

Histological Structure of Testes and Ductus Epididymis of Rusa Deer (*Cervus timorensis*)

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ABSTRACT

Rusa deer (*Cervus timorensis*) raising farm has been highly increasing in numbers during the last decade in Thailand. The velvet is needed in the ancient Chinese medicinal composition and the meat for consumption. The appropriate time of mating in rusa deer of tropical climate as Thailand is still undetermined since various reports indicated that the climate zone influenced the different mating period. This study was mainly aimed to preliminary observed the histological structure of testes and epididymis of rusa deer in the rutting period. Testes of 2 mature rusa deer age 2 and a half years old were collected during October - November 2005 at the slaughter house of Kasertsart University, Kampaeng Saen Campus. The tissue embedded in paraffin blocks were then cut approximately 5mm in thickness and stained with Hematoxylin-Eosin (H&E). The samples were examined under light microscope. The testes were covered by fibrous connective tissue of the tunica albuginea. The testicular tissue consisted of packed seminiferous tubules. The interstitium composed of loose areolar connective tissue surrounding basement membrane of seminiferous tubules. The lumen of seminiferous tubules displayed various spermatogenic cells, spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids. Male genital ducts were divided into 4 regions namely, Rete testis, Ductuli efferentes, Ductuli epididymides and Ductus epididymis. In the part of Ductus epidymis are pseudostratified stereocilliated columnar epithelium cell.

Key words: testes, epididymis, rusa deer

INTRODUCTION

The rusa deer (*Cervus timorensis*), is a seasonal breeding animal characterized by a short rutting period during hard antler (Lincoln, 1978). Therefore, testicular and epididymal structures could indicate the sperm maturation which appear in the spermatogenesis. In comparision other species that are non-seasonal breeding animals

have been reported to demonstrate continuous spermatogenesis. Thus, this species could be a model for investigation of spermatogenesis in seasonal breeding animals through the structure of morphometric and cellular configuration based on the histological technique. Male germ cells or spermatozoa are produced in the process called spermatogenesis, within the seminiferous tubules (Sharpe, 1994). The sperms move from the

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seminiferous tubule within each testes, through the rete testis and efferent ductules, to the epididymis. The passage through the epididymis contributes to sperm maturation (Barth and Oko, 1989). The rutting period was indicated as the time of breeding of the male deer which could be observed the morphology of seminiferous tubules and epididymis. For this reason, this study is aimed at bringing technique of histology to demonstrate the stage of sperm maturity of rusa deer for a good understanding of sperm development during hard antler.

MATERIALS AND METHODS

Animals

Two 2 years and six months old rusa deers raised under semi-free ranging condition in Nakornpathom Province, Thailand. They were used in this study during the rutting period in the middle of October 2006.

Specimens collection

Testes and head of epididymis from the slaughtered bucks for meat were collected and put into the neutral buffer formalin fixative. The specimens were trimmed and fixed overnight at room temperature. The size of specimens was in cube shape of 1×1×1 cm. The long section of testes and cross section of epididymis were done.

Processing of testes and epididymis tissues

The samples were labeled and washed in running tap water for at least 2 hours before processing in serial alcohol, xylene and melting paraffin using automatic tissue processor (Shandon™). The serial steps were 70% ethanol, 80% ethanol, 2 times of 95% ethanol, 2 times of absolute alcohol, equal volume of absolute alcohol in xylene, 2 times of xylene and 2 times of paraffin. The duration used in each step was 1 hour 30 minutes. The processed tissues were then embedded in the paraffin block.

Histological study

The paraffin- embedded testis and epididymis specimens were sectioned at 5mm by the microtome (Shandon™). The sectioned tissues were then stained with Hematoxylin – Eosin (H&E). The characteristics and morphology of the Sertoli's cells, spermatogonia, primary spermatocyte, secondary spermatocyte and spermatid were observed under 400x light field microscope (Olympus®) to characterize the stage of spermatogenesis.

RESULTS

The testes of rusa deer are pair ovoid shape, and each one is enclosed in fibrous capsule of dense connective tissue (tunica albuginea). The connective tissue of tunica albuginea extended through the parenchyma of testis and separated into lobules. Each lobule composed of several tightly coiled seminiferous tubules and interstitial cells (Figure 1). The interstitium are loose areolar connective tissue which surrounded the seminiferous tubules. There are many kind of cells can be observed such as interstitial (Leydig) cell, blood vessel and myoid cell (Figure 2). In this species, Leydig cells are much less prominent and do not form clusters. Also present in scant interstitium close to basement membrane of the seminiferous tubules are myoid cells. These cells are spindle shaped fibrocytes.

