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Estrogen level and cervical mucus of Timor hind (*Rusa timorensis*) after mineral block supplementation during estrous cycle

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Abstract. The aim of this research was to observe the effect of mineral block containing Selenium, Magnesium and Zinc to the length of estrous of Timor hind based on estrogen level and cervical mucus (potential hydrogen (pH)) and natrium chloride (NaCl)). The materials used were ten Timor hinds with range of Body Condition Score (BCS) 2.75 ± 0.25 with first parity. Timor hinds were grouping into 2 groups, it was T0 = without mineral block supplementation and T1 = with mineral block supplementation for 8 weeks. After treatment, the estrous cycle of Timor hind was synchronized using vaginal sponge that contained 20 mg medroxy progesterone acetate (MPA) for 16 days. Cervical mucus and blood were collected at 0, 24, 72, 84, 96, 108, 120 and 144 hours after releasing of vaginal sponge. The cervical mucus was measured for pH and percentage of NaCl. The estrogen level was analysed by radio immunoassay (RIA). Data were analysed using non parametric statistics Mann-Whitney U test. The result showed significantly different was found on 120 and 144 hours of pH of cervical mucus. pH of cervical mucus on 120 and 144 hours were T0 = 8.46 and T1 = 8.30; T0 = 8.46 and T1 = 8.28, respectively. NaCl level on 72, 84, 96 and 108 hours were also significantly different in cervical mucus and estrogen level. The NaCl level of cervical mucus for 72, 84, 96 and 108 hours were T0 = 0.00181% and T1 = 0.00236%; T0 = 0.00179% and T1 = 0.00227%; T0 = 0.00178% and T1 = 0.00202%; T0 = 0.00156% and T1 = 0.00177%, respectively. The estrogen level for 72, 84, 96 and 108 hours were T0 = 8.21 pg/mL and T1 = 12.90 pg/mL; T0 = 7.54 pg/mL and T1 = 12.33 pg/mL; T0 = 4.56 pg/mL and T1 = 12.09 pg/mL; T0 = 3.76 pg/mL and T1 = 10.55 pg/mL, respectively. Estrous length of Timor hinds in group T0 and T1 was 48 and 84 hours, respectively. In conclusion, mineral block supplementation leads to longer estrous in Timor deer than those not given supplementation.

Keywords: estrogen level; mineral supplementation; cervical mucus; Timor deer

8 Introduction

Timor deer (*Rusa timorensis*) is one of endemic cervus spp. In Indonesia. Based on body size, Timor deer belonging to the medium size. Chest circumferences of male Timor deer is 88.00 to 94.70 cm [1]. Timor deer was classified to vulnerable because the number of animal is lower than 10.000 in wild. Increasing population was occurred in captivity. In Central Java Province, Indonesia around 800 heads of Timor deer were reared in captivity on 2018. Unfortunately, inbreeding was one of the problems that affected by limitation of male Timor deer.



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Artificial insemination is one of the problems solving for inbreeding in captivity of Timor deer. Artificial insemination is the mating method by depositing semen into the vagina with human support [2]. Artificial insemination needs proper observation for estrous sign of female Timor deer. On other hand, Timor deer breeder still found difficulty to observe the estrous sign [3]. So does other species deer such as spotted deer [4]. The easiest way to find out estrous sign is through estrous behaviour [5]. But, estrous behaviour needs to confirm with physiological signs such as pH of cervical mucus, NaCl level in cervical mucus and estrogen level to reach the high percentage of pregnancy. In the other hand, silent heat also affected by deficiency of some minerals (selenium, magnesium and zinc) as cofactor [6]. Previous research in Timor deer with different body condition score (2.75 to 3.25) conducted by [7] showed that supplementation of mineral block containing selenium, magnesium and zinc did not affected to pH and NaCl level of cervical mucus. They also could not find the dynamics of estrous cycle, because they did not complete with estrogen level data. Therefore, we need to observe about effect of mineral block (containing selenium, magnesium and zinc) to the pH of cervical mucus, NaCl level of cervical mucus and estrogen level.

