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Reproductive anatomy, manipulation of ovarian activity and non-surgical embryo recovery in suni (*Neotragus moschatus zuluensis*)*

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Summary. Marked disparity in the uterine horn dimensions and relative degrees of caruncle development in suni suggested that exclusive or predominant dextral implantation occurs in association with bilateral ovulatory activity. Daily urinary measurements of pregnanediol-3α-glucuronide revealed an oestrous cycle of ~21 days in length. Ovarian activity was controlled for synchronization of oestrus by using progestagen-impregnated intravaginal sponges and multiple ovulations were induced by using exogenous gonadotrophin therapy. An effective transcervical uterine catheterization technique was developed for the non-surgical collection of embryos. The efficiency of embryo recovery performed 5 days after sponge removal was 50·0%.

Keywords: suni; antelope; reproductive anatomy; placentation; oestrous cycle; superovulation; embryo collection

Introduction

Suni (*Neotragus moschatus zuluensis*) are small (4–6 kg) antelope which are indigenous to the arid regions of East Africa. Although not officially designated an endangered species, Zulu suni are declining in number in their native habitat and are represented in captivity by isolated and highly inbred populations. All captive Zulu suni in the United States are descended from one pair of wild-caught animals. Difficulties in capturing animals from the wild and excessive mortalities incurred during transport through federally required quarantines preclude the introduction of new individuals into captive populations.

Artificial insemination and embryo transfer technologies provide alternative strategies for increasing genetic diversity in captive populations by transporting genetic material in the form of gametes or embryos. To develop effective programmes, however, an understanding of basic reproductive processes is needed in addition to developing methodologies for monitoring ovarian activity during hormonal manipulation of the oestrous cycle. Evaluation of reproductive anatomy would facilitate the design and development of appropriate instrumentation for non-surgical artificial insemination, embryo collection or embryo transfer. A knowledge of comparative placentation would aid in the selection of suitable species to serve as recipients for interspecific embryo transfers.

The objective of this report is to provide the groundwork for the application of artificial reproductive technology in suni.

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Materials and Methods

Animal housing conditions. The suni used in this investigation were housed at the Dallas Zoo in outdoor exhibits (4·7 × 9·1 m) that contained brick shelter buildings (21·1 m^2) with concrete floors. Females were maintained in groups of 2–4. For breeding purposes, males were transferred into the female groups or individual females were placed with individually housed males. Females were returned to the female groups after all laparoscopies and non-surgical embryo collection procedures.

Reproductive tract morphology. Reproductive tracts were collected at necropsy, over a 2-year period, from 5 multiparous suni. These animals died for a variety of reasons not directly attributable to this investigation. One of the 5 uteri examined was gravid, containing a male fetus (crown–rump length 5 cm) in the right uterine horn. The tracts were removed within 24 h after death and stored at −20°C until processed. After thawing at 37°C, various features of the reproductive tracts were categorized, measured and photographed.

Placentome histology. Placentomes were obtained from partial placentas recovered from 3 animals *post partum*. The samples were fixed in 10% buffered formalin until processed using standard histological techniques of embedding in paraffin wax and staining with haematoxylin–eosin (Humason, 1972). Sections (5–6 μm) were cut in longitudinal and transverse planes, with respect to the direction of the chorioallantoic villi and evaluated using bright-field optics (× 31–312).

Serum progesterone immunoreactivity. To identify the length of the oestrous cycle in suni, whole blood samples (3 ml each) were withdrawn from each of 2 adult, parous females for 30 and 35 consecutive days, respectively. The selection of these sampling intervals was based on an anticipated oestrous cycle length similar to that of small domestic ruminants (i.e. 17–21 days; Morrow, 1986). Serum was stored at −20°C until assayed for progesterone concentrations (C. Munro, University of California, Davis, CA, USA). After thawing, serum samples (100 μl) were extracted with 2·0 ml petroleum ether (Nanograde; Mallinckrodt, St Louis, MO, USA). Progesterone immunoreactivity was measured using a solid-phase microtitre plate enzyme immunoassay (EIA) The EIA competitive reaction involved progesterone 3-O-carboxymethyloxime–horseradish peroxidase as the label and an antiserum raised in rabbits against progesterone 11α-hemisuccinyl–bovine serum albumin. Descriptions of the methodology and assay validation for a wide variety of species, including domestic ruminants, have been previously reported (Munro & Stabenfeldt, 1984).

