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Observation of Male Reproductive Organ in Korean Water Deer (*Hydropotes inermis argyropus*)

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ABSTRACT

The Korean water deer which is endemic in China and Korea, is currently over-populated in some region in South Korea. In order to accumulate basic reproductive information for wildlife management of this species, male reproductive organs were anatomically observed in different periods. The male reproductive organs from water deer roadkills were macroscopically and histologically observed. The location and shape of testes and accessory reproductive glands (ampulla, vesicular gland, prostate gland and bulbourethral gland) of the water deer showed a resemblance to other small ruminant species. The size of the testes and the status of spermatogenesis were different between two periods which were from October to December and from April to July. It appears that the breeding season of the male Korean water deer is from October to December.

Key words: Korean water deer, male reproductive organ, anatomy, spermatogenesis, seasonality, cervidae

INTRODUCTION

The Korean water deer, *Hydropotes inermis argyropus*, belongs to the cervidae family which can be classified into three subfamilies: Hydropotinae, Odocoileinae and Cervinae. The water deer is the only species that belongs to Hydropotinae and is different from other subfamilies by the phylogenic evidences (Cap *et al*., 2002; Kuznetsova *et al*., 2005), the lack of antlers and the existence of well-developed upper canine in males. This species is distributed all over South Korea and in limited areas in around East China. The water deer has been introduced to Europe in the 1900's (Ward, 2005). Resulting captive and feral populations remained viable but remarkably stable in number (Hofman *et al*., 1988; Kirkwood *et al*., 1988). Although the population of this species in China is designated as the endangered situation (Fang and Wan, 2002; Zhu *et al*., 2004), the over-populated situation in some regions in South Korea has caused various conflicts to human life, including severe agricultural damages. The studies on this species had been ecologically (Dubost *et al*., 2008, 2011; Zhang, 2000) and genetically (Hu *et al*., 2006; Koh *et al*., 2009) performed and the growth and reproduction of water deer have been investigated in the introduced population in Europe (Dubost *et al*., 2010; Kirkwood *et al*., 1988; Mauget *et al*., 2007). Although, many reproductive anatomical studies have been reported in some cervidae species including sika deer (*Cervus nippon*) (Hayakawa *et al*., 2009, 2010) and roe deer (*Capreolus capreolus*)

(Göeritz *et al.*, 2003), there have been no reports on the reproduction of the endemic population of these species in Korea or China. The accumulation of the basic information on reproduction is meaningful for both the conservation and management of water deer in Korea and China. In this study, anatomical characteristics of male reproductive organs of the Korean water deer were analyzed both macroscopically and histologically.

MATERIALS AND METHODS

Water deer roadkills were collected from Gyeonggi, Chungbuk and Chungnam wild animal rescue centers and Cheorwon center of the Korean association for bird protection in Korea. Twenty-five individuals were obtained within the periods of October to December of 2010 and April to June of 2011. Male reproductive organs, including testes and accessory reproductive organs, were excised and fixed in 10% formalin solution until observation. The size and weight of the testes and accessory reproductive organs were measured and observed. Afterwards, they were dissected and dehydrated in a graded series of ethanol, cleared in xylene and embedded in paraffin. These embedded organs were then sectioned at 4 μm and placed on silane-coated glass slides. After deparaffinization, these sections were stained with Hematoxylin and Eosin (HE). Eruption and abrasion of the molar teeth were examined in order to ensure that the animals used in this study were fully grown. The statistical differences of the size and weight of the testes and accessory reproductive organs between two periods (October to December of 2010 and April to June of 2011) were analyzed using student's t-test. We considered differences to be significant if $p<0.05$.

RESULTS

Upon the observation of the molar teeth, all individuals used in this study were classified as adults.

Testis: The testis was found outside of the body, positioned vertically between the legs. Each testis had an oval shape and was attached to the epididymis within the scrotum. There were no significant differences regarding the location and shape of the testes between two periods in which the studies were performed. The volume and weight of the testes as well as the existence of spermatids in the convoluted seminiferous tubules are shown in Table 1. The volume and weight of the testis were different between two periods which were from October to December and from April to July (Fig. 1). Testes observed during the former period were larger and heavier than those observed in the latter (Fig. 1). The spermatids in the convoluted seminiferous tubules were only observed in the specimens collected from October to November (Fig. 2a, b).

