Hematologic Profile and Semen Quality of Male Timor Deer (Rusa timorensis) at Various Hierarchies

by Daud Samsudewa

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D. Samsudewa[1]*, S. S. Capitan[2], C. C. Sevilla[2], R.S.A. Vega[2] and P. P. Ocampo[3]

[1]Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia
[2] Institute of Animal Science, College of Agriculture & Food Science, University of The Philippines Los Banos, Los Banos, Laguna, Philippines
[3]Institute of Biological Sciences, University of The Philippines Los Banos, Los Banos, Laguna, Philippines

Corresponding E-mail: daudreproduksi@gmail.com

Abstract

The aim of this research was to observe hematologic profile i.e. erythrocyte count, hemoglobin and hematocrit and semen quality, i.e. semen volume, sperm motility and sperm abnormality of a-male, þ-male and subordinate male Timor deer raised under captivity. Twelve males (51 ± 6 months old; 68.29 ± 8.41kg body weight) at similar antler stages were use in this study. Before and after 43 days of establishment of dominance hierarchy blood were sampled after sedation for erythrocyte count, hemoglobin (mg/dL), and hematocrit (%). Likewise, semen was collected using electroejaculator and were analyzed for semen volume (ml), sperm motility (%) and sperm abnormality (%) to compare male deer at various heirarchies. Wilcoxon signed ranks test and Kruskal-Wallis H test of non-parametric analysis was done. Significant difference was tested with Mann- Whitney U test. The results showed that highest count of erythrocyte shown on a and þ- male (1.60 million per μL). The highest increase in hematocrit was observed in þ-male (5%) and then followed by S2-male (4%). S2-male had the highest increase in hemoglobin (0.13 g/dL). The highest increase in semen volume was observed in a –male (0.75 ml). Social stress affected negatively the sperm motility and abnormality ($P<0.05$). The highest decrease was observed in S2-male.

1. Introduction

The Timor deer (*Cervus timorensis* Blainville) is one of the medium size deer in Indonesia with 56.80 ±11.63 kg male body weight and 80.04 ±5.93 cm shoulder height [1]. Timor deer are currently being reared in captivity in Indonesia. But, increasing population of Timor deer in Indonesian captivity is still low. The captive farm in Kudus regency reported increasing population 0.5 head per year [1]. This research suggest that the productivity of Timor deer in captivity can be improved if some key aspects of management such as feeding, mating, aggressive male behavior and maternal care are addressed.

Aggressive male behavior was common in captive farm of Timor deer, to form dominance hierarchy. Aggressive male behavior during establishment of dominance hierarchy causes unnecessary stress i.e. partitioning nutrients towards fighting and wound healing. This social stress may lead to poor health of Timor deer and reproductive performance. Social stress has adverse effects on the reproduction system and health of both males and females [2]. Understanding and managing social stress of Timor deer will help establihsh the best management system esspecially in improving reproduction efficiency.

The research about understanding the effect of establishing dominance hierarchy of Timor deer on the reproduction was limited. Therefore this study was conducted to know the effect of dominance hierarchy on hematologic profile and semen quality of Timor deer.

2. Materials and Methods

2.1. Care & Management of Timor Deer

This research was conducted in H. Yusuf Wartono Timor deer captivity, Margorejo, Dawe, Kudus, Central Java, Indonesia. Blood and semen samples of 12 male Timor deer (51 ± 6 months old; 68.29 ± 8.41kg body weight) at similar antler stages were used in this research. Timor deer were placed in three communal stalls (23.5 x 21.5m). Four male Timor deer in every stall will form dominance hierarchy by fighting for about one week. Finally, Timor deer in every stall will form a- male (Superior male), þ-male (Second rank male), subordinate-1 (S1) male (Third rank male) and subordinate-2 (S2) male (Fourth rank male). Hematologic profile and semen volume and quality were obtained before and at 43 days after establishment of dominance hierarchy. Seven kilogram napier grass (Pennisetum puprpureum) was fed for each individu per day to fullfil consumption. Water was prepared ad libitum.

2.2. Blood Collection and Assay

Blood samples were taken by jugular venepuncture from each male after ketamine and xylazine sedation. Blood analysis was done to obtain the hematologic picture (hematocrit, haemoglobin and erythrocyte).

