Haryana Vet. (June, 2022) **61(1),** 51-55

Research Article

HISTOENZYMIC LOCALIZATION OF DEHYDROGENASES IN CYCLIC CORPUS LUTEUM OF INDIAN BUFFALO

KRITIMA KAPOOR[1]*, OPINDER SINGH[2] and DEVENDER PATHAK[3]
[1]Department of Veterinary Anatomy, College of Veterinary Sciences, Rampura Phul,
[2,3]Department of Veterinary Anatomy, College of Veterinary Sciences, Ludhiana,
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, India

Received:04.06.2021; Accepted:23.07.2021

ABSTRACT

Studies were conducted on 24 corpus luteum (CL) of healthy buffalo ovaries collected from local slaughter houses; classified into early luteal (stage I), mid luteal (stage II), late luteal (stage III) and regressing phase (stage IV), based on the gross morphological structure of ovaries. Localization of succinate dehydrogenase (SDH) was weak in early luteal, strong in mid luteal and moderate to weak in regressing phases of CL. Weak to moderate activity of β-Hydroxy steroid dehydrogenase (3β HSD) enzyme was observed in developing small and large luteal cells respectively, in early luteal phase. The enzyme reactivity followed a specific pattern in mid luteal phase. In late luteal and regressing phase, activity of 3β-HSD was reduced considerably as weak localization of the enzyme. Weak G-6-PD activity was localised in cytoplasm of developing luteal cells in initial stage of CL, strong and intense in small and large luteal cells of mid luteal phase, respectively. No reaction was observed in central degenerating area in late luteal phase while most of the immune cells recruited in corpus albicans had strong activity of G-6-PD. Therefore, histoenzymic studies revealed the localization of dehydrogenases in different phases of cyclic CL of buffalo and their subsequent role in steroidogenesis.

Keywords: Buffalo, Corpus Luteum, Dehydrogenases, Histoenzyme, Reproduction

How to cite: Kapoor, K., Singh, O. and Pathak, D. (2022). Histoenzymic localization of dehydrogenases in cyclic corpus luteum of Indian buffalo. *Haryana Vet.* **61(1)**: 51-55.

Buffalo is an important livestock of Indian economy as it plays significant role by contributing alone more than half (i.e., 54%) of total milk produced in country and 12% of the world's milk production (Singh *et al.*, 2014). They are maintained chiefly for milk production thus reproductive performance is a major determinant in its efficient production. Under optimal conditions, their puberty is attained at 15 to 18 months (Warriach *et al.*, 2015) and reproductive performance depends upon several factors, one among them being formation and development of corpus luteum (CL) in ovary which is critical for successful establishment and maintenance of early pregnancy in mammals.

CL is a transitory endocrine gland with well-regulated mechanisms for its development, maintenance and regression, detected more often in right ovary in buffaloes (Devender *et al.*, 2013). In bovine ovary, granulosa and thecal cells of ruptured follicles, following ovulation under the influence of luteinizing hormone undergoes luteinisation and the follicle thus gets transformed into CL (Kapoor *et al.*, 2018). Luteinization involves two major simultaneous processes *viz*., extensive tissue remodelling and acquisition of luteal function. The hypertrophy of granulosa cells indicates the increased metabolic activity which is required for synthesis of hormone progesterone by luteal cells. Therefore, several enzymes play essential roles in metabolism of luteal cells and dehydrogenases are one of them. Among dehydrogenases, Succinate dehydrogenase (SDH), Lactate dehydrogenase (LDH), Glucose-6-phosphate dehydrogenase (G-6-PD), an enzyme of glycolytic pathway, and β-Hydroxy steroid dehydrogenase (3β HSD), a mitochondrial enzyme involved in steroidogenesis, were chosen for present study. Due to scarcity of literature available for these enzymes in CL of buffaloes, the present study was undertaken.

