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# Faecal steroid metabolites for non-invasive assessment of reproduction in common warthogs (*Phacochoerus africanus*), red river hogs (*Potamochoerus porcus*) and babirusa (*Babyrousa babyrussa*)

Eva M. Berger [a], Kristin Leus [b], Paul Vercammen [c], Franz Schwarzenberger [a,*]

[a] *Department of Natural Sciences - Biochemistry, University of Veterinary Medicine, Veterinärplatz 1, A-1210 Vienna, Austria*

[b] *Centre for Research and Conservation, Royal Zoological Society of Antwerp, Koningin Astridplein 26, B-2018 Antwerp, Belgium*

[c] *Breeding Centre for Endangered Arabian Wildlife, PO Box 29922, Sharjah, United Arab Emirates*

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**Abstract**

The objectives of this study were to analyse faecal steroid metabolites in African and South East Asian pig species kept in European zoos. Species studied were the warthog (*Phacochoerus africanus*), the red river hog (*Potamochoerus porcus*) and the babirusa (*Babyrousa babyrussa*). Faecal samples were collected 1–3 times per week from non-pregnant and pregnant captive female warthogs ($n = 9$), red river hogs ($n = 7$) and babirusas ($n = 5$). Enzyme-immunoassays for faecal progesterone, androgen, and oestrogen metabolites, were tested for their ability to determine follicular and luteal phases. In all three species, oestrous cycles could be monitored with 20α-OH- and 20-oxo-pregnane assays. In contrast, oestrogens and androgens were not useful in characterising follicular activity during the oestrous cycle in any species. Faecal 20α-OH- and 20-oxo-pregnane values were significantly correlated. Faecal pregnane concentrations revealed species-specific differences. Luteal phase values

* Corresponding author. Tel.: +43 1 250774104; fax: 43 1 250774190.
*E-mail address:* Franz.Schwarzenberger@vu-wien.ac.at (F. Schwarzenberger).


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of 20α-OH-pregnanes were considerably higher than 20-oxo-pregnanes; 20α-OH-pregnanes were in the range of 3–10 μg/g in warthogs and red river hogs, whereas concentrations were 30–200 μg/g faeces in the babirusa. Regular oestrus cycles had a length of about 35 days in all three species studied. Results indicated a seasonal influence on the occurrence of reproductive cycles in the warthog with anoestrous periods in the European summer. The red river hog was found to be a seasonal and poly oestrous breeder; oestrus cycles started by January and continued until summer. In contrast, the babirusa showed non-seasonal ovarian cyclicity. In pregnant red river hogs, progesterone metabolites were comparable to luteal phase values of the oestrous cycle during the first 3 months of gestation, but did further increase during the last month of pregnancy. Oestrogens and 17-oxo-androstanes were significantly elevated during the second half of gestation. In summary, the reproductive biology of three exotic pig species was studied using non-invasive faecal steroid analysis and these methods were used for comparative investigations of oestrous cycles, pregnancy and seasonality.


*Keywords:* Pig species; Reproductive cycles; Faecal steroid metabolites; Pregnancy; Seasonality

## 1. Introduction

Within the order Artiodactyla, the suborder Suiformes includes two superfamilies, the Anthracotheroidea (including the family Hippopotamide), and the Suoidea, respectively. The later includes the family Dicotylidae (including three species of peccaries), and the family Suidae. Within the Suidae there are three subfamilies, the Suinae (the true pigs), the Phacochoerinae (the warthogs), and the Babirousinae (Groves and Grubb, 1993). Species included in this study belong to the later three subfamilies and were the red river hog (*Potamochoerus porcus*), the common warthog (*Phacochoerus africanus*) and the babirusa (*Babyrousa babyrussa*).

The common warthog and the red river hog are widespread, locally abundant and relatively non-threatened Afrotropical suids, although both species are now often restricted to protected areas. Warthogs are being more widespread and numerous than red river hogs. Warthogs occur in savannah grasslands, whereas red river hogs are found in swamps, thickly wooded areas and forests. (Radke, 1991; Seydack, 1991; Vercammen and Mason, 1993; Vercammen et al., 1993; Somers et al., 1995; Anderson et al., 1998; D'Huart and Grubb, 2001; Randi et al., 2002; Muwanika et al., 2003). The range of the warthog extends through sub-Saharan west, central and east Africa and southwards to northern Namibia and South Africa. Results of genetic analysis suggest, that the common warthog exists in three genetically distinct groups; one southern, one western and the other in Eastern Africa (Muwanika et al., 2003). According to Groves and Grubb (1993) and Vercammen et al. (1993), two species of 'bush pigs' are recognized: the red river hog (*Potamochoerus porcus*) and the bushpig (*P. larvatus*). The red river hog occurs only in equatorial West Africa, from Senegal to south eastern Zaire, whereas the bushpig has a relatively wide range extending from Somalia to the cape province of South Africa (Seydack, 1991; Vercammen et al., 1993).

Adult male warthogs are usually solitary or live in small, loose bachelor groups, unattached to the small family units, which generally comprise one to three adult females and their offspring. Males fight for females but are not known to defend a territory. The mating system is promiscuous with males mating with numerous females (Radke, 1991;

Vercammen and Mason, 1993; Somers et al., 1995; Plesner-Jensen et al., 1999). In contrast, red river hogs live in small family groups of usually 4–10 individuals consisting of a monogamous breeding pair and their offspring; they are usually territorial (Seydack, 1991; Vercammen et al., 1993). Sometimes, red river hogs aggregate into large groups but it is not yet known what causes these aggregations (Vercammen et al., 1993).

