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Intensive management of the Burmese brow-antlered deer *Cervus eldi thamin* for effective captive breeding and conservation

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The Burmese brow-antlered or Eld's deer *Cervus eldi thamin* is a sub-tropical endangered subspecies that is primarily distributed in central Myanmar (3° to 25°N); approximately 5000 are believed to remain in the wild (Salter & Sayer, 1986). The North American captive population consists of 140 individuals mainly distributed among three herds separated by as much as 4500 km. This species, and these populations, typify the challenges associated with genetically managing captive hoofstock populations. Eld's deer reproduce well in captivity but their excitable temperament increases the risk of stress and injury during long-distance transport. The deer have a reputation for displaying self-destructive behaviours in captivity, which has decreased their popularity as exhibit animals and discouraged their routine transport among institutions. As a consequence, the carrying capacity for the species has been reached in North American zoos and the deer have become inbred within breeding facilities.

Zoo managers and biologists now face a dilemma. How can genetic diversity be maintained without increasing animal numbers? Germ plasm cryopreservation, combined with artificial insemination, has potential for overcoming management problems while providing insurance against further losses in genetic diversity as a result of additional inbreeding, disease or other, unforeseen, catastrophes. The Eld's deer is a prime candidate for a demonstration of how 'assisted reproductive biotechnology' can be integrated with sound animal husbandry to manage and preserve genetic diversity within fragmented captive ungulate populations. However, the prerequisite for successfully applying these biotechnologies is the development of a strong biological database. Such information can only be obtained when animals are sufficiently tractable to permit detailed scientific studies to be conducted under controlled conditions. In this paper we describe the intensive management approach used at

the National Zoological Park's Conservation and Research Center and provide an overview of the scientific information we have accumulated using this strategy.

BASIC MANAGEMENT APPROACHES

Several strategies have been used for managing the North American populations of Eld's deer. Maintaining animals in a herd within large pastures (2–20 ha) is an option but such animals generally revert to 'wild' excitable behaviour patterns. Under this scheme even the simplest manipulations necessary for routine health screening or animal translocations can be difficult and unsatisfactory. Deer must be monitored from all-terrain vehicles and generally observed through binoculars. Essentially all procedures require remote anaesthetic delivery under field conditions, increasing time, expense and the incidence of injury and mortality.

A semi-intensive management approach involves the maintenance of intermediate-sized herds (ten to 20 individuals) within paddocks, holding yards or pastures (0·5–2 ha). The deer can be conditioned to voice commands or to feeding regimens that enable them to be routinely shifted between yards. Individual hinds or stags can be moved between groups for breeding purposes or can be mustered into squeeze chutes for routine health-related procedures, such as tuberculin skin-testing or blood sampling. This approach is similar to that found in commercial deer farming but is not often used successfully in zoological institutions. Although useful for propagation, exhibition or simple behavioural studies, this approach generally is inadequate for conducting detailed biological investigations requiring intensive 'hands-on' manipulations, or repeated collection of biological materials, such as blood, urine and faeces, or morphometric measurements. We have therefore developed an alternative intensive management strategy involving hand-rearing and conditioning which permits detailed scientific investigations while minimizing stress to the animals.

HOUSING

Cervid species can be housed safely within a managed barn facility in most zoo settings. Because deer typically are excitable and exhibit a strong flight response when threatened, confinement, as opposed to housing in large areas, can actually simplify the safe manipulation of the animals. The CRC Ungulate Research Facility houses 13.19.2 Eld's deer. Within the complex there are two types of barn layout, both incorporating specialized design characteristics.

Rivinus Barn is a double-winged complex with office and laboratory facilities located between the two wings (Fig. 1). The North (13 stalls) and and South (14 stalls) wings consist of individual $3{\cdot}4 \times 4{\cdot}6$ m stalls, each of which provides access to an outdoor run of $3{\cdot}6 \times 36{\cdot}6$ m. Centre aisles permit vehicle access for stall cleaning and loading of animals into livestock trailers. In most areas chain-link fencing is used but in the North wing the stalls are partitioned with solid galvanized steel providing a visual barrier to minimize inter-♂ aggression during rut. The substrate is earth and/or fine gravel to improve traction and prevent hoof overgrowth. Each wing contains an electronic 'walk-on' platform scale (accurate to 0·1 kg), as well as hay and food pellet storage areas. Skylights provide exposure to natural fluctuations in daylength. Although the animal areas are not centrally heated, dual infra-red heat lamps are fitted within each stall. The laboratory area contains materials for food preparation and storage, as well as adequate space for performing laboratory analyses and minor non-sterile surgical procedures.

The Rivinus Annex (Fig. 1) is comprised of two parallel rows each of ten $3{\cdot}7 \times 2{\cdot}4$ m stalls with a 4 m wide centre aisle providing vehicle access. The wooden partition walls are removable allowing any stall to be doubled in width.

