



Shiraz University



IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol.25

No.1

Ser. No.86

2024

**IRANIAN
JOURNAL
OF
VETERINARY
RESEARCH**



Short Paper

Effect of season on histoarchitecture of pineal gland in buffalo (*Bubalus bubalis*)

Sharma, A.¹; Kumar, V.¹; Singh, V. K.^{2*}; Patbandha, T. K.³
and Vyas, Y. L.¹

¹Department of Veterinary Anatomy, College of Veterinary Science & A. H., Kamdhenu University, Junagadh-362001, Gujarat, India; ²Department of Veterinary Physiology & Biochemistry, College of Veterinary Science & A. H., Kamdhenu University, Junagadh-362001, Gujarat, India; ³Polytechnic in Animal Husbandry, College of Veterinary Science & A. H., Kamdhenu University, Junagadh-362001, Gujarat, India

*Correspondence: V. K. Singh, Department of Veterinary Physiology & Biochemistry, College of Veterinary Science & A. H., Kamdhenu University, Junagadh-362001, Gujarat, India. E-mail: drvksingh@kamdhenuuni.edu.in



10.22099/IJVR.2024.48516.7084

(Received 6 Oct 2023; revised version 2 Jan 2024; accepted 24 Jan 2024)

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Abstract

Background: The photoperiod and other seasonal variations are the key factors that affect reproduction and production of the animals. The pineal gland secretes melatonin hormone that affects several physiological functions of the body during different seasons. **Aims:** The present study was conducted to study the histoarchitectural and micrometrical changes in the pineal gland of buffalo (*Bubalus bubalis*) during different seasons of the year. **Methods:** Pineal glands of 30 adult female Jaffarabadi buffaloes were collected from the slaughterhouse during the winter, summer, and rainy seasons. Samples were processed by standard histological procedures and stained with various stains for histological and micrometrical observations. **Results:** The pinealocytes constituted a major cellular portion of pineal parenchyma. The pinealocyte nuclei were lightly stained and more euchromatic during the winter season whereas darkly stained and slightly heterochromatic during summer. The calcium deposits occupied a larger area of pineal parenchyma during the summer as compared to the winter season. The pinealocyte density, the nuclear diameter of pinealocytes, and the number of argyrophilic nucleolar organizer regions (AgNOR) were highest during the winter season as compared to the summer and rainy seasons. **Conclusion:** The present study shows the influence of season on the histoarchitecture and histometry of the pineal gland of buffalo and indicated higher pineal activity during the winter season in this species.

Key words: Buffalo, Histoarchitecture, Pineal gland, Season

Introduction

Water buffalo (*Bubalus bubalis*) is an important economic livestock resource for millions of farmers, mainly in developing countries of Asia, and significantly contributes in global milk and meat production. Buffaloes are sensitive to high ambient temperature which negatively affects the reproduction and production (Marai and Haebe, 2010).

The pineal is a solid, pea-shaped endocrine gland, located just in front of two rostral colliculi and attached to the caudo-dorsal aspect of the thalami by a very small stalk (Sharma *et al.*, 2019). The pinealocytes and glial cells are the major components of parenchyma of pineal gland (Eurell and Frappier, 2006). The pineal gland produces melatonin hormone, a derivative of serotonin, which regulates the circadian rhythm, many

physiological functions and behavior of animals. Different animals have different patterns of melatonin secretion which are impacted by environmental factors (Delgadillo *et al.*, 2004).

Limited literature is available on the effect of season on the histoarchitecture of the pineal gland, especially in the buffalo. However, effect of season on gross morphology of pineal gland in buffalo has been recently reported (Sharma *et al.*, 2019). Studying the effect of environmental changes on the microscopic structure of the pineal gland, might help in understanding the impact of season on physiological functions which is important in enhancing the reproduction and production efficiency of buffaloes. The present study was conducted to clarify the histoarchitectural alterations in the pineal gland of the adult female buffalo during different seasons of the year.

Materials and Methods

Sample collection

The present study was conducted on the pineal glands of 30 adult, female Jaffarabadi buffaloes. The age of animals ranged from about 5 to 7 years. The approximate age was estimated by using dentition of animals. The samples for the study were collected from the local slaughterhouse at Junagadh (70.5° east longitude and 21.4° north latitude), India, during winter, summer, and rainy season. The pineal glands of 10 buffaloes were collected during each season.

Processing of tissue for light microscopy

Immediately after collection, the tissue samples were fixed in 10% neutral buffered formalin. After fixation, the tissue samples were processed and embedded in paraffin wax (58-60°C) by using standard tissue processing schedule. Sections with 3-7 µm thickness were prepared by a manual rotary microtome (Leica RM 2125 RTS, Germany). The tissue sections were stained with Hematoxylin and Eosin stain for histomorphology, Masson's trichrome stain for collagen fibers, Von Kossa's method for calcium, Bilchowsky's method for nerve fibers (Luna, 1968), Gridley's method for reticular fiber, Weigert's method for elastic fibers (Sheehan and Hrapchak, 1973), and Silver stain for argyrophilic nucleolar organizer region (AgNOR) (Lindner, 1993).

