Estrus response and pregnancy rate of *Rusa timorensis* following estrus synchronization with prostaglandin analogue

Mahre[2], M.B., Wahid[1*], H., Rosnina[1], Y., and Jesse[1], F.F.A.
[1]Department of Veterinary Clinical studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. [2]Department of Veterinary Physiology, Pharmacology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.
[*]Corresponding author: wahidh@upm.edu.my

Abstract

The purpose of this study was to evaluate the efficacy of cloprostenol in inducing estrus in cycling *Rusa timorensis* hinds by assessing circulating progesterone (P_4) concentration, occurence of estrus and pregnancy rate. Estrus was synchronized in eight cycling hinds with 500 µg cloprostenol given intramuscularly at 10 days apart. Eight other *R. timorensis* hinds were not-synchronized and served as control. Blood samples, collected every three to four days for 29 days from 24 hours prior to the first cloprostenol injection, were analyzed for plasma progesterone concentration. Estrus was recorded based on observation of estrus signs during the period of blood sampling and all hinds were subjected to transrectal ultrasonography to assess pregnancy status. On the basis of changes in plasma progesterone concentration, cloprostenol induced estrus in only 63% of the treated hinds. The other three treated hinds showed progesterone values (0.8 ng/mL) which appeared to be too low to indicate presence of corpus luteum for the drug to act on. Spontaneous estrus occurred in 50% of the untreated hinds. Five of the 8 treated hinds and four control hinds displayed standing estrus. Pregnancy rate to cloprostenol induced estrus following natural mating was good with 50% of the treated hinds conceived. In the present study, estrus synchronization had been successfully achieved using prostaglandin analogue.

Keywords: *Rusa timorensis,* estrus synchronization, cloprostenol, pregnancy rate

Introduction

Rusa deer (*Rusa timorensis*) are polyestrous breeders, with no definite breeding season (Food and Agriculture Organization, 1982), but a tendency for breeding from March to July in tropical Peninsular Malaysia has been established (Mahre *et al.*, 2012; 2013). In deer farming operations, there has been a growing interest in the possibilities of early synchronization of estrus in the breeding season to prevent the deer from giving birth when resources are scarce, predation is heavier or weather conditions become adverse. Additionally, many of the world's species of deer are becoming extremely rare. Twenty-nine species are listed either as endangered or extinct, and species of *R. timorensis* is likely to become threatened and endangered in the near future (IUCN, 2008). Successful artificial breeding of any deer species will require a feasible and effective method of estrus synchronization (Haigh *et al*., 1988) other than artificial insemination.

The ability of prostaglandin $F_{2\alpha}$ to prematurely regress the corpus luteum has been documented in red deer (Fisher *et al*.,1994; Asher *et al*., 1995), wapiti (Glover, 1985), fallow deer (Asher *et al*., 1988; Jabbour *et al*., 1993), Pere David's deer (Curlewis *et al*., 1988), white tail deer

(Haigh, 1984; Magyar *et al*., 1989) and reindeer (Ropstad *et al*., 1996). However, no published information is yet available on the efficacy of cloprostenol in inducing estrus in *R. timorensis.* Therefore, the present study was designed to evaluate the efficacy of cloprostenol in inducing estrus in *R. timorensis* during the estrous cycle, by assessing changes in peripheral progesterone concentrations, occurence of estrus and pregnancy rate.

Materials and Methods

Ethical Consideration

This study was undertaken with the approval of the Animal Care and Use Committee (reference number: UPM/FPV/PS/3.2.1.551/AUP-R141), Universiti Putra Malaysia.

Animals and Management

Sixteen cycling hinds, between five and nine years old, with an average body weight of 65.9 ± 1.19 kg were selected for this study. No stags were placed with these hinds prior to experiment initiation. Each hind was determined to be cyclic prior to the study through blood sampling and analysis as described by Mahre et al. (2013). The hinds were raised at the Universiti Putra Malaysia deer breeding unit (2.995^{o}N, 101.729^{o}E). Each hind was fed with 3.4 kg of commercial hays (16% crude protein), 1.1 kg of concentrates (15% crude protein) daily and fresh water was provided *ad libitum.*

