HISTOENZYMIC STUDIES ON THE OVARY OF INDIAN BUFFALO DURING DIFFERENT REPRODUCTIVE STAGES

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ABSTRACT

Distribution pattern of phosphatases, oxidoreductases and non-specific esterases were studied in ovary of prepubertal, follicular phase, luteal phase and in pregnant animals. In the ovary of prepubertal buffaloes, distribution of phosphatases (AKPase and G-6-Pase) was negligible in the surface epithelium, tunica albuginea and stroma in the cortex region while it was weak during the follicular, luteal and pregnant animals. Its activity was strong in the primordial and primary follicles in all the phases of the reproduction. Strong to intense reaction was observed in the theca cells and granulosa cells. The luteal cells and blood vessels in corpus luteum of luteal phase showed moderate reactions while strong reaction was observed in the pregnant buffaloes. The enzyme reaction of dehydrogenases revealed that the steroidogenic cells were more active during follicular phase, luteal phase and pregnancy while weak activity was observed in the surface epithelium and tunica albuginea. Reactivity of diaphorases were weak to moderate in surface epithelium and tunica albuginea, moderate activity in theca cells and oocyte and intense reaction in granulosa cells of tertiary follicles and weak activity in connective tissue septa and intense in luteal cells in parenchyma of corpus luteum of buffalo. Activity of NSE was strong in the granulosa cells and corona radiate cells of secondary and tertiary follicles while it was moderate in the theca cells. Most of the enzymes were more active in pubertal and pregnant buffaloes compared with prepubertal buffaloes and could be correlated with the steroid synthesis. The difference in the intensity of enzyme in different compartments of ovary also correlated with the cells participating in the hormone synthesis.

Keywords: *Bubalus bubalis*, buffalo, histoenzymic study, ovary, reproductive stages

INTRODUCTION

Buffalo is one of the important livestock resources of India and it plays a vital role in improving the socio-economic status of rural masses. Buffalo can be considered as a pillar for the development of dairy industry in India as the species is a major contributor in country's milk production (56.00% of total milk), despite the fact that they constitute only 34.60% of total bovine population, and this can be attributed to steady increase in buffalo population in India during last two decades, which is about 1.93% (FAO, 2012). Delayed puberty, poor expression of estrus, high incidence of early embryonic mortality, long inter-calving period and seasonal variation in fertility compromise the productivity of buffalo (*Bubalus*

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bubalis). A coordinated relationship exists between hypothalamus, pituitary and ovary of animals and ovary undergoes changes in its activity of cell types based on influences from hypothalamus and pituitary at different stages of reproduction. The location and the reactivity of various enzymes vary during different stages of reproduction and they play important role at each stage of reproductive physiology. The complete study of various enzymes during different stages of reproduction is required to understand the follicular development and steroidogenic activity of ovaries in buffaloes. Thus the present investigation was designed to elucidate phosphatases, oxidoreductases and non-specific esterases in ovary during prepubertal, follicular phase, luteal phase and pregnant buffaloes.

MATERIALS AND METHODS

The ovaries of buffaloes (n = 52) were collected from New Delhi Municipal Corporation Abattoir, Gajipur; M.K. Overseas Pvt. Ltd., Dera Basi, local abattoir at Bareilly and Teaching Veterinary Clinical Complex, GADVASU, Ludhiana. They were classified into four groups, prepubertal (based on the history and age of the animal), follicular phase (presence of a dominant follicle on the surface and absence of corpus luteum), Luteal phase (presence of a fully developed corpus luteum), pregnant (presence of foetus in the uterus).

Fresh unfixed tissues from ovary of Indian buffaloes were collected from slaughter houses immediately after the slaughter of the animals and placed in tissue freezing medium (Leica) and frozen in liquid nitrogen. Cryostat sections of 6 to 7 µm thicknesses at -20°C were obtained on glass slides with cryostat microtome and incubated with different substrates for the demonstration of phosphatases, oxidoreductases, esterases as described in Table 1. The positive and negative controls were carried out wherever possible.

RESULTS AND DISCUSSION

Distribution of phosphatases, oxidoreductases and non-specific esterases were studied in ovary of prepubertal (Table 2), follicular phase (Table 3), luteal phase (Table 4) and in pregnant animals (Table. 5).

