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Reproductive physiology of the female greater bilby (*Macrotis lagotis* Thylacomyidae): evidence for a male-induced luteal phase

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Abstract. Endocrinology of the oestrous cycle, pregnancy and early lactation was investigated in captive Western Australian greater bilbies (*Macrotis lagotis*). Initially, six females were monitored for changes in urogenital cytology, plasma progestogen, pericloacal and pouch morphology in the absence of a male. This was followed by the introduction of a male and a reproductive assessment through mating, gestation and early lactation. In the absence of a male, there was no cyclical pattern of urogenital cytology, pericloacal or pouch development, and progestogen concentrations remained basal. Within 5 days of the introduction of a male, all females had a karyopycnotic index of 100%. Spermatozoa were present in the urogenital smear within 3 days of male introduction in all five females that gave birth. Five to 9 days after the introduction of a male, there was an increase in plasma progestogen concentration that remained elevated for 14–19 days. Six of the seven females gave birth approximately 3 days after reaching peak plasma progestogen concentrations. Gestation length ranged between 14 and 17 days. Plasma progestogen concentrations of the postpartum and early lactation period were lower ($P < 0.0001$) than during gestation, but greater ($P < 0.0001$) than those recorded before the introduction of a male. One female that gave birth early in the study that was examined until weaning of the pouch young showed a cyclical pattern of plasma progestogen secretion that ended at weaning. This study provides evidence that the luteal phase in the greater bilby is induced by the presence of a male. Similar to female reproductive physiology in the Peramelidae, elevated progestogen concentration in the greater bilby was extended into lactation.

Additional keywords: copulation, faecal, gestation, lactation, plasma, progestogen, urogenital cytology.

Introduction

The greater bilby (*Macrotis lagotis*) is a small omnivorous, nocturnal, solitary marsupial inhabiting arid and semi-arid regions of central Australia (Pavey 2006). Once moderately abundant and widespread over most of mainland Australia (Watts 1969), predation by introduced carnivores, competition with feral herbivores and environmental degradation have reduced the bilby population to approximately 20% of its former range (Kennedy 1992; Southgate 1994). Listed on the Red Species list of the International Union for Conservation of Nature (IUCN) as being vulnerable to extinction, the bilby now occupies only a small isolated area in south-western Queensland and a larger, low-density area in the north-western deserts of the Northern Territory and Western Australia (Moritz *et al*. 1997). In response to the current endangered status of the bilby, the National Recovery Plan for the Greater Bilby (Pavey 2006) was developed. An important objective of this plan was to establish the coordinated management of *ex situ* populations designed for intensive breeding and reintroduction.

Given the central role of captive breeding to the successful conservation of the bilby, it is remarkable that knowledge of the reproductive physiology of this species is limited (McCracken 1986, 1990; Johnston *et al*. 1995; Curnow *et al*. 2001). Previous studies have found that the captive bilby is physiologically capable of breeding throughout the year (McCracken 1983; Southgate *et al*. 2000) and that conception may occur before pouch young are weaned (Southgate *et al*. 2000). Although McCracken (1986) attempted to characterise the oestrous cycle of the bilby by means of urogenital cytology, the cycle lengths varied widely (12–37 days) and no attempt was made to relate cytology to reproductive hormones. Curnow *et al*. (2001) examined changes in faecal steroid hormones to investigate oestrous cycle activity in

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three animals, but the interpretation of faecal hormone profiles was equivocal. With the exception of the latter account, there is currently no published information on the endocrinology of the oestrous cycle, gestation or lactation period in the female bilby. This information is fundamental for understanding key reproductive events and in the validation of behaviour and changes in urogenital cytology as indicators of oestrus.

Presumably because of the close phylogenetic relationship between the Thylacomyidae and Peramelidae, both families were placed in the same reproductive pattern, 'Group 2' marsupials, as defined by Tyndale-Biscoe and Renfree (1987). Members of this group are polyoestrus, polyovular and have an ultrashort gestation period occupying less than the luteal phase, which is prolonged into early lactation. The aim of the present study was to attempt to characterise the reproductive cycle of the bilby in terms of endocrinology and investigate whether its luteal phase is also extended into lactation.

Materials and methods

Animals

Sexually mature female ($n = 7$) and male ($n = 2$) Western Australian bilbies located in the Peron Captive Breeding Centre (Monkey Mia, WA, Australia; latitude 25°43′S, longitude 113°43′E) were used in the present study from early May to late July 2007. Females were aged between 9 months (F3, F4, F5 and F6) and 2 years (F1 and F2); males were 2–3 years of age. The F3, F4, F5 and F6 females were nulliparous, whereas F1 and F2 females had given birth previously; F7 entered the study approximately 2 days before giving birth. None of the animals had any history of reproductive disease and remained clinically healthy throughout the study period. This study was conducted in parallel with the bilby captive breeding programme based at Francois Peron National Park (WA, Australia). Within 3 weeks of giving birth, six of the seven female bilbies were released in Lorna Glen National Park, WA, as part of the reintroduction programme. Access to the animals was given by the Department of Environment and Conservation (Western Australia State Government CE001652) and experimental procedures were approved by The University of Queensland Animal Ethics Committee (SAS/208/07).