*Corresponding author: kritimakapoor89@gmail.com

MATERIALS AND METHODS

The tissue samples of CL from healthy buffaloes ovaries (n=24) were collected from local slaughter houses. The extracted CL were cleaned, carefully observed and based on their gross morphology categorised into early luteal (stage I, 1 to 5 days), mid luteal (stage II, 6 to 11 days), late luteal (stage III, 12 to 16 days) and regressing phase (stage IV, 17 to 20 days) having 6 ovaries in each group. The fresh unfixed CL collected from different stages of cyclic CL were immediately stored in liquid nitrogen and subjected to cryostat sectioning. Sections of 10-12 μm thickness were obtained on clean glass slides in double. The positive and negative controls were carried out wherever possible. The sections were incubated with different substrates to study the distribution pattern of dehydrogenases i.e., SDH, LDH, 3βHSD and G-6-PDas described in Table 1.

RESULTS AND DISCUSSION

Dehydrogenases: Luteal cells in parenchyma of CL were observed for distribution and localization of dehydrogenases

i.e., SDH, LDH, 3β-HSD and G-6-PD in its different phases of cyclicity of CL.

a) Succinate dehydrogenase (SDH): In early luteal phase i.e., corpus haemorrhagicum, SDH activity was diverse in developing luteal cells. It varied from weak to moderate granular type in developing luteal cells throughout the parenchyma. However, in its subsequent stages, most of the developing large luteal cells and occasional small luteal cells depicted moderate granular activity (Fig. 1a). Afew large luteal cells had strong and blood capillaries had weak to moderate enzyme reaction. Conversely, the SDH activity was negligible in connective tissue septa that divided the parenchyma into lobules.

SDH localization was observed as uniform strong enzyme activity inluteal cells of mid luteal phase. However, its expression followed a specific pattern as the luteal cells towards the centre had strong activity whereas those at periphery had moderate or mixed enzyme activity. The connective tissue capsule that encapsulated the parenchyma and septae arising from it had negligible to weak enzyme activity. The reactivity of enzyme was primarily strong, perinuclear and granular in both large and small luteal cells (Fig. 1b). However, it was moderate or slightly less strong in small luteal cells that comprised the peripheral parenchyma. Similarly, developing granulosa luteal cells in human CL depicted moderate SDH reactivity whereas theca cells depicted no significant staining (Wiley and Esterly, 1976). The higher enzymic activity may correspond to higher secretory activity of luteal cells. SDH plays important role in steroidogenesis as it being closely linked to cytochrome system (Motta and Hafez, 1980).

In late luteal phase CL, SDH expression was observed to be moderate to weak in the luteal cells of parenchyma. In initial stages of this phase, its activity was weak in centre of parenchyma since the process of regression initiated from centre. SDH activity gradually became weak as the luteal cells followed regression throughout the parenchyma in subsequent stages of this phase. Few luteal cells at periphery depicted moderate to strong histoenzymic expression. Wiley and Esterly (1976) also reported that the reactivity for SDH decreased during involution of human CL. Corpus albicans was devoid of SDH activity as the parenchyma mostly comprised of regressed luteal cells and connective tissue (Table 2). Similar findings have been reported by Singh and Roy (1996) in buffalo CL.

b) Lactate dehydrogenase (LDH): LDH activity was weak in developing luteal cells in corpus haemorrhagicum. However, after subsequent development of luteal cells, it was observed as moderate reaction in some large luteal cells (Fig. 2a). In guinea pig CL, LDH activity, involved in final step catalysis in glycolytic pathway, washigh in early stages of estrous but decreased toward the end of the cycle. However, these glycolytic enzyme activities were not significantly correlated to progesterone concentrations in their study (Sasaki and Hanson, 1974).

Table 1

Histoenzymic techniques applied on cryosections of cyclic corpus luteum in buffalo

Sr. No.	Enzyme	Substrate	Method	Reference	Incubation Time
Dehydrogenases					
(i)	Succinic Dehydrogenase (SDH)	Di-Na-succinate	Standard method of bound enzyme by nitro BT method	Pearse (1972)	15 min
(ii)	Lactate Dehydrogenase (LDH)	Na-DL-lactate	Standard method of bound enzyme by nitro BT method	Pearse (1972)	30 min
(iii)	3β-Hydroxy steroid dehydrogenase (3βHSD)	DHA	Standard method of bound enzyme by nitro BT method	Pearse (1972)	30 min
(iv)	Glucose -6-phosphate dehydrogenase (G-6-PD)	Di-Na glucose-6-phosphate	Standard method of bound enzyme by nitro BT method	Pearse (1972)	30 min

The developed luteal cells that comprised the major steroidogenic cells of mid luteal CL depicted moderate to strong cytoplasmic microgranular perinuclear reaction (Fig. 2b). Brinkworth (1978) demonstrated the LDH activity in CL of rat and mouse to establish that it was much greater ingranulosa cells of mouse CL than in rat. LHD activity might be correlated with the synthesis of steroid hormone by luteal cells. In late luteal phase, its activity was very weak to feeble granular enzyme reaction in the cytoplasm of large luteal cells mainly and few small luteal cells. However, it was almost nil in regressed luteal cells observed throughout the parenchyma. Similar observations were made by Singh and Roy (1996).

