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Research Article

HISTOENZYMIC DISTRIBUTION OF PHOSPHATASES, OXIDOREDUCTASES AND ESTERASES IN TESTIS OF BUFFALO FOETUS

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ABSTRACT

The present study was conducted on testis of buffalo foetuses collected from the abattoir immediately after sacrifice and the age of foetus was determined by measuring the crown rump length. The cryostat sections of 10-12 µ thickness were obtained at -23^{0}C and were incubated in different substrates for demonstration of phosphatases, oxidoreductases and esterases. The study revealed a variable activity of phosphatases (alkaline phosphatase, glucose-6-phosphatase), oxidoreductases (succinic dehydrogenase, lactate dehyrodenase, glutamate dehydrogenase, glucose-6-phosphate dehydrogenase**,** reduced nicotinamide adenine dinucleotide phosphate diaphorase, reduced nicotinamide adenine dinucleotide diaphorase) and esterases (non-specific esterases) in different components of testis during early stages of gestation. The enzymic activity has been correlated with their physiological function.

Key words: Buffalo fetus, histoenzymic activity, testis

Testis is a primary male sex organ performing both exocrine and endocrine functions. The knowledge of development of testis is prerequisite for clinical evaluation of abnormalities occurring during sexual differentiation. The studies have been conducted in post-natal life in buffalo (Singh, 1996) whereas prenatal studies have been reported in sheep (Hochereau de Reviers *et al.*, 1995), goat (Farooqui *et al.*, 2012) and buffalo (Kaur, 2006). The present study was conducted to correlate the components of testis with its functional activity.

MATERIALS AND METHODS

The present study was conducted on testis of six buffalo foetuses obtained from a local abattoir. The foetal body length was measured as curved line in centimetre with the help of an inelastic thread along the vertebral column between the most anterior part of frontal bone to the rump at ischiatic tuberosity and designated as crown rump length (Edward 1965). The approximate age of the foetuses was calculated by using the formula given by Soliman (1975) and foetuses were divided into three groups based on their curved crown rump length (CVRL). The fresh tissue samples from different foetuses were collected immediately after slaughter and subjected to cryostat sectioning at -23^{0}C. The sections of 10-12 µ thickness were cut and incubated for demonstration of various enzymes viz; alkaline phosphatase (AKPase) and G-6-Phosphatase (G-6-Pase) by coupling azodye method (Barka and Anderson, 1963), succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), glutamate dehydrogenase (GLD), reduced nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase), reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-diaphorase) and glucose-6-diaphorase (G-6-PD) by nitro BT method (Pearse, 1972) and non-specific esterase (NSE) by Naphthol acetate method (Barka and Anderson, 1963).

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RESULTS AND DISCUSSION

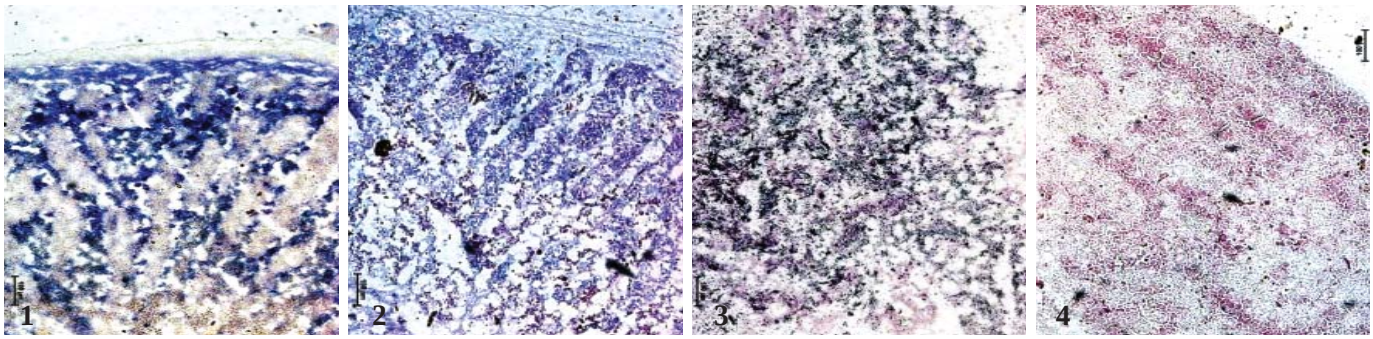
The histoenzymic activities of different phosphatases, oxidoreductases and esterases of testis are summarized in Table 1.