Erythrocyte was counted using blood containing EDTA. Sipped the blood into a standard blood dilution pipette to the 0.5 mark, followed by methyl violet 2B diluents to the 1.01 mark. The final dilution was 1 : 200. Shaked the pipette briefly, place drops to the counting chamber of a neubauer haemocytometer. Let it settled for a few minute, the red cell count was determined under high-dry magnification (40 x objectives and 10 x ocular). Erythrocytes appearing in the center and four corner squares were counted and the total multiplied by 10^4 to give erythrocyte count per microliter [3].

Haemoglobin was determined at the start by filling the Sahli tube with HCl 0.1 N up to 2 mark. Blood was drawn into a haemoglobin pippete up to the 20 mark and filled the Sahli tube. Blood was stirred for 3 minutes until hematin acid appeared. Placed the Sahli tube in comparator block. Water filled the Sahli tube and it was stirred until it obtained the same colour as the standard in the comparator block. Scale was read in Sahli tube [3].

Blood was collected in tubes with ethylenediamine tetraacetate (EDTA) and then fill in microcapillary tubes. Sealed the microcapillary tubes with wax and centrifuged at 16,000 rpm for 5 minutes and read using a standard microhematocrit graphic reader [3].

2.3. Semen Quality

Semen was collected from each male after ketamine and xylazine sedation with use of electroejaculator with 15 V and 500 mA. Increase of voltage was done gradually until 15 V [4]. Semen was analyzed macroscopically (volume) and microscopically (motility and sperm abnormality). Volume of semen was measured in ml by using tulip scale. Measurement range of tulip scale was 0-12 ml with the sensitivity of 0.1 ml.

Motility of spermatozoa was determined by the following steps: Mixed the semen of Timor deer by inverting vial 2-3 times. Placed a drop of the Timor deer semen and spread on the warm slide. Covered with cover slip. Examined the semen immediately for motility, using the low power objective of the microscope. Observe the edge of the semen to ascertain an approximation of the percentage of live and motil spermatozoa. Graded The semen based on the scale 0-100% [5].

Determination of spermatozoa abnormality was done using the following steps: Smear the semen on object glass and stained with eosin-negrosin. Examined the preparation under high objective (1000x) of the microscope. The sperms were observed for morphological abnormalities such as head abnormalities (giant, small, double etc.), middle piece abnormalities (double midpieces, swollen midpieces, coiled midpieces etc.) and tail abnormalities (coiled tails, absent tails, broken tails etc.). Minimum 200 sperms were counted using random fields on the different parts of the slide. Count the abnormality and convert it to percent (%) [5].

2.4. Data Analysis

Wilcoxon signed ranks test was used in comparing before and at 43 days after establishment of hierarchy for all parameters. The comparison of various hierarchy (Superior, 2nd, 3rd and 4rth rank) was accomplished using Kruskall-Wallis H Test for non-parametric analysis for this research. Mann whitney U test of non-parametric analysis was done when different rank were found significant [6].

3. Results and Discussion

3.1. Erythrocyte count

Average values of erythrocyte count of a, þ, S1-male and S-2 male of Timor deer before and at 43 days after establishment of dominance hierarchy are shown in Table 1.

Table 1. Average values of erythrocyte count of male Timor deer at various hierarchies before and 43 days after establishment of dominance hierarchy

FACTOR	HIERARCHY			
	a-Male	þ-Male	S1-Male	S2-Male
	.....Million per μL.....			
Before	5.42	5.66	6.85	6.61
After	7.77	7.05	5.57	6.68
Z	1.60	1.60	1.07	0.00

Wilcoxon signed ranks test for erythrocyte showed no significant difference ($P \leq 0.05$) in median values before and 43 days after establishment of dominance hierarchy for all hierarchy levels. The non-parametric Kruskal-Wallis H test showed no significant difference ($P \leq 0.05$) among the hierarchies before ($z^2 = 3.21$) and 43 days after ($z^2 = 6.64$) establishment of dominance hierarchy in terms of erythrocyte count.

