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Enzyme histochemistry of uterine tube of buffalo during follicular and luteal phase: A seasonal variation

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ABSTRACT

Enzyme histochemistry on uterine tube of buffalo during summer and winter season was conducted in different phases of estrus cycle in relation to reproductive efficiency. Fresh tissues of buffalo uterine tubes were collected from infundibulum, ampulla and isthmus immediately after slaughtering. Cryostat sections were obtained and incubated to find variation in localization of various enzymes. AKPase showed strong to intense activity in winter and strong during summer season in the *lamina epithelialis* whereas negligible to weak reaction was found in *lamina propria*, *propria submucosa*, *tunica muscularis* and *tunica serosa* of uterine tube during both the seasons. Succinic dehydrogenase showed strong reaction in winter and moderate to strong during summer in the *lamina epithelialis* of uterine tube whereas reaction was moderate to strong in *tunica muscularis*, weak in the tunica serosa and negligible to weak in the *lamina propria* of uterine tube during both the seasons. G-6-P-D showed strong to intense reaction during winter and summer in the *lamina epithelialis* of uterine tube whereas reaction was negligible to weak in *lamina propria*, weak in *propria submucosa*, moderate to strong in the *tunica muscularis* and negligible to weak in the *tunica serosa* of uterine tube. NADH-diaphorase and NADPH- diaphorases showed intense reaction during both summer and winter in lamina epithelialis, moderate to strong reaction in *tunica muscularis*, weak to moderate reaction in *lamina propria* and *propria submucosa*, and weak reaction in *tunica serosa* of infundibulum, ampulla and isthmus during both the seasons. The activity of the enzymes was found to be more in winter and in luteal phase as compared to summer and follicular phase.

Keywords: Buffalo, Histoenzyme, Summer uterine tube, Winter

Milk production in India was 230.58 million tons during 2022-23 which increased by 3.83% from the year 2016-17 to 2022-23 (BAHS 2023). Buffaloes are considered as sluggish breeders and their productivity is hampered by many factors including late maturity, poor expression of estrus cycle, silent heat, poor conception rate and increased inter-calving intervals (Madan 1988). Although buffaloes are polyestrous animals and show estrous throughout the year yet they exhibit distinct seasonal pattern (Madan 1990). Reproductive performance of buffaloes is highly declined during summer and it has been observed that temperature humidity index (THI) above 75 had negative effect on the reproductive performance of buffaloes (Vale 2007). The bovine uterine tube is divided into three parts, i.e. infundibulum, ampulla and isthmus. The epithelium lining of uterine tube was consisted of ciliated and non-ciliated cells. The ciliated cells help in transport of gamete and embryo, whereas the secretory cells are involved in secretion of the uterine fluid (Mokhtar 2015). The success rate of fertilization and early embryonic development is dependent upon optimum environment which facilitates sperm transport and capacitation, fertilization and early cleavage (Hollis *et al.* 1984). Alkaline phosphatase is a lysosomal enzyme that catalyzes various reactions in the body and is involved in the active transport of protein and DNA turnover in the nucleus (Mishra *et al.* 2003). Several enzymes like dehydrogenases are involved in the cellular conversion of cholesterol into progesterone. Dehydrogenases of glycolytic and tricarboxylic acid (TCA, i.e. Krebs cycle) pathways play an essential role in providing the energy needed for various metabolic activities of somatic and germ cells. SDH has an important role in steroidogenesis as it is being closely linked to cytochrome system (Motta and Hafez 1980). The activity of NADH – diaphorase is correlated with mitochondrial activity within the cytoplasmic electron transport system (Chayen *et al.* 1969). The localization and reactivity of different enzymes vary during different phases of estrus cycle and during different seasons and they have significant role in reproductive physiology. So the present study was carried out to explore different enzymes during summer and winter seasons.