Induction of Estrus

Estrus synchronization treatment was timed to coincide with the approximate onset of the breeding season of *R. timorensis* hinds in tropical Peninsular Malaysia as determined previously by Mahre *et al.* (2012; 2013). Hinds were randomly divided into two equal groups. In the treatment group, each hind received two intramuscular injections of 500 µg cloprostenol (Estrumate®, Schering-Plough Animal Health Division of Intervet Australia) 10 d apart. Eight other hinds received two intramuscular injections of 2 ml distilled water to serve as control. No stags were placed with the hinds until 24 h after the second injection of cloprostenol. In this study, estrus was considered to have occurred in both the control and treated hinds based on cyclic basal progesterone levels, standing estrus and the observations of the estrus signs of swollen and moistened external genitalia. Luteal and follicular phases of the estrous cycle were determined according to the methods of Mahre *et al.* (2012; 2013).

Collection of Blood

The hinds were physically restrained, and no drugs were used. Two mL of blood samples were collected into heparinized vacutainer tubes using 18 gauge needles and venoject tubes by jugular venepuncture, from 0900 to 1100, twice weekly, at 3- to 4-d intervals for one mo. This was done to relate the occurrence of estrus with progesterone data. Blood samples were centrifuged at 1500 g for 15 min. The plasma obtained was aspirated into labelled microvials and stored at – 20 ^{o}C until assay.

Estrus Response

During the period of blood sampling, observations on estrus and mating were conducted visually with the aid of a 126 m/1000 m field 7.2º binoculars (Sakura, Japan). Males were placed in the pen 24 h after the second injection of cloprostenol. Both the control and treated hinds were housed in the same pen. The hinds were mated by two active males during this period, and between the 2 males, they took turn to

breed the females every 24 h. The males were fitted with crayon mating-harness that left marks on the females after mounting. Behavioural signs of estrus such as chin resting, frequent licking or sniffing of the vulva, vocalization and mounting behaviours of both the stags and hinds were also visually observed during the period of blood sampling. Before blood sampling, the external genitalias were examined closely for changes in size of the vulva and presence of moistness and mucus discharge. Time to onset of induced estrus was the period from cessation of treatment to beginning of estrus, while the duration of estrus was considered to be the period from the onset of estrus to the end of estrus.

Vulvar Biometry

The diameter of the vertical vulva (distance between superior and inferior commissure) and horizontal vulva (at the broadest horizontal curvature) were measured using Vernier calipers before each blood sampling.

Pregnancy Determination

Transrectal ultrasonography was performed using a real-time ultrasound scanner (Aloka,500 SSD, Japan), with a 7.5 MHz transrectal linear probe on 33 and 43 d (Myreal *et al.,* 1992; Asher *et al.,* 1995) after mating, to determine the pregnant hinds. Embryonic vesicle and the embryo could be detected if the hinds were pregnant at these stages (Myreal *et al.,* 1992).

Plasma Progesterone Assay

Plasma progesterone concentrations were measured using a commercial radioimmunoassay (RIA) kit (Immunotech®, France). The assay quality control samples containing high and low hormone concentrations were included at the beginning and at the end of each assay. Intra–assay and inter–assay coefficient of variations were 6.5% and 7.2%, respectively. The progesterone assay specificity was 100% (17–hydroxyprogesterone 0.3%, 20 α–dihydroprogesterone 2.0%) and sensitivity was 0.02 ng/ml.

Statistical Analysis

Data were analysed using statistical software IBM SPSS Statistic 21. Data were expressed as mean ± standard error of the means. Data on the intervals from treatment to the onset of estrus and plasma progesterone concentrations following administration of the second injection of cloprostenol were analysed by analysis of variance (ANOVA). The time of standing estrus and vulva biometry as well as days of blood sampling were also related to progesterone data by analysis of variance. Differences are statistically significant when $P < 0.05$.

Results and Discussion

On the basis of changes in plasma progesterone concentrations, cloprostenol induced estrus in only 63% of the treated hinds (Figure 1). The interval between the last cloprostenol injection to estrus was 78 h. Sixty three percent of the treated hinds displayed standing estrus 78 h after the last cloprostenol injection (Table 1). No mounting behaviour was observed in the other three treated hinds. Other behavioural signs of estrus were not observed. However, the vulvas of all the hinds that displayed standing estrus were slightly swollen and moistened within 60 h during estrus detection (Table 2). In this study, the other three treated hinds showed progesterone values (0.8 ng/ml) which appeared to be too low to indicate presence of a corpus luteum for the

