ELSEVIER

Animal Reproduction Science 72 (2002) 245–257

ANIMAL REPRODUCTION SCIENCE

www.elsevier.com/locate/anireprosci

Faecal progesterone metabolites and behavioural observations for the non-invasive assessment of oestrous cycles in the common wombat (*Vombatus ursinus*) and the southern hairy-nosed wombat (*Lasiorhinus latifrons*)

M.C.J. Paris [a,*], A. White [b], A. Reiss [b], M. West [a], F. Schwarzenberger [c]

[a] *Monash Institute for Reproduction and Development, 27-31 Wright Street, Melbourne, 3168 Vic., Australia*
[b] *Melbourne Zoo, P.O. Box 74, Parkville, Melbourne,Vic. 3052, Australia*
[c] *Institut für Biochemie and L. Boltzmann-Institut für Veterinärmedizinishe Endokrinologie, Veterinärmedizinishe Universität Wien, Veterinärplatz 1, A-1210 Vienna, Austria*

Received 2 January 2002; received in revised form 1 May 2002; accepted 7 May 2002

Abstract

Wombats belong to Australia's unique marsupial species. Two of the three remaining species, the common wombat (*Vombatus ursinus*) and the southern hairy-nosed wombat (*Lasiorhinus latifrons*) are abundant. The third species, the northern hairy-nosed wombat (*Lasiorhinus krefftii*) has only about 115 individuals left in the wild. This study aimed to gain further insight into the basic reproductive biology of wombat species and evaluate the value of faecal progesterone metabolites and behavioural patterns as a means for non-invasive monitoring of the oestrous cycle in common and the southern hairy-nosed wombats. In an initial study, three different faecal steroid assays showed that 20α-OH-pregnanes were the main progesterone metabolites. These metabolites were examined in captive female common wombats ($n = 5$) and southern hairy-nosed wombats ($n = 2$). In one female common wombat and in one male common wombat oestrous related behavioural data were obtained. Individual cycling females exhibited a significant relationship between plasma progesterone and faecal pregnanes. In the common wombat, the values for faecal pregnanes showed an oestrous cycle length of 55.1 ± 11.7 days with a follicular phase of 25.6 ± 6.3 days and a luteal phase of 28.2 ± 12.7 days. The data for faecal pregnanes obtained in the southern hairy-nosed wombat during the breeding season gave an oestrous cycle length of 41.1 ± 12.8 days with a

* Corresponding author. Present address: Department of Gastroenterology and Clinical Nutrition, Royal Children's Hospital, Flemington Road, Parkville, Melbourne, Vic. 3052, Australia. Tel.: +61-3-93455060; fax: +61-3-93456240.
E-mail address: parism@murdoch.rch.unimelb.edu.au (M.C.J. Paris).

0378-4320/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0378-4320(02)00088-X

follicular phase of 27.9 ± 12.3 days and a short luteal phase of 13.3 ± 1.1 days. The behavioural data show that the faecal sniffing behaviour of the male, tended to increase around the time that oestrous was found. In conclusion, monitoring of 20α-OH-pregnanes in wombat faeces could be a useful methodology to monitor reproductive cycles in the wombat, and can possibly be applied to monitor the endangered northern hairy-nosed wombat.
© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Wombat; Faecal steroid analysis; Behaviour; Progesterone; Pregnanes; Marsupial

1. Introduction

Australia is home to three large herbivorous, burrowing, nocturnal, pouched mammal (marsupial) species, the common wombat (*Vombatus ursinus*), the southern hairy-nosed wombat (*Lasiorhinus latifrons*), and the northern hairy-nosed wombat (*Lasiorhinus krefftii*). The common wombat is found in Victoria, New South Wales, Tasmania, and parts of South Australia. The southern hairy-nosed wombat is confined to South Australia and parts of Western Australia, but both species are locally highly abundant in areas of suitable habitat. Due to their burrowing behaviour they are seen as a pest in pastoral areas and culling programs are in place for population control (Temby, 1994). At present, licensed culling is the authorised management strategy.

In contrast, the northern hairy-nosed wombat is among Australia's rarest marsupial and is one of the world's most endangered mammals (Gordon et al., 1985; Horsup, 1996). Currently, the remaining individuals are restricted to one single population within Epping Forest National Park in central Queensland. At best estimate, 115 animals, including approximately 20 breeding females, are believed to survive in the wild (Horsup, 1996). No northern hairy-nosed wombats are kept in captivity. Despite an ongoing recovery program for the northern hairy-nosed wombat over the last decade, it is now suggested that natural breeding even in conjunction with habitat protection and restoration, can not guarantee the survival of this endangered marsupial species. Assisted reproductive technologies may be the only way to protect the existing gene-pool from further loss, and to help generate a sustainable captive population, which could be reintroduced to a suitable habitat.

A basic understanding of the reproductive biology of a species is essential before attempts can be made to manipulate or assist fertility. Although several studies have investigated aspects of the biology of wombat species, there is very little data published on female wombat reproduction (Gaughwin, 1979; Moritz et al., 1998; Peters and Rose, 1979; Triggs, 1996; Tyndale-Biscoe and Renfree, 1987; West et al., 2001). The reticence of wombats to breed in captivity makes it difficult to study reproduction in captive animals and their cautious, nocturnal nature makes field studies challenging.

