Hormonal changes during luteal regression in farmed fallow deer, *Dama dama*

G. W. Asher, A. J. Peterson and W. B. Watkins*

*Ruakura Agricultural Centre, Ministry of Agriculture and Fisheries, Private Bag, Hamilton, New Zealand; and *Postgraduate School of Obstetrics and Gynaecology, University of Auckland, National Women's Hospital, Epsom, Auckland, New Zealand*

Summary. Concentrations of progesterone, oxytocin and PGFM (pulmonary metabolite of PGF-2 α) were measured in plasma from peripheral blood samples collected from 5 fallow does every hour or 2 h for 12-h periods on Days 15–20 inclusive of the oestrous cycle (i.e. luteolysis). For 3 does that exhibited oestrus on Day 21, plasma progesterone concentrations fluctuated between 3 and 10 ng/ml on Days 15–18 inclusive. Thereafter, values declined progressively to attain minimum concentrations of <0.5 ng/ml on Day 20. Basal concentrations of plasma oxytocin and PGFM fluctuated between 5 and 20 pg/ml and 10 and 100 pg/ml respectively. Episodic pulses of plasma oxytocin (>300 pg/ml) occurred on Days 15 and 16, whereas pulses of plasma PGFM (>400 pg/ml) occurred on Days 19 and 20. There was little apparent correlation between episodic pulses of the two hormones. For 2 does that exhibited oestrus on Day 22, plasma progesterone concentrations declined to minimum values of 1.0–1.5 ng/ml by Day 20. One of these does showed very high levels of oxytocin secretion throughout the sampling period while the other showed an apparent paucity of oxytocin secretory periods. Two does hysterectomized on Day 13 of their second oestrous cycle failed to exhibit further oestrous cycles. Continual elevation of plasma progesterone concentrations (2–6 ng/ml) for an 8-month period indicated persistence of the corpus luteum after hysterectomy. It is concluded that luteolysis in fallow deer involves episodic secretion of both oxytocin and PGF-2 α .

Keywords: fallow deer; *Dama dama*; reproduction; luteolysis; prostaglandin; oxytocin

Introduction

The non-pregnant fallow doe is seasonally polyoestrous, exhibiting regular 21–22-day oestrous cycles characterized by well delineated follicular, luteal and luteolytic phases (Asher, 1985). The remarkable uniformity of the oestrous cycle and the reliability of synchronization techniques for oestrus (Asher *et al.*, 1986) indicate that the fallow doe is useful for studying the endocrine control of various phases of the cervid oestrous cycle. Recent studies have investigated temporal hormonal relationships around oestrus (Asher *et al.*, 1986) but endocrine events during luteal regression have not been studied previously in fallow deer or any other cervid species.

It is well established that the regression of the corpus luteum in many domestic ruminants is due to the episodic release of prostaglandin (PG) F-2 α from the uterine endometrium following an obligatory period of progesterone priming (Scaramuzzi *et al.*, 1974; Baird *et al.*, 1976). PGF-2 α is almost completely inactivated in the peripheral circulation through the lungs (Piper *et al.*, 1970) and most probably exerts its luteolytic effect on the ovary by local counter-current transfer via the venous-arterial plexus in the ovarian pedicle (McCracken *et al.*, 1972). Several studies have shown that oxytocin is also luteolytic in various ruminants (Armstrong & Hansel, 1959; Cooke & Knifton,

1981). The corpora lutea of sheep and cows contain high concentrations of oxytocin (Wathes & Swann, 1982; Flint & Sheldrick, 1983; Fields *et al.*, 1983) and secrete it into the ovarian vein (Flint & Sheldrick, 1982, 1983). In the ewe there is a close temporal relationship between secretory pulses of ovarian oxytocin and uterine PGF-2 α during spontaneous luteolysis (Flint & Sheldrick, 1983; Hooper *et al.*, 1986), leading to the hypothesis that these two hormones engage in a positive feedback loop during luteolysis, resulting in a more rapid demise of the corpus luteum (Flint & Sheldrick, 1982, 1983).

The present study on fallow deer was designed to test the hypotheses that (1) the main luteolysis in fallow does is of uterine origin and (2) luteolysis involves interactions between the secretion of PGF-2 α and oxytocin.