Husbandry

Bilbies (females and males) were housed in separate adjacent thick-wire meshed enclosures (4 × 2 m and 2 m high). A common wire-framed roof fixed with synthetic shade cloth stretched over two rows of naturally lit enclosures. Aligned enclosures shared a common wall, the bottom half boarded with sheets of galvanised iron, with thick wire mesh that continued to the roof. There was no visual contact between bilbies, but olfactory and auditory contact was possible. The floor of the enclosure was wire mesh covered in 15 cm of a course sandy substrate; a wooden nest box measuring 1 × 0.5 m and 0.5 m high was provided in each enclosure. Bilbies were fed a daily mix that contained ¼ cup seed mix (Thompson and Redwood Produce Supplies, Upper Swan, WA, Australia), 25 g assorted fruit (banana, apple, grape, kiwi, mandarin and pear) and vegetable (carrots, sweet potato and cucumber), ¼ cup 'Pedigree Advance–Puppy' dog biscuits (Masterfoods, Mars Petcare, Wodonga, Vic., Australia), 1 teaspoon mealworms and 1 teaspoon insectivore mix (Wombaroo Food Products, Adelaide, SA, Australia), supplemented with 5 g cheese, 5 g boiled egg or 5 g 'bilby meat mix' (mince, insectivore mix, sunflower oil, balanced calcium) (Mavlab, Slacks Creek, Qld, Australia), horsepower vitamin E (Equine Nutrition Systems, Vineyard, NSW, Australia) and Divetelact (Sharpe Laboratories Animal Health Division, Ermington, NSW, Australia). Water was freely available at all times.

Anaesthesia

Following capture by hand into soft cotton bags, bilbies were brought into an air-conditioned laboratory in preparation for anaesthesia. Anaesthesia was induced using a face mask with 5% isoflurane (Bomac Animal Health, Hornsby, NSW, Australia) in 1½ L oxygen per min and maintained for venipuncture using 1.5% isoflurane in 1½ L oxygen per min. Induction of anaesthesia took 1–2 min, after which bilbies where maintained for 2 min for blood and urogenital sample collection. All bilbies where fully recovered within 10 min of induction.

Venipuncture

Blood samples (0.5 mL) were collected from the mid-ventral tail using a 25-g needle attached to a winged infusion set and a 1-mL syringe (Becton Dickinson, North Ryde, NSW, Australia). Blood was placed into 1-mL heparinised tubes (Becton Dickinson) and centrifuged at 1600*g* for 5 min. It was possible to collect a blood sample from the mid-ventral tail vein every 3 days over a 3-month period without any evidence of tissue trauma.

Urogenital cytology

Urogenital smears were collected from conscious animals by securing the bilby in a soft cotton bag and exposing the hindquarters. Smears were taken from the anterior extremity of the urogenital sinus using the method described previously by McCracken (1986). Cells from the urogenital epithelium were then smeared onto a microscope slide, air-dried, fixed in methanol and stained with Diff Quick stain (Harleco, Philadelphia, PA, USA). Five cell types were recognised in the urogenital smear: squamous, superficial, intermediate, parabasal and leucocytes (Fig. 1*a*, *b*). Smears were then examined at ×400 magnification and a total of 400 cells was analysed. The karyopycnotic index (KI) was then calculated as described by Peters and Rose (1979). For the present study, the KI (0–1) was defined as the number of squamous + superficial + intermediate cells divided by the total number of cells counted. When females were paired with males, the entire smear was also scanned for the presence of spermatozoa (Fig. 1*a*).

Examination of external genitalia and pouch condition

The pericloacal region was examined for any evidence of engorgement or tumescence. Previous observations by Lyne (1976) had noted swelling of the lips of the urogenital opening (pericloacal) during oestrus in the bandicoot, so this phenomenon was also examined in the bilby. Pouch condition