In corpus albicans phase, its activity was almost negligible in regressed luteal cells and connective tissue that comprised the major component of parenchyma in this phase. However, it was observed in occasional regressing luteal cells and thicker blood vessels (Table 2).

c) 3β-Hydroxy steroid dehydrogenase (3β HSD): In corpus haemorrhagicum, 3β-HSD activity was weak to moderate in most of the developing small and large luteal cells. However, it was moderate to strong in some of the developed large luteal cells and few small luteal cells (Fig.

Table 2

Histoenzymic distribution of Dehydrogenases in cyclic corpus luteum of buffalo

S. No.	Group	Corpus haemorrhagicum (CL–H)			Mid luteal corpus luteum (LCL)			Late luteal corpus luteum			Corpus albicans (CL-AB)		
		LCs	Ct Septa	BVs	LCs	Ct Septa	BVs	LCs	Ct Septa	BVs	LCs	Ct Septa	BVs
1	SDH	+/++	0/+	0/+	+++	++	+	+	+	0	0	0/+	0
2	LDH	+	0	+	++/+++	+	+	+/++	0	0	+	0	+
3	3βHSD	+/++	+	0/+	+++	+	+	+	0/+	0	0	0	0
4	G-6-PD	++	0	0	+++	0	0	0/+	0	0	0	0	0

LCs: Luteal Cells; Ct: Connective tissue; BVs: Blood Vessels
0 Not observed; + Weak; ++ Moderate; +++ Strong

3a). Its weak histoenzymic expression depicted that a lesser amount of enzyme was present in this phase which is requisite for conversion of precursor into progesterone. In CL of hamster, the concentration of 3β HSD was found to be highest on day 1 of cycle with a gradual decline over the subsequent three days (Chatterjee and Greenwald, 1976).

In mid luteal phase, 3βHSD localization followed a specific pattern. It was strong within cytoplasm of both small and large luteal cells located at periphery just adjacent to capsule covering the parenchyma. Conversely, the central parenchyma had weak to moderate activity in most of theluteal cells, although, some of the central luteal cells had strong enzyme localization as a granular perinuclear reaction in their cytoplasm (Fig. 3b). This pattern of cytoplasmic expression of enzyme is attributed to its localization in mitochondria and endoplasmic reticulum of luteal cells and it was correlated with distribution of lipids as its physiological role is to convert lipids present within luteal cells in the form of cholesterol to progesterone (Kapoor *et al.*, 2018). The biological process of progesterone synthesis necessitated two enzymatic steps i.e., initially the conversion of cholesterol to pregnenolone catalyzed by P450 side chain cleavage (P450 scc), found on inner mitochondrial membrane and subsequently, conversion of pregnenolone to progesterone catalyzed by 3β-HSD present in smooth endoplasmic reticulum (SER) (Christenson and Devoto, 2003). In bovine luteal cells, progesterone is involved in its own production not only by increase of 3β-HSD activity, but by influence on the de novo synthesis of 3β-HSD and cytochrome P450 scc enzyme proteins, on days 5-10 and 11-16 of CL. (Rekawiecki *et al.*, 2005).

