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Research Article

HISTOCHEMICAL STUDIES ON THE TESTICULAR STROMA, SEMINIFEROUS TUBULES AND THE LEYDIG CELLS IN ASSAM GOATS

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ABSTRACT: The present study was conducted on eighteen male Assam goats of age ranging from day 1/day old to 10 months divided into six post natal age groups *viz*. group-I (0-day), group-II (2 months), group-III (4 months), group-IV (6 months), group-V (8 months) and group-VI (10 months) consisting of three animals in each group. Tissue pieces were collected from three different regions of the testis *viz*., upper, middle and lower and subsequently fixed in Bouin's solution. All the tissues were processed for paraffin sections and stained for various stains for demonstration of various histochemical substances *viz.* McManus method for glycogen, Alcian Blue method at pH 1.0 for acid mucopolysaccaharides, Fuelgen reaction for nucleic acids and Mercuric Bromophenol Blue method for Protein. The connective tissue of the capsule and septula testis was weakly reactive to basic proteins in day-old kids (group-I) and moderately reactive in 2 months old kids (group-II). The nuclei of the spermatogenic cells more particularly of the primary spermatocytes showed a strong reaction for basic proteins which were probably due to their increased activity during meiotic phases of spermatogenesis at 4 to 8 months of age (groups-II to V). The basement membranes of the seminiferous tubules were strongly PAS reactive in day-old kids (group-I), moderate in 2 months old kids (group-II) and weak reaction was seen in the older goats, where as the Sustentacular or Sertoli cells showed a very weak reaction in all the age groups under study. The capsule and septula testes showed a faint reaction for nucleic acids in the male goats in all the age groups. The nuclei of various spermatogenic cells were Fuelgen positive and the intensity of reaction increased with the advancing age of the kids. The connective tissue of the capsule and septula testis showed faint reactions for acid mucopolysaccharides in all the age groups. The Leydig cells were also weakly reactive in the kids up to 2 months of age (group-II), but showed moderate reactions in older kids.

Key words: Testis, Post natal development, Histochemistry, Assam goat.

INTRODUCTION

Attaining an early puberty in male is important from the economic point of view. It has a direct effect on selection of goats for progeny testing. Testis is the main organ of male reproductive system and the testicular parenchyma is composed of seminiferous tubules from where spermatozoa are produced and Leydig cells produce testosterone which is responsible for male sexuality and secondary male sex characteristics (Dellmann and Wrobel 1987, Hafez 2000). Quantitative testicular histology has been used to determine daily sperm production in the boar (Kennelly and Foote 1964) and short horn bull (Swienstra 1966).

Goats play an important role in socio-economic condition of the rural people. Post natal anatomical studies on the male genital system at various ages, particularly the testis and its tubular system are important to know the anatomical growth and development. A few anatomical studies on testes are reported in goats *viz.* post natal development of testes in Malabari goat from 4 to 11 months of age (Bilaspuri and Singh 1992), biometry of the testes in Sirohi goats (Mishra *et al*. 1984), testicular measurements in Assam local X Beetal goats (Sarmah *et al.* 1998) and testicular growth in British Saanen, Alpine and Toggenburg breeds of bucks (Ahmad and Noakes 1996). Some works were also conducted in other ruminants elucidating morphology and biometry of the testes such as buffalo (Pal and Bharadwaj 1983) and rams (Sergeev and Zaboloskii 1976). This work is the first of its kind reporting the histochemistry of the testis in Assam

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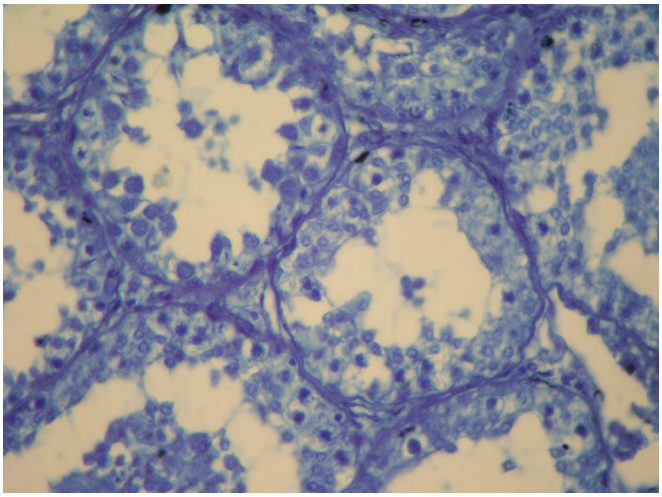