The Babirusa is known only from the Indonesian islands Sulawesi, the Togian islands, and the Sula Islands Taliabu, Mangole and Buru. The babirusa inhabits tropical rainforests on the banks of rivers and ponds abounding in water plants (Macdonald, 1991, 1993). The babirusa is seriously threatened over most of its remaining range by deforestation and hunting pressure (Macdonald, 1991, 1993; Clayton et al., 2000; Milner-Gulland and Clayton, 2002). Babirusa social organization comprises solitary adult males and matriarchal groups of one or a few adult females and their immature young (Macdonald, 1991, 1993; Leus et al., 1992).

Pigs are attractive and charismatic animals for display and education, kept in several European zoos. The major problems with these captive populations are the small numbers of founder animals; the captive red river hog and the babirusa populations are thought to descend from four to three founder animals, respectively (Macdonald, 1991, 1993; Vercammen et al., 1993). Import of new founder animals is restricted or almost impossible due to epizootic diseases (Macdonald, 1991, 1993; Vercammen and Mason, 1993; Vercammen et al., 1993; Anderson et al., 1998). The captive generations of warthogs and red river hogs breed well in captivity; however, a number of wild caught warthog founder animals did not reproduce. The recommendation for captive breeding of the babirusa in European zoos in recent years was to limit breeding, until captive born offspring from new founders from Indonesia have been imported (Macdonald, 1991, 1993; Clayton et al., 2000).

This comparative study aimed to gain insight into the basic reproductive biology of African and South East Asian Suidae species kept in European zoos. A better understanding of their reproductive biology will benefit further breeding management of these exotic pig species. We established non-invasive techniques for monitoring endocrine profiles in warthogs (*Phacochoerus africanus*), red river hogs (*Potamochoerus porcus*) and babirusas (*Babyrousa babyrussa*). These methods were used for comparative investigations of oestrous cycles, pregnancy and seasonality.

## 2. Materials and methods

### 2.1. Animals and sample collection

Faecal samples for this study were collected in several European zoos (Table 1) from sexually mature female warthogs ($n = 9$), red river hogs ($n = 7$) and babirusas ($n = 5$). According to studbook data the first age of breeding for the species studied is about 2 years and the reproductive age range is between 10 and 15 years. Since the study animals were kept in different zoos, the husbandry protocols varied slightly. During the period of faecal sample collection breeding was intended in all warthogs and red river hogs included in this study, whereas in the babirusa it was attempted only in the animal from Edinburgh (Bb #1). Freshly defecated faecal samples were collected 1–3 times per week over periods of 3–22 months. Samples were stored frozen at $-20\,^{\circ}\mathrm{C}$ until analysis.

Table 1
Animals included in this study

| Species and animal ID | House name | Location | Year of birth | Age (years) at the beginning of this study | Sample collection $n$ = months | Captive/ wild born | Proven breeder in relation to study period[c] |
|---|---|---|---|---|---|---|---|
| Warthogs | | | | | | | |
| Wh #1 | Mientje | Antwerp | 1995 | 2.5 | 17 | Wild | No |
| Wh #2 | Truudje | Antwerp | 1995 | 2.5 | 17 | Wild | During |
| Wh #3 | Zina | Antwerp | 1998 | 1.5 | 3 | Captive | After |
| Wh #4 | Thais | Arnhem | 1992 | 7 | 4 | Wild | No |
| Wh #5 | Tischa | Arnhem | 1992 | 7 | 4 | Wild | After |
| Wh #6 | Piggy | Beekse Bergen | 1995 | 4 | 6 | Wild | No |
| Wh #7 | Porky | Beekse Bergen | 1995 | 4 | 6 | Wild | No |
| Wh #8 | Suelin | Dvur Kralove | 1996 | 3 | 22 | Captive | After |
| Wh #9 | Beatrix | Stuttgart | 1982 | 16 | 11 | Captive | Before |
| Red river hogs | | | | | | | |
| Rrh #1 | Frau Nagel | Munich | 1992 | 6 | 11 | Captive | During |
| Rrh #2a[a] | Heidi I | Munich | 1997 | 1 | 10 | Captive | After |
| Rrh #3 | Dickes Kind | Munich | 1997 | 1 | 8 | Captive | After |
| Rrh #4a[b] | Lisa | Rotterdam | 1994 | 4 | 10 | Captive | After |
| Rrh #4b[b] | Lisa | Rotterdam | 1994 | 6 | 7 | Captive | After |
| Rrh #5a[b] | Mona | Rotterdam | 1994 | 4 | 10 | Captive | During |
| Rrh #5b[b] | Mona | Rotterdam | 1994 | 6 | 7 | Captive | During |
| Rrh #6 | Klara | Salzburg | 1998 | 2 | 12 | Captive | During |
| Rrh #2b[a] | Heidi II | Salzburg | 1997 | 3 | 12 | Captive | During |
| Rrh #7 | Susi | Wuppertal | 1991 | 7 | 8 | Captive | No |
| Babirusas | | | | | | | |
| Bb #1 | Muhibah | Edingburgh | 1993 | 10 | 5 | Captive | No |
| Bb #2 | Bega | Munich | 1991 | 12 | 12 | Captive | No |
| Bb #3 | Bettina | Stuttgart | 1975 | 14 | 13 | Captive | Before |
| Bb #4 | Rifka | Vienna | 1990 | 3.5 | 4 | Captive | After |
| Bb #5 | Beroga | Wuppertal | 1990 | 14 | 14 | Captive | Before |

[a] During the course of this study female Rrh #2a was transferred from Munich to Salzburg.
[b] Samples from these animals were collected during subsequent years with a break of 1 year between sample collections.
[c] Proven breeder: bred during, before or after the course of this study.

