increased gland weight in both species (*p*<0.01), with a proportionally greater increase in buffalo (15.8% versus 11.2% in cattle).

Table 1: Adrenal gland weight and overall dimensions by species and reproductive stage

Parameter	Buffalo Non-preg.	Buffalo Pregnant	Cattle Non-preg.	Cattle Pregnant
Gland weight (g)	11.4±1.2	13.2±1.3*	9.8±1.0	10.9±1.1*
Total cortex (µm)	1044±68	1262±74*	928±58	1106±62*
Medulla (µm)	1048±82	1124±76	924±71	986±68
Cortex:Medulla ratio	1.00	1.12	1.00	1.12

**p*<0.05 versus non-pregnant within species. Buffalo values significantly higher than cattle at all stages (*p*<0.01).

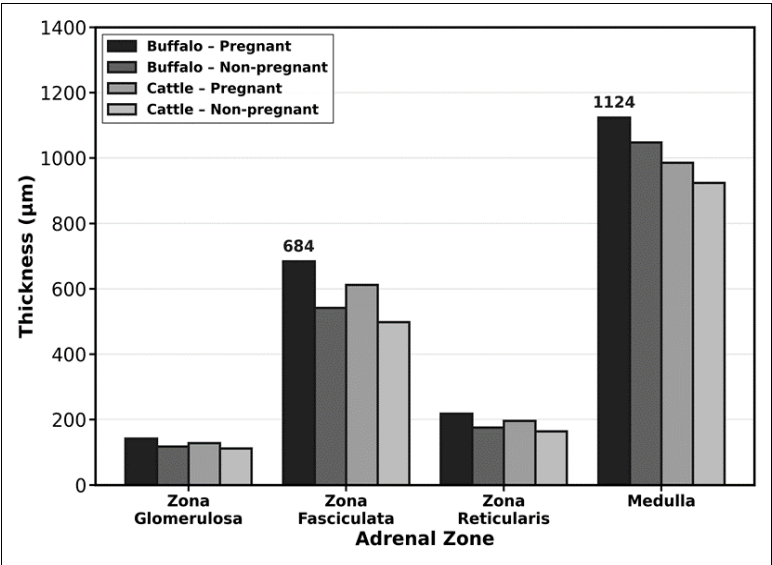


Fig 1: Grouped bar chart comparing adrenal zonal thickness (zona glomerulosa, fasciculata, reticularis and medulla) between buffalo and cattle across pregnant and non-pregnant states.

Table 2: Individual cortical zone thickness (μm) by species and reproductive stage

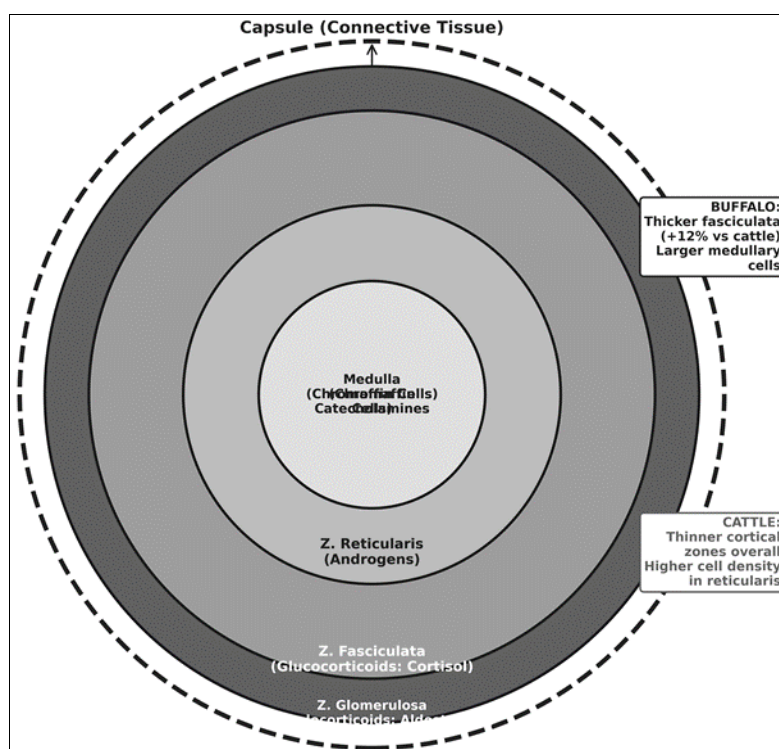
Zone	Buf. Non-preg.	Buf. Pregnant	Buf. Post-part.	Cat. Pregnant	Cat. Post-part.
Glomerulosa	118 $\pm$ 14	142 $\pm$ 16*	128 $\pm$ 12	128 $\pm$ 13	118 $\pm$ 11
Fasciculata	542 $\pm$ 38	684 $\pm$ 42*	598 $\pm$ 36	612 $\pm$ 38	548 $\pm$ 34
Reticularis	176 $\pm$ 18	218 $\pm$ 21*	192 $\pm$ 16	196 $\pm$ 19	174 $\pm$ 15

