

## HISTOCHEMICAL STUDIES ON THE TESTIS AND EPIDIDYMIS OF CHAMURTHI HORSE

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## ABSTRACT

The present histochemical study was conducted on the testes and epididymis of 10 Chamurthi horses. The histochemical reactions were observed mainly in the capsule, seminiferous tubules, interstitial tissue and luminal contents of the testis and in the capsule, peritubular connective tissue layer, basement membrane, lining epithelium, supra nuclear zone, stereo cilia and luminal contents of the epididymis, which showed the presence of glycogen, sulphated mucosubstances, lipids and cholesterol. The capsule of the testes and epididymis showed moderate concentrations of glycogen and weak to mild concentrations of mucopolysaccharides, respectively.

**Keywords:** Chamurthi horse, Epididymis, Histochemistry, Testes

Chamurthi (Hill) horse is one of the six recognized breeds of horses in India. It is the animal of choice for the military personnel (Anonymous, 2003). Breeding and reproduction in Chamurthi horses is regulated through village panchayats without any scientific interference. Very scanty literature is available regarding histochemical reactions in testes and epididymis of Chamurthi horses (Sudhakar and Sharma, 1993) hence the present study was taken up to elucidate the histochemical localization of carbohydrates and lipids

## MATERIALS AND METHODS

The tissue pieces of 3-5 mm size collected from the testis and epididymis of 10 apparently healthy Chamurthi horses were fixed in neutral buffered formalin and processed with alcohol-benzene schedule for paraffin technique. Sections cut at 5-6  $\mu$ m thickness were then subjected to PAS (Periodic-acid-Schiff reaction) and Best's Carmine method for glycogen and Alcian Blue method (pH 2.5) for weakly acidic sulphated mucosubstances (Luna, 1968). For the demonstration of lipids, the tissue samples were taken and quickly frozen in the cryostat microtome chamber. Cryostat sections of 10  $\mu$ m thicknesses were obtained at -20 °C and subjected to Schultz method for cholesterol and Sudan Black- B method for fat (Luna, 1968).

## RESULTS AND DISCUSSION

## TESTES

**Carbohydrate histochemistry:** The testicular capsule showed moderate concentration of glycogen same as reported by Uppal *et al.* (2003) in the testis of donkeys, however, Sudhakar and Sharma (1993) and Pyne and Sinha (1989) observed strong and mild reaction, respectively for glycogen in the testes of Spiti ponies and goats. The interstitial tissue revealed moderate concentration

of glycogen (Fig. 1). In the cytoplasm of the Leydig cells, an intense reaction for glycogen was observed. Sudhakar and Sharma (1993) in Spiti ponies and Uppal *et al.* (2003) in donkeys found slightly PAS positive reaction in the cytoplasm of the Leydig cells whereas Goyal and Dhingra (1973) recorded the absence of glycogen in the interstitial tissue but observed a moderate activity in the walls of arterioles and venules. The presence of varied amounts of glycogen was also reported by Dellmann and Eurell (1998) in the interstitial cells of stallion.

The peritubular cells and the basement membrane of the seminiferous tubules (Fig. 1) showed strong glycogen reactions. The lining epithelium was PAS positive. Elongated and matured spermatids showed comparatively stronger reactions for glycogen than in the spermatogonia, spermatocytes and spherical spermatids. The Sertoli cells and luminal contents revealed a moderate glycogen concentration (Fig. 2). These carbohydrates might serve as an endogenous source of energy as glycogen of Sertoli cells supplies the nutrients to the germinal cells (Johnson *et al.*, 1970). Dellmann and Eurell (1998) have reported a varying amount of glycogen in the peritubular cells of stallion. In donkeys, the mass of the sperms in the seminiferous tubules was PAS positive (Uppal *et al.*, 2003). Gofur *et al.* (2008) stated that in buffaloes, the basement membrane of seminiferous tubules, spermatid and spermatozoa showed positive affinity whereas spermatogonia, primary spermatocytes and secondary spermatocytes revealed negative affinity to PAS stain. Blood vessels in the capsule and in the interstitial space in Chamurthi horses showed strong glycogen concentration.

**Alcianophilia:** In the testis, the capsule showed a weak concentration of acidic mucopolysaccharides. The basement membrane of the seminiferous tubules and peritubular cells showed a mild type of reaction with Alcian blue pH (2.5). In the lining epithelium, spermatogonia

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and spherical spermatids revealed weak reactions whereas spermatocytes, sertoli cells, elongated spermatids, matured spermatids and luminal contents showed mild concentrations of mucopolysaccharides.

In the interstitial tissue, mild type of reaction was observed in the Leydig cells. Pyne and Sinha (1989) in goats observed a mild reaction for acid mucopolysaccharides in the stroma, basement membrane and peritubular cells of the seminiferous tubules. Uppal *et al.* (2003) in donkeys and Kishore *et al.* (2008) in rams reported that the epithelium of the seminiferous tubules showed a weak to moderate concentration of acid mucopolysaccharides.

