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Immunolocalization of 3β-Hydroxysteroid Dehydrogenase (3β-HSD) in the Testis of Lesser Mouse Deer (*Tragulus javanicus*)

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ABSTRACT

The present study describes the location of 3β-hydroxysteroid dehydrogenase in the testis of lesser mouse deer (*Tragulus javanicus*) using immunoperoxidase technique. Testicular samples were obtained from 4 adult male lesser mouse deer; two samples were collected during the dry and the other 2 samples during the wet season. All the samples were subjected to immunoperoxidase staining technique to determine the localization of 3β-hydroxysteroid dehydrogenase (3β-HSD), an enzyme that plays a central role in biosynthesis of steroid hormones, including androgen and oestrogen and an indicator for spermatogenesis activity. The positive reactions were observed in the Leydig, Sertoli, and spermatogonia cells of all animals. The number of positive Leydig cells was significantly ($p<0.05$) higher in the testes sampled during the dry season while the number of positive Sertoli cells was significantly ($p<0.05$) higher in the testes of animals that were sampled during the wet season. However, the total number of cells showing positive reactions remained insignificantly ($p<0.05$) different in all the animals.

Keywords: 3β-Hydroxysteroid dehydrogenase (3β-HSD), lesser mouse deer

INTRODUCTION

Lesser mouse deer (*Tragulus javanicus*, family Tragulidae) is the smallest ruminant that inhabits the tropical rain forest of the Southeast Asia (Medway, 1969). Currently, its population is decreasing. In order to prevent its extinction, conservation and breeding strategies which include management and knowledge on the reproductive system are important. However, only limited information on the reproductive physiology of the male lesser mouse deer is currently available.

In male animals, the pattern of reproductive activity can be observed by measuring the testosterone levels (Payne and Youngblood, 1995). It is important to note that the synthesis of steroid hormones, such as androgen (testosterone) and oestrogen, requires the activity of steroidogenic enzymes like cholesterol side chain cleavage cytochrome (P450scc), 3ßhydroxysteroid dehydrogenase (3ßHSD), and cytochrome 17-a hydroxylase (P450c17) (Bhasin *et al*., 2003; Donnel, 2003).

The enzyme 3 β-hydroxysteroid dehydrogenase/isomerase (3β-HSD) catalyses an essential step in the biosynthesis of steroid hormones, which include androgen and oestrogen production, and it is widely distributed

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in the peripheral steroid target organs (Vidal *et al.,* 2000; Prisco *et al*., 2007). Nonetheless, the distribution of 3β-HSD enzyme in mammals is not only expressed in the steroid-producing organs (Penning, 1997), it is also found in several peripheral tissues including the skin (Dumont *et al*., 1992), breast and liver (Zhao *et al*., 1990, Zhao *et al*., 1991), lung (Milewich *et al*., 1977), kidney (Bain *et al*., 1991), endometrium (Bonney *et al*., 1985), prostate (El-Alfy *et al*., 1999), and brain (Ukena *et al*., 1999).

Thus, investigation on the immunolocalization of 3β-hydroxysteroid dehydrogenase can be used to characterize the sites of biosynthesis of androgen intervening in spermatogenesis. This is because the knowledge on the distribution and appearance of steroidogenic enzymes in the testis will lead to a better understanding of the synthesis and proportion of steroid hormones in a species. This study investigated the locations of 3β-hydroxysteroid dehydrogenase (3β-HSD) enzyme, which is important in biosynthesis of androgens within the testis of male lesser mouse deer.

MATERIALS AND METHODS

Animals

Four sexually mature male lesser mouse deer (*Tragulus javanicus*), that were kept individually in single cages, were used in this study. Unilateral castration was made on each animal; two were during the wet season of October, while the remaining two were done during the dry season of July. All the experiments were conducted in accordance to the Ethical Committee, Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Immunohistochemistry