Phosphatases

Alkaline phosphatases (AKPase)

In the ovary of prepubertal buffaloes, distribution of AKPase was negligible in the surface epithelium, tunica albuginea and stroma in the cortex region while it was weak during the follicular, luteal and pregnant animals. Its activity was strong in the primordial and primary follicles in all the phases of the reproduction (Figure 1(a)). AKPase is known for synthesis and transport of substances across the cell membrane and thus it might be required for growth and differentiation of ovarian follicles. Roy and Uppal (2005) discussed that this enzymes occurred in cells specialized for endocytosis and pinocytosis. This enzyme has been found to be involved in energy transfer reactions (Freeland and Szepesi 1971). Strong to intense reaction was observed in the theca cells and granulosa cells (Figure 1(b)). Weak reaction was observed in the oocyte and zona pellucida while intense reactions were noted in the corona radiate cells in all the stages of reproductive cycle (Figure 1(c)). The distribution of this enzyme was moderate in the ovarian medulla during prepubertal period while weak to moderate during all pubertal stages.

Table 1. Histoenzymic techniques used on cryostat sections of ovary.

Sr. No	Enzyme	Substrate	Method	Incubation Time (minute)	Reference
A. Phosphatases					
i.	Alkaline phosphatise (AKPase)	Naphthol AS-MX phosphate disodium salt in combination with Fast Blue RR	Simultaneous coupling azo dye method using substituted naphthol	30	Barka and Anderson (1963)
ii.	Glucose-6- phosphatase (G-6-Pase)	Glucose-6-phosphate and lead nitrate	Lead nitrate method of Wachstein and Meisel (1956)	20	Barka and Anderson (1963)
B. Oxidoreductases					
iii.	Succinic Dehydrogenase (SDH)	Di-NA-succinate	Standard method of Bound enzyme by Nitro BT method	15	Pearse (1972)
iv.	Lactate dehydrogenase (LDH)	Na-DL-lactate	Standard method of Bound enzyme by Nitro BT method	30	Pearse (1972)
v.	Glutamic dehydrogenase (GLD)	Na-L-glutamate	Standard method of bound enzyme by Nitro BT method	15	Pearse (1972)
vi.	Glucose-6-phosphate dehydrogenase (G-6-PD)	Di-Na-Glucose-6-Phosphate	Standard method of bound enzyme by Nitro BT method	30	Pearse (1972)
vii.	Reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-diaphorase)	Co-enzyme (NADPH)	Standard method of Bound enzyme by Nitro BT method	35	Pearse (1972)
vi.	Reduced nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase)	Co-enzyme (NADH)	Standard method of Bound enzyme by Nitro BT method	35	Pearse (1972)
vii.	Monoamine oxidase (MAO)	Tryptamine hydrochloride	Standard method of Bound enzyme by Nitro BT method	60	Pearse (1972)

Table 1. Histoenzymic techniques used on cryostat sections of ovary. (Continue)

Sr. No	Enzyme	Substrate	Method	Incubation Time (minute)	Reference
A. Phosphatases					
C. Esterases					
viii.	Non-specific esterase (NSE)	Alpha naphthol Acetate	Naphthol acetate method	10	Barka and Anderson (1963)

Table 2. Enzyme histochemistry on the ovary of prepubertal buffalo.

Enzyme/Ovarian components	Cortex												Medulla				
					Follicle												
					Secondary				Graafian/Tertian								
	SE	TA	CS	P	TH	MG	ZP	O	TH	MG	ZP	O	IGC	MS	BV	RO	HG
AKPase	O	O	O	+++	+++/+++	++	-	+	+++/++++	++	-	+	++/+++	O	+++	+/++	-
G-6-Pase	+	+	+	+/++	+	+	-	-	+	+	-	-	+	+	+	+	-
G-6-PD	O/+	O/+	O/+	+	+/++	+	-	-	+/++	+	-	-	+	O/+	+	+	-
SDH	+	+	O/+	+	O/+	O/+	-	-	O/+	O/+	-	-	+	O/+	+	+	-
GLD	+	+	O/+	+	+	+	-	-	+	+	-	-	++	O/+	+	+	-
LDH	++	+	+	++	+	++	-	-	O/+	+/++	-	-	+/++	+	+	O/+	-
NADH diaphorase	+	O/+	+	++++	++++	+++	O	+	+++/++++	++	O	+	++	+	+	++	-
NADPH diaphorase	+	O/+	O/+	+++	+++	+	O	+	+++	+	O	+	++	O/+	+	+	-
MAO	O/+	O/+	O/+	++	+	+	-	-	+	+	-	-	+	O/+	O/+	+	-
NSE	+	+	O/+	+	O/+	+	-	-	O/+	+	-	-	+	O/+	+	+	-

- Not Observed, O-Absent; + Weak; ++ Moderate; +++ Strong, ++++ Intense

SE- Surface epithelium, TA-Tunica albuginea, CS -cortical Stroma, P-Primordial and Primary follicle

TH- theca, MG-Membrana Granulosa, ZP- Zona Plellucida, O-Oocyte

IG-Interstial gland, MS- Medullary Stroma, HG-Hilar Gland, BV- Blood vessel

Table 3. Enzyme histochemistry on the ovary of buffalo during follicular phase.