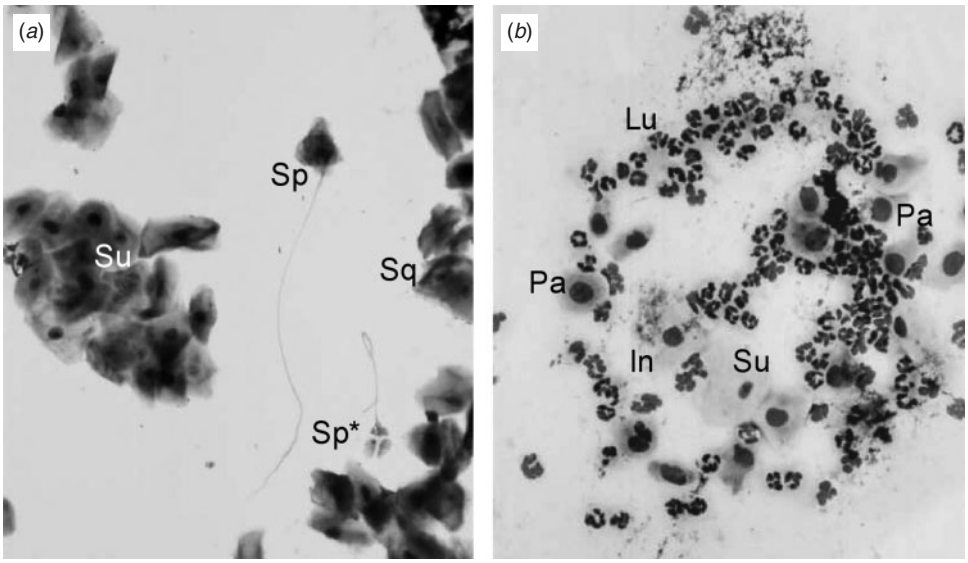

Fig. 1. Bilby urogenital smears. (*a*) Urogenital smear taken from a female bilby 3 days after introduction of a male bilby, containing 100% cornified epithelium and spermatozoa. (*b*) Urogenital smear taken from a female bilby before introduction of a male. In, intermediate epithelial cell; Lu, leucocyte; Pa, parabasal epithelial cell; Sp, spermatozoa; Sp*, spermatozoa with decondensed sperm head; Sq, squamous epithelial cell; Su, superficial epithelial cell.

throughout the oestrous cycle and gestation was also documented subjectively and categorised into one of five pouch morphologies (Fig. 2) as follows: (*1*) Stage 0 or nulliparous pouch, non-glandular, non-vaginated, pale pink in colour; (*2*) Stage 1 development, non-glandular, loose folds in skin, slightly swollen appearance, pale pink in colour; (*3*) Stage 2 development, shiny appearance, vaginated, deep pink in colour; (*4*) Stage 3 full development of pouch in preparation for neonate, deeply vaginated, glandular, purple in colour; and (*5*) Stage 4 parous pouch, vaginated, non-glandular, deep pink in colour.

Progestogen assay

Plasma concentrations of progestogen were determined by enzyme immunoassay (EIA) using a monoclonal antibody to progesterone (CL425; Coralie Munro, University of California at Davis, Davis, CA, USA). The antibody cross-reacts with a broad range of progestogens (Munro and Stabenfeldt 1984), including progesterone (100%), 4-pregnen-3β-ol-20-one (172%), 4-pregnen-3α-ol-20-one (188%), 4-pregnen-11α-ol-3,20-dione (147%), 4-pregnen-11β-ol-3,20-dione (2.7%), 5α-pregnan-3α-ol-20-dione (64%), 5α-pregnan-3β-ol-20-one (94%), 5α-pregnan-3,20-dione (55%), 5β-pregnan-3,20-dione (8%), 5β-pregnan-3α-ol-20-one (2.5%) and 5β-pregnan-3β-ol-20-one (12.5%). Standards for the assays were prepared using progesterone (P8783; Sigma-Aldrich, St Louis, MO, USA). The intra- and interassay coefficients of variation were both $<12\%$. Serial dilutions showed parallelism to the standard curve (Fig. 3).

Experimental design

Commencing in May 2007, six sexually mature females were housed individually without male contact for approximately 2 months. This period was based on the duration of at least three presumptive oestrous cycles (mean ($\pm$ s.e.m.) 21 ± 7 days), as defined by McCracken (1986). Blood samples were collected every 3 days throughout this period, with urogenital smears taken every 1–3 days. Changes in pouch condition and pericloacal morphology were also noted during this period. In late June and early July, a male bilby was progressively introduced into each of the female enclosures. Dates of joining were as follows: F5, 16–25 June; F6, 22–27 June; F4, 26 June–1 August; F2, 28 June–5 August; F1, 2–7 August; F3, 6–21 August. Because all animals were part of a controlled breeding programme, only two males were used for mating. The male was removed upon the consecutive appearance (4–5 days) of a very high proportion of cornified cells and the presence of spermatozoa in the urogenital smear. Blood samples (every 3 days) and urogenital smears (every 1–3 days) were collected following the introduction of the male for approximately 3 weeks after the male had been removed. This period represented the potential gestation and early lactation periods. In order to determine the first presence of spermatozoa in the female reproductive tract, urogenital cytology was conducted daily until the male was removed from the female. An additional female gave birth early in the study to two live pouch young and this female was subsequently sampled every 3 days throughout the entire lactation period through to weaning.

Statistical analyses

Concentrations of plasma progestogen collected from five females before the introduction of males, during gestation and early postpartum were analysed using a repeated-measures analysis using the MIXED procedure in SAS version 8.2 (SAS Institute, Cary, NC, USA). Data were transformed to natural logarithms before analysis. A first-order autoregressive error structure provided the most appropriate model. Least-squares means for time period were compared using *t*-tests.