Alkaline Phosphatase: The activity of AKPase was weak and granular in the surface epithelium and

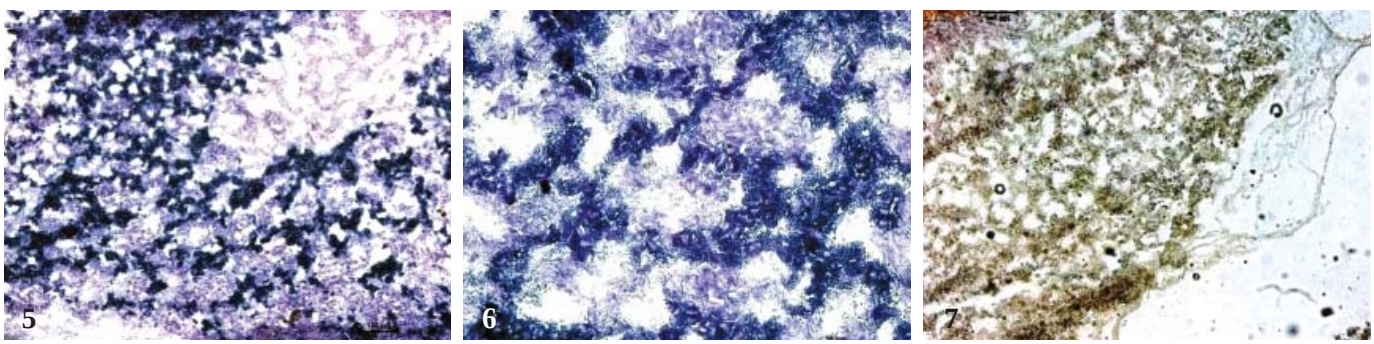
Table 1
Histoenzymic activities in testis of buffalo foetus

Enzyme	Surface epithelium	Testicular cords	Interstitial tissue	Mediastinum testis
AKPase	0/+	+/++	++	0/+
G-6-Pase	0/+	++	++	0/+
SDH	+	++	+/++	+
LDH	0/+	++	+/++	0/+
NADPH	+	++/+++	+/++	+
NADH	+	+++/++++	+++	+/++
G-6-PD	+	++/+++	++/+++	+/++
GLD	0/+	++	+++	+
NSE	0	+/++	+/++	+

AKPase=Alkaline phosphatase; G-6-Pase=G-6-Phosphatase; SDH= Succinate dehydrogenase; LDH=Lactate dehydrogenase; GLD= Glutamate dehydrogenase; NADH=Reduced nicotinamide adenine dinucleotide diaphorase; NADPH=Reduced nicotinamide adenine dinucleotide phosphate diaphorase; G-6-PD=Glucose-6-phosphate dehydrogenase; NSE=Non-specific esterase.



Figs 1-4. 1. Weak AKPase reaction in surface epithelium, moderate in tunica albuginea, testicular cords and interstitial tissue. Azo dye method ×100; 2. Weak SDH reaction in surface epithelium, weak to moderate reaction in testicular cords and interstitial tissue. Nitro BT method ×100; 3. Moderate reaction of G-6-PD in the testicular cords and weak reaction in the mediastinum testis. Nitro BT method ×100; 4. Moderate reaction of GLD in the testicular cords and weak reaction in the mediastinum testis. Nitro BT method ×100.



Figs 5-7. 5. Strong to intense NADH reaction in the testicular cords and strong reaction in the interstitial tissue. Nitro BT method ×400; 6. Strong to intense NADH reaction in the rete cords and weak to moderate reaction in the mediastinum testis. Nitro BT method ×400; 7. Very weak NSE in the surface epithelium and weak to moderate reaction in the testicular cords and interstitial tissue. Naphthol acetate method ×400.

mediastinum testis of buffalo foetus (Fig. 1). A weak to moderate granular AKPase reaction was also observed in the basement membrane and cellular components of the testicular cords. The interstitial tissue also exhibited moderate AKPase activity. However, Farooqui *et al.* (2012) noticed intense AKPase reaction in the basement membrane of convulated sex cords and rete testis from 116 days of gestation onwards in goat fetuses. The localization of AKPase at the basement membrane and the cellular components of the sex cords may be related with the transportation of ions across the membrane and its presence in the interstitial cells may be due to the sterioidogenic activity of the Leydig cells (Singh 1996).

Glucose-6-Phosphatase: The G-6-pase activity was weak in the surface epithelium and mediastinum testis of the buffalo foetuses. Moderate activity was observed in the sex cords and interstitial tissue (Table 1). The presence of G-6-Pase is indicative of glucose metabolism in the fetal testis. Similar findings have been reported in the neonatal buffalo calves by Singh (1996). However, doubtful G-6-Pase activity was observed in the seminiferous tubules of buffalo testis by Roy and Singh (1994).

The oxidoreductases enzymes are concerned with biological oxidation and reduction and include dehydrogenases and diaphorases.

Succinic Dehydrogenase: Weak activity of SDH was observed in the surface epithelium and mediastinum testis. Moderate reaction of SDH in the sex cords and weak to moderate in the interstitial tissue was observed in this study (Fig. 2). The activity in the interstitial tissue may be related with the synthesis of the steroid hormones and proliferation of tissue during foetal life. Similarly, Singh (1996) reported fine SDH activity in the basal and luminal compartments of the seminiferous tubules in neonatal buffalo calves.