Urinary pregnanediol-3α-glucuronide immunoreactivity. Daily urine samples (~ 1 ml each) were collected between 06:00 and 08:00 h by perineal massage from each of 3 hand-raised female suni. Immediately after collection, the urine samples were frozen without preservatives at −20°C until processed. Pregnanediol-3α-glucuronide (PdG) immunoreactivity was measured in unextracted urine by radioimmunoassay, according to the method described by Loskutoff *et al.* (1982) for the okapi. Urine was diluted (1:200) in phosphate-buffered saline; 0·2 ml was then taken directly for assay.

Tritiated PdG and PdG antiserum were provided by Dr P. Samarajeewa (Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, UK). Stock tritiated-PdG was diluted to 6000 c.p.m./0·1 ml (sp. act. 43 Ci/mmol) in phosphate-buffered saline for use in the radioimmunoassay. Antiserum, raised in a rabbit against PdG, demonstrated 6·7% cross-reactivity with free pregnanediol and less than 0·01% cross-reactivities with all other conjugated and free steroids tested (P. Samarajeewa, personal communication). Stock PdG antiserum was diluted (1:10 000) in phosphate-buffered saline containing 1·0 g gelatin/l for use in the radioimmunoassay. Serial dilutions of pooled suni urine (1:40–1:2560) resulted in displacement curves parallel to that obtained with PdG standards over the range 0·04–2·50 ng/tube. The sensitivity of the assay was 51·5 pg/tube or 10·3 ng/ml. Intra- and inter-assay precision was assessed by repeated measurements of a pooled suni urine sample with a mean (± s.e.m.) PdG concentration of 0·64 ± 0·01 μg/ml and were expressed by coefficients of variation of 4·4% and 9·5%, respectively.

To compensate for variations in fluid intake and output (Erb *et al.*, 1970), urinary creatinine concentrations were measured by the method described by Taussky (1954). The intra- and inter-assay coefficients of variation were 3·1% and 7·6%, respectively. PdG concentrations were divided by the concentrations of creatinine and are reported as mass (ng)/mg creatinine.

Synchronization of oestrus. Progesterone-impregnated intravaginal sponges were tested in 12 attempts in 6 suni females for synchronization of oestrus. Progestagen (6-methyl-17-acetoxyprogesterone, MAP; Depo-Provera, Upjohn Co., Kalamazoo, MI, USA) was suspended (20 mg) in 3·0 ml 95% ethanol; foam rubber cylinders (~ 30 × 15 mm) were saturated and then air dried. Gelatin capsules were frequently used to facilitate sponge insertion intravaginally. The sponges were threaded with synthetic surgical suture (size 0; 3·5 metric) which facilitated retrieval.

In the initial attempts at synchronization of oestrus and induction of multiple ovulation, sponges were inserted on Day 1 (a.m.) of the treatment protocol and removed on Day 11 (p.m.). The basis for selection of this treatment interval was an anticipated oestrous cycle length similar to that observed in small domestic ruminants. Subsequent to the identification of the actual oestrous cycle length in suni, as determined by daily urinary PdG measurements, the treatment protocol was modified such that the sponges were inserted on Day 1 (a.m.), replaced with fresh sponges on Day 8 (a.m.) and removed on Day 15 (p.m.). Ovarian responses to the treatment protocols were assessed via laparoscopy under halothane anaesthesia 5–8 days after sponge removal.

Induction of multiple ovulation. The efficacy of an exogenous gonadotrophin preparation (FSH-P; Schering Corp., Kenilworth, NJ, USA) in stimulating follicular recruitment was evaluated in a total of 22 attempts in 10 mature suni females. Total dosages were administered intramuscularly in a decreasing regimen over 4 days. Five treatment protocols were evaluated, based on the total dosage and frequency of FSH-P administered: single *versus* twice daily FSH-P injections and length of MAP treatment (11 *vs* 15 days; Table 1). Ovarian responses to the treatment regimens were evaluated laparoscopically 5–8 days after sponge removal.

Mating and non-surgical embryo collection. Female suni were treated with an FSH-P treatment regimen (III, IV or V; Table 1) and housed with males on the day of sponge removal. Mating was expected to occur 24–36 h after sponge removal based on the results of preliminary studies in which breeding activity was continuously observed in one pair of animals for 72 h after sponge removal and in 3 pairs of animals for 36 h at 12–48 h after sponge removal. On a separate occasion, breeding activity was observed in 3 additional pairs of animals by video surveillance for a similar time frame as in the latter study.