Accessory reproductive organs

Macroscopic observation: Four accessory reproductive organs (ampulla, vesicular glands, prostate glands and bulbourethral glands) were identified (Fig. 3). The ampulla is an enlarged part of the deferent duct before it reaches the genital fold. The duct narrows as it moves away from the ampulla and dips under the body of the prostate gland. The paired vesicular glands are the largest of the accessory genital glands in this species. It is a round-shaped, hard, compact organ with an uneven surface. The free ends of the gland are directed cranially and slightly laterally and they lie in the genital fold which is dorsal to the bladder. The terminal segments of the ureters and

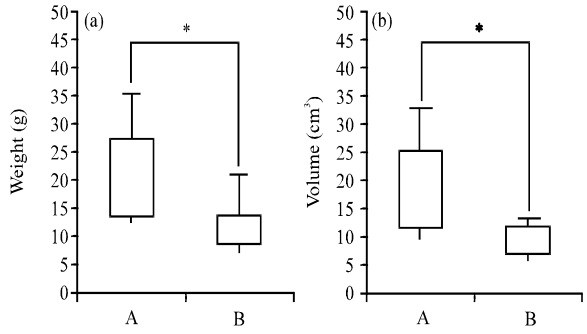

Fig. 1(a-b): The comparison of the (a) weight and (b) volume of testis in two different periods. (A: October to December, B: April to July, *p<0.05). Boxes range the standard deviations and whiskers indicate the highest and lowest values

Table 1: The volume and weight of testis and the existence of spermatid in the convoluted seminiferous tubules

Specimen number	Collected date	Volume of testis (cm^3)*	Weight of testis (g) (R/L)	Spermatid
KJ0572	2010-10-30	10.45	6.4/ 5.9	+
KJ0573	2010-11-03	22.04	12.8/11.8	+
KJ0574	2010-11-05	10.35	5.2/7.4	+
KJ0576	2010-11-29	16.93	8.6/ 8.6	+
KJ0577	2010-12-01	32.51	18.7/16.5	+
KJ0578	2010-12-05	19.28	9.9/11.3	+
KJ0580	2010-11-21	9.27	5.2/5.1	+
KJ0581	2011-12-02	13.81	8.1/9.1	+
KJ0582	2010-12-03	12.46	7.9/ 8.0	+
KJ0583	2010-12-03	19.12	11.5/11.2	+
KJ0585	2010-12-16	25.72	14.4/14.0	+
KJ0587	2010-12-19	19.96	10.1/11.0	+
KJ0589	2010-12-19	23.88	14.6/13.0	+
KJ0590	2010-12-19	26.49	14.9/12.2	+
KJ0591	2010-12-24	9.86	5.3/5.9	+
KJ0592	2010-12-24	17.89	10.2/10.7	+
KJ0594	2011-04-01	9.16	5.3/5.9	-
KJ0595	2011-05-18	5.76	3.4/3.4	-
KJ0597	2011-05-18	11.44	3.8/6.6	-
KJ0599	2011-06-18	7.51	4.3/5.5	-
KJ0608	2011-06-19	8.96	5.9/6.0	-
KJ0609	2011-06-21	9.65	5.8/6.0	-
KJ0610	2011-07-04	13.12	7.6/7.9	-
KJ0611	2011-07-04	10.20	6.7/6.3	-
KJ0612	2011-07-06	5.79	5.3/4.5	-

*Volume of testis was calculate as follows: V: $4/3\pi abc$ (a: long axis; b: short axis, c: width of testis). R: right, L: left. +: spermatids were discovered in the convoluted seminiferous tubules, -: spermatid were not discovered in the convoluted seminiferous tubules

deferent ducts are positioned between the left and right glands. The body of prostate gland lies transversely on the dorsal surface of the urethra, just caudal to the vesicular gland. The

