Monitoring reproductive steroids in feces of Arabian oryx: toward a non-invasive method to predict reproductive status in the wild

Stéphane Ostrowski, Caroline Blanvillain, Pascal Mésochina, Khairi Ismail, and Franz Schwarzenberger

Abstract We measured metabolites of progesterone (progestins) in fecal samples collected from captive Arabian oryx (*Oryx leucoryx*) females in postpartum ($n=8$), nonpregnant ($n=9$), and pregnant ($n=8$) reproductive stages between 1996 and 1998. We analyzed progestins using enzyme-immunoassays for pregnanediol and 20-oxo-pregnanes, respectively. Progestin concentrations were elevated for 3 days after parturition and then decreased to basal anestrous concentrations. Ovarian cyclicity resumed 25±2.4 days after parturition in 5 of the 8 females monitored. In nonpregnant females, excretion of fecal progestins followed a cyclic pattern increasing 6- to 12-fold from the follicular to the luteal phase. Fecal progestin concentrations allowed discrimination between pregnant and nonpregnant females after 3 months of gestation ($P<0.01$), mean concentration of the tested hormone metabolites being at least 3 times higher during mid and later stages of gestation (>3 months) than during early pregnancy (0–3 months). These data were subsequently used to set criteria for designation of a cow as pregnant in 55 free-ranging Arabian oryx in the reserve of Mahazat as-Sayd, Saudi Arabia sampled in 1998–1999 and 2003. The proportion of pregnant and nonpregnant oryx correctly identified by the test was 81% and 83%, respectively, when using both progestin assays. Despite a limited sample size, our results provide evidence that fecal progestin analysis is a reliable non-invasive method to determine the reproductive status of captive Arabian oryx and that it also can provide reasonably accurate physiological indices of pregnancy status in free-ranging specimens.

Key words Arabian oryx, fecal progesterone metabolites, *Oryx leucoryx*, pregnancy diagnosis, progestin, reproductive monitoring

During the last decade, the measurement of fecal steroid metabolites as a non-invasive method to assess reproductive status has become a routine procedure in a variety of nondomestic mammals (Schwarzenberger et al. 1996*a*). It has proved successful in monitoring endocrine cycles and pregnancy diagnosis in a number of ruminants, including the muskox (*Ovibos moschatus*) (Desaulniers et al. 1989), caribou (*Rangifer tarandus*) (Messier et al. 1990), bison (*Bison bison*) (Kirkpatrick et al. 1992), moose (*Alces alces*) (Monfort et al. 1993, Schwartz et al. 1995), scimitar-horned oryx (*Oryx*

Address for Stéphane Ostrowski, Pascal Mésochina, and Khairi Ismail: National Wildlife Research Center, P.O. Box 1086, Taif, Saudi Arabia; present address for Ostrowski: Université Claude Bernard, Lyon-1, Laboratoire de Physiologie, UMR 5123, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France; e-mail: stephane.ostrowski@laposte.net. Address for Caroline Blanvillain: Laboratoire de Conservation des Espèces Animales, Ménagerie du Jardin des Plantes, 57 rue Cuvier, 75005 Paris, France. Address for Franz Schwarzenberger: Department of Natural Sciences - Biochemistry, University of Veterinary Medicine, Veterinärplatz 1, A-1210 Vienna, Austria.

dammah) (Shaw et al. 1995, Morrow and Monfort 1998), elk (*Cervus elaphus nelsoni*) (White et al. 1995, Garrott et al. 1998), bighorn sheep (*Ovis canadensis*) (Borjesson et al. 1996), pudu (*Pudu puda*) (Blanvillain et al. 1997*b*), sable antelope (*Hippotragus niger*) (Thompson et al. 1998, Thompson and Monfort 1999), white-tailed deer (*Odocoileus virginianus*) (Kapke et al. 1999), okapi (*Okapia johnstoni*) (Schwarzenberger et al. 1999), sika deer (*Cervus nippon*) (Hamasaki et al. 2001), and Mohor gazelle (*Gazella dama mhorr*) (Pickard et al. 2001). However, most of these studies were done in captivity, where confounding factors that could influence fecal steroid metabolite concentrations (Berger et al. 1999) such as stress (Plotka et al. 1983), body condition (Cook et al. 2002), or variation of dietary constituents (Wasser et al. 1993) are limited. More recently the method was used with success to determine pregnancy in a number of seasonally breeding Nearctic ungulates in their natural environment (White et al. 1995, Garrott et al. 1998, Stoops et al. 1999, Cook et al. 2001, Garrott et al. 2003). However, it still remains unknown to which extent the method can be used in aseasonal breeders and in hyperarid habitats where forage quality and appended environmental stress fluctuate unpredictably.

Nonseasonal breeders and desert-adapted ungulates are interesting models to test the accuracy of fecal steroid predictions as they have adopted a more opportunistic and less predictive reproduction strategy (Skinner et al. 1974). In accordance with this hypothesis, observations made on free-ranging and captive specimens (Stanley Price 1989, Sempéré et al. 1996) showed that the Arabian oryx (*Oryx leucoryx*), an 80–100-kg antelope that lives in the harshest Arabian deserts, has the capacity to breed year-round, and that environmental factors could be implicated in the patterns of body conditions of females and in the timing of reproductive events (Spalton 1995, Sempéré et al. 1996).

Because Arabian oryx number <1,500 free-ranging specimens worldwide (Mallon and Kingswood 2001), investigations that intend to document the physiological mechanisms that control reproduction in this species have been mostly confined to captive specimens (Sempéré et al. 1996, Blanvillain et al. 1997*a*, Ancrenaz et al. 1998). However, our knowledge about the environmental cues that could determine the success and synchronization of reproduction effort among free-living oryx remains limited and would be markedly improved should we be able to determine, preferably with a non-invasive method, the pregnancy status of free-ranging females.