The present study showed that establishment of dominance hierarchy did not influence the number of erythrocyte. This result contradicts the research on red deer stags that showed lowest red blood cell count (RBC) count during rutting period, since RBC count in this research (5.42 to 7.77 x $10^6/\mu$L) still falls under the normal range (6.2 to 11.7 x $10^6/\mu$L) [7]. However, these values are still low compared with those reported in Ragunan Zoo in Jakarta, 8.12 to 9.89 x $10^6/\mu$L with an average of 9.01 x $10^6/\mu$L ± 0.93 x $10^6/\mu$L [8], and those found in male Rusa Deer (*Cervus timorensis russa*) in Thailand, 11.22 x 10^6 ±1.19 x $10^6/\mu$L [9].

3.2. Hemoglobin

Average values of hemoglobin (Hb) of a, þ, S1-male and S-2 male of Timor deer before and 43 days after establishment of dominance hierarchy are shown in Table 2.

Table 2. Average values of male Timor deer hemoglobin at various hierarchies before and 43 days after establishment of dominance hierarchy

FACTOR	HIERARCHY			
	a-Male	þ-Male	S1-Male	S2-Male
	.....g/dL.....			
Before	11.80	11.80	12.07	12.61
After	11.60	11.40	11.13	13.00
Z	0.45	0.82	1.07	0.00

In this parameter, Wilcoxon signed ranks test for hemoglobin showed no significant difference ($P \leq 0.05$) in median values before and 43 days after establishment of dominance hierarchy groups for all male groups. Furthermore, the non-parametric Kruskal-Wallis H test showed no significant difference ($P \leq 0.05$) among the hierarchies both before ($z^2 = 1.56$) and 43 days after ($z^2 = 2.61$) establishment of dominance.

Hb in this study (11.60 to 13.00 g/dL) still falls below the nomal range (7.9 to 17.4 g/dL) [7], but is still low compared with those found in Ragunan Zoo, 14 to 17.6 g/dL with an average of 16.25 ± 1.63 g/dL [8], but comparable with that measured on Timor deer in Thailand, 12.5 ± 1.7 g/dL by [9].

3.3. Hematocrit

Average values hematocrit of a, þ, S1-male and S-2 male of Timor deer before and 43 days after establishment of the hierarchy are shown in Table 3.

Table 3. Average values of male Timor deer hematocrit at various hierarchies before and 43 days after establishment of dominance hierarchy

FACTOR	HIERARCHY			
	a-Male	þ-Male	S1-Male	S2-Male
	..... %			
Before	31.67	31.67	34.00	35.00
After	35.33	36.67	35.00	39.00
Z	1.63	1.60	0.82	1.63

Wilcoxon signed ranks test showed no significant difference ($P \leq 0.05$) in median values before and 43 days after establishment of dominance hierarchy for all male groups. The non-parametric Kruskal-Wallis H test showed no significant difference ($P \leq 0.05$) among the groups both before ($z^2 = 2.62$) and 43 days after ($z^2 = 2.82$) establishment of dominance hierarchy in terms of percent hematocrit.

Hematocrit in this study (31 to 39%) falls under the normal range (26 to 46%) [7], but is still low when compared with those reported in Ragunan Zoo, Jakarta (40.5 to 43.5%) [8], and that estimated on Timor deer in Thailand which were 41.75 ± 1.32% [9].

The results of the present work on the relationship between dominance hierarchy and hematologic profile varies. Although not statistically significant the highest increase of hematocrit after establishment of hierarchy was shown by þ-male (5%) and followed by S2-male (4%). Hemoglobin, in S2-male showed the highest increase (0.13 g/dL). Both male groups had increased hematocrit and hemoglobin content which are probably due to stress related to dominance hierarchy establishment. Research on Roe deer showed that during the mating season stags need physical strength and endurance to be able to cope with the huge energy demands [10]. Further, they stated that since increased hematocrit improves oxygen supply to the muscles, it seems likely that during the rut,

males with high testosterone level (the dominant male) will be able to endure more intense physical exercises during male-to-male combats. Furthermore, metabolic efficiency is needed to study for copulation with and in defense for females. The a-males had increased hematocrit (3.67 g/dL) and erythrocyte content (2.35 million x $10^6/\mu$L). This condition indicates the fitness of a-male.

3.4. Semen volume

Average values of semen volume of a, þ, S1-male and S-2 male of Timor deer before and 43 days after establishment of dominance hierarchy are shown in Table 4.