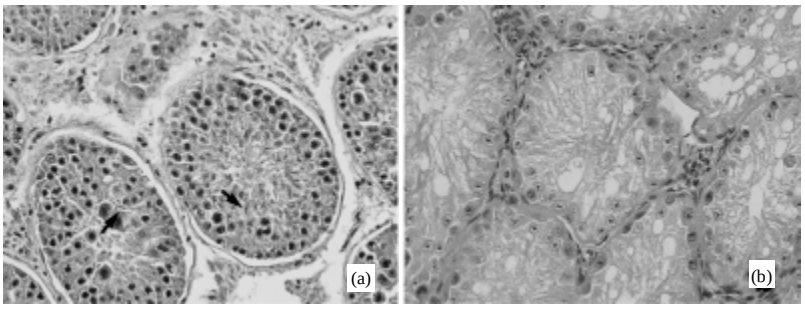

Fig. 2(a-b): The convoluted seminiferous tubules in December 6th 2010 (a) and July 7th 2011 (b) Note the spermatids (arrows) in seminiferous tubules (a) (H.E. staining) (x 400)

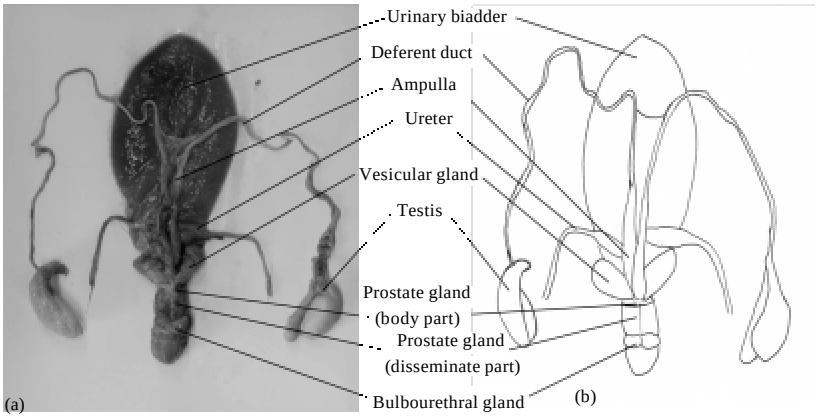

Fig. 3(a-b): (a) Photograph and (b) schematic drawing of the male reproductive organs in the Korean water deer

disseminated part surrounds the urethra completely. The bulbourethral gland consists of two round-shaped, independent lobes. They lie on the dorsal surface of the urethra opposite to the ischiatic arch and are covered by the proximal part of the thick bulbospongiosus muscle. Volumes of these four accessory reproductive organs are written in Table 2. The difference in the volumes of these four accessory reproductive organs between the two studies was not significant (Fig. 4).

Histological observation: In the period of October to December, the luminal secretions from secretory epithelia of all accessory reproductive organs were observed, but not in the periods of April to July. The epithelia of the secretory alveoli were lined by single columnar cells in the ampulla and by single cuboidal cells in the whole parts of prostate glands in the both periods of October to December and April to July. In the bulbourethral and vesicular glands, the epithelia of the secretory alveoli in the periods of October to December were lined by single cuboidal cells and in the periods of April to July by single columnar cells (Fig. 5).

