



Histoenzymic studies on testis following oral zinc supplementation in Assam male goats

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Key role of zinc in spermatogenesis, sperm motility and morphology and their metabolism, tubular secretion of male hormones etc. is well documented. It is necessary to see the role of dietary zinc in regulating the activities of enzymes like LDH, MDH, Δ^5 -3 β -HSD and 17- β -HSD which are known to be associated with the functioning of the male genital system towards maturity. Taking the above background into consideration and as no earlier study of this kind was conducted in Assam goat, so this present study was undertaken to elucidate the effect of oral zinc supplementation on histoenzymology of the testis in this local goat species.

Assam local male kids (36) of 3 months of age and between 3 and 4 kg of body weight were included in this experiment. They were divided into 3 groups, viz. group 1 (control group), kids grew without zinc supplementation; group 2 (inorganic group), kids grew with inorganic zinc supplementation; and group 3 (organic group), kids grew with organic zinc supplementation. All the animals were reared under semi-intensive system in the Experimental Animal Shed belonging to Department of Veterinary Physiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati. Standard managerial practices were adopted with regards to rearing of experimental animals during the period of experiment. The experimental goats received concentrate mixture @ 50 g/day/goat up to 5 months and then @100 g/day/goat up to 7 months in addition to their free range grazing. Group 1

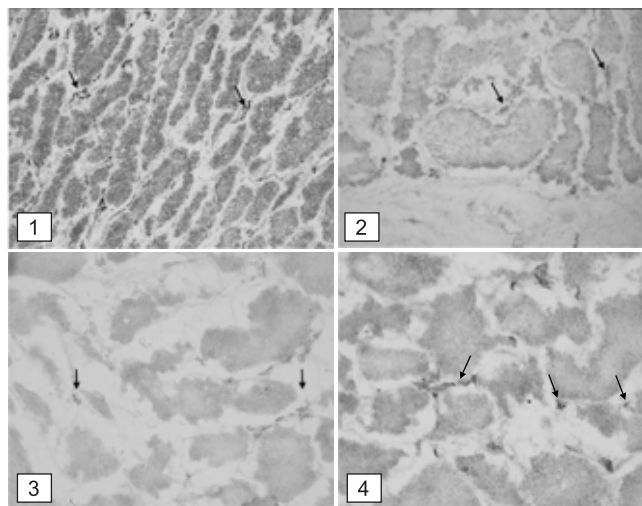
(control) animals received no zinc supplementation while the groups 2 and 3 animals received inorganic and organic form of zinc supplements, respectively, along with concentrate mixture. The aforesaid concentrate mixture contained 2% mineral mixture – without zinc which was supplied by AICRP project. The kids belonging to group 2 and 3 received 120 mg of inorganic zinc supplement (zinc sulphate) and 40 mg of organic zinc supplement respectively.

For histoenzymic studies, tissue samples from testis and epididymis of the animals from each group were collected and sectioned at -20°C using a cryostat and incubated for the histoenzymic localization of lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and Δ^5 -3 β -hydroxysteroid dehydrogenase (Δ^5 -3 β -HSD) as per Pearse (1980), and 17- β -hydroxysteroid dehydrogenase (17- β -HSD) as per (Barka and Anderson 1965). The slides were studied and photographed using an image analyser.

Lactate dehydrogenases (LDH): Histoenzymic studies revealed slight variations of LDH activity in both zinc-supplemented and control groups. The activity in the LDH positive granules (Formazan granules) was mild to moderate in the seminiferous epithelial cells of the testis at 4 months of age and moderate to strong at 6 and 7 months of age in the control group. It indicated that there was age-related increase in the histoenzymic activities of LDH in this tissue. The Leydig cells also showed a similar trend since mild activity at 4 months of age was found to be strong and intense at 6 and 7 months kids (Fig.1). These findings are in good agreement to the earlier studies being carried out by Bilashpuri and Guraya (1983) and Shen and Lee (1993). However, the present study showed slightly higher activities of this enzyme in testicular tissues of zinc-supplemented groups as compared to the control. This higher histoenzymic activity of LDH in zinc-supplemented groups in the present study might be due to stimulatory effect of zinc on steroidogenesis as enzyme co-factor.

