Original Article

Effects of immunization against gonadotropin releasing hormone on reproductive functions in male rusa deer (*Rusa timorensis*)

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Abstract

We performed an evaluation of the effect of gonadotropin releasing hormone (GnRH) vaccine on male rusa deer reproductive function. Mature male rusa deer (*Rusa timorensis*) were divided into two groups: untreated group (n=3) and treated group (n=7). The treated rusa deer were administered with two ml of the GnRH-protein conjugate vaccine (Improvac®; 200 µg/ml). The vaccine was injected subcutaneously into the treated group three times at four-week intervals (April, May and June) and an additional single dose booster was given after a decrease in GnRH antibody levels was detected (October). Blood testosterone concentrations and GnRH antibody titer were determined before the vaccination. Following that, a series of blood collection and evaluation was done for one year. In addition, we conducted a series of semen collection and evaluation, testicular size measurement and antler development recording in all deer for one year. Our results indicated that the level of GnRH antibody significantly increased (p<0.05) compared with the pre-vaccinated level. The antibody titer reached peak concentrations after the third vaccination. The high level was maintained for twelve weeks and then gradually declined to baseline. The antibody titer increased again after the fourth vaccination. The testosterone concentrations had no significant difference between the groups and times. However, the testosterone level in the treated group showed a negative correlation with GnRH antibody titer (R=-0.25). The average of testicular size of the treated group was smaller than that of the untreated group (p<0.05). The semen quality of the treated group started to decline after the third vaccination (p<0.05). Then, after the fourth vaccination, azoospermia was detected. In this study, six stags in the treated group shed their hard antlers immediately after the third vaccination. Although regrowth of velvet was observed, it did not harden. In conclusion, the GnRH vaccination in male rusa deer resulted in the increase in GnRH antibody titer, which negatively correlated with blood testosterone. The decrease in blood testosterone might be involved in the lower semen quality and poor antler development.

Keywords: GnRH vaccine, male rusa deer, semen quality, testosterone

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Introduction

In Thailand, zoos and wildlife breeding centers often experience problem of overpopulation of wildlife, particularly members of the cervidae family. Negative impact of the high number of cervids in captivity includes crowded conditions, lack of feed, and sexual and aggressive behavior during the breeding season. Some populations rise from bottleneck founders, resulting in low genetic diversity. Traditionally, sterilization of animals has been achieved through surgical methods for population management (Kutzler and Wood, 2006). However, in some species, this procedure has significant disadvantages such as trauma, production setbacks and potential death (D'Occhio, 1993). Immunocontraceptive vaccination is one of the alternative methods for fertility control (Fagerstone et al., 2002). This vaccine has low risk of complications compared to anesthesia and surgery. It is also a reversible method; the treated animals can recover and become fertile (Godfrey et al., 1996; Kutzler and Wood, 2006). Gonadotropin-releasing hormone (GnRH) is widely used in domestic animals (Donald and Thompson, 2000) and wildlife species (Herbert and Trigg, 2005). GnRH is produced from cell bodies of neurosecretory neurons in hypothalamus and secreted via the hypophysial portal system to the capillary plexus within the adrenohypophysis. This hormone plays a central role in the development and maintenance of reproductive function in vertebrates (Somoza et al., 2002). GnRH proteins are identified according to their original form and specific peptide sequences. They are divided into three paralogous forms, i.e. GnRH1 (mammalian GnRH), GnRH2 (chicken GnRH) and GnRH3 (salmon GnRH) (Okubo and Nagahama, 2008). They are decapeptides which conserve structure in mammals. They conserve amino acid at 1, 4, 9 and 10 positions (Somoza et al., 2002). The GnRH vaccine stimulates antibody production to inactivate endogenous GnRH in the hypophyseal portal blood and subsequently to suppress the synthesis and secretion of gonadotropin and steroid hormone. It also arrests gametogenesis and gonadal atrophy (Miller et al., 1997; Herbert and Trigg, 2005). The GnRH vaccine was studied in male animals for suppressing reproductive performance such as goat (Godfrey et al., 1996), swine (Miller et al., 2003), cat (domestic short hair cat) (Levy et al., 2004; Robbins et al., 2004), lambs (Earl et al., 2006), stallions (Turkstra et al., 2005; Janett et al., 2009) and mice (*Mus musculus*) (Ganaie et al., 2011). Moreover, it was studied for wildlife population control such as in White-tailed deer (*Odocoileus virginianus*) (Killian et al., 2005) and feral swine (Killian et al., 2003). In the male white-tailed deer, GnRH vaccine reduced the reproductive capacity and antler development. However, the use of GnRH vaccine has not been done in rusa deer. The purpose of this study was to investigate the efficacy of GnRH vaccine (Improvac®) in rusa deer (*Cervus timorensis*) on antibody concentrations, plasma testosterone concentration and semen quality.