The objective of this study was to measure fecal metabolites of progesterone in captive Arabian oryx females during the various stages of their reproductive activity, and to explore the potential of using these steroid metabolites as a pregnancy diagnosis in free-ranging specimens.

Methods

Captive females

To establish the mean values of fecal progestin concentrations during different reproduction states of Arabian oryx, we separated 25 adult females, captive-raised at the NWRC, into 3 groups; postpartum ($n=8$), nonpregnant ($n=9$), and pregnant ($n=8$). We individually marked all studied females with ear notches and numbered ear-tag. Postpartum females were individually kept in 0.8-ha enclosures, and we collected their feces twice a week during the month following parturition ($n=81$ samples). We maintained the nonpregnant females in one herd in a 150-ha enclosure and collected their feces twice a week for 35 days ($n=87$ samples). Finally we divided the pregnant females in 2 herds of 4 housed in adjacent 5-ha enclosures, and collected their feces once ($n=3$) or twice ($n=5$) per week from the supposed time of conception as determined by ultrasound examination (Delhomme and Ancrenaz 1994) until parturition ($n=344$ samples). We fed all oryx with dry hay (Katambora Rhodes Grass, 91% dry matter, 14% crude protein) ad libitum and supplemented pregnant females with 200 g/day of a commercial dairy pellet (18% crude protein; 18 MJ/kg, Arasco, Saudi Arabia). Saltlicks and water were available ad libitum. Our experimental protocol carried out between 1996 and 1998 was approved by the National Commission for Wildlife Conservation and Development, Riyadh, Saudi Arabia.

Free-ranging females

We conducted the study on free-ranging females in the reserve of Mahazat as-Sayd, a 2,244-km^2 protected area in the open steppe desert of west-central Saudi Arabia (28°15'N/41°40'E). Captive-reared oryx reintroduced in Mahazat as-Sayd between 1990 and 1993 acclimatized quickly to wild conditions without supplemental food and water; and the population increased to 700–800 individuals by

2003 (Mésochina et al. 2003). We collected the feces from 55 marked adult oryx in 1998–1999 and 2003. We located females once every 10–14 days for 10 months after feces collection and recorded calving events. We recognized late abortions when, in the absence of a newborn calf, females showed significant bloodstains around genitalia and exteriorized placental membranes. For the retrospective interpretation of the reproductive status of free-ranging females, we used 255 days as the average gestation period for the species (Sempéré et al. 1996) as well as the calving date prior to feces collection when available.

Feces collection, steroid extraction, and assay

We collected fecal samples from the ground on average 1–3 min after observing oryx defecating; in Mahazat as-Sayd, usually when they left their shade tree in late afternoon (Seddon and Ismail 2002). When the targeted female was in a group, we only sampled it when it defecated <40 m from us, a reasonable proximity to avoid collection mistakes. We brushed away adhering soil from fecal pellets and placed 8–10 g of fresh feces in airtight labeled plastic bags in a cool box at 4–7°C, until they were stored at -20°C about 5–10 min later at the NWRC and 15–120 min later at Mahazat as-Sayd.

Extraction and processing of fecal progesterone metabolites from oryx feces were done as described by Schwarzenberger et al. (2000). Because previous works on herbivores had shown that drying of fecal samples was not necessary to study progesterone metabolites in feces, we performed extractions from wet feces (see Schwarzenberger et al. 1996*a* for a review). We extracted hormone metabolites with 4 ml methanol and mixed 1 ml of double-distilled water from 0.5 g (±0.01 g) of a homogenized sample of feces. After vortexing for 30 min, we centrifuged the sample (2,500g, 10 min) and mixed 1 ml of the methanol phase with 5 ml of diethyl ether and 0.25 ml of a 5% solution of sodium bicarbonate ($NaHCO_3$) and centrifuged again (2,500g, 10 min). After separation the ether phase was evaporated, the dry extract re-dissolved in 0.5 ml assay buffer and then analyzed after appropriate dilution (1:10 to 1:100).

We assayed fecal progestin metabolites using enzyme immunoassays (EIA) (Schwarzenberger et al. 2000). Studies using chromatography in combination with immunoassays have shown in a large array of species that unmetabolized progesterone is nearly absent from feces as it is metabolized to several 5α- and 5β-reduced pregnanes prior to its excretion (Schwarzenberger et al. 1996*a*). Steroid antibodies used in this study were group-specific antibodies raised in rabbits and the EIA were performed in microtiter plates coated with sheep anti-rabbit IgG. Antibodies used were those against 5α-pregnane-3 β-ol-20-one 3CMO:BSA (20-oxo-pregnane), and 5β-pregnane-3α,20α-diol 3-gluc:BSA (pregnanediol). These antibodies showed significant cross-reactivity with different 5-reduced pregnanes containing either a 20-oxo or a 20α-OH-group and were, therefore, termed group-specific (Schwarzenberger et al. 1996*b*). Serial dilutions of the methanol phase from fecal extractions over the range 1:10 to 1:100 gave displacement curves parallel to that obtained with the standard curves. The intra- and inter-assay coefficients of variation for these assays were between 10 and 15%. We expressed hormone concentrations as mass units of hormone per gram of wet feces.