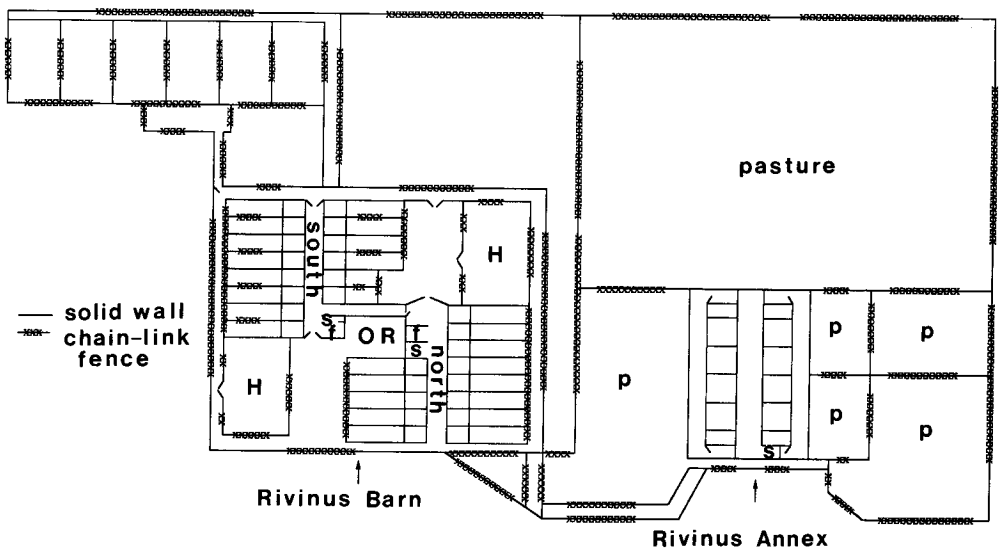

Fig. 1. Schematic representation of the Conservation and Research Center's Ungulate Research Facility (not to scale). OR. office, research laboratory and rest rooms; H. holding yard; p. small to intermediate-sized paddocks; s. platform scale; f. hay store. The eight larger stalls in the Rivinus Annex are shown in their double-width configuration, divided into two they give a possible 20 stalls.

Skylights provide excellent exposure to natural fluctuations in daylight. The entire facility is centrally heated and six ceiling fans assist air circulation. Stalls and runway are covered with a rubberized non-skid flooring and stall floors are pitched slightly towards centre drains for the collection of urine specimens. A 1·5 m wide earth-floor outer perimeter corridor allows animals to be shifted from either the front or rear of a stall to any other stall or exit in the building. The corridor contains an electronic platform scale, on which the animals can be easily confined for routine weighing. The animals are exercised daily in small to intermediate-sized outdoor paddocks (Fig. 1).

During the winter, the rutting season or for most research studies, ♂♂ are housed singly indoors. While research projects are in progress, ♀♀ are usually housed in pairs or singly within barn stalls. At other times, deer are maintained on pastures in single sex groups to prevent unwanted matings. Hinds can be grouped with minimal inter-animal aggression because dominance hierarchies are established quickly. Males can be maintained together except during the rutting season (January to May). A system of interconnecting 1·5 m wide shift corridors and gates permits deer to be moved between the Barn and Annex and the adjacent 0·2 ha holding yards or 0·8 ha pastures.

HUSBANDRY

Diet Excellent body condition is maintained by feeding good quality alfalfa hay together with alfalfa pellets (12·5% protein) in daily rations consisting of 2–3% body weight. Within individual stalls, hay is suspended from a rack at a height of *c.* 1·5 m and pellets (1 kg total ration) are placed in a ground-level feed pan. Grouped deer are fed at multiple feeding stations to prevent monopolization of food by dominant individuals. The diet is supplemented with natural browse, partly to prevent boredom, and trace mineral salt blocks and fresh water are provided *ad libitum*.

Lactation and growth Milk composition, intake and neonatal growth have been

studied in nine mother-young pairs by C. Wemmer and S. Crissey. Fat (10·5–11%), protein (5·5–7·7%) and lactose (3·6–4·5%) content in the dam's milk remains relatively constant through the first 21 weeks of lactation. The average amount of time the young spent suckling in a 24-hour period was 52 minutes during the first week of lactation, declining to three minutes by seven weeks of age. The average total milk intake per day was 640 ml during the first week of life and gradually declined to less than 200 ml immediately before weaning at *c.* 25 weeks of age. There were no sex-related weight differences between fawns up to 13 weeks of age. Fawns weighed 4–5 kg at birth and *c.* 22 kg by 13 weeks. By 27 weeks the mean weight of ♂♂ (36·3 g) exceeded that of ♀♀ (33·1 kg) ($P<0·05$).

