RELATION OF ANTLER STAGES AND REPRODUCTIVE ACTIVITY OF BAWEAN DEER (*Axis kuhlii*) STAGS

Adji Santoso Dradjat[1]

ABSTRACT

Reproductive activity during antler stages were studied in three Bawean deer (*Axis kuhlii*) stags kept in Lombok island of Indonesia. The antler of there stags were casted in September. Neck girth was minimum during antler cast 30.6 ± 0.52 cm, and maximum during hard antler 32.4 ± 0.51 cm. Testicular circumference was smaller during antler cast 13.0± 0.11 cm and maximum during hard antler 14.1 ± 0.17 cm. Volume of ejaculated semen was found 0.13 ± 0.05 ml during antler cast and velvet and the greater was 0.30 ± 0.2 ml during hard antler. Concentration of spermatozoa/ml during antler cast, velvet and hard antler stages were 102 ± 3.60 x 10^7, 220 ± 14.20 x 10^7, 308 ± 4.04 x 10^7 respectively. Score of spermatozoa motility, percentage of live of spermatozoa and normality of spermatozoa were maximum during hard antler 5.4 ± 0.3, 93.66 ± 2.08 %, 94.33 ± 1.52 % respectively and decrease 3.3 ± 0.13, 68.33 ± 2.08 %, 67.66 ± 5.13 % during velvet respectively and minimum during antler shading 2.1 ± 0.13, 38.33 ± 2.51 %, 19.66 ± 4.04 % respectively. It could be concluded that there was a synchrony antler cycle of three Bawean stags and there was testicular cycle of antler stages in male Bawean deer.

(Key words: Bawean deer, Reproduction, Antler stages).

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[1] Faculty of Animal Husbandry Mataram University, Mataram.

HUBUNGAN ANTARA STATUS RANGGAH DAN AKTIVITAS REPRODUKSI PADA RUSA BAWEAN (*AXIS KUHLII*) JANTAN

INTISARI

Aktivitas perkembang biakan selama siklus ranggah diamati pada tiga ekor rusa Bawean (*Axis kuhlii*) jantan yang dipelihara di P.Lombok. Ranggah rusa tersebut luruh pada bulan September. Lingkar leher kecil pada saat ranggah luruh yaitu sebesar 30.6 ± 0.52 cm, dan terbesar pada saat ranggah keras yaitu 32.4 ± 0.51 cm. Lingkar testis terkecil pada saat rangah luruh yaitu 13.0± 0.11 cm dan terbesar pada saat ranggah keras yaitu 14.1 ± 0.17 cm. Volume semen sebesar 0.13 ± 0.05 ml pada saat ranggah luruh dan volume terbesar pada saat ranggah keras yaitu sebesar 0.30 ± 0.2 ml. Konsentrasi spermatozoa/ ml pada saat ranggah luruh, tahap pertumbuhan ranggah dan ranggah keras adalah berturut-turut 102 ± 3.60 x 10^7, 220 ± 14.20 x 10^7, 308 ± 4.04 x 10^7. Skor motilitas, persentase spermatozoa hidup dan spermatozoa normal terbesar didapatkan pada saat ranggah keras yaitu berturut-turut 5.4 ± 0.3, 93.66 ± 2.08 %, 94.33 ± 1.52 % , nilai tersebut turun menjadi 3.3 ± 0.13, 68.33 ± 2.08 %, 67.66 ± 5.13 % pada saat pertumbuhan ranggah dan turun lagi menjadi minimum pada saat ranggah luruh yaitu 2.1 ± 0.13, 38.33 ± 2.51 %, 19.66 ± 4.04 %. Akhirnya dapat disimpulkan bahwa terdapat keseragaman siklus ranggah pada rusa Bawean jantan dan siklus testis yang berkaitan dengan siklus ranggah.

(Kata kunci: Rusa Bawean, Reproduksi, Status ranggah).