Enzyme/ Ovarian components	Cortex												Medulla				Corpus Luteum
					Follicle												
					Secondary				Graafian/Tertian								
	SE	TA	CS	P	TH	MG	ZP	O	TH	MG	ZP	O	IG	MS	BV	HG	
AKPase	O	O	O/+	+++	+++/++++	++/+++	O	+	+++/++++	++/+++	-	+	++	O/+	+++	+	++
G-6-Pse	+	+	+	++	++	+	-	-	++	+	-	-	++	+	+	+	++
G-6-PD	O/+	O/+	O/+	+++	+++	++	O	+	+++	++	-	-	++	O/+	+/++	-	++++
SDH	+	+	+	+	++	++	-	+	+/++	+/++	-	+	+/++	+	O/+	-	+++/++++
GLD	+	+	+	++	++/+++	+	-	-	++/+++	++	-	-	++	+	++	-	+++
LDH	++	+	++	++	++	++	-	+/++	++	++	-	+/++	++/+++	++	++	-	+++
NADH diaphorase	+	O/+	+	++++	+++	++	O	+	+++/++++	++	O	+	++	+	++	++	+++
NADPH diaphorase	+	+	+	++++	+++	++	-	+	++++	++	-	+	+++	+	+	-	++/+++
MAO	+	+	+	++	++	++	-	-	+	+	-	-	+++	+	++	-	++
NSE	O/+	O/+	O/+	++	+/++	+++	-	-	+++	+++	-	-	++++	O/+	++	-	++++

- Not Observed, O-Absent; + Weak; ++ Moderate; +++ Strong, ++++ Intense

SE- Surface epithelium, TA-Tunica albuginea, CS -cortical Stroma, P-Primordial and Primary follicle

TH- theca, MG-Membrana Granulosa, ZP- Zona Plellucida, O-Oocyte

IG-Interstial gland, MS- Medullary Stroma, HG-Hilar Gland, BV- Blood vessel

Table. 4. Enzyme histochemistry on the ovary of Buffalo during luteal phase.

Enzyme/ Ovarian components	Cortex												Medulla				Corpus luteum
					Follicle												
					Secondary				Graafian/Tertian								
	SE	TA	CS	P	TH	MG	ZP	O	TH	MG	ZP	O	IGC	MS	BV	HG	
AKPase	+	+	O/+	+++/++++	++/+++	++	-	+	++/+++	++	-	+	++	O/+	+++	+/++	+++/++++
G6Pase	+	+	O/+	+++/++++	++/+++	++	-	+	++/+++	++	-	+	++	O/+	+++	+/++	+++/++++
G-6-PD	+	+	+	++/+++	++/+++	++	-	-	++/+++	++	-	-	++	+	O/+	-	+++
SDH	O/+	O/+	O/+	+	+/++	+	-	-	+/++	+	-	-	+++	O/+	+/++	-	+++
GLD	+	O/+	+	++	+	++	-	-	+	+	-	-	+	+	O/+	-	+++
LDH	+	+	+	++	+	++	-	+/++	+	++	-	+/++	++	+	+	-	+++
NADHd	+	+	+	+	+++	++	-	-	+++	++	-	-	++	+	+/++	-	++/+++
NADPHd	+	+	+	+	+++	++	-	-	+++	++	-	-	++	+	+	-	+++/++++
MAO	+	+	+	+	+	+	-	-	+	+	-	-	+++	+	+/++	-	++++
NSE	O/+	O/+	O/+	+++	O	+++	-	-	+	+++	-	-	++++	O/+	+	-	++++

- Not Observed, O-Absent; + Weak; ++ Moderate; +++ Strong, ++++ Intense

SE- Surface epithelium, TA-Tunica albuginea, CS -cortical Stroma, P-Primordial and Primary follicle

TH- theca, MG-Membrana Granulosa, ZP- Zona Plellucida, O-Oocyte

IG-Interstial gland, MS- Medullary Stroma, HG-Hilar Gland, BV- Blood vessel

Table. 5. Enzyme histochemistry on the ovary of pregnant buffalo.

