



## Comparing ovulation synchronization protocols for artificial insemination in the scimitar-horned oryx (*Oryx dammah*)

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### Abstract

Ovarian response and pregnancy success in scimitar-horned oryx ( $n = 28$ ) were compared, following treatment with two synchronization protocols and fixed-time artificial insemination (AI) with frozen-thawed semen. Each oryx received two injections of 500  $\mu\text{g}$  of prostaglandin- $\text{F}_{2\alpha}$  analogue ( $\text{PGF}_{2\alpha}$ -only) 11 days apart, and half received  $\text{PGF}_{2\alpha}$  in combination with an intravaginal progesterone-releasing device (CIDR11 +  $\text{PGF}_{2\alpha}$ ). Semen was collected by electroejaculation from anaesthetised adult oryx and cryopreserved. Anaesthetised females were transcervically inseminated  $56.0 \pm 1.1$  h ( $\pm$ S.E.M.) after  $\text{PGF}_{2\alpha}$  injection and/or device withdrawal using  $28.0 \pm 1.5 \times 10^6$  motile thawed sperm. Ovarian endocrine response was monitored in 20 females by analysing faecal oestrogen and progesterone metabolites. Periovarian oestrogen peaks were detected in 19/20 (95%) females after synchronization. There were no between-treatment differences in oestrogen concentrations or peak characteristics ( $P > 0.05$ ). Luteal development after synchronization was delayed in half the progesterone treated (CIDR11 +  $\text{PGF}_{2\alpha}$ ) females, and faecal progestin excretion profiles indicated that the ovulatory follicle associated with synchronization either failed to ovulate or to fully lutenise. Pregnancy was diagnosed by ultrasonography and/or rectal palpation and was monitored by faecal progestin excretion. More

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( $P = 0.013$ ) pregnancies resulted from the  $\text{PGF}_{2\alpha}$ -only treatment (37.5%, 5/14) than from the CIDR11 +  $\text{PGF}_{2\alpha}$  treatment (0/14), and four healthy scimitar-horned oryx calves were born, three after gestation intervals of 247 days and one after 249 days. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Scimitar-horned oryx; Artificial insemination; Faecal steroid; Ovulation induction; Ultrasound

## 1. Introduction

Scimitar-horned oryx (*Oryx dammah*) are a large (130–180 kg) African antelope that historically inhabited the Sahel, the semi-arid transition zone south of the Sahara, and the northern edge of the Sahara (Newby, 1988). The IUCN Red List status of the scimitar-horned oryx has recently been changed to Extinct in the Wild (East, 1999). Survival of the scimitar-horned oryx and the maintenance of genetic diversity are obligatorily tied to the success of ex situ programmes. Genome resource banking and artificial insemination (AI) have been recognised as tools for genetically managing captive populations of endangered species (Wildt, 1992; Holt et al., 1996b).

AI was initially developed in domestic species to: (i) avoid the transmission of sexually transmitted diseases; (ii) safely and rapidly distribute desirable genotypes; and (iii) increase reproductive efficiency (Jöchle, 1993). Despite the potential application of AI in the management of zoo-maintained populations particularly in obviating the risk and expense of transporting live animals, there are only a small number of reports documenting successful AI in any antelope species. AI of scimitar-horned oryx was attempted previously (Garland et al., 1992; Shaw et al., 1995; G. Asher, personal communication), but to date, only two calves have been born (Garland et al., 1992).

Previous scimitar-horned oryx studies have investigated the ovarian cycle and ovulation induction (Durrant, 1983; Pope et al., 1991; Schiewe et al., 1991; Shaw et al., 1995; Bowen and Barrell, 1996; Morrow, 1997; Morrow and Monfort, 1998; Morrow et al., 1999), as well as semen collection and cryopreservation (Foxworth et al., 1989; Loskutoff et al., 1989; Durrant and Patton, 1991; Schiewe, 1991; Garland et al., 1992; Roth et al., 1998, 1999). These studies have generated a useful database for developing an AI programme. Successful AI depends on consistent induction of ovulation combined with an ability to place viable semen in the female reproductive tract at the optimum time and site. Further, the ability to tightly regulate ovulation allows insemination of females at a pre-determined time, thereby maximising labour efficiency.

Methods of synchronising oestrus and ovulation have been reported in the scimitar-horned oryx (Pope et al., 1991; Schiewe et al., 1991; Shaw et al., 1995; Bowen and Barrell, 1996; Morrow and Monfort, 1998), but there is a lack of data on the assessment of fertility after treatments. Garland et al. (1992) reported 60% (3/5) of oryx exhibiting signs of oestrus and two resulting pregnancies after females were treated with a combination of exogenous progesterone for 13 days and prostaglandin- $\text{F}_{2\alpha}$  analogue ( $\text{PGF}_{2\alpha}$ ) on day 13. In previous studies (Morrow, 1997), we determined that oestrus and ovulation in scimitar-horned oryx could be reliably induced using  $\text{PGF}_{2\alpha}$  administered 10 days apart, or in combination with exogenous progesterone. Both of these treatment regimens resulted in a high incidence of oestrus and ovulation.

The present study was designed to evaluate pregnancy rate after fixed-time transcervical insemination with frozen-thawed semen in females in which ovulation had been induced using a combination of exogenous progesterone and PGF<sub>2α</sub> or PGF<sub>2α</sub> alone.

## 2. Methods and materials

### 2.1. Location

This study was conducted during October at two locations, the National Zoological Park's Conservation and Research Center (CRC) near Front Royal, VA, USA (38°53' N, 78°9' W) and at the Wilds, a wildlife-conservation centre located near Cumberland, OH, USA (40°N, 82°W).