Data analysis

In nonpregnant captive oryx, we considered that a progestin nadir followed by a clear increase was representative, respectively, of the follicular and luteal phases of the estrous cycle. We regrouped hormone concentration data and determined cut-off values to discriminate between follicular and luteal phases based on 99% confidence intervals (CI). The upper limit of the 99% CI of the mean concentration value for follicular phase was just below the lower limit of the 99% CI of the mean concentrations for the luteal phase. Because 20-oxo-pregnanes and pregnanediol showed parallel patterns, pregnanediol concentrations during anestrous, follicular, and luteal phases were determined according to the phase distinction established for 20-oxo-pregnanes. We subsequently used these cut-off values to discriminate between pregnant and nonpregnant cows in Mahazat as-Sayd. In these animals a fecal progestin concentration above the upper limit of the 99% CI of the mean luteal phase concentration indicated pregnancy and a fecal progestin concentration below the upper limit of the 99% CI of the mean follicular phase concentration indicated nonpregnancy (Messier et al. 1990). Because progestin concentrations in captive oryx overlapped between nonpregnant females in luteal phase and pregnant females in early gestation, we categorized free-ranging oryx as equivocal when

progestin concentrations were between these 2 tolerance limits. Although 20-oxo-pregnanes had the highest detection power, preliminary measurements made in captive animals showed that the inclusion of pregnanediol increased the accuracy and reliability of pregnancy status detection. We, therefore, used both progestin concentrations in wild females. We assumed that a female was pregnant (or nonpregnant) when both progestin concentrations indicated pregnancy (or nonpregnancy) or when one progestin indicated pregnancy (or nonpregnancy) and the other was equivocal. The animal was equivocal when both progestins indicated an equivocal status.

Feces of oryx in captivity consisted of 60–65% water and are known to vary between 40 and 65% according to hydration state (S. Ostrowski, unpublished data). Similar to almost all desert ungulates, Arabian oryx under summer free-ranging conditions can reabsorb water from their digestive tract to produce feces with water content as low as 40% (Grenot 1992). Therefore, we also evaluated predictive pregnancy results in free-ranging oryx after applying a 25% correction factor on steroid metabolite concentrations.

Values are presented as means±SEM. We tested data for normality with Kolmogorov-Smirnov goodness of fit test and for homoscedasticity with Levene's test (Zar 1996). We compared log-transformed progestin and estrogen concentrations using analysis of variance (ANOVA). We used an ANOVA for repeated measures when testing for differences between hormone mean concentrations within different reproductive stages and an ANOVA for nonrepeated measures when comparing hormone concentrations between different reproductive stages. We ran post-hoc Newman-Keuls multiple-range tests to define the heterogeneous groups within different reproductive stages. We performed analyses on STATISTICA (StatSoft 1999).

Results

Captive oryx

20-oxo-pregnane and pregnanediol fecal concentrations measured in postpartum females ($n=8$) were elevated for 3 days after parturition and then decreased to basal anestrous concentrations (respectively; $F_{1,7}=111, P<0.001$, and $F_{1,7}=80.3, P<0.001$) (Table 1). Both progestins increased in 5 animals that resumed ovarian cyclicity after 25±2.4 days. Progestin concentrations did not differ between 0–3 days after parturition and during this first bout of ovarian activity. The other 3 females did not resume ovarian cyclicity during the 38 days of postpartum sampling.

In nonpregnant females excretion of fecal progestins followed a cyclic pattern. From the follicular to the luteal phase 20-oxo-pregnanes and pregnanediol increased 12- and 6-fold, respectively (Table 1). The upper 99% confidence limits for 20-oxo-pregnane and pregnanediol concentrations were 15,500 ng/g feces and 14,500 ng/g feces, respectively, in females in luteal phase and 2,150 ng/g feces and 2,050 ng/g feces, respectively, in females in follicular phase.

Table 1. Pooled steroid concentrations (ng/g wet fecal matter) of 20-oxo-pregnanes and pregnanediol collected from captive Arabian oryx (n = 25) held at the National Wildlife Research Center, Taif, Saudi Arabia between 1996 and 1998.

	20-oxo-pregnanes			Pregnanediol		
Variable	Mean	SEM	n	Mean	SEM	n
Postpartum						
0–3 days	7,237.2	1,391.8	8	4,632.4	718.1	8
Anestrous phase	650.3	139.9	8	823.1	130.6	8
Estrous resumption	8,854.9	931.4	5	5,217.2	406.7	5
Nonpregnant						
Follicular phase	721.5	73.4	9	1,297.5	221.1	9
Luteal phase	8,701.8	464.2	9	7,911.6	519.5	9
Unknown phase	3,789.0	224.6	9	3,981.8	356.2	9
Pregnant						
0–3 months	8,973.3	1,658.3	8	6,103.9	1,286.2	8
3–6 months	31,370.4[a]	6,236.2	8	18,249.4[a]	5,137.9	8
6–8.5 months	35,700.9[a]	9,137.4	8	19,134.5[a]	5,547.0	8

[a] Fecal steroid concentrations annotated with a superscript (a) were significantly higher ($P < 0.01$) in pregnant females as compared to other classes.