Tools to monitor the reproductive status non-invasively may assist in increasing the general knowledge in wombat reproduction by allowing easy monitoring of captive animals but also provide a field application. Schwarzenberger et al. (1996a, 1997) have developed faecal steroid assays to monitor the progesterone metabolites present in the female's faeces for a wide range of wildlife species. These methods may be suitable for non-invasive monitoring of the wombat species. Once the reproductive cycle of a wombat is characterised in detail, studies investigating reproduction in free ranging animals can be designed and

manipulation of fertility (fertility control in the two abundant species and assisted reproduction in the endangered northern hairy-nosed wombat) can be further investigated.

A long-term study of southern hairy-nosed wombats shows that they are seasonal breeders and suggests that environmental factors might influence the rate of reproduction (Gaughwin and Wells, 1978). Hamilton et al. (2000) examined the endocrinology of male southern hairy-nosed wombat using faecal androgens. This study showed that faecal testosterone is a valid indicator of male reproductive status in this species, with significant correlations between faecal testosterone, plasma testosterone, and prostate and bulbourethral gland weights.

Reproduction in common wombats appears to be less strictly seasonal (Moritz et al., 1998). The occurrence of females with pouch young (ranging in age from newborn to 10 months old) in summer, autumn, and winter and ovarian follicular activity in collected reproductive material (Paris et al., unpublished data) suggests that there is no distinct seasonal reproductive pattern. In addition, histological analysis has shown that the common wombat is monovular with ovulations alternating between ovaries (Moritz et al., 1998). In 1977, one study documented the oestrous cycle of captive common wombats, indicating an oestrous length of 32–34 days as analysed using vaginal smear patterns (Peters and Rose, 1979). We recently established a captive colony of common wombats and studied the progesterone profile in those animals. From those data, we concluded that the common wombat is poly-oestrus and that the oestrus cycle ranges from 45 to 51 days (West et al., 2001). In addition, one captive female was monitored after pouch young removal and it was found that it may take up to 1 year to return to oestrus. This suggests that the common wombat may not be seasonally reproductive but has a long lactational and postlactational anoestrus period.

Over the last few decades several reports have been published on reproductive and social behaviour of both common and southern hairy-nosed wombats in captivity (Triggs, 1996; Condor, 1970; Crowcroft and Soderlund, 1977). One study reported (Peters and Rose, 1979) that female captive common wombats became very active at oestrous, and would pace continually, one animal becoming very vocal and aggressive. In addition, a flehmen response (Triggs, 1996; Gaughwin, 1979) has been reported. Flehmen occurs in many mammal species, and is very common in ungulates. Flehmen response in wombats occurs when the male sniffs very closely the scats or urine of the female. The male raises his head in the air, pulling the upper lip back in a series of rapid movements exposing the teeth (Triggs, 1996).

The goal of this study was to investigate the value of faecal pregnanes and behaviour as non-invasive monitoring tools for female common wombats and the southern hairy-nosed wombats to increase understanding of wombat reproductive biology. In a primary data set, different assay systems for the measurement of faecal progesterone metabolites have been tested for their suitability to follow the oestrous cycle. In addition, faecal results in the common wombat were validated by the determination of plasma progesterone.

2. Materials and methods

2.1. Animals

Two female common wombats (*V. ursinus*) were captured using cage traps for basic female reproduction studies as permitted by a DNRE permit, and approved by the AEESC from the

University of Melbourne, Australia. Another two female common wombats previously held for parasite studies were transferred to this experiment. All four animals (CW1–4) were individually housed at the Department of Veterinary Sciences, University of Melbourne, Werribee. They were fed daily and had access to water ad libitum. Another female common wombat (CW5), one male common wombat (CW6) and two female southern hairy-nosed wombats (SHN1-2) were held at Melbourne Zoo.

2.2. Blood collection

In the four common wombats (CW1–4) held at the Department of Veterinary Sciences, peripheral heparinized blood samples were collected, alternately from one of the superficial leg veins. Samples were collected three times per week between July 2000 and March 2001. For this procedure, sedation was needed using intramuscular Zoletil (4 mg/kg). Blood samples were centrifuged at 1500 rpm for 5 min. Plasma was subsequently stored at −20 °C until analysis.

2.3. Faecal collection

From the four common wombats (CW1–4) held at the Department of Veterinary Sciences, fresh faecal samples were collected on average three times per week at 8 a.m. between July 2000 and March 2001. Samples from the Melbourne Zoo wombats (CW5, and SHN1-2) were collected on average three times per week between June 1996 and May 1997. Care was taken to collect fresh samples that were not contaminated by urine. All samples were immediately stored at −20 °C, shipped to Vienna, Austria and kept at −20 °C until analysis.