drug to act on (Figure 2). Although the data (Figure 2) showed progesterone levels were lowered after the administration of the second cloprostenol injection, no behavioural signs of estrus were observed in these three treated hinds. This is in agreement with the findings of Garcia *et al.* (2003), who reported that a corpus luteum is only considered to be functional if it showed a progesterone value above 1 ng/mL. This finding also concurs with earlier studies on *Cervus canadensis* (Glover, 1985) and *Cervus elaphus* (Fisher *et al.*, 1994), both of which demonstrated that the corpus luteum was not fully responsive to the luteolytic action of cloprostenol when administered on days 10 – 11 of the estrous cycle. Glover, (1985); Fisher *et al.*(1994) and Mahre *et al.* (2014) reported that the luteal phase started on day 4 of the estrous cycle and ended on day 16 of the estrous cycle in *Cervus canadensis*, *Cervus elaphus and Rusa timorensis* but the luteolytic action of cloprostenol in *Rusa timorensis* was incomplete in some animals probably due to differences in hypothalamic-pituitary support of the corpora lutea. Macmillan *et al.* (1985) and Henderson and McNatty (1977) reported that pituitary support of the corpus luteum in other species determined the sensitivity of luteal tissue to the luteolytic effects of endogenous or exogenous prostaglandins. The issue of refractoriness in *Rusa timorensis* requires further clarification if prostaglandins are to be used for induction of luteolysis without reference to stage of the estrous cycle.

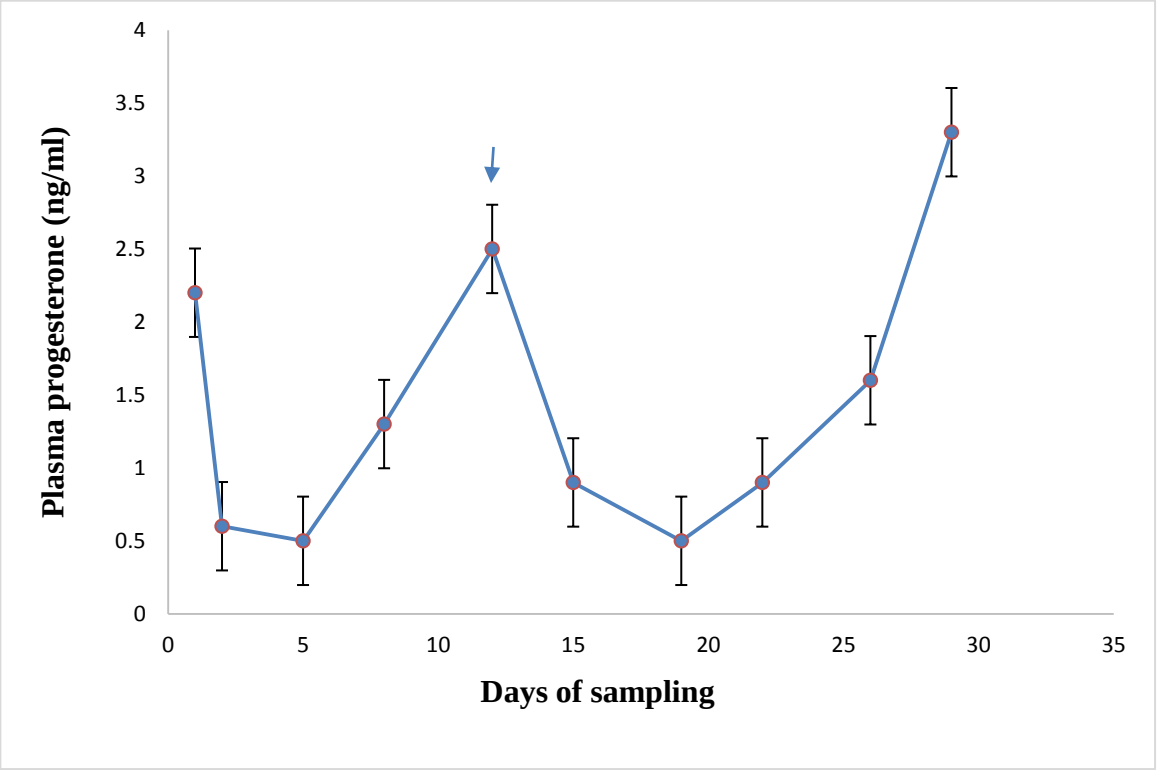

Figure 1. Mean P_4 concentrations of Rusa deer hinds (n=5) treated with two injections of 500 µg cloprostenol given at 10 d apart showing complete luteolysis. Arrow denotes the time of second cloprostenol injection.