Fig. 1. Photomicrograph of the testis in a ten months old buck showing reactions to basic proteins. Bromophenol Blue stain, 400 X.

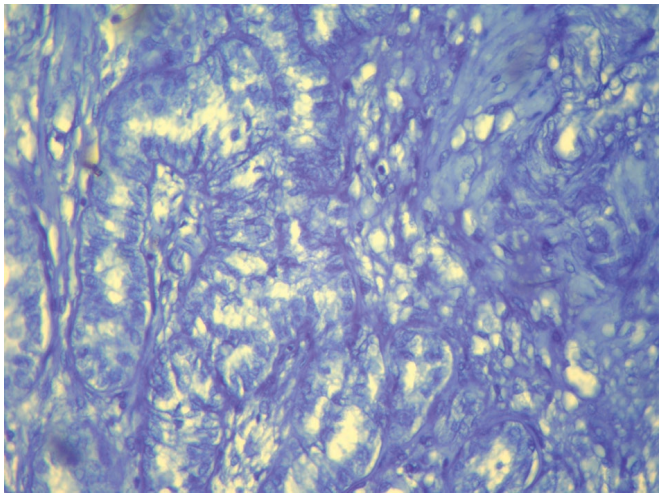

Fig. 2. Photomicrograph of basement membrane of the tubuli recti and rete testis in a four months old male kid showing reactions to basic proteins, Bromophenol Blue stain, 400 X.

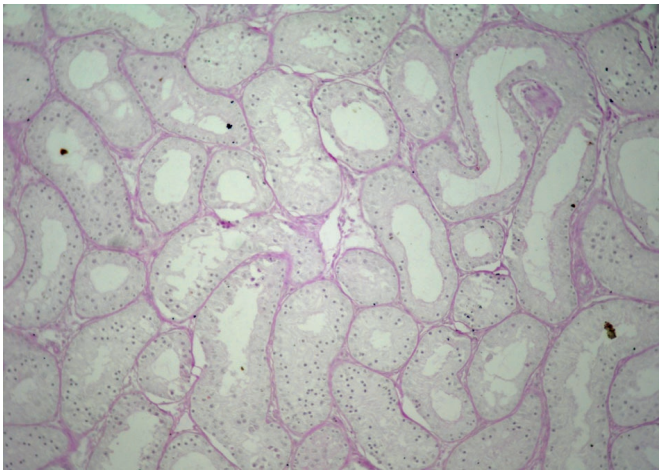

Fig. 3. Photomicrograph of the testis in a two months old male kid showing PAS reaction in the basement membranes of the seminiferous tubules. Mc manus Method, 100 X.

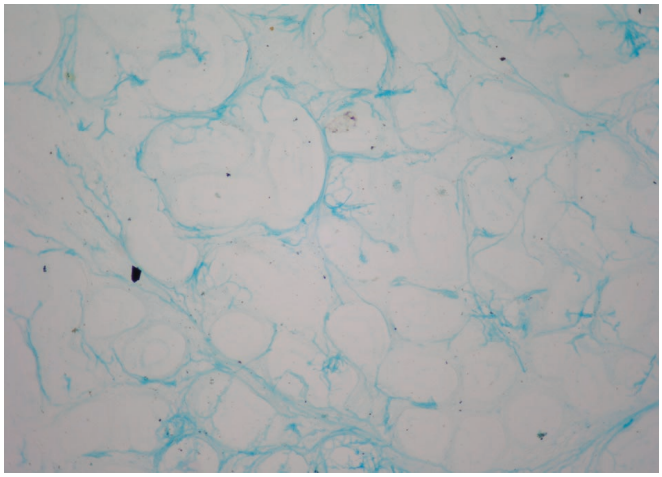

Fig. 4. Photomicrograph of seminiferous tubules in a two months old male kid showing weak reactions to acid mucopolysaccharides. Alcin Blue method (pH 1.0), 100 X.

goat at various ages during post natal development which will provide valuable information to the biological scientists.

