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THE ANNUAL REPRODUCTIVE CYCLE OF THE TSESSEBE *DAMALISCUS LUNATUS LUNATUS* (BURCHELL)

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The annual reproductive cycle of the tsessebe

Damaliscus lunatus lunatus (Burchell)

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Damaliscus lunatus lunatus (Burchell)

by

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ABSTRACT

In the present study, the general status and biology of the rare tsessebe antelope is described, as are the effects of stress due to confinement and physical restraint on antelope. The effects of semi-intensive management, as well as the presence or separation (by a fence only) of the bull from the cows on reproductive patterns in tsessebe are examined. Neither these nor the physical restraining technique employed to collect faecal and blood specimens appeared to influence the reproductive cycles of the tsessebe. Cows in good body condition showed reproductive cyclicity throughout the year. The oestrous cycle length was approximatly 21 days long. No significant differences in haematological and plasma biochemical parameters were observed between the winter and summer months, nor were they significantly different from blood collected from a range of other ungulate species.

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CHAPTER 1

Introduction

Natural History of Tsessebe

Tsessebe, Damaliscus lunatus lunatus, one of seven subspecies of Damaliscus lunatus (Ansell, 1972), occurs in the southern region of the continent of Africa (Skinner & Smithers, 1990). These antelope are classified as "scarce" in the South African Red Data Book - Terrestrial Mammals (Smithers, 1986), although the Mace & Lande (1991) criteria for classification used for the IUCN's Red data list, places the tsessebe in the "safe" category. The distribution of this species has declined considerably in historical times. A survey carried out in the Transvaal estimated that there were about 1160 tsessebe in the Kruger National Park, while an additional 1000 animals were found in areas surrounding the Park borders, and about 70 animals have been reported to occur in the Pilanesburg National Park (Carr, 1986). There are approximatly 80 tsessebe in the Percy Fyfe Nature Reserve in the Northern Province (Howard, M. pers. comm.). Translocations have taken place, including the importation of animals from Zimbabwe into South Africa. Tsessebe have been relocated to Kwazulu/Natal and Northern Cape Provinces, the Etosha National Park, Namibia as well as to farms in the Transvaal.

The habitat requirements of tsessebe can be summarized as open woodland, where they are dependant on open water (Garstang, 1982). In Botswana, they favour the fringes of grasslands that form abrupt ecotones with woodlands, the importance of which appears to derive more from the availability of shade rather than for concealment. Tsessebe prefer to keep potential danger in sight and will take to the open frequently when disturbed (Child,

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Howard, M. Chief conservator, Percy Fyfe Nature Reserve, Potgietersrus, Northern Province.

Robbel & Hepburn, 1972). Tsessebe are almost exclusively grazers, and in the drier grasslands show a preference for grass up to 0,6 m tall (Child, Robbel & Hepburn, 1972). They also make persistent use of areas cleared by burning.

Tsessebe are inquisitive and curious, and will stand in the open even after a number of members of the herd have been shot (Skinner & Smithers, 1990). This fact, as well as others such as habitat destruction and fragmentation, bush encroachment and environmental catastrophes, have contributed to their disappearance from much of their former distributional range.

Tsessebe are gregarious animals and are typically found in small herds. Males establish and maintain territories which they patrol regularly (Joubert, 1972). Females in harems remain permanently associated with their territorial male (Joubert, 1972; Grobler, 1973; Garstang, 1982;). Yearling males are evicted from the harem herds by territorial males soon after the commencement of the calving season (October). This activity gains momentum as the autumn rut approaches (Skinner & Smithers, 1990).

Tsessebe are seasonal short-day breeders. In the Transvaal, rutting behaviour commences in early January and peaks in March. The bulk of the calf crop is born in October, with occasional births in November and early December (Fairall, 1968; Garstang, 1982). In northern Botswana, the rut peaks in March to April and calves are born in October through December (Child, Robbel & Hepburn 1972); the bulk of the calf crop being born within a 40 day period .