Introduction

Bawean deer (*Axis kuhlii*) is indigenous deer of Indonesia which originally lives in Bawean island. Since human population increases in early this century, population of Bawean deer has decreased and has been included as an endangered species (Blough and Atmosoedirdjo, 1987) as only between 300 to 500 animals were detected in Bawean island with small numbers of this deer were raise in some zoos. Early studies indicated that reproductive activity of temperate species deer related to the antler cycle and influenced by season and photoperiod (Lincoln, 1985). The deer showed seasonal breeding pattern, male rutting behavior, growth and casting antler associate with testicular cycle which indicate by testosterone production (Chaplin and White, 1972; Gosch and Fischer, 1989; Bubenik *et al.*, 1983; Haigh *et al.*, 1984; Hochereaude Reviers and Lincoln, 1978). In tropical areas however, as there is no fluctuation of photoperiod, consequently mechanism of pineal gland to testicular cycle to influence antler cycle and testicular cycle of tropical species in tropical areas is not clearly investigated. While temperate species of deer in temperate areas display testicular quiescence with azospermia (Haigh *et al.*, 1984; Hochereau-de Reviers and Lincoln, 1978; Loudon and Curlewis, 1988; Gosch and Fischer, 1989), tropical species in tropical areas may produce fertile spermatozoa at any stages of antler cycle. It was hypothesized although tropical species of deer has antler and testicular cycle, these deer still produce fertile spermatozoa any time of the year. In addition there is no information available of antler cycle, testicular cycle and sperm production of Bawean deer. Hence, the present study was performed to evaluate the antler cycle stages to the reproductive activity of Bawean stags.

Material and Method

Three mature Bawean stags with live weights of 34,5 kg, 36 and 37 kg, were used in this study. They were raised at Mataram University farm (Lingsar) and were trained to enter the yards and crush. They were fed grass with cut and carry system and supplemented with concentrate mix of rice bran, coconut mill and grinded corn, as necessary. These stags were placed in separate paddock with the hinds.

Semen was collected following antler casting, during velvet stages or a month after casting and during hard antler stages or a month after velvet rubbing. Collection of semen was performed using electro-ejaculation under general anesthesia, using a Ram probe (Ruakura).

General anesthetic used in the present study, previously has described (Mylrea, 1992; Haigh *et al.*, 1993), by using one mg/kg BW Xylazine hydrochloride (Xylazil-20, Troy Laboratories Pty Australia) combined with two mg/kg BW Ketamine hydrochloride (Ketamil, Troy Laboratories Pty Australia) (English, 1988) injected intramuscularly, with stags restrained in a crush. The stags were then allowed to stay in a darkened pen until they fell into lateral recumbency. Neck girth and testicle diameter were measured using standard measuring tape.

The penis was then pulled out from the prepuce, by pushing the upper leg forward, and, to prevent the penis from retracting into the prepuce a piece of woven bandage was used to hold it. A bell test tube was warmed by hand and held in front of the penis. The lubricated electro-ejaculator probe was inserted into the anus, with longitudinal electrodes on its ventral surface. The stimulation of the electro-ejaculator was applied for 5 seconds, with rest intervals, by pushing off the electro ejaculator for 5 seconds. Once the semen was ejaculated, or after two minutes of stimulation, electric induction was stopped. Reverzine (Yohimbin HCl, Parnell Laboratories Aust. Pty Ltd, Silverwater, NSW, Australia), 0.4mg/kg BW was injected intramuscularly, after semen collection, to reverse the effect of anesthesia (English, 1988). Then the stags were allowed to recover under shade, before being returned to their paddocks.