## 2.2. *Faecal extraction*

Extraction of faecal samples was done using methanol and diethylether as described and used for several other mammalian species (Schwarzenberger et al., 2004, and references herein). Wet faeces was mixed with 4.5 ml aqueous methanol (80%), vortexed, and after centrifugation 1 ml of the supernatant methanol was transferred into a new vial, mixed with 0.5 ml of a 5% $NaHCO_3$ in water solution, and re-extracted with 3.0 ml diethyl ether. The ether phase was transferred into a new vial, and evaporated. The residue was re-dissolved and diluted with assay buffer (1:10 to 1:1.000 according to concentrations) and aliquots were analysed by enzyme-immunoassays (EIA) as described previously (Schwarzenberger et al., 2004).

### 2.3. Enzyme-immunoassays

The extracts were analysed using group-specific EIAs for immuno-reactive progesterone, androgen and oestrogen metabolites (Schwarzenberger et al., 2004). Antibodies used in the EIAs were raised in rabbits (Schwarzenberger et al., 1997). Assays used included 20α-OH-pregnanes (5β-pregnane-3α-20α-diol 3HS:BSA; trivial name pregnanediol), 20-oxo-pregnanes (5α-pregnane-3β-ol-20-one 3HS:BSA), 17-oxo-androgens (5α-androstane-3,17-dione 3-CMO:BSA; trivial name epiandrosterone), and total oestrogens (oestradiol-17β-OH 17-HS:BSA). Intra- and inter-assay coefficients of variation in these assays were below 10 and 15%, respectively (Schwarzenberger et al., 2004).

### 2.4. Data analysis

Definition of an-oestrus periods, the follicular (FP) and luteal (LP) phases of the reproductive cycle were based on the analysis of faecal pregnane concentrations taking into consideration results of both faecal pregnane assays. As samples were not collected every day, the interval between two consecutive values was divided by 2 and resulting values were added to the FP and the LP, respectively. The length of the oestrous cycle was defined as the interval between two subsequent onsets of the LP. Data in the results are presented as mean ± S.E.M.

The length of the luteal phase was calculated on the basis of 20α-OH-pregnanes and 20-oxo-pregnanes. Firstly, luteal activity was defined as the periods when 20α-OH-pregnanes had increased and remained over 200, 1000 and 3000 ng/g faeces for warthogs, red river hogs and babirusas, respectively. Based on this, mean values of 20α-OH-pregnanes during anoestrus periods were calculated. This resulted in mean ± S.D. concentrations of 100.0 ± 61.6, 312.5 ± 302.4 and 1771.8 ± 1094.5 ng/g for warthogs, red river hogs and babirusas, respectively. Based on these results, we defined the LP when 20α-OH-pregnane concentrations were three standard deviations above the mean. This resulted in values of 284.8, 1219.7 and 5055.3 ng/g for warthogs, red river hogs and babirusas, respectively. The final 20α-OH-pregnane threshold for the definition of the onset and the end of the LP was set at 300, 1300 and 5000 ng/g for warthogs, red river hogs and babirusas, respectively (Table 2).

In accordance with the calculations of the 20α-OH-pregnanes, also the 20-oxo-pregnane values for the onset and the end the LP were calculated. The mean ± S.D. concentrations during anoestrus periods were 32.6 ± 26.1, 52.1 ± 41.2 and 108.8 ± 51.3 ng/g for warthogs, red river hogs and babirusas, respectively. For the definition of the LP, 20-oxo-pregnane concentrations of three standard deviations above the mean were calculated; this resulted in 110.9, 175.6 and 262.7 ng/g for warthogs, red river hogs and babirusas, respectively. Accordingly, the final 20-oxo-pregnane thresholds for the definition of the LP were 110, 180, and 270 ng/g for warthogs, red river hogs and babirusas, respectively (Table 2).

A pregnancy in one warthog (Wh #2) and five pregnancies in four red river hogs (Rrh #1, #2b, #5a, #5b and #6) were confirmed by farrowing records. As mating records were not available, gestation lengths in the red river hogs were calculated on the basis of the luteal

Table 2
Summary of results

| | Warthog | Red river hog | Babirusa |
|---|---|---|---|
| Threshold for onset of LP 20α-OH-Pregnanes; 20-oxo-pregnanes | 300 ng/g faeces; 110 ng/g faeces | 1300 ng/g faeces; 180 ng/g faeces | 5000 ng/g faeces; 270 ng/g faeces |
| Length of oestrous cycle | 34–36 days | 34–37 days | 37 days |
| Length of gestation | 165 days | 126.5 ± 1.85 days | 161–164 days[a] |
| Seasonality | Seasonal effect on oestrous cyclicity; decline of ovarian activity during European summer | Seasonal breeding; oestrous cycles during European winter until early summer | Non-seasonal breeder |

[a] According to Chaudhuri et al., 1990 and Houston et al., 2001.

phase at the beginning of gestation. Pregnancy length was calculated from four pregnancies. The fifth pregnancy was not included in the calculation, since the sampling interval during the presumed time of conception was too long. Concentrations of 20α-OH-pregnanes, 20-oxo-pregnanes, oestrogens and 17-oxo-androgens of pregnant red river hogs were aligned to the day of parturition and grouped in 2-week interval. The Pearson coefficient of correlation and linear regression were calculated between the concentrations of fecal 20α-OH- and 20-oxo-pregnanes.