Table 1. Descriptions of 5 FSH-P regimens evaluated for multiple ovulation induction in suni

Regimen	MAP treatment interval (days)	FSH-P dosage (mg a.m./mg p.m.) on day of MAP treatment							Total FSH-P (mg)
		9	10	11	12	13	14	15	
I	11	0·75/0	0·62/0	0·50/0	0·37/0				2·25
II	11	1·50/0	1·25/0	1·00/0	0·75/0				4·50
III	11	1·50/1·50	1·25/1·25	1·00/1·00	0·75/0				8·25
IV	11	0·75/0·75	0·62/0·62	0·50/0·50	0·37/0				4·12
V	15					0·75/0·75	0·62/0·62	0·50/0·50	3·75

Non-surgical embryo collections were performed in multiparous suni using a modification of the procedure described by Coonrod *et al.* (1986) and illustrated by Kraemer (1989) for small ruminants. At 5–8 days after sponge removal, the females were captured and physically restrained before the induction of general anaesthesia using halothane gas. Each animal was then placed in dorsal recumbency and intubated with a 6·0 mm endotrachial tube; anaesthesia was maintained with halothane and oxygen. Excess faecal material was removed and perineal regions were cleansed thoroughly.

Instruments used for non-surgical embryo collection were previously sterilized in ethylene oxide and aerated for at least 3 days. During the procedure, the instruments were soaked in chlorhexidine diacetate solution (0·2% v/v) when not in use and rinsed throughly with sterile saline before use. The cervical os in each animal was visualized using a Plexiglass cylindrical speculum (length, 13 cm; diameter, 1·5 cm), with a bevelled and fire-polished end. Laparoscopic grasping forceps (4 mm) were inserted through the speculum to retract the cervix. After grasping the cervical os, the speculum was removed and the cervix was gently retracted. A nasal speculum was then used to visualize the cervical os and the grasping forceps were replaced with a pair of Allis tissue forceps. Dilatation of the cervical lumen was achieved by sequentially introducing stylets of increasing diameter (22–18-gauge) which were gently manipulated in a circular motion. A sterile, gloved finger, inserted into the vagina by the hand holding the tissue forceps, aided in guiding the stylets through the cervical lumen. A 16-gauge Verres cannula was then inserted transcervically into the uterine body. A three-way stopcock, attached to a 20-ml syringe (Air-Tite; Products Co., Inc., Vineland, NJ, USA) containing medium, was secured onto the Verres cannula. The medium used for embryo collection was phosphate-buffered saline with 0·2 ml heat-inactivated newborn calf serum/ml (American Embryo Systems, Grand Prairie, TX, USA), 100 U penicillin G/ml, 100 μg streptomycin/ml and 0·25 μg amphotericin B/ml.

Uterine lavage was performed initially in the uterine body, then in the individual uterine horns. Using a finger intravaginally to guide and position the tip of the Verres cannula, the uterus was filled with medium in 3–5 ml increments, which were collected by gravitational flow into sterile, disposable collection dishes. Medium recovery was facilitated by palpation of the caudal abdomen. The total amount of medium used for transcervical uterine lavage in the suni was approximately 80 ml. To ascertain the efficiency of the non-surgical embryo collection procedure, laparoscopy was performed after embryo recovery in each female to document the number of corpora lutea present.

Statistical analysis. Averages are reported as mean ± s.e.m., range of values and number of animals (N). When appropriate, comparisons between treatment groups were made using the Student's *t* test ($\alpha = 0{\cdot}01$).

Results

Reproductive anatomy

A composite diagram of a reproductive tract of the mature, non-pregnant suni is illustrated in Fig. 1. Overall, the genital organs of the suni were similar to those found in domestic ruminants.

The ovaries were oval and symmetrical, with an average (mean ± s.e.m.) length of 11·5 ± 1·2 mm (9–5 mm; N = 4) and width of 6·9 ± 0·6 mm (5–8 mm; N = 4). The mesosalpinx formed a conical ovarian bursa which covered the lateral surface of each ovary. The bipartite uterus was composed of a relatively large uterine body with an average length of 27·2 ± 2·9 mm (21–35 mm; N = 4) and two asymmetrical uterine horns which have also been observed *in situ* via laparoscopy in a total of 12 multiparous and nulliparous animals. The right uterine horn, with an average length of 81·6 ± 10·4 mm (50–101 mm; N = 4), was larger than the left uterine horn, with an average length of 48·2 ± 7·9 mm (26–63 mm; N = 4). Uterine caruncles, prominent exclusively in the right uterine horn, were round to oval and arranged in 4 irregular rows. There was no internal septum present in the uterine body. The internal cervical os was indistinct and the average length of the cervical lumen was 29·2 ± 1·5 mm (26–35 mm; N = 4). Three rigid, regularly positioned plicae circulares projected into the cervical lumen. Prominent fornices surrounded the medial plica circularis and the dorsolateral aspect of the external cervical os; a conspicuous fold of cervical tissue was present on the ventral aspect. The vagina had a thick, muscular wall with prominent longitudinal folds and an average length of 49·5 ± 5·8 mm (34–62 mm; N = 4). The vestibule was relatively shorter, with an average length of 29·2 ± 2·4 mm (25–34 mm; N = 4); two rows of minor vestibular glands were present on the ventral aspect. The labia of the vulva and glans clitoris were not prominent.

