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Research Article

SEASONAL VARIATION IN HISTOENZYMIC LOCALIZATION IN BUFFALO CORPUS EPIDIDYMIS

VEERPAL KAUR, VARINDER UPPAL*, NEELAM BANSAL and ANURADHA GUPTA
Department of Veterinary Anatomy, College of Veterinary Sciences
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, India

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ABSTRACT

The study was conducted on corpus epididymis of twelve buffaloes collected from abattoir during four seasons of year;Group-I Spring season (February-April), Group-II Summer season (May-July), Group-III Autumn season (August-October) and Group-IV Winter season (November-January). The cryostat sections of 10 µm thickness were cut at -20 °C and incubated in different substrates for demonstration of phosphatases, oxidoreductases and esterases. The study revealed a higher activity of phosphatases (AKPase, G-6-Pase), oxidoreductases (SDH, LDH, G-6-PD), diaphorase (NADH, NADPH) and esterases (NSE) in body of epididymis during winter season, followed by autumn, spring and summer. The increased activity of these enzymes may be correlated with increased reproductive activity of buffalo bulls during winter season.

Keywords: Buffalo, Epididymis, Histoenzyme, Seasonal study

The epididymidis provides a favorable milieu for the morphologic and physiologic maturation of the spermatozoa. It is the site where re-absorption of the testicular fluid and secretory activities occur, benefiting the maturational process of the spermatozoa (Cooper, 2007). The morphology and function of the epididymis in seasonally reproducing animals change analogue to the testicular tissue (Aguilera-Merlo *et al.*, 2005). Seasonal variations of genitalia could represent an interesting aspect affecting fertility, which might influence the quality of semen and, in turn, the chance of cryopreservation (Yasuo *et al.*, 2006). In literature histoenzymic distribution of phosphatases and oxidoreductases in epididymis have been reported in buffalo (Singh and Dhingra, 1971; Goyal and Dhingra, 1974 and Singh, 1989), goat (Karmore *et al.*, 2015), donkey (Uppal *et al.*, 2002 and Bansal *et al.*, 2003), ram (Kishore *et al.*, 2012) and camel (Ahmed *et al.*, 2013) but very few reports (Kaur *et al.*, 2018) could be traced out regarding seasonal variation in histoenzymic activity in epididymis, so the present work was planned.

MATERIALS AND METHODS

The study was conducted on corpus epididymis of twelve adult buffalo bulls collected from slaughter house during different seasons of the year divided into four groups i.e. Group-I Spring season (February-April), Group-II Summer season (May-July), Group-III Autumn season (August-October) and Group-IV Winter season (November-January). The tissues were collected immediately after slaughter and cryostat sections of 10 µm thickness of fresh tissues were cut at -20 °C. The sections were incubated in different substrates for demonstration of enzymes viz; alkaline phosphatase (AKPase), glucose-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 (NADPHdiaphorase) (Pearse, 1972) and non-speciûc esterase (NSE) by naphthol acetate method (Barka and Anderson, 1963).

*Corresponding author: v.uppal@yahoo.com

RESULTS AND DISCUSSION

The histoenzymic activity of different phosphatases, oxidoreductases and esterases in corpus epididymis during spring, summer, autumn and winter season has been summarized in Table 1.

Phosphatases

Alkaline phosphatase

Tunica albuginea of corpus epididymis was weak to moderately positive in all the seasons. Activity in epithelium of corpus epididymis was moderate to strong in summer (Fig.1) but strong during autumn and winter seasons (Fig. 2). While the blood vessels showed strong activity during winter season. Singh and Dhingra (1971) observed that AKPase activity was more intense in the head and body than in tail region of the epididymis. The activity was more pronounced during the winter season followed by autumn, spring and summer season which suggested the high reproductive activity in the winter months when the epithelium of epididymis was actively involved in the process of fluid reabsorption and transport across membranes. Connective tissue was weakly positive in all the seasons as reported by Goyal and Dhingra (1974) in buffalo epididymis. Earlier Tingari and Moniem (1979) reported that sub epithelial tissue, blood vessels, stereocilia and luminal contents showed highest activity of