The testicular samples were fixed for 24 h in 8 ml of Bouin's fixative, followed by 70% ethanol until processing. The testicular samples were sectioned between 3 and 4 µm, de-waxed, rehydrated in graded ethanol, and washed in distilled water. The sections were subjected to antigen retrieval by microwaving in 0.01 M citrate buffer (pH 6) on a full power (boiling point) for 10 min. After that, the sections were allowed to cool at room temperature for 30 min before they were washed, three times for 5 min each with distilled water and subsequently incubated in 3% H_2O_2 in PBS for 10 min to block endogenous peroxidase. After 3 washes for 5 min in PBS, the sections were preincubated with blocking solution 3% normal goat serum in PBS for 60 min to prevent non-specific immunostainning with secondary antibody. The sections were washed in PBS (10 min) and incubated with primary antibody, the rabbit polyclonal to 3 β-HSD1 (Abcam Cambridge, UK #ab65156) 1:400 in 1% BSA. The reactions were detected with a biotin-conjugate secondary antibody (Biotinylated anti-rabbit IgG (H+L), Vector, USA # BA-1000) 10 ug/ml in 10 mM Phosphate, pH 7.8, 0.15M NaCl and ABC complex using DAB as chromogen. Counterstaining was performed with Mayer's hemalum. Negative controls were performed by omitting the primary antibody. On the contrary, the positive controls were performed on the paraffin sections of the rats' testis.

The samples were then examined under light microscopy. The numbers of Leydig, Sertoli, and spermatogonia cells found in five microscopic fields at 400 magnifications with positive reactions were counted.

Statistical Analysis

All the data were statistically analysed using student t-test.

RESULTS

Positive reactions were observed in the Leydig, Sertoli and spermatogonia cells (*Fig. 1*), with the most significant ($p<0.05$) positive reactions observed among the Leydig cells of the animals sampled during the dry season. In contrast, significantly ($p<0.05$) more positive reactions were observed in the Sertoli cells of the animals sampled during the wet season (*Fig. 2*). The numbers of spermatogonia, showing positive reactions, were insignificant ($p>0.05$) between the animals that were sampled during the dry

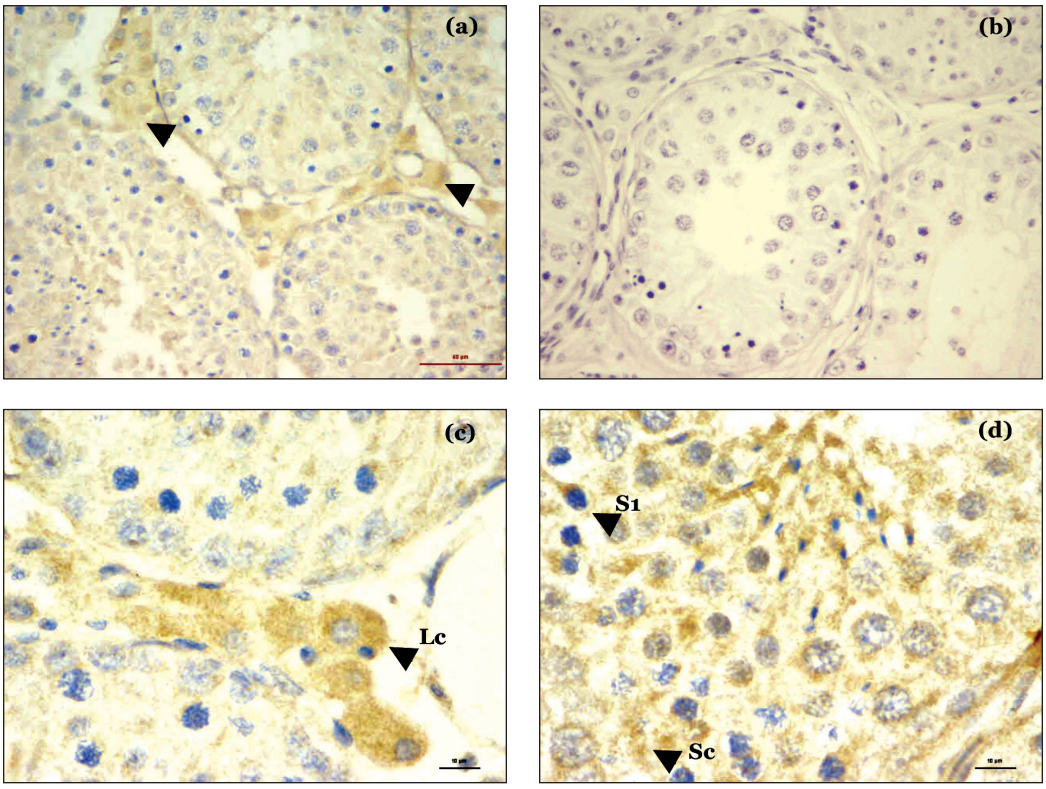

Fig. 1: Photomicrographs showing the immunohistochemistry staining for 3β-hydroxysteroid dehydrogenase (3βHSD) enzyme in the testis of lesser mouse deer. (a) Positive reaction against 3β-HSD and (b) negative sample. (c) The distribution of 3βHSD enzyme in Leydig cells, Lc and (d) in Sertoli cells Sc and spermatogonia S1

or wet season. However, the total numbers of cells showing positive reaction revealed insignificant ($p<0.05$) differences between all the four animals.

