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Full Length Article

Seasonal Effects of Castration on the Male Gonads in Elk Deer (*Cervus canadensis*)

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Abstract

Elk deer (*Cervus canadensis*) is an economically important species valued for its antlers. The aim of this study is to evaluate the effects of castrating season on antler development in elk deer. The testes were surgically collected from the following four groups of six-year-old captive bulls during the non-mating season: in March, before casting of the antler stump; in April, after casting, that is, 50 days into the period of early velvet growth; in June, during antler removal when the antler growth peaked, that is, 85 days into the velvet antler period; and in October, during slaughter. The testis size and weight increased during the velvet antler growth period, from April to October, whereas, in April, the testis weight decreased significantly. Furthermore, the sperm concentration and motility increased significantly from March to October. There was no significant difference in the plasma testosterone concentration between the pre- and post-castration groups, although it was slightly higher in the pre-castration group. The plasma testosterone concentration in the castrated animals was lower than 0.025 ng mL^{-1}, whereas that in the control group was much higher (10.53 ng mL^{-1}). Therefore, our results suggest a possible correlation between the velvet antler growth and castration timing in elk deer. © 2019 Friends Science Publishers

Keywords: Castration; Elk deer; Sperm; Testosterone; Testes

Introduction

The sexual activity of most wild animals occurs at a specific season of the year in order to give birth to and nurture the newborns under favorable environmental condition. Therefore, it is often related to seasonal changes, where changes in weather, such as ambient temperature and food availability, can affect offspring survival. The evolution of seasonal breeding may have been influenced by environmental factors, such as seasonal rainfall cycle, fluctuations in food supply, local resource competition, and predation pressures (Asher and Thompson, 1989). Seasonal breeders, such as elk deer (*Cervus canadensis)*, have an estrous cycle, and they breed in autumn when the daylight hours are short and ambient temperature is low and do not deliver until spring or early summer (Suttie *et al*., 1984; Schnare and Fischer, 1987). In general, elk deer breeds from September to March, with the main breeding time occurring during the first few months of the mating season (Garcia *et al*., 2002).

In order to inform methods of assisted reproduction, a detailed understanding of the reproductive physiology of wildlife is essential. Season can modify sperm availability and quality, and there are several hormonal changes associated with growth, sexual maturity, and breeding in elk deer. The size and spermatogenic activity of the testes in male deer increase during the breeding season (September to February) and decrease during the non-breeding season, which leads to estrus synchronization in females to maximize the fertilization capacity of high-quality sperm from dominant males. Such periodic changes in seminal vesicles are regulated by testosterone secreted by the pituitary gland (Schnare, 1991).

The function of the epididymis is also regulated by testosterone (Goeritz *et al*., 2003; Pereira *et al*., 2005). During the growth of antlers, after the breeding season, the plasma testosterone concentration is low (Lincoln, 1971). An increase in the testosterone concentration prior to the mating season causes spermatogenesis and keratinization of the bones that form antlers (Muir *et al*., 1988; Asher and Thompson, 1989). Thus, seasonal changes in blood testosterone levels regulate the annual antler cycle (Goss, 1983; Fennessy and Suttie, 1985; Bubenik *et al*., 1990; Rolf and Fischer, 1990).

The testes and accessory sex glands have an important role in testosterone production by facilitating the optimal

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control of semen production, ensuring good quality and number of spermatozoa during the short breeding season. Testosterone secretion increases before mating, leading to intra-sexual fights and competitions to establish hierarchies. The higher-ranking males often possess larger antlers and are more successful in mating. The blood testosterone secretion in males generally increased 50–60 days before the mating period. The growth of antlers and the changes in the breeding cycle can be attributed to individual variations, such as age, genetics, and seasonal influences.