Reproductive Physiology of Ungulate Species

In general, the reproductive or oestrous cycle of sexually mature ungulates is characterised by regular waves of ovarian follicular growth and atresia which are mediated by the interaction of several hormones and growth factors. Functionally and for convenience sake, the endocrine events of the oestrous

cycle may be sub-divided into two phases, namely, the follicular and luteal phases. The follicular phase is associated with the period of luteal regression during which time a new cohort of follicles is recruited to develop under the influence of follicle stimulating hormone (FSH). At some point, the dominant growing follicle becomes independent of FSH for continued development and begins to secrete inhibin, a hormone that inhibits the further secretion of FSH, which consequently results in the atresia of the younger, FSH-dependent growing follicles. The increasing levels of follicular-derived oestrogens eventually results in a surge release of luteinizing hormone (LH) from the anterior pituitary gland and, consequently, follicular rupture and release of the mature oocyte at ovulation. The luteal phase commences as the post-ovulatory follicle cells luteinise, immediately following ovulation, and functionally convert to progesterone production (Yadav, Walton, & Leslie, 1988).

In the domestic cow (Bos taurus), the concentrations of circulating progesterone begin to increase two to three days post-oestrus (p.o.) and peak on approximately day 10 p.o. (Hansel, & Convey, 1983). The degeneration of the corpus luteum, which in the domestic cow occurs around day 18 p.o., is associated with a decline in circulating progesterone levels. The lifespan and function of the corpus luteum are prolonged when the animal becomes pregnant. Levels of faecal progesterone metabolites appear to closely mimic those of the circulating plasma progesterone in cyclic, anoestrous and pregnant cattle (Desaulniers, Goff, Betteridge, Rowell & Flood, 1989.).

Elevated circulating progesterone concentrations, therefore, indicate either pregnancy, or the luteal phase of the oestrous cycle. During pregnancy, the elevated progesterone levels are maintained by the corpus luteum, placenta, or both. During pregnancy, progesterone has a negative feedback effect on gonadotrophin releasing hormone (GnRH), which prevents the release of FSH and LH and, therefore, ovulation as well.

In the domestic cow, the oestrous cycle length is somewhat variable; however, it is generally accepted that, on average, the cycle length in heifers and cows is 20 and 21 days, respectively (Hansel, 1959). The average estimated cycle length for blesbok (Damaliscus dorcas phillipsi) is 28-32 days (Marais, 1988), for springbok (Antidorcas marsupialis) 16 days (Liversidge & de Jager, 1984), for blackbuck (Antilope cervicapra) 17 days (Holt, Moore, North, Hartman, & Hodges, 1988), for suni (Neotragus moschatus zuluensis) 21 days (Loskutoff, Raphael, Nemec, Wolfe, Howard, & Kraemer, 1990), for giraffe (Giraffa camelopardalis) and okapi (Okapia johnstoni) 15 days (Loskutoff, Walker, Ott-Joslin, & Lasley, 1986), and for scimitar-horned oryx (Oryx tao) 22 days (Loskatoff, Ott-Joslin, & Lasley, 1983).

Haematology and Biochemistry of Antelope

Information concerning baseline haematological and serum / plasma biochemical values in wild animals is important for the study and management of both captive and free-ranging populations. Certain variables have been suggested as reliable indices of physical and nutritional states of individuals or groups, and changes in these may prove useful in the investigation of pathological conditions, including those manifested by stress.

Although various studies have been carried out which report normative ranges for various blood constituents in African antelope (e.g., Cooper, 1992; Drevemo, Grootenhuis, & Karstad, 1974; Bush, Smith, & Custer, 1981; Pospisil, Kasa, Vahala, & Mouchova, 1984a, b, c, d; Peinado, Viscor, & Palomeque, 1990;), limited information exists regarding the genus Damaliscus and data are not currently available for the tsessebe.

Health Assessments of Game Species

A number of factors may influence body condition, such as season, nutritional status, sex, age, physiological status,

disease and stress. The assessment of body condition was considered to be important for this study, in view of the fact that a deterioration of body condition could adversely affect the health and reproductive status of the animals (Clarke, & Tilbrook, 1992).