**Cholesterol reaction:** The lipids form the structural components of membranes help in storage and transport of metabolic fuel (Lehninger, 1975). A strong reaction of cholesterol was observed in the basement membrane and Leydig cells of the testes of Chamurthi horse (Fig. 3). Cholesterol positive secretions were also observed in the interstitial space and in the spermatogenic cells. In Gaddi goats and sheep, strong reactions for cholesterol were observed in Leydig cells, intertubular tissue and spermatogenic cells (Anonymous, 2003). No reaction was observed in the capsule of the testis and a weak to mild type of reaction for cholesterol was observed in the lining epithelium of the seminiferous tubule.

**Lipid histochemistry (Sudanophilia):** The capsule of the testis showed no reaction for the lipids, however Pyne and Sinha (1989) stated that the capsule of the testes showed a mild reactivity for lipids in goats. A moderate concentration of lipids was observed in the basement membrane of the tubules where as a weak to mild type of reaction was observed in the lining epithelium (Fig. 4). In the Leydig cells, strong concentration of lipids was present. Blood vessels in the capsule and in the interstitial tissue showed a mild concentration of lipids (Fig. 4). Posalaki *et al.* (1968) demonstrated sudanophilic lipids in spermatogenic cells of rat. Dellmann and Eurell (1998) reported varying amounts of lipid inclusions in the Sertoli cells of testes of domestic animals. Bilaspuri and Guraya (1983) observed lipids in seminiferous epithelial cycle of buffalo, goat and ram. Roy and Singh (1994) reported weak sudanophilia in spermatogonia and increased to moderate in spermatocytes and moderate to strong in spermatids.

## EPIDIDYMIS

**Carbohydrate histochemistry:** The glycogen reaction was moderate in tunica albuginea, mild in peritubular connective tissue and intense in the basement membrane of the epididymis (Fig. 5). This is in agreement with the findings of Sudhakar *et al.* (1990) in Spiti ponies. The lining epithelium, subapical surface of the epithelium and

the masses of sperms found in the lumen of the epididymis showed strong reaction but stereocilia showed moderate reaction for glycogen. The blood vessels in the capsule and in the intertubular connective tissue revealed strong concentration of glycogen. Pal and Bhardwaj (1986) in buffaloes observed a mild PAS positive reaction in the sperm mass.

**Alcianophilia:** Acid mucopolysaccharides enhance the fertilizing capacity of spermatozoa (Johnson *et al.*, 1970). The tunica albuginea of the epididymis showed mild concentration of acidic mucopolysaccharides and a moderate reaction was observed in the connective tissue. The interlobular connective tissue showed moderate concentration of acid mucopolysaccharides and was comprised of moderately Alcian blue reactive loosely arranged collagen and reticular fibres (Fig. 6). The strong concentration of acidic mucopolysaccharides was observed in the basement membrane and subapical region of the ductules. Cilia showed a moderate reaction with Alcian blue whereas in the luminal contents of the epididymis, a mild reaction was observed. These findings were similar to the observations of Sudhakar *et al.* (1990) in Spiti ponies. Bansal *et al.* (2003) reported that the stereocilia and luminal contents were positive for neutral and acid mucopolysaccharides in donkeys.

**Cholesterol reaction:** In the epididymis, weak cholesterol reaction was observed in the basement membrane. Cholesterol positive droplets were seen in the interstitial space. The columnar cells lining the mucosa of the epididymis showed a weak to moderate reaction. The sperms in the lumen and the apical surface of the columnar cells showed cholesterol positive droplets of varying sizes (Fig. 7). In the epididymis of Gaddi goat and sheep, moderate to intense reaction for cholesterol was observed in the lining epithelium and cholesterol positive droplets were observed in the interstitial space and in the lumen (Anonymous 2003). No reaction was observed in the capsule of the epididymis.

**Lipid histochemistry (Sudanophilia):** In the capsule of the epididymis, no presence of lipids was observed. The basement membrane of the ductules showed a moderate concentration of lipids whereas peritubular connective tissue, subapical surface and luminal contents revealed a mild reaction and the lining epithelium, stereocilia showed a weak concentration of lipids. Blood vessels in the connective tissue showed a moderate presence of lipids (Fig. 8). Nicander (1958) observed that the vacuolated cytoplasm of the lining cells contained lipid droplets in the ductus epididymidis in stallion. Goswami *et al.* (1993) stated that in camel small lipid granules were scattered