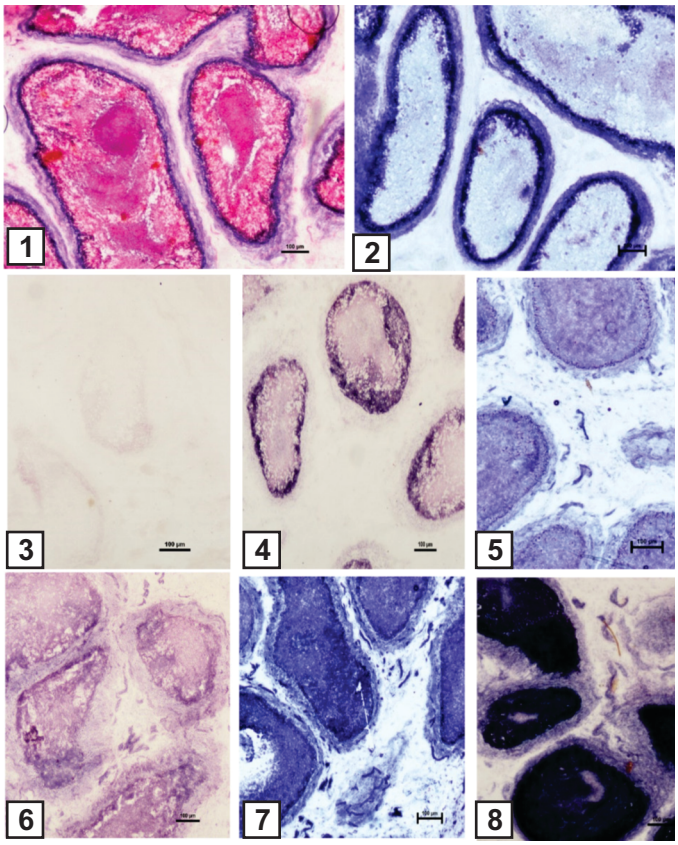

Fig. 1-8. Photomicrographs of cryostat sections of corpus epididymis showing epithelium (EP), peritubular muscle layer (PTML) and blood vessel (BV) : **1.** AKPase activity during summer season x 100; **2.** AKPase activity during winter season x 100; **3.** SDH activity during summer season x 100; **4.** SDH activity during winter season x 100; **5.** LDH activity during autumn season x 100; **6.** NADH activity during summer season x 100; **7.** NADH activity during winter season x 100; **8.** NADPH activity during winter season x 100

AKPase in the proximal part of the middle segment of the camel epididymis. In the present study, luminal spermatozoa were weakly positive in all the seasons whereas Alsum and Hunter (1978) reported that luminal contents of the ductuli efferentes and epididymis of rhesus monkey had a high alkaline phosphatase activity.

Glucose -6- phosphatase

A weak activity of G-6-Pase in the tunica albuginea was observed in all seasons. The activity was slightly more in epithelium during winter season. Whereas connective tissue, blood vessels, peritubular muscle layer and luminal contents in all the seasons were weakly positive as reported earlier by Singh (1989) in buffalo epididymis whereas Kanai *et al.* (1981) reported a moderate activity in principal cells of mouse epididymis and Rajani *et al.* (2008) observed higher activity in basal cells of rat epididymis.

Oxidoreductases

Succinate Dehydrogenase

The activity of SDH was increased in epithelium during winter season which suggested the active role of the epithelium during the period of highest epididymal activity (Figs. 3, 4). SDH is a mitochondrial enzyme and is involved in kreb's cycle. It functions not only in mitochondrial cell respiration and energy generation, but also plays a role in oxygen level sensing and tumor suppression. All other structural components were weakly positive as earlier reported by Blackshaw and Samisoni (1967), Erkman (1971) and Singh (1989) in buffalo epididymis.

Lactate Dehydrogenase

LDH is an enzyme of glycolysis and its variation in different components of epididymis is concerned with the conversion of pyruvate to lactate in those particular regions. During winter season activity was increased in epithelium as compared to summer, spring and autumn seasons (Fig. 5) whereas connective tissue, muscle layer and blood vessels were weakly positive. Singh (1989) also observed a weak activity of LDH in buffalo epididymis. Earlier it had been reported in rats that chronic exposure to constant light promotes a reduction of fertilizing ability and indicates that continuous lighting reduces the total LDH activity possibly due to moderate aging of spermatozoa with LDH activity of rat epididymis and spermatozoa in the duct by lengthening of the sperm transit through the epididymis (Ponce *et al.*, 2001).

Glucose-6- phosphate dehydrogenase

Intertubular connective tissue, blood vessels, muscle layer and tunica albuginea were weakly positive for G-6-PD while weak to moderate activity was observed in the epithelium during all seasons as observed by Singh (1989) in buffalo epididymis. The epithelium was weak to moderately positive during all the season. The tunica albuginea, inter tubular connective tissue and blood vessels exhibited a weak activity during all the seasons. Luminal contents of corpus and cauda were weakly positive in summer season and moderately positive in spring, autumn and winter season. Singh (1989) also observed a weak activity of G-6-PD in buffalo bull epididymis. G-6-PD controls the flux into the pentose phosphate pathway and is the main cytoplasmic source of reduced nicotinamide adenine dinucleotide phosphate (NADPH). Generation of NADPH by G-6-PD fuels antioxidant systems, and thus decreases reactive oxygen species (ROS) (Jain *et al.*, 2004).