MATERIALS AND METHODS

A total of 18 male Assam goats procured from in and around Khanapara, Guwahati varying in age from 0-day to 10 months were used in the present study. The animals were divided into six age groups *viz.* group-I (Day old/ day1), group-II (2 months), group-III (4 months), group-IV (6 months), group-V (8 months) and group-VI (10 months) consisting of three animals in each group. The age of the goats was estimated from birth records. Each animal was weighed using Spring Balance to record the body weight. The animals were sedated by giving intramuscular injection of Siquil (Triflupromazine Hydrochloride) @ 1mg/Kg body weight and subsequently anaesthetized by administering intravenous injection of Intraval Sodium (Pentobarbital Sodium) @ 15 mg/Kg body weight (Hall *et al*. 2000). After induction of proper level of anesthesia, the animals were sacrificed. The technical program involving animals was approved by Animal Ethics Committee, AAU.

After sacrificing the animals, the testes were collected by dissecting the scrotum and tissue pieces from it were taken from three different regions of the testis *viz*., upper, middle and lower and subsequently fixed in Bouin's solution prepared. All the tissues were processed for paraffin sections by alcohol- xylene method using cedarwood oil (Luna 1968). Sections were cut at 5 µ thickness using a Rotary Microtome (Thermo, Germany)

and stained for various stains for demonstration of various histochemical substances *viz.* McManus method for glycogen, Alcian Blue method at pH 1.0 for acid mucopolysaccharides, Fuelgen reaction for nucleic acids and Mercuric Bromophenol Blue method for Protein (Humason 1967).

RESULTS AND DISCUSSION

In the present study, the connective tissue of the capsule and septula testis was weakly reactive to basic proteins in day-old kids (group-I) and moderately reactive in 2 months old kids (group-II). A moderate to strong reaction exhibited by the basement membranes of the seminiferous tubules indicated presence of a fair amount of basic proteins at various ages. These findings were in corroboration with the observations of Pyne and Sinha (1989) in goats, and Singh (1996) in buffaloes. The nuclei of the spermatogenic cells more particularly of the primary spermatocytes showed a strong reaction for basic proteins which were probably due to their increased activity during meiotic phases of spermatogenesis at 4 to 8 months of age (groups-II to V). Bilaspuri (1978) also reported that the activity of protein in the spermatocytes increased from leptotene to deplotene stages in buffalo. Further, Rajani *et al*. (2001) observed that the reaction for basic proteins in all the tissues of rat testis increased with the advancement of age as also seen in the present study. A weak reaction observed in the nuclei and cytoplasm of the lining epithelium of the tubuli recti and rete testis at various ages in the study might be due to their less activity as compared to the spermatogenic cells of the seminiferous epithelium.

The connective tissue stroma of the capsule of the testes showed weak to moderate Periodic Acid Schiff (PAS) reaction in all the age groups indicative of its less glycogen content, which might be a specific characteristic in regard to this breed of goat. Chandra Pal and Bharadwaj (1989) also reported that the connective tissue fibres of the testes of buffalo calves at different ages exhibited mild to moderate PAS reaction. Again, basement membranes of the seminiferous tubules were strongly reactive in day-old kids (group-I), moderate in 2 months old kids (group-II) and weak reaction was seen in the older goats. The weak reactivity of the basement membranes in older subjects as compared to the younger ones was reported earlier in buffalo calves (Goyal and Dhingra 1973), Black Bengal goats (Pyne and Sinha 1989). Also, Razi *et al.* (2012) also reported that the first three layers of germ cells showed weak reactions to PAS in adult Wister rats. This might be due to the utilization of the glycogen from the basement membrane by the developing spermatogenic cells much more in adult goats as compared to the young ones. Gofur *et al*. (2008) stated that the basement membrane of seminiferous tubules, spermatids and spermatozoa showed positive affinity for PAS stain whereas the spermatogonia, primary and secondary spermatocytes and Sertoli cells showed negative affinity in bull above one year of age. The Sustentacular or Sertoli cells showed a very weak reaction in all the age groups under study. As regard to the reactivity of the Sertoli cells to PAS reaction, Goyal and Dhingra (1973) reported that the Sertoli cells completely lacked PAS active materials in the testis of buffalo. On the contrary, Dhingra (1980) found a positive reaction for PAS active carbohydrates in the Sertoli cells of goats. This variation in the reactivity of the Sertoli cells to PAS reaction might be due to breed or species variations.

