Animal Reproduction Science 141 (2013) 148–153

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Plasma progesterone changes and length of oestrous cycle in Rusa Deer (*Rusa timorensis*)

M.B. Mahre[a,b], H. Wahid[a,*], Y. Rosnina[a], F.F.A. Jesse[a], C.A. Azlan[a], K.C. Yap[a]

[a] *Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

[b] *Department of Veterinary Physiology, Pharmacology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri, P.M.B. 1069 Maiduguri, Borno State, Nigeria*

ARTICLE INFO

Article history:
Received 27 March 2013
Received in revised form 16 July 2013
Accepted 30 July 2013
Available online 12 August 2013

Keywords:
Rusa timorensis
Progesterone
Estrus cycle
Rusa deer

ABSTRACT

A study was conducted to profile the plasma progesterone (P_4) concentrations and establish the length of oestrous cycle in the *Rusa timorensis* during the breeding season. Five healthy hinds were selected for peripheral blood sampling twice weekly to gauge the P_4 levels by radioimmunoassay, at the start of the breeding season indicated by rutting behaviours of sexually active males. The hinds were polyestrous as proven by the cyclic trend of P_4 levels. After the presumptive oestrus indicated by the lowest P_4 concentrations (0.20 ± 0.09 ng/ml), this ovarian hormone was markedly elevated on day 7 of the cycle (0.78 ± 0.20 ng/ml), reached its peak (2.61 ± 0.23 ng/ml, $P < 0.05$) on day 14, and then declined to the basal level in the subsequent oestrus. The mean oestrous cycle length in *R. timorensis* during the breeding season was 19.2 days with a range of 18–21 days, and the pattern of circulating progesterone during the oestrous cycle of the *R. timorensis* is similar to those of other deer species. It was also observed that the length of oestrous cycle of *R. timorensis* determined by gauging the progesterone levels and observation of the oestrous behaviours as well as changes in the cellular pattern of vaginal epithelial cells are highly consistent.

1. Introduction

The Rusa deer, *Rusa timorensis*, is a species native to the Indonesian Archipelago and it has been introduced to South-East Kalimantan, New Guinea, the Bismarck Archipelago, New Caledonia, Australia and New Zealand (Food and Agriculture Organization, 1982). The *R. timorensis* hinds are aseasonal polyestrous breeders. Both the hinds (females deer) and the stags (males deer) attained sexual maturity at 18 months old (Food and Agriculture Organization, 1982) and the body weight of Rusa hinds at first mating, when they are 18–20 months of age, is approximately 46 kg (Van Mourik, 1986).

Rusa deer are long day breeders without delayed implantation (Van Mourik and Stelmasiak, 1985). Under appropriate environmental conditions and proper nutrition, the major breeding activity observed in *R. timorensis* in Malaysia is between the month of March and July (Mahre et al., 2012).

The progesterone (P_4) levels in peripheral blood of mammals provide valuable information about their reproductive status (Liu et al., 2002). Plasma P_4 concentrations have been measured in several deer species including red deer (Adam et al., 1985; Kelly et al., 1985; Asher et al., 1997, 2000, 2011; Garcia et al., 2002, 2003), fallow deer (Asher, 1985; Asher et al., 1986, 1988), white-tail deer (Plotka et al., 1980), Pere Davids deer (Curlewis et al., 1988) and Chita deer (Chapple et al., 1993) during the breeding season. However, no information is available on the pattern of circulating levels of P_4 during the breeding season of *R. timorensis*. Moreover, to our knowledge, the reproductive

* Corresponding author. Tel.: +60 3 89468272; fax: +60 3 89468333.
E-mail addresses: wahid@vet.upm.edu.my, wahidharon@gmail.com (H. Wahid).

0378-4320/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.anireprosci.2013.07.012

biology of the *R. timorensis* deer hinds have not been well documented, especially the duration and hormonal profiles of the oestrous cycle. Therefore, the objectives of this study were to depict the changes of circulating levels of P_4 and determine the length of oestrous cycle in the *R. timorensis* hinds during their breeding season.

