The Use of Progesterone in Inducing Estrous Synchronization in Chital Deer (*Axis Axis*) Examined by Progesterone Radio Immunoassay

(PENGGUNAAN PROGESTERON UNTUK SINKRONISASI BIRAHI PADA RUSA CHITAL (*AXIS AXIS*) DIEVALUASI MENGGUNAKAN *PROGESTERONE RADIO IMMUNOASSAY*)

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ABSTRACT

The aim of the present study was to assess the estrous synchronization techniques by using progesterone radio-immunoassay. Twenty Chital hinds aged between three and ten years old, and one vasectomised stag aged of eight years, were used in this study. The trials Controlled Internal Drug Release (CIDR) treatment for 14 days and the CIDR was replaced by day seven. Estrous detection was studied using a vasectomised stag fitted with a crayon harness. The blood samples were collected during the CIDR removal, and two days (48 hours) after. Radio immunoassay was used to measure progesterone concentrations. The results indicated that progesterone concentration immediately before CIDR removal was 3.57 ± 0.61 ng/ml and 48 hours after CIDR removal was 0.62 ± 0.48 ng/ml. Although progesterone concentrations decreased sharply following estrous synchronization, only three hinds (15%) showed estrous exhibition.

Key word: Chital deer, progesterone, estrous synchronization.

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ABSTRAK

Penelitian ini bertujuan untuk melakukan evaluasi sinkronisasi birahi menggunakan teknik *radio-immunoassay* terhadap kadar hormon progesteron. Dua puluh rusa Chital betina umur antara tiga sampai sepuluh tahun, dan satu rusa Chital jantan yang telah di vasektomi berumur delapan tahun digunakan dalam penelitian ini. Rusa-rusa betina tersebut diberi hormon progesteron *Controlled Internal Drug Release* (CIDR) secara intravaginal selama 14 hari dan diganti setelah tujuh hari digunakan. Deteksi birahi dilakukan menggunakan rusa jantan vasektomi yang dipasangi (*crayon harness*). Contoh darah diambil pada saat CIDR diambil dan 48 jam sesudahnya. Radio immunoassay digunakan untuk deteksi kadar hormon progesteron dalam darah. Hasil penelitian menunjukkan bahwa kadar progesteron pada saat CIDR diambil adalah 3.57 ± 0.61 ng/ml dan 48 jam setelah CIDR diambil adalah 0.62 ± 0.48 ng/ml. Walaupun kadar progesteron turun dengan cepat setelah sinkronisasi tetapi hanya tiga ekor (15%) yang menunjukkan tingkah laku birahi.

Kata kunci: rusa Chital, progesteron, sinkronisasi birahi.

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INTRODUCTION

Chital deer are known as a nervous and flighty animal (Anderson, 1984). This species is indigenous to India, Sri Lanka, Bangladesh and Nepal (Schaller, 1984) and has been relocated successfully into Hawaii (Graf and Nichols, 1966), Texas (Ables, 1977), California (Wehausen and Elliott, 1982), and was introduced to Queensland in 1863 (Bentley, 1978), before being transferred to NSW in about 1970's (Chapple, 1989). This deer is farmed successfully in Australia for the production of venison (English, 1992). Chital deer can be trained not only for normal farm management practices but also for tolerance to frequent blood sampling and non invasive procedures (Chapple *et al.*, 1991; Mylrea, 1992).

Estrous synchronization is a technique to induce estrous and ovulation, by mimicking the decrease of progesterone concentrations at the end of the luteal phase. The progesterone treatment was used to suppress, temporarily, ovarian activity or to prevent estrous and ovulation and therefore artificially extend the lifespan of corpus luteum (Asher *et al.*, 1988b). The sharp decrease of progesterone level can be induced by maintaining high progesterone concentrations before the progesterone devices are removed. This technique not only mimics the decrease of progesterone but also induces luteal regression. Consequently, not only exogenous but also endogenous progesterone would decrease. The decrease of progesterone level induced negative feed back to luteinizing hormone (LH) and produced positive feed back effect to estrogen and LH surge. Finally ovulation occurred at the end of estrous behavior (Monfort *et al.*, 1990; Asher *et al.*, 1992). The success of estrous synchronization occurs when the progesterone concentrations reaches the basal levels, resulting in obvious estrous behavior and ovulation.