Neonatal examination and treatments Every fawn is given a complete physical examination 24–48 hours after birth. The umbilicus is treated with antiseptic (Neo-Violet), and 1500 IU tetanus antitoxin and 3 ml clostridium bacterin-toxoid (Clostridium Chauvoei-Septicum-Novyi-Sordellii-Perfringens types C and D) injected subcutaneously. Vitamin E (3 ml, 1500 IU) and 2·5 ml prophylactic long-acting antibiotics (penicillin G benzathine and penicillin G procaine, 300 000 IU/ml) are also administered subcutaneously. The eyes are flushed with sterile saline and a broad-spectrum ophthalmic antibiotic ointment (gentamicin sulphate without steroids) is placed in each eye. Blood samples are also obtained by jugular venepuncture and used to generate a complete blood count and serum chemistry profile. A sulphate turbidity test (McEwan *et al.*, 1970; Parkinson *et al.*, 1982) is routinely performed to check for failure of passive transfer of maternal immunoglobulins. The fawn is weighed, ear tagged for permanent identification and gross morphometric measurements are obtained (in centimetres), including: (1) length from tip of nose to tip of tail; (2) shoulder height from apex of scapula to dorsal hoof margin; (3) chest girth caudal to shoulder; (4) chest girth at point of last rib; (5) tail length; (6) upper neck circumference just below angle of mandible; (7) lower neck circumference cranial to scapulae; (8) circumference of cranium; (9) ear length. When the procedures are complete the newborn is reunited with the dam, or fawns for hand-rearing are placed in a heated stall bedded with straw.

Hand-rearing Information on sire and dam identification, whether the fawn suckled, weight at removal and general physical condition are recorded on the hand-rearing record. Ingestion of colostrum within the first 24 hours is critical for survival (Robbins *et al.*, 1987). The absorption of immunoglobulins is maximal at 12 hours after birth and is completed by 24 hours (Robbins *et al.*, 1987). If failure of passive transfer of maternal immunoglobulins is suspected and less than 24 hours has elapsed since birth, frozen conspecific colostrum or soluble colostrum powder is provided. The fawn's weight is recorded daily for the first three weeks and daily records of food intake, weight gain and general health include date, time, body weight, amount and composition of milk formula offered, amount of milk consumed and occurrence of urination or defecation. The condition of the stool is graded on a scale of one to five (1 = liquid to 5 = hard). Activity patterns (sleeping, recumbent or standing, and active or inactive) are also recorded at the time the animal handler enters the fawn's stall for feeding.

Formula consists of Carnation canned condensed milk mixed with equal parts of previously boiled and cooled water. Milk is offered in a three-litre bottle and supplemented with four to five drops of a soluble multivitamin (Poly-Vi-Sol), 0·25 g of table salt and 400 IU Vitamin E per day. During the first week of life, 180 ml lukewarm milk mixture (3–4% of body weight) is offered four times per day (0730, 1130, 1530 and 1930 hours). The first feeding after separation from the

dam, is delayed several hours to increase the fawn's appetite and improve bottle acceptance. Conversion to a bottle can be difficult, often requiring considerable encouragement such as expressing small amount of milk from the rubber nipple into the fawn's mouth. This is done cautiously to avoid potential problems associated with tracheal aspiration and secondary pneumonia. While the fawn is nursing, a warm damp sponge is used to stroke the ano-genital region to stimulate suckling, urination and defecation reflexes.

The total daily food ration is equivalent to 18–20% of the fawn's body weight. After the first week of life, the proportion of milk is gradually increased by 15–20% per week, until by three weeks, only undiluted condensed milk with vitamins and salt is fed. If the proportion of milk in the formula or the amount of formula offered are increased too rapidly and diarrhoea occurs, the proportion and amount of formula are returned to their previous levels. A paediatric electrolyte solution can be substituted for water in the formula to facilitate rehydration and electrolyte replacement. Although there is individual variation, fawns typically receive 300–400 ml per feed (four times per day) at one month of age, 400–500 ml per feed (three times a day) at two months and 700–800 ml per feed (twice a day) at three months. Alfalfa pellets and hay are offered to fawns *ad libitum* beginning at one week of age but solid food consumption does not generally begin until about three weeks. At this time 50–60 g of alfalfa pellets, pre-soaked in warm water are mixed with milk formula to give a puree which is fed by bottle. Bottles and nipples are sterilized between feeds, and different animal handlers rotate nursing duties to enhance fawn socialization with humans. Fawns are encouraged to lick a trace mineral block and are not prevented from consuming small amounts of earth that can provide a valuable source of supplemental iron and rumen microflora (Robbins *et al.*, 1987).

TRAINING

Conditioning to the barn setting and training of movement routines begins whenever a neonate or new adult is added to the collection. Before 1988, the deer were exposed to only a small number of animal keepers, and other personnel and visitors were excluded from the premises. This strategy was designed to isolate them from outside disturbances and avoid triggering self-destructive behaviours. Unfortunately, when unfamiliar individuals such as maintenance and research staff, or unusual noises or equipment, were encountered the animals became excited. In 1988, a new approach was gradually implemented with the goal of acclimatizing animals to novel stimuli and minimizing the flight responses. Initially, an unfamiliar keeper spent 30–60 minutes per day positioned immediately outside each animal's indoor stall, separated by chain-link fencing. Confinement within high-wall stalls minimized the chance of a flight response and precluded serious injury. A soothing voice combined with vegetable treats, such as carrots and apples, was used as positive reinforcement. Acclimatization gradually occurred over four to six weeks. When an individual became over-excited the keeper simply moved away. Eventually, the deer began to exhibit a reduced flight distance and within four to six weeks, the keeper was able to work within the stall while the animal had the option of remaining or moving to the outside run. Once a deer was used to the primary caretaker, it was exposed regularly to other keepers, volunteers, researchers and maintenance personnel. The deer were also increasingly exposed to other stimuli including vehicles, horse trailers, lawn mowers, wheel barrows and associated noises. Finally radio music was played at a medium volume 24 hours a day to acclimatize the deer to background noises.