Table 1. Incidence of estrus and conception in hinds in the treated and control groups

Parameter	Treated (n=8)	Control (n=8)
No. of hinds with moist, swollen vulva	5	4
No. of hinds that displayed estrus	5	4
Interval between second cloprostenol injection and estrus signs (h)	60 ± 4.8	-
Duration of estrus (h)	24 ± 2.6	24 ± 2.2
No. of hinds conceived	4	4
Estrus response	5/8 (63%)	4/8 (50%)
Pregnancy rate	4/8 (50%)	4/8 (50%)

Table 2. Means (± SEM) for vulva biometry during the estrous cycle of *R. timorensis*

Vulva diameter	Follicular phase	Luteal phase	P-value
Vertical (cm)	1.78 ±1.28	1.20 ± 0.18	P < 0.05
Horizontal (cm)	1.10± 0.52	0.78±0.04	P < 0.05

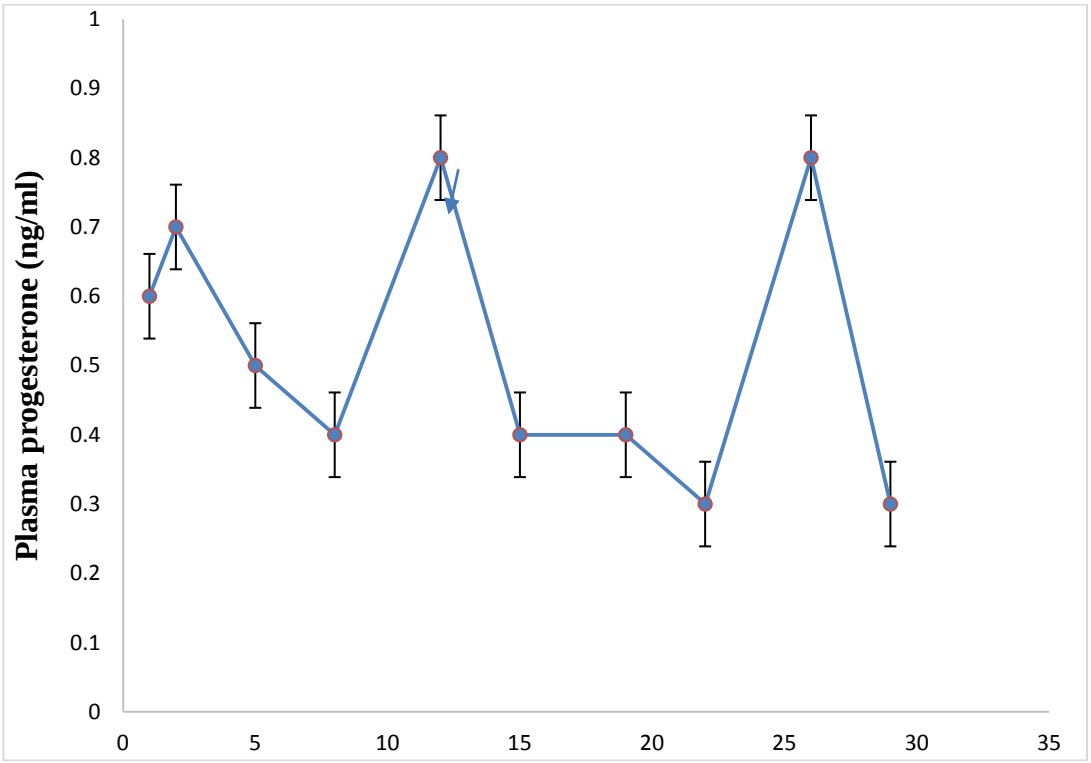

Figure 2. Mean P_4 concentrations of the other 3 treated hinds that showed progesterone values (0.8 ng/ml) which appeared to be too low to indicate presence of a corpus luteum for the drug to act on. Arrow denotes the time of second cloprostenol injection.