The aim of the study was to assess the success of estrous synchronization by determining progesterone concentrations.

MATERIALS AND METHODS

Animals.

Twenty Chital hinds aged between three and ten years , and one vasectomised stag aged of seven years, were used in this study. They were farmed in Sydney University (Camden, N.S.W) and were trained to enter the yards and crush. They were allowed to graze in the paddock with supplementation of hay and pellets during summer and winter, as necessary.

Estrous synchronization.

Progesterone treatment was performed by intravaginal device Controlled Internal Drug Release (CIDR) insertion. The hinds were restrained in a drop floor crush and the CIDR type G (goat, Riverina artificial breeder, Albury NSW Australia) was inserted by a lubricated applicator (Obstetrical lubricant, Parnell Lab. Aust. Pty. Ltd. Alexandria NSW). The CIDR insertion lasted for 14 days and the replacement of a new CIDR was performed on day seven.

Estrous detection.

Estrous detection was studied using a vasectomised stag fitted with a crayon harness. The hinds were determined to be in estrous when the crayon marks were found on the hinds' rumps. Observation

of estrous behavior was done twice a day, until five days following CIDR removal.

Blood collection.

The hinds were restrained in a drop floor crush and a blind folder was used to cover the eyes. The head was held by an assistant while the blood samples were collected from the jugular vein, using an evacuated 15 ml draw blood collecting tube with 2.5 cm, 18 gauge needle (Vacutainer, Becton Dickinson, Rutherford, New Jersey, USA). The blood samples were collected during the CIDR removal, and two days (48 hours) after. It was then centrifuged at 3200 g for 15 minutes, and the serum was poured into a container and stored at -20°C until it was assayed.

Radioimmunoassay procedure.

The samples and the reagents were introduced at the same temperature (room temperature). After identification of the tubes, 50 ml of each standard, controls, samples and non specific binding were pipetted into the antibody coated tubes. All the tubes were then added with 500 ml of 80% diluted 125I labeled progesterone and were shaken, using vortex mixer for 30 seconds. These tubes were then covered with plastic film and incubated for two hours in room temperature. The solution in the tubes was then decanted, and the tubes were tapped onto the absorbent paper. The inverted tubes were allowed to drain against absorbent paper for ten minutes. The binding of the radioactive to the antibody was counted by setting the inverted tubes onto the gamma counter.

Assay validation.

Serum progesterone levels were estimated by Radio-immunoassay kit (Spectra, progesterone^{125}I, Orion diagnostica, P.O. Box 83 SF-02101 ESPOO FINLAND). This assay was validated for the Chital deer serum. The recovery study was done by serial dilution's of progesterone, from 10, 5, 2.5, 1.25 and 0 ng/ml, and it was then added to the stag's serum with the endogenous progesterone levels 1.07 ng/ml. The addition of exogenous steroid hormone to the serum which contained endogenous steroid hormone was done as reported by Friedrich *et al.*, (1980). The straight line with coefficient correlation $R^2 = 0.998$, and equation of $Y= 1.03 + 1.06x$, indicated that there was no systematic error in the assay. The average of the percentage recovery was calculated by subtraction of the progesterone value from the progesterone contained in the diluent (Stag serum) and it was 102 ± 3.79 % (means ± SEM).
The detection limit and upper limit calculated by Assay zap (Taylor, 1988) were 0.25 ng/ml and 25.02 ng/ml respectively. The sensitivity of the assay by using the calculation of Munro and Stabenfeldt, (1984) was 0.37 ng/ml. The significant cross reaction of several steroids was only Progesterone (100 %), Pregnenolone (3.90%), 5β -Dihydro-progesterone (0.75%) and 5a-Dihydroprogesterone (0.22%). Precision of the assay. Precision of the intra-assay was calculated by using three pools of progesterone concentrations; high, medium and low, (n= 4), with means concentrations of 28.81, 6.41 and 1.02 ng/ml respectively. The coefficients of variations were 2.3, 4.5 and 6.6 % respectively (one assay). Non Specific Binding (NSB) in the present study was 2.5% of the total count, and the binding was 42.2%.

Analysis of data.

Progesterone concentrations were analyzed by calculating the average standard deviation.