Materials and Methods

Animals: The breeding season of rusa deer is from January to June and the non-breeding season is from July to December (Nikorn Thongtip, unpublished data). Ten male rusa deer (2.5-3 years old, 40-50 kg body weight) were used for the experiment. The animals were housed at Kasetsart University, Kamphaeng Sean Campus, Thailand (14°N latitude). All deer were healthy. They were housed in stocking rate and exposed to natural photoperiod. They were fed *ad libitum* fresh Paragrass (*Brachiaria mutica*), commercial pellet, mineral block and water supply from March 2011 until the end of the experiment (April, 2012). The stags were separated from hinds throughout the experiment. Before vaccination, all experimental deer had hard antlers and normal testicular size compared with other rusa deer.

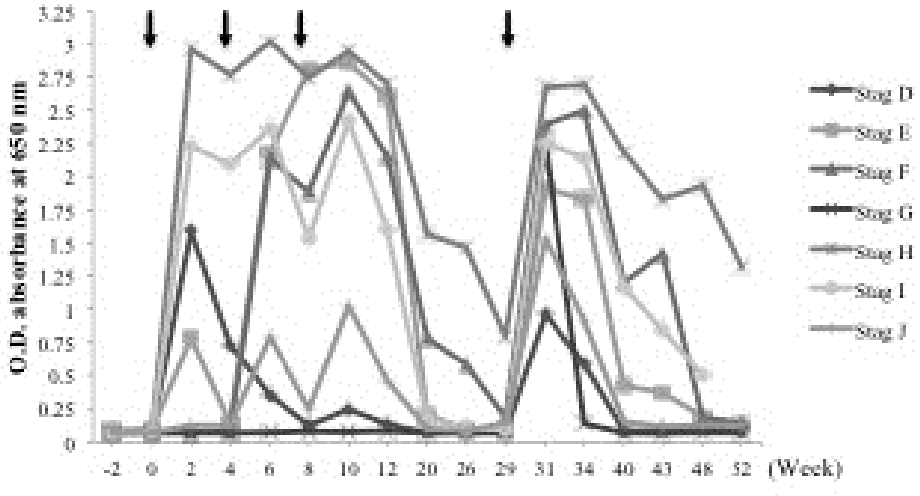


Figure 1 GnRH antibody response in 7 stags (treated group) after immunized with Improvac®

Treatment: The rusa deer stags were divided into two groups. Seven stags received vaccine (Improvac® Pfizer Animal Health, Australia) and three stags were untreated. Each ml of the vaccine contained 200 µg GnRH-protein conjugate. Two ml was injected into subcutaneous tissue at the base of the neck. Each male

in the treatment group received 3 doses of the vaccine at 4-week intervals and a single dose booster after the GnRH antibody level decreased. In March, 10 ml of whole blood were collected from all males before the vaccination. The treatment group was given the primary vaccination in early April. Then, whole blood was collected on weeks 0, 2, 4, 6, 8, 10, 12, 20, 26, 29, 31, 34, 40, 43, 48 and 52. The blood samples were centrifuged at 8000 rpm for 5 min. Serum samples were kept and stored at -20°C until analysis.

GnRH antibody titers: Antibody was estimated by using modified indirect ELISA (Zamaratskaia et al., 2008). Briefly, 96-well microtitre plates (Maxisorb, Nunc, Denmark) were coated overnight at 4°C with 1.18233 µg/ml LHRH human acetate salt (Sigma-Aldrich®) in bicarbonate coating buffer pH 9.6. The plates were washed twice with wash buffer (PBS containing 0.05% Tween20) and blocked with 1%BSA 150 µl/well for 90 min at 37°C, then the blocking solution was poured out. The rusa deer serum samples were assayed in duplicate. The serum was diluted 1:100 in blocking solution and incubated 100 µl/well for 60 min at 37°C. Two negative controls were run on each plate: one negative control was buffer without rusa deer serum and the other was non-vaccinated rusa deer serum. The high-titer rusa deer serum served as positive control. The plates were washed five times with wash buffer. Then, horseradish peroxidase (HRP) conjugated Protein A (KPL, USA) was diluted (1:5,000) in blocking solution and 100 µl of protein A–HRP was added to each well. The specimens were incubated for 30 min at 37°C. Then, the incubation solutions were removed and discarded. The washing steps were repeated twice. Then, addition of 3, 3', 5, 5'-tetramethylbenzidine (TMB) (SureBlue™, KPL, USA) 100 µl to each well was performed and color development was observed. Optical density (O.D.) was determined at 650 nm. GnRH antibody titers were reported as absorbance values. Samples that had higher absorbance than the cut-off values were classified as positive samples.