2.4. Behavioural observations

Detailed behavioural data were collected daily for one female (CW5) and one male common wombat (CW6), housed separately, over the period March 1996–1997. For the female, CW5, the following were noted at 09:00 each day: amount of food consumed overnight, amount of overnight digging, general level of activity, behaviours indicative of heightened aggression or agitation (including attacking enclosure furniture, vocalisations, and attitude toward keeper), and sleeping and defecation sites. Each morning the male, CW6, was first locked in his night den. A sample of the female's faeces was collected from her enclosure and placed on a concrete surface in the male's enclosure in the same position each day. Then, the male was released, and his reaction to the female's faeces was examined in detail including: (a) whether the male came out of the den on his own accord (within 5 min), (b) whether he approached the faeces immediately, after some time (time recorded), or not at all, and (c) time spent sniffing the faeces and presence of other behaviours (raising head/sniffing air, pawing at faeces, snorting, putting nose in faeces, salivating, vocalizing, and curling lip/baring teeth). Observations ended 5 min after the male moved away from the faeces.

2.5. Blood progesterone analysis

The assay for measuring progesterone values (nmol/l) was an automated chemilluminescence system (Chiron Diagnostics ACS:180 Progesterone kit), which is a competitive

immunoassay using direct chemiluminescent technology and an acridiunium ester-labeled mouse monoclonal anti-progesterone antibody. This assay has a high specifivity with a cross-reactivity of less than 0.95% with other compounds. The progesterone concentrations can be measured within the range of 0.1–60 ng/ml. The interassay coefficient variation was 8.8% and the intra-assay coefficient variation was 4.1%. Serial dilution of wombat plasma showed linearity.

2.6. Faecal steroid analysis

Faecal samples were extracted before analysis by enzyme-immunoassays (EIA). For the extraction, 0.5 g of wet faeces was mixed with 0.5 ml distilled H_2O and 4 ml methanol. This mixture was shaken for 30 min at room temperature whereafter centrifuged for 15 min. The supernatant was subsequently diluted to 1 in 10 in assay buffer and used for EIA. The assays performed for measuring the faecal steroids have been previously developed (Schwarzenberger et al., 1993, 1997). In brief, the steroid antibodies used in this study were group specific antibodies raised in rabbits and the EIAs were performed in microtiter plates coated with sheep anti rabbit IgG.

In a primary data set, three different EIAs were tested for their suitability to follow estrous cycles in wombat faeces. Antibodies tested were those against 5β-pregnane-3α,20α-diol 3-gluc:BSA (trivial name pregnanediol; 5β-20α-OH-pregnanes; Schwarzenberger et al., 1993); 5α-pregnane-20α-ol-3-one 3CMO:BSA (5α-20α-OH-pregnanes) and against 5α-pregnane-3β-ol-20-one 3HS:BSA (20-oxo-pregnanes; Schwarzenberger et al., 1996b). These antibodies showed significant crossreactivities with different 5-reduced pregnanes containing either a 20-oxo or a 20α-OH-group and were therefore termed group-specific (Schwarzenberger et al., 1997). Cross-reactivities for the 5β-20α-OH-pregnane and for the 20-oxo-pregnane assays were described previously (Schwarzenberger et al., 1993, 1996b). The most significant cross-reactivities with the 5α-20α-OH-pregnane assay were those with a series of 5α reduced pregnanes, namely 5α-pregnane-20α-ol-3-one (384%); 5α-pregnane-3β,20α-diol (40%); 5α-pregnane-3α,20α-diol (8.9%); 5α-pregnane-20β-ol-3-one (6.4%); the only significant cross-reactivity with steroids from the 5β-pregnane series was those against 5β-pregnane-20α-ol-3-one (26.3%).

After initial testing of a series of samples, all other samples were only analysed using the 5β-20α-OH-pregnane assay and further results obtained with this assay are termed 20α-OH-pregnanes. This assay was further validated by demonstrating parallelism between standard curves and serial dilutions of the faecal extracts and by showing that faecal values followed the same trend as the values obtained with the plasma progesterone assay. The intra- and interassay coefficients of variation for the assays tested were similar to those described previously and were between 10 and 15%.

2.7. Data analysis

Data are presented as mean ± S.E. oestrous cycle lengths, follicular phase lengths and luteal phase lengths were calculated for individual animals showing oestrous cycles and mean values were also calculated for the combined values in both wombat species. Definition of the follicular phase and luteal phase was based on blood progesterone and faecal

pregnanes values, respectively. The luteal phase for blood values was defined as starting with the first point that the concentration of progesterone increased and remained above 1.5 ng/ml and ending when values had dropped and stayed below this value. The in between time was defined as the follicular phase. The time from the start of a luteal phase till the start of the next luteal phase was defined as the oestrus cycle length.

The onset of the luteal phase for faecal pregnanes was defined as the first point after which values had increased above 120 ng/g in faeces and remained at >120 ng/g faeces for at least two consecutive values and ending when values dropped and stayed below this value. The in between time was defined as the follicular phase. The time from the start of a luteal phase till the start of the next luteal phase was defined as the oestrus cycle length.

All faecal and blood data were transformed using a logarithm transformation, and regression analysis was performed to estimated the correlation between the transformed blood progesterone and values for faecal pregnanes measured on the same day. To compare the male's sniffing response with the values found for faecal pregnanes in the female, a non-parametric correlation coefficient was calculated using the Spearman Rank correlation test.