### 2.2. Animals

Sixteen female scimitar-horned oryx (4–12 years of age; 5 parous, 11 nulliparous; liveweight  $152.0 \pm 2.5$  kg ( $\pm$ S.E.M.)) were maintained at CRC in two 0.2-hectare pastures with natural shade and access to a barn (22 m<sup>2</sup>). A 9-year old, vasectomised male oryx was housed with the females throughout the study. Oryx had access to fresh water, mineral block, pasture and good quality meadow hay ad libitum, and were supplemented with a 12.5% protein concentrate (Washington National Zoo Herbivore Maintenance Pellet, Agway, Syracuse, NY, USA). At the Wilds, 12 female oryx (7–15 years of age; 7 parous, 5 nulliparous) were maintained in groups of four in 56 m<sup>2</sup> enclosures with access to a barn (37 m<sup>2</sup>). A vasectomised male oryx was not available at the Wilds. Oryx were provided with fresh water, good quality hay, and a 14.3% protein concentrate (Mazuri ADF-#25 Herbivore Pellet, Ralston Purina, St. Louis, MO, USA). Oryx at both locations were not on public exhibit and were individually identified by colour plastic ear tags and North American Regional Studbook numbers (Rost, 1994). All females were separated from intact males before, during, and after the study, and there was no opportunity for natural breeding. Before anaesthesia, food and water were withdrawn for 48 and 24 h, respectively. The Institutional Animal Care and Use Committee of the CRC National Zoological Park approved the research proposal.

### 2.3. Induction of ovulation

Oryx, at each location, were assigned to one of two treatment groups that were balanced for age and parity. Each oryx received two i.m. injections of PGF<sub>2α</sub> (500 μg cloprostenol sodium; Estrumate, Miles, Shawnee Mission, KS, USA) 11 days apart (PGF<sub>2α</sub>-only), and half received PGF<sub>2α</sub> in combination with an intravaginal progesterone releasing CIDR-B<sup>®</sup> device (10% w/v, 1.9 g progesterone; InterAg, Hamilton, New Zealand) (CIDR11 + PGF<sub>2α</sub>). Oryx were inseminated at  $56.0 \pm 1.1$  h (range 54.0–58.4 h) after CIDR device withdrawal and/or the second PGF<sub>2α</sub> injection. The timing of ovulation relative to the onset of behavioural oestrus is not documented for the scimitar-horned oryx. The time interval to insemination was selected based on results of

previous oestrous synchronization studies and interpolation from other livestock species. The average interval between  $\text{PGF}_{2\alpha}$  administration and onset of oestrus of  $32.8 \pm 0.75$  h in oryx [(mean  $\pm$  S.E.M), Morrow, 1997], and between oestrus and ovulation (24 h) in sheep, red deer, and fallow deer (Cumming et al., 1973; Asher et al., 1990, 1992) provided the best estimate for timing the insemination 56 h after the second  $\text{PGF}_{2\alpha}$  injection in this study.

#### 2.4. Sample collection and faecal steroid extraction

Blood samples were collected by jugular venipuncture (10 ml; Serum Separator Vacutainer, Becton-Dickenson and Co., Franklin Lakes, NJ, USA) while oryx were individually restrained unsedated in a handling device (The Tamer<sup>®</sup>, Fauna Products, Red Hook, NY, USA). Samples were collected at the time of each  $\text{PGF}_{2\alpha}$  injection and during the insemination procedure. Samples were stored on ice until transferred to the laboratory where they were centrifuged ( $500 \times g$ , 15 min) and the serum stored at  $-20^\circ\text{C}$  until radioimmunoassay (RIA) for luteinising hormone (LH) and progesterone.

Faecal samples (10–12 g) were collected directly from the rectum during restraint or from the enclosure substrate (after observing defaecation) and stored without preservative at  $-20^\circ\text{C}$ . Faeces (3–7 samples/animal/week) were collected from 20/28 oryx (16 at CRC; 4 at the Wilds) throughout treatment and for 46–49 (CRC) and 15 (the Wilds) days after AI. Faecal collection continued throughout gestation and for 21 days after parturition for pregnant oryx at CRC.

Faecal samples were dried in a Speedvac Rotary Evaporator (Savant Instruments, Forma Scientific, Marietta, OH, USA), and steroids were extracted from 25 mg of dried, pulverised faeces using a technique adapted from Wasser et al. (1994) and described and validated for oryx faeces (Morrow and Monfort, 1998). Final hormone concentrations were corrected for individual procedural losses and expressed as nanogram (oestrogens) or microgram (progestins) per gram dry faeces.

#### 2.5. Radioimmunoassays (RIA)

All assays were previously validated by demonstrating (1) parallelism between serial dilutions of oryx serum or faecal extract and the standard curve and (2) recovery of exogenous hormone added to oryx serum or faecal extract (Morrow and Monfort, 1998).

Oestrogen excretion was measured in duplicate 250  $\mu\text{l}$  aliquots of faecal extract (diluted 1:80 in steroid diluent) using the ImmuChem<sup>™</sup> Total Estrogens Kit (ICN Biomedical, Costa Mesa, CA, USA). Assay sensitivity was 1.25 pg/tube, and inter- and intra-assay coefficients of variation for oryx control samples were 13.5% and 8.9%, respectively.

Serum LH concentrations were measured in duplicate 100  $\mu\text{l}$  aliquots of unextracted serum using a heterologous ovine LH RIA developed by Brown et al. (1991) and modified for oryx serum (Morrow and Monfort, 1998). The antiserum (PKC-231A) was used at a final dilution of 1:80,000 and had little or no cross-reactivity with other pituitary hormones (Brown et al., 1991). Assay sensitivity was 0.2 ng/ml, and all samples were included in a single assay.

Serum progesterone concentrations were measured in duplicate 100  $\mu$ l aliquots of unextracted serum using a solid-phase  $^{125}$ I RIA (Coat-A-Count Progesterone, Diagnostic Products, Los Angeles, CA, USA) validated for oryx serum (Morrow and Monfort, 1998). Assay sensitivity was 0.1 ng/ml, and all samples were included in a single assay.