2. Materials and Methods

2.1. Care and Management of Timor Deer

This study was carried out in H. Yusuf Wartono Timor deer breeding captivity in Margorejo village, Dawe district, Kudus regency, Central Java province of Indonesia. Ten hinds (first parity with body condition score (BCS) 2.75 ± 0.25) and 2 bucks (BCS 3.00) were used in this research. Timor deer placed in two stall (5m x 5 m) with 1 buck and 5 hinds for each stall. Two treatments (T0 and T1) were applied. One stall was treated by mineral block containing with selenium (1 ppb), magnesium (510 ppb) and zinc (30 ppb) for 8 weeks (T1) and the other stall was not treated by mineral block (T0). Estrous synchronization used 20 mg MPA on sponge vagina [2] was done for 16 days after treatment of mineral block. Chemical in cervical mucus and blood of hinds were collected on 0, 24, 72, 84, 96, 108, 120, 144 hours after removing of sponge vagina. Elephant grass (7 kg) (*Pennisetum purpureum*) and 2 kg cassava (*Manihot utilisima*) was fed each day for hinds as the main feed.

2.2. Chemical of cervical mucus

Cervical mucus was collected used catheter after sedation with 0.5 mL Acetyl Promazine (ACP). Cervical mucus was used for obtaining data potential hydrogen (pH) and NaCl level.

2.2.1. *pH of cervical mucus.* pH of cervical mucus was collected used pH universal indicator with range 6.2-8.4 (sensitivity 0.3). Cervical mucus was smeared on pH universal indicator and compare to the standard.

2.2.2. *NaCl Level of cervical mucus.* Cervical mucus was diluted with propanol up to 1 mL. Titration of Cr (*Chromium*) and AgNO₃ (*Silver nitrate*) was done up to colour changing. NaCl level (%) of cervical mucus was counted used equation [7]:

$$\text{Kadar NaCl (\%)} = \frac{(V_1 \cdot N) \times B \times P \times 100\%}{V_2 \times 1.000}$$

Additional information:

- V₁ = Volume AgNO₃
- N = Normality AgNO₃
- B = MR NaCl (58,46)
- P = dilution factor
- V₂ = Sample volume

2.3. Estrogen level of hinds

Blood samples were taken by jugular venepuncture from hinds after 0.5 mL ACP sedation. Blood analysis was done to obtain the estrogen level used RIA.

2.4. Data Analysis

Mann Whitney U test as the alternative of independent test on statistic non parametric was used for statistical analysis of data. Supporting program for statistical analysis was SPSS 16.0 for windows.

3. Results and Discussion

3.1. pH of Cervical Mucus of Timor Deer Hinds

Mean value of pH of cervical mucus Timor deer hinds with and without supplementation of mineral block shown in Table 1. Mann-Whitney U test for pH of cervical mucus Timor deer hinds showed no significant different ($P \geq 0.05$) from 0 to 108 hours. Significantly different ($P < 0.05$) was found on 120 and 144 hours.

Table 1. Mean pH of Cervical Mucus with and without Supplementation of Mineral Block on Timor Deer Hind.

Time range after removing sponge vagina	Treatment (mean \pm SD)	
	T ₀	T ₁
0	8.58 \pm 0.29	8.38 \pm 0.22
24	7.82 \pm 0.45	7.96 \pm 0.13
72	8.06 \pm 0.46	8.08 \pm 0.16
84	8.20 \pm 0.41	8.16 \pm 0.23
96	8.32 \pm 0.24	8.20 \pm 0.21
108	8.44 \pm 0.40	8.30 \pm 0.31
120	8.46 \pm 0.25 ^a	8.30 \pm 0.31 ^b
144	8.46 \pm 0.37 ^a	8.28 \pm 0.40 ^b

Table 1. showed that during diestrous (0 hours) to proestrous (24 hours) pH was fell down on two treatments. Decreasing pH during diestrous to proestrous is affected by increasing level of NaCl on cervical mucus [8]. Meanwhile, the data of pH cervical mucus was fell down on normal range compared to the normal pH cervical mucus of livestock is 5.5-8.6.