3. Results

In Fig. 1, the faecal test results are shown in CW5 and SHN 1, where the specificity for faecal 5β-20α-OH-pregnanes, 20-oxo-pregnanes, and 5α-20α-OH-pregnanes, using antibodies against these progesterone metabolites, was tested. The results clearly show that the most suitable assay for the analysis of wombat faeces is to measure the amount of 5β-20α-OH-pregnanes present. Therefore, in all other collected faecal samples, only the assay for 5β-20α-OH-pregnanes was used after this initial testing with the three assays.

In Fig. 2, all obtained blood and faecal samples have been graphed from CW1 to CW4. From these data, it was concluded that two common wombats, CW1 (graphed in Fig 2a) and CW3 (graphed in Fig. 2b), showed regular cycling patterns and two other wombats remained anoestrus during the time period studied. From the data collected from animals CW1 and CW3, it was calculated that cycling common wombats have an oestrous cycle of 43.8 ± 6.4 days (CW1: 38.7 ± 6.4, and CW3: 46.8 ± 4.4) with a follicular phase of 13 ± 3.7 (CW1: 12.3 ± 2.9, and CW3: 13.4 ± 4.3) days and a luteal phase of 30.4 ± 5.7 (CW1: 26 ± 6, and CW3: 33 ± 4.3) days when measuring the blood progesterone values. The oestrous cycle length measuring faecal pregnanes in common wombats was calculated in CW3 and CW5 as 52.3 ± 8.2 days (CW3: 45.6 ± 6.0, and CW5: 60.8 ± 10.8) with a follicular phase of 25.3 ± 9.7 days (CW3: 30 ± 6.5, and CW5: 22.7 ± 4.5) and a luteal phase of 27 ± 6.3 days (CW3: 17.5 ± 5.1, and CW5: 36.8 ± 9.8). The obtained cycle lengths for CW3 for progesterone and pregnanes are comparable. In the cycling female, CW1, oestrous length could not be determined by fecal pregnanes as the pregnane values did not return to basal values. Data shown in Fig. 2, show that a lag time between blood and faecal samples is not clearly present, even in cycling females.

In Fig. 3a and b, all data obtained with the collected faecal samples are graphed from SHN1 and SHN2. In these animals, measuring faecal pregnanes, a comparable total oestrous cycle length of 41.1 ± 12.8 days was found, with a follicular phase of 27.6 ± 12.5 days and

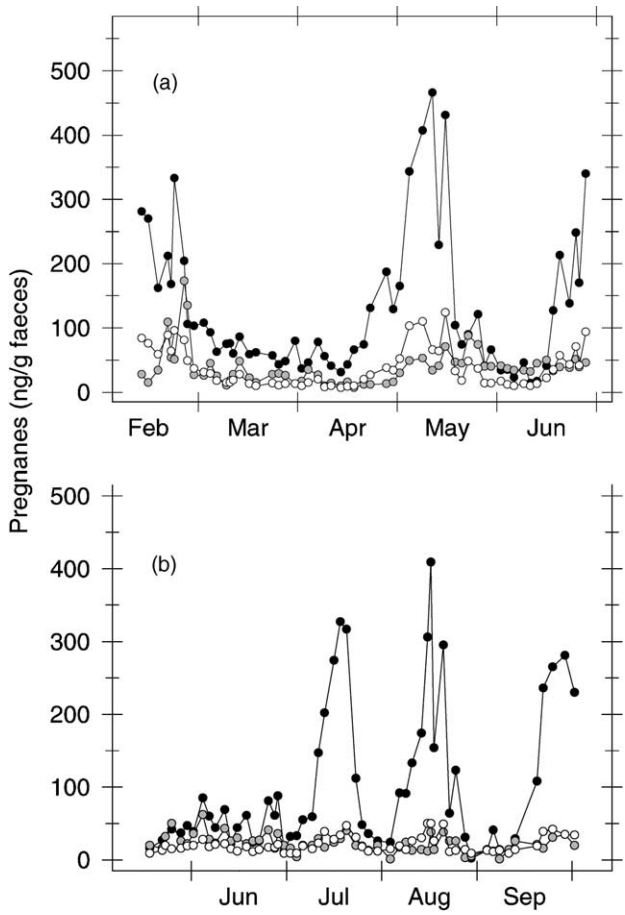

Fig. 1. Testing of three different assays for their suitability to follow estrous cycles in wombat faeces: (a) common wombat (CW5), and (b) southern hairy-nosed wombat (SHN1). Antibodies used in the different assays were those against 5β-20α-OH-pregnanes (●), 20-oxo-pregnanes (◍), and 5α-20α-OH-pregnanes (○).

a relatively short luteal phase of 13.3 ± 1.1 days. In the common wombats cycling patterns were found year round while in the two southern hairy-nosed wombats the luteal phases were only observed between the end of June and the beginning of January.