In late luteal phase, the enzyme activity was reduced considerably and therefore, detected as weak localizationin regressing luteal cells. Deane *et al.* (1966) reported that there was a decline in 3β-HSD enzyme activity that could be responsible for elevation inlipid content at this stage. This observation was correlated with the fact that the absence of this enzyme consequently inhibited the production of progesterone from cholesterol and thus corresponds to increased level of lipids. The enzyme activity was altogether absent in parenchyma of corpus albicans phase, thereby indicating no synthesis of progesterone hormone in this phase. It was nil in blood vessels and other connective tissue stromal components (Table 2).

d) Glucose-6-phosphate dehydrogenase (G-6-PD): The developing luteal cells had weak G-6-PD activity within their cytoplasm in initial stages of corpus haemorrhagicum (Fig. 4a). G-6-PD was more marked in small luteal cells as compared to large luteal cells in the proliferating CL. Subsequently, in the same phase, overall enzyme activity was moderate to strong in developing luteal cells but it followed a mixed type of reaction pattern in such luteal cells. At higher magnification, it was strong in most of the developed small and large luteal cells but moderate in few luteal cells that were in proliferating stage (Fig. 4b). In guinea pig CL, G-6-PDH was found to increase during the proliferation of luteal cells as its increase lead to NADPH production along with ribose provision for nucleic acid synthesis (Sasaki and Hanson, 1974). However, the luteal cells specifically located adjacent to septae and blood capillaries depicted strong activity of enzyme (Fig. 4b).

In mid luteal phase, the parenchyma as a whole had developed small and large luteal cells with strong and intense microgranular reaction within their cytoplasm and nil in the connective tissue septa dividing the parenchyma (Fig. 4c). Sasaki and Hanson (1974) reported that the enzyme activity in guinea pig CL probably peaked from 5 to 10 days when the progesterone concentration stayed at a