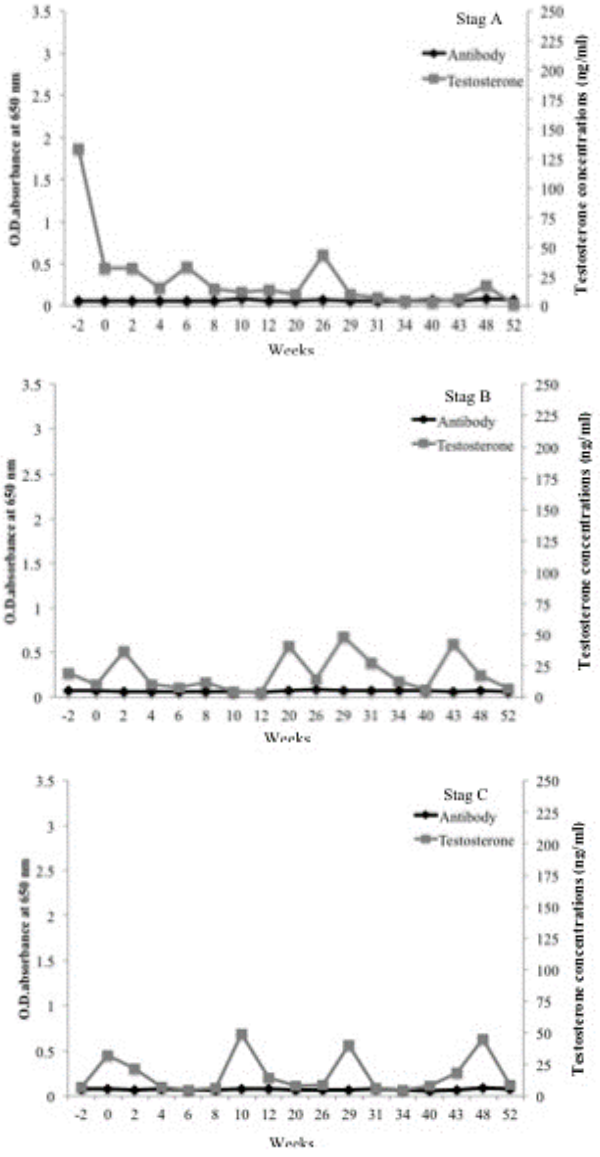


Figure 2 Individual changes in testosterone patterns and GnRH antibodies in the untreated group (Stags A, B and C)

Testosterone analysis: Concentration of testosterone in the serum was determined by using competitive enzyme immunoassay according to Brown et al. (2004). The antibody for testosterone analysis (poly clonal anti-testosterone R 156/7, 1:16,700 dilution) was obtained from Jo Corbin (Department of Population Health and Reproduction, Clinical Endocrinology Laboratory, Ca, USA). The standard ranged from 23 pg to 6,000 pg/ml. Sample serum was diluted 1:5-1:16 in assay buffer. The intra- and inter-assay coefficients of variation were 8.65% and 11.78% (n=8 plates).

