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Immunohistochemical localization of inhibin/activin subunits in adult Asian elephant (*Elephas maximus*) testes

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ABSTRACT. Immunolocalization of inhibin-α and inhibin/activin βA and βB subunits in the testes of Asian elephant was determined. Testicular sections were immunostained with polyclonal antisera against inhibin subunit-α and inhibin/activin βA and βB using the avidin-biotin-peroxidase complex method. Positive immunostaining against inhibin-α subunit was strongly present in Sertoli cells, and positive immunostaining for the inhibin/activin βA and βB subunits was observed in both Sertoli and Leydig cells. These results indicated that while Sertoli cells are the predominant source of inhibin and activin secretions in the testes of adult male Asian elephant, Leydig cells are a source of activin but not inhibin.

KEY WORDS: Asian elephant, immunohistochemistry, inhibin/activin subunits, Leydig cell, Sertoli cell

Inhibin and activin are non-steroidal glycoprotein hormones involved in feedback controls between the gonads and pituitary gland [2, 14, 17]. Both hormones belong to the transforming growth factor-β (TGF-β) group. Inhibins have a disulfide-linked heterodimer structure, composed of an α-subunit and either a β_A (inhibin A) or β_B (inhibin B) subunit. Activins have either a homo- or heterodimer structure, in which only the β-subunits are linked to form activin A (β_A-β_A), activin B (β_B-β_B), and activin AB (β_A-β_B) [1, 14]. In mammals, inhibin is principally produced in the testes and ovaries, the endocrinal functions of which are regulated by gonadotropins secreted by the pituitary gland. The secretion of pituitary follicle-stimulating hormone (FSH) is suppressed by inhibin and stimulated by activin [4].

The Asian elephant (*Elephas maximus*) is the largest terrestrial mammal native to Asia, which spans 13 countries, including Thailand. To date, there has been a rapid decline in the population of Asian elephants in captivity, because of low birth rates and high mortality [21]. Several studies have been conducted to understand elephant reproductive biology and hormonal physiology, in order to improve the management of captive breeding strategies and development of assisted reproduction technology.

Although research regarding the role of inhibin in both African and Asian female elephants has been previously reported [7, 13, 24], only males in the African species have been studied [8, 12]. The present study determined the immunolocalization of inhibin/activin subunits in the testes of adult male Asian elephants.

The testes of a 20-year-old Asian elephant in its non-musth state were collected after its accidental death, and preserved in 4% paraformaldehyde prior to use in the experiment. Testicular tissues were dehydrated in a graded series of ethanol and then

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embedded in paraffin wax. In order to prepare samples for histological observation using hematoxylin and eosin (HE) stain, 5 μm sections were cut, mounted onto slides coated with poly-L-lysine (Sigma-Aldrich, St. Louis, MO, U.S.A.), deparaffinized, and then rehydrated using the routine procedure.

In order to unmark antigen site, the 5 μm serial sections of the Asian elephant testes tissue were deparaffinized and then treated by autoclaving in 0.01 M citrate buffer (pH 6.0) at 120°C for 10 min. A solution of 3% H_2O_2 was applied for 30 min in order to block any endogenous peroxidase activity, followed by washing with phosphate-buffered saline (PBS) three times. In order to reduce background staining of secondary antibodies, the sections were incubated with 10% normal goat serum; they were then incubated with the primary antibodies for 12 hr at 4°C. The antibodies against each inhibin subunit were anti-[Tyr30]-porcine inhibin α chain (1–30)-NH_2 conjugated to rabbit serum albumin, acetyl anti-porcine inhibin βA (81–113)-NH_2 (#305-24D), and anticyclic acetyl human inhibin βB (80–112)-NH_2 (#305-25D). The secondary antibody analysis was performed using goat anti-rabbit IgG conjugated with biotin, and peroxidase with avidin (Vectastain ABC Rabbit IgG Kit, Vector Laboratories, Burlingame, CA, U.S.A.). Antibody binding was visualized using 3–3′ diaminobenzidine tetrahydrochloride (Histofine DAB-3S kit, Nichirei Biosciences, Tokyo, Japan). Normal rabbit serum was used as a substitute for the primary antisera in the experimental controls.

HE staining was used to observe the different stages of spermatogenic cells in the seminiferous tubules, including the spermatogonia, spermatocytes, round spermatids, and elongated spermatids; the male elephant used in the study was determined to have normal spermatogenesis (Fig. 1A). The immunohistochemistry analysis revealed positive immunostaining against the inhibin-α subunit, a result that was observed strongly in the Sertoli cells (Fig. 1B); however, immunoreactivity for the inhibin/activin-βA and βB subunits was detected in both the Sertoli and Leydig cells (Fig. 1C and 1D). No immunostaining reaction was observed within the control tissues stained with normal rabbit serum (Fig. 1E).

It has been suggested that the paracrine and autocrine functions of the inhibin subunits are involved in spermatogonial development, Sertoli cell proliferation, and steroid biosynthesis in male mammals [23], with Sertoli cells generally being considered the major source of endogenous inhibin. Nevertheless, cellular sources for inhibin expression and secretion might differ, depending on the animal species [3, 6]. In human, and in some monkey, bull, and rat species, localization of inhibin subunits is predominantly observed in the Sertoli cells [4, 5, 9–11, 16, 19]. In some other mammals, the Leydig as well as the Sertoli cells are capable of secreting inhibin, i.e., horse, Shiba goat, dog, pig, Japanese black bear, golden hamster, and muskrat [6, 15, 18, 20, 22, 23]. According to the present study, and also a previous study by Li *et al*. [12], the sources of inhibin secretion also differ between elephant species. The results of the present study suggested that Sertoli cells are the most probable predominant source of inhibin and activin in male Asian elephants, whereas Leydig cells probably secrete activin but not inhibin. However, study of the different sources of inhibin production in African elephant has found that both Sertoli and Leydig cells can synthesize inhibin and activin [12]. Moreover, the inhibin secretory pattern in male African elephants has been previously reported by Kaewmanee *et al*. [8], who found that the level of circulating immunoreactive (ir)-inhibin in male African elephants was correlated with the degree of musth and testosterone level, with the latter being higher in the musth than in the pre- and non-musth stages. The authors suggest that the increasing level of (ir)-inhibin and testosterone during the musth stage resulted from the stimulation of high luteinizing hormone (LH) and FSH during the pre-musth stage [8].