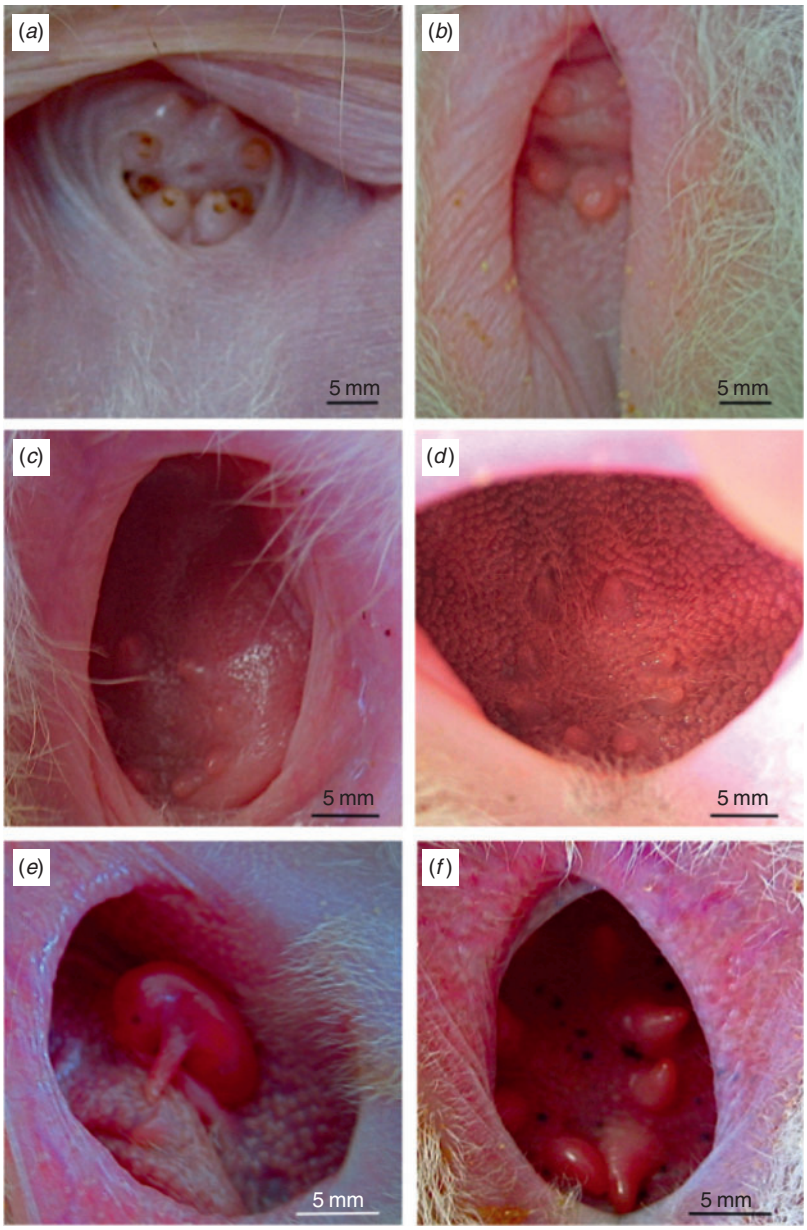

Fig. 2. Bilby pouch morphology. (*a*) Stage 0 or nulliparous pouch: non-glandular, non-vaginated, pale pink in colour. (*b*) Stage 1 development: non-glandular, loose folds in skin, slightly swollen appearance, pale pink in colour. (*c*) Stage 2 development: shiny appearance, vaginated, deep pink in colour. (*d*) Stage 3 full development of pouch in preparation for neonate: deeply vaginated, glandular, purple in colour. (*e*) Active pouch with 1-day-old neonate. (*f*) Stage 4 parous pouch: vaginated, non-glandular, deep pink in colour.

Results

Female reproductive physiology before the introduction of the male

Changes in urogenital cytology and concentrations of progestogen in six females before the introduction of the male are shown in Fig. 4. There was no evidence of regular cyclical patterns in urogenital cytology (KI) during the 2 months before the introduction of a male. Plasma progestogen concentrations before the introduction of a male remained basal in all females. There were also no consistent patterns of change in the appearance of the clitoris, pericloacal region, teats or pouch.

