Immunolocalization of Steroidogenic Enzymes in the Fetal, Neonatal and Adult Testis of the Shiba Goat

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Abstract: The localizations of steroidogenic enzymes (P450scc, 3βHSD, P450c17 and P450arom) in testes of Shiba goats were investigated by immunohistochemistry. P450scc, 3βHSD, P450c17 and P450arom were detected in all Leydig cells of adults. P450scc and P450c17 were observed in most Leydig cells in the fetus (90 days) and neonate (15 days). 3βHSD and P450arom were found in some Leydig cells of the fetus with weak immunostaining but the numbers of immunopositive Leydig cells and intense immunostaining were increased in Leydig cells of the neonate. These results suggest that Shiba goat testes have the ability to synthesize progestin, androgen and estrogen in the fetus, neonate and adult, and synthesis of these steroid hormones showed an age-related rise.

Key words: goat testes, immunohistochemistry, steroidogenic enzymes

Two hormones are required for the sexual differentiation of the mammalian fetus, testosterone and Mullerian-inhibiting hormone. Testosterone plays a critical role in mammalian reproduction; it is essential for maintaining sexual function, germ cell development, and accessory sex organs [19]. Estrogen is an important hormone regulating part of the male endocrine system [23]. Previous studies have indicated that various processes important for spermatogenesis are impaired by inappropriate exposure to estrogens during fetal and pubertal development, as well as in adulthood, highlighting the importance of a normal balance between androgen and estrogen for male fertility [5].

The synthesis of androgens and estrogen from cholesterol requires the activity of steroidogenic enzymes. Cholesterol side-chain cleavage cytochrome P450 (P450scc), 3β-hydroxysteroid dehydrogenase (3βHSD) and cytochrome 17α-hydroxylase P450 (P450c17) are required to convert cholesterol to testosterone. Cytochrome P450 aromatase (P450arom) is responsible for the formation of estrogen from testosterone [2, 5]. The precise location of steroidogenic enzymes inside the
testes has been studied in recent decades. Immunolocalization of various steroidogenic enzymes in the testes has been reported in several species, including humans [10], mice [18], bears [24], raccoon dogs [25] and deer [9], but data on the pattern of expression of steroidogenic enzymes necessary for androgen and estrogen production in developing testis, especially in large experimental animals, are limited.

The shiba goat is a Japanese miniature goat. It is a non-seasonal breeder under natural daylight and its inbred strain has been established for experimental use [11]. The age of puberty in Shiba goats is about 7 months and male adult Shiba goats are virile throughout the year. They have the ability to copulate continuously throughout the year [22]. The aim of the present study was to investigate localization of steroidogenic enzymes and to determine the developmental pattern of steroidogenic enzymes in fetal, neonatal and adult testicular tissues of Shiba goats.

Four Shiba goats maintained at Tokyo University of Agriculture and Technology were used in this study. Testicular tissues were obtained from one fetus of 90 days, one neonate of 15 days old and two adult Shiba goats (both 3 years old). The testes were excised immediately from animals after sacrifice by ketamine overdose. The testes obtained were immediately fixed in 4% paraformaldehyde (Sigma Chemical Co., St. Louis, MO, USA) in 0.05 M PBS, pH 7.4 for immunohistochemical observation. All procedures were carried out in accordance with the guidelines established by Tokyo University of Agriculture and Technology.

The serial sections of testes were incubated with 10% normal goat serum to reduce background staining caused by the second antibody. The sections were then incubated with primary antibodies (1:500 or 1:1000) raised against bovine adrenal P450scc, human placental 3βHSD, porcine testicular P450c17, and human placental P450arom for 12 h at room temperature. The sections were then incubated with a second antibody, goat anti-rabbit IgG conjugated with biotin and peroxidase with avidin, using a rabbit ExtrAvidin™ staining kit (Sigma, St. Louis, MO, USA), followed by colouring with 30 mg 3,3-diaminobenzidine (Wako, Tokyo, Japan) solution in 150 ml of 0.05 M Tris-HCl L–1 buffer, pH 7.6, plus 30 µl H2O2. Finally, the reacted sections were counterstained with haematoxylin solution (Merck, Tokyo, Japan). The control sections were treated with normal rabbit serum (Sigma, St. Louis, MO, USA) instead of the primary antisera.