There are several methods currently employed for estimating the quality of body condition in ruminants. Some of these were unsuitable because the animal has to be slaughtered in order to determine variables such as the relative proportion of kidney fat to total body weight (Ransom, 1965; Monro, & Skinner, 1979), adrenal indices, bone marrow fat content and bone marrow fat indices (Sinclair, & Duncan, 1972). Non-invasive methods, which do not require slaughter of the animal, include body weight determination, measurement of blood variables such as blood urea nitrogen concentrations, lipids, or the assessment of outward appearance. None of these methods provide an accurate, quantitative measure of body condition; however, certain techniques may possibly be used to provide an indication of the trend in body condition over a specified period of time. Assessment of outward morphological appearance was selected as the most effective method for monitoring the trend in body condition alterations during the present study since it would require minimal disturbance to the antelope.

Effects of Stress on Antelope

When capturing, maintaining in paddocks, or restraining free-ranging antelope, certain exertional and psychological stressors may result in physiological manifestations. Some of these include reproductive dysfunction (Clarke & Tilbrook 1992) and/or the ultimate survival of the animals.

An agent inducing stress is referred to as a stressor. To be regarded as a stressor, a stimulus must be quantitatively excessive and induce changes which threaten the integrity of the organism. If it persists for a long enough period, the stress

cannot be controlled by natural negative feedback or other mechanisms normally present for maintaining homeostasis.

Various authors have attempted to define stress in terms of physiological manifestations (Selye, 1973, 1976; Moberg, 1985; Dantzer, & Mormede, 1985). Riley (1981) defines stress as the psychoneuroendocrine influence on the physiology and biochemistry of the organism. Stress can often result in a complex interaction of biochemical pathways with many intricate feedback control systems affecting many physiological functions of the organism. Also, individual organisms may respond differently to the same stressor (Hattingh, 1986).

Catecholamines are released in response to a stressor within seconds, as their release is under direct neurogenic control (Moberg, 1985). Corticosteroids are released at a slower rate than catecholamines, also in response to a stressful situation. Because these reactions are stimulated in part by emotional experience, the manner in which the animal perceives a stressor is important (Frankenhaeuser, 1986).

Work performed by Murray, Lewis, & Coetzee, (1981), on evaluating capture techniques for research on impala (Aepyceros melampus melampus), showed that confinement in a crush led to 30% mortalities and restraint with tranquillisation resulted in only 22% mortalities. Most of the deaths were caused by "fatal stress" during physical restraint, according to the authors. They concluded that results obtained from animals manually restrained after arousal show that this procedure constitutes a "severe form of stress". This is in accordance with the findings of Presidente, (1973), Drevemo, & Karstad (1974), Wesson, Scanlon, Kirkpatrick & Mosby (1979a).

Hattingh, Pitts, & Carlston, (1990) found that the manual restraint of impala maintained in either their natural habitat or bomas resulted in what was apparently a physiological response to a stressor, but that the reactions observed were different

depending on whether the animals were naive to the stimulus or not. Similar conclusions were made by Wesson et al (1979a), while working on white-tailed deer (Odocoileus virginianus borealis). Their analyses of physiological variables appear more closely related, and better explained, by the relative stress of handling itself, rather than an effect due to the handling method. Data for the adrenocortical response of physically restrained deer by Wesson, Scanlon, Kirkpatrick, Mosby, & Butcher, (1979b) showed a marked similarity to values for impala held in bomas. However, values given for captive deer killed at close range by rifle (Wesson, Scanlon, Kirkpatrick, & Mosby, 1979c) were similar to those for physically restrained animals, whereas impala killed by rifle were found to have cortisol values in the range of those determined at time zero for wild animals caught at night (Hattingh, 1988; Hattingh, Pitts & Carlston, 1990). This suggests that the captive white-tailed deer that were shot at close range may have expressed a similar anticipatory conditioned response proposed previously for impala (Hattingh 1988).