Female reproductive physiology after introduction of the male

Changes in urogenital cytology and concentrations of progestogen in six females after the introduction of a male are shown in

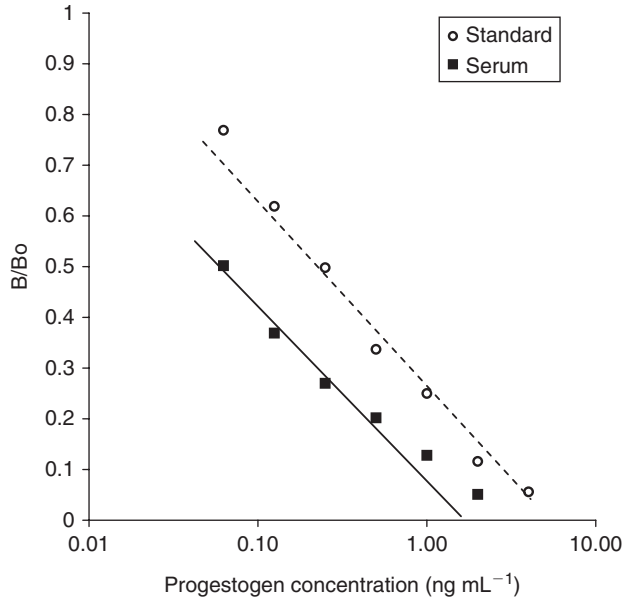

Fig. 3. Parallelism of pooled bilby plasma progestogen with the assay standard.

Fig. 4. Approximately 3.0 ± 0.7 days (mean $\pm$ s.e.m.) after the introduction of a male there was a marked change in urogenital cytology, with most females showing a KI of 1. Spermatozoa were found in the urogenital smears of all six females either on the day the male was introduced (F1 and F3) or the day after (F4), 3 days after (F2 and F5) or 6 days after (F6). The day of parturition was observed for F1, F4 and F5. Based on the first presence of spermatozoa in the reproductive tract, the respective gestation periods were 16, 14 and 17 days. Pouch young were also observed in the F2 and F3 females 2 days after the last pouch check, so that the approximate gestation periods of these animals ranged from 12 to 14 and from 15 to 17 days, respectively.

Five (F1), 6 (F2) and 9 (F3, F4, F5 and F6) days after the introduction of a male bilby, there was a steady rise in plasma progestogen concentrations. The mean ($\pm$ s.e.m.) of the two highest plasma progestogen concentrations for those bilbies that gave birth (F1–F5) was 3.95 ± 0.81 ng mL^{-1} ($n = 10$ samples), which was significantly greater ($P < 0.05$) than their overall mean basal concentration of 0.33 ± 0.02 ng mL^{-1} ($n = 100$ samples). All bilbies gave birth during a period of elevated plasma progestogen; female F2 gave birth to one young, the F3, F4 and F5 females gave birth to two young and F1 gave birth to three young. The average of the two peak luteal plasma progestogen concentrations of the female (F6) that failed to give birth (8.77 ng mL^{-1}) were substantially higher than those in the females that gave birth.

There were marked changes in pouch development once the male was introduced and these were most marked in nulliparous females, which possessed only a rudimentary pouch at the time the male was introduced (Fig. 2*a*). Nulliparous pouches were pink in appearance, non-glandular and non-vaginated so that teats were fully exposed to the exterior. The developed pouches of the two parous females were pink in appearance, non-glandular and vaginated so that teats were fully enclosed within the pouch (Fig. 2*f*). In preparation for the housing of neonates, the pouch became highly glandular, purple in colour, mucoid in appearance and deeply vaginated (Fig. 2*e*). The progressive development of the pouch (Fig. 2*a–d*) was consistent with a rise in progestogen concentration. There was some variability between individuals as to when, in the luteal phase, the pouch showed changes in development. For two females (F4 and F5) these changes occurred 10 days before birth and in one female (F3) this phenomenon occurred 6 days before birth. A fourth nulliparous female (F6) that failed to give birth also showed pouch development similar to those females that did give birth. Serial observations of the pericloacal region showed no evidence of engorgement or tumescence after introduction of the male.

Combined plasma progestogen profiles of the gestation and early lactation periods of the five females (mean $\pm$ s.e.m.) that gave birth are shown in Fig. 5. Following parturition, progestogen concentrations declined over the next 3 days and then appeared to plateau during early lactation. The combined progestogen concentration during early lactation (>3 days postpartum) of all five females that produced pouch young ($n = 23$; mean 1.31 ng mL^{-1}; 95% confidence interval (CI) 1.03–1.67 ng mL^{-1}) was 2.8-fold lower ($P < 0.0001$) than peak progestogen concentrations during gestation ($n = 14$; mean 3.68 ng mL^{-1}; 95% CI 2.78–4.87 ng mL^{-1}), but 4.4-fold higher ($P < 0.0001$) than the basal concentrations of progestogen before the introduction of the male ($n = 100$; mean 0.30 ng mL^{-1}; 95% CI 0.26–0.34 ng mL^{-1}). The early post-luteal progestogen profile of the female (F6) that failed to produce pouch young ($n = 7$, 0.81 ± 0.05 ng mL^{-1}) was less than that of the females that did give birth.

Female reproductive physiology from parturition to weaning

Female F7 gave birth 2 days after commencing the study and was subsequently examined every third day until the pouch young were weaned for changes in urogenital cytology and plasma progestogen (Fig. 6). Although there appeared to be regular cycles of slightly elevated plasma progestogen every 11–12 days postpartum, the peak postpartum progestogen concentrations (approximately 2 ng mL^{-1}) of female F7 were substantially less than the concentration measured 2 days before parturition (9.5 ng mL^{-1}). After female F7 had weaned the pouch young (81 days), the plasma progestogen concentration declined to basal concentrations. The KI of female F7 showed little evidence of regular cycles during the lactation period, although there was a high KI (0.98) observed 6 days before weaning.

