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Age related studies on the dehydrogenases of the testis in Assam goat

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ABSTRACT

Male Assam goats (18) varying in age from 0-day to 10 months were used in the present study. The animals were divided into groups, viz. group 1 (0-day), 2 (2 months), 3 (4 months), 4 (6 months), 5 (8 months) and 6 (10 months) consisting of 3 animals in each group. The cryostat sections were incubated for the histoenzymic demonstration of various enzymes viz. glucose-6-phosphate dehydrogenase (G-6-PDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), Δ5-3 ß-hydroxysteroid dehydrogenase (Δ5-3 ß-HSD) and 17-ß-hydroxysteroid dehydrogenase (17-ß-HSD). The seminiferous tubular epithelium of the testis in day-old (group 1) and 2-month old (group 2) kids showed mild activity of LDH enzyme which increased to moderate at 4 months of age (group 3) and moderate to strong in 6- and 8-month old goats (groups 4, 5). The activity of MDH remained weak in the seminiferous epithelium up to 4 months of age (group-3), increased to moderate in 6- month-old bucks (group 4) and strong in older goats. The activity of G-6-PDH was weak in the seminiferous epithelium at all the ages except at 8 and 10 months of age (groups-5, 6), in which a moderate activity of this enzyme was seen. The activities of 17-ß-HSD and Δ5-3ß-HSD were absent in the seminiferous epithelium in all the animals. These enzymes showed moderate to intense activities in the leydig cells from 6–10 months of age (groups 4 to 6). Similarly, LDH, MDH and G-6-PDH exhibited moderate activity in the Leydig cells of male kids at 4 month of age and the activity increased with advancing age of the male goats.

Key words: Goat, Histoenzymology, Interstitial tissue, Seminiferous epithelium, Testis

India possesses 122.92 millions goats of which 29.06 lakh are found in Asom (Anonymus 2003). The histological and histoenzymic studies are necessary to evaluate the growth process of the male genital organs in terms of cellular activities concerned with the spermatogenesis and the biochemical steps involved in the male hormone production during steroidogenesis. It is essential to observe the activity of enzymes like oxidoreductases, Δ5-3-ß-HSD and 17-ß-HSD in the tissues during the study of growth process of the male genital tract towards maturity. Histoenzymic studies on the testis were conducted in rat (Polaski 1965), boars (Hay and Deane 1966), bulls (Blackshaw and Samisoni 1967), camel (Osman *et al.* 1978) and in buffalo (Singh *et al.* 1996). The present investigation is the first report on histoenzymic localization of various dehydrogenases in the testis at different postnatal ages in Assam goat.

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MATERIALS AND METHODS

Male Assam goats (18) varying in age from 0-day to 10 months were used in the present study. The animals were divided into groups 1 (0-day), 2 (2 months), 3 (4 months), 4 (6 months), 5 (8 months) and 6 (10 months) consisting of 3 animals in each group. The age of the goats were estimated from birth records. The animals were sedated by giving intramuscular injection of triflupromazine hydrochloride @ 1mg/kg body weight and subsequently anaesthetized by administering intravenous injection of intravel sodium (pentobarbital sodium) @ 15 mg/kg body weight (Hall *et al*. 2000). After induction of proper level of anesthesia, the testicles were incised out by performing open method of castration as per standard protocol (permitted by Institutional Animal Ethics Committee, AAU). Subsequently, the animals were given proper post operative treatment and care.

Representative tissue samples from testes of the animals of various age groups were collected and sectioned at 10 µ thicknesses using a cryostat. The cryostat sections were incubated for the histoenzymic demonstration of various enzymes as given in Table 1.

Table 1. Histoenzymic techniques used for the demonstration of different enzymes

Enzyme	Substrate used	Incubation time	Method and source
Glucose-6-phosphate dehydrogenase(G-6-PDH)	Di-Na-glucose-6-phosphate	30 min	Nitro- BT method (Pearse 1980)
Lactate dehydrogenase (LDH)	Sodium-DL-lactate	30 min	Nitro- BT method (Barka and Anderson 1965)
Malate dehydrogenase (MDH)	L-malic acid	30 min	Nitro- BT method (Barka and
Δ5-3 ß- hydroxysteroid dehydrogenase (Δ5-3 ß-HSD)	Dehydroepi-androsterone	30 min	Anderson 1965) Nitro- BT method (Barka and Anderson 1965)
17-ß- hydroxysteroid dehydrogenase (17-ß-HSD)	Estradiol	45 min	Nitro- BT method (Barka and Anderson 1965)

The activity of the above enzymes was recorded in all the tissues.