As a result of this programme, deer can now be routinely moved through aisles and shift corridors to interconnected holding yards, between barns, onto

weighing platforms and even into horse trailers often with the use of only simple voice commands. For example, deer being temporarily maintained on adjacent pasture can be called into the barn and segregated into individual stalls within five to ten minutes. A similar approach is used to confine ♂♂ within 1 × 2 m urine collection enclosures within their stalls. Urine samples of ♀♀ are routinely collected by placing a cup attached to a 1 m pole into the urine stream or by aspirating a sample from urine pooled on the ground. Urine specimens from both sexes are used to track long-term reproductive-endocrine rhythms (Monfort *et al.*, 1990). Additionally, anaesthetics, vaccinations or worming medications generally can be administered by hand-syringe or using a stick/pole device.

Our overall management strategy minimizes self-destructive behaviours by conditioning animals to a diversity of stimuli. We strive towards behaviourally conditioned deer that do not become imprinted on humans or as adults exhibit abnormal social attachment to humans. Fawns are group reared when possible and are maintained within visual, auditory and olfactory proximity to adults of both sexes. Our training regimen begins during the hand-rearing programme. For example, the bottle is used as inducement to move fawns from their stalls to a weighing platform or other areas of the barn. Conditioning and training initiated at an early age are easily continued into adulthood and results in tractable and socially normal barn-maintained deer. Consistency of demeanour and verbal commands among barn workers is critical for effective control of deer of all ages.

PREVENTATIVE MEDICINE

Routine herd health screening includes twice-yearly assessment of faecal samples for endoparasites and appropriate treatment with antihelminthics. Pre- and post-shipment quarantine procedures include intradermal tuberculin skin-testing using avian and mammalian tuberculin (PPD) injected at two sites on shaved regions of the cervical neck, and mammalian old type (OT) administered in the caudal tail fold. Neck inoculation sites are visually inspected daily and the skin thickness overlying the inoculation sites measured using callipers 72 hours post-inoculation; the tail inoculation site is palpated. All animals are vaccinated annually using clostridium bacterin-toxid (2·5 ml s.c.), tetanus toxoid (1 ml i.m.), rabies (Imrab, 2 ml i.m.) and dewormed prophylactically with 1% ivermectin (Ivomec, 1·0 ml s.c.). Generally, Eld's deer are vaccinated while physically restrained, however, sedation also can be used for vaccine administration, tuberculin skin-testing, hoof trimming or blood sampling using xylazine hydrochloride (Rompun, 0·3–0·5 mg/kg i.m.). A surgical plane of anaesthesia is achieved in tame Eld's deer using combined ketamine hydrochloride (Ketaset, 2–3 mg/kg body weight) and xylazine hydrochloride (0·3–0·4 mg/kg body weight) administered intermuscularly. This drug combination has been used successfully for minor surgical procedures, placement of chronic indwelling catheters, electroejaculation and laparoscopic intrauterine insemination (Monfort *et al.*, in press a, b, c).

LIFE HISTORY PATTERNS

Eld's deer are moderately sexually dimorphic; although both sexes stand 80–90 cm tall at the shoulder ♂♂ weigh 90–120 kg and ♀♀ 60–90 kg (Wemmer, 1987). Both sexes have a greyish-brown coat during the winter and the spring moult results in a reddish-brown summer coat. In adult stags, long thick hair forms a prominent neck mane during winter and spring. Eld's deer antlers are simplified structurally in comparison to other *Cervus* sp, being bow- or lyre-shaped in a frontal perspective, and sweeping in a continuous curve from the brow-tine to the beam. The main antler beam generally is palmated with several small snags at the junction of the brow-tine and main beam (Wemmer, 1987). Both sexes have prominent preor-

bital scent glands below the inner margin of the eyes and subcaudal scent glands below the base of the tail (Wemmer & Montali, 1988). Stags wallow in mud and tend to utilize latrines for depositing urine and faeces (Wemmer & Montali, 1988).

Rut occurs during late winter and early spring in South-east Asia (Lekagul & McNeely, 1977; Salter & Sayer, 1986). During the rut, normally solitary ♂♂ are found in herds averaging three ♂♂ and eight ♀♀ of various age classes (Salter & Sayer, 1986). Two analyses of zoo data indicated that 80–90% of all births in captivity occur from September to November in France (48°N) (Prescott, 1987) and the United States (38°N) (Wemmer & Grodinsky, 1988). A report that October and November are the peak birth months in captive Eld's deer at Delhi Zoo (29°N), within the species' natural range (Desai & Malhotra, 1978), suggests that there is no latitudinal shift in the timing or duration of the birth season.