Processing of tissue for scanning electron microscopy

For scanning electron microscopy, the tissue samples were washed in chilled 0.1 M phosphate buffer (pH 7.2). Washed samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.2) for 4-6 h. Fixed samples were washed in 0.1 M phosphate buffer. Thereafter, the samples were dehydrated in ascending-grade acetone solutions at 4°C. The specimens were then placed in a critical point drying apparatus (Uppal *et al.*, 2014). Thereafter, tissue samples were coated with a sputter coater (Emitech SC7620) with 35 nm thick layer of gold. The tissue samples, were viewed under the scanning electron microscope (Zeiss EVO-18, Germany).

Microscopy

The stained sections were analyzed using an optical microscope (Zeiss Primo Star, Germany). Digital histological photographs were captured with the help of microscopic camera (Zeiss Axiocam ERC 5s, Germany). Micrometry was performed using microscopic image analysis software (Carl Zeiss Zen 2 blue edition, Germany). The diameter of the nucleus of pinealocytes, number of pinealocytes and glial cells (per mm²), and number of Argyrophilic Nucleolar Organizer Region (AgNOR) in each nucleus of pinealocytes were recorded. All the micrometric observations were recorded in ten randomly selected fields of the stained tissue section from each sample of different seasons.

Statistical analysis

The obtained micrometrical data are presented as mean±standard error (SE). The effect of seasons on different parameters was analyzed using one-way ANOVA (analysis of variance) with SPSS software. The pair-wise mean differences were compared by Tukey's test. After the analysis of data, the differences were considered statistically significant at P<0.05.

Results

Histoarchitecture

Histologically, the pineal glands of the buffalo were composed of a capsule and the gland parenchyma. The outer surface of the capsule was folded with numerous fine blood vessels and nerve fibers between these folds (Fig. 1A). The secondary folds were also detected at higher magnification (Fig. 1B). The capsule was mainly consisted of collagen and reticular fibers but the elastic fibers were not observed (Figs. 1C-D). Distinct differences were not observed in the histoarchitecture of the capsule during different seasons.

The parenchyma was composed of pinealocytes, glial cells, fibers, and blood vessels. The connective tissue trabeculae present in the parenchyma were mainly consisted collagen fibers, reticular fibers, nerve fibers, and blood vessels (Figs. 2A-C). Few elastic fibers were also observed in the wall of the large blood vessels (Fig. 2D). The glandular parenchyma was richly supplied with small blood vessels surrounded by collagen fibers. During the winter season, the large blood vessels were surrounded by a huge amount of homogenous material that was intensely stained with aniline blue in Masson's trichrome method. The large blood vessels also showed eosinophilic material in their lumen with vacuoles during winter. However, a negligible difference was observed in the amount of collagen around the smaller capillaries (Figs. 3A-D).

The pinealocytes constituted a major cellular portion of the pineal gland and were organized in the form of bunches and irregular strings, though some individually scattered pinealocytes were also observed (Fig. 4A). Pinealocytes were characterized by the presence of large, rounded, and euchromatic nuclei with a distinct nucleolus. The cytoplasm of pinealocytes was slightly eosinophilic and the cell boundary was not distinct. The pinealocytes nuclei were lightly stained during the winter season and comparatively darker during the summer season (Figs. 4B-C). The processes of pinealocytes intermingled with each other and with the processes of glial cells. This network of processes was the distinctive feature of pineal parenchyma (Fig. 4D).

The glial cells were observed between the pinealocytes, in trabeculae, and around the blood vessels. Different types of glial cells were characterized by varying shapes of nucleus (Fig. 5A). The cells with elongated nuclei were mainly observed along the blood capillaries. Some cells, with small, rounded, and darkly stained nuclei were also observed, especially in the