Malate dehydrogenases (MDH): An age-related rise of histoenzymic activity of MDH in the seminiferous epithelium was observed in the present study. This epithelial tissue

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Figs 1–4. **1.** Photomicrograph of the testis in a 7 month-old kid (Organic group) showing activities of LDH enzyme in the Leydig cells (arrows), 100 ×. **2.** Photomicrograph of the testis in a 7-month old kid (inorganic group) showing activities of MDH enzyme in the Leydig cells (arrows). 100 ×. **3.** Photomicrograph of the testis in a 6- month-old kid (Organic group) showing activities of Δ^5 -3 β HSD enzyme in the Leydig cells (arrows), 100 ×. **4.** Photomicrograph of the testis in a 6 month old kid (inorganic group) showing activities of 17- β -HSD enzyme in the Leydig cells (arrows), 100 ×.

exhibited a weak reaction at 4 months of age with a rise to moderate activity at 5 and 6 months of age in both control and treated groups, while a strong activity was observed at 7 months of age. Leydig cells also showed higher MDH activity at 6 and 7 months of age (Fig.2). It could be assumed that as the kids grew, the operation of MDH dependent TCA cycle in the seminiferous tubule might rise to higher optimum level to meet up energy demand of the steroidogenic cells in the seminiferous tubule. Shen and Lee (1993) recorded strong MDH activity in the foetal Leydig cells and at the time of birth in mouse and the activity was widely distributed in the Leydig cells after puberty. The present study did not reveal any marked increase of MDH activity in the zinc-supplemented groups as compared to control group. It was perhaps due to the fact that the zinc had least or no effect on MDH in testicular tissues.

Delta 5 -3 beta hydroxysteroid dehydrogenase (Δ^5 -3 β -HSD): The activity of this steroidogenic enzyme in the Leydig cells of the testis increased with advancing age in all the groups (Fig.3). However, comparatively higher activities of this enzyme were observed in the zinc-supplemented groups than that of control. Since this enzyme helps in the conversion of progesterone into male hormones

androstenedione and testosterone, the present study indicated that zinc supplementation had positive effect on testosterone production in growing kids approaching puberty. Conversion of Δ^5 steroid to Δ^4 steroid by Δ^5 -3 β -HSD was also reported in the ovary of goat (Goswami 1989).

17- β - hydroxysteroid dehydrogenase (17- β -HSD): Our findings in regards to activity of 17- β -HSD indicated that zinc supplementation had stimulatory effect on this enzyme since 17- β -HSD was comparatively higher in treated groups than that of control in the Leydig cells from 4 months onwards (Fig. 4). Further the study revealed that the histoenzymic activity of this enzyme increased with advancing age of kids, being highest at 7 months of age. Stimulatory effect of zinc on the steroidogenic activity of the testis could be possible reason, because 17- β -HSD involved in different steps of steroidogenesis in the Leydig cells including the conversion of androstenedione to testosterone. Literature also indicates that at birth the 17- β -HSD activities was low and the activity increased at the time of maturity in mouse testis (Shen and Lee 1993). However, latest references could not be found in the available literature to discuss with.

SUMMARY

Studies were conducted to ascertain the effect of organic and inorganic forms of oral zinc supplementation on the histoenzymology of the testis in Assam goats of 3 to 7 months of age. It revealed that the histoenzymic activity of different tissue enzymes like LDH, MDH, Δ^5 -3 β - hydroxysteroid dehydrogenase and 17- β -hydroxysteroid dehydrogenase in the Leydig cells of the testis increased with advancing age in all the groups, but comparatively higher activities of this enzyme were observed in the zinc-supplemented groups than that of control.

REFERENCES

- Barka T and Anderson J. 1965. *Histochemistry*. pp. 237-316. Hoeber Medical Division, Harper and Row, New York.
- Bilaspuri G S and Guraya S S. 1983. Histochemical localization of glycolytic enzymes, alcohol and secondary alcohol dehydrogenase in the testes of buffaloes, goats and rams. *Journal of Agricultural Science* **101**: 457–62.
- Goswami J. 1989. 'Effect of progestagen-PMSG treatment of certain ovarian characteristics and hormonal profile in anestrus local goats (*Capra hircus*) of Asom.' Ph.D. thesis. Assam Agricultural University, Khanapara, Guwahati, India.
- Pearse A G E. 1980. *Histochemistry. Theoretical and Applied*. Pp. 1342–48. Churchill Livingstone, New York.
- Shen, R and Lee, I P. 1993. Developmental pattern of enzymes in mouse testes. *Journal of Reproduction and Fertility* **48**: 301–5.