Semen was evaluated as soon as collected, by the following criteria: volume, abnormalities, live sperm and concentrations of sperm, as described (Mylrea, 1992; Haigh *et al.*, 1993). After collection, the volume of ejaculate was measured in a graduate test tube. The rate of motility was studied by dropping fresh semen (0.05 ml) onto a warmed slide, under a microscope with 40x magnification. The proportion of live, motile cells was scored at the edge of the drop, with a scoring system from 0 to 5 (Shackell, 1989; Haigh *et al.*, 1993). Examination of morphology was done to estimate the percentage numbers of normal cells, immature (protoplasmic droplet), abnormality during spermatogenesis (head) and post-spermatogenesis, as well as artifacts of the sperm. Semen of 0.02 was added to 0.1 ml of Nigrosin Eosin stain and smeared onto a warmed slide (36°C) and dried. The Nigrosin Eosin stain used in this study, was made of Eosin (water soluble) 1.67 gr, Nigrosin (water soluble) 10.0 gr, in 100 ml distilled water. These chemicals were mixed, filtered and warmed to 36°C before being used (Mylrea, 1992). Sperm morphology was examined under the microscope at 100x magnification. This staining was also used to estimate the ratio of live and dead spermatozoa (Mylrea, 1992). The sperm concentration was counted by using a haemocytometer pipette and counting chamber, under the microscope, after the samples settled for 5 minutes (Mylrea, 1992).

Result and discussion

As there is limited information on antler cycle of an endangered species of tropical deer in tropical areas, the present study was conducted in Bawean deer of Indonesia. Early studies seasonal breeding of antler and testicular cycle in deer were mostly undertaken in temperate deer in temperate areas in fallow deer (Chaplin and White, 1972; Gosch and Fischer, 1989), in white tailed deer (Bubenik *et al.*, 1983), in wapiti (Haigh *et al.*, 1984), in red

deer (Hochrereau de- Reviers and Lincoln, 1978) or tropical deer in temperate areas in chital deer (Loudon and Curlewis, 1988, Mylrea, 1992).

Results of the present study showed that there is synchrony of antler casting in Bawean deer, the antler was casted between 19 to 30 September 1998. This indicated that antler casting in Bawean deer was in certain time and there is unknown entraintment which induce the antler casting. Study in deer has been established in temperate species that photoperiod influence annual antler cycle and testicular cycle (Chaplin and White 1972) and melatonin treatment disrupt seasonal antler cycle (Fennessy *et al.*, 1985). However there is no effect of melatonin in tropical species deer in temperate areas (Loudon and Curlewis, 1988). It is important to considered that only three stags were used in the presents study, which may not represent the actual population of Bawean deer in their own range.

The results of neck girth measurement indicated that neck girth varies during antler stages, smaller during antler cast, and larger during hard antler stages (Figure 1). Neck girth (mean ± SD) minimum during antler cast 30.6 ± 0.52 cm, increase during velvet and maximum during hard antler stages 32.4 ± 0.51 cm. There is increasing neck girth of Bawean deer from casting antler, velvet stages and hard antler. The increase of the neck girth related to testosterone production (Loudon and Curlewis, 1988), in which influence secondary sexual characteristic of stag. This was shown from the results that there are variation of neck girth during antler stages; smaller during antler casting and increase during velvet and maximal during hard antler stages.

Scrotal circumference was measured during antler cycle, indicated that testicular diameter was smaller during antler cast and bigger during hard antler (Figure 2). Testicular circumference (mean ± SD) was smaller during antler cast 13.0 ± 0.11 cm, increase during velvet and maximum during hard antler 14.1 ± 0.17 cm. The results also indicated that testicle diameter was smaller during antler casting and biggest diameter was found during hard antler. Increasing size of testicle indicated reproductive activity. It was reported in red deer, in temperate areas that during sexual season the testicular size increases to 3 fold than during quiescent period (Hochrereau de-Reviers and Lincoln, 1978). It seems that there are antler cycles both in tropical species or temperate, however, increasing testicular size in tropical deer is not as dramatic reported in temperate deer. Haigh *et al* (1984), reported in wapiti that scrotal circumference decline gradually during the season, however testosterone concentration decrease sharply.

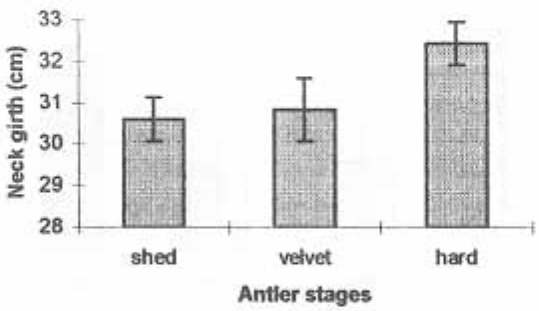

Figure 1. Neck girth during antler cycle.