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MATERIALS AND METHODS

The tissue samples of uterine tube (n=12) of adult buffalo aged between 3 to 7 years were collected from slaughter house and post-mortem hall, GADVASU, Ludhiana. Six samples were collected during summer (Mid April to Mid July) and six during winter season (Mid November to Mid February). Out of six samples, three were in follicular and luteal phase each. Groupings were done based on the ovarian structure. Ovaries with well-developed *corpus luteum* were classified into luteal phase and ovaries with dominant follicle without a *corpus luteum* were grouped into follicular phase samples. The uterine tube was examined for any gross pathological lesions before collection. Fresh unfixed tissues of buffalo uterine tubes were collected from infundibulum, ampulla and isthmus immediately after slaughter. Cryostat sections (10-12 µm thickness) were obtained at -20°C on clean glass slides and were incubated to observe distribution of enzymes such as Alkaline phosphatase (Barka and Anderson 1963), Glucose-6 phosphate dehydrogenase, Succinic dehydrogenase, reduced Nicotinamide adenine dinucleotide phosphate diaphorase and reduced Nicotinamide adenine dinucleotide diaphorase (Pearse 1972). The qualitative grading of enzyme reaction was done on the basis of intensity of enzyme localization and it was interpreted and graded as follows: negligible to weak reaction (0/+), weak reaction (+), moderate reaction (++), strong reaction (+++) and intense reaction (++++).

RESULTS AND DISCUSSION

Alkaline phosphatase (AKPase): The lamina epithelialis in all the parts of the uterine tube gave positive reaction to AKPase during follicular and luteal phases in both the seasons (Table 1). Abdalla (1968) also detected the AKPase activity at free border of the tubal epitheliumin goat. Bjorkman and Fredricsson (1961) suggested that the presence of this enzyme might be responsible for resorptive function of the ciliated cells. The lining epithelium of the mucosal folds of infundibulum gave intense reaction to AKPase during the winter luteal phase (Fig. 1A) and strong to intense reaction during the winter follicular phase, strong during the summer luteal phase (Fig. 1B) and moderate to strong during the summer follicular phase. In case of ampulla and isthmus, the lining epithelium showed intense reaction to AKPase during the winter luteal phase, strong

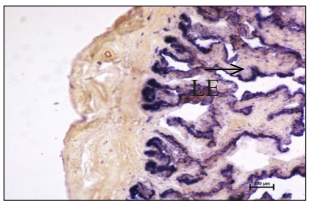

Fig. 1(A). Intense AKPase reaction in the lining epithelium (LE) of infundibulum of buffalo during winter luteal phase. Azo dye method ×100

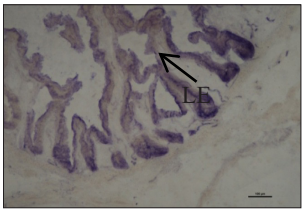

Fig. 1(B). Strong AKPase reaction in the lining epithelium (LE) of infundibulum of buffalo during summer luteal phase. Azo dye method ×100

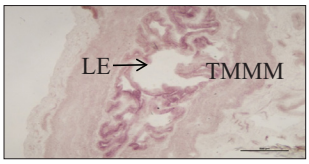

Fig. 1(C). Intense reaction of SDH in the lining epithelium (LE) and moderate reaction in the *tunica muscular* is (TMM) of isthmus of buffalo during winter luteal phase). Nitro BT method×40

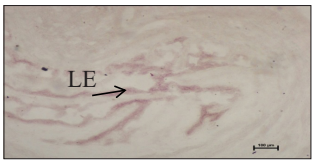

Fig. 1(D). Strong to intense reaction of SDH in the lining epithelium (LE) of isthmus of buffalo during summer luteal phase. Nitro BT method×100

Table 1. Histoenzymic localization of Alkaline phosphatase in buffalo uterine tube during follicular and luteal phase during summer and winter

Compartment	Layer	Alkaline phosphatase			
		Winter follicular	Winter luteal	Summer follicular	Summer luteal
Infundibulum	*Lamina epithelialis*	+++/++++	++++	++/+++	+++
	Lamina propria	+	+	0/+	0/+
	Propria submucosa	+	+	0/+	0/+
	Tunica muscularis	0/+	0/+	0/+	0/+
	Tunica serosa	0/+	0/+	0/+	0/+
Ampulla	*Lamina epithelialis*	+++/++++	++++	+++	+++/++++
	Lamina propria	+	+	+	+
	Propria submucosa	+	+	+	+
	Tunica muscularis	+++	0/+	++/+++	0/+
	Tunica serosa	0/+	0/+	0/+	0/+
Isthmus	*Lamina epithelialis*	+++/++++	++++	+++	+++/++++
	Lamina propria	++	++	+/++	++
	Propria submucosa	+/++	+/++	+	++/++
	Tunica muscularis	0/+	0/+	0/+	0/+
	Tunica serosa	0/+	0/+	0/+	0/+