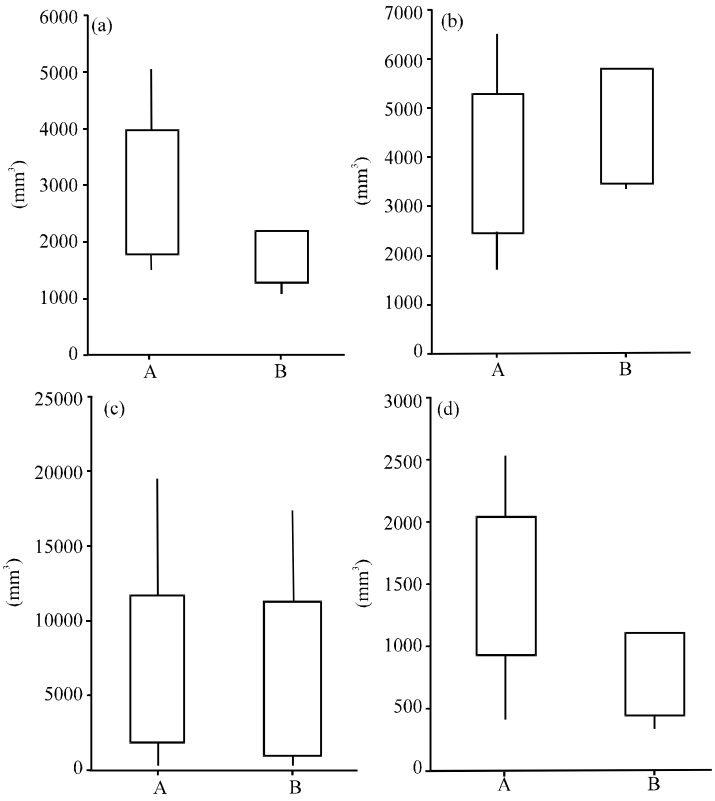

Fig. 4(a-d): The comparison of the volume(mm^3) of accessory reproductive organs (ampulla (a) vesicular gland (b) prostate gland (c) bulbourethral gland (d) in the two periods (A: October to December, B: April to July) boxes range the standard deviations and whiskers indicate the highest and lowest values

Table 2: The volume of accessory reproductive organs in the Korean water deer

Specimen number	Collected date	Volume of ampulla (cm^3)	Volume of vesicular gl. (cm^3)	Volume of prostate gl.-1 (cm^3)	Volume of prostate gl.-2 (cm^3)	Volume of bulbourethral gl. (cm^3)
KJ0572	2010-10-30	1.52	3.25	0.92	8.81	1.38
KJ0573	2010-11-03	2.02	2.02	2.04	11.99	0.56
KJ0574	2010-11-05	4.17	1.74	0.34	18.17	1.01
KJ0576	2010-11-29	4.99	4.39	0.54	12.36	1.76
KJ0577	2010-12-01	2.15	6.51	0.74	13.67	1.50
KJ0578	2010-12-05	2.03	4.79	0.89	9.89	1.52
KJ0582	2010-12-03	3.66	3.72	0.73	19.40	2.24
KJ0583	2010-12-03	2.44	4.92	0.45	10.50	1.92
KJ0585	2010-12-16	2.49	4.84	0.82	17.74	2.54
KJ0589	2010-12-19	2.02	2.09	0.46	17.87	1.41
KJ0590	2010-12-19	2.86	3.48	0.58	18.47	1.48
KJ0592	2010-12-24	4.03	4.52	1.42	15.45	1.39
KJ0594	2011-04-01	1.12	4.15	0.24	10.91	1.17
KJ0595	2011-05-18	2.12	3.33	0.53	14.93	0.83
KJ0597	2011-05-18	1.75	5.45	0.71	11.72	1.08
KJ0599	2011-06-18	2.01	5.73	1.02	16.23	0.49

*Volume of accessory reproductive organs (ampulla, vesicular, prostate (body, disseminate part, bulbourethral) was calculate as follows: V: $4/3\pi abc$ (a: long axis; b: short axis, c: width of accessory reproductive organs). Prostate gl.-1; body part, prostate gl.-2; disseminate part