The high progesterone concentration recorded in the treated hinds on the day of cloprostenol treatment indicated a high proportion of hinds with a functioning corpus luteum on that day could be the reason for successful synchrony resulting from the 2-injection cloprostenol regime. In these respects, the responsiveness of *R. timorensis* hinds to cloprostenol in this study was similar to that observed for other deer species such as the red deer (Fisher *et al.,* 1994; Asher *et al.,* 1995) and fallow deer (Asher and Thompson, 1989) as well as other domestic ruminants such as sheep (Acritipoulou and Haresign, 1980), goats (Hearnshaw *et al.,* 1974) and cattle (Rowson *et al.,* 1972; Roche, 1974).

The mean interval from the time of administration of cloprostenol to the onset of estrus in *R. timorensis* hinds was found to be 60 ± 4.8 h. This probably aroused from a slow reduction in progesterone secretion to basal concentration as a consequence of higher initial luteal activity at the time of cloprostenol treatment (Mahre *et al.,* 2014). A similar phenomenon was also observed by Asher *et al.* (1995) in red deer, although, the interval from the last cloprostenol treatment to onset of estrus was longer for *R. timorensis* hinds as compared to the red deer which was 57.4 ± 1.8 h. The interval from cloprostenol treatment to onset of estrus for fallow deer was found to be 55.2 ± 1.2 h which was shorter than that observed for red deer (Asher and Thompson, 1989; Asher *et al.,* 1995) and *R. timorensis* hinds in the current study. Reasons for variation between species remain to be resolved (Asher *et al.* 1995).

Based on the observation of standing estrus, moist and swollen vulvas (Table 2) as well as progesterone data (Figure 3), spontaneous estrus occurred in four of the eight untreated hinds (Figure 3). The progesterone levels for the 4 control hinds during the 30-d blood sampling indicating the corpus luteum was at its peak luteal phase (2 ng/mL) on day 12 of blood sampling and started to regress on d 15 (above 1 ng/mL) and continued to decline rapidly before the beginning of the next estrus which was d 22 of blood sampling. In this experiment, estrus was considered to have occurred on the day when progesterone level was at its lowest level (below 1 ng/mL). This finding indicates that *R. timorensis* hinds have a tendency to exhibit natural estrus synchrony relative to treated hinds. This finding corresponds closely with the findings of Shelton (1960), Clutton-Brock *et al.* (1982) and Glenn and Guinness (1985) who reported that the enclosing together of female mammals and the subsequent introduction of a male produced synchronous breeding. The induction and synchronization of estrus among unisexual grouped females in the presence of a male (the Whitten effect; Whitten, 1966) and the male induced failure of implantation and return to the estrous cycle (the Bruce effect; Bruce, 1960) are among the best examples of male to female pheromone effects in female mammals.

Although, cloprostenol can synchronize estrus in several species, the fertility of the induced estrus is often lower than that of an unsynchronized or progestagen-synchronized estrus (Glover, 1985; Fisher e*t al.,* 1994). This is in contrast with the findings in the current study in which estrus response and pregnancy rate following estrus synchronization with cloprostenol and natural mating were moderate with 63% estrus response and 50% pregnancy rate, respectively. These differences were most likely the result of subtle variation in luteotrophic support of the corpus luteum reflecting differences between species (Henderson and McNatty, 1977; Macmillan *et al.,* 1985).