2. Materials and methods

2.1. Ethical consideration

These studies were undertaken after the approval of the Animal Care and Use Ethics Committee, Universiti Putra Malaysia as required in Malaysia by the Animal Welfare Act (2012). All procedures were conducted by fully trained staff from the Faculty of Veterinary Medicine, Universiti Putra Malaysia.

2.2. Animals and management

Five adult and healthy *R. timorensis* hinds, age 4–5 years, with average live body weight of 55 ± 3.41 kg were selected for this study. In the present study, oestrus was not synchronised, but the natural oestrous cycle was monitored. The animals were raised at the Universiti Putra Malaysia deer breeding unit (2.99° N, 101.7° E). Since the animals were accustomed to blood sampling and experienced no behavioural handling stress, the hinds were physically restrained by hands and no drugs were used.

Eight hundred grams of the concentrates, containing 15% crude protein, was supplied to each hind per day, half of the feed given in the morning and other half given in the afternoon. The concentrates were goat pellets manufactured by Miba-mansura Trading Sdn. Bhd. Malaysia. In addition, hays containing 16% crude protein and fresh water were provided ad libitum.

2.3. Sampling period

Sampling was conducted between 5th April and 2nd July 2012 (breeding season) as indicated by the antler cycle and rutting behaviours of the sexually active males. During this period, no adult males were housed together with the females.

2.4. Blood sampling

Blood samples were collected in heparinized vacutainer tubes from all hinds by jugular venepuncture in the morning (09:00 to 10:00 h), twice weekly at 3–4 days interval and were centrifuged at 1000 rpm for 10 min. The plasma obtained was aspirated and stored in labelled micro vials at −20 °C until assay.

2.5. Observation of oestrus signs and vaginal smear sampling

Homosexual Mounting behaviours of the five hinds were visually observed twice daily at 07:30 and 17:00 for 1.5 h each time. Before blood sampling, the vulva was examined for size and moistness as well as mucus secretion from the vagina. Vaginal cytology was performed to confirm the stages of the oestrous cycle and was applied according to Mahre et al., 2012. Vaginal smears were collected twice weekly from 5th April until 2nd July 2012 by gently swabbing the vagina with a sterile cotton swab. Smears were transferred to 1–5 slides, air dried, and immediately fixed with 100% ethanol and stained with 5% Giesma. Smears were examined under a light microscope at magnification of 400×. Two hundred epithelial cells from each slide were evaluated and classified. Cells were counted in 10 different fields with the aid of a lens graticule and Grunert's criterion was used to classify the vaginal cells into superficial, intermediate and parabasal cells (Schutte, 1967). The number of each cell type was then expressed as a percentage.

2.6. Plasma progesterone analyses

Progesterone concentrations in plasma were measured using a commercial radioimmunoassay (RIA) kit (Immunotech®, France). The assay quality control samples containing high and low hormone concentrations were included in the beginning and at the end of each assay. Intra-assay and inter-assay coefficient of variation were 11% and 10%, respectively. The progesterone assay specificity was 100% (17-hydroxyprogesterone 0.3%, 20 α-dihydroprogesterone 2.0%) and sensitivity was 0.02 ng/ml.

2.7. Criteria to determine the oestrous cycle length and the oestrus stage

The length of the oestrous cycle was determined from the basal progesterone levels that included the increase corresponding to the development of a corpus luteum. The onset of the first oestrous cycle was considered to be the day of lowest progesterone concentration before a steady increase in this hormone, whereas the end of the last cycle was considered to be the day when blood progesterone returned to basal levels.

2.8. Statistical analysis

Data were analysed using statistical software SPSS version 21. Progesterone concentrations are expressed as mean ± SEM. A one way analysis of variance (ANOVA) was used to test the differences between specific pairs of the Progesterone in the samples obtained from various phases during the rutting season. Differences are shown when $P < 0.05$ and Spearman correlation was used to test the correlation between progesterone and vaginal cytology.

3. Results

3.1. Progesterone profiles of the oestrous cycles

All five individual hinds exhibited cyclic patterns of P_4 levels with the basal level of P_4 in cyclicity every 18–21 days during the sampling period in their rutting season, between April, and July (Fig. 1). There were transitory, slight elevation and soon diminished P_4 concentrations at