Discussion

The results of the present study represent the first endocrine description of reproductive physiology in the female greater bilby based on plasma progestogen and have revealed some novel reproductive biology in this species. Although female bilbies were adjacent to males and had auditory and olfactory access, they were visually and physically separated during the first part of the study. During this period, there was no evidence of regular cyclical changes in the cell types of the urogenital epithelium and

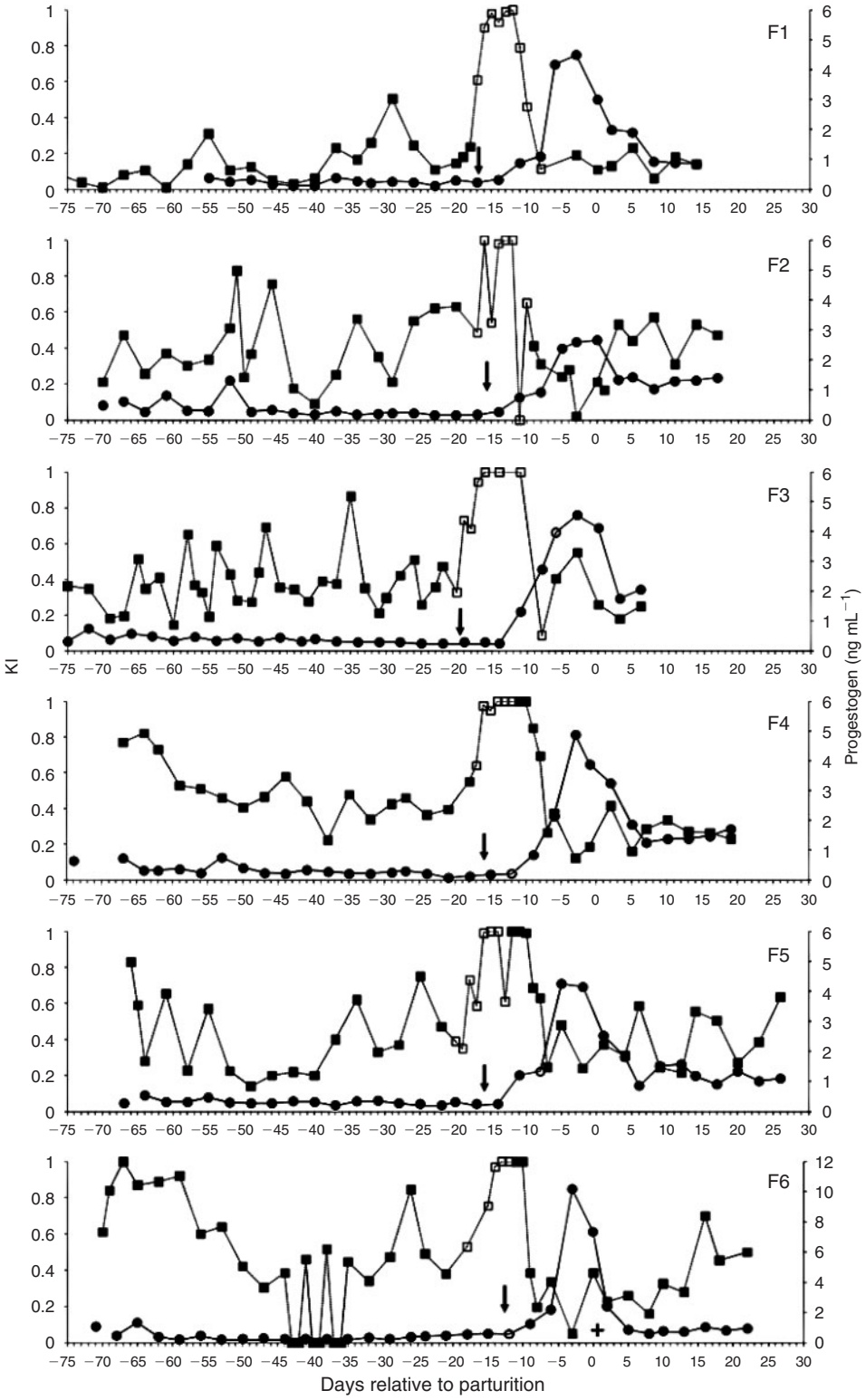

Fig. 4. Relationship between plasma progestogen concentrations (ng mL^{-1}) in female bilbies (F1–F6) and the karyopycnotic index (KI; 1 = 100%) before and after the introduction of males. (●), plasma progestogen concentration; (■), KI; (□), housed with male; (○), initial changes in pouch development; ↓, first presence of spermatozoa in the urogenital smear; +, no pouch young found.