Enzyme / Ovarian components	Cortex												Medulla				Corpus Luteum
	SE	TA	CS	P	Follicle												
					Secondary				Graafian/ Tertian								
					TH	MG	ZP	O	TH	MG	ZP	O	IGC	MS	BV	HG	
AKPase	+	+	O/+	+++/++++	++/+++	++	-	+	++/+++	++	-	+	++	O/+	+++	+/++	+++/++++
G6Pase	+	+	O/+	+++/++++	++/+++	++	-	+	++/+++	++	-	+	++	O/+	+++	+/++	+++/++++
G-6-PD	+	+	+	++/+++	++/+++	++	-	-	++/+++	++	-	-	++	+	O/+	-	+++
SDH	O/+	O/+	O/+	+	+/++	+	-	-	+/++	+	-	-	+++	O/+	+/++	-	+++
GLD	+	O/+	+	++	+	++	-	-	+	+	-	-	+	+	O/+	-	+++
LDH	+	+	+	++	+	++	-	+/++	+	++	-	+/++	++	+	+	-	+++
NADH D	+	+	+	+	+++	++	-	-	+++	++	-	-	++	+	+/++	-	++/+++
NADPH D	+	+	+	+	+++	++	-	-	+++	++	-	-	++	+	+	-	+++/++++
MAO	+	+	+	+	+	+	-	-	+	+	-	-	+++	+	+/++	-	++++
NSE	O/+	O/+	O/+	+++	O	+++	-	-	+	+++	-	-	++++	O/+	+	-	++++

- Not Observed, O-Absent; + Weak; ++ Moderate; +++ Strong, ++++ Intense

SE-Surface epithelium, TA-Tunica albuginea, CS-cortical Stroma, P-Primordial and Primary follicle

TH-theca, MG-Membrana Granulosa, ZP-Zona Plellucida, O-Oocyte

IG-Interstial gland, MS-Medullary Stroma, HG-Hilar Gland, BV- Blood vessel

Similar observations were recorded by Bhardwaj and Roy (2001) in Indian buffalo at different ages.

The luteal cells and blood vessels in corpus luteum of luteal phase showed moderate reactions while strong reaction was observed in the pregnant buffaloes (Figure 1(d)). The higher activity in the pregnant buffaloes may be correlated with its secretory activity. Similar findings were reported by Boos *et al.* (1988) in cows and Singh and Roy (1999) in buffaloes.

Glucose-6- phosphatase (G-6-Pase)

The distribution of G-6-Pase in ovarian surface epithelium, tunica albuginea and cortical stroma of prepubertal and pubertal buffaloes was weak while it was moderate in the primordial and primary follicles. Follicular cells of primary follicle and granulosa cells of secondary follicles showed strong to intense reaction (Figure 1(e)). Granulosa cells showed strong activity while the theca cells exhibited weak activity (Figure 1(f)). The activity of this enzyme was weaker in the prepubertal buffaloes than that during follicular, luteal and pregnant animals. The activity of this enzyme was weak to moderate in the ovarian medulla cells. The findings of the present study were supported by the observations of Bhattacharya and Saigal (1990) in goats.

The luteal cells of corpus luteum exhibited moderate reaction both during luteal phase and in pregnant buffaloes. The moderate activity of this enzyme indicated better glucose metabolism in cells undergoing steroid synthesis. G-6-Pase enzyme is responsible for glucose metabolism and may be associated with steroid synthesis of the cells.

Oxidoreductases and dehydrogenases

Glucose-6-phosphatase dehydrogenase (G-6-PD)

The surface epithelium, tunica albuginea and cortical stroma exhibited weak activity of G-6-PD while moderate reaction was observed in the primordial and primary follicles during all the stages of buffaloes (Figure 2(a)). The membrana granulosa cells exhibited strong reaction of G-6-PD while theca cells showed weak to moderate reaction during follicular and luteal phases of the estrous cycle (Figure 2(b)). The enzyme is responsible for conversion of glucose to acetyl coenzyme-A, which is utilized for the active synthesis of fatty acids and steroids. Similar observations were recorded by Bhardwaj and Roy (2001a) in buffalo ovary at different age groups.

The capsule, stroma and blood vessels were devoid of G-6-PD activity. Micro granular reaction of moderate intensity was exhibited by the luteal cells during luteal phase and in pregnant animals (Figure 2(c)). The observations of the present study were in consonance with that of Roy and Saigal (1985) in pregnant sheep ovary.

Succinate dehydrogenase (SDH)

The activity of SDH enzyme was not appreciable in surface epithelium, tunica albuginea and cortical stroma of prepubertal buffalo ovary and weak reaction was observed during pubertal stage (Figure 2(d)). Reaction was moderate in the primordial and primary follicles. In the growing and tertiary follicles during follicular phase and luteal phase of estrous cycle, theca cells exhibited weak reaction during prepubertal period while it was moderate during the pubertal stages whereas granulosa cells exhibited strong granular reaction (Figure 2(e)). SDH plays important role in steroidogenesis as it being closely linked to

cytochrome system (Motta and Hafez, 1980).

Luteal cells of buffalo ovary exhibited moderate to strong reaction of SDH in the luteal phase of estrous cycle while it was strong in the pregnant animals (Figure 2(f)). The reaction was perinuclear and granular in nature. Similar findings were made by Singh and Roy (2001) in buffalo corpus luteum. The higher enzyme activity may correspond to the higher secretory activity of the luteal cells.