Progestin excretion was measured in duplicate 100  $\mu$ l aliquots of faecal extract (diluted 1:500 in phosphate buffered saline, pH 7.4) using a progesterone RIA developed by Brown et al. (1994) and modified for scimitar-horned oryx faecal extracts (Morrow and Monfort, 1998). Extracts from samples collected during gestation were diluted 1:5,000 in phosphate buffered saline. Assay sensitivity was 3 pg/ml, and inter- and intra-assay coefficients of variation for scimitar-horned oryx control samples were 5.8% and 8.7%, respectively.

### 2.6. Semen collection and cryopreservation

Semen from two scimitar-horned oryx (7 and 15 years; 166 and 144 kg liveweight, respectively) was collected by electroejaculation using a standardised protocol (Roth et al., 1998). Males were anaesthetised using an i.m. combination of etorphine (3.5 mg M99; Wildlife Pharmaceuticals, Fort Collins, CO, USA), ketamine hydrochloride (100.0 mg Ketaset; Fort Dodge Laboratories, IA, USA), and xylazine (40.0 mg Xyla-ject; Phoenix Pharmaceuticals, St. Joseph, MO, USA) delivered by projectile dart. An electrostimulator (AC, 60 Hz) with a 3.9 cm rectal probe was used to administer stimuli that increased incrementally from 2 to 6 V, separated by several 5-min rest intervals (Roth et al., 1998). Upon completion of the procedure, anaesthesia was reversed with i.v. diprenorphine (7.0 mg M50/50; Wildlife Pharmaceuticals) and yohimbine chlorohydrate (48.0 mg, Sigma, St. Louis, MO, USA) administered i.m. and i.v.

Semen was collected into warm, plastic vials and volume was measured. A microscope was used to assess percent motile spermatozoa and progressive status (forward progressive motility on a scale of 0 = no movement to 5 = rapid, steady forward movement; Howard et al., 1986). Sperm concentration per milliliter of ejaculate was assessed using a standard haemocytometer procedure. A 10  $\mu$ l aliquot of each semen sample was fixed in 0.3% glutaraldehyde for later evaluation of sperm morphology (Howard et al., 1986) and acrosomal integrity (Roth et al., 1998). After collection, semen was diluted to  $400 \times 10^6$  spermatozoa/ml in prewarmed (37°C) equine (EQ) extender without glycerol (composition; 300 mM glucose monohydrate, 13 mM sodium citrate dihydrate, 1 mM EDTA-disodium salt, 14 mM sodium bicarbonate, 305 mM lactose, 250 IU/ml polymyxin B-sulfate, 0.25% sodium tri-ethanolamine lauryl sulphate, and 20% egg yolk in distilled water) and allowed to cool in a waterbath to 4°C over 1.5 h (Roth et al., 1999). Semen was diluted further (1:1) using cooled EQ (with 10% glycerol) for a final concentration of 5% glycerol and  $200 \times 10^6$  spermatozoa/ml. Semen was equilibrated at 4°C for 1 h, and then loaded into pre-labeled 0.5 ml straws (IMV International, Minneapolis, MN, USA) that were heat-sealed. Straws were placed directly on a block of dry ice for 10 min before being plunged and stored in liquid nitrogen. One straw per ejaculate was thawed in a 37°C waterbath for 30 s and an aliquot assessed for percent live spermatozoa, post-thaw progressive motility, sperm morphology, and acrosomal integrity.

### 2.7. Insemination

A combination of medetomidine (0.05 mg/kg bodyweight, range 0.03–0.06 mg/kg; Wildlife Pharmaceuticals), ketamine hydrochloride (3.1 mg/kg bodyweight, range 2.4–3.8 mg/kg), and atropine sulfate (0.02 mg/kg bodyweight, range 0.01–0.02 mg/kg; Vedco, St. Joseph, MO, USA) was administered i.m. to each oryx. After anaesthesia induction, oryx were intubated and placed in lateral recumbency. Animals were supplemented with i.v. ketamine to maintain an appropriate level of anaesthesia. Faeces were manually removed from the rectum, and the cervix was stabilised by palpation per rectum. A 5  $\mu$ l aliquot of thawed semen was assessed for post-thaw motility and status. The straw was loaded into a bovine inseminating pipette (0.5 ml, IMV International) and manipulated through the cervix into the caudal aspect of the uterine lumen. Half the semen was deposited into each uterine horn. The oryx has a duplex uterus, and the cervix bifurcates at the second or third annular ring (Hradecky, 1982; Mossman, 1989). The degree of bifurcation among oryx in this study varied from being completely bifurcated to being bifurcated at the internal cervical os. Ovarian structures were not palpated at insemination to avoid disrupting the oviductal fimbriae. Upon completing insemination atipamezole (0.17 mg/kg bodyweight, range 0.09–0.28 mg/kg; Wildlife Pharmaceuticals) was administered i.v. to reverse anaesthesia.

### 2.8. Pregnancy diagnosis

A real time,  $\beta$ -mode, diagnostic ultrasound scanner (Aloka 500 V; Corometrics Medical Systems, Wallingford, CT, USA) equipped with a linear-array, transrectal 5 MHz transducer was used to examine the reproductive tract 46–49 (CRC) and 53–55 (the Wilds) days after AI. Oryx were restrained unsedated in the handling device for ultrasound examination. Conception to AI was determined by the presence of a 5 cm spherical echogenic image representing the fluid-filled uterine horn and the presence of a foetus. Images of the uterine lumen and foetus were freeze-framed on the scanner screen, measured using the system's electronic calipers, and printed. Conception was also confirmed when faecal progesterin concentrations remained elevated ( $> 15 \mu\text{g/g}$  faeces) for 45 days after insemination (CRC oryx only). Conversely, non-pregnant oryx were identified by longitudinal faecal progesterin excretion patterns indicative of a return to cyclic ovarian activity. Rectal palpation and ultrasound examination of the reproductive tract 113 days after AI was used to confirm pregnancy in oryx at the Wilds.