STRESS PHYSIOLOGY

Our deer have become sufficiently acclimatized to allow frequent serial blood sampling from fully conscious composed animals using a remote catheterization technique (Monfort *et al.*, in press b, d). Adrenal hormones, such as cortisol, are commonly used to provide a physiological index of animal stress. However, most studies have relied on infrequent, single blood samples, often collected while animals were anaesthetized or physically restrained. Our results clearly illustrated the need for frequent blood sampling to ensure accurate assessment of adrenal status in Eld's deer. Cortisol may only be an appropriate indicator of stress if the secretory dynamics, including pulse amplitude and frequency, are thoroughly evaluated. Deviation from a regular episodic secretory pattern may suggest physiological stress but infrequent blood sampling, especially when animals are physically restrained or anaesthetized, could easily lead to erroneous conclusions regarding adrenal status.

REPRODUCTION

Environmental factors act as proximate cues modulating onset and cessation of reproductive activity in most seasonally breeding mammals. Although subjected to an extended rainy season (mid May to mid October), Eld's deer are not exposed to wide seasonal oscillations in photoperiod in their native habitats (Wemmer & Grodinsky, 1988). There is a general consensus that cervid species from between 20°N and 20°S experience little seasonality and remain reproductively aseasonal even when translocated to temperature zones (Lincoln, 1985; Loudon & Brinklow, 1992). However, Eld's deer appear to be unique in demonstrating a seasonal reproductive rhythm in a sub-tropical habitat and failing to exhibit a latitudinal shift in the timing or duration of their annual reproductive rhythms when translocated to temperate latitudes. We have been interested in studying the similarities and differences in endocrine regulatory mechanisms between this sub-tropical species and temperate cervids.

Female reproduction Females can conceive as yearlings (mean age = 469 days) and the interbirth interval is *c.* 380 days regardless of whether fawns are produced and reared in the previous year (Wemmer & Grodinsky, 1988). Monitoring urinary oestrogen conjugates and/or pregnanediol-3α-glucuronide (PdG) (Fig. 2) has confirmed that hinds are seasonally polyoestrous, spontaneous ovulators with onset of oestrus occurring in late winter or early spring (January to March) followed by a seasonal and/or lactational anoestrus beginning in autumn (August to October). Cyclic fluctuations in PdG correspond to progesterone concentrations measured in peripheral blood circulation. The average duration of the oestrous cycle is 18·5 days (based on urinary PdG profiles) and, if conception does not occur, hinds may cycle for up to eight months (Fig. 2). Some ♀♀ can exhibit prolonged oestrous cycles, as long

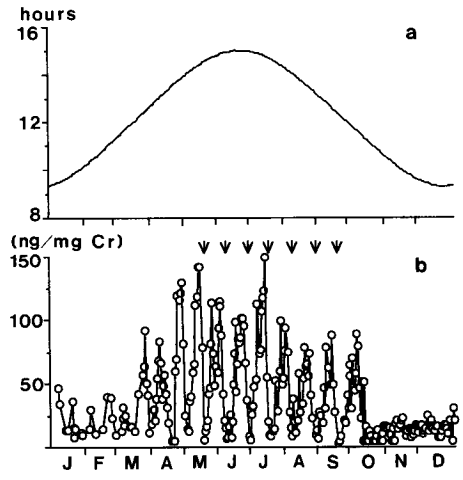

Fig. 2. Annual non-conception urinary PdG profiles from a representative Eld's deer *Cervus eldi thamin* **hind sampled three to five times per week for one year. a. hours of daylight; b. urinary pregnanediol-3α-glucuronide levels (in ng/mg creatinine). Arrows indicate observations of behavioural oestrus. (Adapted from Monfort** *et al.* **1990.)**

as 62 days, presumably reflecting abnormally prolonged corpus luteum progesterone secretion. Hinds exhibit behavioural oestrus consisting of scent marking the stag, animal handlers or inanimate objects with preorbital scent glands. In one study, overt signs of oestrus were observed in 42 of 65 oestrous cycles (65%) and always corresponded with intercyclic troughs (two to five days in duration) in PdG excretion (Monfort *et al.*, 1990). Oestrus usually lasts 12–24 hours and hinds may exhibit increased activity patterns and a clear to milky vaginal discharge (Wemmer & Grodinsky, 1988). Copulation is characterized by an ejaculatory thrust in which the ♂'s rear feet leave the ground (Wemmer & Grodinsky, 1988). The mean gestation lasts about eight months (33·5±0·4 weeks). Nearly all captive births (>97%), are of singletons and sex ratios do not deviate significantly from 1:1 (Prescott, 1987; Wemmer & Grodinsky, 1988). Pregnancy can be diagnosed by markedly elevated urinary PdG excretion by week 12 of gestation and urinary oestrogen conjugates can be used to estimate the expected data of parturition during the final month of gestation (Fig. 3) (Monfort *et al.*, 1990).