## 3. Results

### 3.1. Determination of suitable assays

Representative profiles of faecal 20α-OH-pregnanes, 20-oxo-pregnanes, oestrogens and 17-oxo-androstanes in the three species studied are shown in Figs. 1–3. In all three species LP and thus oestrous cycles could be monitored with both pregnane assays used. In contrast, oestrogens and 17-oxo-androstanes were unsuitable for the determination of the FP. Faecal steroid metabolite concentrations revealed species-specific differences (Figs. 1–3). LP values of 20-oxo-pregnanes were 0.5–3.0 μg/g faeces in all three species and thus were comparable between species studied. In contrast, LP values of 20α-OH-pregnanes were considerably higher. Values were in the range of 3–10 μg/g in warthogs and red river hogs, whereas concentrations were 30–200 μg/g faeces in the babirusa. Faecal 20-oxo- and 20α-OH-pregnane values were significantly correlated ($p < 0.001$). The correlation coefficients and regression equations between these two hormones were $r = 0.62$, $y = 879.3 + 1.9x$, $n = 322$ in the warthog; $r = 0.78$, $y = 2180.4 + 1.4x$, $n = 550$ in the red river hog; and, $r = 0.84$, $y = 1131.0 + 59.7x$, $n = 244$, in the babirusa.

### 3.2. Reproductive pattern in warthogs

Samples over periods of 11–22 months were collected from four females (Table 1); these females had several oestrus cycles each year (Figs. 1 and 4(a and b)). Additional results

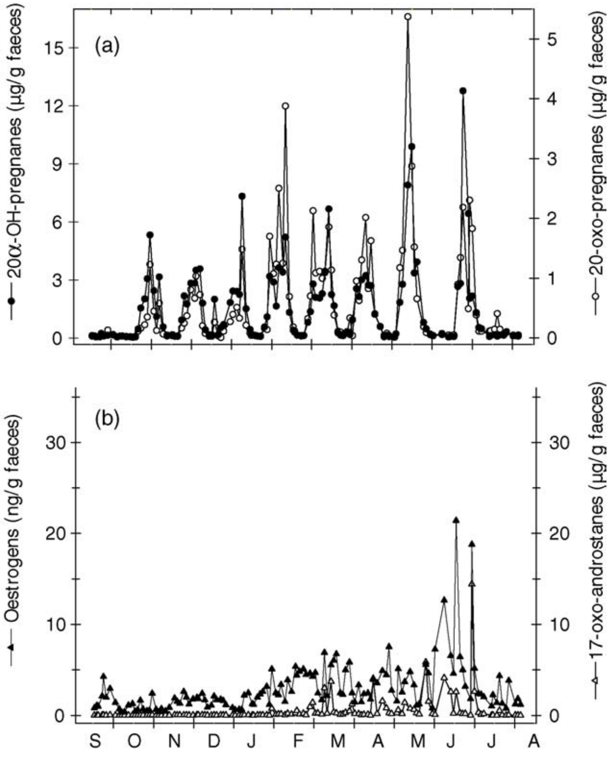


Fig. 1. Representative profiles of (a) faecal 20α-OH-pregnanes (●) and 20-oxo-pregnanes (○); (b) faecal oestrogens (▲) and 17-oxo-androstanes (△) during oestrous cycles in a female common warthog (Wh #1).

from animals listed in Table 1 are not included in the graphs. Results indicate a seasonal influence on oestrous cycles. In the female Wh #8 (Fig. 4a) anoestrus periods occurred between April and October in the first year, whereas it occurred in September in the second year of study. Regular oestrus cycles between September and December were also observed in Wh #4 and Wh #5. Oestrus cycles had a length of 34–36 days. The LP ($n = 26$) showed a mean length of $22.4 \pm 0.42$ days.

Samples during gestation were available from one animal (Wh #2) during the first 2 months and the last month of pregnancy (Fig. 4b). Pregnancy length was 165 days. Oestrogen levels started to increase around 25 days after conception and reached values of about 300 ng/g faeces by the end of the second month of pregnancy. Oestrogen values were in the range of 300–500 ng/g faeces during the last months of gestation; peak values of about 3000 ng/g were observed around 2 days before parturition.

Other warthogs in this study had irregular or missing luteal activity (results not included in the graphs). The animal Wh #9, which had irregular luteal activity was already 16 years. Periods of missing luteal activity during April and May occurred in two other animals (Wh #6 and #7). In addition, missing luteal activity in October, after a luteal phase in September, occurred in Wh #3.

