The Haryana Veterinarian (June, 2023) **62(1),** 114-117 ISSN 0033-4359 (P) **Research Article**

ENZYME HISTOCHEMISTRY OF MAMMARY GLAND OF GOAT DURING POSTNATAL DEVELOPMENT

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ABSTRACT

The present study was conducted on the mammary gland of goat during prepubertal, lactating and non-lactating age groups. Fresh cryostat sections of 8-10 µm thickness were obtained and incubated for the demonstration of phosphatases, dehydrogenases and esterases. It was observed that the activity of AKPase, NADH and NADPH was strong to intense reaction in the alveolar epithelium, blood vessels but moderate to strong reaction in duct system. The reaction of G-6-Pase, SDH, LDH, G-6-PD and NSE was reported to be moderate in the alveolar epithelium, weak to moderate in the duct system and weak in the blood vessels, muscles and adipose tissue. The myoepithelial cells showed strong AKPase activity, moderate NADH and NADPH reaction, but very weak activity for dehydrogenases. The stroma was devoid of any activity for phosphatases, but showed weak reaction for oxidoreductases and non-specific esterase. Based on present investigation, it was concluded that the activity of various enzymes was more in the lactating animals, followed by non-lactating and prepubertal ones. The increase in the enzymatic reaction in lactating goats may be correlated with the functional activity and proliferation of glandular tissue during lactation stage.

Keywords: Enzyme, Goat, Mammary gland, Postnatal development

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India is second in the population of goats with the average milk yield per day per animal at National level during 2018-19 for goat is 0.45 kg/day. Mammary gland is the largest exocrine gland in the animal body which plays an important role in milk synthesis, secretion, and involution to prepare the gland for subsequent lactation. Enzyme histochemistry can be used as a tool to study the secretory activity of mammary gland during prepubertal, lactating and non-lactating stages. Some of the histoenzymatic studies have been reported on the mammary gland of goat (Roy *et al.*,1997), buffalo (Singh, 2000; Challana *et al.*, 2016) and sheep (Paramasivan *et al.*, 2014), but there is scarcity of the literature on the enzyme histochemistry of mammary gland in goat during different stages of development during postnatal life. Therefore, the present study was undertaken to explore the histoenzymic changes in the mammary gland of goat during prepubertal, lactating and non-lactating age groups.

MATERIALS AND METHODS

The present study was conducted on mammary glands of nine goats divided into three groups i.e. Group I: Prepubertal, Group II: Pubertal (lactating) and Group III Pubertal (non- lactating). The fresh tissue samples were collected immediately after slaughter and subjected to cryostat sectioning at -20º C. The sections of 8-10 µm thickness were obtained and incubated for demonstration of various enzymes *viz*; alkaline phosphatase (AKPase), G-6 phosphatase (G-6-Pase) by coupling azodye method (Barka and Anderson, 1963), succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), Glucose-6-phosphate dehydrogenase (G-6-PD), reduced nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase), reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-diaphorase) by nitro BT method (Pearse 1972) and non-specific esterase by naphthaol acetate method (Barka and Anderson, 1963). The intensity of various enzymes was determined by the coloured affinity at the site of reaction and denoted as intense, strong, moderate, weak and negligible.

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RESULTS AND DISCUSSION

The histoenzymatic activity of different phosphatases, oxidoreductases and non-specific esterase of mammary glands in goat (Table 1).

1. PHOSPHATASES

(i) Alkaline Phosphatase (AKPase): The reaction of AKPase was found to be weak to moderate in the alveolar epithelium and duct system, but moderate to strong in the basement membrane, blood vessels, subepithelial contents during prepubertal stage (Fig. 1). The activity was increased in lactating animals as strong to intense in the basal lamina, alveolar epithelium and blood vessels, moderate to strong in duct system, but declined in non-lactating group as strong activity in alveolar epithelium, moderate to strong in blood vessels and moderate in duct system (Figs. 2 & 3). Paramasivan *et al.* (2014) also reported moderate to strong reaction of AKPase and ACPase in the luminal secretions in sheep. Parmar et al. (1986) also observed more AKPase in

mammary gland of lactating animals as compared to the non-lactating ones. The myoepithelial cells showed moderate to strong reaction, whereas the stroma exhibited negligible reaction in prepubertal, lactating and non-lactating groups. The stronger activity in myoepithelial cells may be responsible for better cellular communication required for synthesis of milk. Similarly, Katiyar, (1982) also noticed the moderate to intense alkaline phosphatase activity in myoepithelial cells. The localization of AKPase at the basal lamina and alveolar epithelium may be related with the transportation of ions across the basement membrane of the alveoli which plays an important role in the secretion of milk during lactation.