Lactate Dehydrogenase: In the present study, a very weak LDH activity was seen in the surface epithelium and mediastinum testis. The testicular cords showed a moderate reaction which increased towards the centre of the tubules (Table 1). However, the activity varied from weak to moderate in the interstitial tissue. The LDH is an enzyme of glycolysis and is concerned with the conversion of pyruvate to lactate. Roy and Singh (1994) also reported increased activity of LDH towards the centre of tubules in buffalo testis. The increased activity may be associated with the spermatogenesis and redistribution of subcellular components of developing tissue.

Glucose-6-phosphate Dehydrogenase: The activity of this enzyme was found to be moderate to strong in the testicular cords, intertubular tissue and mediastinum

testis (Fig. 3). However, a weak reaction was observed in the surface epithelium. Singh (1996) reported this enzyme to be an interstitial enzyme, however, Bilaspuri (1978) found its localization primarily in the seminiferous tubules. Thus the activity of this enzyme is co-related with the proliferative activity of the testis.

Glutamate Dehydrogenase: The activity of GLD was found to be negligible to weak in the surface epithelium, moderate in the testicular cords and mediastinum testis and strong in the intertubular tissues (Fig. 4). This may be due to the fact that this enzyme plays an important role in the oxidative deamination during steroidogenesis.

Reduced Nicotinamide Adenine Dinucleotide Phosphate Diaphorase: The activity of NADPH was found to be strong in the basement membrane and cellular components of the sex cords (Table 1). The activity decreased from strong to moderate towards the centre of the tubules. A weak reaction of NADPH-diaphorase was observed in the surface epithelium and mediastinum testis. Similar findings have been reported in the buffalo testis during postnatal life by Roy and Singh (1994) and Singh (1996).

Reduced Nicotinamide Adenine Dinucleotide Diaphorase: A weak activity of NADH-diaphorase was observed in the surface epithelium. Its activity was more in the luminal compartment of the sex cords. This may be due to the proliferation of gonocytes and pre-sertoli cells to establish future spermatogenesis in adult animals. The intertubular tissue also showed strong NADH activity (Fig. 5). In the rete testis the reaction was more at the peripheral part than the centre (Fig. 6). Strong NADH activity had been reported in the Leydig cells of buffalo testis during postnatal life by Bilaspuri and Guraya (1983) and in pig fetal testis by van Vorstenbosch *et al.* (1986).

Esterases: A negligible reaction for NSE was observed in the surface epithelium of the testis in the buffalo foetus. The activity was found to be weak to moderate in the testicular cords and interstitial tissue whereas it was weak in the mediastinum testis (Fig. 7). Similar findings have been reported by Singh (1996) in the buffalo foetal testis.

REFERENCES

Barka, T. and Anderson, A.J. (1963). Histochemistry, Theory. Practice and Bibliography. Harper and Rao Publishers Inc., New York.

Bilaspuri, G.S. (1978). Morphological, histochemical and biochemical studies on the testis of some farm animals. Ph. D. thesis, Punjab Agricultural University, Ludhiana, India.

Bilaspuri, G.S. and Guraya. S.S. (1983). Histochemical localization of glycolytic enzymes, alcohol and secondary-alcohol dehydrogenases in the testes of buffaloes, goats and rams. *J. Agric. Sci.* **101**: 457-462.

Edward, M.J. (1965). Observations on the anatomy of reproductive organs of cows with special reference to those features sought during examination per rectum. *New Zealand Vet. J.* **13(2)**: 25-37.

Farooqui, M.M, Chandrapal, A. and Prakash, A. (2012). Histological and histochemcial studies on the prenatal development of testis in goat (*Capra hircus*). *International J. Morphol.* **30**: 1408-1421.

Hochereau de Reviers, M.T., Perreau, C., Pisselet, C., Locatelli, A. and Bosc, M. (1995). Ontogenesis of somatic and germ cells in sheep fetal testis. *J. Reprod. Fert.* **103(1)**: 41-46.

Kaur, M. (2006). Studies on histogenesis and organogenesis of the testis in buffalo *(Bubalus bubalis)*. M.V.Sc. thesis. Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Pearse, A.G.E. (1972). Histochemistry: Theortical and Applied (3rd edn.), Churchill Livingstone, London.

Roy, K.S. and Singh, B. (1994). Spermatogenic cell histochemistry in buffalo bull during winter. *Indian J. Anim. Sci.* **64**: 708-711.

Singh, P. (1996). Histological, histochemical and histoenzymic studies on the post natal development and differentiation of seminiferous epithelium and Leydig cells in buffalo testis. M.V.Sc. thesis, Punjab Agricultural University, Ludhiana, India.

Soliman, M.K. (1975). Studies on the physiological chemistry of the allantoic and amniotic fluid of buffaloes at various periods of pregnancy. *Indian Vet. J.* **52**: 111-117.

van Vorstenbosch, C.J., Spek, E., Colenbrander, B. and Wensing, C.J.G. (1984). Sertoli cell development of pig testis in the fetal and neonatal period. *Biol. Repd.* **31**: 565-577.