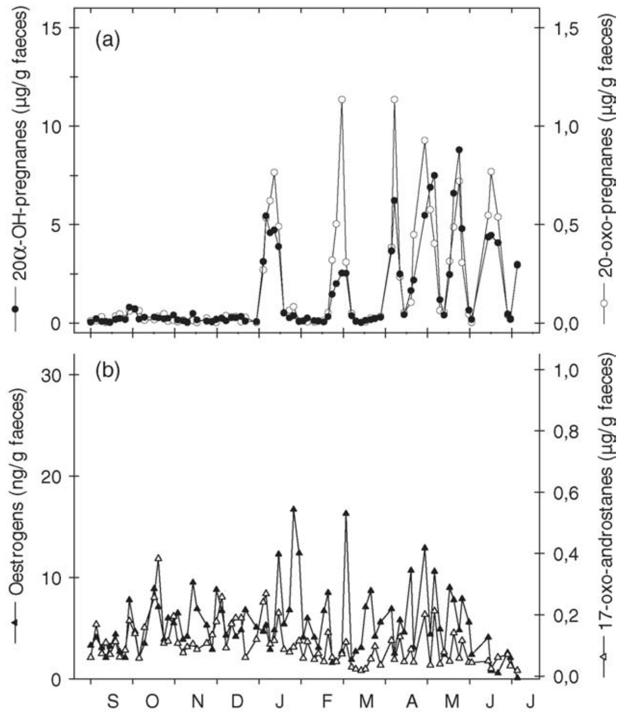


Fig. 2. Representative profiles of (a) faecal 20α-OH-pregnanes (●) and 20-oxo-pregnanes (○); (b) faecal oestrogens (▲) and 17-oxo-androstanes (△) during oestrous cycles in a female red river hog (Rrh #4a).

### 3.3. Reproductive pattern in red river hogs

Our results indicate that red river hogs are seasonal and poly oestrous breeders (Figs. 2 and 5). Oestrus cycles started in late December and continued until summer. Sample collection was stopped between April and July, and therefore the beginning of the anoestrous period observed during late summer and autumn could not be exactly determined. The length of the oestrous cycle was 34–37 days.

Samples from pregnant red river hogs were available from five pregnancies in four individual animals (Rrh #1, 2b, 5a, 5b and 6). Animals #4 and #5 were kept together, but only animal #5 (Fig. 5b) was pregnant two times, although animal #4 had regular oestrous cycles (Fig. 2). Three animals (Rrh #1, 5a and 5b) conceived by mid of January (a representative profile is shown in Fig. 5b). The other two pregnant red river hogs in this study (Rrh #2b and #6) had irregular oestrus cycles commencing by December/January. These animals conceived by mid of March (Rrh #2b) and beginning of May (Rrh #6; Fig. 5c). As mating records were not available, gestation lengths were calculated on the basis of the luteal phase at the beginning of gestation; the range was 121–129 days (126.5 ± 1.85). Progesterone metabolite concentrations during the first 3 months of gestation were comparable to luteal phase values of the oe-

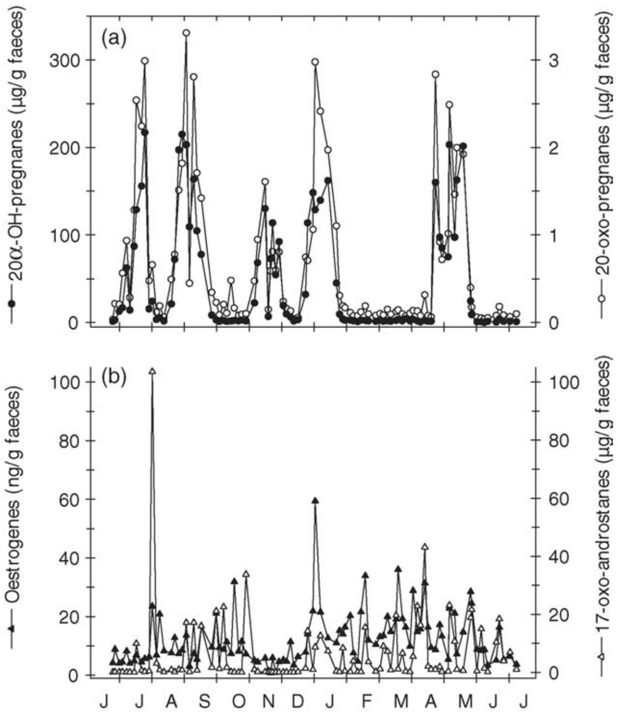


Fig. 3. Representative profiles of (a) faecal 20α-OH-pregnanes (●) and 20-oxo-pregnanes (○); (b) faecal oestrogens (▲) and 17-oxo-androstanes (△) during oestrous cycles in a female babirusa (Bb #3).

strous cycle, but did further increase during the last month of pregnancy. Oestrogens and 17-oxo-androgens were significantly elevated during the second half of gestation (Fig. 6).

### 3.4. Reproductive pattern in babirusas

Faecal 20α-OH-pregnane concentrations in the babirusa were considerably higher than in the other two species studied (Figs. 3 and 7). Oestrous cycles in the babirusa did occur throughout the year. Only four animals in our study showed luteal activity (Figs. 3 and 7(a and b)), whereas Bb #4 had low faecal pregnane concentrations indicating missing luteal acitivity (Fig. 7c). Regular oestrous cycles of $36.9 \pm 2.03$ days ($n = 5$) occurred between January and June in Bb #5, whereas the period before these cycles was characterized by persistent luteal activity (Fig. 7a). Luteal phases in Bb #3 (Fig. 3) were separated by extended follicular phases; the length of the luteal phases in this animal was $39.3 \pm 1.34$ days ($n = 5$). Regular luteal activity was also observed in Bb #1 (Fig. 7b), but progesterone metabolite concentrations in this animal were considerably lower than those of the other babirusas studied. There were no pregnancy data available for babirusa.