Male reproduction Stags are fertile at one year of age and exhibit sexual and aggressive rutting behaviours during late winter and early spring in South-east Asia (Salter & Sayer, 1986) and the USA (Wemmer & Grodinsky, 1988; Monfort *et al.*, in press a). Unlike most temperate 'short-day' breeding cervids, maximum

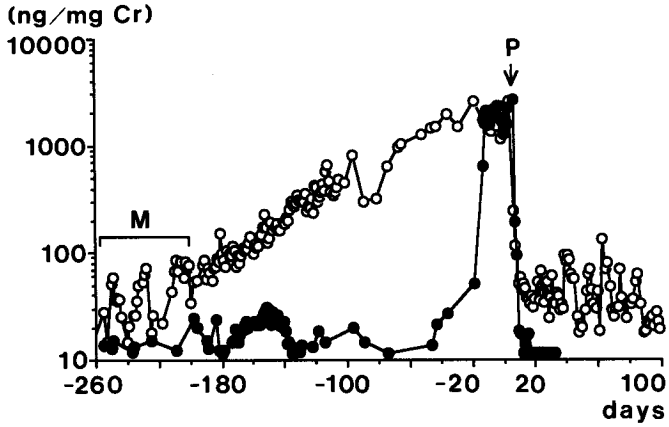

Fig. 3. Urinary oestrone conjugates (open circles) and PdG concentrations (closed circles) (in ng/mg creatinine) in a representative hind sampled from 260 days before parturition until 100 days post partum. All values are aligned to the day of parturition. P. parturition; M. paired with ♂. (Adapted from Monfort *et al.* **1990.)**

antler development and behavioural aggression (Fig. 4a), body weight gain and testicular growth (Fig. 4b) occur in Eld's deer during the winter and spring as daylength increases (Monfort *et al.*, in press a, b).

Blood samples have also been collected weekly for 52 weeks from six adult stags exposed to normal fluctuations in photoperiod (38°N) (Monfort *et al.*, in press a). Serum luteinizing hormone (Fig. 4d) concentrations peaked in the autumn (October), three months before follicle-stimulating hormone and testosterone (Fig. 4c) peaked in early winter (January). Marked circannual variations in circulating prolactin (Fig. 4d) also suggested that Eld's deer may use photoperiodic cues to modulate seasonal fertility. Although antlerogensis corresponded to circannual fluctuations in LH, FSH and testosterone, the hormonal rhythms were shifted six months out of phase relative to most temperate deer species (Suttie *et al.*, 1984). Antler length, body weight and chest girth were maximal during pre-rut (December to January). Maximal scrotal circumference and combined testes volume were observed in mid winter (February), whereas peak neck girth and behavioural aggression occurred one to three months later (March to May). The seasonal cycle in body weight, neck and chest girths observed in Eld's deer was qualitatively similar to that observed in Red deer *Cervus elaphus* (Suttie *et al.*, 1984), Chital *Axis axis* (Loudon & Curlewis, 1988) and Fallow deer *Dama dama* (Asher *et al.*, 1989). This seasonal pattern in morphometric measures presumably reflected endogenous metabolic processes that occur independently of gonadal steroid secretion and *ad libitum* food intake (Loudon & Brinklow, 1992). Typical of cervids, Eld's deer stags exhibit a notable increase in voluntary food intake during the autumn and winter that presumably maximizes their body condition before the spring rut.

Semen samples also have been collected quarterly by electroejaculating anaesthetized ♂♂ using a standardized protocol (Monfort *et al.*, in press a). Motile sperm were produced in all seasons but the number of motile sperm/ejaculate

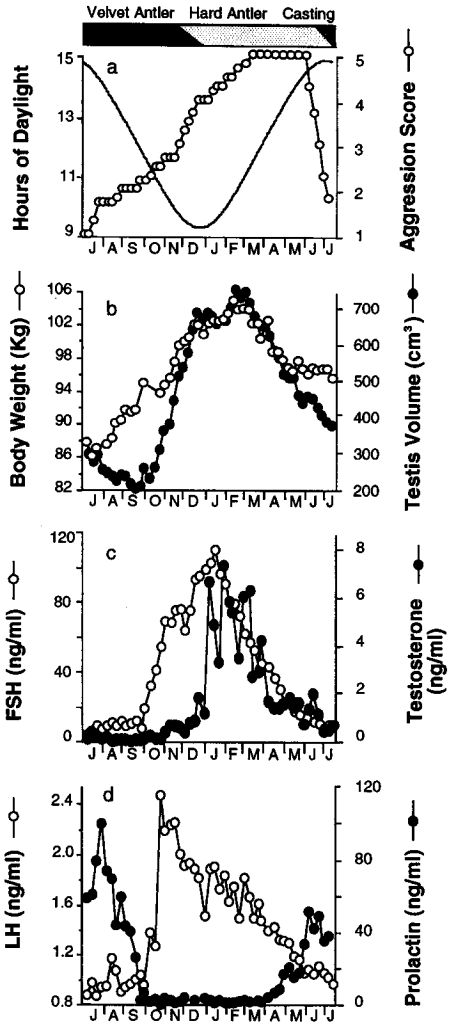

Fig. 4. Data from six adult Eld's deer stags sampled for one year: a. (solid line) hours of daylight (at 38°N), (open circles) weekly mean behavioural aggression score towards handler on scale 1–5 (1 timid to 5 aggressive threatening 'head-down' posture), (top) antler development throughout year; b. (open circles) body weight in kg, (closed circles) testis volume calculated as combined volumes both testes in cm³; c. (open circles) serum FSH from pituitary, (closed circles) testosterone from testis both in ng/ml; d. (open circles) LH, (closed circles) prolactin both from pituitary in ng/ml. (Adapted from Monfort *et al.* in press a.)