Knox, Hattingh, & Raath, (1992) evaluated certain physiological variables of impala held in bomas that were subjected to repeated capture. They found that the non-surviving group of impala had a similar hyper- or hypo-response for certain physiological parameters, and suggested that these criteria may be used to identify individuals which are unlikely to survive confinement in a boma and repeated handling procedures. A similar conclusion was made by Kock, Clark, Franti, Jessup, & Wehausen, (1987) who investigated the effect of capture on various biological parameters in bighorn sheep (Ovis canadensis).

The use of tranquillisers to facilitate the adaptation of antelope to boma-confinement, transport, new environments and physical handling has been suggested by various authors (Ebedes, du Toit, & van Rooyen, 1989; Knox, Hattingh, & Raath, 1990; Bartels 1992). Knox et al. (1990) found that the administration of tranquillisers was not effective for reducing excitability and panic behaviour in impala subjected to procedures requiring

handling. Observations of tranquillised antelope indicate that they are less excitable and resume feeding and other behaviours soon after their initial confinement. Therefore, these authors suggest that the use of appropriate drugs for facilitating acclimitisation of wild impala to captive conditions is recommended during the initial confinement period since it is at this time that deleterious, stress-related responses are expected to occur most frequently. In situations where animals are subjected to repeated handling procedures for blood sampling or other purposes, however, the advantages of tranquillisers are largely negated.

Wesson et al. (1979b) found that when manually restraining white-tailed deer, circulating progestins and progesterone levels correlated well with elevated corticoid levels. Cross-reactivities between progestins and progesterone to corticosteroids were found to be absent. It is possible that the adrenal gland may also be producing progestins and progesterone under stressful conditions. Further evidence for progestogen activity by the adrenals has also been reported by Butcher, (1977) and Green & Moore, (1977).

Rationale and Objectives

Baseline data on the unique reproductive biologies of mammalian species are essential for formulating effective management plans. Because of the lack of published data available on the reproductive physiology and blood composition of wild ungulate species, there is a need for further scientific research in this area to develop and optimize conservation breeding strategies.

The results of the present study will serve to expand the existing information available describing ranges of various blood constituents and progesterone in African antelope when maintained and treated as described below, and will provide the only known source of such data for the tsessebe.

In order to meet the objectives of this study, the maintenance of, and procedures administered to, the antelope had to be structured in such a way as to minimize the stress caused by handling.

Therefore, the objectives of this study were to:

1. Elucidate the annual plasma progesterone patterns in pregnant and non-pregnant tsessebe cows.
2. Provide information on ranges for specific blood constituents and variables in tsessebe; thereby, expanding the data base available for African antelope species.
3. Formulate a model for reproductive research and assist in the development of a management protocol for medium-sized wild ungulates in intensive management systems.
4. Form the basis for further reproductive research which may result in the development of an *in situ* as well as an *ex situ* conservation plan for endangered medium to large sized antelope.

The following key questions were addressed:

1. What is the oestrous cycle length of the tsessebe cow?
2. Does the tsessebe cow exhibit reproductive cyclic activity if restrained by a body clamp on a regular basis throughout the year?
3. Does the tsessebe cow exhibit cyclic reproductive activity throughout the year if maintained in the presence (but not physical contact) of a bull in a paddock system and given supplementary feed?
4. Can the breeding season of tsessebe cows be extended if they are maintained under these same conditions?
5. Is the corpus luteum of the tsessebe the main source of progesterone maintaining pregnancy?
6. Does the tsessebe bull remain fertile?

7. Is the blood composition for various haematological and biochemical parameters in tsessebe similar to that of other African antelope species?

CHAPTER 2

Materials and Methods

Study Area

The present study was conducted at the Tompi Seleka Agricultural College in the Northern Province. The average rainfall for the area is 450 mm per annum. The habitat of the study area falls within the vegetation type "mixed bushveld" (Acocks, 1953), with the predominant tree species being Combretum spp., silver cluster-leaf (Terminalia sericea), sickle bush (Dichrostachys cinerea), round-leaved teak (Pterocarpus rotundifolius) and Acacia spp.