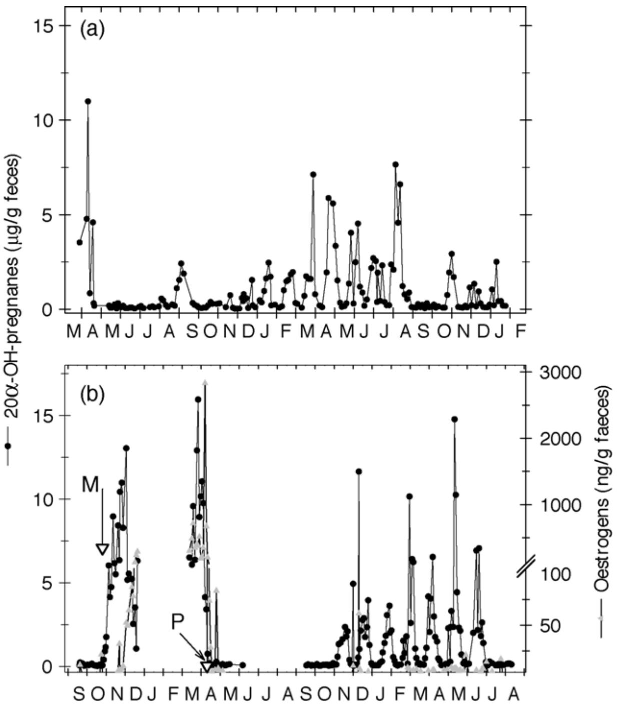


Fig. 4. Profiles of 20α-OH-pregnanes (●) in individual warthogs: (a) Seasonal effect on oestrous cyclicity (Wh #8); (b) pregnancy and regular oestrous cycles (Wh #2); the profile of oestrogens (▲) is included; parturition (P) occurred at the time of peak oestrogen concentration in April in the first year of study; (M) presumed time of mating.

## 4. Discussion

Results of our comparative endocrine studies on reproductive function in two African pig species, the warthog and the red river hog, and the South East Asian babirusa suggested that reproductive monitoring in these species is possible using faecal pregnane assays. This finding is comparable to results in the domestic pig (Sanders et al., 1994; Moriyoshi et al., 1997; Hulten et al., 1999) and to several other mammalian species studied to date (Schwarzenberger et al., 1996, 1997, 2004). In domestic sows, results of faecal hormone metabolites have been demonstrated to correlate to plasma progesterone levels during oestrous cycles and early pregnancy (Choi et al., 1987; Van de Wiel et al., 1992; Moriyoshi et al., 1997; Hulten et al., 1999; Ohtaki et al., 1999; Isobe and Nakao, 2004). As suggested in domestic pigs our results in faecal steroid analysis could be used as a management tool to confirm oestrus and cyclicity, and for monitoring growth, maintenance and regression of corpora lutea. In non-domestic pig species, however, the easier sample collection will make faecal steroid analysis the method of choice for further endocrine studies (Schwarzenberger et al., 1996, 1997).

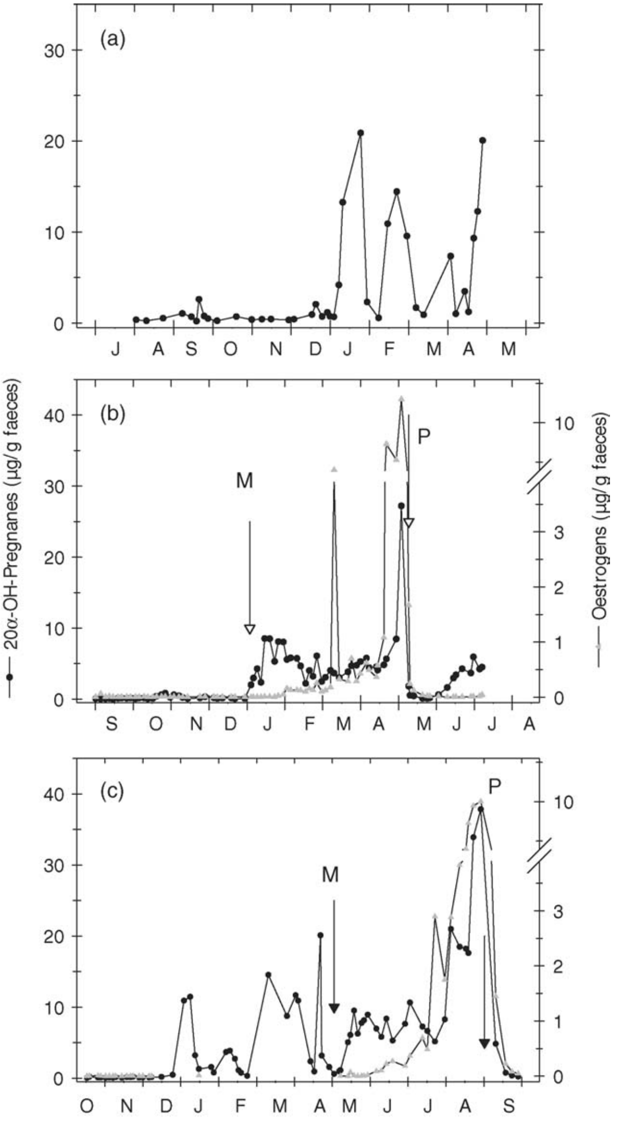


Fig. 5. Profiles of 20α-OH-pregnanes (●) in a non-pregnant red river hog (part a; Rrh #2a); 20α-OH-pregnanes (●) and faecal oestrogens (▲) during gestation in the red river hogs Rrh #5a (part b) and Rrh #6 (part c). Arrows indicate time of presumed mating (M) and time of parturition (P).

