Plasma concentrations of prolactin in brushtail possums (*Trichosurus vulpecula*) in different physiological states

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Abstract

Prolactin (Prl) has been implicated in reproduction in many mammalian species and is illustrated by the distinctive patterns of secretion during the breeding season, the oestrous cycle and lactation. The recent development of a homologous RIA for measuring the circulating Prl concentrations in brushtail possums has facilitated the reliable measurement of Prl in plasma during different physiological states in this species for the first time. Determination of Prl concentrations during lactation involved the collection of weekly blood samples from eight female possums from the time of parturition through either one or two consecutive lactational cycles. Prl was at baseline levels during early lactation (weeks 0–14 post-partum), and then increased markedly to maximum concentrations at weeks 19–21 before returning to nadir levels at a time coincident with the weaning of pouch young (weeks 23–27). The profile of Prl secretion over the oestrous cycle and in particular at the time of the preovulatory LH surge was obtained from 14 possums during the reproductive cycle, in which preovulatory follicle development and ovulation were monitored by laparoscopy. There was no distinct daily pattern

of Prl secretion during the oestrous cycle; however, in 3/4 possums in which a typical preovulatory LH surge was measured, a biphasic preovulatory Prl surge was also observed. The preovulatory Prl surge commenced 2–6 h prior to, and had returned to baseline close to the onset of, the preovulatory LH surge, and a second surge of Prl occurred concomitantly with the delayed preovulatory FSH surge. Seasonality of Prl levels was established from weekly blood samples collected from six barren female possums, and concentrations of Prl were lower during the breeding season compared to the non-breeding season. Additionally, a circadian pattern of Prl secretion was evident in both female and male possums, with Prl levels higher in the morning compared to the afternoon. In conclusion, interpretation of endogenous secretory patterns suggests that Prl may be important during late lactation and at impending ovulation, but the involvement of the circannual rhythm of Prl in the regulation of seasonality in the brushtail possum remains to be determined.

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Introduction

Prolactin (Prl) has been reported to play a role in reproduction, in particular ovarian function, and lactation in many mammalian species (for reviews, see [Sinha 1995](#), [Freeman *et al.* 2000](#)). However, it was not until the recent production of Prl-related knockout (KO) mice models ([Thomas & Capecci 1987](#)) that it was established that Prl does indeed play a fundamental role in both reproductive and lactational processes. A decline in ovulation rate, reduced fertilisation, almost complete arrest of preimplantation development and implantation failure of the uterus culminates in complete sterility in Prl receptor homozygous KO female mice (PRLR^{-/-}) ([Bole-Feysot *et al.* 1998](#)). In the male, Prl has been implicated in testosterone production by Leydig cells ([Kelly *et al.* 1991](#)), sperm capacitation ([Shah & Sheth 1979](#)) and function of accessory reproductive glands

([Bartke 1980](#), [Costello & Franklin 1994](#)), but surprisingly, PRLR^{-/-} male mice are fully fertile although puberty is delayed ([Bole-Feysot *et al.* 1998](#)).

Prl is involved in maintenance of the corpus luteum (CL) by stimulating the expression of both oestrogen and luteinising hormone (LH) receptors ([Gibori 1993](#)) and by suppressing the activity of 20 α -hydroxysteroid dehydrogenase, thereby inhibiting progesterone (P₄) catabolism to an inactive steroid ([Albarracin *et al.* 1994](#), [Telleria *et al.* 1997](#)) in rats. Prl has also been implicated in both parturition ([Hearn 1974](#)) and maintenance of embryonic diapause ([Hearn 1974](#), [Curler *et al.* 1986](#)) in several marsupial macropodid species, thereby suggesting a role in luteal function in marsupials. The widespread expression of Prl receptor mRNA (L Haydon & DC Eckery, unpublished observations) in those ovarian cell types that also express mRNA for steroid acute regulatory protein, P450 side-chain cleavage, 3 β -hydroxysteroid

dehydrogenase, and P450 aromatase (Eckery *et al.* 2002, Whale *et al.* 2003) in the brushtail possum implies that Prl plays a significant role in ovarian function and in particular steroidogenesis in this species. The recent development of a homologous Prl RIA for measuring Prl in brushtail possums (Crawford *et al.* 2005) has enabled the endogenous secretory patterns of Prl during lactation, the oestrous cycle and throughout the breeding and non-breeding seasons to be elucidated for the first time in this species.