(ii) Glucose-6-Phosphatase (G-6-Pase): G-6-Pase showed moderate activity in the alveolar epithelium and duct system in lactating animal whereas weak to moderate activity was reported in these components during non-lactating and prepubertal stages (Fig. 4). The blood vessels showed mild activity of G-6-Pase and stroma was devoid of any activity in all the groups studied. It was also noticed that amount of G-6-Pase was more in the lactating group which decreased in non-lactating and further declined in prepubertal goats. The present findings indicated that G-6-Pase activity was higher in functionally active cells with well-developed endoplasmic reticulum (Shugyo *et al.*, 1986). The localization of G-6-Pase in goat mammary gland may also be related with glucose metabolism required for secretory activity of alveolar cells.

2. OXIDOREDUCTASES

(I) DEHYDROGENASES

(i) Succinate Dehydrogenase (SDH): A weak to moderate SDH activity was observed in alveolar epithelium, myoepithelial cells and duct system, very weak reaction in stroma of mammary gland in goat of all the age groups studied (Fig. 5). As SDH is a mitochondrial enzyme which plays an important role in the oxidation of biological tissues, so its localization in the mammary gland of goat may be related with the proliferation and secretion of tissue. Similarly, Lenhardt *et al.* (1994) reported weak SDH reaction in alveolar epithelium, myoepithelial cells and blood vessels in adult cattle and (Challana *et al.*, 2016) in buffalo foetus.

(ii) Lactate Dehydrogenase (LDH): Moderate to strong fine granular LDH reaction was noticed in the alveolar epithelium weak to moderate in duct system and weak reaction in the stroma of lactating animals. The LDH reaction was reported to be weak in the blood vessels, muscles and adipose tissue in prepubertal and non-lactating animals (Fig. 6). Similarly, LDH activity was observed in the glandular epithelium and stroma of goat (Roy *et al.*, 1997) and buffalo (Singh, 2000). Challana *et al.* (2016) also noticed moderate to strong LDH activity in mammary gland during prenatal life. The increased LDH activity was indicative of anaerobic cellular respiration in the alveolar cells which may be related with the increase in protein synthesis in active cells (Sarangi *et al.*, 2019).

(iii) Glucose-6-Phosphate Dehydrogenase (G-6-PD): The reaction of G-6-PD was reported to be moderate to strong in alveolar epithelium, weak to moderate in duct system and weak in stroma of mammary gland in lactating goats (Fig. 7), however the reactivity was comparatively lesser in non-lactating and prepubertal animals. The increased G-6-PD activity may be due to the increased glucose utilization and protein synthesis in adult animal (Darzi *et al.*, 1998). G-6-PD is important for pentose phosphate shunt which is required for cellular differentiation during development of tissue.