Materials and Methods

Animals and management. Five parous 5-year-old fallow does had their first oestrus of 1986 artificially synchronized. A single silicone elastomer device impregnated with 0.5 g progesterone (12% CIDR-type S; N.Z. Dairy Board, Hamilton, N.Z.) was inserted intravaginally into each doe on 11 April and removed 15 days later. After CIDR removal the does were run with a vasectomized buck for the duration of the breeding season (April–October).

The deer were contained in high-fenced paddocks within 150 m of an enclosed observation platform. They were grazed on ryegrass–clover pastures and offered supplements of meadow hay and whole-kernel maize.

Detection of oestrus. The vasectomized buck was fitted with a ram mating harness throughout the breeding season (after Asher, 1985). Crayons were replaced every 2nd or 4th day and twice daily observations were made to detect crayon mating marks on the does.

Blood sampling. (a) The does were mustered into a covered handling shed, individually restrained in a cradle and blood sampled (jugular venepuncture into 10-ml heparinized Vacutainers) every 2 days from 15 April (CIDR insertion) until 3 July, then every 4 days until 3 October and thereafter every 7 days until 5 February 1987.

(b) On Days 15–20 of the first oestrous cycle (i.e. 14–19 May inclusive) the does were separated from the buck and held indoors for 12 h from 06:00 h to 18:00 h each day. At each blood sampling they were individually restrained in the cradle. They were blood sampled every hour on Days 15–18 inclusive and every 2 h on Days 19 and 20. Samples (~5 ml) were withdrawn from the right external jugular vein into heparinized Vacutainers. Skin covering the vein had been shaved previously and was cleaned with antiseptic solution after each sampling. Each night the does were reunited with the buck.

Blood samples were centrifuged immediately after collection and the plasma was stored at -10°C pending assay.

Mid-cycle hysterectomy. Two of the does (W2 and W7) were hysterectomized on Day 13 of the second oestrous cycle (i.e. 3 June). They were fully anaesthetized with an i.m. injection of an aqueous mixture of 5 mg ketamine hydrochloride (Ketaset; Bristol-Meyers Co., Syracuse, NY, U.S.A.) and 5 mg xylazine hydrochloride (Rompun; Bayer Leverkusen, West Germany) per kg liveweight. Anaesthesia was maintained during surgery with 1.0% halothane vapour (Halothane B.P.; May & Baker Ltd, Dagenham, U.K.). The reproductive tract was exteriorized via a mid-ventral incision and the uterus removed without disruption of the ovarian vascular system. Recovery from anaesthesia after completion of surgery was unaided.

Hormone assays. Plasma progesterone concentrations were determined in duplicate using an extraction radioimmunoassay similar to that described by Fairclough *et al.* (1975) and which has been validated previously for fallow deer serum (Asher, 1985). The antiserum was raised in a rabbit against progesterone-11-BSA conjugate and used at a final dilution of 1:3000. The only major cross-reaction of a wide range of steroids tested in the assay was cholesterol (1.5%). The sensitivity of the standard curve was 0.3 ng/tube (0.15 ng/ml plasma) and the intra- and inter-assay coefficients of variation were 9.2% and 11.0% respectively.

Plasma oxytocin concentrations were determined using the radioimmunoassay described by Robinson (1980) following extraction of oxytocin from plasma (1.0 ml) using Sep-Pak cartridges (C₁₈; Waters Associates, Milford, MA, U.S.A.) as described by Hooper *et al.* (1986). The extraction efficiency, determined from the recovery of trace amounts of radioactive ligand added to plasma samples, was >85%. The antiserum (RIII 5; National Institute for Medical Research, London, U.K.) was raised in a rabbit against oxytocin–thyroglobulin conjugate and used at a final dilution of 1:280 000. High specificity of the antiserum has been demonstrated by Robinson (1980). The inter-assay coefficient of variation for a 20-pg/ml control sample was 7.4% ($n = 5$ assays). The intra-assay coefficient of variation for multiple determinations of the same control sample was 3.8%. The sensitivity of the standard curve was 1.25 pg/tube (3.13 pg/ml).