Materials and Methods

Animals and management

Possums were live-captured in the Wellington (latitude 41°S) region of New Zealand. At the time of capture, each animal was screened for health status and only apparently healthy animals were included in this study. Animals were housed in the AgResearch Wallaceville possum facility under a group housing system (McLeod *et al.* 1997). A mixed diet of fresh fruits and vegetables, bread and cereal-based pellets was provided along with selective browsing of *Pinus radiata* branches. Fresh water was always available.

All experimental procedures were approved by the AgResearch Wallaceville Animal Ethics Committee in accordance with the Animal Welfare Act of NZ, 1987.

Hormonal profiles during lactation

To determine the endogenous secretory patterns of reproductive hormones during lactation, groups of three reproductively cycling adult female possums and one adult male possum were housed together as breeding groups. In March 2003, pouches of eight female possums (3.25 ± 0.11 kg bodyweight) were checked daily for confirmation of the exact day of parturition. Blood samples were collected via jugular venepuncture from anaesthetised possums (induced and maintained by inhalation of a gaseous mixture of oxygen:isofluane; ICI Pharmaceuticals, Cheshire, UK) on the day of parturition, then weekly thereafter until either removal of a joey or natural weaning (19–31 weeks). In four of these animals, weekly blood samples were continued for a second consecutive lactational cycle (13–27 weeks). Joeys that displayed signs of ill health were immediately excluded from the study.

Hormonal profiles during the oestrous cycle

To determine the endogenous secretory patterns of reproductive hormones over an oestrous cycle, particularly at the time of impending ovulation, 20 adult female possums with joeys were divided into two replicates (for logistical purposes). This experiment was completed in one breeding season (2003). For the collection of blood samples, an indwelling catheter was inserted surgically in the jugular vein (Curlewis *et al.* 1985) of anaesthetised possums (Zoletil

100; Virbac, Techvet Laboratories, Auckland, New Zealand). Blood samples were taken daily by jugular venepuncture from anaesthetised (isofluane) possums when the catheter failed. During the intensive (hourly) blood sampling period, packed cell volume was monitored every 4 h and red blood cells (resuspended in sterile saline at 37 °C) were replaced via the catheter after each sample was collected. No individual haematocrit value dropped below 82% of the initial value at any time during the sampling period. Repeated laparoscopic examination of the ovaries was carried out at infrequent intervals (2–4 days) in anaesthetised (isofluane) possums to monitor follicle development (Crawford *et al.* 1997). Not more than four laparoscopic examinations were performed on any individual animal. All joeys were removed and rendered unconscious either by immersion in ice water to induce hypothermia (<60 days old cannot regulate body temperature) or isofluane (≥ 60 days old) before being killed by decapitation.

Replicate 1 A group of ten possums (2.86 ± 0.10 kg) with indwelling catheters had pouch young removed (RPY) (estimated age ranging from 18–47 days) in mid-breeding season (May). Daily blood samples were collected from –4 to 23 days after RPY. Laparoscopic examinations were carried out at infrequent intervals from 6 days after RPY until the observed day of ovulation (presence of a stigma point on the preovulatory follicle). Blood samples were collected at hourly intervals for 48 h in individual animals in which the presumptive preovulatory follicle appeared to be within 48 h of ovulation (Crawford *et al.* 1997).