### 2.9. Data analysis

Standard descriptive statistics, including mean and standard error of the mean (S.E.M.), are presented. Mean hormonal profiles for each treatment are presented with S.E.M. for each sample time. Features of the hormonal profiles (e.g., length of induced ovarian cycle, luteal phase, characteristics of the periovulatory oestrogen peak) and changes in concentrations over different phases of the cycle were analysed by analysis of variance (ANOVA) (Statview, Version 1.1; Brainpower, Calabasas, CA, USA) for treatment effects. Because only four oryx were monitored using faecal steroid excretion

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at the Wilds, location was not included in the models. Pregnancy data was analysed by chi square analysis.

### 3. Results

Three oryx lost the CIDR device, which was replaced on the day of loss in two animals; the remaining female lost the CIDR device 1 day before scheduled withdrawal. Post-AI hormonal data from two oryx at CRC (PGF<sub>2α</sub>-only treatment) were excluded from analysis because they were isolated for veterinary treatment resulting from injuries incurred on the day of insemination.

Faecal excretion data is presented for the 10 oryx monitored in each treatment (8 at CRC, 2 at the Wilds), serum concentration is presented for all 14 oryx in each treatment.

Based on serum progesterone concentrations (> 1 ng/ml), a functional corpus luteum (CL) was present in 13/14 (93%) PGF<sub>2α</sub>-only and 9/14 (64%) CIDR11 + PGF<sub>2α</sub> oryx at the time of initial PGF<sub>2α</sub> injection.

Faecal progestin concentrations (Fig. 2a) in PGF<sub>2α</sub>-only oryx declined ( $P < 0.05$ ) from  $10.0 \pm 2.4$  μg/g at the time of initial PGF<sub>2α</sub> injection to baseline ( $1.9 \pm 0.3$  μg/g) before increasing ( $P < 0.05$ ) to luteal phase concentrations ( $9.2 \pm 1.9$  μg/g) at the time of the second PGF<sub>2α</sub> injection. Based on serum progesterone concentrations at the second PGF<sub>2α</sub> injection, 12/14 (86%) PGF<sub>2α</sub>-only oryx had a functional CL.

In CIDR11 + PGF<sub>2α</sub> oryx, faecal progestin concentrations (Fig. 2b) declined ( $P < 0.05$ ) from  $15.2 \pm 4.2$  μg/g at the time of initial PGF<sub>2α</sub> injection to  $6.9 \pm 0.6$  μg/g (concentrations normally associated with the exogenous progesterone released by the CIDR device; Morrow, 1997). Based on faecal progestin excretion profiles, ovulation was inhibited in 8/10 (80%) of these oryx. The remaining two CIDR11 + PGF<sub>2α</sub> oryx had elevated faecal progestin concentrations (17.3 and 17.0 μg/g, respectively), indicating active luteal tissue present at the time of CIDR device withdrawal/PGF<sub>2α</sub> injection. In both cases, the second PGF<sub>2α</sub> injection caused progestin concentrations to decline to nadir (3.5 and 3.8 μg/g) within 48 h.

Serum progesterone was < 0.7 ng/ml for all oryx at insemination, and serum LH was > 5 ng/ml in 7/14 (50%) PGF<sub>2α</sub>-only and 3/14 (21%) CIDR11 + PGF<sub>2α</sub> oryx.

Distinct periovulatory faecal oestrogen peaks were detected in 19/20 (95%) oryx after CIDR device withdrawal and/or PGF<sub>2α</sub> injection (Fig. 1). Peak faecal oestrogen concentrations (baseline oestrogen concentration + 2 SD) were detected 1, 2, and 4 days after CIDR device withdrawal/PGF<sub>2α</sub> in 23%, 65%, and 12% of oryx, respectively. There were no differences ( $P > 0.05$ ) in faecal oestrogen peak characteristics (mean peak concentration, day of peak, duration of elevated oestrogen concentrations) between treatments. The single oryx failing to exhibit a peak in faecal oestrogen concentrations (PGF<sub>2α</sub>-only group) was anovulatory after the first PGF<sub>2α</sub> injection. There was no treatment difference ( $P > 0.05$ ) in faecal oestrogen during the 11-day ovulation induction interval (PGF<sub>2α</sub>-only,  $217.5 \pm 47.1$  ng/g versus CIDR11 + PGF<sub>2α</sub>,  $188.2 \pm 38.9$  ng/g), or during the 12 days after AI (PGF<sub>2α</sub>-only,  $320.9 \pm 42.2$  ng/g versus CIDR11 + PGF<sub>2α</sub>,  $245.3 \pm 26.0$  ng/g).