This relation is not only detected in temperate species in temperate areas but also tropical species in temperate areas. Loudon and Curlewis (1988) reported that in chital deer (*Axis axis*) testis diameter minimum 1 to 2 months after antler cast and maximum was detected during hard antler. Loudon and Curlewis (1988) also concluded that body weight, neck circumference, testosterone concentration parallel to testis diameter.

Volume of ejaculated semen (mean ± SD) (Figure 3) was found 0.13 ± 0.05 ml similar during antler cast and velvet, while the greatest volume was found 0.30 ± 0.2 ml during hard antler. The results of the present study indicated that volume of semen was varied widely during antler stages, the greater volume was found during hard antler. Similar pattern was also found in sperm production such as motility, live and normal spermatozoa was higher, lower and minimum during antler cast, velvet and hard respectively. Similar finding was reported in fallow deer (*Dama dama*), a temperate species that there are relations of testicle volume, ejaculate volume, concentration spermatozoa per ejaculate and percentage of normal spermatozoa (Gosh and Fisher, 1985). Temperate species deer in temperate areas during velvet stages, there is a quiescent periods where the stags produce azospermia (Jaczewsky et al., 1984), however, Bawean deer in tropical areas produce motile spermatozoa at any time of the year. In temperate areas, rutting expression occurs at the end of maximal size of testis (Lincoln, 1985), in Bawean deer is still undetected.

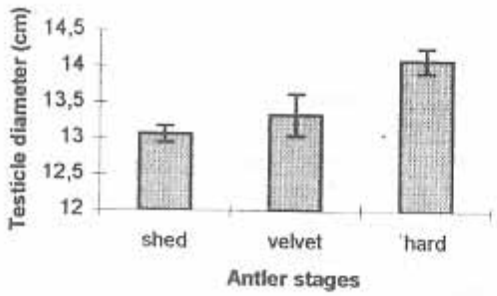

Figure 2. Scrotal circumference during antler cycle.

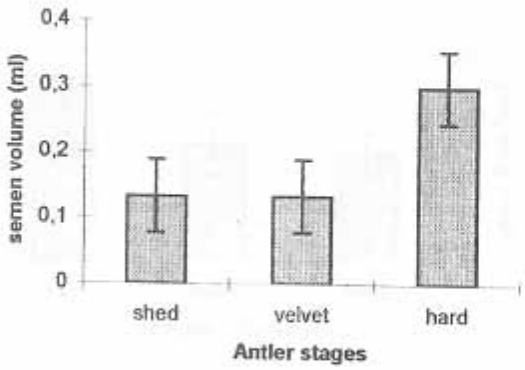

Figure 3. Volume semen during antler cycle.

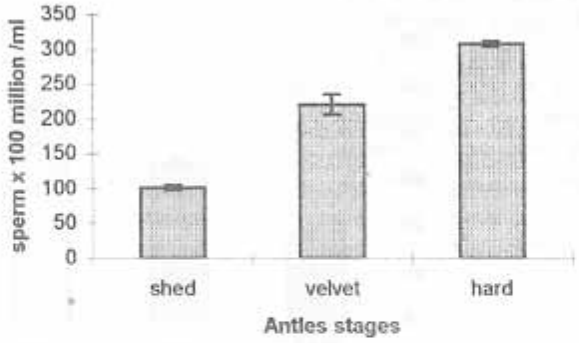

Figure 4. Sperm production during antler cycle

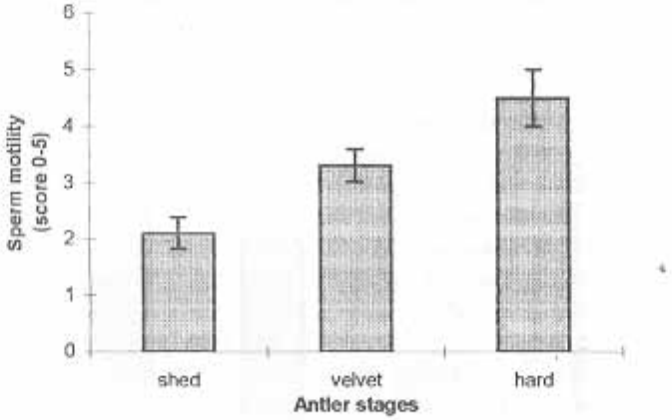

Figure 5. Score of sperm motility during antler cycle.