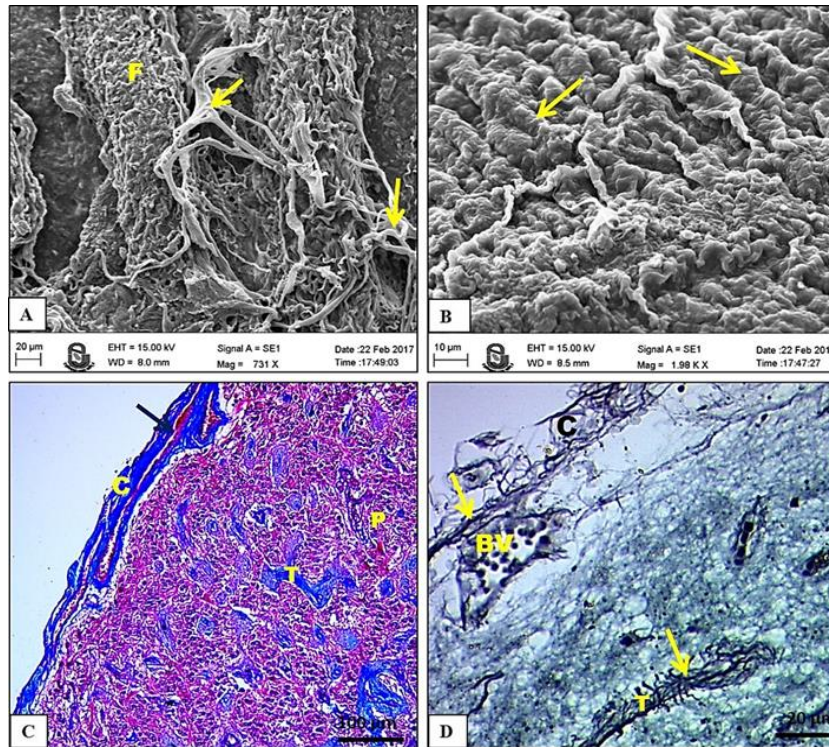


Fig. 1: Photomicrographs of the capsule of pineal gland of buffalo. (A) folds (F) on the outer surface of the capsule with fine blood vessels and nerve fibers (arrow), (SEM, scale bar: 20 μm), (B) Secondary folds (arrows) on the outer surface of capsule, (SEM, scale bar: 10 μm), (C) Collagen fibers (blue colored) in capsule (C), around the blood vessels (arrow), and in parenchyma (P) of gland in connective tissue trabeculae (T), (Masson's trichrome, scale bar: 100 μm), and (D) reticular fibers (arrow) in capsule (C), trabeculae (T), and around the blood vessel (BV), (Gridley's, scale bar: 20 μm)

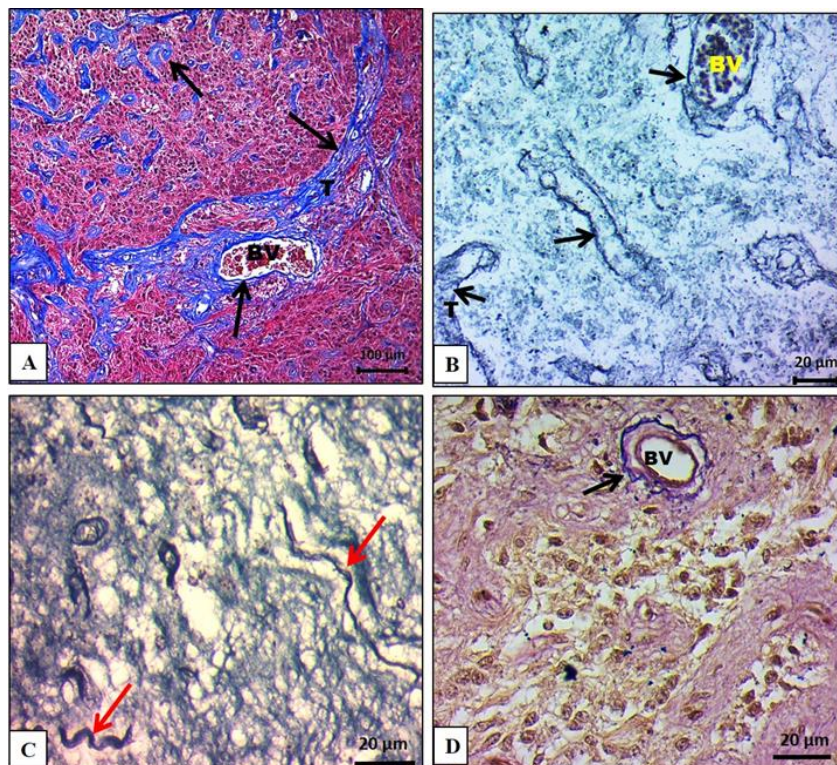


Fig. 2: Photomicrographs showing connective tissue fibers in pineal parenchyma. (A) Collagen fibers (arrow) in trabeculae (T) and surrounding the blood vessels (BV), (Masson's trichrome, scale bar: 100 μm), (B) Fine reticular fibers (arrow) in trabeculae (T), surrounding the blood vessels (BV), (Gridley's staining, scale bar: 20 μm), (C) Nerve fibers (arrow) in pineal parenchyma, (Bilchowsky's staining, scale bar: 20 μm), and (D) Elastic fibers (arrow) in the wall of blood vessels (BV), (Weigert's staining, scale bar: 20 μm)