Replicate 2 A group of ten possums (2.86 ± 0.14 kg) with indwelling catheters had RPY (estimated age ranging from 27–115 days) in the late breeding season (August). Daily blood samples were collected from –2 to 34 days post-RPY. Seven days after RPY, laparoscopic examination of ovaries and consequent intensive (hourly) blood sampling regimes of individual animals commenced as described earlier.

Additionally, the plasma samples from one possum, in which a preovulatory LH surge was captured in an intensive blood sampling window from an identical experiment carried out at AgResearch Invermay in 1997, was included in this study.

Hormonal profiles during the breeding and non-breeding seasons

To determine endogenous secretory patterns of reproductive hormones over an entire breeding and non-breeding season, six barren adult female brushtail possums were divided into two groups and each group was housed with a vasectomised male possum. Vasectomies were carried out under general anaesthetic (Zoletil 100), although, as a precaution, local anaesthetic (Lignocaine hydrochloride BP 2%; Ethical Agents Ltd, Auckland, New Zealand) was also administered s.c. at the two incision sites. An injection (i.m.) of antibiotic

(Streptopen; A/S Rosco, Denmark; Bomac Laboratories Ltd, Auckland, New Zealand) was given as a prophylactic measure against infection. A small incision was made on one side of the scrotum using aseptic techniques and the vas deferens was located, cut and the ends ligated. The incision site was closed and the procedure was repeated on the other side.

The blood samples were collected via jugular venepuncture from anaesthetised (isofluane) female possums at weekly intervals from March 2004 to May 2005. Reproductive cyclicity was monitored in individual possums at daily intervals by analysis of urine for cytology (Curlewis *et al.* 1985, Duckworth *et al.* 1998).

Circadian hormonal profiles

Changes in endogenous circadian secretory patterns of Prl were determined in female possums in the mid-late follicular phase and reproductively active male possums. Blood samples were collected at 0800 h and 1700–2000 h for either 2 ($n=11$) or 3 ($n=8$) consecutive days in males and 3 ($n=14$) consecutive days in females. The blood samples were collected either via jugular venepuncture under anaesthesia (isofluane) or an indwelling jugular cannula in July 2004 (males) and May–July 2003 (females).

Hormone RIA

Plasma concentrations of LH and follicle-stimulating hormone (FSH) were measured by heterologous RIA using procedures described previously for the possum (Moore *et al.* 1997a,b). Within this study, the limits of detection (80% of zero binding) were 0.2 and 0.5 ng/ml for LH and FSH respectively. The intra- and interassay coefficients of variation (CV) for LH RIA were <12.5 and <10.3% respectively. The intra- and interassay CV values for FSH RIA were <11.7 and <8.1% respectively.

Plasma Prl concentrations were measured by homologous RIA using a previously described method (Crawford *et al.* 2005). Within this study, the limit of detection of the assay was 0.2 ng/ml, and the intra- and interassay CV values were <11.5 and <14.1% respectively.

Plasma P_4 levels were measured by direct RIA as described previously (Crawford *et al.* 1997). The limit of detection was 0.5 ng/ml, and the intra- and interassay CV values were <11.9 and <9% respectively.

Statistical analyses

Plasma Prl, LH, FSH and P_4 concentrations over one or two consecutive lactational cycles and during the oestrous cycle in female possums, and plasma Prl concentrations in the morning and afternoon in female and male possums, were log-transformed prior to analysis using a one-way ANOVA. Weekly plasma Prl, LH, FSH and P_4 concentrations over 15 months in female possums were log-transformed prior to

analysis using two-way ANOVA. Differences over time were calculated by an unbalanced design using Genstat regression.