3.2. NaCl Level of Cervical Mucus of Timor Deer Hinds

Cervical mucus must be adequate during estrous. Cervical mucus secretion affected by level of estrogen and progesteron. Inadequate of cervical mucus will affected to the sperm penetration and fertility [9]. Mean value of NaCl level of mucus servix Timor deer hinds with and without supplementation of mineral block shown in Table 2.

Mann-Whitney U test for NaCl level of cervical mucus Timor deer hinds showed no significant different ($P \geq 0.05$) from 0 to 24 and 120 to 144 hours. Meanwhile, the significantly different ($P < 0.05$) was found on 72 to 108 hours.

Highest NaCl level of cervical mucus found in proestrous phase (24 hours after release of sponge vagina). This result was linier with estrogen level of Timor deer hinds. Highest estrogen level found in proestrous phase [6]. But, in proestrous phase (0 to 24 hours after release of sponge vagina) the statistical analysis shown non-significant different. Significance difference shown in estrous phase (72 to 108 hours after release of sponge vagina). It is mean that mineral block supports the consistency of estrous phase. Selenium, magnesium and zinc support the granulosa cell to produce estrogen. The level of NaCl of cervical mucus will be back to the normal level after diestrous phase (120 and 144 hours after discharge of sponge vagina).

Table 2. Mean NaCl Level of Cervical Mucus with and without Supplementation of Mineral Block on Timor Deer Hind.

Time range after removing sponge vagina	Treatment (Mean \pm SD) (%)	
	T ₀	T ₁
0	0.00147 \pm 0.00118	0.00187 \pm 0.00078
24	0.00229 \pm 0.00063	0.00242 \pm 0.00025
72	0.00181 \pm 0.00038 ^a	0.00236 \pm 0.00084 ^b
84	0.00179 \pm 0.00055 ^a	0.00227 \pm 0.00021 ^b
96	0.00178 \pm 0.00065 ^a	0.00202 \pm 0.00030 ^b
108	0.00156 \pm 0.00048 ^a	0.00177 \pm 0.00059 ^b
120	0.00133 \pm 0.00021	0.00160 \pm 0.00021
144	0.00134 \pm 0.00021	0.00160 \pm 0.00057

3.3. Estrogen Level of Timor Deer Hinds

Mean value of estrogen level of Timor deer hinds with and without supplementation of mineral block shown in Table 3.

Table 3. Mean Estrogen level of Cervical Mucus with and without Supplementation of Mineral Block on Timor Deer Hind.

Time range after removing sponge vagina	Treatment (Mean \pm SD) (%)	
	T ₀	T ₁
0	2.77 \pm 0.88	4.45 \pm 0.78
24	9.32 \pm 0.88	13.13 \pm 0.73
72	8.21 \pm 0.79 ^a	12.90 \pm 0.94 ^b
84	7.54 \pm 0.32 ^a	12.33 \pm 0.55 ^b
96	4.56 \pm 0.84 ^a	12.09 \pm 0.48 ^b
108	3.76 \pm 0.61 ^a	10.5 \pm 0.94 ^b
120	3.30 \pm 0.53	7.81 \pm 0.39
144	2.90 \pm 0.39	6.48 \pm 0.79

Mann-Whitney U test for estrogen level of Timor deer hinds showed no significant different ($P \geq 0.05$) from 0 to 24 and 120 to 144 hours. Meanwhile, the significantly different ($P < 0.05$) was found on 72 to 108 hours.

Estrogen level of Timor deer after supplementation of mineral block (containing with selenium, magnesium and zinc) was higher than previous research (7.06-11.81 pg/mL) [6]. Increasing estrogen level was affected by supplementation of mineral block that support reaction of androgen to estradiol. Significantly different was found on 72 to 108 hours after removing sponge vagina. The difference of length estrous between T₀ and T₁ was 48 hours and 84 hours, respectively.

4. Conclusion

Mineral block affected to pH of cervical mucus, NaCl level of cervical mucus and estrogen level of Timor hind. Supplementation of mineral block leads to longer estrous in Timor deer than those not given supplementation.

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