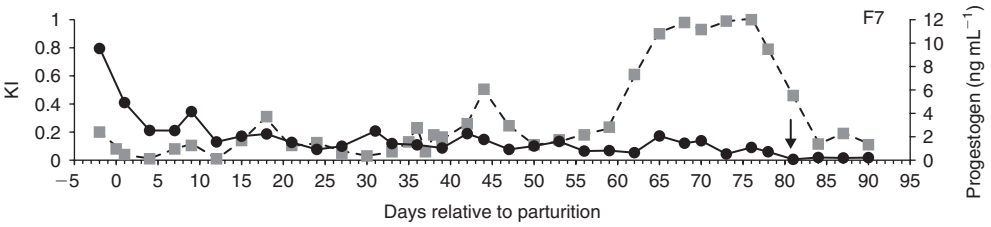

Fig. 5. Relationship between plasma progestogen concentrations (ng mL^{-1}) in female bilby F7 and the karyopycnotic index (KI; 1 = 100%) throughout the peripartum period, lactation and weaning (•), plasma progestogen concentration; (■), KI; ↓, pouch young exit.

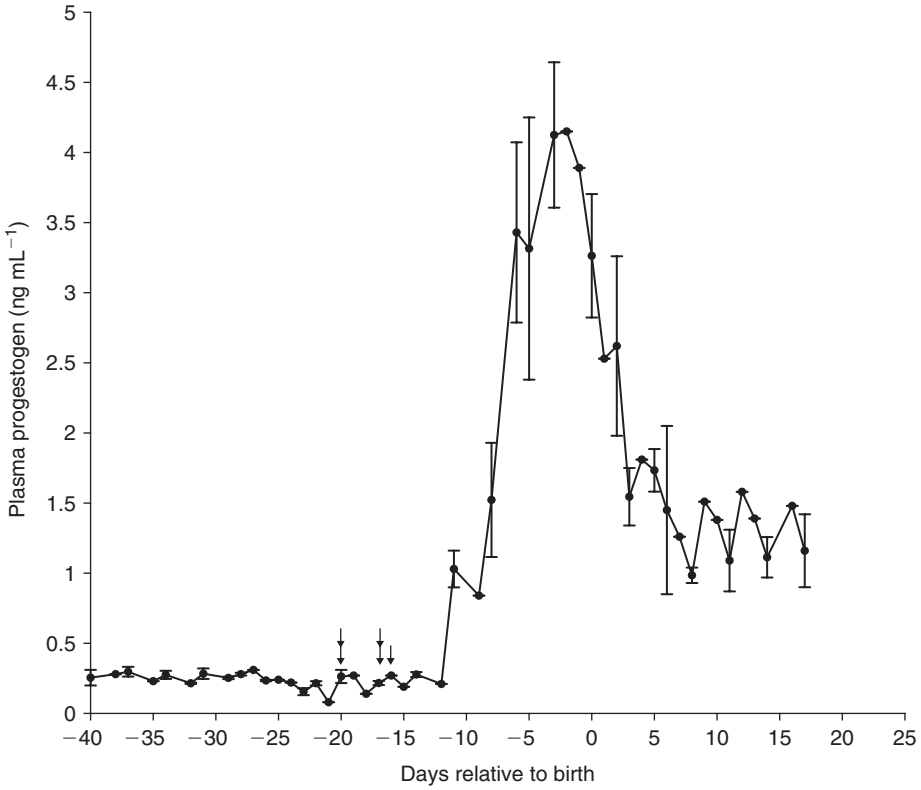

Fig. 6. Mean (± s.e.m.) progestogen concentrations (in ng mL^{-1}+; •) in the five females that ultimately gave birth. ↓, introduction of the male bilby into the female enclosure.

plasma progestogen concentrations remained basal. There was also no evidence of behavioural oestrus or changes in the pouch or external genitalia. This was despite the fact that females were housed separately without male contact from 55 to 75 days, sufficient time to allow the observation of at least one and possibly up to five periods of oestrus (McCracken 1986).

Although captive bilbies are physiologically capable of breeding throughout the year (McCracken 1983; Southgate *et al.* 2000), there is some evidence from wild populations that the breeding season may vary in accordance with environmental conditions (Jones 1924; Hulbert 1972). Perhaps the variability in breeding season reported by these earlier studies was dependant on rainfall and food availability; consequently, captive bilbies with sufficient food resources are capable of breeding throughout the year. Given the previous breeding history of the bilby population at the Peron Captive Breeding Centre and the fact that the males were introduced to females in a staggered manner over a 3-week period, it is highly unlikely that introduction of the male bilbies in the present study coincided with an acute photoperiodic trigger in seasonal reproduction.