In contrast to progesterone metabolites, oestrogens and their precursors, androgens, were unreliable for oestrous cycle monitoring, probably because the swine ovary is producing large numbers of follicles without showing a wave like pattern of follicle development like ruminant species (Evans, 2003). Faecal steroid metabolite concentrations revealed species-specific differences. Faecal LP concentrations of 20-oxo-pregnanes were comparable in

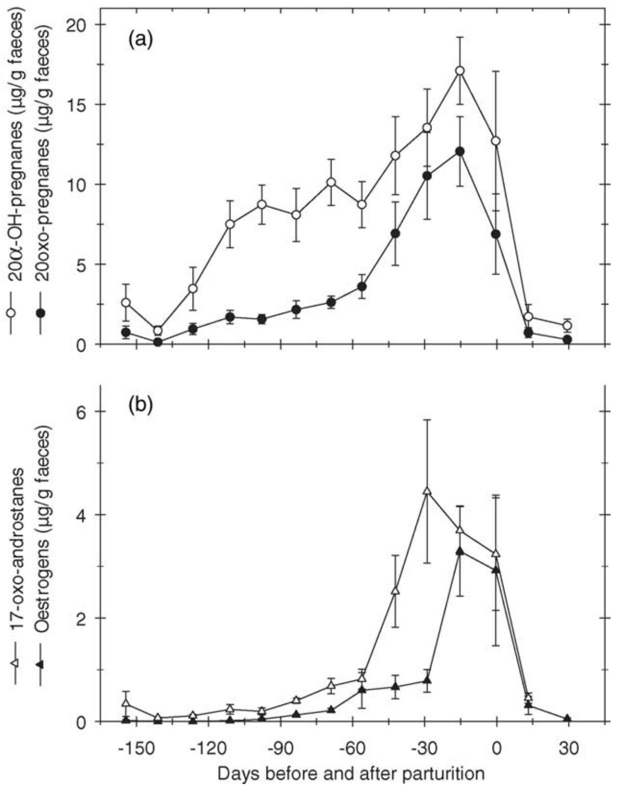


Fig. 6. Composite profiles of (a) faecal 20-oxo-pregnanes (◯) and 20α-OH-pregnanes (●), and (b) oestrogens (▲) and 17-oxo-androstanes (△) during gestation of red river hogs ($n = 4$). Data were aligned to the day of parturition (Day 0) and are presented as mean ± S.E.M. Gestation length was 126.5 ± 1.85 days.

all three species; however, 20α-OH-pregnanes were an order of magnitude higher in the babirusa, as compared to the warthog and red river hog. Studies on the excretion of radiaoactively labelled steroids would help to clarify further questions on steroid metabolism in non-domestic pig species. A study in domestic pigs demonstrated that 34% of progesterone and only 4% of oestrone were excreted via faeces (Palme et al., 1996).

The oestrus cycle length is considerably different between the domestic pig and the three species studied. Oestrus cycle length is between 18 and 21 days in domestic pigs (Geisert, 1999), whereas our results revealed a length of around 35 days in warthogs, red river hogs and babirusas. Our result on the oestrus cycle length in the babirusa is comparable to the range of 28–42 days reported in the literature (Chaudhuri et al., 1990; Macdonald, 1993). In comparison, average cycle length in the collared (*Tayassu tajacu*) and the white-lipped (*T. pecari*) peccary were reported to be about 28 and 30 days, respectively (Mauget et al., 1997). Fecal progestagen analysis indicated a cycle length of 35 days in the hippopotamus (Graham et al., 2002).

According to literature, pregnancy length in warthogs and in red river hogs is around 170 and 120 days, respectively. Therefore, our findings in one warthog and five red river

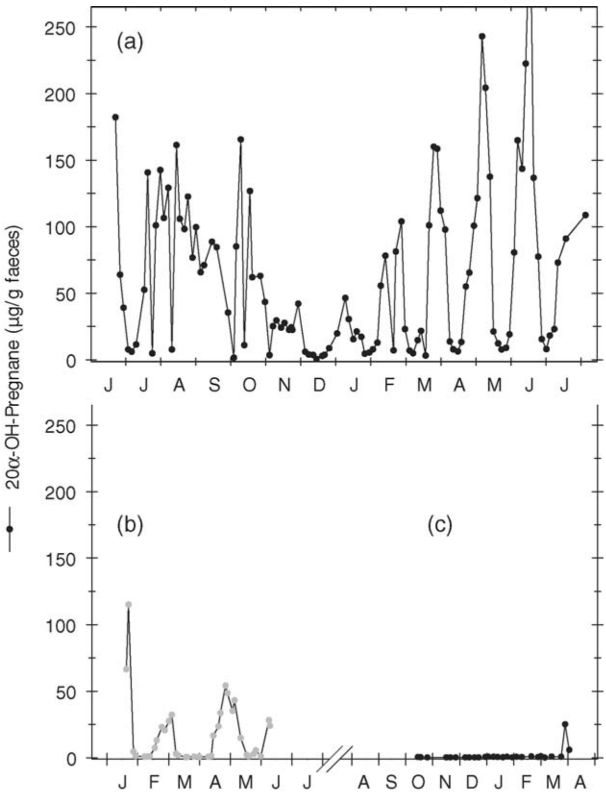


Fig. 7. Profiles of 20α-OH-pregnanes (●) in different babirusas: (a) persistent luteal activity followed by regular oestrous cycles in Bb #5, (b) regular oestrous cycles with low luteal phase concentrations in Bb #1, and (c) low faecal pregnane concentrations indicating missing luteal activity in Bb #4.