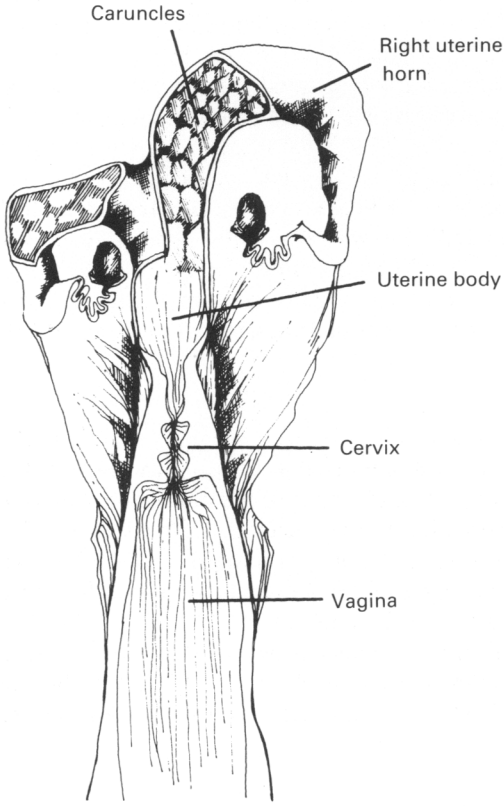

Fig. 1. Composite diagram (derived from 4 females) of the reproductive tract of a mature, non-pregnant female suni.

Placentation

Morphological features of the suni placenta classify it structurally as cotyledonary and epitheliochorial, as is typically found in the domestic cow, as well as other antelope species. Histological evaluation of suni placentome sections disclosed highly branched chorioallantoic villi which were markedly corrugated towards the chorionic surface of the placentome. This pattern is similar to those reported for related species such as the steenbok (*Raphicerus campestris*; Hradecky, 1986), Kirk's dik-dik (*Madoqua kirki*; Wislocki, 1941) and oribi (*Ourebia ourebi*; Kellas, 1966). The villi had a mesenchymal core with an extensive vessel network and were covered with a continuous single layer of squamous to cuboidal epithelium. The villi were separated by thin maternal septa. The maternal crypts were lined with a thin, continuous syncytium.

Oestrous cycle characterization

Serum progesterone immunoreactivity was measured in serial blood samples collected from each of 2 adult suni females for 30 and 35 days, respectively. Peak progesterone concentrations averaged 2·28 ± 0·12 ng/ml (1·10–5·70 ng/ml; $n = 54$) and baseline progesterone concentrations averaged 0·39 ± 0·07 ng/ml (0·02–0·91 ng/ml; $n = 11$). Complete oestrous cycles were apparently not enclosed within either sampling period. Further extension of the sampling interval was not considered since the health of these animals appeared to be detrimentally affected (i.e. weight loss) by the daily physical manipulations. Alternatively, urinary progesterone metabolites, as measured by PdG immunoreactivity, were evaluated to elucidate the length of the oestrous cycle in suni.

Three female suni calves were hand-raised to ensure the consistent procurement of daily urine samples. The initiation of pubertal ovarian activity (N = 3) was detected at an average age of 350·0 ± 23·1 days (307–386 days), as defined by the first appearance of elevated PdG concentrations, reflecting the completion of an ovulatory event. Cyclic patterns of PdG excretion were observed with baseline or trough concentrations averaging 0·07 ± 0·01 μg/mg creatinine (0·03–0·14 μg/mg; $n = 41$). Peak PdG concentrations, defined as those values significantly ($P < 0·01$) greater than average baseline PdG concentrations, averaged 0·49 ± 0·01 μg/mg creatinine (0·24–0·96 μg/mg; $n = 156$). Creatinine concentrations, which were used to index the random urine samples, averaged 1·8 ± 0·06 mg/ml (0·4–4·0 mg/ml; $n = 100$). A representative PdG profile of an adult female suni is illustrated in Fig. 2. The estimated length of the oestrous cycle in suni (N = 3), determined by counting the days between the initial baseline concentrations in the troughs throughout the cycling patterns of 3 suni, averaged 21·3 ± 0·4 days (20–25 days; $n = 15$).