Table 4. Average values of male Timor deer semen volume at various hierarchies before and 43 days after establishment of dominance hierarchy

FACTOR	HIERARCHY			
	a-Male	þ-Male	S1-Male	S2-Male
	ml			
Before	1.00	1.70	1.83	1.13
After	1.75	1.62	2.07	1.43
Z	1.07	0.00	0.27	1.07

Wilcoxon signed ranks test for semen volume showed no significant difference ($P \leq 0.05$) in median between before and 43 days after establishment of dominance hierarchy groups for all males. Kruskal-Wallis H test showed significant differences ($P<0.05$) among the groups before ($z^2 = 8.75$) establishment of dominance hierarchy in terms of semen volume. On the other hand, no significant difference ($P \leq 0.05$) was found after ($z^2 = 0.64$) the establishment of dominance hierarchy. The significant difference ($P<0.05$) before establishment of dominance was found in a-male compared with other males.

Alpha-male had the largest increase in semen volume (0.75 ml). Increasing semen volume in a-male is related with the rutting status of the animal. The a-males are the most active Timor deer in terms of mating. The semen volume non-rutting Eld deer stags was 2.11 ± 0.61 ml and during rutting Eld deer stags have a significant difference in ejaculate semen volume, around 3.49 ± 0.36 ml [11]. Semen volume of male Timor deer in this study (0.30 to 3.50 ml) was still within range compared with semen volume of Timor deer in Ragunan Zoo, Jakarta (2.06 ± 0.63 ml) [12].

3.5. Sperm motility

Average values of sperm motility of a, þ, S1-male and S-2 male of Timor deer before and 43 days after establishment of dominance hierarchy are shown in Table 5.

Table 5. Average values of male Timor deer sperm motility at various hierarchies before and 43 days after establishment of dominance hierarchy

FACTOR	HIERARCHY			
	a-Male	þ-Male	S1-Male	S2-Male
	%			
Before	70.00	69.17	70.00	75.00
After	60.83	46.67	46.67	33.33
Z	1.60	1.60	1.34	1.07

Wilcoxon signed ranks test showed no significant difference in median values before and after establishment of dominance hierarchy groups for all male groups. Kruskal-Wallis H test for showed

no significant difference (P≤0.05) among the groups before ($z^2 = 1.77$) and after ($z^2 = 0.12$) establishment of dominance hierarchy in terms of sperm motility.

Establishment of dominance hierarchy among male groups did not affect motility of spermatozoa. Sperm motility of the present study (33.33 to 75.00%) is lower compared the sperm motility of Timor deer in Ragunan zoo (75.83 ± 3.76%) [12].

3.6. Sperm abnormality

Average values of sperm abnormality of a, þ, S1-male and S-2 male of Timor deer before and 43 days after establishment of dominance hierarchy are shown in Table 6.

Table 6. Average values of male Timor deer sperm abnormality at various hierarchies before and after establishment (43 days) of dominance hierarchy

FACTOR	HIERARCHY			
	a-Male	þ-Male	S1-Male	S2-Male
	..... %			
Before	8.31	8.46	6.78	10.13
After	9.98	19.79	17.93	44.58
Z	0.00	1.60	1.60	1.07

Wilcoxon signed ranks test was showed no significant difference (P≤0.05) in median values before and 43 days after establishment of dominance hierarchy for all male groups. The non- parametric Kruskal-Wallis H test showed no significant difference (P≤0.05) among the groups before ($z^2 = 1.97$) and 43 days after ($z^2 = 2.54$) establishment of dominance.

Establishment of dominance hierarchy did not affect the proportion of sperm abnormality. Sperm abnormality of Timor deer in this research (8.31 to 44.58%) is higher than Timor deer in Ragunan zoo (7.31 ± 2.99%) [12]. Sperm abnormality found in this research includes sperms without tail, double head, double tail, small head and giant head. S2 male shown higher of sperm abnormality was connected with social stress and inhibition of mating for S2 male compared to other male. Eld's deer stags had lower number of normal sperm outside of rutting season (18.2 ± 5.9%) and stags in rutting season had the highest number of normal sperm (85.7 ± 5.4%) [11].

4. Conclusion

Dominance hierarchy was affected by decrease in sperm motility and increase in sperm abnormality esspecially to subordinate 2 (S2) male. Beta-male and S2-male had increased hematocrit and hemoglobin content which are probably due to stress affected by aggressive male behavior during dominance hierarchy establishment. The a-male is the fitness of male Timor deer compare with other hierarchy level.

Acknowledgments

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