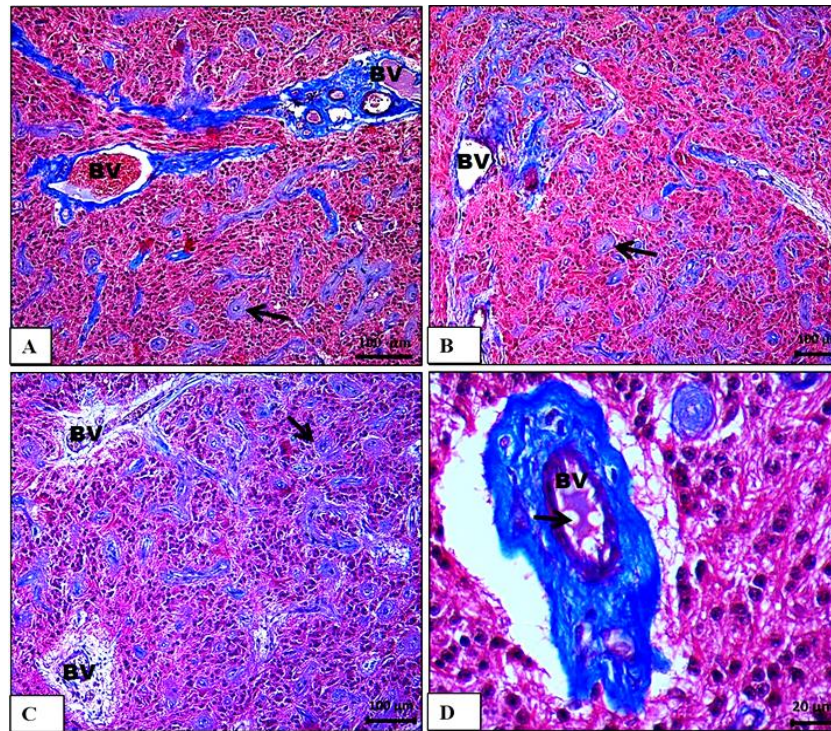


Fig. 3: Photomicrographs show collagen fibers in pineal parenchyma during different seasons. (A) Collagen fibers surrounding the larger blood vessels (BV), and small capillaries (arrow) during the winter season, (Masson's trichrome, scale bar: 100 μm), (B) Collagen fibers surrounding the large blood vessels (BV), and small capillaries (arrow) during the rainy season, (Masson's trichrome, scale bar: 100 μm), (C) Very low amount of collagen fibers surrounding the large blood vessels (BV), slight reduction in amount of collagen fibers surrounding the small capillaries (arrow) during the summer season, (Masson's trichrome, scale bar: 100 μm), and (D) Large amount of collagenous material surrounding the blood vessels (BV) and lumen of blood vessels showing vacuoles (arrow) during winter season, (Masson's trichrome, scale bar: 20 μm)