Plasma concentrations of 13,14-dihydro-15-keto prostaglandin F (PGFM), the primary pulmonary metabolite of PGF-2 α , were determined in duplicate using the extraction radioimmunoassay procedure described by Fairclough &

Payne (1975). The antiserum used in the assay (560-JCC-146; Upjohn Company, Kalamazoo, MI, U.S.A.) was raised against PGFM-BSA conjugate and was used at a final dilution of 1:1500. The antiserum had a 20% cross-reaction with 15-keto-PGF-2 α , and <1% cross-reaction with all other prostaglandins tested. It did not distinguish between 13,14-dihydro-15-keto PGF-1 α and 13,14-dihydro-15-keto PGF-2 α and the results are expressed as PGFM equivalents. The extraction efficiency, calculated as the mean ($\pm$ s.d.) recovery of radioactive ligand added to fallow deer plasma ($n = 10$ determinations), was $86.8 \pm 1.5\%$. The inter-assay coefficients of variation for determinations of low (mean concentration = 91 pg/ml), medium (459 pg/ml) and high (896 pg/ml) control samples in each assay ($n = 5$) were 17.0%, 14.1% and 7.2% respectively. The intra-assay coefficient of variation for multiple determinations of the medium control sample was 8.9%. The sensitivity of the standard curve was 5 pg/tube (25 pg/ml plasma).

A hormone secretory pulse was defined as a marked increase in peripheral plasma concentrations such that 2 consecutive samples exceeded 2 standard deviations (s.d.) above the doe mean or a single sample exceeded 3 s.d. above the doe mean.

Results

Hormone changes during luteolysis

All 5 does exhibited oestrus between 46 and 64 h after CIDR removal (mean $\pm$ s.d. = 58 ± 8 h). Three does (W39, W49 and W67) returned to oestrus on or about Day 21; the remaining 2 does (W2 and W7) returned to oestrus on or about Day 22. Plasma progesterone, oxytocin and PGFM concentrations are presented in Fig. 1 (Day 21 returns) and Fig. 2 (Day 22 returns).

For individual does, plasma progesterone concentrations fluctuated between 3 and 10 ng/ml on Days 15–18 inclusive. However, on Days 19 and 20 all does exhibited a progressive decline of progesterone concentrations. Day 20 values were lower (< 0.5 ng/ml) for does exhibiting oestrus on Day 21 (Fig. 1) than for the 2 does returning to oestrus 1 day later.

During the intensive blood sampling period plasma concentrations of both oxytocin and PGFM exhibited sporadic pulses, although there appeared little relationship between the pulse episodes of the two hormones.

Certain similarities featured in the 3 profiles presented in Fig. 1. Basal plasma oxytocin values fluctuated between 5 and 20 pg/ml, but pulses exceeding 300 pg/ml occurred on Day 15 (W39 and W67) or Day 16 (W49). Lower amplitude pulses (100–200 pg/ml) also occurred between Days 15 and 17 (e.g. doe W67). Basal plasma PGFM values fluctuated between 10 and 100 pg/ml. In contrast to oxytocin, high amplitude pulses of PGFM (> 400 pg/ml) generally occurred between Days 18 and 20, somewhat later in the oestrous cycle. Lower amplitude pulses (100–300 pg/ml) were also apparent on Days 17 and 18 for Doe W67.

Plasma oxytocin and PGFM profiles presented in Fig. 2 (Day 22 returns) differ in several respects from the profiles described for Does W39, W49 and W67. The profile of Doe W2 is unusual in that marked increases in plasma oxytocin concentrations were not apparent until Days 19 and 20 (later than in Fig. 1) and a pulse (135 pg/ml) that occurred on Day 19 was coincidental with a pronounced pulse (450 pg/ml) of plasma PGFM.

The profile of Doe W7 showed frequent fluctuations of both plasma oxytocin and PGFM concentrations throughout the sampling period. Pulses of plasma oxytocin were particularly apparent on Days 15, 17 and 19, with those occurring on Days 15 and 19 being of unusually long duration (6–8 h). The apparent pulses of plasma PGFM were generally of low amplitude (< 200 pg/ml) when compared with those observed in other profiles.

The effect of mid-cycle hysterectomy on luteal regression

Plasma progesterone profiles and dates of oestrus of the 5 does, for the 10-month period from April 1986 to February 1987, are presented in Fig. 3. Plasma progesterone concentrations of the two hysterectomized does remained elevated (> 2 ng/ml) for almost the entire period of sampling after hysterectomy and oestrus was not observed during this time. Plasma progesterone concentrations declined gradually towards the end of the sampling period.