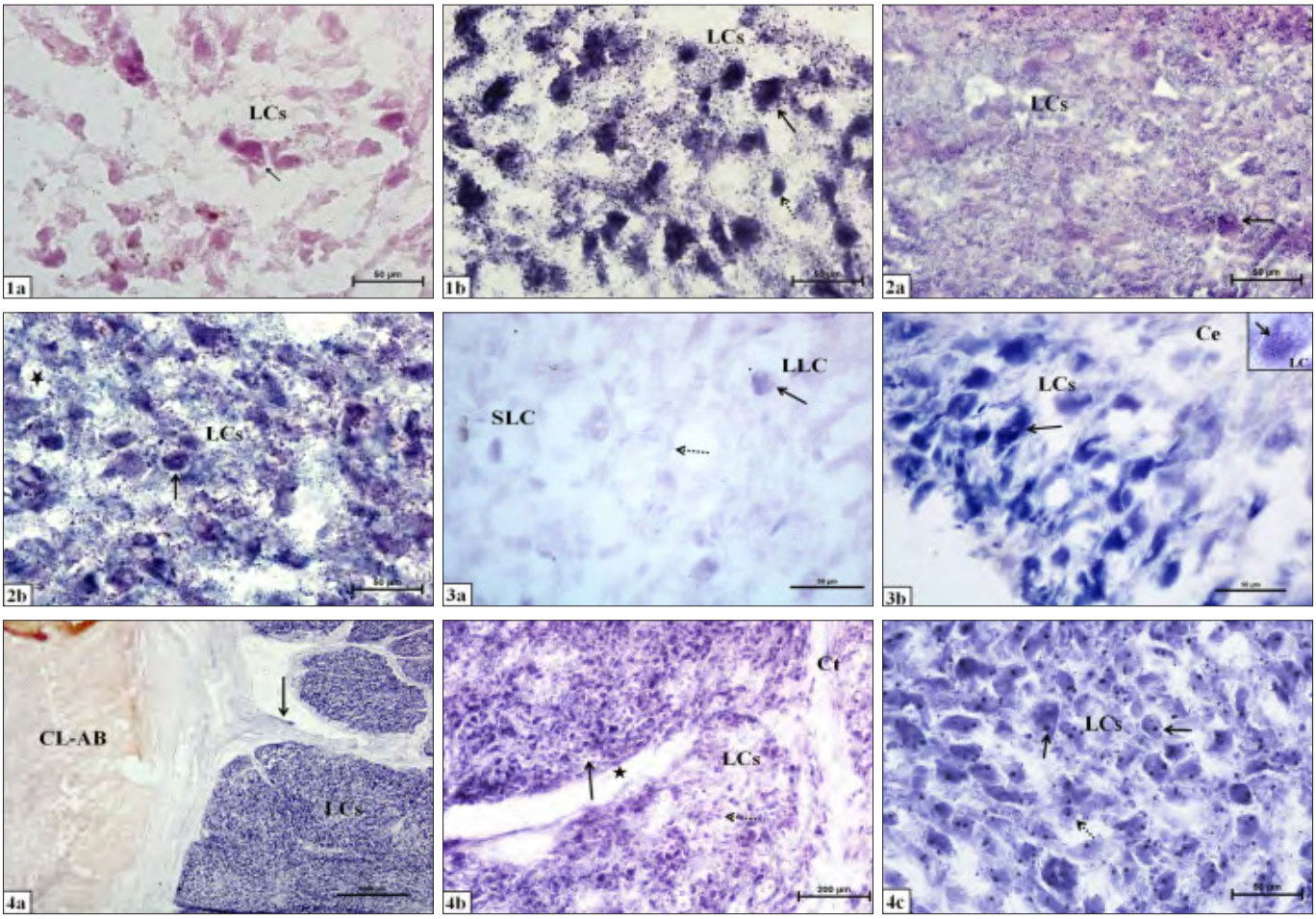

Fig 1a-4c. **(1a)** Photomicrograph of corpus haemorrhagicum showing weak SDH activity in most of the developing luteal cells, moderate activity in few luteal cells (LCs, arrow). Nitro BT method X400. **(1b)** Photomicrograph showing mid luteal CL showing strong SDH activity in perinuclear region of large (arrow) and small (dotted arrow) luteal cells (LCs). Nitro BT method X400. **(2a)** Photomicrograph of corpus haemorrhagicum parenchyma showing weak LDH activity (arrow) in developing luteal cells (LCs). Nitro BT method X400. **(2b)** Photomicrograph of mid luteal CL showing moderate to strong microgranular LDH activity (arrow) in cytoplasm of luteal cells (LCs) and stromal cells, blood vessels (star) had weak reaction. Nitro BT method X400. **(3a)** Photomicrograph of corpus haemmorhagicum depicting weak to moderate 3âHSD activity in developing small (SLC), large luteal cells (LLC) and weak in connective tissue (dotted arrow). Nitro BT method X400. **(3b)** Photomicrograph of mid luteal phase showing strong 3âHSD in luteal cells (LCs, arrow) at periphery and weak in center (Ce). [Inset: Luteal cell (LC) with cytoplasmic granular perinuclear reaction (arrow)] Nitro BT method X400. **(4a)** Photomicrograph of corpus haemorrhagicum showing moderate to strong G-6-PD activity in luteal cells (LCs), weak to moderate in septae (arrow) and no activity in corpus albicans (CL-AB). Nitro BT method X20. **(4b)** Photomicrograph of same stage CL showing strong G-6-PD activity in developed small and large luteal cells (LCs), strong in luteal cells adjacent to septae, weak in some LCs (dotted arrow), connective tissue septae (Ct) and blood capillaries (star). Nitro BT method X100. **(4c)** Photomicrograph of mid luteal CL showing intense cytoplasmic microgranular G-6-PD enzyme reaction in small (dotted arrow) and large luteal cells (LCs; arrow), nil in connective tissue septa. Nitro BT method X400.

high level. In later stages of the same phase, enzyme activity was more intense at periphery and mixed moderate within the central part of parenchyma. Its activity in most of the small and large luteal cells of the central area was intense granular nuclear reaction while it was strong in their cytoplasm. However, some of the luteal and non luteal cells i.e., immune cells depicted moderate activity of the enzyme.

In late luteal phase, G-6-PD was nil in the central degenerating area occupied mainly by connective tissue. In initial part of this phase, its activity was observed as mixed type since the luteal cells near centre depicted weak activity whereas others had moderate activity. Progressively, rest of the parenchyma with luteal cells in regressing phase had weak activity although its activity was still observed as strong reaction in remaining intact luteal cells. Boos (1988) observed that G-6-PD activity was high in small luteal cells during formation and low in large luteal cells of bovine CL and during advanced and late regression no strongly reacting luteal cell was visible. Subsequently, it progressed to corpus albicans phase, the intact luteal cells decreased considerably and thus the strong G-6-PD activity was observed mostly in immune cells recruited at this stage for regression.

The overall G-6-PD activity was reduced considerably in corpus albicans phase. As most of the luteal were

regressed, its activity was feeble to negligible. However, some of the intact luteal cells at periphery had feeble to moderate activity. In complete scar tissue stage,G-6-PD activity was nil in parenchyma. Majumdar and Nandy (1987) proposed that the regressing CL had reduced G6PDH activity in the degrading luteal cells of bubaline cyclic CL.