Glutamate dehydrogenase (GLD)

The activity of GLD was negligible in the surface epithelium, tunica albuginea and cortical stroma. A weak reaction was observed in the primordial and primary follicles during all the stages of reproduction. The theca cells and membrana granulosa cells showed moderate to strong reaction during follicular while luteal phase of pubertal buffaloes (Figure 2(g)). The ovarian medulla showed moderate GLD activity while the blood vessels revealed weak activity. The luteal cells showed weak activity of GLD both during luteal phase and in pregnant animals. Similar observations have been recorded by Bhardwaj and Roy (2001b) in buffaloes.

Lactic dehydrogenase (LDH)

The activity of LDH was weak in the surface epithelium, tunica albuginea and cortical stroma was weak to moderate reaction was observed in the primordial and primary follicles during all the stages of buffaloes (Figure 2(h)). The membrane granulosa cells and theca cells exhibited moderate reaction of LDH during follicular and while weak during luteal phase of the estrous cycle (Figure 2(i)). The enzyme is responsible for conversion of glucose to acetyl coenzyme-A, which is utilized for the active synthesis of fatty acids and steroids. The observations of the present study are in consonance with that of Roy and Saigal (1985) in sheep.

Luteal cells showed micro granular reaction of LDH both during luteal phase and in pregnant buffaloes. The capsule, stromal cells and blood vessels had negligible reaction. Similar observations were made by Singh and Roy (1996). The activity of this enzyme may be correlated with the synthesis of steroid hormone by luteal cells.

Diaphorases

NADH diaphorases

The activity of NADH-diaphorase was weak to moderate in the surface epithelium, tunica albuginea and cortical stroma in prepubertal buffaloes whereas moderate reaction in the pubertal animals (Figure 3(a)). Moderate reaction was observed in the primordial and primary follicles during the pubertal period while a strong activity was exhibited in the ovary during follicular phase.

Strong to intense activity was recorded in the theca cells and membrane granulosa cells of growing and tertiary follicles during follicular phase (Figure 3(b)). The medulla of ovary showed moderate reaction while the blood vessels showed weak reaction. Similar observations were made by Bhardwaj and Roy (2003). The enzyme is related with the steroidogenic activity. Guraya (1985) reported that the inner layer of granulosa cells of preantral follicles had more activity than the outer layer.

The luteal cells exhibited strong to intense reaction of NADH-diaphorase in pregnant animals while moderate to strong reaction was observed in the corpus luteum during luteal phase (Figure 3(c)). The weak to moderate activity was also observed in the connective tissue components and blood vessels. Similar observations were recorded by Singh and Roy (1996) in Indian buffaloes.

NADPH diaphorases

The activity of NADPH-diaphorase was negligible in the surface epithelium, tunica albuginea and cortical stroma in all stages of buffaloes. Moderate to strong reaction was observed in the primordial and primary follicles during the prepubertal, follicular phase and luteal phase but was weak in pregnant animals (Figure 3(d)).

Moderate activity was observed membrana granulosa cells of growing and tertiary follicles during follicular and luteal phase while strong activity was seen in the theca cells (Figure 3(e)). Similar observations were made by Bhardwaj and Roy (2001b). The ovarian medulla of ovary showed moderate reaction while the blood vessels showed weak reaction. Roy and Saigal (1985) also reported weak activity in blood vessels in ovarian medulla of sheep. The enzyme is related with the steroidogenic activity. The enzyme is responsible in steroidognesis by converting cholesterol to progesterone (Sorensen and Singh, 1973).

Moderate to strong reaction was observed in the luteal cells in corpus luteum during luteal phase and in pregnant animals (Figure 3(f)). As reported by Singh and Roy (1996) in buffaloes and Roy and Saigal (1985) in sheep. The increased activity of NADPH during luteal phase and prengnancy might be correlated to increased secretory activity of cells as the enzyme is responsible for conversion of cholesterol to progesterone (Sorensen and Singh, 1973) and fatty acid synthesis (Hoyer 1980).

Monoamine oxidase

The MAO activity was negligible in the surface epithelium, tunica albuginea and cortical stroma and weak in the primordial and primary follicles in prepubertal and whereas it was weak to moderate in pubertal buffaloes (Figure 4(a)). The granulosa cells and theca cells during follicular and luteal phase of estrous cycle exhibited weak to moderate activity of MAO. Strong MAO activity was observed in the interstitial gland present in the medulla of ovary during follicular and pregnant animals (Figure 4(b)). Yoshimoto *et al.* (1986) recorded highest activity in blood vessels of proestrous rat and postulated its role in ovulation.