RESULTS AND DISCUSSION

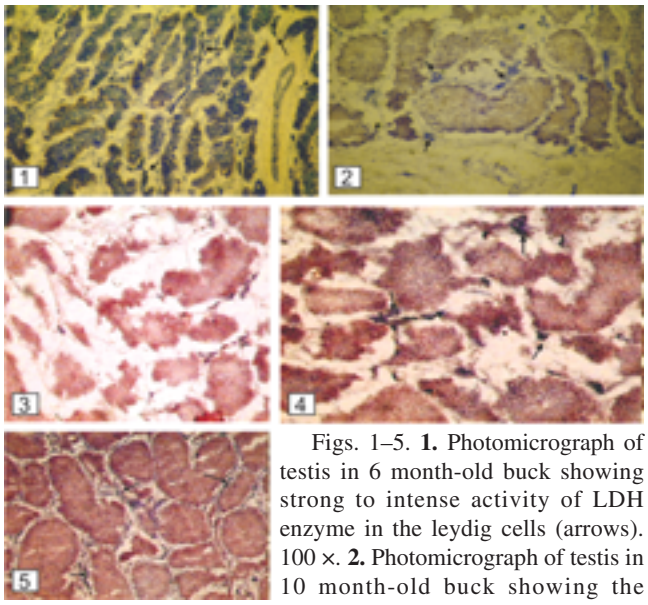
The histoenzymic activities of various tissue enzymes viz. LDH, MDH, G-6-PDH, 17-ß HSD and Δ5–3 ß HSD were found concentrated mostly in the seminiferous epithelium and Leydig cells. In the present study, the seminiferous epithelium in day-old (group 1) and 2 months old kids (group 2) showed mild activity of LDH enzyme that increased to moderate at 4 months of age (Fig. 1). LDH positive granules with moderate to strong intensity of activity were seen in the various spermatogenic cells of the seminiferous epithelium in 6–8 months old bucks (group 4–5) and strong activities of the enzyme were observed in the spermatids of 10 months old goats (group 6). Strong LDH activity in the spermatids indicated higher glycolysis in these cells as also observed in buffalo bulls (Roy and Singh 1994). Zinkham *et al.* (1964) demonstrated isoenzyme of LDH (LDH-X) associated with the process of spermatogenesis. Histoenzymic activity of LDH observed in the seminiferous epithelium of the testes in Assam goats of various ages was mainly in the spermatogonia. Similar findings were also reported by Blackshaw and Samisoni (1967) stating that the LDH showed a marked activity in the spermatogonia of the bull testis.

No activity of LDH was seen in the Leydig cells in the male kids up to 2 month of age (group 2). However, the Leydig cells in 4-month old kids (group 3) showed moderate activity of LDH and the activity varied from strong to intense in older bucks. This enhanced localization of the LDH in the Leydig cells in older bucks might be to perform the catalytic functions in the process of steroidogenesis for the production of increased testosterone hormone by these cells at this age. These findings were in close agreement with the observations of Hitzeman (1962) narrating that the Leydig cells of the mouse testis exhibited intense activities of LDH after puberty. Strong activity of LDH in the Leydig cells was also reported in bull (Blackshaw and Samisoni 1967) and in buffalo bulls (Singh *et al.* 1996).