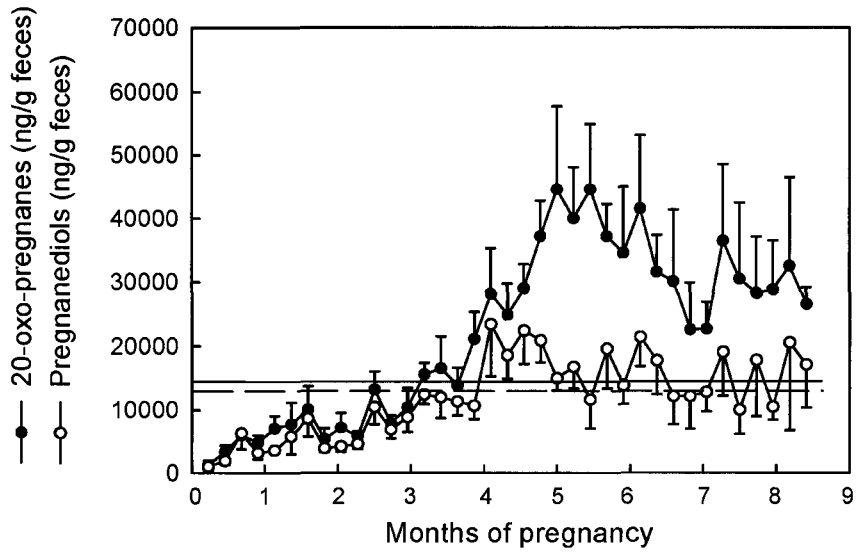


Figure 1. Mean concentrations (ng/g feces) of immunoreactive 20-oxo-pregnanes (+SE) and pregnanediol (–SE) during the gestation period of 8 female Arabian oryx, at the National Wildlife Research Center, Taif, Saudi Arabia. The solid and dashed pointed lines represent respectively the upper limit of 99% tolerance limit (*t*-value ± SD) for the mean concentrations of fecal 20-oxo-pregnanes and pregnanediol in nonpregnant females during the peak luteal phase of their estrous cycle.

During gestation 20-oxo-pregnane and pregnanediol concentrations in feces varied significantly (respectively; $F_{2,14}=51.7, P<0.001$, and $F_{2,14}=8.7$, $P<0.01$). Mean concentration of the 2 tested hormones was 2 to 3 times lower during early pregnancy (0–3 months) than during mid and later stages of gestation (>3 months) (Figure 1). Although progestin concentrations slightly decreased during the last month of pregnancy as compared to the seventh month of pregnancy, the difference was not significant ($P>0.05$). Fecal progestin concentrations were not different in early pregnant (0–3 months) and nonpregnant females in luteal phase ($P>0.06$). However, 20-oxo-pregnane and pregnanediol fecal concentrations allowed a discrimination between pregnant and nonpregnant females during mid (3–6 months; $F_{1,15}=35.8, P<0.001$ and $F_{1,15}=9.2, P<0.01$) and late (6–8.5 months) pregnancy for the 20-oxo-pregnanes ($F_{1,15}=26.7, P<0.001$). Fecal progestins also allowed a discrimination between mid- and late-pregnant and postpartum females ($F_{1,14}>5.2, P<0.05$).

Free-ranging oryx

Based on the results of the fecal progestin analysis of captive oryx, we selected a pregnancy detection cutoff value for 20-oxo-pregnanes and pregnanediol of 15,500 ng/g feces and 14,500 ng/g feces, respectively. Thirty-seven females were retrospectively confirmed pregnant upon sampling and 18 were nonpregnant. Two oryx aborted and 35 produced calves throughout the year, with calves born during all months except September. The proportion of pregnant females correctly identified by the test (i.e. test accuracy) was 81% (30/37) when using both progestins (Table 2). We obtained the 6 equivocal

Table 2. Accuracy of pregnant and nonpregnant predictions based on steroid concentration of 20-oxo-pregnanes and pregnanediol in feces of 55 free-ranging Arabian oryx sampled in 1998–1999 and 2003 in Mahazat as-Sayd protected area, Saudi Arabia.

	Pregnancy			Nonpregnancy		
Steroids	Correct[a]	Equivocal[b]	Incorrect[c]	Correct	Equivocal	Incorrect
20-oxo-pregnanes	25/37	11/37	1/37	15/18	1/18	2/18
Pregnanediol	20/37	16/37	1/37	13/18	3/18	2/18
20-oxo-pregnanes + pregnanediol	30/37	6/37	1/37	15/18	1/18	2/18

[a] Number correctly classified per actual number in group.

[b] Equivocal results refer to progestin concentrations in feces of free-ranging oryx that did not allow to discriminate between pregnancy and nonpregnancy because of an existing overlap of progestin concentrations measured during luteal phase and early pregnancy.

[c] Number incorrectly classified per actual number in group.

results in pregnant females from samples collected <75 days after the presumed date of conception and measured the false negative case in a female sampled 6 days before parturition. The proportion of nonpregnant females that were correctly identified by the test (i.e., test reliability) was 83% (15/18) when using both progestin metabolites (Table 2). One of the 2 false positive cases was measured in a female sampled 4 days after parturition. Reanalysis of results after correcting for difference in water content of feces did not give different conclusions.

Discussion

Successful use of fecal steroid metabolites to determine pregnancy in the Arabian oryx is based on previous works with related Artiodactyla species. However, to our knowledge, this is the first report of use of fecal reproductive steroids to diagnose pregnancy in a free-ranging antelope. In earlier studies fecal reproductive steroids have been measured to detect ovarian activity in captive scimitar-horned oryx, a species phylogenetically and physiologically related to the Arabian oryx (Shaw et al. 1995, Morrow and Monfort 1998), but pregnancy detection in the wild was never attempted in this species due to the lack of viable, free-ranging populations (International Union for Conservation of Nature and Natural Resources [IUCN] 1996). Testing the accuracy of fecal progestin monitoring in a recently established, wild population of Arabian oryx also is likely to be ecologically relevant to any re-establishment project of scimitar-horned oryx, a critically endangered African antelope (IUCN 1996).

As a preliminary step, we have demonstrated that monitoring of fecal steroids could be used to assess the pregnancy status of captive Arabian oryx. The concentration of progestins in feces provided an accurate indicator of mid and late pregnancy (i.e.> Day 90) in captive animals. The level of fecal progestins of pregnant females steadily increased after conception and remained high for the remainder of the gestation period. Because progestin concentrations overlapped between nonpregnant oryx in luteal phase and pregnant females before 90 days of parturition, the analytical method we used was not reliable to detect early pregnancy in this species, a result seen in several other ungulate species in which steroids are produced by the placenta (Schwarzenberger et al. 1996*a*, 2000).