Fig. 1. Profiles of plasma progesterone, oxytocin and PGFM concentrations on Days 15–20 of the oestrous cycle of 3 fallow does exhibiting a 21-day cycle.

By contrast, the other 3 does exhibited cyclic fluctuations in plasma progesterone concentrations corresponding to oestrous cycles, until at least late August. Thereafter, they became anoestrous, exhibiting low plasma progesterone values (< 1 ng/ml) until December. All 3 does showed

Fig. 2. Profiles of plasma progesterone, oxytocin and PGFM concentrations on Days 15–20 of the oestrous cycle of 2 fallow does exhibiting a 22-day cycle.

a slight rise in progesterone values during the last 2 months of sampling but there was no evidence of oestrous cyclicity.

Discussion

These results demonstrate that the luteolytic phase of the fallow deer oestrous cycle has a number of physiological similarities to luteolysis in several domestic ruminant species. The apparent failure of luteal regression after mid-cycle hysterectomy, as indicated by the persistent continuous secretion of progesterone over the subsequent 8-month period, clearly implies an obligatory role of the uterus for luteolysis in the fallow doe. The observation that episodic fluctuations in the peripheral plasma concentrations of PGFM, the pulmonary metabolite of PGF-2 α , occurred during luteal regression in entire fallow does indicates that PGF-2 α is the most likely uterine luteolysin in this species. This is substantiated by the observation that administration of a prostaglandin analogue (cloprostenol: Estrumate, Imperial Chemical Industries PLC, Cheshire, U.K.) on Days 13 or 14 of the oestrous cycle results in a rapid decline in plasma progesterone concentrations and the occurrence of oestrus between 42 and 50 h (G. W. Asher, unpublished data).

Fig. 3. Profiles of plasma progesterone concentrations of 5 fallow does for the 10-month period from April 1986 to February 1987. Open circles represent values after hysterectomy (hst) and arrows indicate oestrus.

The pulsatile pattern of PGF-2 α secretion in fallow does, as indicated by sporadic surges in peripheral plasma concentrations of PGFM on Days 18–20 of the oestrous cycle, is similar to that reported in a number of studies on sheep in which pulses of PGF-2 α secretion were more pronounced during the later, rather than earlier, stages of luteolysis (Baird *et al.*, 1976; Flint & Sheldrick, 1983). Similarly, the occurrence of sporadic surges of peripheral plasma oxytocin concentrations early in the luteolytic phase of the oestrous cycle of at least 3 fallow does is in accordance with recent studies on sheep (Fairclough *et al.*, 1980; Flint & Sheldrick, 1983; Hooper *et al.*, 1986), although peripheral plasma concentrations of the hormone appeared to be considerably higher in fallow deer than in sheep (Flint & Sheldrick, 1983), cattle (Vighio & Liptrap, 1986) and goats (Homeida & Cooke, 1983) during the same stage of the cycle.

In sheep there is a temporal relationship between secretory episodes of oxytocin (or oxytocin-related neurophysin) and PGF-2 α (or PGFM) during luteolysis (Wathes, 1984). There was little apparent correlation between peripheral plasma pulses of the 2 hormones in the present study on fallow deer. However, the hourly or 2-hourly sampling regimen may have lacked sufficient precision to detect correlated pulses. The data indicate that in this species the mean surges in oxytocin concentrations generally occur at an earlier stage of luteolysis than those of PGFM. It is possible that peripheral plasma concentrations of the 2 hormones (or metabolites) as reflected by our sampling regimen do not reflect adequately their true secretory pattern or changing sensitivity of the target organs. A closer temporal relationship between oxytocin and PGF-2 α may exist at the utero-ovarian level because not only does PGF-2 α exert its luteolytic effects on the ovary by local counter-current transfer (e.g. sheep: McCracken *et al.*, 1972), but also oxytocin of luteal origin is transferred in the ovarian counter-current system and may exert a local effect on the uterine endometrium (e.g. sheep; Schramm *et al.*, 1986).

We have not demonstrated a luteal source of oxytocin in fallow deer. However, the corpus luteum is a major source of oxytocin during luteal regression in sheep and cattle (Wathes, 1984) and it is reasonable to assume that the peripheral plasma concentrations of oxytocin observed in the present study reflect ovarian secretion of the hormone.

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