Results

Hormonal profiles during lactation

The profiles of mean plasma Prl, LH and FSH concentrations were similar in the first and second consecutive lactation cycles (Fig. 1). Mean Prl levels were generally basal from 0 to ~14 weeks post-partum, when pouch young lacked fur and were mostly confined to the pouch. The intermittent exit of the furred young from the pouch after approximately 12–16 weeks of age was coincident with an initial rise ($P<0.01$) in plasma Prl concentrations. Mean Prl concentrations peaked at 19–21 weeks post-partum, when pouch young were observed to reside permanently outside the pouch and ride on the back of the adult. Plasma Prl concentrations declined ($P<0.01$) thereafter, and reached basal levels at 23–27 weeks post-partum coincident with the observed regression of the mammary glands and weaning. Mean weekly plasma LH concentrations were at basal levels throughout the first and second consecutive lactation cycles (Fig. 1). Similarly, mean weekly plasma FSH concentrations did not change significantly during lactation (Fig. 1).

Representative profiles of plasma Prl, LH, FSH and P_4 concentrations in individual possums over one or two consecutive lactational cycles are illustrated in Fig. 2. Possums with pouch young, which were weaned naturally (Fig. 2a and c, first cycle; Fig. 2d, second cycle) or became ill and either died or were euthanised at the time of normal weaning

Figure 1 Mean maternal plasma concentrations of (LH ○), (FSH □) and prolactin (PrL ●) in relation to time after parturition in female brushtail possums with healthy suckling pouch young over one ($n=8$) or two consecutive ($n=3$) lactational cycles. Values are depicted as means $\pm$ S.E.M. Asterisks denote values that are significantly different ($P<0.05$).

Figure 2 Representative maternal plasma profiles of LH (○), FSH (□), Prl (●) and progesterone (P₄; ■) in relation to time after parturition in individual female brushtail possums over one (a and c) or two consecutive (b and d) lactational cycles. Arrows depict the birth of pouch young and arrowheads represent the termination of suckling by pouch young due to either weaning (open) or removal due to illness or death (closed). Note that weeks 0–33 are during the breeding season and weeks ~33–49 are during the non-breeding season.

(Fig. 2b, first cycle), had a normal secretory pattern of Prl (cf. Fig. 1) and ovulated either during or after elevated P₄ plasma concentrations (Fig. 2). However, Prl concentrations remained low throughout lactation in animals that had pouch young in ill-health that either died, or were euthanised, prior to natural weaning (Fig. 2c, second cycle; Fig. 2d, first cycle). Animals with pouch young that died prematurely in the breeding season ovulated thereafter as represented by high P₄ levels (Fig. 2d, first cycle). There was no distinct pattern of plasma LH or FSH secretion in individual lactating possums; however, plasma FSH concentrations declined ($P < 0.05$) prior to a marked and sustained increase in plasma P₄ levels (Fig. 2).

Hormonal profiles over the oestrous cycle

Fourteen possums that had RPYP were observed by laparoscopic examination to develop a preovulatory follicle, which progressed to ovulation, while the other five possums remained anoestrus. The daily pattern of mean plasma Prl,

LH, FSH and P₄ concentrations over an oestrous cycle was similar in animals in Replicate 1 (Fig. 3a) and Replicate 2 (Fig. 3b). There was no distinct pattern in mean Prl levels during the follicular phase of the oestrous cycle. Mean Prl concentrations were lower ($P < 0.05$) on days 1–6 post-ovulation than those observed on 17–22 days post-ovulation. The between-animal variation was large in both the replicates.

Mean LH concentrations were at baseline throughout the sampling period, except on the day of ovulation (day 0), when LH concentrations had increased ($P < 0.05$). On the day of ovulation, the between-animal variation was large for both the replicates.