Progestin concentrations measured in feces of nonpregnant Arabian oryx also have the potential to be used to monitor ovarian cyclicity. In 5 out of 8 monitored females, the timing of postpartum estrous estimated from fecal steroid metabolites was in the range of previous descriptions for the species (i.e., 16–35 days; Sempéré et al. 1996). However, the fact that 3 individuals did not resume estrous cycle by 38 days after parturition suggest, in the absence of lactational anestrous reported in this species, that environmental factors could influence estrous resumption. Results from the nonpregnant and postpartum animals also suggest that fecal progestin concentrations matched earlier results of ovarian cyclicity based on measures of progesterone in the plasma (Sempéré et al. 1996, Blanvillain et al. 1997*a*).

Based on single-sample analysis, we predicted pregnancy in free-ranging Arabian oryx with 82% accuracy, a result in the range of what has been reported for wild herbivores (i.e. 55 to 100%) depending on the species, extraction method, and assay procedures (Desaulniers et al. 1989, Messier et al. 1990, Monfort et al. 1993, Kirkpatrick et al. 1993, Borjesson et al. 1996, Garrott et al. 1998). It was noticeable that the 6 equivocal samples obtained from pregnant females were collected in the first 75 days of gestation, confirming that we could not detect pregnancy in free-ranging oryx during the first third of the gestation. Nonetheless, pregnancy detection was rather reliable after 2.5 months of gestation, since we had no equivocal results after 75 days of pregnancy and since 2 of the 30 pregnant females correctly identified by the test were sampled at 73 and 78 days of gestation.

We correctly predicted nonpregnancy in 15 out of 18 wild oryx (Table 2). Because 9 out of the 15 nonpregnant cows correctly detected were sampled at the end of summer, in August and September, when oryx are often in suboptimal body condition, it is possible that a number of them experienced an anovulatory period rather than a follicular activity. This hypothesis is further supported by the fact that only 1 nonpregnant female showed a progestin level compatible with an ovarian activity, presumably in luteal phase (i.e., an equivocal result). The occurrence and determinism of an anovulatory stage has never been documented in the Arabian oryx and would have important management implications should it be confirmed.

The default of accuracy and reliability in pregnancy detection that we have observed in free-rang-

ing animals was due partly to the fact that the false negative case and 1 of 2 false positive cases were sampled during peripartum (i.e., ±1 week around parturition), a period during which we measured intermittent falls of progestin concentrations below the upper tolerance limit for individual captive females preparing for calving, as well as persistent elevated progestin concentrations during the 3 days following calving. Studies that have reported on the use of fecal progestins to diagnose pregnancy in ungulates have rarely mentioned sampling during peripartum as a source of variability. However during late pregnancy, one might expect progestin concentrations to vary more widely because of the large amount of fetal corticosteroids produced in preparation for delivery (McEwan and Whitehead 1980). We recommend caution when interpreting fecal steroid results from ungulates with suggestive signs of imminent parturition or obvious evidences (i.e., swollen udder and blood marks around vulva and along hind legs) of recent calving.

In conclusion, we correctly addressed the reproductive status of 82% (45/55) of the free-ranging oryx cows. In 13% (7/55) of the cases, we could not determine whether the female was early pregnant or nonpregnant and in luteal phase. Finally in 5% (3/55) of the free-ranging oryx, the test provided incorrect results, a relatively modest level of error. Therefore monitoring of 20-oxo-pregnanes and pregnanediol in feces of free-ranging Arabian oryx offers potential for extensive ecological studies of reproduction success and will provide the opportunity to investigate which environmental cues are the most predictive of the timing and success of reproduction in this nonseasonal desert antelope.

Acknowledgments. We wish to express our appreciation to the National Commission for Wildlife Conservation and Development (NCWCD), Riyadh, Saudi Arabia for support during our research efforts. Wildlife research programs at the National Wildlife Research Center (NWRC) have been made possible through the initiative of HRH Prince Saud Al Faisal and under the guidance of A. H. Abuzinada. We thank J. Renaud for the logistical support throughout the study. We thank E. Bedin and the Mammal Department staff at the NWRC for assistance with sampling and animal care. The ranger staff of Mahazat as-Sayd provided invaluable help in locating animals. The help of A. Aichinger, E. Leitner, and E. Möstl at the Department of Natural Sciences, University of Veterinary Medicine, Vienna, Austria in the assay of fecal steroids is warmly acknowledged. Funding for this study was received from NCWCD/NWRC.

Literature cited

ANCRENAZ, M., C. BLANVILLAIN, A. DELHOMME, A. GRETH, AND A. SEMPÉRÉ. 1998. Temporal variation of LH and testosterone in Arabian oryx from birth to adulthood. General and Comparative Endocrinology 111:283–289.

BERGER, J., J. W. TESTA, T. ROFFE, AND S. L. MONFORT. 1999. Conservation endocrinology: a noninvasive tool to understand relationship between carnivore colonization and ecological carrying capacity. Conservation Biology 13:980–989.

BLANVILLAIN, C., M. ANCRENAZ, A. DELHOMME, A. GRETH, AND A. SEMPÉRÉ. 1997*a*. The presence of the male stimulates puberty in captive female Arabian oryx (*Oryx leucoryx*, Pallas, 1777). Journal of Arid Environment 36:359–366.