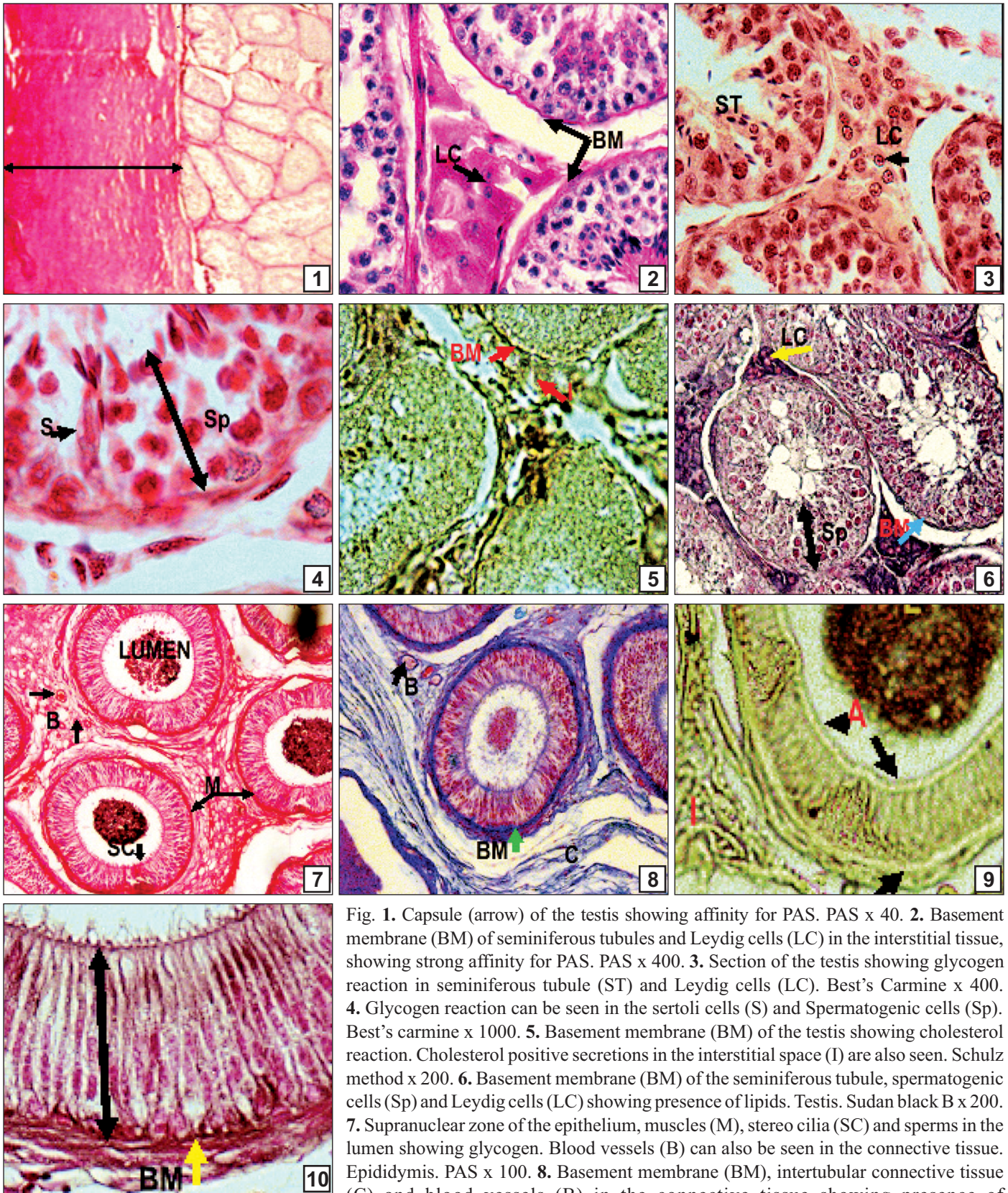


Fig. 1. Capsule (arrow) of the testis showing affinity for PAS. PAS x 40. 2. Basement membrane (BM) of seminiferous tubules and Leydig cells (LC) in the interstitial tissue, showing strong affinity for PAS. PAS x 400. 3. Section of the testis showing glycogen reaction in seminiferous tubule (ST) and Leydig cells (LC). Best's Carmine x 400. 4. Glycogen reaction can be seen in the sertoli cells (S) and Spermatogenic cells (Sp). Best's carmine x 1000. 5. Basement membrane (BM) of the testis showing cholesterol reaction. Cholesterol positive secretions in the interstitial space (I) are also seen. Schulz method x 200. 6. Basement membrane (BM) of the seminiferous tubule, spermatogenic cells (Sp) and Leydig cells (LC) showing presence of lipids. Testis. Sudan black B x 200. 7. Supranuclear zone of the epithelium, muscles (M), stereo cilia (SC) and sperms in the lumen showing glycogen. Blood vessels (B) can also be seen in the connective tissue. Epididymis. PAS x 100. 8. Basement membrane (BM), intertubular connective tissue (C) and blood vessels (B) in the connective tissue showing presence of mucopolysaccharides. AB (pH 2.5) x 100. 9. Cholesterol positive droplets on apical surface (A), interstitial space (I) and basement membrane (BM). Positive reaction in the lumen (L) can also be seen. Epididymis. Schultz's method x 400. 10. Lining epithelium (arrow) and basement membrane (BM) showing weak and moderate reaction for lipids respectively. Epididymis. Sudan black B x 400.

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throughout the cytoplasm of the columnar cells of the epididymis. The basal cells revealed coarse sudanophilic positive lipid droplets. Circular smooth muscle fibres of tunica muscularis revealed a mild lipid deposition.

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