MAO activity was negligible in the connective tissue capsule. Luteal cells exhibited weak activity during luteal phase while the reaction was moderate in pregnant animals (Figure 4(c)). MAO is involved in the process of vasodilation (Raekallio, 1970). Its role in the corpus luteum of pregnant animals may be related to efficient delivery of this hormone. It is also involved in the biological oxidation and reduction for the energy production which is required for steroidogenesis (Singh and Roy, 1996)

Non Specific Esterases (NSE)

The surface epithelium, tunica albuginea and cortical stroma exhibited negligible NSE activity and the primordial and primary follicles showed weak reaction in in prepubertal and pubertal buffaloes. The NSE activity was moderate in the theca cells of tertiary follicles during follicular phase, luteal phase and in pregnant buffaloes while it was weak in the prepubertal buffaloes (Figure 5(a) and 5(b)). The reactivity was micro granular in nature. The granulosa cells exhibited strong reactivity in the follicular and luteal phase of the estrous cycle. Bhatttacharya and Saigal (1990) also observed moderate to strong activity in the thecal cells of goat ovarian follicles. Ovarian medulla showed moderate to strong reaction while the blood vessels showed weak reaction.

Luteal cells during luteal phase and pregnant buffaloes exhibited strong reaction

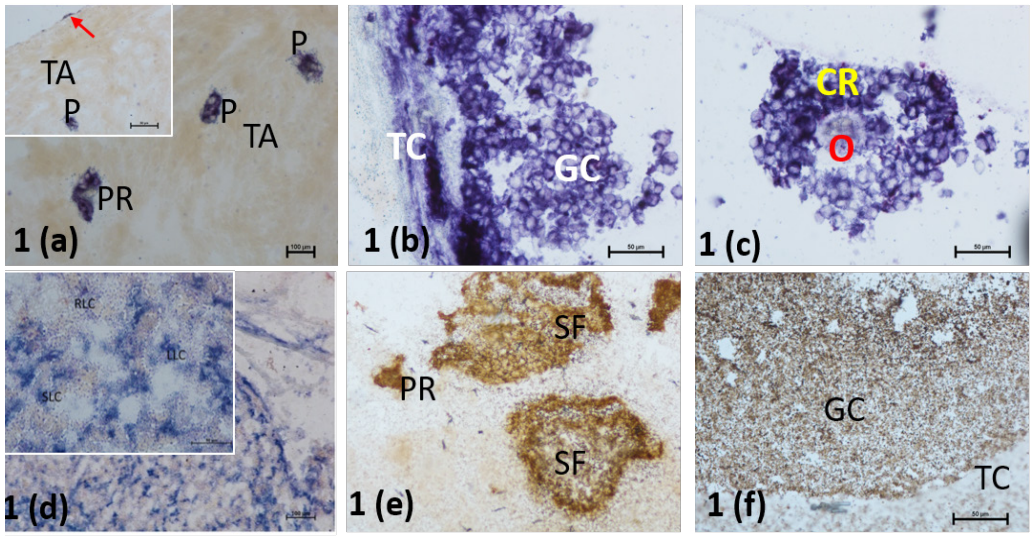

Figure 1. Representative cryostat sections showing phosphatase enzyme activity, Azo Dye method for AKPase and. (a) AKPase activity in surface epithelium (arrow), negligible activity in tunica albuginea (TA) and strong reaction in primordial (P) and primary follicles (Pr) of buffalo ovary during follicular phase. Original magnification. X 100; inset with AKPase activity in surface epithelium (arrow), tunica albuginea (TA) and strong reaction in primordial (P). Original magnification. X 400. (b) Strong to intense reaction of AKPase in granulosa cells (GC) and theca cells (TC). Original magnification x 400. (c) Strong to intense reaction of AKPase in oocyte (O) and corona radiata cells (RC). Original magnification x 400. (d) Strong reaction of AKPase in corpus luteum. Original magnification x 100. Inset showing strong reaction in large luteal cells (LLC) and small luteal cells (SLC) and weak reaction in regressing luteal cell (RLC). Original magnification x 400. (e) Glucose 6-Pase activity in primary follicle (PR) and secondary follicle (SF). Original magnification x 100. (f) Glucose 6-Pase activity in granulosa cells of tertiary follicles (GC) and weak reaction in theca cells (TC). Original magnification x 400.