The seminiferous tubule consisted of stratified epithelium located on a basement membrane. The epithelium was described as the important location for spermatogenic cells and Sertoli's cells. The Sertoli's cells were found in less number than spermatogonia. Sertoli cells extend from the basement membrane to the luminal surface of the seminiferous epithelium. The nucleus of each Sertoli cell is ovoid and lightly stained. In the present study, the histological aspect of the spermatogenesis of rusa deer in rutting period was shown. The seminiferous epithelial

cycle can be distinguished in 3 stages which are 1) spermatogonia 2) primary spermatocyte and 3) spermatid (Figure 3). The spermatogonia cells lie on the lamina of tubule. They have a round nucleus with chromatin granules of variable sizes. In some part, primary spermatocyte appears as larger cells than spermatogonia. In cross-section of seminiferous tubules a large number of primary spermatocytes can be observed. The spermatids can be distinguished in the lumen of seminiferous tubule. They are very small in diameter with eccentric nucleus. The chromatin is condensed and

stained dark blue.

The structure of ductus epididymis formed from highly coiled ducts which have big lumen and thick wall (Figure 4), lay embedded in collagenous connective tissue. The Figure 5, showed the detail of epithelium of epididymis lined by a very tall pseudostratified columnar epithelium. Most cells of the epithelium, also called principal cells, have long stereocilia projection from the surface. In addition, the spermatozoa were obviously found with normally formed morphology of head and tail.

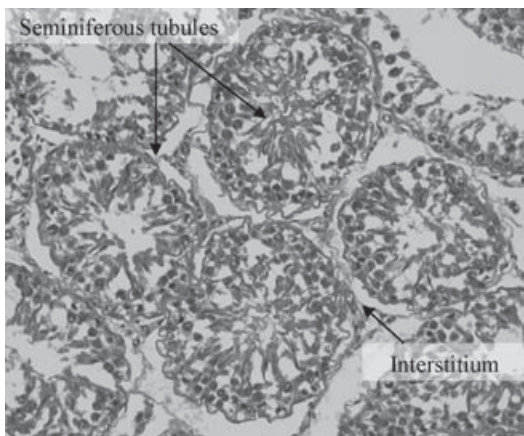


Figure 1 Seminiferous tubules and the scant interstitium (H&E, 400x).

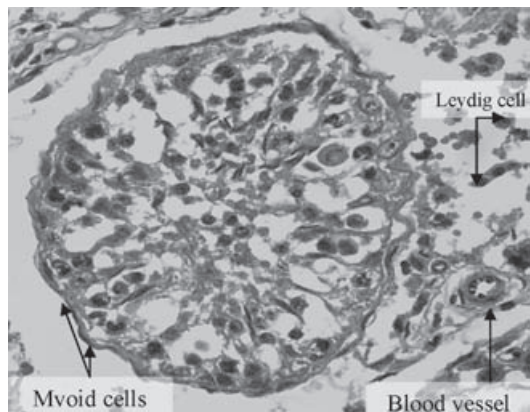


Figure 2 Seminiferous tubules and the scant interstitium (H&E, 1000x).

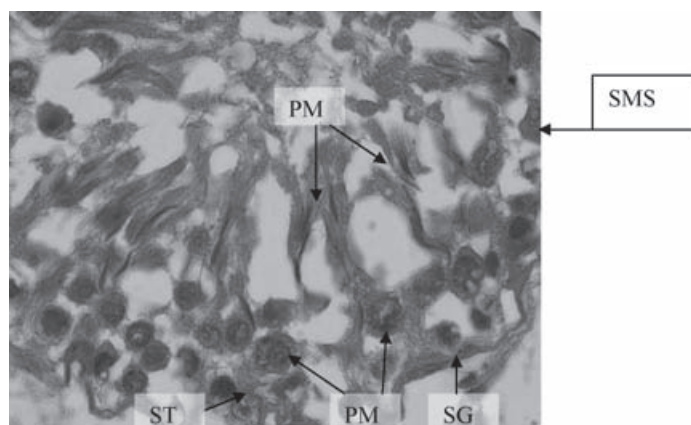


Figure 3 The spermatogenesis in seminiferous tubule of mature rusa deer composed of ST=Sertoli's cells, SG=spermatogonia, PM= primary spermatocytes, SMS=spermatids (H&E, 1000x).