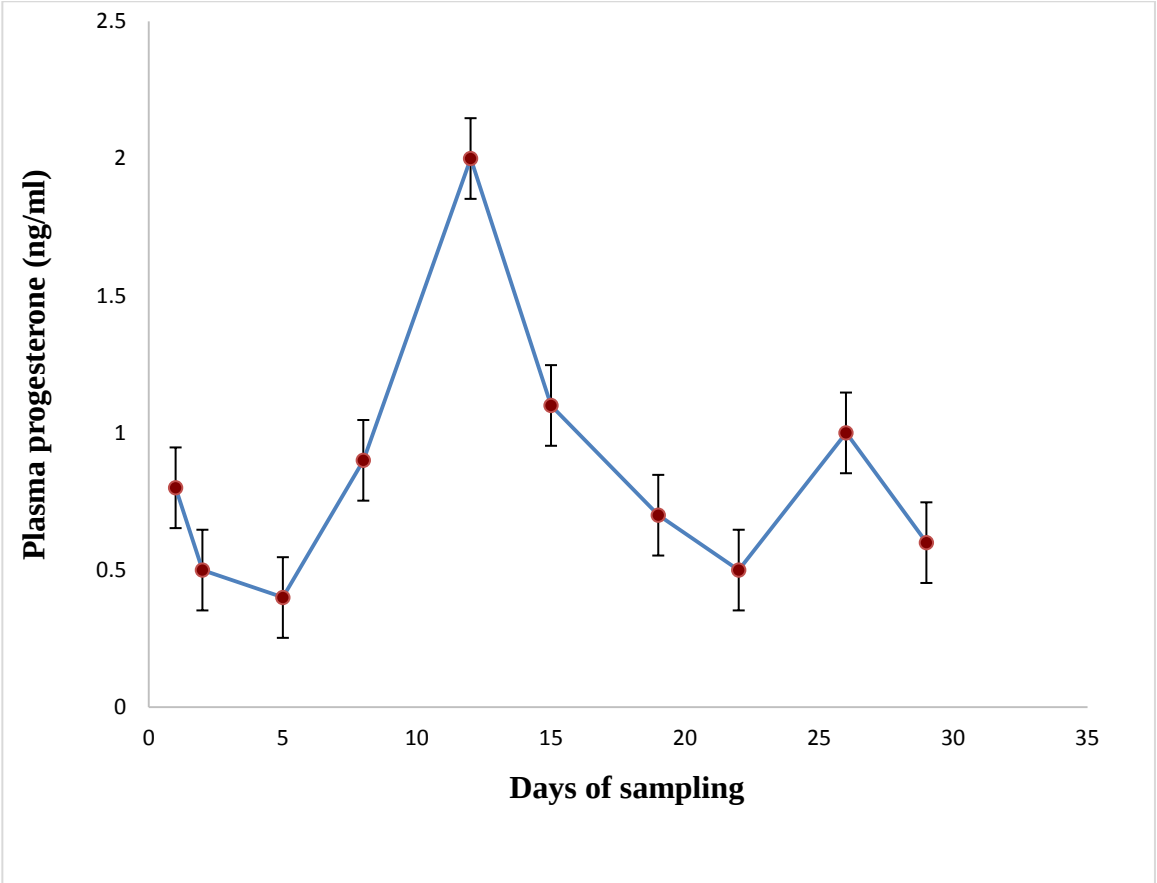

Figure 3. Mean P_4 concentrations of the untreated rusa deer hinds (n=4) that exhibited natural estrus sychrony.

Conclusion

Estrus synchronization had been successfully achieved using prostaglandin analogue. However, future studies need to refine synchronization regimes in order to increase estrus synchrony and pregnancy rate.

Acknowledgement

This research was supported by a grant from Research University Grant Scheme (RUGS), UPM Project 9301400.

References

Acritopoulou, S. and Haresign, W. 1980. Response of ewe to a single injection of an analogue of PGF-2α given at different stages of the estrous cycle. *J. Reprod. Fertil*. 58: 219-223.

Asher, G.W., Peterson, A.J. and Watkins, W.B. 1988. Hormonal changes during luteal regression in farmed fallow deer *(Dama dama). J. Reprod. Fertil*. 84: 379-86.

Asher, G.W. and Thompson, J.G.E. 1989. Plasma progesterone and LH concentrations during oestrous synchronization in female fallow deer (*Dama dama*). *Anim. Reprod. Sci*. 129: 143-453.

Asher, G.W., Fisher, M.W., Berg, D.K., Veldhuizen, F.A., and Morrow, C.J. 1995. Luteolytic potency of a prostaglandin analogue at different stages of the oestrous cycle in red deer hinds. *J. Reprod. Fertil*. 103(2): 307-14.

Bruce, H.M. 1960. Bruce effects. *J. Reprod. Fertil*. 1: 96-103.

Curlewis, J.D., Loudon, A.S.I. and Coleman, A.P.M. 1988. Oestrous cycles and the breeding season of the Pere David's deer hind, *Elaphurus davidianus*. *J. Reprod. Fertil*. 82:119-126.

Clutton-Brock, T.H., Guinness, F.E. and Albon, S.D. 1982. Red Deer. Behaviour and Ecology of Two Sexes. University of Chicago Press, Chicago.

Fisher, M.W, Asher, G.W, Meikle, L.M, and Newman, R.E. 1994. Cloprostenol induced luteolysis in the red deer hinds: The effects of age, dose, number of corpora lutea and the later stages of the oestrous cycle. *Anim. Reprod. Sci*. 35: 99-109.

Food and Agriculture Organization. 1982. Animal Production and Health Paper 27: 3-4.