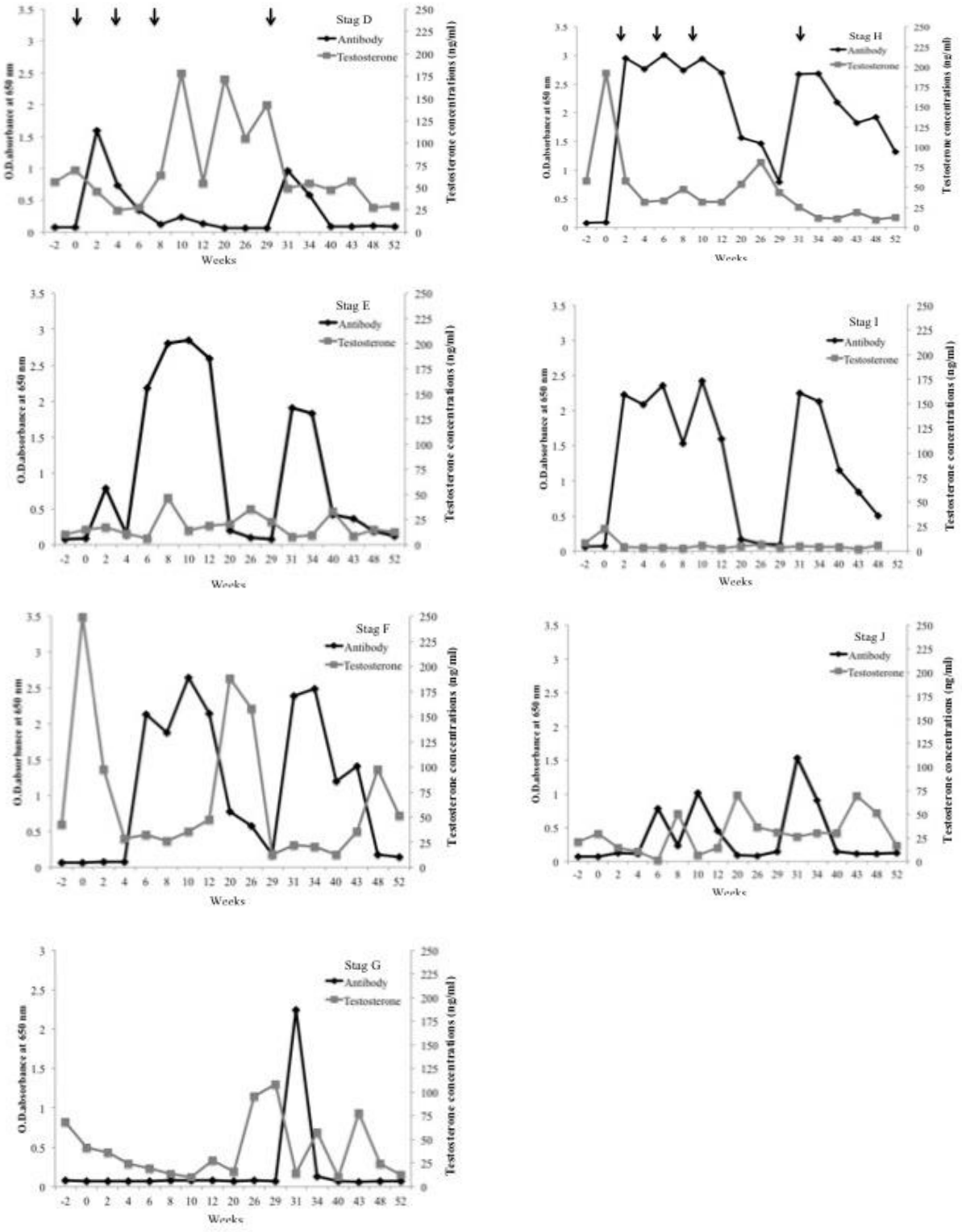


Figure 3 Individual changes in pattern of testosterones and variation of GnRH antibodies in the treated group (Stags D, E, F, G, H, I and J) before and after vaccination

Semen collection and evaluation: The first semen collection was performed in the breeding season (March 2011; two weeks before the first vaccination) and the series attempts were performed on June 2011 (four weeks after the second vaccination), January 2012 (thirty-five weeks after the third vaccination) and April

2012 (forty-four weeks after the third vaccination). Semen was not collected during the non-breeding season (July-December 2011). All stags were caught using a deer crush and anesthetized by using a combination of 1 mg/kg xylazine HCl (Ilium Xylazil-100®, Troy laboratories PTY Limited, Smithfield NSW, Australia) and 5 mg/kg ketamine HCl (Calypsol®, Gedeon Richter Ltd., Budapest, Hungary) intramuscularly. Semen was collected using electro-ejaculation (Monfort et al., 1993). The ejaculates were analyzed for color, volume, pH, mass movement progressive motility and sperm viability. Sperm concentration was determined by using 1% paraformaldehyde solution and standard hemacytometer. Sperm viability was evaluated by using eosin-nigrosin staining and with phase contrast microscopy (CX31, Olympus). A total of 200 spermatozoa were classified as live and dead sperm (Tamuli and Watson, 1994).

Volume of testis: Testicular measurements were taken during the 1st (two weeks before the first vaccination), 2nd (four weeks after the second vaccination), 3rd (thirty-five weeks after third vaccination) and 4th (forty-four weeks after the third vaccination) semen collections. Longitudinal and transversal radius of testis was measured by using a vernier caliper. Volume was calculated using formula of a prolate spheroid: $V = \frac{3}{4}\pi ab^2$ (a=longitudinal, b=transversal radius) (Gosch and Fischer, 1989).

Antler development: Information about antler development was recorded while the observer was waiting for stags' defecation. Antler status was divided into three categories: cast, velvet and hard antlers (Li et al., 2004). Photographs of antlers were taken throughout the experiment.