From 12 days prior to ovulation, mean FSH concentrations continuously declined ($P < 0.01$) to nadir levels during the late follicular phase. An increase ($P < 0.01$) in FSH concentrations was observed on the day of ovulation. Mean FSH concentrations were higher ($P < 0.05$) from days 3 to 18 post-ovulation in possums, compared to those in the late follicular stage (–4 to –1 days post-ovulation). Due to the

Figure 3 Mean daily plasma concentrations of LH (○), FSH (□), Prl (●) and P₄ (■) in reproductively cycling female brushtail possums in relation to the day of ovulation. For the logistics of this study, the animals were allocated into either Replicate 1 (a; $n=8$) or Replicate 2 (b; $n=6$). Note that blood sampling was continued for 10 days longer in some animals in Replicate 2 compared to those in Replicate 1. Values are denoted as means $\pm$ S.E.M.

longer sampling period in Replicate 2 animals, mean FSH concentrations continuously declined from 16 to 19 days post-ovulation depicting the progression of these animals into the next follicular phase.

Mean P₄ levels were at baseline throughout the follicular phase of the oestrous cycle. The concentrations increased significantly ($P<0.01$) by day 9 post-ovulation, peaked at 13–14 days post-ovulation and returned to nadir levels by 15–17 days post-ovulation.

Hormonal profiles during the peri-ovulatory period

Representative hourly profiles of plasma LH, FSH and Prl concentrations in individual possums are illustrated in Fig. 4a–i. The hormonal profiles varied markedly between the eight possums in which the intensive blood-sampling window was completed 1–4 days prior to the observed day of ovulation (stigma observed on the preovulatory follicle). Four of these animals had basal LH concentrations, low FSH concentrations and either high- (Fig. 4a) or low-amplitude (Fig. 4b) fluctuations in the concentrations of Prl. The remaining four possums had basal plasma levels for all three hormones (Fig. 4c). A preovulatory LH surge was measured, within the intensive blood-sampling window, in four possums in this study and in one possum from an identical experiment undertaken at AgResearch Invermay, where possums were sampled near the day of ovulation. In these five possums, a surge (5–8 h in duration) of plasma LH and a coincident, but slightly delayed surge (6–9 h in duration) of FSH, were observed (Fig. 4d–h). In three of these five animals, a surge (3–5 h in duration) of Prl was also observed 2–6 h before the preovulatory LH surge and a second Prl surge (5–9 h in duration) occurred coincidentally with the delayed preovulatory FSH surge (Fig. 4d–f). In the remaining two possums, plasma Prl remained at basal levels (Fig. 4g and h) and one of these animals exhibited two LH and FSH surges that were 12 h apart (Fig. 4h). Plasma Prl concentrations were baseline during this 48 h window in these five animals, excluding the time of preovulatory Prl surge. In two possums, the intensive blood-sampling window was initiated one day after the day of ovulation. In these animals, the plasma concentrations of LH were low and of Prl were variable, whereas those of FSH increased gradually over time (Fig. 4i).

Hormonal profiles during the breeding and non-breeding seasons

Weekly profiles of plasma P₄, LH, FSH and Prl concentrations in six individual possums over a 15-month period are illustrated in Fig. 5. Analysis of these results included the data from March to October (entire breeding season 2004), December to February (entire non-breeding season 2004–2005) and April to May (partial breeding season 2005). Data outside these months were considered to be in the transition time between the breeding and non-breeding seasons and thus were excluded from the analysis. The overall timing of the breeding and non-breeding seasons in individual

Figure 4 Representative profiles of plasma concentrations of LH (○), FSH (□) and Prl (●) in individual adult female brushtail possums at (a)–(c) 1–4 days prior to ovulation, (d)–(h) near the time of ovulation and incorporating the preovulatory surges and (i) 1–2 days after ovulation. Note the abnormal double surge of LH and FSH in one animal (h).

possums was confirmed by the presence or absence of episodic elevations in P_4 concentrations respectively (Fig. 5a). The majority of the peaks of P_4 concentrations were preceded (1–2 weeks) by a significant influx in the numbers of epithelial cells within the urine of these possums. Plasma LH levels were usually at basal levels in all animals, although infrequent elevations in LH concentrations were detected (Fig. 5b). Both plasma FSH and Prl concentrations fluctuated markedly throughout the breeding and non-breeding seasons in all animals (Fig. 5c and d). Despite wide animal variation, mean plasma concentrations of Prl were lower ($P < 0.05$) and FSH were higher ($P < 0.05$) in the breeding season compared with the non-breeding season. There were no evident seasonal changes in mean weekly plasma LH levels.