Garcia, A.J., Landete-Castillejos, T., Gomez-Brunnet, A., Garde, J.J. and Gallego, J. 2003. Characteristics of the oestrous cycle of Iberian red deer assessed by progesterone profiles. *J. Exp. Zoo.* 298A: 143-149.

Glover, G.J., 1985. Aspects of the reproductive physiology of female wapiti. MSc Thesis. University of Saskatchewan, Canada.

Glenn, R.I., and Guinness, F.E. 1985. Synchrony of estrus and conception date in red deer (*Cervus elaphus* L.). *Anim. Behav*. 33: 1169-1174.

Haigh, J.C. 1984. Artificial insemination of two white-tailed deer. *J. Am. Vet Med Assoc*. 185: 1446–7.

Haigh, J.C., Michael, C., and Sasser, G.R. 1988. Estrus synchronization and pregnancy diagnosis in red deer. *J. Zoo Anim Med*. 19(4): 202-207.

Hearnshaw, M., Restall, B.J., Nancarrow, C.D. and Matter, P.B. 1974. Synchronization of oestrus in cattle, sheep and goats using a prostaglandin analogue. *Proceedings of the Australian Society of Animal Production* 10: 242-245.

Henderson, K.M. and McNatty, K.P. 1977. A possible interrelationship between gonadotrophin stimulation and prostaglandin F_2 inhibition of steroidogenesis by granulosa luteal cells in vitro. *J. Endocrinology*. 73: 71-78.

IUCN 2008. IUCN Red List 2008. Available at www.iucnredlist.org. Accessed 15 April 2012.

Jabbour, H.N., Veldhuizen, F.A., Green, G., and Asher, G.W. 1993. Endocrine responses and conception rates in fallow deer *(Dama dama)* following oestrous synchronisation and cervical insemination with fresh or frozen-thawed spermatozoa. *J. Reprod Fertil.* 98: 495-502.

Magyar, S.J., Biediger, T., Hodges, C., Kraemer, D.C. and Seager, S.W.J. 1989. A method of artificial insemination in captive white-tailed deer *(Odocoileus virginianus)*. *Theriogenology* 31: 1075-80.

Macmillan, K.L., Day, A.M., Taufa, V.K., Peterson, A.J. and Pearce, M.G. 1985. Effects of an agonist of gonadotropin releasing hormone in cattle. II. Interactions with injected prostaglandin $F_{2\alpha}$ and unilateral ovariectomy. *Anim Reprod Sci*. 8: 213-223.

Mahre, M.B., Wahid, H., Rosnina, Y., Jesse, F.F.A. and Azlan, C.A. 2012. Exfoliative vaginal cytology during the oestrous cycle of Rusa deer (*Cervus timorensis). Proceedings of the International Conference on One Health and VAM Congress.* 24: 345-47.

Mahre, M.B., Wahid, H., Rosnina, Y., Jesse, F.F.A, Azlan, C.A. and Yap, K.C. 2013. Plasma progesterone changes and length of oestrous cycle in rusa deer *(Rusa timorensis). Anim. Reprod Sci.* 141: 148-53.

Mahre, M.B. 2014. Oestrus cycle, female reproductive system morphology and temperament and sperm attributes of Rusa deer (*Rusa timorensis*) in captivity. PhD. Thesis, Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Roche, I.F. 1974. Synchronization of oestrus and fertility following artificial insemination in heifers given $PGF_{2\alpha}$. *J. Reprod Fertil.* 37: 135-138.

Ropstad, E., Kindahl, H., Nilsen, T.A.B, Forsberg, M., Sire, J.E. and Pedersen O. 1996.The effect of cloprostenol in non-pregnant and pregnant Norwegian semi-domestic reindeer *(Rangifer tarandus tarandus* L.*). Anim. Reprod Sci*. 43: 205-19.

Rowson, L.E.A., Tervit, R. and Band, A. 1972. The use of prostaglandins for synchronization of oestrus in cattle. *J. Reprod Fertil*. 29: 145 (Abstract).

Shelton, M. 1960. The influence of the presence of a male goat on the initiation of estrus cycling and ovulation in Angora does. *J. Anim. Sci*. 19: 368-375.

Whitten, W.K. 1966. Whitten effects. In Advances in Reproductive Physiology, ed. McLaren, A. (Academic, London), Vol.1, pp. 155-177.