Sperm production was evaluated by counting concentration of electro-ejaculated spermatozoa. The results indicated that concentration of spermatozoa was lower during antler cast, velvet and maximum during hard antler (Figure 4) with concentration of spermatozoa/ ml (mean ± SD) of 102 ± 3.60 x 10^7, 220 ± 14.20 x 10^7, 308 ± 4.04 x 10^7 respectively.

Motility of spermatozoa was maximum during hard antler and minimum during antler casting. Spermatozoa motility (mean ± SD) was maximum during hard antler and decrease during velvet and minimum during antler shading with score of 5.4 ± 0.3, 3.3 ± 0.13. and 2.1± 0.13 respectively

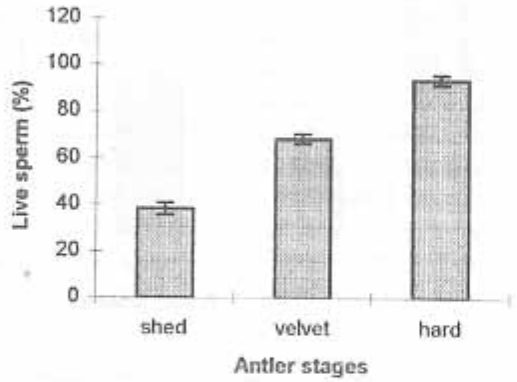

Figure 6. Live spermatozoa during antler cycle

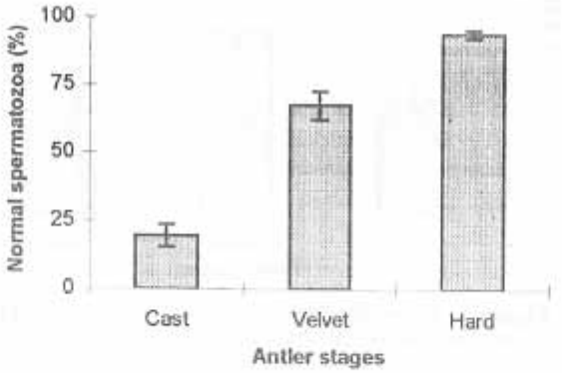

Figure 7. Normality of spermatozoa during antler cycle.

Percentage of live sperm depicted in Figure 6, indicated that live spermatozoa (mean ± SD) was maximum during hard antler and decrease during velvet and minimum during antler shading with percentage of 93.66 ± 2.08 %, 68.33 ± 2.08 % and 38.33 ± 2.51 % respectively.

Number of normal spermatozoa was shown Figure 7. with (mean ± SD) maximum during hard antler 94.33 ± 1.52 % and decrease 67.66 ± 5.13 % during velvet and minimum during antler cast 19.66 ± 4.04 % respectively

Bawean deer is adapted to tropical condition, where there are no significant different of photo periods, the seasonal change such as temperature, rainfall may dictating forage and grass production probably induce the antler cycle. As it was reported (Lincoln, 1985) that fertility of male tropical species of

deer in tropical areas are depressed during dry season, where FSH and LH was produced by anterior pituitary. From Graph 4 to 7 showed that sperm production and their quality related to the antler cycle. It was reported that there are relationship between seasons, LH and testosterone concentration, testosterone concentration maximum during mating season as testicular activity is also maximum in red deer (Lincoln and Kay, 1979).

Conclusion

It can be concluded that Bawean stags have antler cycle which related to their reproductive activities. Bawean stags have maximum reproductive activity during hard antler stages. It was suggested that semen bawean deer should collected for future insemination during hard antler cycle. Further study of reproductive pattern of Bawean deer will be a valuable finding which can be used as consideration to manage endangered tropical deer in tropical areas.

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