0: Negligible, +: Weak, ++: Moderate, +++: Strong, ++++: Intense

to intense during the summer luteal phase, winter follicular phase and strong during the summer follicular phase. The *lamina propria* and *propria submucosa* of infundibulum showed weak reaction to AKPase during both the phases in winter and negligible to weak reaction during summer. Irrespective of the phases of estrus cycle and seasons, the *lamina propria* and *propria submucosa* showed weak reaction to AKPase in ampulla. The lamina propria showed moderate activity during both the phases in winter and summer luteal phase and weak to moderate during the summer follicular phase in isthmus. AKPase activity was weak to moderate in the *propria submucosa* of isthmus during both the phases in winter and during the summer luteal phase. In summer follicular phase, weak reaction was observed in the *propria submucosa* of isthmus. Irrespective of the phases of estrus cycle and seasons, negligible to weak AKPase activity was found in the *tunica muscularis* of the infundibulum and isthmus whereas the ampulla showed strong AKPase reaction during winter follicular phase and moderate to strong during the follicular phase in summer and negligible to weak reaction during luteal phase in both seasons. In the *tunica muscularis*, AKPase was present in the connective tissue surrounding the muscle fiber bundle which could be seen as fine granules in this layer. The *tunica serosa* showed negligible to weak reaction to AKPase in all the parts of the uterine tube during both the seasons in both phases.The mesothelial lining of the tunica serosa and the blood vessels gave positive reaction to AKPase. The pseudoglands underlying the mucosa gave intense reaction to AKPase during both the phases during both the seasons throughout the uterine tube. The reaction was seen in the apical portion and cytoplasm of the glandular epithelium. AKPase activity was found to be more in the distal or the basal parts of the mucosal folds than in the upper parts. The enzyme activity was found to more intense in isthmus than in the other parts of the uterine tube. Bjorkman and Fredricsson (1961) in bovine reported that alkaline phosphatase activity at the free border of the cells was more apparent in the isthmus region than in the ampullary region. They suggested that this activity may be due to the uterine secretions. The intensity of the enzyme reaction was more during winter as compared to summer. The reaction was found to be more during luteal phase as compared to follicular phase. Zamiri and Blackshaw (1979) reported that many physiological processes, including the synthesis of fibrous proteins, DNA turnover in the nucleus, and secretion, have been implicated to alkaline phosphatase.

Bjorkman and Fredricsson (1961) reported that alkaline phosphatase might be concerned with the transport of substances either into or more likely out of the cells. Increase activity of this enzymes during the secretory phase showed that they are directly concerned with secretion of the oviduct, which may be due to the more ionic movement in the endothelium of blood vessels. The present findings are in agreement with Uppal and Roy (2007) in buffalo fetus and Khillare *et al.* (2013) in Japanese quail.

Succinic dehydrogenase (SDH): The lamina epithelialis of infundibulum, ampulla and isthmus gave intense reaction to SDH during the luteal phase in winter (Fig. 1C) and strong to intense reaction during the luteal phase in summer (Fig. 1D). Strong SDH reaction was seen during the winter follicular phase and moderate to strong during summer follicular phase (Table 2). Foraker and Wingo (1956) observed moderate succinic dehdrogenase activity in the epithelium of the mucosal folds of the fallopian tube in rats. They found that the activity was more evident in the cells of the fimbrae than in the rest of the tube.