BLANVILLAIN, C., J. L. BERTHIER, M. C. BOMSEL DEMONTOY, A. J. SEMPÉRÉ, G. OLBRICHT, AND F. SCHWARZENBERG. 1997*b*. Analysis of reproductive data and measurement of faecal progesterone metabolites to monitor the ovarian function in the Pudu (*Pudu pudu*). Mammalia 61:589–602.

BORJESSON, D. L., W. M. BOYCE, I. A. GARDNER, J. DEFORGE, AND B. LASLEY. 1996. Pregnancy detection in bighorn sheep (*Ovis canadensis*) using a fecal-based enzyme immunoassay. Journal of Wildlife Diseases 32:67–74.

COOK, R. C., J. G. COOK, R. A. GARROTT, L. L. IRWIN, AND S. L. MONFORT. 2002. Effects of diet and body condition on fecal progestagen excretion in elk. Journal of Wildlife Diseases 38:558–565.

COOK, R. C., D. L. MURRAY, J. G. COOK, P. ZAGER, AND S. L. MONFORT. 2001. Nutritional influences on breeding dynamics in elk. Canadian Journal of Zoology 79:845–853.

DELHOMME, A., AND M. ANCRENAZ. 1994. Gestation diagnosis by ultrasonography in a captive herd of Arabian oryx (*Oryx leucoryx*). Pages 119–121 *in* Proceedings of the 36th International Symposium of Diseases of Zoo and Wild Animals, Christianland, Norway.

DESAULNIERS, D. M., A. K. GOFF, K. J. BETTERIDGE, J. E. ROWELL, AND P. F. FLOOD. 1989. Reproductive hormone concentrations in feces during the estrous cycle and pregnancy in cattle (*Bos taurus*) and muskoxen (*Ovibos moschatus*). Canadian Journal of Zoology 67:1148–1154.

GARROTT, R. A., L. L. EBERHARDT, P. J. WHITE, AND J. ROTELLA. 2003. Climate-induced variation in vital rates of an unharvested large-herbivore population. Canadian Journal of Zoology 81: 33–45.

GARROTT, R. A., S. L. MONFORT, P. J. WHITE, K. L. MASHBURN, AND J. G. COOK. 1998. One-sample pregnancy diagnosis in elk using fecal steroid metabolites. Journal of Wildlife Diseases 34: 126–131.

GRENOT, C. J. 1992. Ecophysiological characteristics of large herbivorous mammals in arid Africa and the Middle East. Journal of Arid Environment 23:125–155.

HAMASAKI, S., K. YAMAUCHI, T. OHKI, M. MURAKAMI, Y. TAKAHARA, Y. TAKEUCHI, AND Y. MORI. 2001. Comparison of various reproductive status in sika deer (*Cervus nippon*) using faecal steroid analysis. Journal of Veterinary Medical Science 63: 195–198.

INTERNATIONAL UNION FOR CONSERVATION OF NATURE AND NATURAL RESOURCES. 1996. 1996 Red List of Threatened Animals. International Union for Conservation of Nature and Natural Resources, Gland, Switzerland.

KAPKE, C. A., P. ARCESE, T. E. ZIEGLER, AND G. R. SCHEFFER. 1999. Estradiol and progesterone metabolite concentration in white-tailed deer (*Odocoileus virginianus*) feces. Journal of Zoo and Wildlife Medicine 30:361–371.

KIRKPATRICK, J. F., K. BANCROFT, AND V. KINCY. 1992. Pregnancy and ovulation detection in bison (*Bison bison*) assessed by means of urinary and fecal steroids. Journal of Wildlife Diseases 28:590–597.

KIRKPATRICK, J. F., D. F. GUDERMUTH, R. L. FLAGAN, J. C. MCCARTHY, AND B. L. LASLEY. 1993. Remote monitoring ovarian function and pregnancy of Yellowstone bison. Journal of Wildlife Management 57:407–412.

MALLON, D. P., AND S. C. KINGSWOOD. 2001. Antelopes. Part 4: North Africa, the Middle East, and Asia. Global Survey and Regional Action Plans. SSC Antelope Specialist Group, IUCN, Gland, Switzerland.

MCEWAN, E. H., AND P. E. WHITEHEAD. 1980. Plasma progestins levels during anestrus, estrus, and pregnancy in reindeer and caribou (*Rangifer tarandus*). Pages 324–328 *in* E. Reimers, E. Gaare, and S. Skjenneberg, editors. Proceedings of the second international reindeer/caribou symposium, Roros, Norway, 1979. Direktoratet for vilt og ferskvannsfisk, Trondheim, Norway.

MÉSOCHINA, P., S. OSTROWSKI, AND K. ISMAIL. 2003. Arabian oryx reintroduction into Mahazat as-Sayd protected area – Saudi Arabia: from rehabilitation to population management. Pages 29–30 *in* P. S. Soorae, editor. Re-introduction News No. 23. Newsletter of the International Union for Conservation of Nature and Natural Resources /Species Survival Commission Re-introduction Specialist Group, Abu Dhabi, UAE.

MESSIER, F., D. M. DESAULNIERS, A. K. GOFF, R. NAULT, R. PATENAUDE, AND M. CRETE. 1990. Caribou pregnancy diagnosis from immunoreactive progestins and estrogens excreted in feces. Journal of Wildlife Management 54:279–283.

MONFORT, S. L., C. C. SCHWARTZ, AND S. K. WASSER. 1993. Monitoring reproduction in captive moose using urinary and fecal steroid metabolites. Journal of Wildlife Management 57: 400–407.

MORROW, C. J., AND S. L. MONFORT. 1998. Ovarian activity in the scimitar-horned oryx (*Oryx dammah*) determined by faecal steroid analysis. Animal Reproduction Science 53:191–207.