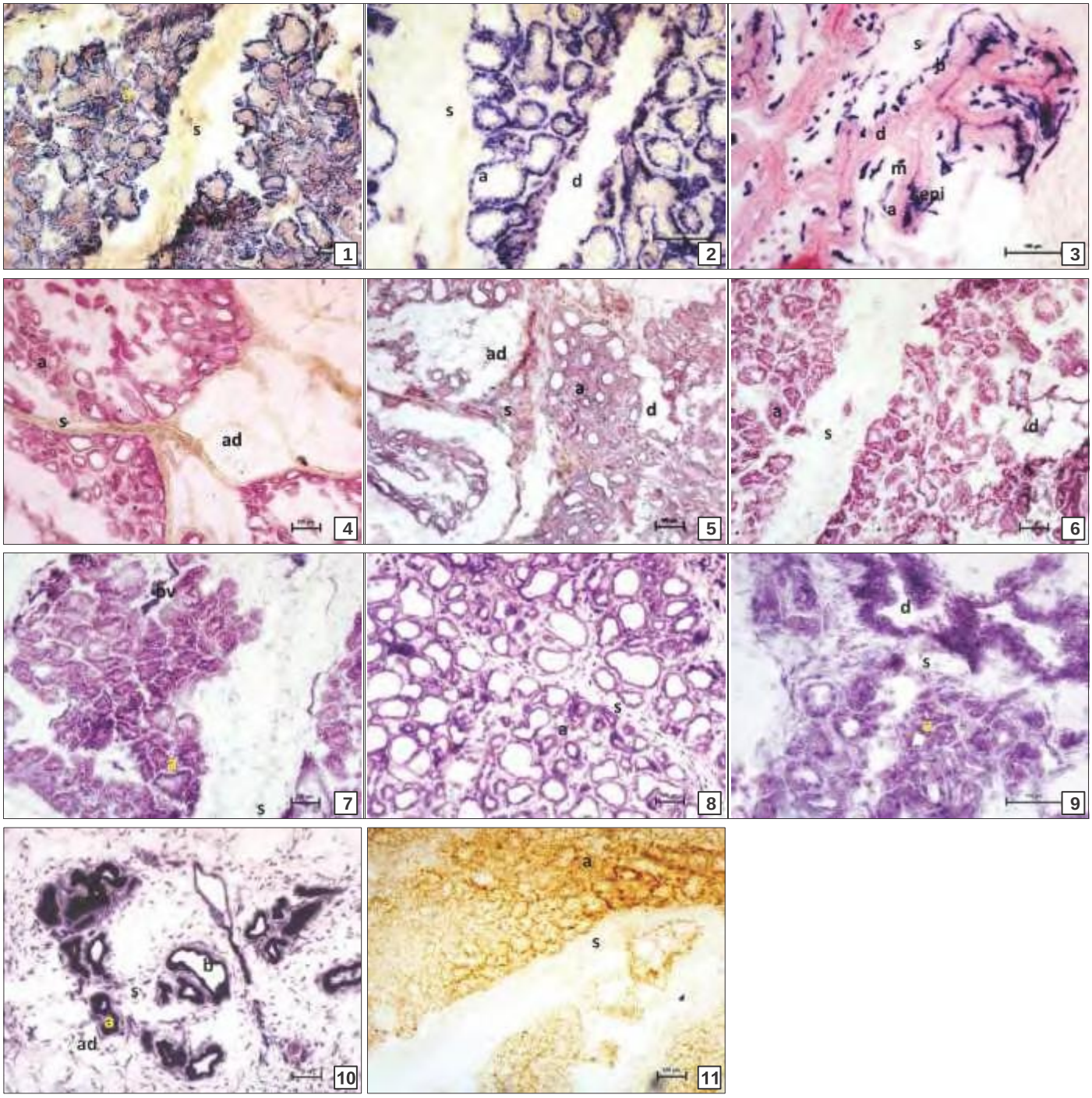
(II) DIAPHORASES

(i) Reduced Nicotinamide Adenine Dinucleotide Diaphorase (NADH-Diaphorase): Strong to intense NADH activity was observed in the alveolar epithelium and strong in duct system in both lactating and non-lactating groups (Figs. 8 & 9). However, stroma, muscles and nerve fibres exhibited weak activity but blood vessels showed moderate reaction in all the age groups. The adipose tissue present in the prepubertal and non-lactating animals showed very weak reaction. In general, the activity of NADH was less in different components of mammary gland and teat in prepubertal animals. Similar findings have been reported by Challana *et al.* (2016) in glandular mammary tissue in buffalo foetus and Singh and Roy (2003) in adult buffalo.

(ii) Reduced Nicotinamide Adenine Dinucleotide Phosphate Diaphorase (NADPH-Diaphorase): Among all the dehydrogenases enzymes studied, NADPH showed intense reaction in alveolar epithelium, duct system in prepubertal goats as compared to adult animals (Figs. 10 & 11). The localization of NADPH in different components of mammary gland may be responsible for reduced coenzyme and oxygen in the hydrogen transport chain. Our findings correlate well with the observations of Sarangi (2022), who also reported intense NADPH reaction in alveolar and ductular epithelium space in mammary gland of buffalo.