Regression analysis showed a strong linear relationship between transformed blood progesterone and faecal pregnanes in CW1 and CW3 with a highly significant relationship (CW1: $r^2 = 64.7\%$, $P < 0.0005$, and CW3: $r^2 = 60.1\%$, $P < 0.0005$). These animals are both showing regular oestrous cycle patterns as defined by the obtained measurements in blood progesterone and faecal pregnanes. This implies that for a cycling common wombat it would be possible to predict the blood progesterone value from the values for faecal pregnanes. On the contrary, the correlation between values of non-cycling females, CW2 and CW4, is not significantly different from zero (CW2: $r^2 = 0.1\%$, $P > 0.1$, and CW4:

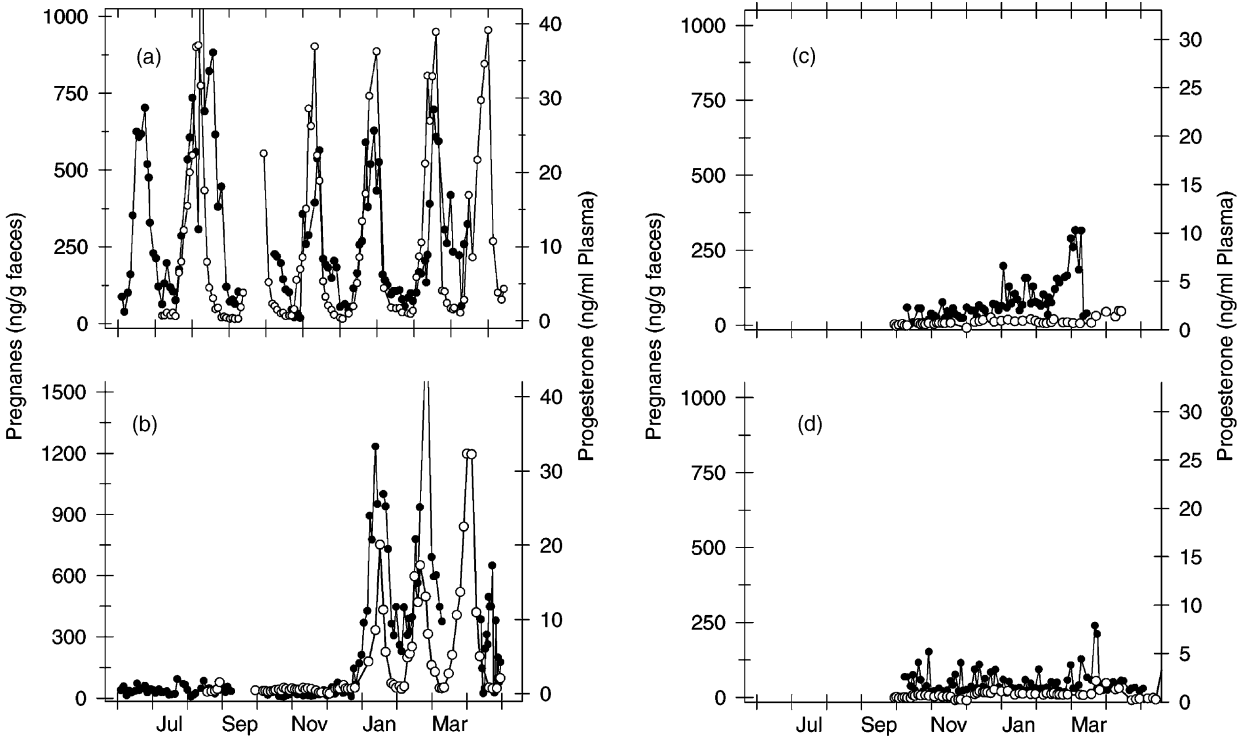

Fig. 2. Plasma progesterone (○) and faecal 5β-20α-OH-pregnane (●) values during oestrous cycles in common wombats: (a) CW3; (b) CW1; (c) CW2; (d) CW4.

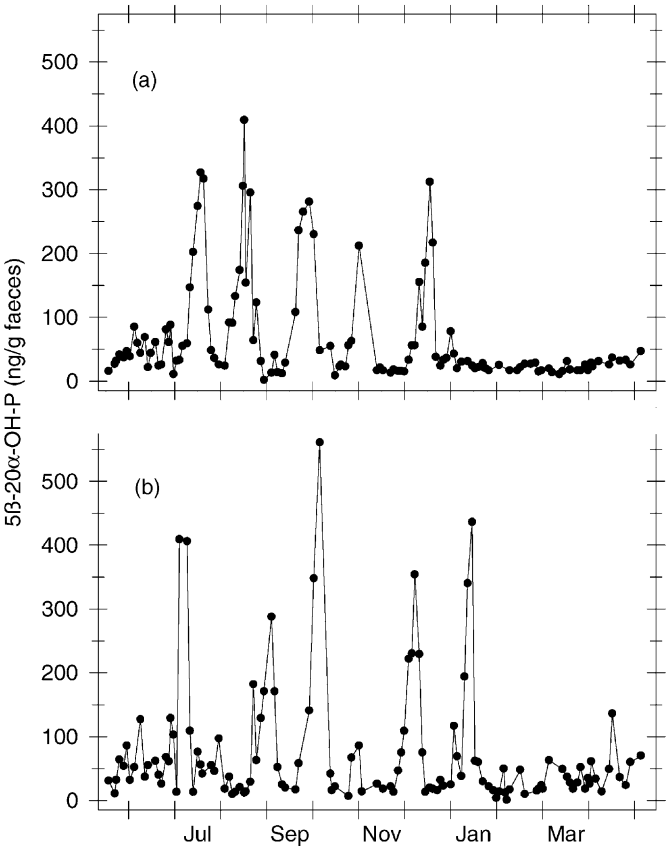

Fig. 3. Faecal 5β-20α-OH-pregnanes (●) in the southern hairy-nosed wombat: (a) SHN1, and (b) SHN2.