Table 1 Semen characteristics (Mean±SE) of the untreated group and the treated group before and after receiving vaccination with Improvac®

Group	Period	Mass activity	Progressive motility (%)	Volume (µl)	Sperm concentration (x 10^6 sperm/ml)	Live sperm (%)
Untreated group (n=3)	2 weeks before 1st vaccine	2±2.64 (1-5)	11.67±7.63 (5-20)	166.67±152.75 (200-300)	691.67±1,180.72 (20-2,055)	49.5±0.86 (48.5-50)
	4 weeks after 2nd vaccine	2±2.64 (1-5)	33.3±49.32 (0-90)	806±342.96 (410-1,008)	356.67±476.48 (10-900)	60.83±21.10 (46-85)
	35 weeks after 3rd vaccine	3.67±1.52[a] (2-4)	66.67±15.27[a] (50-80)	2,043.33±1,380.0 (600-3,350)	700±313.24[a] (400-1,025)	8.33±5.50[a] (83-94)
	44 weeks after 3rd vaccine	3.6±1.35[a] (3-5)	73.33±11.54 (60-80)	2,130±1,426.28 (670-3,520)	1,063.33±197.31[a] (930-1,290)	59.5±22.56 (33.5-74)
Treated group (n=7)	2 weeks before 1st vaccine	4.28±0.95 (3-5)	35.71±26.99 (10-90)	1,400±628.54 (530-2,030)	807.12±656.98 (185-1,825)	58.67±4.7 (52.25-56)
	4 weeks after 2nd vaccine	0.71±1.88 (0-5)	18.57±34.36 (0-95)	781.43±440.53 (400-1,750)	670.71±1,490.03 (20-4,050)	46.41±17.92 (27-71)
	35 weeks after 3rd vaccine	NA	NA	1,407.14±553.76 (0-2,470)	NA	NA
	44 weeks after 3rd vaccine	1±1.09[b] (1-3)	40±32.86 (0-70)	1,234.83±998.65 (52-2475)	119.16±113.99[b] (50-235)	54.41±42.41 (78-90.5)

[a,b] superscripts within column are significantly different ($p<0.05$).
NA; not available

Statistic analysis: Data from repeated sample collection were analyzed with the Statistical Analysis System (SAS Institute, USA, version 9.0). Mean±SE of each treatment (GnRH antibody, testosterone concentrations, volume of testis) was calculated by using repeated measures analysis of variance. Correlations between GnRH antibody titer and testosterone concentrations from all time points were determined by using correlation analysis. Mean±SE of semen characteristics, i.e. mass activity, percentage of progressive motility, semen volume, sperm concentration and percentage of live sperm, of the untreated and treated groups were compared using repeated measures ANOVA.

Results

GnRH antibody titer: The mean±SE of optical density of antibody titer of the untreated and treated groups were 0.06±0.009 and 0.87±0.09, respectively. GnRH antibody titers in all treated stags are shown in Figure 1. Antibody titer level of the untreated and treated groups before vaccination were 0.06±0.004 and 0.07±0.002, respectively. The treated group had a significant increase in GnRH antibody titer before the

second vaccination and after the fourth vaccination (p<0.05) compared with the untreated group. The titer of five stags (D, E, H, I and J) started to rise immediately after the first vaccination, however, the titer of one stag (F) responded to immunization after the second vaccination and the titer of another stag (G) started to rise after the fourth vaccination. Five vaccinated deer antibody titers reached the highest level after the third vaccination. This level was maintained for twelve weeks, then gradually decreased to baseline. After the fourth vaccination, the antibody titer of the treated deer was increased again. The absorbance of treated deer samples ranged from 0.96-2.67. Thereafter, following the fourth vaccination for twelve weeks, the titers (except stags F and H) had trends of decreasing to the baseline again.

Testosterone concentrations: The mean testosterone concentrations and GnRH antibody titer of individual rusa deer in the untreated and treated groups are presented in Figure 2 and Figure 3. The mean testosterone concentration of the untreated group and the treated group before vaccination were 38.99±19.38 and 63.31±18.96 ng/g feces. During two weeks after the first vaccination, the testosterone levels were lower than before vaccination in all stags. Results from ANOVA indicated that the testosterone concentrations were not significantly different between the groups (P=0.10) and times (P=0.08). However, the testosterone concentration in the treated group was negatively correlated with GnRH antibody titer (R=-0.25, p<0.05). Individual untreated deer showed fluctuation of testosterone concentrations (1.42-49.41 ng/ml). This hormone showed regular peaks four times per year (Figure 2).