The tsessebe used in the present study were maintained in a paddock system, which is illustrated in Fig. 1. The dominant grass species for paddock A (13 ha.) were guinea grass (Panicum maximum) and finger grass (Digitaria eriantha), and for paddock B (5,5 ha.) gum grass (Eragrostis gummiflua) and spreading prickle grass (Aristida congesta barbicollis).

Paddocks C (2 ha.), D (0,8 ha.) and E (0,5 ha.) were severely overgrazed with mostly forb species present and, therefore, were not considered as grazing paddocks, but rather as capture paddocks. Supplementary feed in the form of chopped lucerne (Medicago satins) and guinea grass (Pannicum maximum) was supplied ad libitum to all the tsessebe. In addition, antelope cubes (Protein: min. 160 g/kg, Fat: min. 25 g/kg, Fibre: max. 80 g/kg, Calcium: max. 16 g/kg, Phosphorus: min. 7 g/kg, Vitamin A: 10 000 I.U./kg, Moisture: max. 120 g/kg) (Epol, Johannesburg, South Africa) was made available to each animal at 200 g/day.

The design of the paddocks was determined by the prevailing conditions (i.e., geography of the area and college infrastructure). All the paddocks were linked with gates or passages in such a way as to allow the tsessebe to be maintained

in, or moved in or out of, any paddock in the boma. Paddock E was partitioned by two dividing fences (Fig 1). The boma used to separate and restrain the tsessebe consisted of three funnel-shaped game pens (30 m^2), a sorting pen (15 m^2) and a body clamp. The game pens were divided into two halves by cross walls and gates, as illustrated in Fig. 2. A modified cattle body clamp was used to restrain the tsessebe for the collection of all of the necessary specimens (Plate 1).

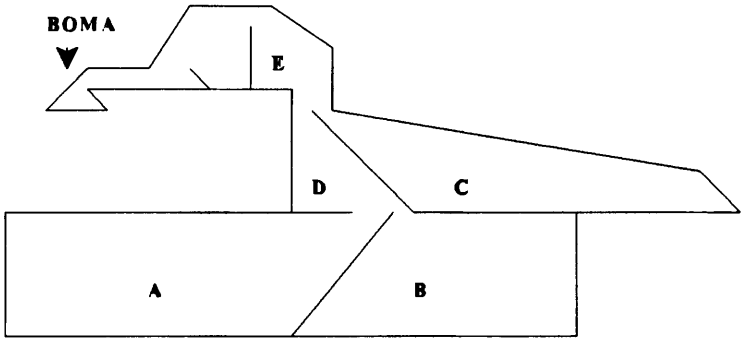

Fig. 1. Diagramatic representation of the paddock system.

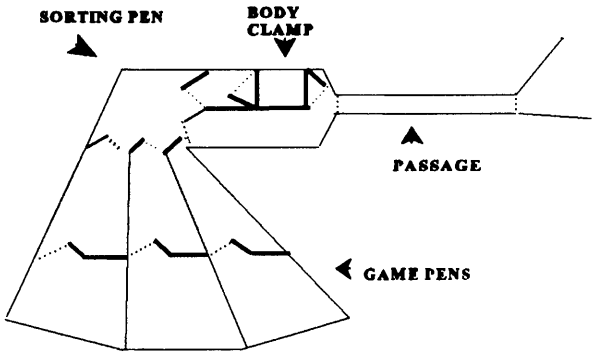

Fig. 2. Diagrammatic representation of the game pens, sorting pen and body clamp used to separate and restrain the tsessebe.