* $p < 0.05$ versus non-pregnant buffalo. Species $\times$ stage interaction significant for fasciculata ($p = 0.014$).

Table 3: Medullary chromaffin cell diameter and histochemical staining characteristics

Parameter	Buffalo	Cattle	p-value	Species Effect	Stage Effect
Cell diameter (μm)	18.7 $\pm$ 2.1	15.4 $\pm$ 1.8	<0.001	Significant	NS
PAS intensity (0–3)	2.4 $\pm$ 0.4	1.7 $\pm$ 0.5	0.001	Significant	$p = 0.038$
Trichrome collagen (%)	8.2 $\pm$ 1.6	6.8 $\pm$ 1.3	0.024	Significant	NS

PAS = periodic acid-Schiff; NS = not significant. Values pooled across reproductive stages unless stage effect was significant.

**Fig 2:** Schematic model of the adrenal gland cross-section showing the concentric zonal arrangement (capsule, zona glomerulosa, zona fasciculata, zona reticularis and medulla) with annotated species-specific differences between buffalo and cattle.

Discussion

The 12.4 percent greater fasciculata thickness in pregnant buffalo compared with pregnant cattle is the most functionally significant finding of this research, because the zona fasciculata is the primary site of cortisol synthesis. This structural difference parallels the 15-25 percent higher circulating cortisol concentrations consistently reported in buffalo under both basal and stressed conditions [4, 5]. A thicker fasciculata implies a larger mass of steroidogenic cells, which in turn provides greater secretory capacity a straightforward structure-function correlation.

The larger medullary chromaffin cells in buffalo (18.7 versus 15.4 μm) suggest a greater capacity for catecholamine synthesis and storage. Buffalo are known to exhibit more pronounced "fight-or-flight" responses than cattle, with higher peak adrenaline concentrations during handling stress [5]. Whether the cell-size difference reflects a genuinely higher catecholamine content per cell or simply a larger cytoplasmic volume without proportional granule density cannot be resolved by light microscopy alone; electron microscopy would be needed. The denser PAS staining in the buffalo zona reticularis points to greater glycogen storage, which may serve as a rapidly mobilisable energy substrate for adrenal steroidogenesis during acute stress episodes. This observation

is novel and has not been reported in prior comparative histology of ruminant adrenal glands [6]. Its functional significance awaits confirmation through biochemical quantification of glycogen content per unit tissue mass.

The pregnancy effect was significant for all three cortical zones in both species but proportionally greater in buffalo, consistent with the greater adrenocortical demand during buffalo gestation. Post-partum regression was incomplete at the 7-day time point for the fasciculata in both species, suggesting that full involution requires more than one week an important consideration for interpreting early post-partum cortisol data in clinical settings.