DISCUSSION

The present investigation showed that the Leydig, Sertoli and spermotogonia cells were positive to 3 beta-hydroxysteroid dehydrogenase (3β-HSD), a bifunctional enzyme that catalyzes the oxidative conversion of Delta (5)-ene-3-beta-hydroxy steroid and the oxidative conversion of ketosteroid, which also plays a crucial role in the biosynthesis of all classes of hormonal steroids (Kowalewski *et al*., 2006). Meanwhile, immunolocalization of 3β-HSD was observed in all the testicular samples but with different cell involvements during the dry (July) and wet (October) seasons. However, the overall number of the positive cells remained insignificantly different between all the four animals, regardless of the season of sampling. This is correlated with the spermatogenesis process in the testis of lesser mouse deer. In particular, the Sertoli and Leydig cells are differently involved in the hormonal control of spermatogenesis, whereby the Sertoli cells play a role before the beginning of meiosis and after spermiation. The Leydig cells involve in the meiosis phase (Prisco *et al*., 2007). The results of this study strongly suggest that both the Sertoli and Leydig cells are involved in the production of androgen hormone needed for spermatogenesis in the testis of lesser mouse

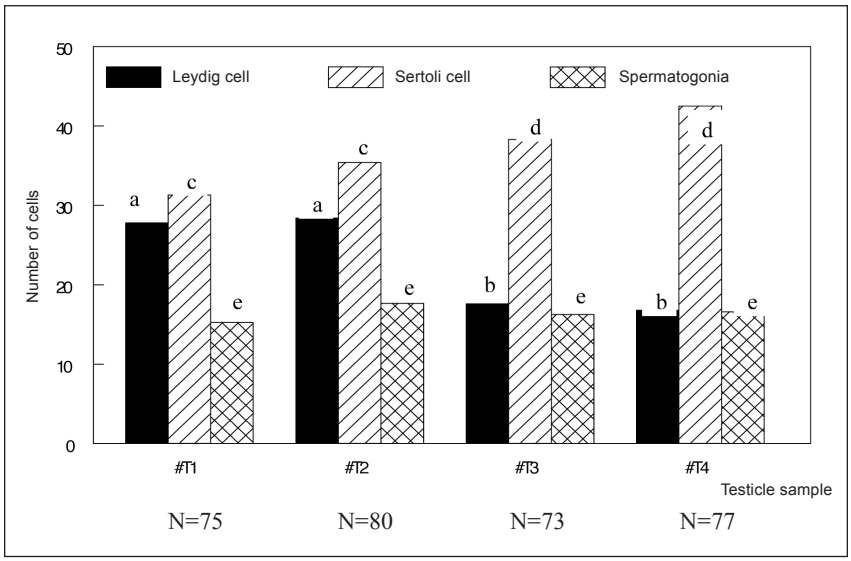

Fig. 2: The number of cells showing positive immunohistochemical reaction for 3β-HSD enzyme. Animals #T1 and #T2 were sampled during the dry season, while animals #T3 and #T4 were sampled during the wet season. Different superscripts indicate significant ($p<0.05$) difference. N=the total number of positive cells for the animals

deer. In addition, the findings also revealed that the Sertoli cells were more prominent than the Leydig cells in controlling and regulating spermatogenic cells, peritubular cells and Leydig cells (Skinner, 1991). Although positive reactions were observed in the spermatogonia, the number was rather low and therefore insignificant.

The findings of this study also suggest that during the dry season, the Leydig cells are more active in producing 3β-HSD enzyme compared to during the wet season. On the other hand, the Sertoli cells are active in producing the enzyme in both the dry and wet seasons. This is correlated well with the concentration of testosterone and mating. Hesterman *et al*. (2005) have reported that during the oestrus cycles, males demonstrate a high level of olfactory interest in their partner's ano-genital area and excreta, affecting the pituitary gland to release luteinizing hormone which regulates testosterone secretion. Subsequently, the relatively high level of testosterone stimulates spermatid maturation (Garner and Hafez, 2000).

In conclusion, this study revealed the presence of immunolocalization of 3β-HSD in the testis of lesser mouse deer in the Leydig cells, Sertoli cells and spermatogenia. Hence, they are considered as androgen-producing cells.