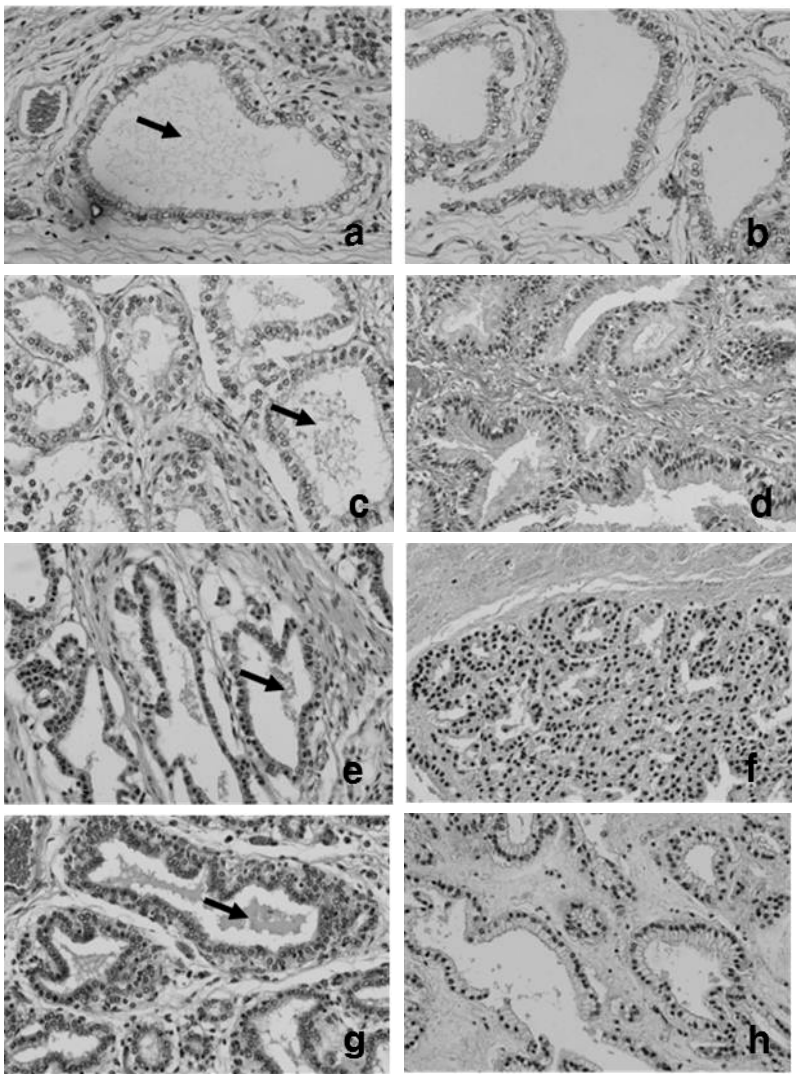

Fig. 5(a-f): Histological views of the accessory reproductive organs of the water deer. (a, b: ampulla, c, d: vesicular gland, e, f: prostate gland, g, h: bulbourethral gland) (a, c, e, g: October to December, b, d, f, h: April to July) (H.E stain, x400) Note the luminal secretion in these four glands on October to December (arrows)

DISCUSSION

There have been very few anatomical papers on the Korean water deer only describing the skull structure (Jung *et al*., 2004) and the branching pattern of aortic arch (Ahn *et al*., 2008). This is the first report in the anatomy of the reproductive organ in Korean water deer. The Korean water deer has the same four typical accessory reproductive glands as other ruminant species (Dyce *et al*., 2010). Their shapes and histological structures also resemble other small ruminant species, including goats and sheep (Bacha and Bacha, 2000; Banks, 1993; Eurell *et al*., 2006). Many cervid species of Northern temperate origin, such as the roe deer (*Capreolus capreolus*), show seasonal production of spermatids (Lincoln, 1992). In the case of Sika deer (*Cervus nippon*) which

inhabits in Japan, the spermatogenic process occurs in July or August and its activity reaches its peak in late October. In February or March, termination of the spermatogenesis was observed (Suzuki *et al*., 1992). According to the results obtained from this study, the spermatogenesis of the water deer also occurs from October to December. Seasonal changes in size and weight of the accessory genital organs were not noted. However, the histological study showed differences in secretory activities between the two seasons, as evidence of luminal secretions was observed. This finding suggests that the breeding season of the animal is from October to December, when the male reproductive functions are most active. The histological characteristics of testis including the classification of the seminiferous epithelial cycle (Hayakawa *et al*., 2009) and immunohistochemical localization of steroidogenic enzymes (Hayakawa *et al*., 2010) have been clearly demonstrated in Sika deer which inhabit in Japan. As these studies, more detailed anatomical studies in Korean water deer is needed in near future.

This study is the first to report that the breeding season of the Korean water deer is from October to November. This evidence will certainly contribute to the wild life management and conservation of this species.

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