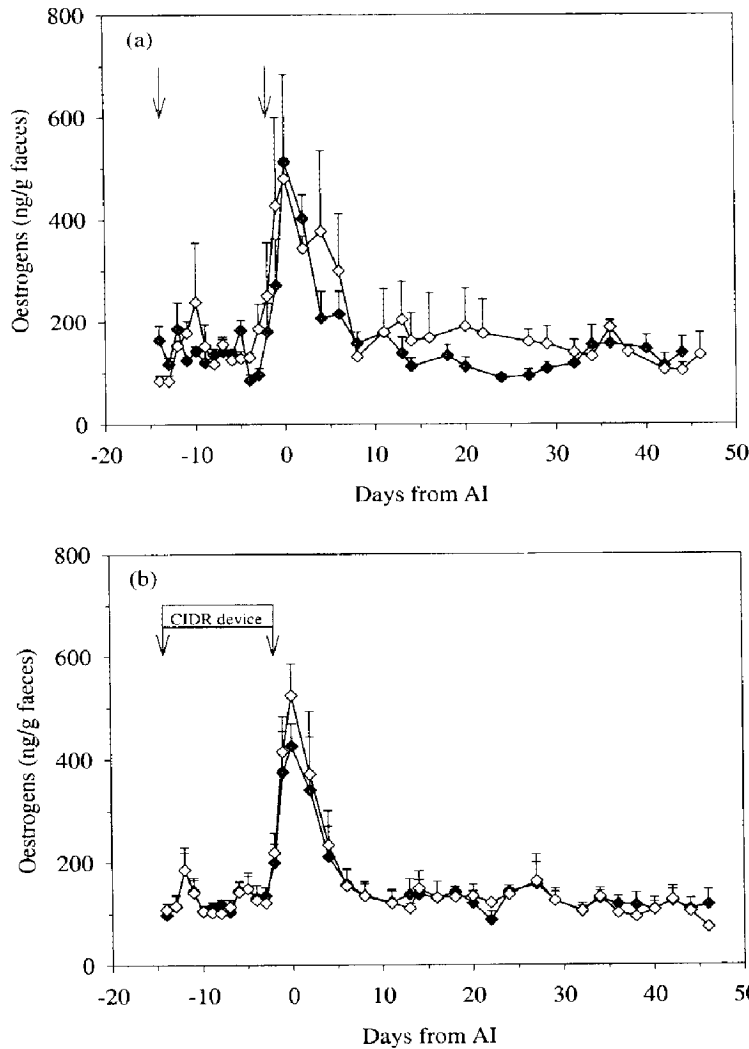


Fig. 1. Mean (+ S.E.M.) faecal oestrogen excretion after (a) two PGF<sub>2α</sub> injections administered 11 days apart (arrows) for scimitar-horned oryx pregnant after AI (◆; *n* = 3) or non-pregnant (◇; *n* = 7) and (b) two PGF<sub>2α</sub> injections combined with insertion of a progesterone releasing CIDR device for 11 days for oryx with normal (◆; *n* = 5) or delayed (◇; *n* = 5) ovarian cycles.

In all cases, periovulatory faecal oestrogen peaks preceded an increase in faecal progestins above basal concentrations (indicative of luteal development). However, the increase in faecal progestins was delayed ( $P < 0.05$ ) in 5/10 CIDR11 + PGF<sub>2α</sub> oryx ( $16.8 \pm 2.5$  days; range 9–23 days) compared to the remaining CIDR11 + PGF<sub>2α</sub> counterparts ( $7.6 \pm 0.7$  days) (Fig. 2b) or to all PGF<sub>2α</sub>-only oryx ( $8.6 \pm 0.8$  days) (Fig. 2a). Similarly, mean faecal progestin concentration during the 12 days after AI was uniformly low in the five oryx with the delayed faecal progestin increase ( $2.5 \pm 0.7$  μg/g;  $P < 0.05$ ) compared to remaining CIDR11 + PGF<sub>2α</sub> ( $6.4 \pm 1.1$  μg/g) (Fig. 2b) and PGF<sub>2α</sub>-only ( $7.8 \pm 0.9$  μg/g) oryx (Fig. 2a).



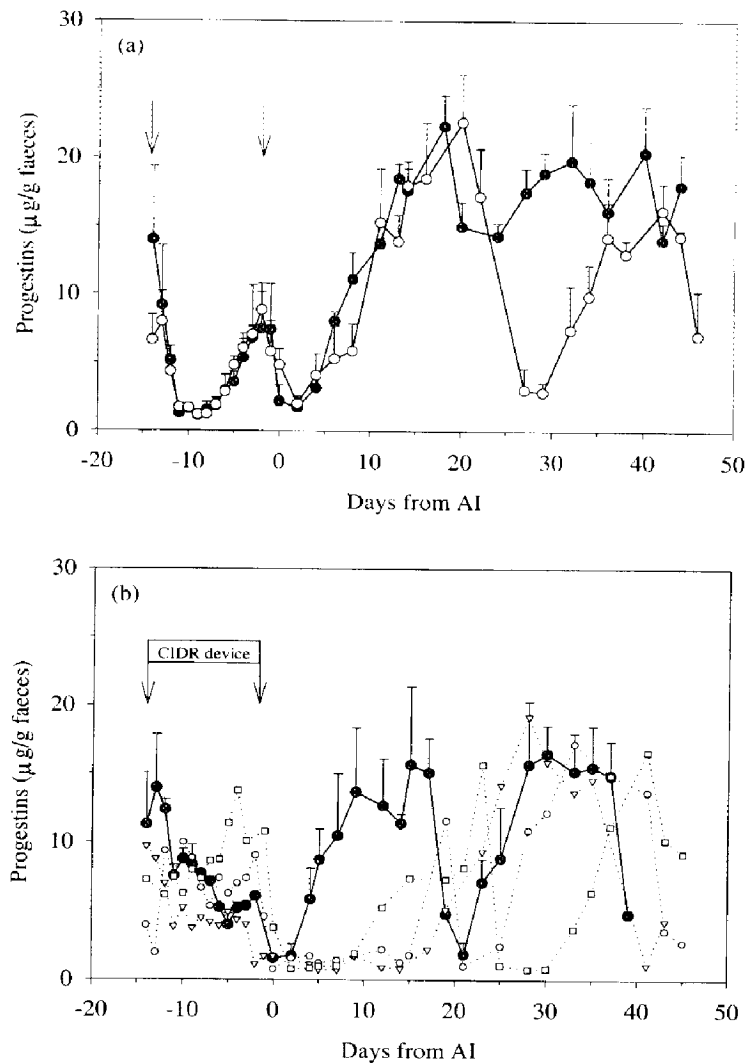


Fig. 2. Mean (+ S.E.M.) faecal progesterin excretion after (a) two  $\text{PGF}_{2\alpha}$  injections administered 11 days apart (arrows) for scimitar-horned oryx pregnant after AI (●;  $n = 3$ ) or non-pregnant (○;  $n = 7$ ) and (b) two  $\text{PGF}_{2\alpha}$  injections combined with insertion of a progesterone releasing CIDR device for 11 days for oryx with normal (●;  $n = 5$ ) or delayed ovarian cycles (dotted lines indicate individual profiles).