$r^2 = 0.3\%$, $P > 0.1$) and this implies that the variable low values found in anoestrous animals make it impossible to confirm a positive correlation.

In Fig. 4, the length of sniffing is shown in correlation to the values for faecal pregnanes in the female CW5. The male's (CW6) most common response to the female's faeces was to sniff them. Only on a small number of occasions did he exhibit any other response. Flehmen was observed three times during the year of observation and always coincided with a relative long sniffing time. On the days of observed flehmen display, the male also investigated the connecting door between the exhibits, and spent some time on the log in his enclosure facing the female's exhibit, sniffing with head raised. A non-parametric correlation coefficient was calculated for the length of sniffing and the faecal pregnane values using the Spearman Rank correlation test. The results for the complete data set ($r_s = -0.095$, d.f. $= 143$, $P = 0.126$) indicate that there was a negative correlation ($r_s = 0.095$) between the behavioural data and the faecal steroid concentrations, with a probability of 12.6% that this was due to chance. This is suggestive of a negative relationship between the male's interest in the faeces, and the levels of pregnanes in the sample, however, the result is not statistically significant.

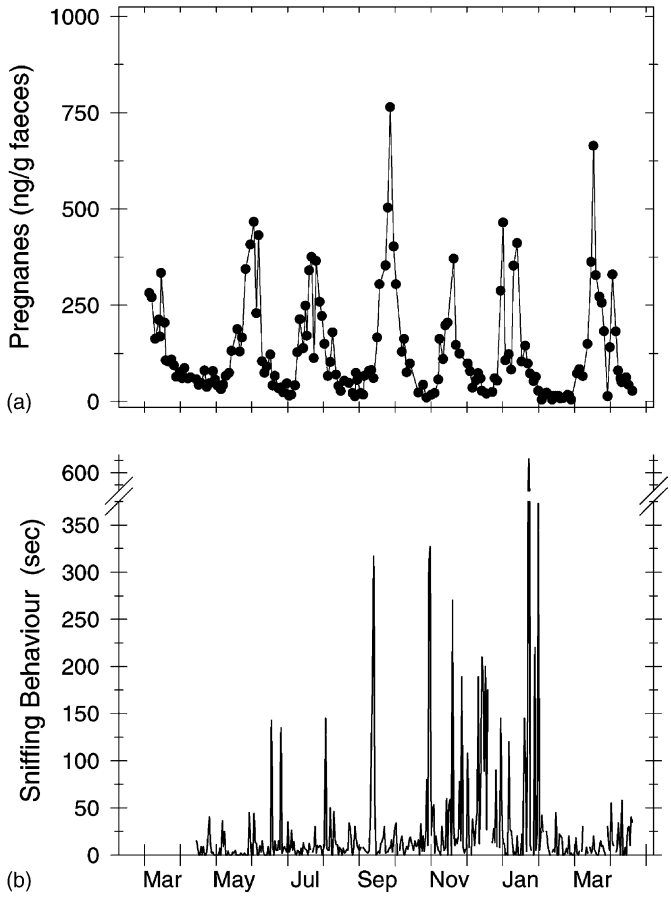

Fig. 4. Faecal 5β-20α-OH-pregnanes (●) in the female common wombat CW5 (a) and length of time (in s) of the male CW6 (b), sniffing the faeces of CW5.

4. Discussion

In both, captive and free-ranging wombats, regular blood or urine collection is very difficult. Faecal steroid analysis is, therefore, at this stage, the only realistic choice for non-invasive reproductive monitoring (Schwarzenberger et al., 1996a,b). In contrast to the wombat species, a lot of basic female reproductive work has been done in other marsupial species (Tyndale-Biscoe and Renfree, 1987), with the tammar wallaby (*Macropus eugenii*), a member of the family of Macropodoidae, being the example of the most well studied species. On the other hand, the basic reproductive cycle has also already been characterised in a number of other marsupial species (Lemon, 1972; Rose and Jones, 1996; Crawford et al., 1997; Fletcher, 1989) and the progesterone profile has also been studied in the koala, a species closely related to the wombat (Johnston et al., 2000). The mean duration of

the non-mated oestrous cycle was reported to be 32.9 ± 1.1 days with the progesterone concentrations remaining at basal values throughout this period due to the fact that this species is an induced ovulator. Following mating, a luteal phase could be recognised, by significant increases in progesterone values, and the cycle length was then reported to be 52.5 ± 0.8 days. The two wombat species studied here had successive luteal phases without mating which suggests spontaneous ovulation. Therefore, the comparison of the duration of the oestrus cycle between the wombat and the koala needs to be based on the oestrous cycle length that includes the luteal phase. This leads to the interpretation that the cycle length of the koala is comparable to that found in the two wombat species.