3. ESTERASES:

(i) Non-Specific Esterase (NSE): A weak NSE activity was observed in the alveolar and ductular epitheliumin the prepubertal, moderate in lactating and weak to moderate in non-lactating goats. However, stroma showed weak to moderate reaction, but negligible NSE



Figs. 1-12. **(1)** Weak AKPase activity in alveolar epithelium (a) and duct system (d), moderate to strong in myoepithelial cells (m), basement memnbrane (b), subepithelial contents (sepi), and negligible in stroma (s) of prepubertal animal. Azodye Method X 200; **(2)** Strong to intense AKPase activity in alveolar epithelium (a), moderate to strong in duct system and negligible activity in stroma (s) in lactating goat. Azodye Method X 100; **(3)** Strong activity of AKPase in alveolar epithelium (a), moderate in duct system (d) and negligible in stroma (s) in non-lactating goat. Azodye Method X 200; **(4)** Moderate G-6-Pase activity in alveolar epithelium (a) and duct system, and negligible in stroma (s) of lactating goat. Lead Nitrate Method X 100; **(5)** Moderate activity of SDH in alveolar epithelium (a), weak in duct system, stroma (s) and adipose tissue (ad) in non-lactating goat. Nitro BT Method X 100; **(6)** Moderate activity of LDH in alveolar epithelium (a), weak in duct system (d), stroma (s), and adipose tissue (ad) in non-lactating goat. Nitro BT Method X 100; **(7)** Moderate to strong activity of G-6-D in alveolar epithelium (a), weak to moderate in duct system (d) and negligible to weak in stroma (s) in lactating goat. Nitro BT Method 100; **(8)** Intense activity of NADH in alveolar epithelium (a) and blood vessels (bv), strong in duct system and weak activity in stroma (s) in lactating goat. Nitro BT Method X 100; **(9)** Strong activity of NADH in alveolar epithelium (a), moderate to strong in duct system and weak activity in stroma (s) in non-lactating goat. Nitro BT Method X 100; **(10)** Strong activity of NADPH in alveolar epithelium (a), moderate to strong in duct system (d) and weak activity in stroma(s) in non-lactating goat. Nitro BT Method X 200; **(11)** Intense NADPH activity in alveolar epithelium and duct system (a, d) and weak activity in stroma (s) and adipose tissue (ad) of prepubertal goat. Nitro BT Method X 100

Table 1. Histoenzymatic distribution of mammary gland of goat at different age groups

ENZYMES	ALVEOLAR EPITHELIUM			DUCT SYSTEM			STROMA		
	L	NL	PP	L	NL	PP	L	NL	PP
AKPase	+++/++++	+++	+/++	++/+++	++	+/++	0	0	0
G-6-Pase	++	+/++	+/++	++	+	+/++	0	0	0
NADH	++++	+++	++	+++	++/+++	++	+	+	0/+
NADPH	++++	+++	++++	+++	++/+++	++++	+	+	+
SDH	++	++	++/+++	+/++	+	+/++	0/+	0/+	+
LDH	++	+	+/++	+/++	+	+/++	0/+	0/+	+
G-6-PD	++/+++	++	+/++	+/++	+	+	0/+	0/+	0/+
NSE	++	+/++	+	+	+	+	+	+	0

(0) Negligible (+) Weak (++) Moderate (+++) Strong (++++) Intense
L-Lactating, NL- Non-lactating, PP-Prepubertal

activity was observed in adipose tissue. The more NSE reaction in lactating animals correlated with the synthesizing and secretory activity of mammary gland parenchyma. The variation in NSE activity in different components of mammary gland may play important role in detoxification, ionic transport, maintenance of tissue size and lipid-ester metabolism (Sarangi, 2022).

It may be concluded from the present study that the activity of different enzymes was found to be more in various components of mammary gland in lactating animals than in non-lactating and prepubertal goats, which may be due to the increased in the functional activity and proliferation of glandular tissue during lactation stage.

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