ACKNOWLEDGEMENT

The authors are thankful to Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana for providing all type of facilities to carry out the study. The funding for this study was provided by College of Veterinary Sciences, GADVASU, Ludhiana.

REFERENCES

Boos, A. (1988). Enzyme histochemistry of bovine luteinized follicular cysts and corpora lutea of estrus cycle. *Zuchthygiene* **23**: 65-77.

Brinkworth, R.I. (1978). On The localization of lactate dehydrogenase in the ovaries and reproductive tracts of rats and mice. *Mech. Ageing Dev.* **8**: 299-310.

Chatterjee, S. and Greenwald, G.S. (1976). Biochemical changes in the corpus luteum of the cyclic hamster. *J. Endocrinol.* **68**: 251-256.

Christenson, L.K. and Devoto, L. (2003). Cholesterol transport and steroidogenesis by the corpus luteum. *Reprod. Biol. Endocrinol.* **1(90)**: 1-9.

Deane, H.W., Hay, M.F., Moor, R.M., Rowson, L.E.A. and Short, R.V. (1966). The corpus luteum of the sheep: relationships between morphology and function during the oestrous cycle. *Acta Endocrinologica.* **51**: 245-263.

Kumar, D., Singh, I. and Singh, U. (2013). Ovarian follicular dynamics in relation to presence of corpus luteum in buffaloes. *Haryana Vet.* **52(2)**: 99-102.

Kapoor, K., Singh, O. and Pathak, D. (2018). Lipid distribution variations in different stages of cyclic corpus luteum of Indian buffalo. *J. Anim. Res.* **8(3)**: 379-385.

Majumdar, A.C. and Nandy, D.K. (1987). Histochemical studies on steroidogenesis in cyclicbubaline corpus luteum. *Indian J. Anim. Sci.* **57**: 1086-1088.

Motta, P.M. and Hafez, E.S.E. (1980). Biology of the Ovary. In: Developments in Obstetrics and Gynecology, 2nd Vol., Springer, Dordrecht. pp. 150-161.

Pearse, A.G.E. (1972). Histochemistry: Theoritical and Applied. (3rd Edn.), Vol. II, Churchill Livingstone, London.

Rekawiecki, R., Nowik, M. and Kotwica, J. (2005). Stimulatory effect of LH, PGE2 and progesterone on StAR protein, cytochrome P450 cholesterol side chain cleavage and 3ß hydroxysteroid dehydrogenase gene expression in bovine luteal cells. *Prostag. Oth. Lipid. M.* **78**: 169–184.

Sasaki, Y. and Hanson, G.C. (1974). Glucose oxidation in corpus luteum and the concentration of sex steroids in systemic plasma during the reproductive cycle of the guinea pig. *Endo.* **95(5)**: 1213-1218.

Singh, O. and Roy, K.S. (1996). Histoenzymic studies on oxidoreductases in cyclic corpus luteum of the Indian buffalo. *Buffalo J.* **3**: 399-403.

Warriach, H.M., McGill D.M., Bush, R.D., Wynn, P.C. and Chohan, K.R. (2015). A Review of recent development in buffalo reproduction. A Review. *Asian Austras. J. Anim. Sci.* **28(3)**: 451-455.

Wiley. C. A. and Esterly, J.R. (1976). Observations on the human corpus luteum: Histochemical changes during development and involution. *Am. J. Obstet. Gynecol.* **125(4)**: 514-519.

CONTRIBUTORS MAY NOTE

- Research/Clinical articles are invited for next issue from the Scientists/Veterinarians engaged in Veterinary Profession.
- Please follow strictly the format of 'The Haryana Veterinarian' for manuscript writing/ submission.
- Please pay processing fee of Rs. 1000/- online in the account of Dean, College of Veterinary Sciences, along with each article.
- After revision, please return the revised manuscript and rebuttal at the earliest.
- Please mention your article reference number in all correspondence for a quick response.
- We solicit your co-operation.
- All correspondence should be addressed to 'The Editor', Haryana Veterinarian, Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences, LUVAS, Hisar-125004.

Editors