Insulin-like growth factor I (IGF-1) is a hormone with a molecular structure similar to that of insulin, a polypeptide hormone. IGF-I and other growth factors provide essential signals for the control of testis development and function. The endocrine, paracrine, and autocrine actions of IGF-1 growth factors represent important temporally active factors in understanding the roles of IGFs in testicular development and function (Griffeth *et al*., 2014). IGF-1 signaling is necessary for effective and successful testis differentiation. Indeed, embryos lacking IGF-1 fail to develop the testes (Griffeth *et al*., 2014). Cortisol is a hormone that plays an important role in restoring homeostasis during or after stress and has often been suggested as a major factor in intervening the effects of stress suppression on reproduction. Further, cortisol regulates reproductive processes by inhibiting testicular androgen secretion and by effecting the development of the gonads, brain–pituitary–gonad axis, and neuroendocrine system (Consten *et al*., 2002). In this study, we observed the effects of castration timing on testis morphology, endocrine hormone secretion, and antler growth.

Materials and Methods

Animal Ethics Statement

This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institute of Animal Science (NIAS). The materials and procedures were approved by the Committee of the NIAS on the Ethics of Animal Use in Experiments.

Experiment Design

The experiment was conducted during the non-mating season using 12 male elk deer aged 6 years and averaging 310 kg. Individuals were randomly assigned to four treatments groups. T1 group was castrated in March, before casting of the antler stump; T2 group was castrated in April after casting, that is, 50 days into the early velvet growth period; T3 group was castrated in June during antler removal, when the antler growth peaked, that is, 85 days into the velvet antler period; and the testes from T4 group were collected during slaughter in October when the antlers harden.

Castration Methods

A local anesthetic Xylazine® (Korea) was administered in the femoral region at a dose of 0.1 mL per kg body weight (3–3.5 mL for each animal). Castration was performed using bloodless castration tools. An incision was made in the scrotum with a scalpel prior to removal of the testes. The central region of the scrotum was disinfected with antiseptic solution Betadine® and an incision was made 5 cm below the skin of the scrotum to reveal the testes, which were then dissected and pulled free after holding the vas deferens using forceps. Hemostasis was achieved by tying the vas deferens and blood vessels. Antiseptics were applied around the scrotum after castration, and a long-term antibiotic Norocillin LA® (1 mL 10 kg^{-1} body weight) and analgesic and anti-inflammatory Metcam 20® (2.5 mL 100 kg^{-1} body weight) were administered to prevent microbial infection.

Testis Size and Weight

After excision, the testes were weighed using a digital balance (Cass, USA). The testis length, width, and circumference were determined using a ruler.

Sperm

The head of the epididymis obtained from castrated elk deer was washed twice in PBS. Capillary blood was oozed out *via* a slight incision into the capillary, and then wiped with antiseptic cotton. About 10 mm of the epididymis head was incised and minced using a sterile scalpel in a 100 mm dish (Falcon) to release the sperm. The released sperm samples were centrifuged at 450 × *g* for 5 min and precipitated as pellets; the supernatant was discarded. Sperm was evaluated using the CASA system (Computer Assisted Sperm Analysis). The CASA system included an optical microscope (Olympus, Japan) and a computer with a sperm class analyzer software (Medical supply Co., Ltd., Korea). Five microliters of sperm samples were used for sperm analysis in a warmed Makler counting chamber (New York Microscope Company Inc., U.S.A.) at 37°C, and the magnification was 10X.

Hormone and Growth Factor Analysis

Hormones and growth factors were measured at the NEODIN Medical Institute (Seoul, Korea). The testosterone concentration was determined using Cobas 8000 e602 (Roche, Mannheim, Germany) with an electrochemiluminescence immunoassay (ECLIA). The IGF-I concentration was determined by ECLIA using an immulite 2000 XPI (Siemens Healthcare Diagnostics, USA). The cortisol concentration was determined *via* a chemiluminescence immunometric assay using Cobas 8000 e602 (Roche, Mannheim, Germany).

Statistical Analysis

Significant differences were assessed with the analysis of variance using the General Linear Model procedure of SAS statistical package (S.A.S. Institute Inc., Gary, N.C., U.S.A.). Differences among treatment means were determined using Duncan's multiple range test. Graphs were prepared using InStat® software (GraphPad, La Jolla, CA, USA). Differences with a *P*-value of less than 0.05 were considered statistically significant.