The *lamina propria* and *propria submucosa* of infundibulum and ampulla showed weak SDH reaction during luteal phase in both winter and summer and negligible to weak reaction during the follicular phase in summer and winter. Weak SDH activity was recorded in

Table 2. Histoenzymic localization of Succinic dehydrogenase in buffalo uterine tube during follicular and luteal phase during summer and winter

Compartment	Layer	SDH			
		Winter follicular	Winter luteal	Summer follicular	Summer luteal
Infundibulum	*Lamina epithelialis*	+++	++++	++/+++	+++/++++
	Lamina propria	0/+	+	0/+	+
	Propria submucosa	0/+	+	0/+	+
	Tunica muscularis	++	++/+++	+/++	++
	Tunica serosa	+	+	+	+
Ampulla	*Lamina epithelialis*	+++	++++	++/+++	+++/++++
	Lamina propria	0/+	+	0/+	+
	Propria submucosa	0/+	+	0/+	+
	Tunica muscularis	++	++	++	++
	Tunica serosa	+	+	+	+
Isthmus	*Lamina epithelialis*	+++	++++	++/+++	+++/+++
	Lamina propria	+	+	0/+	0/+
	Propria submucosa	+	+	+	+
	Tunica muscularis	+/++	++	+	+/++
	Tunica serosa	+	+	+	+

0 : Negligible, + :Weak, ++: Moderate, +++ :Strong, ++++: Intense

the *lamina propria* of isthmus during both the phases in winter and negligible to weak reaction during summer. The *propria submucosa* of isthmus showed weak activity during both winter and summer. Similarly, Foraker and Wingo (1956) found weak SDH activity in the stroma of fallopian tube of rats. The pseudoglands of infundibulum and ampulla gave strong reaction to SDH in all the parts of uterine tube throughout the estrus cycle in both the seasons. The oviductal glands underlying the mucosa of isthmus gave strong positive reaction to SDH during both the phases in both summer and winter. The reaction was seen in the glandular epithelium. The *tunica muscularis* of infundibulum showed moderate to strong SDH activity during the winter luteal phase, moderate activity in winter follicular phase, summer luteal phase and weak to moderate during the summer follicular phase. SDH activity was moderate in *tunica muscularis* of ampulla throughout the estrus cycle in both seasons. In case of isthmus, the *tunica muscularis* showed moderate SDH activity during the winter luteal phase (Fig. 1C), weak to moderate during the winter follicular phase and summer luteal phase and weak reaction during the summer follicular phase. Foraker and Wingo (1956) observed weak succinic dehydrogenase activity in the smooth muscles in the *tunica muscularis* layer in the fallopian tube of rat. The *tunica serosa* was weakly stained with SDH irrespective of season and reproductive phases in all the compartments of uterine tube. The blood vessels gave positive reaction to SDH. The staining intensity of the enzyme was found to be more during winter as compared to winter. Greater activity of SDH was observed during the luteal phase as compared to follicular phase. The variation in activity of SDH in buffalo oviduct in relation to phase and season reflects decreased mitochondrial localization and development of kreb's cycle (Uppal and Roy 2007). SDH has an important role in steroidogenesis as it is being closely linked to cytochrome system (Motta and Hafez 1980).

Glucose-6-phosphate dehydrogenase (G-6-P-D): The lining epithelium of the infundibulum and ampulla gave intense reaction to G-6-P-D during the winter luteal phase strong to intense in summer luteal phase and winter follicular phase and strong during the summer follicular phase (Table 3). The lining epithelium of isthmus gave intense reaction to G-6-P-D during winter luteal phase (Fig. 2A), strong to intense during the summer luteal phase (Fig. 2B) and strong during follicular phase in both winter and summer. Negligible to weak G-6-P-D reaction was detected in the connective tissue fibers of infundibulum and ampulla throughout the estrus cycle in both summer and winter whereas weak reaction in the mucosal folds of isthmus in both seasons. The reaction was weak in *propria submucosa* of infundibulum and ampulla throughout the estrus cycle during both the seasons whereas in case of isthmus it was moderate in follicular phase in both seasons and weak in luteal phase in both seasons. An intense reaction for G-6-P-D was observed in the pseudoglands underlying the mucosa of infundibulum during the luteal phase in both summer and winter and strong reaction during the follicular phase in both summer and winter was observed. The reaction was seen in the glandular epithelium. The pseudoglands gave intense reaction to G-6-P-D during both summer and winter in ampulla.The lining epithelium of the oviductal glands at the base of mucosal folds of isthmus gave intense reaction to G-6-P-D during both the phases in both summer and winter. The *tunica muscularis* gave moderate to strong reaction to G-6-P-D irrespective of estrus cycle and seasons in the entire uterine tube (Fig. 2A-2B). Negligible to weak reaction was noticed in the *tunica serosa* of infundibulum and ampulla regardless of season and phases of estrus cycle. In isthmus, the *tunica serosa* reacted weakly to G-6-P-D during the luteal phase and negligible to weak reaction during the follicular phase in both summer and winter. However, the connective tissue cells and the blood vessels in the *tunica serosa* layer gave