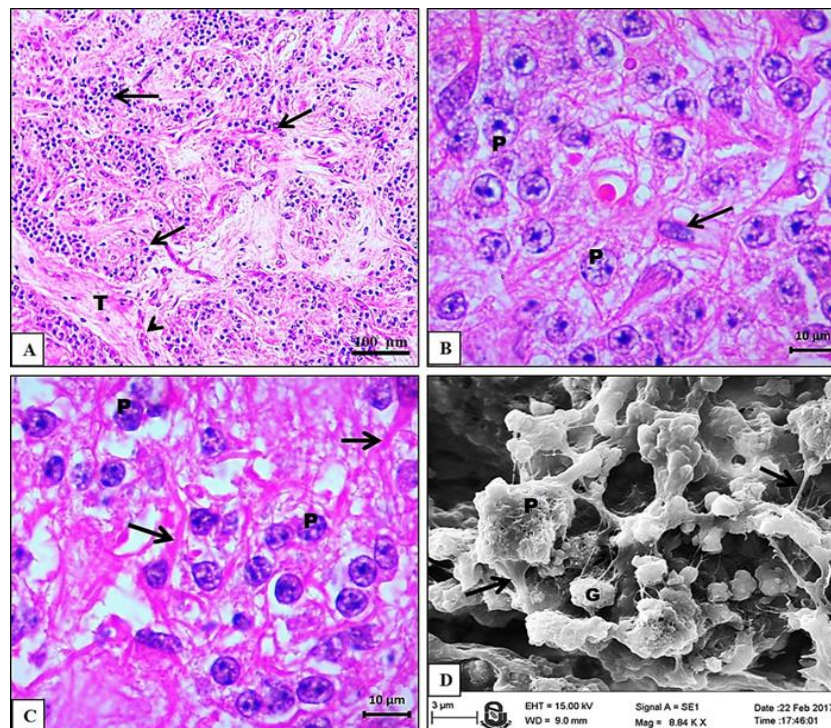


Fig. 4: Photomicrographs showing parenchyma and pinealocytes. (A) Different arrangements of cells (arrow), Trabeculae (T), and blood vessels (arrowhead), (H&E, scale bar: 100 μm), (B) Large, rounded, and lightly stained nuclei of pinealocytes (P), glial cell (arrow) during the winter season, (H&E, scale bar: 10 μm), (C) Smaller, rounded, and darker stained nuclei of pinealocytes (P) and cell processes (arrow) during the summer season, (H&E, scale bar: 10 μm), and (D) Pinealocytes (P) and glial cell (G) with their intermingled processes (arrow) during the winter season, (SEM, scale bar: 3 μm)

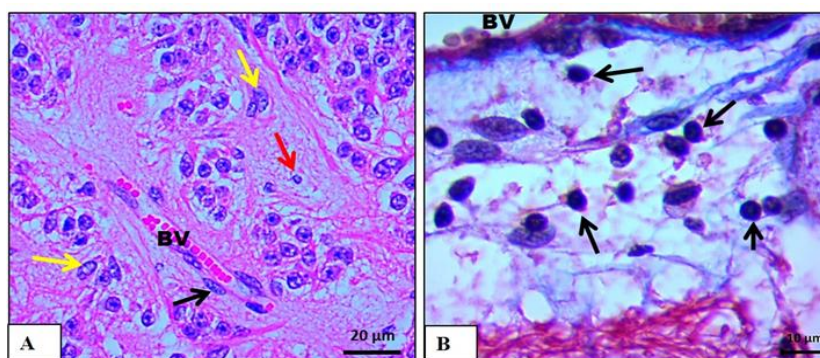


Fig. 5: Photomicrographs showing glial cells. (A) Glial cells with a large oval nucleus (yellow arrow), elongated nucleus (black arrow) along with blood vessels (BV), and with small rounded and darkly stained nuclei (red arrow), during winter season, (H&E, scale bar: 20 μm), and (B) Microglia-like cells (arrow) in perivascular area, during the summer season, (Masson's trichrome, scale bar: 10 μm)

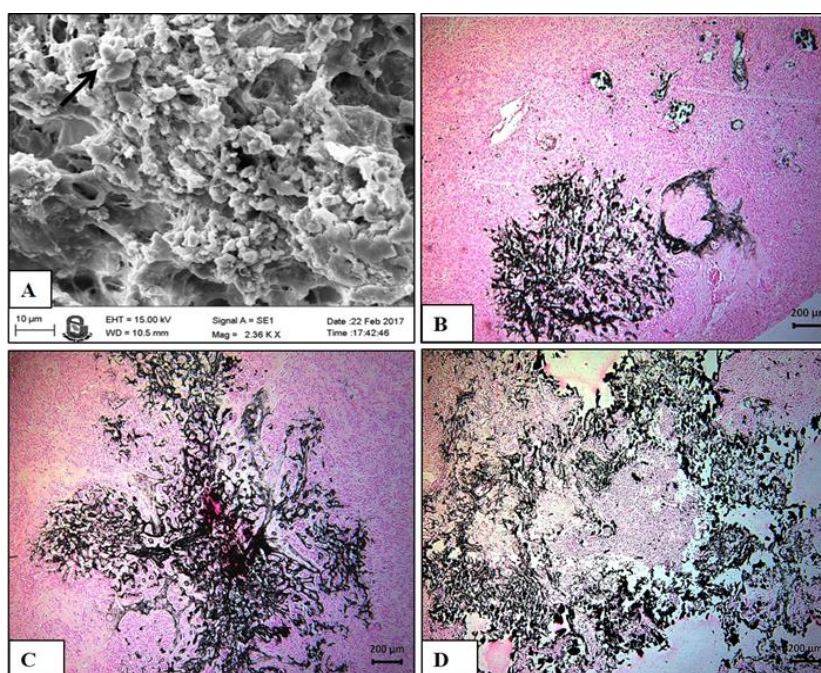


Fig. 6: Demonstration of calcium deposition in pineal parenchyma. (A) Calcium deposits (arrow) during the winter season, (SEM, scale bar: 10 μm), (B) Calcium deposits occupy the lesser area of parenchyma during the winter season, (Von Kossa, scale bar: 200 μm), (C) Calcium deposits occupies the intermediate area of the parenchyma during rainy season, (Von Kossa, scale bar: 200 μm), and (D) Calcium deposits occupies a larger area of parenchyma during the summer season, (Von Kossa, scale bar: 200 μm)

perivascular area. These cells were morphologically similar to the microglial cells and had few processes (Fig. 5B). The microglia-like cells were more frequently observed during the summer followed by rainy season, and were very few in winter.