Peters and Rose (1979) reported in captive common wombats in Tasmania, an oestrous cycle length ranging from 32 to 34 days using repeated vaginal smears. Preliminary data published by our group (West et al., 2001), together with the data presented in this study indicate a longer oestrous cycle length with a mean in these animals of about 45 days. Plasma progesterone data are consistent with patterns found using vaginal smears in these animals (West et al., 2001). The differences between those studies are unclear but may be related to large differences in oestrous cycle length in individual animals, which in our study varied between 40 and 60 days in the common wombat. In contrast, the differences in cycle length were much less pronounced in the southern hairy-nosed wombat.

The data found in this study for the common wombat show that in the cycling wombats a significant linear relationship exists between the values for blood progesterone and faecal pregnanes. This implies that, once the linear relationship for an individual, cycling, wombat has been determined it is possible to predict blood progesterone values from the values obtained for faecal pregnanes. The seasonality of cyclic changes in faecal steroid metabolites seen in the southern hairy-nosed wombat corresponds with the known breeding season in this species (Hamilton et al., 2000). Finding the consistent repetitive pattern in our faecal steroid data, we conclude that monitoring faecal steroids is a reliable tool to assess the reproductive status in both wombat species studied. According to the absolute values and to the differences between the follicular and the luteal phase measured in the three different assays tested, the wombat seems to predominantly excrete 5β-reduced pregnanes containing a 20α-OH group. Results of this study again emphasise the importance of testing different assay systems, when faecal steroid analysis is being developed for a previously non-studied species, as different species show different steroid excretion patterns (Schwarzenberger et al., 1997).

Food retention times in the gut correlate to delayed steroid excretion, however, our results did not reveal a significant lag time between changes in the peripheral blood profiles and corresponding changes in the faecal metabolite profiles. Other studies have previously demonstrated that differences in faecal retention times can be found in both wombat species (Hume and Barboza, 1998) ranging from 35 to 44 h for fluid markers and from 62 to 75 h for particle markers in the common wombat. Retention times in the southern hairy-nosed wombat range from 28 to 49 h for fluid markers and from 52 to 69 h for particle markers. In our study, faecal samples were only collected on average three times per week. The lack of lag time seen in our data may be due to this relatively infrequent recording of the profiles, as lag times may have been more apparent with analysis of daily samples. We do not have data to indicate whether a lag time exists in the southern hairy-nosed wombat but would conclude from our results that daily sampling would also be essential in this species to answer this question.

The behavioural data showed that the male wombat tended to show increased sniffing behaviour in the period before the increases in faecal pregnanes were detected. The observed flehmen response has been described previously by Gaughwin et al. (1979). He suggested that in wombats flehmen may assist in the detection of approaching oestrous. Its occurrence is possibly influenced by the social structure at mating and it is beneficial for the male wombat to detect oestrous while investigating the burrows of females, because he could then remain in the area. In addition, flehmen may be essential to determine the receptivity of a female without too much overt sexual behaviour. Further behavioural observations should be tested as an additional non-invasive method for monitoring wombats that are housed separately in order to determine the timing of introduction of the male to the female for breeding purposes. However, such studies should be accompanied by faecal steroid monitoring.

The results reported here add to the methodology for studying reproduction in the wombat. They show that monitoring faecal pregnanes could be a useful methodology for monitoring reproductive cycles in the wombat and that further observational studies are needed as they might provide valuable tools. The presented results on faecal pregnanes provide further insights in wombat reproductive biology and deliver the basic knowledge required to manipulate fertility. The ability to successfully manipulate reproduction in the three wombat species will have several significant applications. Firstly, wombats breed poorly in captivity, so methods to monitor and induce oestrous and ovulation may be important to assist breeding management of these species. Secondly, increased knowledge of wombat reproduction can be utilised to develop population control strategies based on manipulation of reproduction. Thirdly, and most importantly, increased knowledge of reproductive biology in the southern hairy-nosed wombat and the common wombat will help in the successful development of advanced reproductive technologies for the critically endangered northern hairy-nosed wombat.

Acknowledgements

We thank John Bryant for his assistance in collecting the faecal samples at the Veterinary Department of the University of Melbourne and Lee Skerrat for donating two wombats for our experiments. In addition, we would like to thank Michael Daskalakis for his continued interest in wombat blood samples. We would also like to thank the keeping and veterinary staff of Melbourne Zoo for their assistance and the "Friends of the Zoo" volunteers for collecting behavioural observations. In addition, we are grateful to A. Aichinger and E. Leitner for technical assistance with faecal steroid assays and to E. Möstl (Vienna) for providing steroid antibodies and enzyme labels. We would also like to acknowledge Dr. Malcolm Clark, School of Mathematical Sciences, Monash University for giving statistical assistance. The Monash Institute of Reproduction and Development is acknowledged for financial support.

References

Condor, P., 1970. Breeding of the common wombat in captivity. Vic. Nat. 87, 322.

Crawford, J., Shackell, G.H., Thompson, E.G., McLeod, B.J., Hurst, P.R., 1997. Preovulatory follicle development and ovulation in the brushtail possum (*Trichosurus vulpecula*) monitored by repeated laparoscopy. J. Reprod. Fertil. 110 (2), 361–370.