Immunoreactivities for P450scc, 3βHSD, P450c17 and P450arom were present in cytoplasm of Leydig cells of the adult Shiba goats (Fig. 1i–l). P450scc and P450c17 were expressed in the cytoplasm of Leydig cells of the fetus and neonate (Fig. 1a, c, e, g). 3βHSD and P450arom were found in some cytoplasm of Leydig cells of the fetus, with weak immunostaining (Fig. 1b, d). An increase in the numbers of 3βHSD and P450arom immunopositive Leydig cells were observed in the neonatal testis. The intensity of the immunohistochemical signal for 3βHSD and P450arom appeared to differ between the fetus and neonate, as more intense immunostaining for 3βHSD and P450arom were observed in the neonate (Fig. 1f, h). Immunoreactivities for P450scc, 3βHSD, P450c17 and P450arom were not present in Sertoli cells in the fetus, neonate and adult Shiba goats. No immunostaining was detected in control sections in which normal rabbit serum was substituted in place of the primary antibody (data not shown).

Immunolocalizations of P450scc, 3βHSD and P450c17 were observed for the first time in Leydig cells of the fetal and neonatal Shiba goat testes in this study. These findings are similar to those observed in other species such as rats [6, 14, 20], sheep [21] and pigs [16]. In rats, the onset of testicular testosterone production has been reported to start on Day 15.5 p.c. [6, 14, 20]. Immunohistochemical studies by Goyal et al. [7] have detected androgen receptor in the developing testis (1–19 weeks old) and efferent ducts of the rat. 3βHSD is the predominant regulator of testosterone production in Leydig cells [12, 17]. The present findings on the expression of 3βHSD show an age-related rise in testes, which is in accordance with these earlier reports. Androgen is critical during normal fetal development for male sexual differentiation and postnatally for initiation of spermatogenesis.

Previous studies have shown that estrogens formed locally in the prenatal and early postnatal rat brain from testicular androgens are involved in the irreversible masculinization of an undifferentiated fetal brain [15]. In a recent study, the presence of estrogen receptors in testes and in the associated ducts of the male mouse reproductive tract as early as day 13 of fetal age and throughout fetal development, suggests the possibility that estrogen produced from the fetal testes may play a
role in the development of the male reproductive tract from an early stage of fetal life [8]. Our immunohistochemical evidence here shows that P450arom was present in the fetus and neonate, with an increase in the numbers of immunopositive Leydig cells, suggesting that Shiba goat fetal and neonatal testes have the ability
to synthesize estrogen. This is in agreement with previous studies that estrogen is essential, and plays a role in the development of the male reproductive tract [13].

The present study showed that P450scc, 3βHSD, P450c17 and P450arom were well localized in adult Leydig cells. In some mammals, P450arom is localized in Leydig cells, Sertoli cells and germ cells [12, 24, 25]. However, in rams [3], stallions [1], humans [10] and boars [4], P450arom was localized only in Leydig cells. Our immunohistochemical data indicate that P450arom was localized only in the Leydig cells of Shiba goats. Taken together, these results show that P450arom is located in various areas of testes, with species differences. P450arom is a key enzyme responsible for the formation of estrogen from androgen in the testes and has various sources, suggesting that normal spermatogenesis requires estrogen, and that the timing, amount, and duration of estrogen action must be tightly coordinated for the development and maintenance of normal sperm production [2].

In conclusion, immunoreactions for P450scc, 3βHSD, P450c17 and P450arom were present in Leydig cells of the fetus, neonate and adult Shiba goat. These results suggest that not only androgen but also estrogen are essential for male sexual differentiation, postnatally for initiation and maintenance of spermatogenesis in Shiba goats.

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