Circadian hormonal profiles during the breeding season

Mean plasma Prl concentrations in both female and male possums were significantly higher ($P < 0.01$) at 0800 h compared to that at 1700–2000 h (Fig. 6). The elevation of plasma Prl concentrations in the morning, compared to the

afternoon, was also evident in individual possums that had blood samples collected at intensive intervals near, but not at the time of the preovulatory LH surge (Fig. 4a and i; 0800 h sample corresponded to time 13 and 37 h; Fig. 4b; 0800 h sample corresponded to time 21 and 45 h). Mean plasma LH concentrations remained low regardless of the time of blood collection (data not shown).

Discussion

We report, for the first time, the endogenous secretory patterns of Prl in female brushtail possums during lactation, the oestrous cycle and the breeding and non-breeding seasons, as well as the circadian changes in Prl in female and male brushtail possums.

There was a distinct pattern of Prl concentrations during lactation in the possum in this study, which was similar, but not identical to that previously reported for possums (Hinds & Janssens 1986) and other (Curlewis *et al.* 1986, Hinds & Merchant 1986, Muths & Hinds 1996) marsupial species.

Figure 5 Weekly plasma concentrations of (a) P, (b) LH, (c) FSH and (d) Prl over 15 months incorporating the breeding (shaded areas) and non-breeding (shaded and hatched areas) seasons in individual adult female brushtail possums (i)–(vi) that were housed with vasectomised males. Analysis of these results included data from April to October (entire breeding season 2004), December to February (non-breeding season) and April to May (partial breeding season 2005). The non-shaded areas were considered the transition time between the breeding and non-breeding seasons and were excluded from analysis. Asterisks indicate the day when an increase in epithelial cells was first detected in the urine of individual possums.

Significant mean plasma concentrations of Prl were present only after the 'switch-phase' at 13–17 weeks of lactation. This phase coincided with the development of homeostasis of the pouch young, growth of fur, opening of the eyes and ever-increasing periods of emergence from the pouch, as well as changes in suckling intensity and frequency and in milk composition (Cowan 1989, Crisp *et al.* 1989, Bird *et al.* 1994, Nicholas *et al.* 1997, Demmer *et al.* 1998, Rose *et al.* 2003). In the present study, the levels of Prl were markedly elevated for a much shorter period (i.e. ~6 weeks) compared

to approximately 11 weeks in a previous report (Hinds & Janssens 1986). This difference is likely to be due to the lactational profile being the integration of a series of truncated data from different possums in the Hinds & Janssens study. Elevated Prl concentrations during the later stages of lactation are, at least in part, under dopaminergic control in the brushtail possum (Crawford *et al.* 2005), Bennett's wallaby (Curlewis *et al.* 1986) and Tasmanian bettong (Rose *et al.* 1996). In eutherians, suckling decreases dopamine input to the thyrotroph cells of the anterior pituitary gland resulting

Figure 6 Mean plasma Prl concentrations in female (●; $n=14$) and male (○; $n=8-11$) possums at either 0800 h or 1700–2000 h over three consecutive days. Values are denoted as means $\pm$ S.E.M.