Most of the tissue sections, showed calcium depositions known as corpora arenacea (Fig. 6A). The corpora arenacea were mainly located in the center of the gland parenchyma. The calcium deposition was more abundant during the summer season and occupied a larger area of the gland parenchyma, which was slightly reduced in the pineal gland of rainy season. The calcium deposition was in lesser amount during the winter season and occupied a smaller area of gland parenchyma as compared to summer and rainy seasons (Figs. 6B-D).

Micrometry

The micrometrical data was recorded and presented in Table 1. The nuclei of pinealocytes were significantly larger during the winter season as compared to summer ($P < 0.01$) and rainy ($P < 0.01$) seasons, but the difference between nuclear diameters of summer and rainy seasons were non-significant ($P > 0.05$). The AgNOR stained sections of the pineal gland showed a large spherical nucleus of pinealocytes with brown-black rounded small dots known as nucleolar organizers (Figs. 7A-B). The number of AgNOR were significantly higher during winter than summer ($P < 0.01$) and rainy ($P < 0.01$) seasons. The number of AgNOR differed significantly between summer and rainy seasons ($P < 0.01$). The density of pinealocytes was significantly higher during winter as compared to rainy ($P < 0.01$) and summer

Table 1: Micrometric parameters (mean±SE) of the pineal gland of buffalo during different seasons

Parameters	Seasons		
	Winter	Summer	Rainy
Nuclear diameter of pinealocytes (µm)	7.53 ± 0.17 ^a	6.69 ± 0.07 ^b	6.77 ± 0.08 ^b
Number of AGNOR/nucleus of pinealocytes	1.89 ± 0.08 ^a	0.94 ± 0.02 ^b	1.25 ± 0.06 ^c
Number of pinealocytes/mm ²	4237.50 ± 68.00 ^a	3234.17 ± 16.22 ^b	3772.83 ± 84.75 ^c
Number of glial cells/mm ²	289.83 ± 12.01 ^a	252.17 ± 13.86 ^a	253.33 ± 11.01 ^a

Values with different superscripts within a row differ significantly (P<0.05)

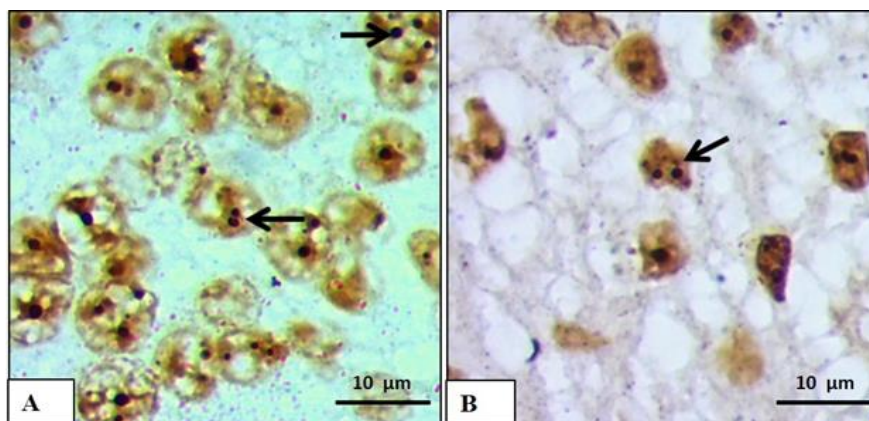


Fig. 7: Showing AgNOR in nuclei of pinealocytes. (A) Numerous AgNOR in the nucleus of pinealocytes (arrow) during the winter season, (AgNOR silver staining, scale bar: 10 µm), and (B) Few numbers of AgNOR in the nucleus of pinealocytes (arrow) during the summer season, (AgNOR silver stain staining, scale bar: 10 µm)

(P<0.01) seasons. The density of pinealocytes also differed significantly between summer and rainy seasons (P<0.01). The number of glial cells was higher during winter than summer and rainy seasons, but this variation was statistically non-significant (P>0.05).

Discussion

The general histomorphology of the pineal gland of buffalo is comparable to the pineal gland of humans (Koshy and Vettivel, 2001), horses (Bolat *et al.*, 2018), donkeys (Ebada, 2012), pigs (Babu and Ramayya, 2014), and viscacha (Busolini *et al.*, 2017). The present study revealed that the pinealocytes constituted about 92.7% to 93.7% of the total cellular content of pineal parenchyma during different seasons. In line with present observation, a high percentage (95%) of pinealocytes were reported in rats (Ibanez *et al.*, 2016).