The length of the induced ovarian cycle for  $\text{PGF}_{2\alpha}$ -only oryx that failed to conceive to AI was  $25.3 \pm 0.3$  days, whereas CIDR11 +  $\text{PGF}_{2\alpha}$  oryx had either normal ( $26.0 \pm 0.7$  days;  $n = 5$ ) that did not differ ( $P > 0.05$ ) from the  $\text{PGF}_{2\alpha}$ -only group, or delayed ovarian cycles (range: 25–41 days;  $35.5 \pm 3.8$  days;  $n = 5$ ;  $P < 0.05$ ). In these non-pregnant oryx, the luteal phase duration (increase in faecal progesterin above baseline to nadir progesterin) was similar ( $P > 0.05$ ) among oryx treated with  $\text{PGF}_{2\alpha}$ -only ( $18.0 \pm 0.6$  days) and oryx having normal ( $20.5 \pm 0.5$  days) or delayed ( $19.8 \pm 1.8$  days) ovarian cycles after CIDR11 +  $\text{PGF}_{2\alpha}$  treatment.

Table 1  
Ejaculate characteristics of two scimitar-horned oryx used as semen donors

ID	Date of collection	Volume (ml)	Sperm			
			Concentration ( $\times 10^6 \text{ ml}^{-1}$ )	Motility (%)	Status <sup>a</sup>	Morphology (% normal)
1896	22 Sep	9.4	888	70	4.0	86
1896	20 Nov	8.4	778	75	3.5	62
831	28 Mar	10.5	883	80	3.0	58
831	21 Sep	17.2	872	80	3.5	82

<sup>a</sup>Forward progression on a scale of 0–5; 0 = no movement, 1 = slight side-to-side movement with no forward progression, 2 = moderate side-to-side movement, 3 = side-to-side movement with slow forward progression, 4 = steady forward progression, 5 = rapid, steady forward progression (Howard et al., 1986).

Characteristics of the semen samples collected from the two male oryx are presented in Table 1. In general, the ejaculate consisted of a large volume (> 8 ml) with a high percentage ( $\geq 70\%$ ) of progressively motile spermatozoa. Post-thaw motility and forward progressive status were reduced after the freeze-thaw procedure (Table 2). Oryx were inseminated with an average of  $27.9 \pm 1.5 \times 10^6$  motile sperm (range,  $17.3\text{--}42.9 \times 10^6$ ). Semen was either deposited into both uterine horns (24/28; 86%) or the anterior cervical region (4/28; 14%).

Three pregnancies were evident by ultrasonography 46–55 days after AI (Table 3). Two oryx were not diagnosed pregnant by ultrasound, but were found to be pregnant on the basis of sustained and elevated faecal progesterin concentrations or by rectal palpation 113 days after AI. The three pregnant oryx at CRC all experienced a transient decline in faecal progesterin concentrations of 4–9  $\mu\text{g/g}$  from Day 20 to Day 24 (Figs. 2a and 3). Progesterin concentrations remained elevated and averaged  $314.4 \pm 91.8 \mu\text{g/g}$  throughout the second half of gestation before declining markedly near parturition. Faecal progesterin concentrations of oryx #1280 declined precipitously from 164.6  $\mu\text{g/g}$  the day before the birth to 20.3  $\mu\text{g/g}$  on the day of birth (Fig. 3). Due to the protective maternal nature of the oryx, it was not always possible to enter the enclosure and collect faecal samples

Table 2  
Post-thaw characteristics (mean  $\pm$  S.E.M.) of semen on the day of insemination, and pregnancy after artificial insemination in scimitar-horned oryx

ID	Date of collection	No. of straws	Post-thaw motility (%)	Post-thaw status <sup>a</sup>	Acrosomes (% intact)	No. of pregnancies (% inseminated)
1896	22 Sep	8	$41.9 \pm 2.1$	$2.8 \pm 0.2$	$58.4 \pm 3.8$	2(25)
1896	20 Nov	6	$41.7 \pm 1.7$	$2.9 \pm 0.1$	$68.3 \pm 1.7$	1(17)
831	28 Mar	10	$53.5 \pm 2.8$	$2.7 \pm 0.1$	$93.0 \pm 0.9$	1(10)
831	21 Sep	4	$45.0 \pm 2.0$	$2.9 \pm 0.2$	$93.8 \pm 1.5$	1(25)
Mean $\pm$ S.E.M.		28 <sup>b</sup>	$46.4 \pm 1.6$	$2.8 \pm 0.1$	$77.9 \pm 3.2$	5 <sup>b</sup> (18)

<sup>a</sup>Forward progression on a scale of 0–5; 0 = no movement, 1 = slight side-to-side movement with no forward progression, 2 = moderate side-to-side movement, 3 = side-to-side movement with slow forward progression, 4 = steady forward progression, 5 = rapid, steady forward progression (Howard et al., 1986).

<sup>b</sup>Total.

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Table 3

Details of scimitar-horned oryx pregnancies following artificial insemination with frozen-thawed semen after synchronization of ovulation using prostaglandin- $F_{2\alpha}$

Dam ID	Age	Parity (calves)	Location	Sire ID	No. of sperm <sup>a</sup> ( $\times 10^6$ )	Pregnancy diagnosis <sup>b</sup>	Gestation (days)	Calf sex	Calf weight (kg)
1280	12	3	CRC	1896	18.4	US, FPC	247	F	8.2
2379	4	0	CRC	1896	24.7	US, FPC	249	F	8.6
2263	5	0	CRC	831	25.6	FPC	247	M	9.1
1645	9	2	the Wilds	831	25.7	US, RP/US	247	M	10.5 <sup>c</sup>
1659	9	0	the Wilds	1896	24.0	RP/US	–	–	–

<sup>a</sup>Number of sperm inseminated = sperm concentration per straw  $\times$  post-thaw motility (%).

<sup>b</sup>US = ultrasound examination on Day 46 (CRC) or Day 54 (the Wilds) of gestation; FPC = faecal progesterin concentrations; RP/US = rectal palpation and ultrasound examination on Day 113 of gestation.