Synchronization of oestrus

The results of the oestrus synchronization attempts using MAP-impregnated intravaginal sponges for 11 and 15 days are summarized as follows. In the first 3 trials, MAP sponges were retained for 11 days without prior knowledge of the oestrous cycle length, or of the age of sexual maturation in suni. No corpora lutea were detected at laparoscopy 5–8 days after sponge removal but an average of 0·7 ± 0·6 small (1–3 mm) antral follicles per trial (range 0–2) was documented. In retrospect, it became evident that 2 of the 3 animals were sexually immature at the time of MAP treatment, as indicated by urinary PdG analysis.

In a total of 9 synchronization attempts using a 15-day MAP treatment period, a single corpus luteum was observed at laparoscopy in each trial (100%), 5–8 days after sponge removal. An average of 0·6 ± 0·3 small (1–3 mm diameter) antral follicles per trial (range 0–2) was also documented.

Induction of multiple ovulation

The results of the multiple ovulation induction attempts, using 5 FSH-P treatment regimens (see Table 1 for descriptions) are summarized in Table 2. Although not statistically significant, the

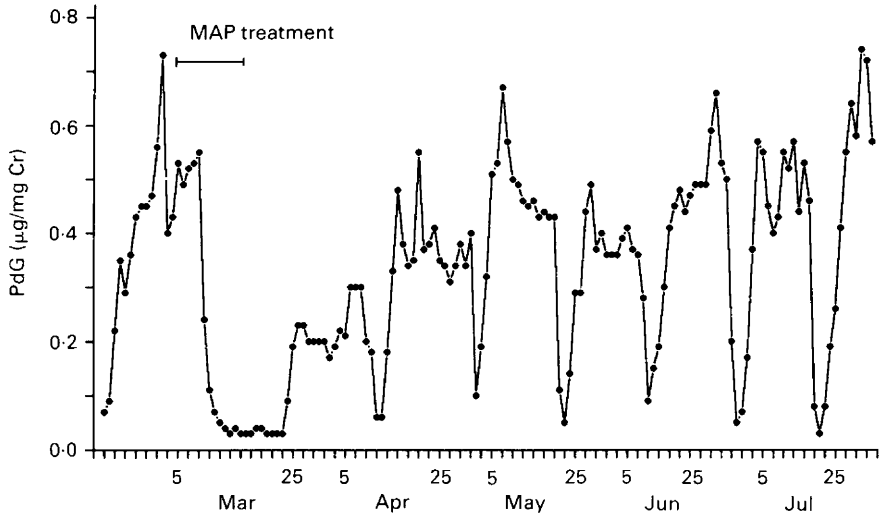

Fig. 2. Immunoreactive profile of pregnanediol-3α-glucuronide (PdG) measured in daily urine samples from a mature female suni (No. H5). The efficacy of a 15-day MAP treatment interval for controlling ovarian activity is illustrated.

highest numbers of corpora lutea as well as the lowest number of follicles were observed 5–8 days after sponge removal using Regimen V. Considering the fact that the total number of injections required for Regimen V was one less than those for Regimens IV and III, these results suggested that Regimen V was the most effective stimulation protocol of the 5 evaluated.

Table 2. Efficacies of 5 FSH-P treatment regimens* for inducing multiple ovulations† in suni

	FSH-P treatment regimens				
	I	II	III	IV	V
Total no. of attempts	4	1	7	2	8
Average no. of CL/animal	1·7 ± 0·8; 0–4	3·0	4·7 ± 1·5; 0–12	5·5 ± 2·5; 3–8	6·6 ± 1·8; 0–14
Luteal diameter (mm)	5·2 ± 0·2; 5·0–6·0	4·0	5·1 ± 0·1; 4·5–8·0	6·7 ± 0·2; 6·0–7·0	Data not available
Average no. of follicles/animal	3·2 ± 0·7; 1–4	6·0	1·3 ± 0·6; 0–4	1·5 ± 0·5; 1–2	0·1 ± 0·1; 0–1
Small antral follicles (1–3 mm)	1·2 ± 0·9; 0–4	0	0·7 ± 0·4; 0–2	1·0 ± 1·0; 0–2	0
Large antral follicles (4–5 mm)	1·0 ± 0·4; 0–2	1·0	1·0 ± 0·4; 0–3	0	0
Preovulatory follicles (6–7 mm)	0·7 ± 0·7; 0–3	0	0	0	0
Luteinized follicles (>8 mm)	0·2 ± 0·2; 0–1	5·0	0	0·5 ± 0·5; 0–1	0·1 ± 0·1; 0–1

Values are mean ± s.e.m.; range.
*See Table 1.
†As assessed by laparoscopy 5–8 days after MAP-impregnated sponge removal.