In the present study, the diameter of the nucleus of pinealocytes varied between different seasons. The nuclear diameter of pinealocyte was largest during the winter season. In the pineal gland of ground squirrels, maximum values of nuclear and nucleolar dimensions were recorded from November to January, whereas minimum values were recorded from April to June (McNulty *et al.*, 1980). Similarly, the diameter of the nucleus of pinealocytes was larger (6.65 ± 0.19) in the rats, exposed to continuous darkness as compared to the rats, exposed to continuous light (5.06 ± 0.06) (Icten *et al.*, 1998). This suggests that the nuclear diameter of the pinealocytes is inversely proportionate to the day length (photoperiod).

An increase in nuclear diameter and cell density during the winter season indicated the augmented activity of pineal gland of buffaloes during this season. The present findings were supported by an experimental study on nuclear morphology of pineal and thyroid glands in rats, treated with natural and synthetic corticoids and antiadrenal agent. The study showed that hyperactivity of the pineal gland was exhibited by a considerable increase in the nuclear diameter of pinealocytes (Sinha *et al.*, 2010). Similarly, Bandyopadhyay *et al.* (2010) observed that the increased activity of the pineal glands is associated with increased cell proliferation and increased nuclear size in mice. The pineal gland of viscacha also exhibited the highest activity during winter whereas minimal activity was observed during summer (Busolini *et al.*, 2017).

Various studies on AgNOR demonstrate that the number of AgNOR in nuclei is positively correlated with the degree of cellular proliferation, cell differentiation, hyperplasia, and transcriptional activity (Uno *et al.*, 1998; Derenzini and Trete, 2001; Aggarwal *et al.*, 2015). In the present investigation, the numbers of AgNOR in the pinealocyte nuclei were highest during the winter season and lowest during the summer season. This elucidates the highest proliferative activity of pinealocytes during winter and the lowest rate of proliferation during the summer season.

Delgadillo *et al.* (2004) observed increased synthesis of melatonin by pinealocytes during winter (short photoperiod) as compared to summer (long photoperiod). However, Xu *et al.* (2018) elucidated that the seasonal changes in melatonin production of hamsters were dependent on the photoperiod as well as temperature

variation. These findings were also supported by the reports in Mediterranean buffaloes which showed maximum concentrations of night-time plasma melatonin during the winter and lowest during the summer (Ramadan, 2017).

The present study revealed that the microglia-like cells were more frequently observed during the summer season as compared to the rainy and winter seasons. It indicates the functional importance of these cells in seasonal glandular activity and modulation of melatonin secretion. Da Silveira *et al.* (2012) suggested that in rats, the microglia-pinealocyte network regulates melatonin output. Similarly, Ibanez *et al.* (2016) observed a complicated connection between microglia, nerves, and blood vessels and reported that the microglia phagocytize only nerve fibers and components of blood vessels, but not pinealocytes.

Consistent with our observations in buffaloes, the calcium deposits (brain sand) are also observed in the pineal gland of humans (Koshy and Vettivel, 2001) and donkeys (Ebada, 2012). However, brain sands are absent in the pineal gland of pigs (Babu and Ramayya, 2014) and horses (Boalat *et al.*, 2018). It is believed that the occurrence of brain sand is generally associated with impaired activity of the gland and the age of the animal. However, Abou-Easa *et al.* (2009) reported a pronounced change in calcium deposition even within a group of buffaloes with the same age. Further, they explained that the obstruction of blood vessels by brain sand was linked with a decrease in pinealocyte count.

In conclusion, based on our findings, the pinealocytes are distributed in the entire parenchyma and constituted a major cellular portion of the pineal gland of Jaffrabadi buffalo. The histomorphological and micrometrical observations showed that the histoarchitecture of the pineal gland was significantly altered during different seasons. A higher cellular density with larger nuclei and increased AgNOR per nucleus of pinealocytes during the winter season indicates that the pineal gland of Jaffrabadi buffalo is morphologically more active during the winter season compared to the rainy and summer seasons.

Acknowledgements

We thank the Dean, College of Veterinary Science & Animal Husbandry and Junagadh Agricultural University, Junagadh for providing research facility and support.

Conflicts of interest

The authors declare that there is no conflict of interest.

References

Abou-Easa, K; Tousson, E and Abd-El-Gawad, M (2009). Involution signs during the postnatal life in the pineal tissue

of buffalo and camel. *Nat. Sci.*, 7: 35-44.

Aggarwal, T; Sawke, G and Sawke, N (2015). Application of AgNOR (argyrophilic nucleolar organizer regions) staining in distinction of non-neoplastic and neoplastic endometrial lesions. *People's J. Sci. Res.*, 8: 23-27.

Babu, AP and Ramayya, PJ (2014). Microanatomical studies on the pineal gland of pig. *Indo-Am. J. Agric. Vet. Sci.*, 2: 67-69.