The seminiferous epithelium showed a weak reaction for MDH in the male Assam goats up to the age of 4 months (group 3). The activity increased from moderate to strong at 6 and 8 months of age (groups 4, 5), respectively. However, the activity of MDH was slightly reduced in the seminiferous epithelium in 10-month old bucks (group 6). Hitzeman (1962) reported a weak activity of MDH in the seminiferous epithelium in bull testis. (Klobucar *et al.* 2000) also observed that the enzymic activity of MDH in the seminiferous epithelium of mice was higher at birth and then declined by 30 days of age and adulthood. In this study, a strong activity of MDH was seen in the Leydig cells (Fig. 2) of male goats from 4 to 10 months of age (groups 3 to 6). This enhanced localization of the MDH in the Leydig cells in older bucks might be to perform stimulatory effect in the process of steroidogenesis for the production of increased testosterone hormone by these cells at this age. An enhanced activity of MDH was reported in the Leydig cells of bull testis (Blackshaw and Samisoni 1967). Again, Hitzeman (1962) also observed that that the MDH activity was widely distributed in the Leydig cells of mouse after puberty.

In the present study, the activity of G-6-PDH was weak, fine granular in the seminiferous epithelium of the testes in all the age groups of Assam goats, except at 8 and 10 months of age (groups 5, 6), in which a moderate activity was noticed. A fairly good G-6-PDH activity was demonstrated in the spermatogonia of bull testis (Blackshaw and Samisoni 1967). G-6-PDH was important in synthetic mechanism and particularly in production of steroid hormones (Roy and Singh 1994). Again, G-6-PDH had its key position in the hexose monophosphate shunt (Blackshaw and Samisoni 1967). The activity of the G-6-PDH in the Leydig cells was moderate to strong in all the male goats at various ages under study (Fig. 3). Strong reactivity of G-6-PDH in the Leydig cells was demonstrated in the bull testis (Blackshaw and Samisoni 1967).

The enzymic activity of Δ5–3 ß-HSD was not seen in the seminiferous epithelium of the testis in all the male goats under study indicating absence of this enzyme in these cells. However, a moderate to strong activity of Δ 5–3 ß-HSD was



Figs. 1–5. **1.** Photomicrograph of testis in 6 month-old buck showing strong to intense activity of LDH enzyme in the leydig cells (arrows). 100 ×. **2.** Photomicrograph of testis in 10 month-old buck showing the activity of MDH enzyme in the leydig cells (arrows). 100 ×. **3.** Photomicrograph of the testis in 6-month-old buck showing the activity of G-6-PDH in the leydig cells (arrows). 100 ×. **4.** Photomicrograph of testis in 8- month-old buck showing the activity of Δ5–3ß HSD enzyme in the leydig cells (arrows). 100 ×. **5.** Photomicrograph of the testis in a 6-month-old buck showing the activity of 17-ß-HSD in the leydig cells (arrows). 100 ×.

observed in the Leydig cells (Fig. 4) from 6 month of age (group 4) onwards in the male Assam goats. The enzymic activity of this enzyme was reported to be variable in different species of animals. Again, localization of Δ 5–3 ß-HSD had been demonstrated in camel (Osman *et al.* 1978). However, in contrast to the present findings, a strong activity of Δ 5–3 ß-HSD was recorded in the leydig cells of adult pigs (Hay and Deane 1966), which could be a species variation.

The activity of 17-ß-HSD was absent in the sex cords and seminiferous tubular epithelium in all the male goats under study. However, (Singh *et al.* 1991) reported that 17-ß-HSD could be found in traces exhibiting fine granular uniform distribution in the cells of the rete testis in adult Jamunapari goats, which might be a breed characteristic. The leydig cells showed no activity of this enzyme up to 2 month of age (group 2). Moderate, strong and intense activities of 17-ß-HSD were observed (Fig. 5) in the Leydig cells in 4-, 6- and 8- and 10- months old goats, respectively. Strong activity of 17-ß-HSD was also reported in buffalo bulls (Roy and Singh 1994).

A strong activity of 17-ß-HSD in the Leydig cells of pubertal goats as observed in this study probably indicated the active role played by this enzyme in steroidogenesis as also reported by Blackshaw and Samisoni (1967) stating that 17-ß-HSD was essential in the production of steroid hormones in bulls. Again, Klobucar (2000) noticed the presence of 17-ß-HSD in the Leydig cells in pigs aged from birth to 40 weeks. In the present study, enzymic activity of 17-ß-HSD was not found in the seminiferous tubular epithelium of the testes.

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