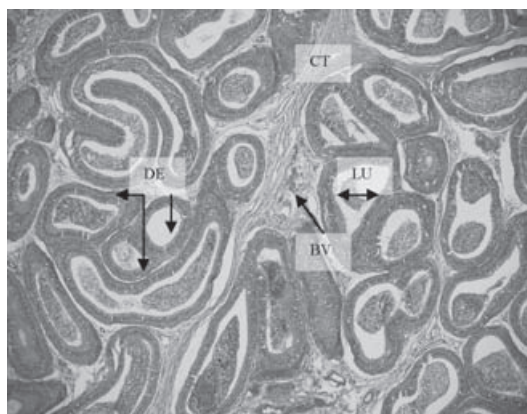


Figure 4 Section of epididymis; DE = ductuli of epididymis, CT=connective tissue, LU=lumen and BV= blood vessel (H&E, 200x).

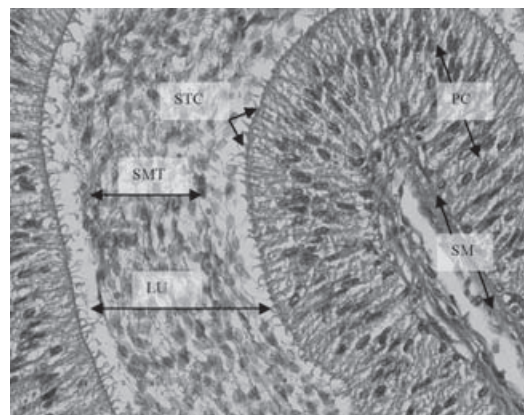


Figure 5 The structure of epididymis; SM = smooth muscle, PC = principal cells, STC = stereocilia, SMT = Spermatozoa, LU = lumen (H&E, 400x).

DISCUSSION

In Europe, the deer families such as roe deer, red deer, sitka deer and rusa deer, demonstrated long rutting period often found in the winter time (October-December) (Scheon *et al.*, 2004), but the shorter period could also be found in Spring (July-September) (Lincoln, 1978). In Thailand which is different climate zone possessing the warmer temperature and shorter period of rutting condition for rusa deer was found in this study (October-November). The information of rutting period and seminiferous epithelial cycle of some deer species which is closely related to rusa deer has been reported. Scheon *et al.* (2004) classified the cycle of roe deer in defined 8 stages, for the one year observation using histological study and Direct Electron Microscopy. However, this study was focused on the important time of mating interval by the simple technique of histology. The complete spermatogenesis or seminiferous epithelial cycle confirmed by histological study of testis and epididymis structure was found. The histology aspect was reported to be a useful tool for morphological study of spermatogenic cells (Ueno

et al., 1990; Sharpe *et al.*, 1994; Scheon *et al.*, 2004). The further step that should be done is increasing number of samples and attempt to define the stage of seminiferous epithelial cycle.

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LITERATURE CITED

- Bubenik, G.A. and J.P. Jacobson. 2002. **Testicular histology of cryptorchid black-tail deer (*Odocoileus hemionus sitkensis*) of Kodiak Island.** Alaska. *Z Jagdwiss* 48: 234-243.
- Barth, A.D. and R.J. Oko. 1989. **Abnormal Morphology of Bovine Spermatozoa.** Iowa State University Press. 281p.
- Lincoln, G.A. 1978. Appearance of antler pedicles in early foetal life in red deer. **J. Embryol Exp. Morph.** 29: 431-437.
- Sharpe, M. 1994. Regulation of spermatogenesis.

- pp.1363-1434. *In* E. Knobi and J.D. Neill (eds.). **The physiology of reproduction**. 2nd ed. Raven Press, New York.
- Schlatt, S., A. Meinhardt and E. Nieschlag. 1997. Paracrine regulation of cellular interactions in the testes: factors in search of function. **Eur J. Endocrinol** 137: 107-117.
- Scheon, J., F. Georitz, J. Streich and S. Bloottner. 2004. Histological organization of roe deer testis throughout the seasonal cycle: variable and constant components of tubular and interstitial compartment. **Anat Embryol** 208: 151-159.
- Ueno, H. and H. Mori. 1990. Morphometrical analysis of Sertoli cell ultrastructure during the seminiferous epithelial cycle in rats. **Biol. Reprod.** 43: 769-776.