Bandyopadhyay, R; Das Gupta, M and Chakraborty, S (2010). Steroid hormone and antihormone induced changes in the pineal and adrenocortical karyomorphology and cell proliferation in mice (*Mus musculus*). *Int. J. Biol.*, 2: 25-39.

Bolat, D; Kurum, A; Bahar, S and Karahan, S (2018). Histomorphometric examination of the pineal gland in foals and adult horses. *Ank. Üniversitesi. Vet. Fakültesi. Derg.*, 65: 205-212.

Busolini, FI; Rosales, GJ; Filippa, VP and Mohamed, FH (2017). A seasonal and age-related study of interstitial cells in the pineal gland of male viscacha (*Lagostomus maximus maximus*). *Anat. Rec.*, 300: 1847-1857.

Da Silveira, CMS; Pinato, L; Tamura, EK; Carvalho-Sousa, CE and Markus, RP (2012). Glia-pinealocyte network: the paracrine modulation of melatonin synthesis by tumor necrosis factor (TNF). *PLoS One*. 7: e40142.

Delgadillo, JA; Cortez, ME; Duarte, G; Chemineau, P and Malpoux, B (2004). Evidence that the photoperiod controls the annual changes in testosterone secretion, testicular and body weight in subtropical male goats. *Reprod. Nutr. Dev.*, 44: 183-193.

Derenzini, M and Trere, D (2001). Silver-stained nucleolar organizer regions (AgNOR). *Pathologica*. 93: 99-105.

Ebada, S (2012). Morphological and immunohistochemical studies on the pineal gland of the donkey (*Equus asinus*). *J. Vet. Anat.*, 5: 47-74.

Eurell, JA and Frappier, BL (2006). *Dellmann's textbook of veterinary histology*. 6th Edn., New York, USA, John Wiley & Sons. PP: 298-319.

Ibanez, RMP; Noctor, SC and Munoz, EM (2016). Cellular basis of pineal gland development: emerging role of microglia as phenotype regulator. *PLoS One*. 11: e0167063.

Icten, N; Karagoz, F and Gunec, KA (1998). The effects of constant darkness and constant light on the pineal gland and thymus morphology in the rats. *Turk. J. Med. Sci.*, 28: 7-12.

Koshy, S and Vettivel, SK (2001). Varying appearances of calcification in human pineal gland: a light microscopic study. *J. Anat. Soc. India*. 50: 17-18.

Lindner, LE (1993). Improvements in the silver-staining technique for nucleolar organizer regions (AgNOR). *J. Histochem. Cytochem.*, 41: 439-445.

Luna, LG (1968). *Manual of histologic staining methods of the Armed Forces Institute of Pathology*. 3rd Edn., New York, USA, McGraw-Hill Book Company.

Marai, IFM and Haebe, AAM (2010). Buffalo's biological functions as affected by heat stress- a review. *Livest. Sci.*, 127: 89-109.

McNulty, JA; Dombrowski, TA and Spurrier, WA (1980). A seasonal study of pinealocytes in the 13-lined ground squirrel, *Spermophilus tridecemlineatus*. *Reprod. Nutr. Dev.*, 20: 665-672.

Ramadan, TA (2017). Role of melatonin in reproductive seasonality in buffaloes. In: Carreira, RP (Ed.), *Theriogenology*. (1st Edn.), Croatia, IntechOpen Press. PP: 87-106.

Sharma, A; Vyas, YL; Tank, PH; Kumar, V and Singh, VK (2019). Gross morphometric study on pineal and pituitary glands of Jaffrabadi buffalo during different seasons of the

- year. *Indian J. Vet. Anat.*, 31: 103-105.
- Sheehan, DC and Hrapchak, BB** (1973). *Theory and practice of histotechnology*. 1st Edn., Saint Louis, MO, USA, The CV Mosby Co.,
- Sinha, BR; Chattapadhyay, R; Chakraborty, N and Chakraborty, S** (2010). Stimulatory response of pineal-thyroid nuclear morphology and function following adrenocortical modulation in rat (*Rattus rattus*). *Proc. Zool. Soc.*, 62: 81-90.
- Uno, T; Hashimoto, S and Shimono, M** (1998). A study of the proliferative activity of the long junctional epithelium using argyrophilic nucleolar organizer region (AgNORs) staining. *J. Periodontal. Res.*, 33: 298-309.
- Uppal, V; Bansal, N; Anuradha; Pathak, D and Singh, A** (2014). Light and scanning electron microscopy studies of quail tongues. *Avian Biol. Res.*, 7: 167-171.
- Xu, X; Liu, X; Ma, S; Xu, Y; Xu, Y; Guo, X and Li, D** (2018). Association of melatonin production with seasonal changes, low temperature, and immuno-responses in hamsters. *Molecules*. 23: 1-12.