Results

Effect of Castration Time on Testis Morphology and Sperm Quality

Compared with the T1 group, in the T2 group, the size and weight of the testes decreased after casting. However, the size and weight of the testes increased significantly from the T2 group (non-breeding season) to T4 group (breeding season) by 2.4- and 1.4-fold, respectively (Table 1, Fig. 1, $P < 0.05$).

The sperm cells were only present in the T1 and T4 groups, but they were not detected in the T2 and T3 groups. The sperm concentration and motility of the deer castrated in March (T1) were 27.9×10^6 (mL^{-1}) and 54.6%, respectively, and those of the deer castrated in October (T4) were 127.8×10^6 (mL^{-1}) and 71.9%, respectively (Table 2).

Effect of Castration Time on Concentration of Hormones and Growth Factors

There was no significant difference in the blood testosterone concentration among the three castrated treatment groups (Fig. 2). However, the overall testosterone concentration was slightly higher before castration. In addition, the pre-castration blood testosterone concentration was higher in the T2 group (0.19 ng mL^{-1}) than in the T1 (0.07 ng mL^{-1}) and T3 (0.1 ng mL^{-1}) groups. The blood testosterone concentration of the T4 group was higher than that of the other groups.

Furthermore, the T2 (206.67 ng mL^{-1}) group showed higher serum IGF-I concentration than that of the T1 (95.23 ng mL^{-1}) and T3 (137.33 ng mL^{-1}) groups before castration. The serum IGF-I concentrations in the T1, T2, and T3 groups were 457.50, 408.50, and 191 ng mL^{-1} (Fig. 3). Its overall concentration according to the growth of the antlers was higher after castration than before castration.

The serum cortisol concentration before castration was higher in the T3 group (2.26 ng mL^{-1}) than in the T1 (1.98 ng mL^{-1}) and T2 (2.08 ng mL^{-1}) groups (Fig. 4). Meanwhile, the serum cortisol concentration after castration was higher in the T2 (2.61 ng mL^{-1}) group than in the T1 (1.71 ng mL^{-1}), T3 (1.30 ng mL^{-1}), and T4 (0.58 ng mL^{-1}) groups.

Table 1: Testis size in elk deer (*Cervus canadensis*) after castration

Castration time*	Testes			
	Diameter (cm)	Length (cm)	Circumference (cm)	Weight (g)
T1	3.17 ± 0.29^{c}	9.17 ± 0.29^{b}	11.93 ± 1.10^{b}	164.00 ± 42.57^{bc}
T2	3.83 ± 0.29^{c}	8.17 ± 0.76^{b}	10.67 ± 0.29^{b}	130.00 ± 11.36^{c}
T3	4.93 ± 0.12^{b}	10.83 ± 2.02^{b}	12.17 ± 1.26^{b}	227.00 ± 51.03^{ab}
T4	6.00 ± 0.71^{a}	14.50 ± 2.12^{a}	15.00 ± 1.41^{a}	310.00 ± 70.71^{a}

[a-c] Different superscript letters indicate statistically significant differences ($P < 0.05$)
The data are expressed as the mean ± standard error of the mean (SEM)
* T1: Casting (March), T2: Velvet growth (April), T3: Velvet antler (June), and T4: Hard antler (October)

Table 2: Concentration and motility of sperms in elk deer (*Cervus canadensis*) after castration

Castration time*	Sperm quality	
	Concentration (mL)	Motility (%)
T1	27.9×10^6	54.6
T2	ND[1]	ND[1]
T3	ND[1]	ND[1]
T4	127.8×10^6	71.9

[1]Not detected
*T1: Casting (March), T2: Velvet growth (April), T3: Velvet antler (June), T4: Hard antler (October)