in hyperprolactinaemia (Ben-Jonathan *et al.* 1980). Although this mechanism is not well understood, a reduced activity of the tuberoinfundibular dopaminergic neurons was associated with a reduction in tyrosine hydroxylase gene expression through a direct feedback mechanism via the activation of the neural afferents that extend from the nipple to the hypothalamus (Wang *et al.* 1993, Li *et al.* 1999, Berghorn *et al.* 2001). Communication between the pituitary and mammary glands is probable in the brushtail possum considering that sensitivity to oxytocin, a pituitary-derived hormone responsible for the effective 'let-down' of milk from the mammary gland (Cross 1977), was marked in small mammary glands supporting immature pouch young compared with larger ones in this species and was facilitated through increased concentration of oxytocin receptors (Sernia *et al.* 1991). However, perhaps the newly born, immature and immune-deficient (Deane & Cooper 1984, Adamski & Demmer 2000, Adamski *et al.* 2000) pouch young is incapable of suckling at an intensity required to elicit an increase in Prl concentrations during the first 13 weeks of lactation, and that hyperprolactinaemia is only necessary for facilitation of larger quantities and/or change in composition of milk that is required to support the older joey (> 13 weeks old). In this study, elevations in Prl concentrations in the later stages of lactation were not observed in animals that had pouch young either die or be removed due to illness. Whether the young were compromised due to deficiencies of the mother (suppressed Prl concentrations) or the joey (inability to suckle) is unknown.

Preparturition events do not appear fundamental to the initiation nor the maintenance of lactation since the attachment of newborn pouch young to teats of non-mated, as well as virgin, female brushtail possums resulted in milk production and subsequent survival and normal growth of transplanted pouch young (Sharman 1962). It may be that the early stages of lactation are partly or wholly under local

autocrine control through feedback inhibition by a milk protein. In ruminants, milk production is regulated via an autocrine mechanism whereby an inhibitory milk protein is synthesised and secreted in milk and acts in a concentration-dependent and reversible manner (Hendry *et al.* 1998). Additionally, alteration in the sucking pattern of young does not influence the milk composition or the rate of milk production in the Tammar wallaby (Nicholas *et al.* 1997, Trott *et al.* 2003). Collectively, these studies suggest that, while the marsupial pouch young is capable of initiating mammatogenesis and lactogenesis, it is the lactating adult that regulates both changes in milk composition and rate of milk production thereafter, and consequently the development and growth of the pouch young.

Whilst there is a distinct pattern of secretion of other pituitary-derived hormones (i.e. LH and FSH) during the follicular phase in the brushtail possum (Crawford *et al.* 1999, this study), there was no obvious discrete profile of Prl secretion during this time. A distinct preovulatory biphasic surge of Prl was observed in three out of four possums in which a typical preovulatory LH surge was detected. However, a biphasic preovulatory surge of Prl was absent in one animal that exhibited a characteristic preovulatory LH surge and, several days later, a postovulatory rise in P_4 concentration, suggesting normal functioning of the CL. From these varied results, it is difficult to conclude that the preovulatory Prl surge is a fundamental event in the late follicular stage in this species. It is interesting to note that Prl secretion appeared to be tightly regulated prior to and immediately after the preovulatory surges in all possums in this study. In rats, ovulation is also preceded by a Prl surge and the onset of this surge usually precedes the onset of the preovulatory LH surge by 2–4 h (Wuttke & Meites 1970, Butcher *et al.* 1974, Hirsch *et al.* 1993). The preovulatory Prl surge in rats has luteotrophic and luteolytic effects in that it causes augmentation of LH-induced luteinisation and associated increased P_4 secretion (Butcher *et al.* 1972, Döhler & Wuttke 1974, Morishige & Rothchild 1974), as well as regression of old CLs (Malven & Sawyer 1966, Wuttke & Meites 1971) through stimulation of collagenases (Hirsch *et al.* 1999). Blockage of the preovulatory Prl surge by dopaminergic agents in rats did not disturb oestrus cyclicity and ovulation (Döhler & Wuttke 1974), but did result in the accumulation of non-steroidogenic CLs (Wuttke & Meites 1971, Döhler & Wuttke 1974). Regulation of the mechanism(s) involved in the generation of Prl surges is reported to be influenced by steroid hormones, and repeated cyclic steroid hormone replacement (oestradiol; $E_2 + P_4$) was capable of producing Prl surges in ovariectomised 6-week-old rats, with multiple cycles resulting in a greater secretory response (Brown *et al.* 2004). In the possum, the follicular phase of the oestrous cycle is prolonged compared to that in the rat and regression of the latest CL was observed prior to the preovulatory Prl surge, suggesting that this surge may only exert luteotrophic effects in this species.