The present study has provided data concerning the localization of inhibin/activin subunits present in Asian elephant testes. In order to better understand the physiological and functional roles of inhibin in male Asian elephants, future studies of circulating inhibin are required, together with observations from research conducted on a wider sample of different individuals.

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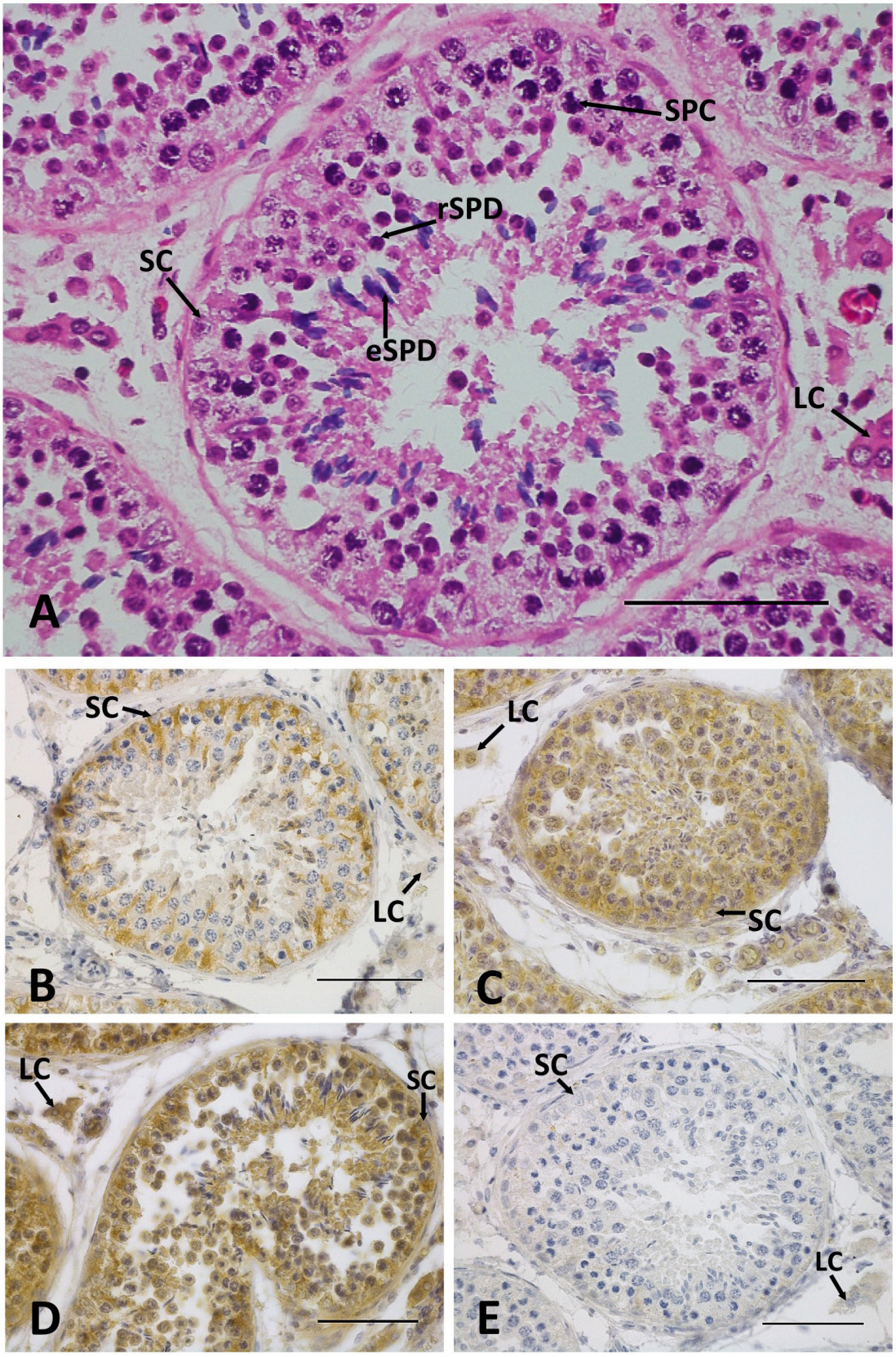

Fig. 1. Histological observation and immunohistochemical localization of inhibin/activin subunits in an Asian elephant's testes. Different stages of spermatogenic cells were observed in the seminiferous tubule (A). Positive immunostaining of the inhibin-α subunit was detected in Sertoli cells (B). In addition, positive immunostainings against the inhibin/activin βA and βB subunits were observed in both the Sertoli and Leydig cells (C and D, respectively). No immunostaining was detected in the control testicular tissue stained with normal rabbit serum (E). The scale bars represent 50 μm. SC: Sertoli cells; LC: Leydig cells; SPC: spermatocytes; rSPD: round spermatids; eSPD: elongated spermatids.

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