Refractoriness to FSH-P treatment was not apparent in this study, based on evaluations of ovarian activity after multiple administrations in individual animals. In one suni (No. G10) that was treated with FSH-P on 8 occasions over a 22-month period, the number of corpora lutea observed at laparoscopy were 16, 1, 8, 0, 9, 14, 11 and 17, respectively. There were no differences ($P > 0{\cdot}10$) between the numbers of corpora lutea observed on the right and left ovaries (Table 3).

Table 3. Laparoscopic evaluations of ovulatory activity in right versus left ovaries in suni treated with MAP for oestrous synchronization of MAP + FSH-P for induction of multiple ovulation

Treatment	No. of animals	Total no. of trials	Average no. of CL/donor	Total no. of CL	No. of CL/ovary (% total)		P*
					Right	Left	
MAP	6	9	1·0 ± 0·0	9	6 (66·7)	3 (33·3)	>0·25
MAP + FSH-P	7	15	6·3 ± 1·1	94	40 (42·5)	54 (57·4)	>0·10

*χ^2 analysis for homogeneity.

Non-surgical embryo collection

The results of the multiple ovulation induction and non-surgical embryo collection attempts on individual suni are summarized in Table 4. In the initial phase of the study, ovulation was anticipated about 3 days after sponge removal, as is expected in small domestic ruminants (Scaramuzzi *et al.*, 1987). Non-surgical embryo collections were, therefore, scheduled on Day 6 (ovulation = Day 1) or 8 days after sponge removal. No embryos or ova were recovered in these attempts, although multiple corpora lutea were observed at laparoscopy. The discovery of zona pellucida fragments in the uterine lavage of one embryo donor suggested that the collection procedure was scheduled during peri-implantation embryonic development.

Table 4. Results of multiple ovulation induction and non-surgical embryo collection attempts on individual suni

Collection date	Regimen	Animal	Days after sponge removal	No. of CL	Recovery
14 May 85	III	E8	8	8	0
27 Feb. 87	III	E8	8	2	0
27 Feb. 87	III	G10	8	12	Zona pellucida fragments
3 Apr. 87	III	E19	6	6	One 2-celled embryo
7 May 87	III	G10	6	1	1 hatched blastocyst
7 May 87	III	E8	6	1	0
13 Jun. 87	III	E13	5	3	0
31 Jul. 87	IV	G10	5	8	3 unfertilized ova
31 Jul. 87	IV	E13	5	3	1 unfertilized ovum
12 Nov. 87	V	G12	5	7	Five 8-celled embryos
12 Nov. 87	V	G10	5	0	0
14 Jan. 88	V	B15	5	4	0
14 Jan. 88	V	G10	5	9	7 unfertilized ova
14 Jan. 88	V	F8	5	—	Eight 8-celled embryos 2 empty zona pellucidae
23 Mar. 88	V	E8	5	3	One 8-celled embryo: 1 infertilized ovum
23 Mar. 88	V	G10	5	14	5 unfertilized ova
23 Mar. 88	V	F8	5	13	Two 8-celled embryos: One 4-celled embryo: Two 3-celled embryos: 4 unfertilized ova

In a preliminary study to determine the applicability of laparoscopic artificial insemination techniques in suni, the ovaries of 4 MAP-treated animals were evaluated 28 h after sponge removal (Raphael *et al.*, 1989). Of these animals, 2 were found to possess single, large (6–7 mm) pre-ovulatory follicles on their ovaries and 2 were found with single corpora haemorrhagica. These observations indicated that ovulation in the MAP-treated suni occurred 26–32 h after sponge removal. Since mating activity was consistently observed 24–36 h after sponge removal, the non-surgical embryo collection procedure was rescheduled from 8 days to 6 days after sponge removal. A hatched blastocyst recovered at that time prompted the further modification of the embryo collection schedule to 5 days after sponge removal.

Non-surgical embryo recovery in suni proved to be effective 5 days after sponge removal. In a total of 10 multiple ovulation induction attempts using 6 suni females, 64 corpora lutea were detected at laparoscopy and 32 embryos or ova were collected, which resulted in an overall recovery rate of 50·0%. An additional eight 8-celled embryos and 2 empty zonae pellucidae, recovered from one donor, were not included in this figure because laparoscopic evaluation for the presence of luteal tissue was not performed. Of the total recovered, 21 (65·6%) were unfertilized ova; however, 15 (71·6%) of these were recovered from one animal (No. G10). The embryos recovered were predominantly (8, i.e. 25·0%) 8-celled and four 3–4-celled stages (12·5%) were recovered in a single trial.