Crowcroft, P., Soderlund, R., 1977. Breeding of wombats (*Lasiorhinus latrifrons*) in captivity. Der Zoologische Garten 47, 313–322.

Fletcher, T.P., 1989. Plasma progesterone and body weight in the pregnant and non pregnant kowari, *Dasyuroides byrnei* (Marsupialia: Dasyuridae). Reprod. Fertil. Dev. 1, 65–74.

Gaughwin, M.D., 1979. The occurrence of flehmen in a marsupial—the hairy-nosed wombat (*Lasiorhinus latifrons*). Anim. Behav. 27, 1063–1065.

Gaughwin, M.D., Wells, R.T., 1978. General features of reproduction in the hairy-nosed wombat (*Lasiorhinus latrifrons*) in the Blanch Town region of South Australia. Bull. Aust. Mamm. Soc. 5, 46–47.

Gordon, G., Riney, T., Toop, J., Lawrie, B.C., Godwin, M.D., 1985. Observations on the Queensland hairy-nosed wombat *Lasiorhinus krefftii*. Biol. Conservat. 33, 165–195.

Hamilton, R.A., Stanton, P.G., O'Donnell, L., Steele, V.R., Taggart, D.A., Temple-Smith, P.D., 2000. Determination of seasonality in southern hairy-nosed wombats (*Lasiorhinus latrifrons*) by analysis of fecal androgens. Biol. Reprod. 63, 526–531.

Horsup, A., 1996. The northern hairy-nosed wombat recovery program: trials and triumphs. In: Stephens, S., Maxwell, S. (Eds.), Back from the brink: refining the threatened species recovery process, Surrey Beatty and Sons: Chipping Norton, Australia, pp. 150–154.

Hume, I.D., Barboza, P.S., 1998. The gastrointestinal tract and digestive physiology of wombats. Wombats, Surrey Beatty, Chipping Norton, Australia, Chapter 7, pp. 67–74.

Johnston, S.D., McGowan, M.R., O'Callaghan, P.O., Cox, R., Nicolson, V., 2000. Studies of the oestrus cycle, oestrus and pregnancy in the koala (*Phascolarctos cinereus*), oestrus and pregnancy in the koala (*Phascolarctos cinereus*). J. Reprod. Fertil. 120, 49–57.

Lemon, M., 1972. Peripheral plasma progesterone during pregnancy and the oestrus cycle in the tammar wallaby, *Macropus eugenii*. J. Endocrinol. 55, 63–71.

Moritz, K., Green, R.H., Selwood, L., 1998. A histological analysis of the reproductive cycle of the common wombat *vombatus ursinus*. In: Wells, R.T., Pridmore, P.A. (Eds.). Wombats, Surrey Beatty, Chipping Norton, Australia, pp. 75–85.

Peters, D.G., Rose, R.W., 1979. The oestrus cycle and basal body temperature in the common wombat (*Vombatus ursinus*). J. Reprod. Fertil. 57, 453–460.

Rose, R.W., Jones, S.M., 1996. The association between basal body temperature, plasma progesterone and the oestrus cycle in a marsupial, the Tasmanian bettong (*Bettongia gaimardi*). J. Reprod. Fertil. 106, 67–71.

Schwarzenberger, F., Francke, R., Göltenboth, R., 1993. Concentration of faecal immunoractive progestagen metabolites during the oestrous cycle and pregnancy in the black rhinoceros (*Diceros bicornis michaeli*). J. Reprod. Fertil. 98, 285–291.

Schwarzenberger, F., Möstl, E., Palme, R., Bamberg, E., 1996a. Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. Anim. Reprod. 42, 515.

Schwarzenberger, F., Tomasova, K., Holeckova, D., Matern, B., Möstl, E., 1996b. Measurement of fecal steroids in the black rhinoceros (*Diceros bicornis*) using group-specific enzyme-immunoassays for 5-reduced 20-oxo-pregnanes. Zool. Biol. 15, 159–174.

Schwarzenberger, F., Palme, R., Bamberg, E., Möstl, E., 1997. A review of faecal progesterone metabolite analysis for non-invasive monitoring of reproductive function in mammals. Int. J. Mamm. Biol., Zeitschrift für Säugetierkunde 62 (Suppl. II), 214–221.

Temby, I., 1994. The law and wombats in Australia. In: Wombats in Australia, Surrey Beatty, Chipping Norton, Australia, Chapter 30, pp. 305–311.

Triggs, B., 1996. The Wombat: Common Wombats in Australia. University of New South Wales Press, Sydney, NSW.

Tyndale-Biscoe, H., Renfree, M. (Eds.), 1987. Book: Reproductive Physiology of Marsupials. Cambridge University Press, Cambridge.

West, M., Paris, M., Galloway, D., Wright, P., Shaw, J., Trounson, A., 2001. The oestrous cycle of the common wombat (*Vombatus ursinus*) assessed by comparing vaginal smears and plasma progesterone concentrations. In: Proceedings of the Australian Society of Reproductive Biology, Brisbane, Australia, 62.