Semen quality: Semen quality of rusa deer during the breeding season [April to July, 2012 (n=2) and April to June 2013 (n=5)] has recently been reported in Malaysia (Mahre et al., 2014). The results revealed some semen parameters such as volume, mass movement, sperm concentration, total motility, progressive motility and viability. The average of each parameters were 2.2±0.3 ml, 3.6±0.2, 886.3±39.7 x 10^6sperm/ml, 78.7±2.0%, 80.8±1.9% and 83.2±0.9%, respectively. Compared with this present study, during before and after the second vaccination, the semen characteristics of both groups remained unchanged. Sperm characteristics, i.e. sperm concentration, percentage of progressive motility, mass activity and percentage of live sperm, decreased significantly (p<0.05) after the third vaccination. Thirty-five weeks after the third vaccination, the low semen quality was clearly observed with azoospermia pattern. The resumption of semen quality was detected forty-four weeks after the third vaccination (p<0.05) (Table 1).

Volume of testis: In this study, the volume of testes of the untreated and treated groups before vaccination were 33.87±10.42 and 29.49±4.10 cm^3. Thirty-five weeks after the third vaccination, the testicular volume of the untreated group was significantly larger than that of the treated group (p<0.05) (Figure 4). Forty-four weeks after the third vaccination, the testicular volume of the treated group (30.13±4.63 cm^3) was also significantly lower than that of the untreated group (55.31±11.21 cm^3).

Antler development: The antler development of the untreated and treated groups are shown in Figure 5. The third vaccine was injected into the treated group in June (late breeding period). Six treated stags shed their hard antlers, but one stag retained hardening and casted its hard antlers later. Both of the untreated and treated groups became velvet in August (non-breeding season). Then, the treated group remained velvet until January (breeding season), whereas the antlers of the untreated group developed to harden. In April, two treated stags remained velvet (on the 44th weeks after the third vaccination).

Discussion

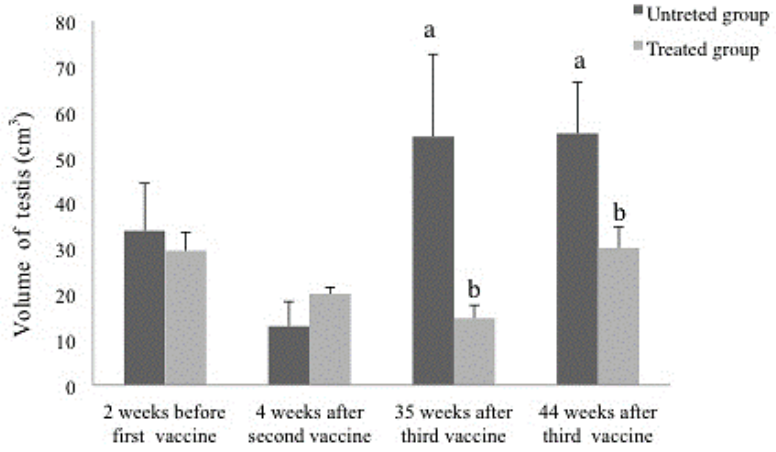
In this study, the GnRH vaccination was effective in male rusa deer. These effects included high GnRH antibody, low blood testosterone level, reduced testicular volume, decreased semen quality and deformed antlers. The results indicated that the treated deer's immune system responded to the vaccine. Antibody response in this study lasted at least 3 months after the third vaccination. Similar to a previous study in lambs immunized with the Improvac® vaccine twice at 3-week intervals, the antibody titer remained for 3 months after the second vaccine (Janett et al., 2003). However, in this study, the reason that some stags failed to produce high antibody titers remains unclear. Previous studies reported that the failure of antibody yield took place because the immune response was deflected by the type and amount of antigen, the carrier protein, the adjuvant, vaccination schedule and the species (Levy et al., 2004; Turkstra et al., 2005). In our study, the serum testosterone concentrations of 6 treated stags were immediately suppressed after the first vaccination. This is in line with results from a previous study that found low levels of testosterone concentration in 4 vaccinated stallions. They were detected after the second vaccination (Janett et al., 2009).

In this study, the antibody titer of the treated group was negatively correlated with testosterone concentration. Each animal exhibited variable immune responses with different antibody titers and testosterone production. The depression of testosterone is likely to have resulted from the GnRH immune response (Turkstra et al., 2005). Moreover, the immune response affect the reduction in semen quality, testicular volume and antler growth. At thirty-five weeks after the third vaccination, azoospermia occurred in the treated stags, whereas the untreated stags exhibited normal sperm concentration. Unfortunately, we did not collect semen from the treated and untreated stags between July-December, which is the non-breeding season. During the non-breeding season, even though the velvet of male deer grows rapidly, they are in the state of reproductive inactivity. In this time, testosterone level is very low and the testicles and accessory sexual organs significantly regress in size (Fennessy and Suttie, 1985; Kierdorf et al., 2009).