Table 3. Histoenzymic localization of G-6-P-D in buffalo uterine tube during follicular and luteal phase during summer and winter

Compartment	Layer	Winter follicular	Winter luteal	Summer follicular	Summer luteal
Infundibulum	*Lamina epithelialis*	+++/++++	++++	+++	+++/++++
	Lamina propria	0/+	0/+	0/+	0/+
	Propria submucosa	+	+	+	+
	Tunica muscularis	++/+++	++/+++	++/+++	++/+++
	Tunica serosa	0/+	0/+	0/+	0/+
Ampulla	*Lamina epithelialis*	+++/++++	++++	+++	+++/++++
	Lamina propria	0/+	0/+	0/+	0/+
	Propria submucosa	+	+	+	+
	Tunica muscularis	++/+++	++/+++	++/+++	++/+++
	Tunica serosa	0/+	0/+	0/+	0/+
Isthmus	*Lamina epithelialis*	+++	++++	+++	+++/++++
	Lamina propria	+	+	+	+
	Propria submucosa	++	+	++	+
	Tunica muscularis	++/+++	++/+++	++/+++	++/+++
	Tunica serosa	0/+	+	0/+	+

0: Negligible, + : Weak, ++: Moderate, +++: Strong, ++++: Intense

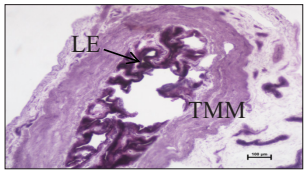

Fig. 2(A). Intense G-6-P-D reaction in the lining epithelium (LE), blood vessels and moderate to strong reaction in the *tunica muscularis* (TMM) of Isthmus of buffalo during winter luteal phase ftt. Nitro BT method×100

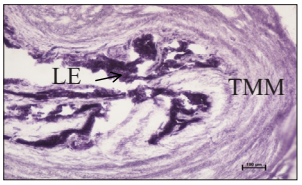

Fig. 2(B). Strong to intense G-6-P-D reaction in the lining epithelium (LE) and moderate to strong reaction in the *tunica muscularis* (TMM) of Isthmus of buffalo during summer luteal phase. Nitro BT method×100

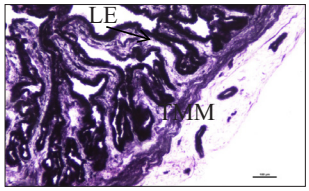

Fig. 2(C). Intense NADH-d reaction in the lining epithelium (LE) and strong reaction in the *tunica muscularis* (TMM) of Infundibulum of buffalo during winter follicular phase. Nitro BT method×200

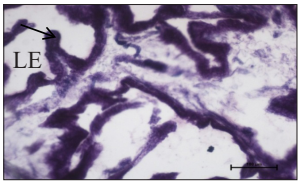

Fig. 2D. Intense NADH-d reaction in the lining epithelium (LE) of Infundibulum of buffalo during summer follicular phase. Nitro BT method×200

strong reaction to G-6-P-D in all the parts of the uterine tube. The mesothelial lining of the *tunica serosa* gave moderate reaction to G-6-P-D. The reaction intensity was strongest in the isthmus than the other parts of the uterine tube. The reaction intensity was more during winter as compared to summer and more during luteal phase. This enzyme is responsible for conversion of glucose to acetyl coenzyme-A which is utilized for the active synthesis of fatty acids and steroids

The presence of G-6-PD enzyme activity indicates the role of pentose phosphate shunt needed for production and utilization of nucleic acids during cell growth. This enzyme is involved in the pentose phosphate pathway and convert Glucose-6-phosphate into 6-phosphogluconolactone which is necessary for the nucleic acid (ribose and deoxyribose) synthesis (Rama Rao 1994).