<sup>c</sup>Liveweight recorded 3 days after birth.

on the day of parturition. In the other two oryx, faecal progesterin concentrations declined from 249.0 and 384.7  $\mu\text{g/g}$  the day before the birth to 51.6 and 27.9  $\mu\text{g/g}$  on the 1 and 4 days after birth (oryx #2379 and 2263, respectively). For all females, faecal progesterin concentrations declined to  $< 15.0 \mu\text{g/g}$  by 5 days after parturition and remained low ( $\leq 15.0 \mu\text{g/g}$ ) until the end of the 21-day post-partum sampling period.

The incidence of pregnancy for  $\text{PGF}_{2\alpha}$ -only oryx (36%; 5/14) was higher ( $\chi^2$ ,  $P = 0.013$ ) than that for CIDR11 +  $\text{PGF}_{2\alpha}$  oryx (0/14). All five pregnancies (three at CRC, two at the Wilds) resulted from intrauterine inseminations and included parous and nulliparous oryx (Table 3). There were no differences ( $P > 0.05$ ) between pregnant and non-pregnant oryx in faecal oestrogen excretion (Fig. 1a) or the number of motile sperm used ( $23.7 \pm 1.2$  versus  $29.1 \pm 1.8 \times 10^6$  motile sperm).

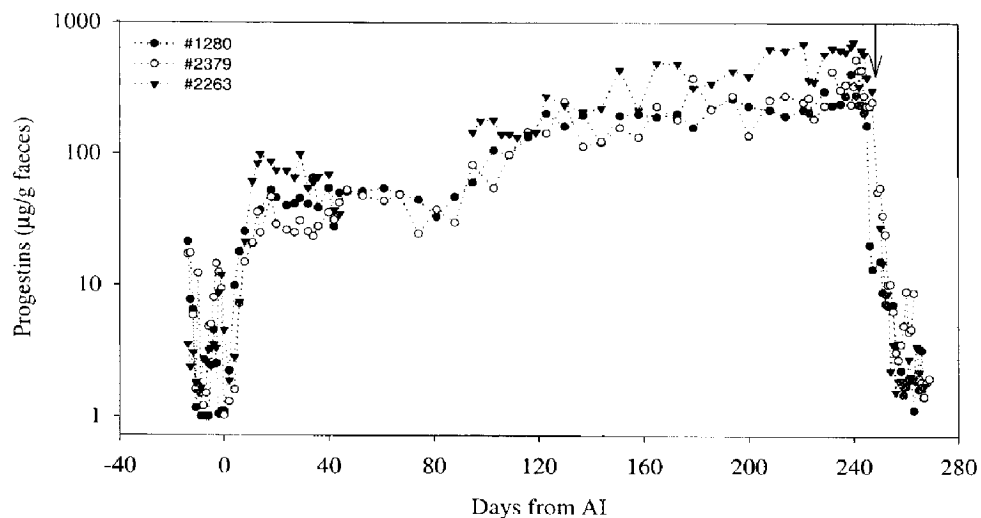


Fig. 3. Faecal progesterin concentrations for the three pregnant scimitar-horned oryx at CRC sampled from 14 days before AI until 21 days post-partum. The vertical axis is presented on a logarithmic scale, and the arrow represents parturition. For oryx #2263 sampling was discontinued after ultrasound examination on Day 46 until analysis of post AI faecal samples confirmed pregnancy and sampling was reinitiated.

Four normal, live scimitar-horned oryx calves were delivered without assistance. Gestation interval, sex, and liveweight are presented in Table 3. Oryx #1659 was confirmed pregnant on day 113 of gestation by rectal palpation and ultrasound images of cotyledons but did not carry the pregnancy to term (Table 3).

#### 4. Discussion

The integrated approach taken in this study generated data of both fundamental and applied benefit to a species that is normally intractable. It demonstrated that transcervical AI with frozen-thawed semen following induction of ovulation using two injections of  $\text{PGF}_{2\alpha}$  can result in the birth of healthy scimitar-horned oryx calves. Results also emphasised the importance of intensive endocrine monitoring because: (1) much of the failure of the exogenous progesterone CIDR treatment to result in pregnancies could be explained by the progestin excretion profile (see below); and (2) a pregnancy not diagnosed by ultrasound was detected using faecal progestin analysis.

AI has been reported for seven antelope species, including Speke's gazelle (*Gazelli spekei*) (Boever et al., 1980), springbok (*Antidorcas marsupialis*) (Boever et al., 1980), addax (*Addax nasomaculatus*) (Densmore et al., 1987; Holt, 1992), blackbuck (*Antelope cervicapra*) (Holt et al., 1988), suni antelope (*Neotragus moschatus zuluensis*) (Raphael et al., 1989), scimitar-horned oryx (Garland et al., 1992; Shaw et al., 1995; G. Asher, personal communication), and the Mhorr gazelle (*Gazella dama mhorr*) (Holt et al., 1996a). These studies, which used a variety of oestrous synchronization and insemination methods with fresh or frozen-thawed semen, produced one Speke's gazelle (Boever et al., 1980), one addax (Densmore et al., 1987), six blackbuck (Holt et al., 1988), and two scimitar-horned oryx calves (Garland et al., 1992). However, despite the success of semen cryopreservation and AI in antelope species, the technology still is not used for managing captive populations (Wildt, 1992; Holt et al., 1996b). The integrated approach used in our study and the production of calves brings us one step closer to using assisted breeding routinely.

Although behavioural oestrus was not monitored in this study, we have previously demonstrated that the incidence of oestrus is high for both  $\text{PGF}_{2\alpha}$ -only (75%) and CIDR10 +  $\text{PGF}_{2\alpha}$  (66%) treatments (Morrow, 1997). Most of the oryx in the present study exhibited clinical signs of oestrus (clear cervical mucus, staining of the anogenital region) and high uterine tone at the time of insemination. Combined with faecal oestrogen and serum LH data, it appeared that insemination was conducted during the periovulatory period for most individuals. Ovarian cycle length in this study was similar to those of spontaneous ovarian cycles and  $\text{PGF}_{2\alpha}$  induced cycles measured previously (Shaw et al., 1995; Bowen and Barrell, 1996; Morrow, 1997; Morrow et al., 1999).