Azoospermia in this study might have occurred from spermatogenesis inhibition after vaccination. Forty-four weeks after the third vaccination, the treated stags resumed the spermatogenesis and improved their semen quality.

In this study, after vaccination, the mean of testicular volume of the treated group was smaller than that of the untreated group. We found that the testicular volume of the treated stags slowly decreased after the second vaccination and apparently reduced at forty-four weeks after the third vaccination. The low testicular volume after vaccination in this study was similar to previous reports in vaccinated male white-tailed deer (Killian et al., 2005), male lambs (Earl et al., 2006), male goats (Godfrey et al., 1996) and stallions (Janett et al., 2009).

In this study, after the third vaccination (in the non-breeding season: June-December), the hard antlers were shed. Following this, the regrown antlers of the treated stags were deformed velvet, whereas those of the untreated stags showed normal velvet (Figure 5a). Furthermore, during the breeding season (January-June), the velvet antlers of the untreated stags continued to grow and harden. Notably, the antlers in some treated stags remained in velvet status (Figs 5b and 5c).



Significant difference (p<0.05) between treatments is marked with a letter (n=7 for the treated group and n=3 for the untreated group).

Figure 4 Volume of testis (Mean±SE) of the untreated group (■) and the treated group (□) before and after receiving vaccinations

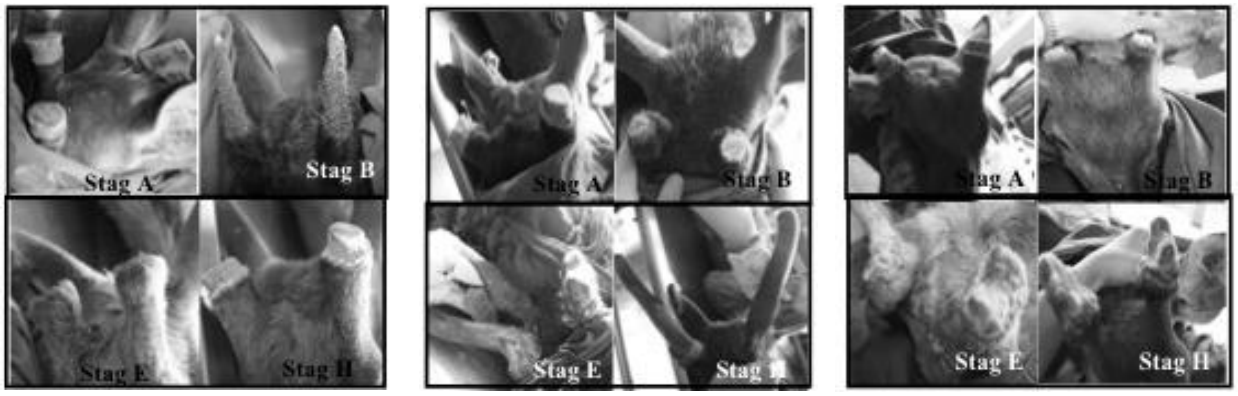


Figure 5 Comparison of characteristics of antlers after vaccinations in the untreated stags (Stag A and B on top part of each picture) and the treated stag (Stag E and H in below part of each picture). (a) = 4 weeks after the second vaccination (mid June), (b) = 35 weeks after the third vaccination (January) and (c) = 44 weeks after the third vaccination (April)

In conclusion, the data in this study demonstrated that the vaccination against GnRH produced GnRH antibodies to suppress serum testosterone production. This low testosterone level was effective in reducing semen quality in male rusa deer through effects such as decreased sperm concentrations and percentage of progressive motility. The vaccine also suppressed testicular size and growth of the antlers. A reverse in the effects of the GnRH vaccine immunization was detected after three months. Our study provides better knowledge of the implementation of immunocontraceptive vaccine in deer species. Our results highlight the potential of the GnRH vaccine for being an important tool for the population control of captive deer in the future.

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บทคัดย่อ

ผลของการใช้วัคซีนคุมกำเนิดต่อต้านฮอร์โมนโกนาโดโทรปิน รีลิสซิ่ง ฮอร์โมนต่อประสิทธิภาพทางการสืบพันธุ์ของกวางรูซ่าเพศผู้ (*Rusa timorensis*)