PICKARD, A. R., T. ABÁIGAR, D. L. GREEN, W. V. HOLT, AND M. CANO. 2001. Hormonal characterization of the reproductive cycle and pregnancy in female Mohor gazelle (*Gazella dama mhorr*). Reproduction (Cambridge) 122:571–580.

PLOTKA, E. D., U. S. SEAL, L. J. VERME, AND J. J. OZOGA. 1983. The adrenal gland in white-tailed deer: a significant source of progesterone. Journal of Wildlife Management 47:38–44.

SCHWARTZ, C. C., S. L. MONFORT, P. H. DENNIS, AND K. J. HUNDERTMARK. 1995. Fecal progestagen concentration as an indicator of the estrous cycle and pregnancy in moose. Journal of Wildlife Management 59:580–583.

SCHWARZENBERGER, F., E. MÖSTL, R. PALME, AND E. BAMBERG. 1996*a*. Faecal steroid analysis for noninvasive monitoring of reproductive status in farm, wild and zoo animals. Animal Reproduction Science 42:515–526.

SCHWARZENBERGER, F., W. RIETSCHEL, B. MATERN, W. SCHAFTENAAR, P. BIRCHER, B. VAN PUIJENBROECK, AND K. LEUS. 1999. Noninvasive reproductive monitoring in the okapi (*Okapia johnstoni*). Journal of Zoo and Wildlife Medicine 30:497–503.

SCHWARZENBERGER, F., W. RIETSCHEL, J. VAHALA, D. HOLECKOVA, P. THOMAS, J. MALTZAN, K. BAUMGARTNER, AND W. SCHAFTENAAR. 2000. Fecal progesterone, estrogen and androgen metabolites for noninvasive monitoring of reproductive function in the female Indian rhinoceros, *Rhinoceros unicornis*. General and Comparative Endocrinology 119:300–307.

SCHWARZENBERGER, F., C. H. SON, R. PRETTING, AND K. ARBEITER. 1996*b*. Use of group-specific antibodies to detect fecal progesterone metabolites during the estrous cycle of cows. Theriogenology 46:23–32.

SEDDON, P. J., AND K. ISMAIL. 2002. Influence of ambient temperature on diurnal activity of Arabian oryx: implication for reintroduction site selection. Oryx 36:50–55.

SEMPÉRÉ, A. J., M. ANCRENAZ, A. DELHOMME, C. BLANVILLAIN, AND A. GRETH. 1996. Length of estrous cycle and gestation in the Arabian oryx (*Oryx leucoryx*) and the importance of the male presence for induction of postpartum estrus. General and Comparative Endocrinology 101:235–241.

SHAW, H. J., D. I. GREEN, A. W. SAINSBURY, AND W. V. HOLT. 1995. Monitoring ovarian function in scimitar-horned oryx (*Oryx dammah*) by measurement of fecal 20alphaprogestagen metabolites. Zoo Biology 14:239–250.

SKINNER, J. D., J. H. VAN ZYL, AND L. G. OATES. 1974. The effect of season on the breeding cycle of plain antelope of the western Transvaal Highveld. Journal of the South African Wildlife Management and Association 4:15–23.

SPALTON, J. A. 1995. Effects of rainfall on the reproduction and mortality of the Arabian oryx (*Oryx leucoryx* Pallas) in the Sultanate of Oman. Dissertation, University of Aberdeen, Scotland, United Kingdom.

STANLEY PRICE, M. R. 1989. Animal re-introductions: the Arabian oryx in Oman. Cambridge University Press, Cambridge, United Kingdom.

STATSOFT. 1999. STATISTICA for Windows. StatSoft, Tulsa, Oklahoma, USA.

STOOPS, M. A., G. B. ANDERSON, B. L. LASLEY, AND S. E. SHIDELER. 1999. Use of fecal steroid metabolites to estimate the pregnancy rate of a free-ranging herd of tule elk. Journal of Wildlife Management 63:561–569.

THOMPSON, K. V., K. L. MASHBURN, AND S. L. MONFORT. 1998. Characterization of estrous cyclicity in the sable antelope (*Hippotragus niger*) through fecal progestagen monitoring. General and Comparative Endocrinology 112:129–137.

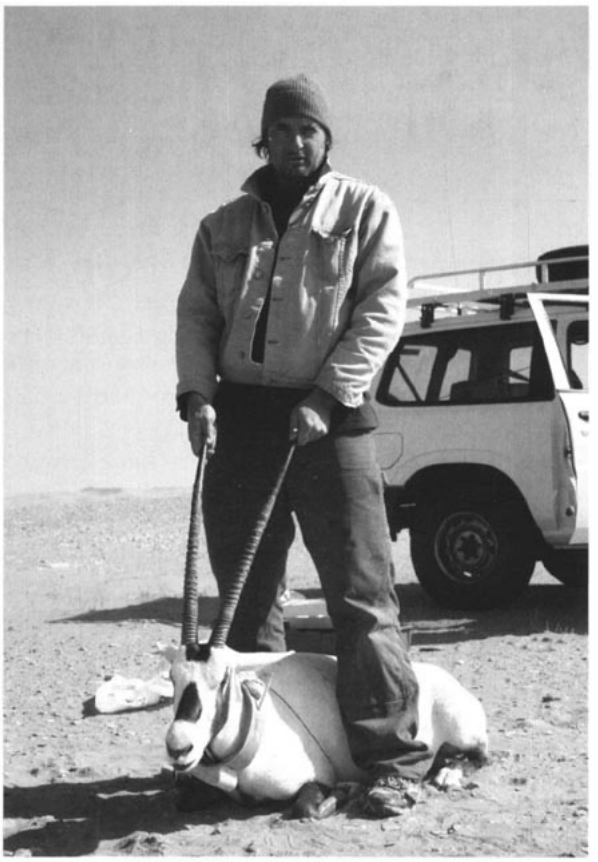
THOMPSON, K. V., AND S. L. MONFORT. 1999. Synchronization of oestrous cycles in sable antelope. Animal Reproduction Science 57:185–197.