RESULTS AND DISCUSSION

As a Chital deer is a nervous animal (Anderson, 1984), they are easily to stress during handling and restraining. It was reported that they can be trained to enter the crush and tolerate to blood sampling (Chapple *et al.*, 1991; Mylrea, 1992). However in the present study, handling of deer was performed such as inserting CIDR, blood samples collection during CIDR removals and blood collection 48 hour after. Progesterone concentrations, during CIDR removal and two days after, are presented in Table 1. The concentration decreased 2.9 ng/ml from 3.57 (immediately before removal) to 0.62 ng/ml (two days after CIDR removal).

Intravaginal insertion of CIDRs has been found successfully to release progesterone concentrations in blood as high as mid luteal phase in Chital deer (Mylrea, 1992). Replacing CIDRs at the middle of the insertion was also found successfully to maintain high progesterone concentration (Fennessy *et al.*, 1990). High concentrations of progesterone at the end of treatment were used to induce a sharp decrease of progesterone, following intravaginal devices withdrawal. The sharp decrease of progesterone induced negative feed back effect to the (LH) luteinizing hormone (Monfort *et al.*, 1990; Asher et al., 1992). Consequently, one of the follicles developed to maturity (dominant). The dominant follicle induced the other follicles to become atretic. Subsequently only one follicle would grow to become an ovulatory follicle. Estrogen was produced and secreted by the ovulatory follicle (Kelly *et al.*, 1982). When the estrogen concentration was at a high level, progesterone, on the other hand, was in the basal level. During this period, the hinds were receptive to the stags. The increase of estrogen induced a positive feed back effect to trigger LH surge (Argo *et al.*, 1992) and this surge brought on ovulation at the end of estrous periods.

In the present study, the progesterone concentration decreased 2.9 ng/ml. Hence, CIDRs released progesterone successfully during these trials. These results are comparable to those reported by Ainsworth and Downey, (1986) in the ewes, that the levels of progesterone increased from 1 to 5 ng/ml after CIDR insertion and decreased from 2 to 0 ng/ml, after intravaginal device withdrawal.

During the induction of estrous and ovulation, the length of progesterone treatment is considered to be an important factor to induce estrous, as it was reported that progesterone produced a synergistic effect from estrogen (Edqvist and Stabenfeldt, 1993).

Table 1 Progesterone concentration during CIDR removal and 48 hours after CIDR removal (Means ± SD ng/ml).

	Progesterone concentration (Means ± SD)
CIDR removal	3.57 ± 0.61 ng/ml
Two days after	0.62 ± 0.48 ng/ml
No. hinds showed crayon marks	3

Progesterone given for 11 to 15 days was considered to be the best treatment for estrous synchronization for deer species (Fennessy *et al.*, 1989a; Mylrea, 1992; Asher *et al.*, 1988a,b; Asher *et al.*, 1992). The sharp decrease of progesterone, in the present study, was not successfully induced estrous. Estrous synchronization treatment produced 15% estrous exhibition.

It was reported that the adrenal gland of white-tailed deer (Plotka *et al.*, 1983; Wood *et al.*, 1986), mule deer (Wood *et al.*, 1986) and fallow deer (Asher *et al.*, 1988a,b) is a significant source of progesterone. It had been speculated that analysis of progesterone concentration from a single sample would make the evaluation (Plotka *et al.*, 1983) of the effect of estrous synchronization difficult, if the adrenal gland is also a major resource of progesterone. Although most of the hinds did not showed estrous behavior, the progesterone concentrations of the deer was categorized in the basal levels two days after CIDR removal. It seems that the adrenal gland of Chital hinds produce low progesterone which enough to inhibit estrous synchronization as in the present study only three out of 20 (15%) Chital hinds showed estrous exhibition. The other possibility is that because of that the present study was performed in December, although the Chital deer are non seasonal polyoestrus, it seems that the season influence reproductivity (Chapple *et al.*, 1991; Mylrea, 1992; English, 1992)

CONCLUSION

Finally, it can be concluded that estrous synchronization using progesterone (CIDR) treatment for 14 days and the CIDR was replaced by day seven successfully induced sharp decrease of progesterone concentrations in Chital deer. The progesterone decreased from 3.57 ± 0.61 ng/ml before CIDR removal to 0.62 ± 0.48 ng/ml 48 hours after CIDR removal. This induction stimulated three out of 20 hinds (15%) showed estrous behavior. Further study need to be performed in tropical conditions.

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