The CIDR device released sufficient exogenous progesterone to inhibit ovulation in the absence of a functional CL. However, luteal development continued in two CIDR11 +  $\text{PGF}_{2\alpha}$  oryx, and it was probable that the developing CL (1–5 days development at CIDR insertion/ $\text{PGF}_{2\alpha}$ ) was unresponsive to the luteolytic effects of the first  $\text{PGF}_{2\alpha}$  injection. This observation reinforces the need for a second  $\text{PGF}_{2\alpha}$  injection at or near the end of short-term progesterone treatments.

The delay in onset of the luteal phase in the CIDR11 + PGF<sub>2α</sub> treated oryx suggested that the ovulatory follicle failed to ovulate or was not fully luteinised after ovulation. The 9–23-day delay most likely represented the time required for a new ovulatory follicle to be recruited, ovulate, and luteinise. We have previously described spontaneously occurring, short ovarian cycles of 8–13 days in scimitar-horned oryx (Morrow et al., 1999). Moreover, we have documented small ( $\leq 5$  mm) and medium (6–9 mm) diameter ovarian follicles in the presence of large ( $\geq 10$  mm) follicles that suggest that scimitar-horned oryx experience follicular activity throughout the cycle (Morrow and Monfort, 1998). In the present study, elevated faecal oestrogen concentrations 1–4 days after CIDR device withdrawal and/or PGF<sub>2α</sub> treatment indicated the presence of oestrogenic follicles. There was no difference in oestrogen excretion between treatments indicating that the oestrogenic follicle did not appear to be adversely affected by short-term progesterone treatment.

The transient decline in progestin concentrations observed 20–24 days after AI in pregnant oryx coincided with luteolysis onset in non-pregnant oryx, and may indicate a minor degree of luteolysis, which is then prevented from proceeding by the presence of a developing embryo. Similar plasma progesterone patterns have been reported for pregnant fallow deer (*Dama dama*) (Asher et al., 1988; Morrow et al., 1995). Subsequent increased progestin concentrations presumably represented increased luteal activity, as it was unlikely that progesterone secreting placental tissue would have formed at such an early stage. The 247–249 day gestation intervals were a few days shorter than the 253- and 257-day intervals for two female calves born following AI in the study by Garland et al. (1992).

Establishment of pregnancies confirmed the biological competence of frozen-thawed scimitar-horned oryx semen in vivo. Frozen-thawed oryx semen (using the same procedure as in the present study) are capable of fertilising heterologous (bovine) oocytes in vitro (Roth et al., 1999) and homologous scimitar-horned oryx oocytes obtained at necropsy (Morrow, 1997). We had planned to inseminate each female with at least  $50 \times 10^6$  live motile sperm. Due to a lower than expected motility post-thaw, only approximately  $28 \times 10^6$  motile sperm were actually inseminated. Thus, the average effective inseminate dose per uterine horn was approximately  $14 \times 10^6$  motile semen. Higher semen concentrations may improve pregnancy rate.

Diagnostic errors in ultrasonographic pregnancy assessment have been reported for blackbuck antelope (du Boulay and Wilson, 1988). The misdiagnosis in our study may have been due to displacement of the reproductive tract during the restraint of the oryx in the handling device. The fail-safe method for diagnosing pregnancy appeared to be measuring sustained elevations in faecal progestin concentrations. This non-invasive technique avoids the stressors that can be imposed on restrained or anaesthetised animals and allows managers to prepare appropriately for impending births.

AI success depends upon response to the ovulation synchronization protocol, timing of insemination, quality and concentration of semen, placement of semen in the tract (cervical versus uterine), longevity of the semen within the tract, and the uterine environment. Further studies are required to determine what factors should be altered to enhance pregnancy success in the scimitar-horned oryx. This study revealed that two injections of PGF<sub>2α</sub> were superior to PGF<sub>2α</sub> combined with the intravaginal CIDR

device for inducing ovulation. The primary research targets now would appear to be: (1) identifying optimal timing of the insemination in relation to the second PGF<sub>2α</sub> treatment; and (2) optimising the concentration of motile semen to be deposited.

The scimitar-horned oryx has thrived in captivity (~1500 oryx are distributed throughout more than 150 locations worldwide), but most individuals are derived from a genetic base of fewer than 40–50 founders captured in Chad in the 1960s (Bertram, 1988; Dixon et al., 1991). Such small, fragmented populations are vulnerable to losses in genetic diversity and fluctuations in size, age, and sex ratios (Holt et al., 1996b). This vulnerability was recognised in the early 1980s, and breeding plans were instituted to maximise genetic variation from different founder lineages. As a result, population management plans for this species operate in North America [Species Survival Plan (SSP)], the United Kingdom and Europe [Europaisches Ehrhaltungszucht Programm (EEP)], and Australasia [Australasian Species Management Plan (ASMP)]. Despite being well organised on a regional and global basis, the conservation of the scimitar-horned oryx presents a paradox to zoo managers and conservation biologists. Existing genetic management strategies necessitate the exchange of valuable breeding stock among geographically disparate populations. However, insufficient funding and enclosure space can sometimes preclude breeding among genetically valuable pairs, and this can adversely impact gene diversity in captive populations. Semen cryopreservation combined with an effective AI programme has potential for overcoming the risk and expense of transporting live animals, and for optimizing the use of limited enclosure space, while simultaneously preserving extant gene diversity. Although the production of multiple offspring after AI was of key importance, the present study also demonstrated that these techniques can be readily adapted to accommodate the diversity of management and husbandry schemes likely to be encountered within zoological institutions and conservation centres worldwide.

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