Discussion

This report describes anatomical and physiological characteristics unique to the reproductive processes of the female suni. Gross anatomical examination of the reproductive tract revealed marked disparity in uterine horn dimensions. Differences in the relative degrees of caruncle formation suggest that the right uterine horn in this species participates exclusively in implantation and embryonic development. Ovarian activity, on the other hand, did not appear to be unilaterally restricted, based on bilateral ovulatory activity observed in suni treated with MAP for oestrus synchronization or MAP and FSH-P for multiple ovulation induction (Table 3).

Exclusive or predominant dextral implantation, associated with bilateral ovulations, has been reported for other ungulate species including the Uganda kob (*Kobus kob*: Buechner, 1961; Stolk, 1963), impala (*Aepyceros melampus*: Mossman & Mossman, 1962; Kennan, 1967; Hofmeyr & Skinner, 1969; Lee *et al.*, 1977), Defassa waterbuck (*Kobus ellipsiprymnus defassa*: Spinage, 1969), Kirk's dik-dik (Kellas, 1955) and common duiker (*Sylvicapra grimmia*: Child & Mossman, 1965; Symington & Paterson, 1970). The mechanism proposed to account for this observed phenomenon is transuterine embryonic migration (Hafez, 1971). Although it is unknown whether embryonic migration occurs in the suni, the relatively large uterine body typically observed in this species corroborates the likelihood of such an event.

The cervical configuration in the suni was similar to that observed in the domestic goat (Schummer *et al.*, 1979). The plicae circulares were positioned in a regular sequence in the cervical lumen, which facilitated the development of an effective transcervical catheterization technique for embryo collection. These anatomical characteristics also support the feasibility of non-surgical embryo transfer in the suni by the transcervical deposition of embryos into the right uterine horns of synchronized recipient animals. These studies are currently in progress.

The epitheliochorial–cotyledonary type of placenta observed in the suni is common to diverse species of antelope (Hradecky, 1986). Differences in this type of placentation are typically observed on a microstructural level in the relative degree of branching and surface corrugation of the chorioallantoic villi and in the overall complexity of the utero–placental junction (Hradecky *et al.*, 1988). These investigators illustrated marked variations in placentome microstructure, ranging from minimal chorioallantoic villous branching with cellular maternal crypt lining, as observed in the Uganda kob and the common duiker, to extensive chorioallantoic villous branching with

syncytial maternal crypt lining, as found in the steenbok and impala. The microstructure of the suni placentome was similar to that reported for the steenbok, Kirk's dik-dik, oribi and impala. It has been suggested that incompatibilities between placental structure may lead to failures in establishing or maintaining gestation (Hradecky *et al.*, 1987). Comparative histological evaluations of placentomes should, therefore, be a prerequisite to the selection of suitable species to serve as recipients for interspecific embryo transfers.

The oestrous cycle in the suni was found to be about 21 days in length, as determined by daily measurements of PdG immunoreactivity. Urinary PdG measurements, indexed by creatinine concentrations, have been shown to reflect circulating progesterone concentrations accurately in a wide variety of ungulates (Loskutoff *et al.*, 1982, 1983, 1986; Holt *et al.*, 1988). The PdG radioimmunoassay offered a simplified technique for the hormonal assessment of serial samples. In addition, serial PdG measurements resulted in steroid profiles that depicted clearer resolutions between peak and baseline concentrations than that observed by serum progesterone measurements (Raphael *et al.*, 1988). Furthermore, the average peak urinary PdG concentrations were 200-fold higher than peak circulating progesterone concentrations which greatly reduced the sample volume required for analysis.

Daily serum progesterone measurements were initially attempted in 2 mature, intractable suni, but the sampling intervals appeared to be insufficient to disclose complete oestrous cycles. However, since the health of these animals appeared to be detrimentally affected by the frequent physical manipulations required for blood sampling, it is possible that the natural endocrine milieu was confounded by the induction of acute adrenal steroidogenic activity in response to stress, as previously demonstrated in domestic species (Moberg, 1976; Welsh & Johnson, 1981).

The duration of oestrus in the suni appeared to be approximately 12 h in length, based on the observation of mating activity 24–36 h after sponge removal. Ovulation was estimated to occur about midway through the oestrous period, based on laparoscopic examination of ovaries 28 h after sponge removal. These results suggest that the suni are unlike domestic ruminant species which not only exhibit longer durations of oestrus but ovulate at the end of, or after, the oestrous period (Hafez, 1987).