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การศึกษาการใช้วัคซีน GnRH ต่อประสิทธิภาพระบบสืบพันธุ์ของกวางรูซ่าเพศผู้ (*Rusa timorensis*) แบ่งกวางรูซ่าเต็มวัยเพศผู้จำนวน 10 ตัวออกเป็น 2 กลุ่ม คือ กลุ่มควบคุม (ไม่ได้ฉีดวัคซีน) (n=3) และกลุ่มที่ฉีดวัคซีน GnRH (n=7) กวางรูซ่าแต่ละตัวในกลุ่มที่ได้รับวัคซีนจะได้รับวัคซีน GnRH ปริมาตร 2 มิลลิลิตร (Improvac®; 1 มิลลิลิตรประกอบด้วย GnRH-protein conjugate 200 μg) ทำการฉีดวัคซีนใต้ผิวหนังทั้งหมด 4 ครั้ง เริ่มฉีดวัคซีนครั้งแรกในเดือนเมษายน ครั้งที่ 2 ในเดือนพฤษภาคม และครั้งที่ 3 ในเดือนมิถุนายน การฉีดวัคซีนแต่ละครั้งห่างกัน 4 สัปดาห์ ส่วนครั้งที่ 4 ฉีดหลังจากตรวจพบว่าระดับแอนติบอดีลดลงสู่ระดับ baseline ซึ่งงานทดลองนี้ฉีดวัคซีนครั้งที่ 4 ในสัปดาห์ที่ 29 ของการทดลอง (เดือนตุลาคม) จากนั้นจึงทำการเก็บซีรัมก่อนฉีดวัคซีน 2 สัปดาห์ และเก็บตัวอย่างหลังฉีดวัคซีนอย่างต่อเนื่อง 1 ปีเพื่อตรวจวัดระดับ GnRH antibody titer ความเข้มข้นของฮอร์โมนเทสโทสเตอโรน และทำการรีดน้ำเชื้อ วัดขนาดของอัณฑะ และสังเกตการพัฒนาของเขากวางรูซ่าทุกๆ 2 เดือน จากการทดลองพบว่าระดับของ GnRH antibody titer เพิ่มสูงขึ้นอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ภายหลังการฉีดวัคซีนเมื่อเปรียบเทียบกับช่วงก่อนฉีดวัคซีน จากนั้นแอนติบอดีเพิ่มระดับสูงขึ้นเรื่อยๆจนถึงระดับสูงสุดหลังจากฉีดวัคซีนครั้งที่ 3 และคงระดับอยู่ในกระแสเลือดเป็นระยะเวลา 12 สัปดาห์ จากนั้นแอนติบอดีค่อยๆลดลงจนถึงระดับ baseline อย่างไรก็ตามแอนติบอดีเพิ่มสูงขึ้นอีกครั้งหลังจากฉีดวัคซีนซ้ำครั้งที่ 4 ในส่วนของความเข้มข้นของฮอร์โมนเทสโทสเตอโรนในกระแสเลือด เมื่อประเมินความแตกต่างในกวางทั้งสองกลุ่มและระยะเวลาที่ทำการตรวจ พบว่ามีความแตกต่างกันอย่างไม่มีนัยสำคัญทางสถิติ อย่างไรก็ตาม พบว่าความเข้มข้นของฮอร์โมนเทสโทสเตอโรนของกวางในกลุ่มที่ได้รับวัคซีนมีความสัมพันธ์เชิงลบ (R=-0.25) กับระดับ GnRH antibody titer ในซีรัมอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ในส่วนของขนาดอัณฑะ กวางรูซ่ากลุ่มที่ได้รับวัคซีนมีขนาดอัณฑะเล็กกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) นอกจากนี้คุณภาพของน้ำเชื้อเริ่มลดลงหลังจากได้รับวัคซีนครั้งที่ 3 และไม่พบตัวอสุจิในน้ำเชื้อหลังจากได้รับวัคซีนครั้งที่ 4 นอกจากนี้กวางรูซ่าบางตัวผลัดเขาแข็งหลังจากได้รับวัคซีนครั้งที่ 3 จากนั้นจึงสร้างเขาอ่อนที่มีรูปร่างผิดปกติและเขาอ่อนที่สร้างขึ้นไม่พัฒนาเป็นเขาแข็ง โดยสรุปการศึกษาผลของการฉีดวัคซีน GnRH ให้กวางรูซ่าเพศผู้พบว่าวัคซีนจะทำให้เกิดการสร้างแอนติบอดีต่อฮอร์โมน GnRH ขึ้น ซึ่งระดับของแอนติบอดีในกระแสเลือดนั้นมีความสัมพันธ์เชิงลบกับฮอร์โมนเทสโทสเตอโรน ซึ่งระดับฮอร์โมนเทสโทสเตอโรนในกระแสเลือดที่ต่ำลงอาจจะเกี่ยวข้องกับคุณภาพน้ำเชื้อที่ลดลงและมีผลต่อการเจริญของเขากวาง

คำสำคัญ: วัคซีน **GnRH** กวางรูซ่าเพศผู้ คุณภาพของน้ำเชื้อ ฮอร์โมนเทสโทสเตอโรน

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