The Leydig cells showed weak to moderate PAS reaction in the testes of 4 months old (group-III) kids. Similar observations were also made by Karmore *et al.* (2001) stating that the Leydig cells showed mild PAS positive reaction in the testis of goat. Moderate PAS reaction as observed in Assam goat might be due to the increased functional activity of the Leydig cells at this age as it was ascertained in the present study itself that the initiation of spermatogenesis started at the age of 4 months in this goat breed. On the contrary, Goyal and Dhingra (1973) reported that the Leydig cells completely lacked PAS reactive material in buffalo, which might draw the same conclusion of species variation. In this study, the basement membranes of the tubuli recti and rete testis exhibited a moderate reaction in younger kids and weak reaction in older goats. These findings were in conformity with that observed in goat (Dhingra 1980) and buffalo (Chandra Pal and Bharadwaj 1990).

In the present study, the connective tissue of the capsule and septula testis showed faint reactions for acid mucopolysaccharides in the male goats of all the age groups as also observed in Black Bengal goats (Pyne and Sinha 1989). However, Chandra Pal and Bharadwaj (1989) reported a mild to moderate reaction for acid mucopolysaccharides in the connective tissue stroma of the testes in Indian buffaloes. Again, a faint reaction to acid mucopolysaccharides was seen in the basement membranes, spermatogenic and Sertoli cells of the seminiferous tubules in the male Assam goats. The Leydig cells were also weakly reactive in the kids up to 2 months of age (group-II), but showed moderate reactions in older kids as also reported by Karamore *et al.* (2001) in goats. In this study, the basement membranes and the lining epithelium of the rete testis and tubuli recti showed weak reactions to acid mucopolysaccharides. On the contrary,

Chandra Pal and Bharadwaj (1990) obtained a mild to moderate reaction for acid mucopolysaccharides in the epithelium of the tubuli recti and rete testis in buffaloes.

The capsule and septulae testes showed a faint reaction for nucleic acids in the male goats in all the age groups. The nuclei of various spermatogenic cells were Fuelgen positive and the intensity of reaction increased with the advancing age of the kids. This might be due to increased mitotic activity of the various spermatogenic cells in growing kids. These findings were in conformity with the observations made in buffalo (Chandra Pal and Bharadwaj 1990) and goat (Kakade and Singh 1990). In this study, the fibrocytes and Leydig cells of the intertubular tissue of the testes showed a mild to moderate Fuelgen reaction. Similar findings in regard to the reactivity of the Leydig cells to Fuelgen reaction was also reported in goats (Bordoloi 1979).

CONCLUSION

The reactivity of the capsule, trabeculae and intratubular connective tissue of the epididymis for basic proteins increased with advancing age of the animals. The nuclei of the spermatogenic cells revealed strong reaction to basic protein at 4 months of age. The basement membranes of the sex cords of the testes in day-old kids were strongly PAS reactive, moderate in 2-month-old kids and showed weak to moderate reaction in older goats. The cytoplasm of the gonocytes was strongly PAS positive at birth while this reaction in the spermatogenic cells diminished with advancing age. The various testicular compartments showed weak to moderate reactions for acid mucopolysaccharides and nucleic acids in the male goats under study.

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