Nicotinamide adenine dinucleotide diaphorase (NADH-D): A very strong reaction for NADH-D was observed in the lining epithelium of infundibulum (Figs 2C-2D), ampulla and isthmus during both the phases in both the seasons (Table 4). However, the reaction was more intense during the luteal phase in all the compartments of uterine tube irrespective of the seasons.

NADH-D activity was negligible to weak in the *lamina propria* of the infundibulum during the winter luteal phase and both the phases in summer whereas weak during the winter follicular phase (Fig. 2C). The *propria submucosa* of infundibulum gave weak reaction to NADH-D during both the phases in winter and negligible to weak during both the phases in summer. Irrespective of the estrus cycle phases and seasons, negligible to weak activity was found in the *lamina propria* and *propria submucosa* of ampulla. Moderate NADH-D activity was observed in isthmus during both the phases in winter and weak activity during both the phases in summer. The apical portion of the pseudoglands of infundibulum and ampulla showed an intense staining reaction regardless of the season and reproductive phases. *Tunica muscularis* showed strong reaction in infundibulum and ampulla during both winter (Fig. 2C) and summer (Fig. 2D). Strong to intense activity was observed in the tunica muscularis of isthmus during the winter luteal phase strong during the winter follicular phase and moderate to strong during both the phases in summer NADH-D activity was very weak in the tunica serosa regardless of the season and phases of estrus cycle). The blood vessels in all the segments of uterine tube stained intensely to NADH-D The connective tissue cells in the *tunica serosa*

Table 4. Histoenzymic localization of NADH-D in buffalo uterine tube during follicular and luteal phase during summer and winter

Compartment	Layers	NADH-D			
		Winter follicular	Winter luteal	Summer follicular	Summer luteal
Infundibulum	*Lamina epithelialis*	++++	++++	++++	++++
	Lamina propria	+	0/+	0/+	0/+
	Propria submucosa	+	+	0/+	0/+
	Tunica muscularis	+++	+++	+++	+++
	Tunica serosa	+	+	+	+
Ampulla	*Lamina epithelialis*	+++	+++	++++	+++
	Lamina propria	0/+	0/+	0/+	0/+
	Propria submucosa	0/+	0/+	0/+	0/+
	Tunica muscularis	+++	+++	+++	+++
	Tunica serosa	+	+	+	+
Isthmus	*Lamina epithelialis*	++++	++++	++++	++++
	Lamina propria	++	++	+	+
	Propria submucosa	++	++	+	+
	Tunica muscularis	+++	+++/++++	++/+++	++/+++
	Tunica serosa	+	+	+	+

0 : Negligible, + : Weak, ++ : Moderate, +++ : Strong, ++++ : Intense

reacted positively to NADH-D. No staining difference was observed in the phases of estrus cycle and in seasons.

Strong activity indicates higher metabolic activity in the cellular proliferation of *lamina epithelialis*. Similar finding have been reported in oviduct of buffalo foetus by Uppal and Roy (2007). This enzyme is related with the steroidogenic activity. The activity of NADH – diaphorase is correlated with mitochondrial activity within the cytoplasmic electron transport system (Chayen *et al.* 1969).

Nicotinamide adenine dinucleotide phosphate – diaphorase (NADPH-D): The lamina epithelialis of infundibulum showed intense reaction to NADPH-D during the winter luteal phase, whereas strong to intense reaction during the summer luteal phase and follicular phase in winter (Fig. 3A) and strong activity during the summer follicular phase (Fig. 3B) (Table 5). The lining epithelium of ampulla showed intense NADPH-D reaction during both the phases in winter (Fig. 3C) and strong reaction during summer (Fig. 3D). Intense staining reaction was detected in the *lamina epithelialis* of isthmus irrespective of the season and reproductive phases. Regardless of the estrus cycle phase and season, very weak reaction was found in the *lamina propria* and *propria submucosa* of infundibulum and ampulla and isthmus during winter. The *propria submucosa* of isthmus showed moderate to strong reaction during the luteal phase in both seasons, moderate reaction in the winter follicular phase and weak to moderate during the summer follicular phase. Strong positive reaction was observed in the epithelium of the pseudoglands in infundibulum and ampulla. The epithelium of the pseudoglands showed an intense staining reaction during the luteal phase in winter, strong to intense during the winter follicular phase and summer luteal phase and strong during the summer follicular phase. In case of ampulla, intense reaction was observed in the glandular epithelium during both the phases in winter and strong during both the phases in summer. NADPH reaction was moderate to strong in tunica muscularis of infundibulum during both the phases in winter (Fig. 3A) and moderate reaction during summer in both the phases (Fig. 3B). Irrespective of the estrus cycle phases and seasons, the *tunica muscularis* reacted moderately to NADPH in ampulla (Fig. 3C-D). In case of isthmus, moderate to strong reaction was seen during the luteal phase in both seasons and moderate during the