The lack of a clear pattern of oestrous cycle activity based on urogenital cytology in the present study is in partial agreement with the observations of McCracken (1986), who observed that only four of eight non-lactating females cycled regularly in the absence of the male, whereas the remaining animals remained in a period of anoestrus for 42–72 days. In the latter study, three of the four anoestrous females eventually commenced cycling. One possible explanation for the difference between

these observations and those of the present study may be that in the study of McCracken (1986), females were housed in groups of four, whereas in the present study females were housed separately in accordance with their naturally solitary behaviour (Southgate *et al*. 2000). In addition, assessment of reproductive status by McCracken (1986) was unaccompanied by any endocrine evidence of a luteal phase.

The most notable feature of ovarian function in the females in the present study before the introduction of the male was the basal secretion of progesterone. This suggests that the physical absence of the male is somehow related to a failure of ovulation and the subsequent lack of formation of a functional corpus luteum. However, it is difficult to ascertain based on information from the present study whether the lack of a luteal phase is associated with a coital- or semen-induced ovulatory pattern, as in the koala (Johnston *et al*. 2000), or whether the physical presence of the male is simply required to initiate ovarian activity after a period of anoestrus. Evidence of male-induced oestrus has been recorded in other female marsupials, such as the gray short-tailed opossum (*Monodelphis domestica*; Fadem 1985; Hinds *et al*. 1992) and the brush-tailed bettong (*Bettongia penicillata*; Hinds and Smith 1992; Smith 1992).

On introduction of the male, there was a marked change in female reproductive physiology. Within 5 days, all females had a KI of approximately 1. In four of the five females that subsequently gave birth, spermatozoa were apparent in the urogenital smear within a day of the male being introduced, indicating that copulation had occurred. This could suggest that all four females were quickly brought into oestrus upon male introduction or, alternatively, that males are capable of subduing, mounting and mating irrespective of female reproductive status. The latter seems unlikely because McCracken (1986) reported that female bilbies typically reject attention by the male and show aggressive behaviour that includes biting the male on the face, neck or forelegs.

Approximately 5–9 days after the introduction of the male, plasma progestogen concentrations began to rise, consistent with the formation and function of a corpus luteum; all four nulliparous females showed elevated plasma progestogen after 9 days. Plasma progestogen concentrations reached a peak within 3 days before parturition. The pattern of plasma progestogen secretion observed during the gestation period of the bilby is similar to that described for the brown bandicoot (Gemmell 1995) in that both species give birth during a period of high progestogen secretion. A minor difference between the bilby and bandicoot is that most bilbies (four of five) gave birth as progestogen secretion began to decrease 3 days after peak progestogen secretion, whereas maximal progestogen secretion in the bandicoot is maintained after birth and into the early lactation period (Gemmell 1995).

The early stage of the bilby luteal phase (gestation) was associated with an extremely rapid development and morphological change of the pouch in all four nulliparous females. The pouches of both nulliparous and parous females became deeply vaginated and glandular in association with peak plasma progestogen concentrations just before parturition, indicating that pouch development was closely linked to the secretion of progestogen.

Following parturition and during early lactation, plasma progestogen concentrations were significantly lower than those during gestation, but greater than the basal progestogen concentrations recorded before males where introduced; these data clearly show that progestogen secretion in the bilby is extended into early lactation. This pattern of post-luteal elevated progestogen concentration was also evident in the female (F6) that failed to give birth and through most of the lactation period in female F7. Although the plasma progestogen concentration in F7 during the lactation appeared to show cyclical peaks above 2 ng mL^{-1} approximately every 11 days for most of lactation, progestogen secretion declined immediately after weaning.

Although the latter observations are limited to only two females, the data may represent preliminary evidence that the suckling stimulus of the bilby pouch young is potentially luteotrophic; a similar profile of progesterone secretion during early lactation has been reported in the brown bandicoot (*Isoodon macrourus*; Gemmell 1979, 1984, 1995). A difference between the bilby and bandicoot is that progestogen concentrations in the bandicoot remain elevated in the early lactation period and then decline steadily to a basal level 19 days after parturition, whereas preliminary data from the present study suggest that progestogen secretion in the bilby declines rapidly to a post-luteal plateau within 5 days of parturition but this level is subsequently maintained throughout lactation.

The present study has provided the first endocrinological description of the female bilby reproductive cycle based on plasma progestogen. Females not in contact with a male did not show oestrous cycle activity, as judged by urogenital cytology and plasma concentrations of progestogen. These findings indicate that ovulation and the luteal phase are dependent on the presence of the male, although it is still not clear whether male introduction was associated with commencement of ovarian activity following a period of anoestrus or induction of ovulation via a copuloreceptive reflex (Johnston *et al*. 2000). Similar to the bandicoot (Gemmell 1979, 1984, 1995) there was evidence in the present study that progestogen secretion is extended into lactation. This study has also shown that it possible to repeatedly collect blood samples from the bilby without any adverse effects. It is recommended that future studies conduct venipuncture on a daily basis in order to document the endocrinology of the oestrous cycle on a finer scale than what has been presented here. Future studies should include the use of remote cameras to document oestrus behaviour, confirm copulation and facilitate a better knowledge of parturition and the gestation period.

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