Table 1
Histoenzymic localization of phosphatases and oxidoreductases in corpus epididymis during different seasons

Season	Spring						Summer						Autumn						Winter					
Parameters	T. alb	EPI	PTML	CT	BV	LU	T. alb	EPI	PTML	CT	BV	LU	T. alb	EPI	PTML	CT	BV	LU	T. alb	EPI	PTML	CT	BV	LC
Enzymes																								
Phosphatases:																								
AKPase	+/++	++/+++	+/++	+	++	0/+	+/++	++/+++	+/++	+	++	0/+	+/++	+++	+/++	+	++	0/+	+/++	+++	+/++	+	+++	0/+
G-6-Pase	+	+/++	+	+	+	+	+	+/++	+	+	+	+	+	++	+	+	+	+	+	++	+	+	+	+
Oxidoreductases:																								
SDH	+	++	+	+	+	++	+	+	+	+	+	+	+	++	+	+	+	+	+	+++	+	+	+	+
LDH	+	+/++	+	+	+	++	+	+	+	+	+	+	+	+/++	+	+	+	+	++	++/+++	+	+	++	+/++
G6PD	+	+/++	+	+	+	+/++	+	+	+	+	+	+	+	+/++	+	+	+	+/++	+	+/++	+	+	+	+/++
NADPH diaphorase	+/++	+/++	+/++	+	+/++	+/++	+/++	+/++	+	+	+/++	0/+	+/++	++/+++	+/++	+	++/+++	++	+/++	+++	++	+	++/+++	+/++
NADH diaphorase	+/++	+/++	++	+	+/++	+/++	+/++	+/++	+	+	+/++	0/+	+/++	++/+++	++	+	++/+++	0/+	+/++	+++	++	+	++/+++	0/+
Esterases:																								
NSE	+	+/++	+	+	+	+	+	+/++	+	+	+	+	+	+/++	+	+	+	+	+	+/++	+	+	+	+

+ Weak, ++ moderate, +++ strong
T alb - Tunica albuginea, Epi – epithelium, PTML - peritubular muscle layer, CT- connective tissue, BV blood vessel, LU – luminal content

NADH diaphorase

In the corpus epididymis, the activity was weak to moderate in tunica albuginea in all the seasons. The epithelium showed a weak to moderate activity in summer, a moderate to strong activity in spring and autumn but a strong to intense activity in winter (Figs 6,7). Blood vessels also exhibited a strong activity in autumn and winter seasons. These finding corroborates well with the earlier findings of Erkman (1971), Singh (1989) and Scala *et al.* (2007) in animals. NADH is a coenzyme which plays critical roles not only in energy metabolism, but also in cell death and various cellular functions including regulation of calcium homeostasis and gene expression (Ying, 2006).

NADPH diaphorase

Epithelium showed a weak to moderate activity in summer and spring but the activity increased during autumn and winter (Fig.8). Blood vessels also exhibited a strong activity in autumn and winter seasons. Luminal contents were moderately positive and the activity was weak to moderate in tunica albuginea in all the seasons. These finding corroborates well with the earlier findings of Singh (1989), Scala *et al.* (2007) and Scala and Maruccio (2012) in buffalo. The intertubular connective tissue exhibited a weak activity in all the seasons. NADP (including NADP+ and NADPH) could belong to the fundamental common mediators of various biological processes, including energy metabolism, mitochondrial functions, calcium homeostasis, antioxidation/generation of oxidative stress, gene expression, immunological functions, aging, and cell death (Ying, 2006). Luminal spermatozoa of corpus region exhibited a strong activity in spring, autumn and winter season while a negligible to weak activity was seen during summer season.

Non-specific esterases

Non-specific esterases are a group of enzymes, of probably diverse, function *in vivo*. Tunica albuginea, peritubular muscle layer, connective tissue, blood vessels and luminal contents exhibited a weak activity of Nonspecific esterases but the epithelium was weak to moderately positive in all the seasons. Similar type of activity was reported earlier by Singh (1989) in buffalo epididymis, Moniem (1980) in mammalian epididymis and Sinowartz *et al.* (1979) in dog epididymis.

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