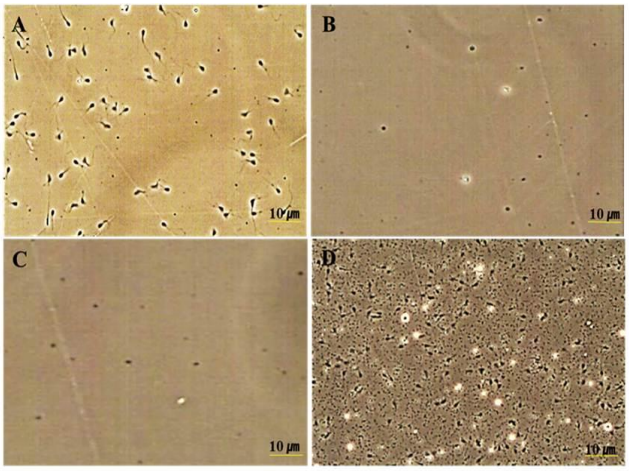

Fig. 1: Photographs of sperm after castration at different time periods in elk deer (*Cervus canadensis*). (**A**): T1, Casting (March); (**B**): T2, Velvet growth (April); (**C**): T3, Velvet antler (June); and (**D**): T4, Hard antler, uncastrated deer (October)

Discussion

By investigating the effects of castration timing on the testis morphology, endocrine hormone secretion, and antler growth, we suggest that routine endocrine monitoring practices are essential to assist reproductive techniques, especially in artificial insemination programs that are important for deer farming. In the present study, the size and weight of the testes increased significantly from April (non-breeding season) to October (breeding season) Seasonal changes in the morphology of the testes were likely caused by the changes in testosterone secretion during lower dorsal horn development in mongrel, which is known to regulate

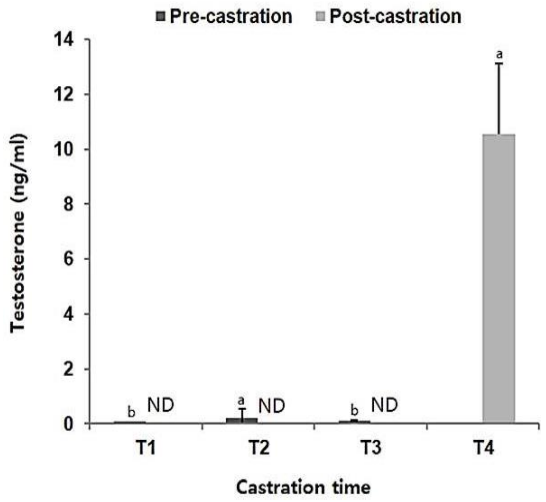

Fig. 2: Testosterone concentration in elk deer (*Cervus canadensis*) before and after castration. T1, Casting (March); T2, Velvet growth (April); T3, Velvet antler (June); and T4: Hard antler (October)
[ND]Not detected. [a–b]
Different superscript letters indicate statistically significant differences ($P < 0.05$)

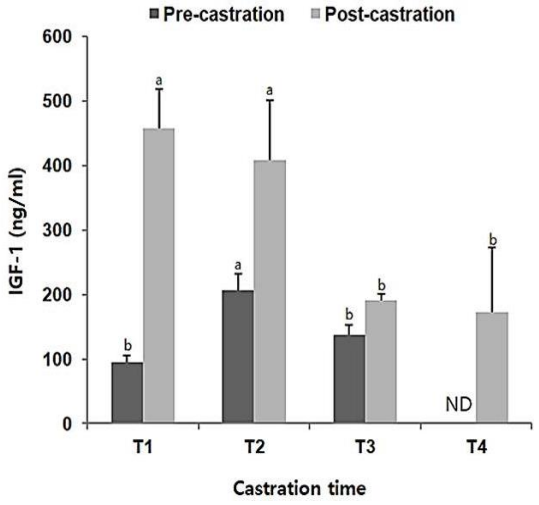

Fig. 3: IGF-1 concentration in elk deer (*Cervus canadensis*) before and after castration. T1, Casting (March); T2, Velvet growth (April); T3, Velvet antler (June); and T4: Hard antler (October)
[ND]Not detected
[a–b] Different superscript letters indicate statistically significant differences ($P < 0.05$)

testosterone secretion (Schnare and Fischer, 1987; Zhang *et al*., 2015).