Changes in photoperiod provide the principal environmental cue to annual reproductive cycles and are exerted on the suprachiasmatic nucleus (rhythm control centre) through the action of melatonin (Lincoln & Short 1980). Melatonin is secreted during darkness and is thought to entrain the organism to the light–darkness changes of the environment. Melatonin acts in the mediobasal hypothalamus to modulate pulsatile gonadotrophin-releasing hormone secretion (Lincoln & Maeda 1992, Malpoux *et al.* 1993) through the modification of the negative E₂ feedback mechanism, as well as driving the distinct annual rhythms of Prl secretion (Lincoln & Clarke 1994) in seasonal mammals. Specifically, Prl secretion peaks during the summer months (long day length) and reaches a nadir in the winter months (short day length) in sheep (Lincoln & Short 1980), deer (Schulte *et al.* 1980, 1981), cattle (Koprowski & Tucker 1971, Karg & Shams 1974) and Tammar wallaby (Tyndale-Biscoe & Hinds 1984, Hinds & Tyndale-Biscoe 1985), but not in the Bennett's wallaby (Curlewis *et al.* 1986, Brinklow & Loudon 1993). We report in this study a similar pattern of seasonal Prl secretion in the female brushtail possum. However, evidence in sheep and deer suggests that Prl is not the mediator of the breeding season, or other functions under seasonal control such as voluntary food intake, body weight, wool growth and pelage changes (Milne *et al.* 1990, Curlewis *et al.* 1991). Although reproductive cyclicity is evident in the brushtail possum, commencing during decreasing day length and terminating during increasing day length (Smith *et al.* 1969, Crawley 1973, Kearle 1984, Cowan 1990, McLeod *et al.* 1999), there is no evidence of a role of Prl in the regulation of seasonal breeding in this, or any marsupial species. Additionally, administration of bromocriptine (dopamine agonist) resulted in reactivation of the suppressed CL and resumption of the development of the quiescent embryo and subsequent birth in the female Tammar wallaby during lactational, but not seasonal, anoestrous (Tyndale-Biscoe & Hinds 1984).

Daily changes in melatonin secretion also result in a prominent circadian rhythm of Prl secretion in the ram during long days; however, the timing of this cycle shifted and the rate of secretion diminished after exposure to short days (Lincoln & Short 1980). Similarly, there is a nocturnal rise in Prl in man (Arendt 1988, Thorner *et al.* 1992) although some studies have attributed this to the occurrence of sleep rather than the concomitant elevation in melatonin levels (Sassin *et al.* 1973). Whilst this study did not include intensive sampling to elucidate the precise circadian rhythm of Prl secretion in male possums, the elevated morning and nadir afternoon levels in both female and male possums suggested that one exists in this species.

In conclusion, measurement of plasma Prl concentrations during lactation in the brushtail possum revealed a short period (6 weeks) of elevation in the later stages, whilst concentrations were low during early lactation suggesting that either Prl is not essential for galactopoiesis or that sensitivity of the mammary gland to Prl is markedly enhanced during the early stages of lactation. We also report a distinct biphasic

preovulatory Prl surge, which is unlikely to be involved in luteolysis, but may exert luteotrophic effects in this species although this remains to be determined. Finally, the characteristic circannual and circadian patterns of Prl secretion observed in many mammalian seasonal breeders was also observed in the brushtail possum in this study, but the role of Prl in the regulation of seasonality in this species is unknown.

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