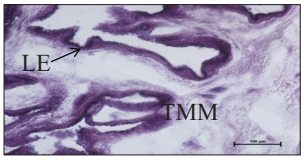

Fig. 3(A). Strong to intense NADPH-d reaction in the lining epithelium (LE) and moderate reaction in the *tunica muscular* is (TMM) of Infundibulum of buffalo during winter follicular phase. Nitro BT method×100

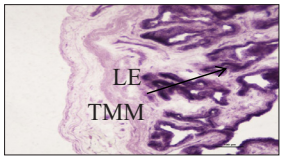

Fig. 3(B). Strong NADPH-d reaction in the lining epithelium (LE) and moderate reaction in the *tunica muscular* is (TMM) of Infundibulum of buffalo during summer follicular phase. Nitro BT method×200

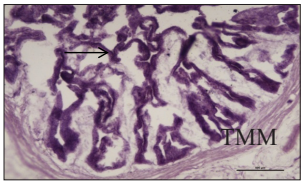

Fig. 3(C). Intense NADPH-d reaction in the lining epithelium (LE) and moderate reaction in the *tunica muscular* is (TMM) of ampulla of buffalo during winter luteal phase. Nitro BT method× 200

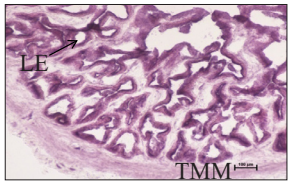

Fig. 3(D). Strong NADPH-d reaction in the lining epithelium (LE) and moderate reaction in the *tunica muscular* is (TMM) of ampulla of buffalo during summer luteal phase. Nitro BT method×100

Table 5. Histoenzymic localization of NADPH-D in buffalo uterine tube during follicular and luteal phase during summer and winter

Compartment	Layer	NADPH-D			
		Winter follicular	Winter luteal	Summer follicular	Summer luteal
Infundibulum	*Lamina epithelialis*	+++/++++	++++	+++	+++/++++
	Lamina propria	0/+	0/+	0/+	0/+
	Propria submucosa	0/+	0/+	0/+	0/+
	Tunica muscularis	++/+++	++/+++	++	++
	Tunica serosa	+	+	+	+
Ampulla	*Lamina epithelialis*	++++	++++	+++	+++
	Lamina propria	0/+	0/+	0/+	0/+
	Propria submucosa	+	+	+	+
	Tunica muscularis	++	++	++	++
	Tunica serosa	+	+	+	+
Isthmus	*Lamina epithelialis*	++++	++++	++++	++++
	Lamina propria	+	+	0/+	0/+
	Propria submucosa	++	++/+++	+/++	++/+++
	Tunica muscularis	++	++/+++	++	++/+++
	Tunica serosa	+	+	+	+

0 : Negligible, + :Weak, ++: Moderate, +++ :Strong, ++++: Intense

follicular phase in both seasons The tunica serosa reacted weakly to NADPH-D in all the compartments of uterine throughout the estrus cycle in both summer and winter. Irrespective of the estrus cycle phases and seasons, the blood vessels) and the connective tissue cells in the *tunica serosa* layer showed positive reaction to NADPH in the uterine tube. Similar activity was reported by Sukhdave and Bansal (2017) in quail.

While comparing both the seasons it was found that the staining intensity was more during winter as compared to summer. The reaction was more in luteal phase as compared to follicular phase. The increased activity of NADPH during luteal phase might be correlated to the increased secretory activity of the cells as the enzyme is responsible for conversion of cholesterol to progesterone (Sorenson and Singh 1973).

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