The results of the synchronization of oestrus trials using MAP-impregnated intravaginal sponges clearly demonstrated the efficacy of a 15-day treatment interval in controlling ovarian activity in the suni. In each case, the treatment resulted in the observation of a single corpus luteum, 5–8 days after sponge removal. Coupled with FSH-P treatment, these data further illustrate the efficacy of exogenous gonadotrophin therapy in stimulating multiple ovulations in the suni. Although marked variations in ovarian responses to different protocols were noted, an effective FSH-P treatment regimen was selected, consisting of a total of 3·75 mg FSH-P administered in a decreasing regimen over 3 days (0·75, 0·62 and 0·50 mg twice/day on Days 13, 14 and 15 of MAP treatment, respectively).

Non-surgical embryo collection in the suni was most effective 5 days after sponge removal. The efficiency of ovum or embryo recovery from individual donor animals ranged from 0 to 77·8%. Embryos were predominantly at the 8-celled stage of development. Studies are in progress to determine whether the embryos recovered from superovulated suni donors are capable of establishing viable pregnancies and developing normally to term. Unfertilized ova constituted the major proportion of the total recovered (61·8%); however, the majority of these (71·6%) were recovered from a particular animal (No. G10; Table 4).

In domestic sheep, MAP or prostaglandin F-2α treatment for synchronization of oestrus have been associated with reduced fertilization rates, apparently due to an inhibition of both sperm transport and the establishment of sperm reservoirs in the cervix (Hawk *et al.*, 1987). These investigators also observed lowered fertilization rates in domestic sheep after FSH-P treatment; however, results were not conclusive since high fertilization rates following exogenous gonadotrophin therapy have been demonstrated in previous studies (Evans & Armstrong, 1984). In this investigation, the majority of unfertilized ova were recovered in several collection attempts from a suni

which responded to FSH-P treatment with an average of 9·3 ± 2·6 corpora lutea (0–17; $n = 7$) per trial, yet which had consistently failed to yield embryos. It is, therefore, possible that the reason for the preponderance of unfertilized ova in this study was due to a disruption of natural behavioural patterns which affected the mating behaviour of this female rather than to adverse effects directly resulting from hormonal therapy.

The reasons for the inability to recover embryos from superovulated suni donors later than 5 days after sponge removal are not known. In domestic goats treated with PMSG for multiple ovulation induction, a high incidence of premature luteal regression was associated with low embryo recovery rates when surgical collections were performed later than 5 days after the onset of oestrus (Armstrong *et al.*, 1983, 1987). These investigators suggested that the inability to recover embryos from does exhibiting premature luteal regression reflected a perturbation of embryo transport that resulted from endocrine abnormalities due to luteal failure. In the present study, it was not possible to determine whether premature luteal regression was a factor that could have contributed to the low recovery rates of suni embryos later than 5 days after MAP withdrawal since laparoscopic measurements of corpora lutea were performed at variable intervals (5–8 days) following MAP sponge removal.

Studies in laboratory animals have shown that exogenous gonadotrophin treatment can accelerate the tubal transport of ova or embryos into the uterus (Miller & Armstrong, 1981) followed by prompt expulsion through the cervix (Doyle *et al.*, 1963; Bennett, 1970). This rapid loss of ova or embryos in superovulated laboratory animals has been attributed to elevated oestradiol concentrations subsequent to ovulation (Greenwald, 1967; Miller & Armstrong, 1981) which may result from asynchronous ovulatory activity.

A preliminary study was recently conducted to collect embryos 6 days after sponge removal of evaluate the influence of technical inexperience on the previous inability to recover embryos later than 5 days after sponge removal. In these trials, 0–2 unfertilized ova were recovered from each of 3 suni with 2–17 corpora lutea per female. At the time of attempted embryo recovery, large (5–8 mm) antral follicles were also detected and transcervical uterine catheterizations were performed with minimal effort, suggesting that oestrogenic activity persisted during the post-ovulatory period. In domestic sheep, oestrogenic activity has been shown to increase cervical compliance typical of parturition even in the presence of high concentrations of progesterone (Stys *et al.*, 1980).

The information provided by this investigation may be used to enhance the breeding management of the suni, which are represented as isolated and highly inbred populations in captivity. Furthermore, these results demonstrated the feasibility of recovering embryos from small antelope by using non-invasive procedures which may be utilized for the captive propagation of endangered species.

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