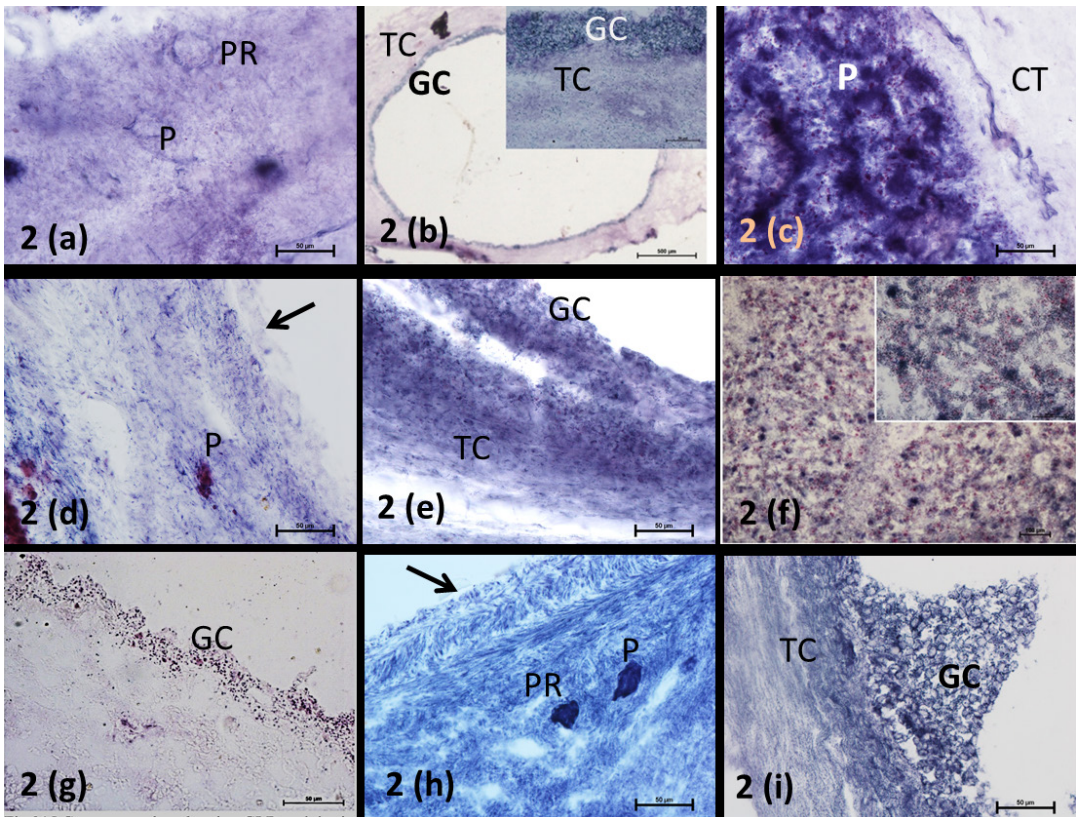

Figure 2. Representative cryostat sections showing activity of dehydrogenases, Nitro BT method. (a), (b) and (c) showing G-6-PDenzyme activity; moderate enzyme reaction in primordial (P) and primary follicles (PR), weak activity in theca cells (TC) and intense reaction in granulosa cells (GC) of tertiary follicles; and weak activity in connective tissue septa and intense in luteal cells in parenchyma of corpus luteum of buffalo. Original magnification (a) X 400; (b) x40, inset x 400 and (c) x400; (d), (e) and (f) showing SDH enzyme activity; Weak activity in surface epithelium (arrow), tunica albuginea and moderate to strong in primordial follicle (P) and primary follicles, strong to intense in the membrane granulosa cells (GC) and moderate in the theca cells (TC), intense reaction in the luteal cells in corpus luteum. Original magnification (d) X 400; (e) x400, inset x 400 and (f) x100 and inset x400. (g) Showing GLD enzyme activity in the membrane granulosa cells (GC). Original magnification x400. (h) and (i) showing LDH enzyme activity; Weak activity in surface epithelium (arrow), moderate to strong in primodial follicle (P) and primary follicles, strong to intense in the membrane granulosa cells (GC) and moderate in the theca cells (TC), intense reaction in the luteal cells in corpus luteum. Original magnification (d) X 400; (e) x400, inset x 400 and (f) x100 and inset x400.