($213 \pm 131 \times 10^6$/ml) was lowest during the autumn and peaked in the winter (winter 1603 ± 370; spring 800 ± 302; summer $760 \pm 246 \times 10^6$ ml) ($P < 0{\cdot}05$). The percentage of structurally normal spermatozoa per ejaculate was more than four-fold higher in the winter (86%) and spring (92%) compared to the autumn (18%) ($P < 0{\cdot}05$). Histological assessments of the regressed testis (July) revealed decreased numbers of germ cells undergoing spermatogenesis and an increased incidence of degenerating and abnormal cell types. In summary, Eld's deer stags exhibit a circannual hypothalamic-pituitary-gonadal cycle with onset of pituitary activation occurring during the autumn and winter, whereas gonadal activity peaks during the winter and spring as day lengths are increasing.

Artificial insemination Captive propagation is important for preserving rare taxa when *in situ* conservation efforts are incomplete, fragmented or likely to fail (Soulé, 1991). When *ex situ* tactics are used to manage small populations, a primary goal is to maintain adequate genetic variability and to avoid inbreeding depression. Artificial insemination, *in vitro* fertilization and/or embryo transfer, can be used to enhance captive breeding of rare species, although as yet these 'assisted techniques', have not been consistently useful for producing offspring from any endangered mammalian species (Wildt, 1992; Wildt *et al.*, in press). Artificial insemination is potentially valuable for: (a) ensuring reproduction between genetically valuable but behaviourally incompatible pairs; (b) eliminating the risks of animal transport; (c) providing an avenue for infusing genes between wild stocks and captive populations, many of which are genetically stagnant (Wildt, 1989).

For artificial insemination to be successful, detailed prerequisite information must include: (a) an understanding of the ♀'s reproductive cycle to allow for the identification or manipulation of oestrus/ovulation; (b) safe and reliable methods for the collection and storage of viable spermatozoa; (c) methods for proper deposition of sperm at the optimal time and site within the ♀. We have now fulfilled these criteria in the Eld's deer (Monfort *et al.*, in press c).

In 1992, nine pregnancies were produced by artificial insemination using frozen-thawed spermatozoa (Monfort *et al.*, in press c). Sperm donors were selected by prospective analysis of their genotypic contribution to the North American population. Three ♂♂ were selected and semen was collected by electroejaculation and stored frozen. Intravaginal progesterone-releasing devices (CIDR-type G, 9% progesterone) were used to synchronize oestrus and ovulation in 20 adult hinds. The devices were removed after 14 days and each ♀ was anaesthetized 70 hours later for transabdominal, intrauterine insemination ($7{\cdot}5$–10×10^6 motile sperm per uterine horn) under laparoscopic observation. Nine hinds (45%) delivered ten offspring after a mean gestation of 241 days (range 235–245 days). This represents the largest number of pregnancies produced in a single AI trial for an endangered mammal.

Our success was built upon techniques that are routinely applied to farmed species such as Red and Fallow deer (Asher *et al.*, 1992). Based upon our preliminary studies, we now have developed the capability routinely to obtain Eld's deer spermatozoa by electroejaculation, and maintain excellent sperm motility and structural integrity after long-term low temperature storage. We can also successfully synchronize oestrous cycles among ♀♀ and monitor the efficacy of such efforts using non-invasive urinary hormone monitoring techniques (Monfort *et al.*, in press c).

This success provides an excellent example of how readily an assisted reproduction technique can be applied to an endangered species if the procedure is already working well in a taxonomically

related 'model'. Although some refinements may be necessary because of unique species' attributes, we anticipate that this approach may have broad application to other cervid species. It is important to emphasize that this study was preceded by the establishment of a strong database for both sexes before AI was attempted. Certainly detailing basic life-history variables, behaviour, seasonality, oestrous cyclicity, gametogenesis and semen freezing (Wemmer & Grodinsky, 1988; Monfort *et al.*, 1990, in press a, b, c, d) helped to ensure a higher rate of success. For example, detailed information on seasonality ensured that hinds were not inseminated too early or late in the breeding season, a factor that severely decreases AI success in Fallow deer (Asher, 1986). Similarly, seasonal evaluation of ♂♂ indicated the time of year most likely to result in peak semen quality and sperm freezability (Monfort *et al.*, in press a).

Our results clearly established the utility of urinary hormone monitoring as an important adjunct to this assisted reproduction strategy. Repeated anaesthesia or restraint for blood collection and ultrasonography is usually unpractical in deer in zoos. In fact, for many species, urinary and/or faecal steroid monitoring are the only alternatives for assessing longitudinal endocrine rhythms. These approaches provide considerable promise for improving success rates of artificial breeding in other stress-susceptible species. Monitoring urinary hormonal metabolites permitted tracking the efficacy of oestrous synchronization and diagnosing pregnancy by week 12 of gestation.