The sperm concentration and motility of the deer castrated in March were significantly lower than that of the intact deer, suggesting that these variables decreased during the non-breeding season (October) and probably increased after the breeding period (April). However, this conclusion cannot be confirmed, as sperms were not detected in the T2 and T3 groups. Haigh (1984) also did not find sperms in the semen collected in June and July, except for a small number

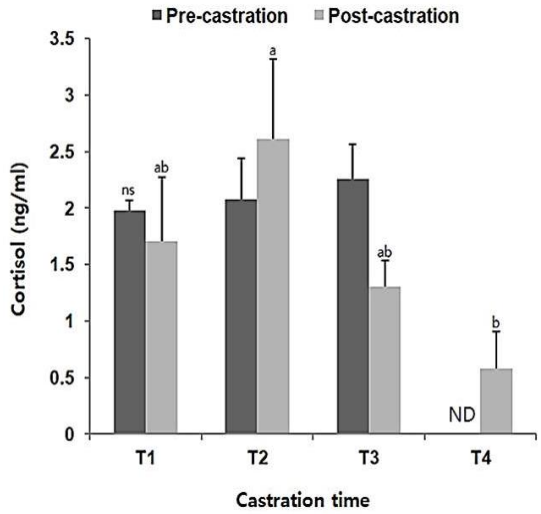

Fig. 4: Cortisol concentration in elk deer (*Cervus canadensis*) before and after castration. T1, Casting (March); T2, Velvet growth (April); T3, Velvet antler (June); and T4: Hard antler (October)
[ND]Not detected
[ns]Means not significant
[a–b] Different superscript letters indicate statistically significant differences ($P < 0.05$)

of spermatozoa, and the number of spermatozoa increased significantly between September and March.

The blood testosterone concentration in the T1 group was 0.07 ng mL^{-1}. This finding is different from that of previous studies of red deer (Suttie *et al*., 1991) and sika deer (Kim *et al*., 2005); both these studies showed concentrations below 5 ng mL^{-1}, which was higher than that observed in the present study. Moreover, the blood testosterone concentration at 6–8 weeks after antler casting (T2 group) was 0.19 ng mL^{-1} in our study, whereas that in red deer was 1.6 ng mL^{-1} (Suttie *et al*., 1995) and in sika deer was 3.0 ng mL^{-1} (Kim *et al*., 2005). These results suggest that the testosterone concentration in the blood should decrease to below 0.07 ng mL^{-1} for the casting of antler. The inconsistency in the concentrations may be due to the specificity of the species and the genetic diversity among individuals due to the breeding environment.

The IGF-I concentration in the T2 group was 3.3 times lower than that in sika deer (1.366 ng mL^{-1}; Kim *et al*., 2005) during the same period. However, the growth curves and surge in the blood IGF-I concentration after castration observed in elk deer were consistent with those observed in sika deer (Kim *et al*., 2005). IGF-I stimulates horn growth, and its receptors are present at the end of the horn (Suttie *et al*., 1985). Based on *in vitro* model experiments, Elliott *et al*. (1993) reported that high testosterone concentrations reduced IGF-binding. In addition, testosterone inhibits IGF-I, thereby enabling cell division and subsequently supporting antler growth (Bubenik, 1966). We observed lower concentrations of blood hormones and an increase in IGF-1 after surgical castration. Antlers regenerate in

response to IGF-I stimulation, and the secretion of IGF-I during the antler growth period indicates a significant correlation with the antler growth in elk deer.

While the current findings add substantially to our understanding of the relationship between castration time and reproductive system of elk deer, it may be instructive to note some limitations of this study. Because it is well known that anesthetics have a direct effect on the testosterone concentration, castration procedures may have affected our results, necessitating studies with alternative approaches. Furthermore, it was difficult to compare our findings directly to those in white-tailed deer (*Odocoileus virginianus*), and there is a limited elk deer population with species specificity (Bartos *et al*., 2000). Further studies are recommended because of genetic variation with species specificity among breeds.

Conclusion

Ensuring and understanding the reproductive health of wild animals is crucial for wildlife as well as livestock management. Our study provides information on seasonal variation in spermatogenesis and fertility in elk deer. Routine endocrine monitoring practices are essential to assist reproductive techniques, especially in artificial insemination programs that are important for deer farming. The results of this study will inform sperm collection timing, promote wildlife conservation programs, and preserve the genetic diversity of elk deer.

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