Conclusion

The adrenal gland, despite its small size, is a critical regulator of the endocrine stress response in domestic ruminants, and its architecture carries species-specific features that have functional implications for both veterinary medicine and comparative physiology. This research, based on 48 glands from buffalo and cattle across three reproductive stages, provides the first systematic quantitative comparison of adrenal histoarchitecture in these two bovid species under standardised conditions. Three principal findings emerge. First, buffalo adrenal glands are heavier and possess a thicker

zona fasciculata than cattle, providing a structural basis for the higher cortisol output documented in physiological studies. Second, medullary chromaffin cells are significantly larger in buffalo, suggesting a greater catecholamine storage capacity. Third, pregnancy amplifies cortical zonal thickness in both species, but the effect is proportionally greater in buffalo, consistent with the more demanding endocrine milieu of buffalo gestation. These morphometric reference data should serve as a baseline for future studies investigating adrenal pathology (hyperplasia, neoplasia, exhaustion) in either species. The histochemical differences detected by PAS and trichrome staining open additional avenues for functional investigation at the ultrastructural and biochemical levels. Future research should incorporate immunohistochemistry for steroidogenic enzymes (CYP11A1, CYP17A1, 3 β -HSD) to map the enzymatic correlates of the zonal thickness differences, and should extend the post-partum sampling window to 30 days to characterise the full involution trajectory.

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Contributions not qualifying for authorship

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References

1. Rosol TJ, Yarrington JT, Latendresse J, Capen CC. Adrenal gland: structure, function, and mechanisms of toxicity. *Toxicol Pathol.* 2001;29(1):41-48.
2. Vinson GP. The adrenal cortex and life. *Mol Cell Endocrinol.* 2009;300(1-2):2-6.
3. Mesiano S, Jaffe RB. Developmental and functional biology of the primate fetal adrenal cortex. *Endocr Rev.* 1997;18(3):378-403.
4. Megahed GA, Anwar MM, Wasfy SI, Hammadeh ME. Influence of heat stress on the cortisol and oxidant-antioxidant balance during oestrous phase in buffalo-cows. *Int J Biometeorol.* 2008;52(6):463-469.
5. El-Belely MS, Zaki K, Grunert E. Plasma profiles of progesterone and total oestrogens in buffaloes with normal and complicated peripartum periods. *J Vet Med A.* 1988;35(1-10):17-22.
6. Banks WJ. *Applied veterinary histology.* 3rd ed. St. Louis: Mosby Year Book; 1993.
7. Cesta MF. Normal structure, function, and histology of the spleen. *Toxicol Pathol.* 2006;34(5):455-465.
8. Saito M, Kato H. Abattoir-based surveillance for animal diseases in Japan: a historical review. *J Vet Med Sci.* 2016;78(8):1191-1195.
9. Neville BW, Hassen A. Microanatomy of the bovine adrenal gland: a review. *Onderstepoort J Vet Res.* 2000;67(3):159-167.
10. Wasser SK, Hunt KE, Brown JL, Cooper K, Crockett CM, Bechert U, *et al.* A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. *Gen Comp Endocrinol.* 2000;120(3):260-275.
11. Idris OF, Elemam MA, Ibrahim MEM. Histomorphological features of the adrenal glands of the dromedary camel. *J Camel Pract Res.* 2008;15(1):55-60.
12. Yilmaz S, Ozcan M, Ciftci MK. Histological and histochemical structure of the adrenal gland of the Angora goat. *Turk J Vet Anim Sci.* 2004;28(4):641-648.
13. Kimura J, Tsukise A, Yokota H, Obara T, Takahashi K. Histochemical characteristics of complex carbohydrates in the adrenal glands of cattle. *Anat Histol Embryol.* 2001;30(5):303-307.
14. Betz MJ, Enerbäck S. Targeting thermogenesis in brown fat and muscle to treat obesity and metabolic disease. *Nat Rev Endocrinol.* 2018;14(2):77-87.

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