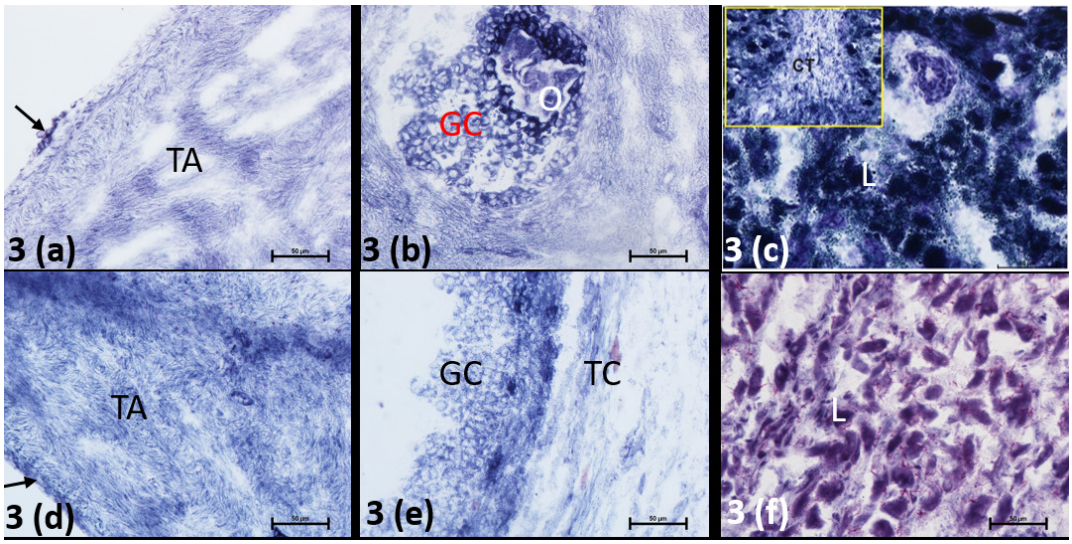

Figure 3. Representative cryostat sections showing activity of diaphorases, Nitro BT method. (a), (b) and (c) showing NADHd activity and (d), (e) and (f) showing NADPHd enzyme activity; weak to moderate enzyme reaction in surface epithelium (arrow) and tunica albuginea (TA), moderate activity in theca cells (TC) and oocyte (O) and intense reaction in granulosa cells (GC) of tertiary follicles; and weak activity in connective tissue septa (CT) and intense in luteal cells(L) in parenchyma of corpus luteum of buffalo. Original magnification (a) X 400; (b) x40, inset x 400 and (c), (d) and (f) x400.

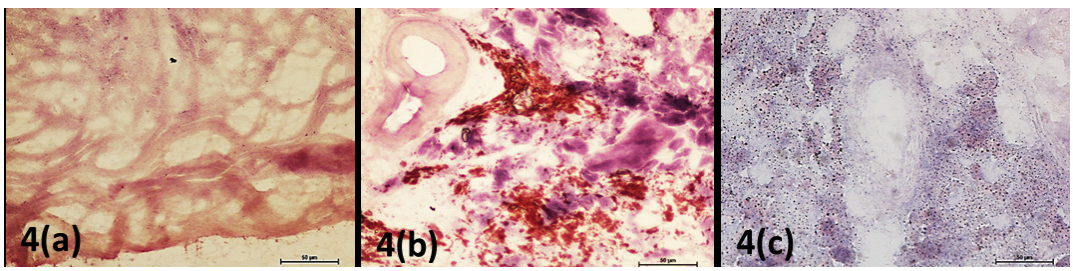

Figure 4. Representative cryostat section of buffalo ovary showing weak MAO activity in cortical zone (a) moderate to strong in medullary zone (b) strong activity in luteal cells in pregnant buffalo (c). Nitro BT method. Original magnification x400.

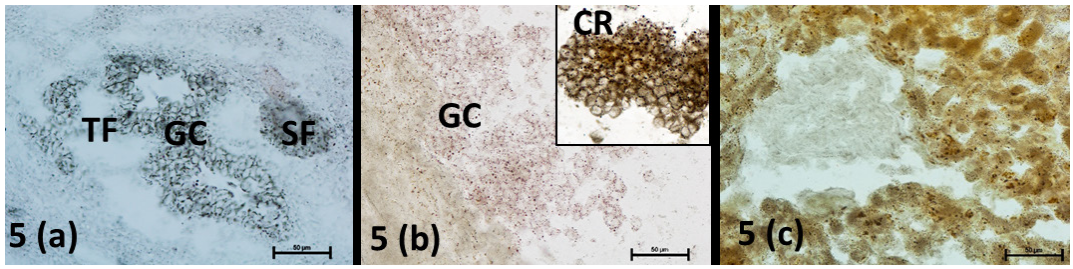

Figure 5. Representative cryostat sections showing activity of NSE activity in ovary of buffalo. Naphthol acetate method. (a) Strong reactions in granulosa cells (GC) of secondary (SF) and tertiary follicles (b) strong reaction in granulosa cells and intense reaction in corona radiate cells (CR); strong to intense reaction in the luteal cells (c). Original magnification (a), (b) and (c) x400.

for NSE while the connective tissue exhibited negligible or weak reactions. The reactivity was micro granular in nature in the cytoplasm of the luteal cells. Similar observations were reported by Bhattacharya and Saigal (1990) in sheep and Bhardwaj and Roy (2003a) in buffaloes.

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