hogs are comparable to the results described by other authors (Radke, 1991, Seydack, 1991; Vercammen and Mason, 1993; Vercammen et al., 1993). Gestation length in the babirusas was reported between 161 and 164 days (Chaudhuri et al., 1990; Houston et al., 2001). In contrast to the species studied, it is 114 days in the domestic pig (Geisert, 1999), and around 150 days in the collared peccary (Hellgren et al., 1985).

In several studies in domestic pigs, oestrogen metabolite concentrations in blood, urine and faeces were used for pregnancy diagnosis. Oestrogens in pregnant sows increase during the period of implantation around 28 days after mating and this increase has been used for pregnancy diagnosis (Cunningham et al., 1983; Atkinson and Williamson, 1987; Choi et al., 1987; Van de Wiel et al., 1992; Szenci et al., 1997; Ohtaki et al., 1999; Vos et al., 1999; Isobe and Nakao, 2004). A peak in faecal oestrogen values around 25 days after conception was noted in the pregnant warthog in this study, whereas no such peaks were observed in the pregnant red river hogs. To what extent this early oestrogen peak is a common phenomena during implantation in warthogs requires further studies.

Oestrogens were significantly elevated during the second half of gestation in the red river hog and thus were suitable indicators of pregnancy. This finding is comparable to results in domestic pigs (Geisert, 1999; Isobe and Nakao, 2004). Moreover, in pregnant collared peccaries plasma oestrogen levels were significantly elevated between Days 90 and 145 of gestation (Hellgren et al., 1985), and urinary hormone analysis in the babirusa demonstrated a rise of conjugated oestrogen during pregnancy reaching maximum levels just before parturition (Chaudhuri et al., 1990). Peak values of oestrogens were observed 2 days before parturition in the warthog and 2 weeks before parturition in red river hogs, suggesting that these metabolites could be used as a diagnostic indicator for impending parturition.

The study animals were kept in West and Central European zoos, between 48 and 53°N. Both African species studied showed a seasonal influence on their reproduction, whereas the babirusa is a non-seasonal breeder. Missing luteal activity in the warthog occurred during the European summer months, whereas red river hogs mate from winter to summer and birth mostly during the European summer and early autumn. The natural distribution of the three species studied is around the equator. The range of the different warthog subspecies is from about 15°N to 20°S (Vercammen and Mason, 1993). The range of the red river hog (*Potamochoerus porcus*) is from about 15°N to 5°S, whereas the closely related bush (*P. larvatus*) pig ranges from 5° to 25°S (Vercammen et al., 1993). The natural range of the Asian babirusa is only around the equator (Macdonald, 1993). Pig species living under seasonal climate conditions like domestic pigs and the European wild boar are seasonal breeders regulated primarily by photoperiod. Seasonality in domestic pigs is often manifest by a reduction in fertility in the summer to autumn period (Peltoniemi et al., 2000). In their natural habitat warthogs and red river hogs are also seasonal breeders, regulated primarily by rainfall patterns and thus nutrition. Warthogs breed throughout the year in equatorial regions, but under seasonal climatic conditions farrowing is synchronized with the end of the dry season (Radke, 1991; Vercammen and Mason, 1993; Somers et al., 1995). Reproduction in red river hogs in their natural habitat is reported to be apparently seasonal with most piglets being born towards the end of the dry season or coinciding with the onset of the raining season (Seydack, 1991; Vercammen et al., 1993). Nearly 75% of births in the closely related southern Bushpig (*Potamochoerus larvatus*) in the Cape Province in South Africa occurred during spring (African September to October; Seydack, 1991). In comparison, reproduction, pregnancies and birth in white-lipped and collared peccaries in the Peruvian Amazon was reported to be equally distributed throughout the year (Gottdenker and Bodmer, 1998). However, the Chacoan peccary in its natural habitat in Paraguay, northern Argentina and eastern Bolivia, was reported to have a spring birth peak clearly correlated to rainfall, although births occur throughout the year (Yahnke et al., 1997).

This study was undertaken to better understand the reproductive biology of warthogs, red river hogs and babirusas and to assist further breeding management of captive populations. Although warthogs and red river hogs born in captivity seem to reproduce well, a number of wild caught warthog founder animals did not bred. Some of these animals were included in this study and these animals had regular oestrous cycles over periods of 6–8 months and were kept with boars. Thus a behavioural component, possibly correlated to acclimatization to captive conditions is a likely cause for their failure in reproduction. Warthogs seem to be facultative co-operate breeders, having a social control of reproduction (Plesner-Jensen et al., 1999). The methods established in this study will benefit further studies on behavioural, nutritional or seasonal factors influencing reproduction in warthogs, red river hogs and babirusas.

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