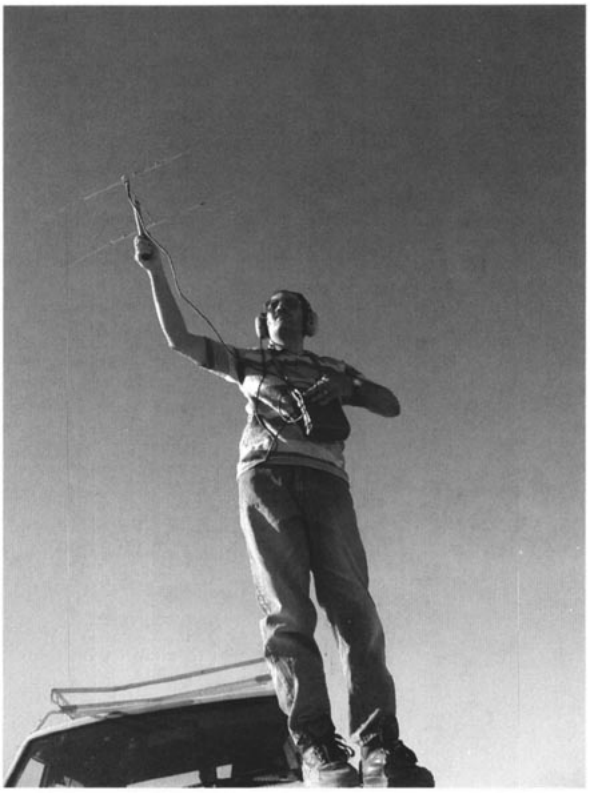
WASSER, S. K., R. THOMAS, P. P. NAIR, C. GUIDRY, J. SOUTHERS, J. LUCAS, D. E. WILDT, AND S. L. MONFORT. 1993. Effects of dietary fiber on faecal steroid measurements in baboons (*Papio cynocephalus cynocephalus*). Journal of Reproduction and Fertility 97:569–574.

WHITE, P. J., R. A. GARROTT, J. F. KIRKPATRICK, AND E. V. BERKELEY. 1995. Diagnosing pregnancy in free-ranging elk using fecal steroid metabolites. Journal of Wildlife Diseases 31:514–522.

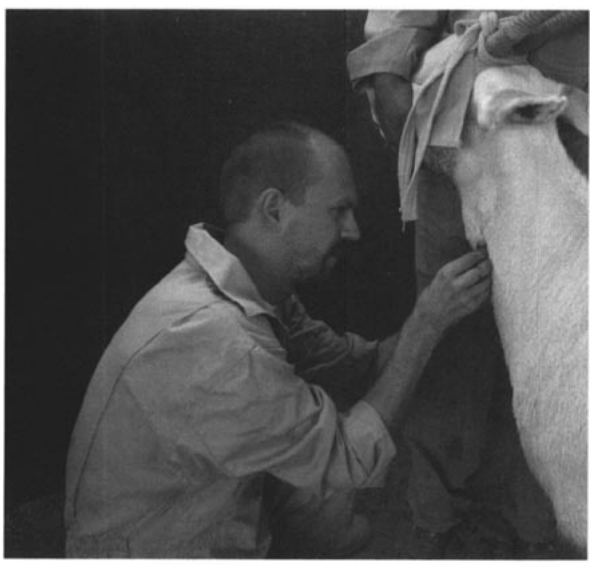
ZAR, J. H. 1996. Biostatistical analysis. Prentice Hall, Englewood Cliffs, New Jersey, USA.

Stéphane Ostrowski (top left) was a wildlife veterinarian with the National Wildlife Research Center (NWRC) of Taif in Saudi Arabia, responsible of the Arabian oryx conservation projects until December 2004. He received a D.V.M. from the Veterinary





School of Lyon, France in 1996 and is currently a Ph.D. candidate in ecophysiology at the University of Lyon 1, France. ***Caroline Blanvillain*** (not shown) holds a D.V.M. from the Veterinary School of Maisons-Alfort, France, and since 1998 a Ph.D. in conservation from the Natural History Museum of Paris, France. Caroline studied the reproductive physiology of Arabian oryx as her doctoral work. Her main post-doctoral professional commitments concerned the conservation of the endemic avifauna of French Polynesia. ***Pascal Mésochina*** (below) holds a Ph.D. in animal science from the Institut National de la Recherche Agronomique, France. Pascal was mammal curator of the Arabian oryx collection at the NWRC between 2001 and 2004. ***Khairy Ismail*** (above) holds a B.S. from the King Abdulaziz University, Jeddah, Saudi Arabia. He is a field biologist based at the NWRC, in charge of the monitoring of Arabian oryx in the reserve of Mahazat as-Sayd since 1990. ***Franz Schwarzenberger*** (below) received a D.V.M. from the School of Veterinary Medicine of the University of Vienna, Austria in 1989. Habilitated for Veterinary Biochemistry in 1997, he is currently an associate professor at the Institute of Biochemistry, University of Veterinary Medicine, Vienna, Austria. During the past 15 years Franz has studied reproductive cycles and pregnancies in a variety of wild and domestic animals using the fecal metabolite enzyme-immunoassays he developed in his laboratory.



Associate editor: *Crête*