The offspring produced by AI in our trial were all considered genetically valuable based on inbreeding coefficients $<0{\cdot}25$ (0·000, $n=6$, 0·031, 0·062 and 0·250). Six of these offspring were hand-reared and at four months of age were transported successfully to the Singapore Zoological Gardens to establish a breeding population. Here is a clear example of how advanced reproductive biotechniques can be combined with sound management and husbandry to produce genetically valuable offspring. Offspring have now been produced from 32 mammalian species using AI with frozen-thawed spermatozoa (Wildt *et al.*, in press). Seven species have been cervids (White-tailed deer *Odocoileus virginianus,* Fallow deer, Red deer, Wapiti *Cervus elaphus canadensis,* Reindeer *Rangifer tarandus,* Axis deer and Eld's deer). Thus, the technology now exists to implement genetic management plans for maximizing the genetic diversity of small captive populations of rare cervid species.

Responsible zoo management must focus on efforts to maintain or enhance genetic diversity within captive populations. Unfortunately, traditional captive breeding/management of wild ungulates has often failed to integrate even regional zoological collections into a single population for genetic and demographic management. For stress-susceptible ungulates, an approach that combines traditional husbandry and management with advanced reproductive biotechniques may be the only realistic hope of maintaining genetic diversity without increasing animal numbers to unmanageable levels. Although performed on a limited scale, the present study represents one of the first examples in which prospective sire and dam selection, germ plasm banking, AI and urinary hormone monitoring have been used for a specific goal in an endangered species. These results were made possible by an intensive management scheme that permitted detailed physiological studies to be conducted under controlled conditions.

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PRODUCTS MENTIONED IN THE TEXT

Carnation milk: canned condensed milk, manufactured by Carnation Co., Los Angeles, CA, USA.
CIDR-type G: controlled internal drug releasing device, 9% progesterone, manufactured by Agricultural Division, CHH Plastic Products Group Ltd, Hamilton, New Zealand.
Clostridium bacterin-toxoid: Clostridium Chauvoei-Septicum-Novyi-Sordellii-Perfringens types C & D bacterin-toxoid, manufactured by Mobay Corporation, Animal Health Division, Shawnee, KS 66201, USA.
Gentocin: gentamicin sulphate ophthalmic ointment, manufactured by Schering Corporation, Kenilworth, NJ 07033, USA.
Imrab: killed virus rabies vaccine, manufactured by Pitman Moore, Inc., Mundelein, IL 60060, USA.
Ivomec: 1% ivermectin solution, manufactured by Merck & Co. Inc., Rahway, NJ 07065, USA.
Ketaset: ketamine HCl, manufactured by Aveco Company, Fort Dodge, IA 50501, USA.
Neo-Violet: gentain violet and neomycin, manufactured by Life Sciences Products, St Joseph, MO, USA.
Penicillin: penicillin G benzathine and penicillin G procaine, manufactured by G. C. Hanford Manufacturing Company, Syracuse, NY 13201, USA.
Poly-Vi-Sol: multivitamin with iron, manufactured by Bristol-Myers Squibb Company, Evansville, IN, USA.
Rompun: xylazine hydrochloride, manufactured by Mobay Corporation, Animal Health Division, Shawnee, KS 66201, USA.
Soluble colostrum powder: manufactured by Quality Plus Essar Corporation, Fort Dodge, IA, USA.
Tetanus antitoxin: manufactured by CEVA Laboratories, Inc., Overland Park, KS, USA.
Tetanus toxoid: manufactured by Mobay Corporation, Animal Health Division, Shawnee, KS 66201, USA.
Vitamin E: manufactured by Stuart Products, Bedford, TX, USA.

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The Vietnamese sika

Cervus nippon pseudaxis

conservation project

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Vietnam is a country approaching an ecological crisis. Forest cover is rapidly dwindling and, with less than 18% of the total area still forested, there is a corresponding loss of fauna. This comparatively small area contains a wealth of endemic forms, as well as a number of species which are threatened on a world scale. Several of these, including the Javan rhinoceros *Rhinoceros sondaicus,* Kouprey *Bos sauveli* and the recently rediscovered Tonkin snub-nosed monkey *Rhinopithecus avunculus* (Ratajszczak, unpubl.), are unlikely to survive beyond the year 2000 unless a concentrated effort is made by the international community.

Captive breeding has a very important role to play in this venture for by the time the nature conservation movement is powerful enough there may well be no large animal species left to preserve in Vietnam. As a first step in the implementation of the large-scale breeding and conservation programme which is necessary for the preservation of the genetic resources of Vietnamese fauna, work has begun on the conservation of Vietnamese sika *Cervus nippon pseudaxis.*

Little information is available on the biology and status of *C. n. pseudaxis.* The status of the taxon as a threatened form was not recognized until the late 1980s when it appeared for the first time on the IUCN red list in the category Endangered (IUCN, 1990) and by that time, the species had probably been extirpated in