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REFERENCES

Bain, P.A., Yoo, M., Clarke, T., Hammond, S.H. and Paine, A.H. (1991). Multiple forms of mouse 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4 isomerase and differential expression in gonads, adrenal glands, liver, and kidneys of both sexes. *PNAS*, *88*, 8870-8874.

Bhasin, G., Henry, H.L. and Norman, A.W. (2003). In H.L. Henry and A.W. Norman (Eds.), *Encyclopedia of Hormones*. California: Academic Press.

Bonney, R.C., Reed, M.J., Beranek, P.A. and James, V.H. (1985). Metabolism of adrenal androgens by human endometrium and adrenal cortex. *Journal of Steroid Biochemistry*, *23,* 347-352.

Donnel, L.O. (2003). In H.L. Henry and A.W. Norman (Eds.), *Encyclopedia of Hormones*. California: Academic Press.

Dumont, M., Luu-The, V., Dupont, E., Pelletier, G. and Labrie, F. (1992). Characterization, expression, and immunohistochemical localization of 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4 isomerase in human skin. *Journal of Investigative Dermatology*, *99*, 415-421.

El-Alfy, M., Luu-The, V., Huang, X.F., Berger, L., Labrie, F. and Pelletier, G. (1999). Localization of type 5 17 beta-hydroxysteroid dehydrogenase, 3 beta-hydroxysteroid dehydrogenase, and androgen receptor in the human prostate by in situ hybridization and immunocytochemistry. *Endocrinology*, *140*, 1481 - 1491.

Garner, D.L. and Hafez, E.S.E. (2000). Spermatozoa and seminal plasma. In E.S.E. Hafez and B. Hafez (Eds.), *Reproduction in farm animals*, *7*. Lippincott Williams and Wlkins.

Hesterman, H., Wasser, S.K. and Cockrem, J.F. (2005). Longitudinal monitoring of fecal testosterone in male Malayan sun bears (*U. malayanus*). *Zoo Biology*, *24*, 403-417.

Kowalewski, M.P., Mason, J.I., Howie, A.F., Morley, S.D., Schuler, G. and Hoffmann, B. (2006). Characterization of the canine 3 beta-hydroxysteroid dehydrogenase and its expression in the corpus luteum during diestrus. *Steroid Biochemistry and Molecular Biology*, *101*, 254-262.

Medway, L. (1969). *The Wild Mammals of Malaya and Offshore Islands Including Singapore*. Kuala Lumpur: Oxford University Press.

Milewich, L., Winters, A.J., Stephens, P. and MacDonald, P.C. (1977). Metabolism of dehydroisoandrosterone and androstenedione by the human lung *in vitro*. *Journal of Steroid Biochemistry*, *8*, 277-284.

Payne, A.H. and Youngblood, G.L. (1995). Regulation of expression of steroidogenic enzymes in Leydig cells. *Biology of Reproduction*, *52*, 217-225.

Penning, T.M. (1997). Molecular endocrinology of hydrixysteroid dehydrogenases. *Endocrine Reviews*, *18*, *2*81-305.

Prisco, M., Liguoro, A., Ricchiari, L., Giudice, G.D., Angelini, F. and Andreuccetti, P. (2007). Immunolocalization of 3 beta- HSD and 17 beta-HSD in the testis of the spotted ray *Torpedo marmorata. General and Comparative Endocrinology*, *155*, 157-163.

Ukena, K., Kohchii, C. and Tsutsui, K. (1999). Expression and activity of 3 beta-hydroxysteroid dehydrogenase/delta5-delta4-isomerase in the rat Purkinje neuron during neonatal life. *Endocrinology*, *140*, 805-813.

Vidal, S., Roman, A., Moya, L. and Kovacs, K. (2000). Expression of 3Beta-hydroxysteroid dehydrogenase/isomerase in the female rat pituitary. *Journal of Endocrinology*, *166*, 95-101.

Zhao, H.F., Rheaume, E., Trudel, C., Couet, J., Labrie, F. and Simard, J. (1990). Structure and sexual dimorphic expression of a liver-specific rat 3 beta-hydroxysteroid dehydrogenase/isomerase. *Endocrinology*, *127*, 3237-3239.

Zhao, H.F., Labrie, C., Simard, J., de Launoit, Y., Trudel, C., Martel, C., Rheaume, E., Dupont, E., Luu-The, V. and Pelletier, G. (1991). Characterization of rat 3 beta-hydrixysteroid dehydrigenase/delta 5-delta 4 isomerase cDNAs anddifferential tissue-specific expression of the corresponding mRNAs in steroidogenic and peripheral tissues. *Journal of Biological Chemistry*, *266*, 583 - 593.