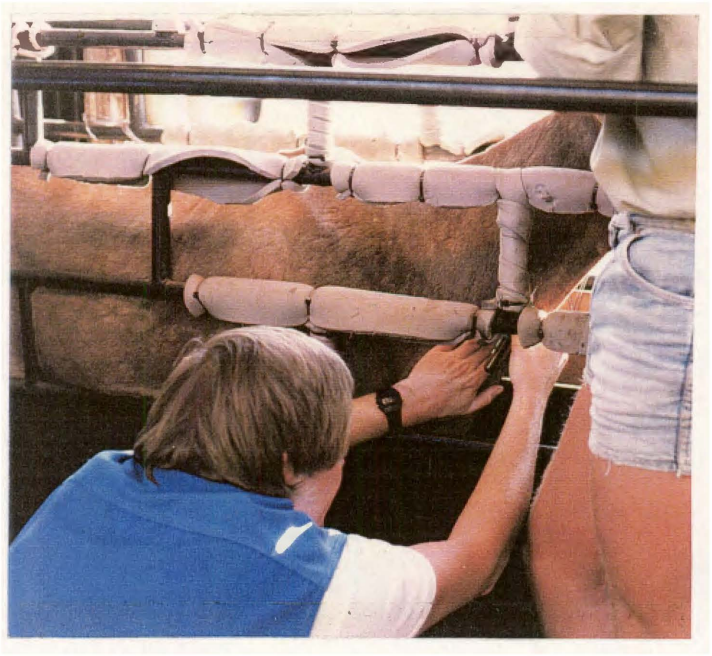

Plate 1. Body clamp and technique used to restrain the tsessebe for blood sampling.

Study Animals

Six tsessebe cows and one bull were obtained from the Potgietersrus Breeding Station of the National Zoological Gardens of South Africa (NZG). An additional three cows were obtained from the Percy Fyfe Nature Reserve (PF) near Potgietersrus.

All the tsessebe were maintained, tranquillised and confined to separate pens at the boma during the first two months, as a means to enhance adaptation to their new environment. Tranquillisation was performed by the weekly administration of 150 mg perphanazine enanthate (Trilafon, Sherag (Pty) Ltd, Johannesburg) and 100 mg zuclopenthixol acetate (Acuphase, H. Lundbeck, Johannesburg), via intramuscular injection. One week before their release into the paddock system, the tsessebe were chemically immobilised to perform a variety of husbandry procedures including:

1. Removing at least 2,5 cm from the tip of each horn, as a precautionary measure to minimise possible injuries

to personnel and tsessebe alike.

2. Attaching ear tags for individual identification. In addition, marks were etched on horns using a saw blade as a back-up identification system, should ear tags become lost.
3. Controlling external and internal parasites. Each animal was treated for worm infestation using injectable Ivermectin (Lagos, Agvet, Johannesburg) at a dose of 1 ml/50 kg. For external parasite control, each animal was treated topically with Flumethrin (Bayer A.H., Johannesburg), which was poured on at a dose of 1 ml/10 kg.

The chemical immobilisations were achieved by darting the cows with a combination solution of 3 mg etorphine hydrochloride (M99; Kruger-Med Pharmaceuticals (Pty) Ltd, Johannesburg) and 10 mg xylazine (Rompun 2%; Bayer S.A., Johannesburg) using a gas powered darting rifle (Model 2V.310; Telinject S.A., Johannesburg). At the completion of the husbandry procedures, the cows were injected intravenously with 6 mg diprenorphine hydrochloride (M50-50 solution; Kruger-Med Pharmaceuticals (Pty) Ltd, Johannesburg) to reverse the effect of the M99.

The tsessebe from the NZG arrived as two groups: the first group consisted of two cows and one bull and the second group consisted of four cows. The second group arrived approximately one year after the first group. Two of these cows died before the study commenced. Three additional cows from PF arrived six months after acquiring the last tsessebe from the NZG; one of these cows did not survive and the remaining two cows calved three months later. Seven months after the last calf was born, the calves were removed and all the tsessebe cows were placed together and maintained in paddock B.

Experimental Approach

Animal restraint for blood and faecal sampling

Most previous studies of this nature have made use of chemical immobilisation or tranquillisation, manual restraint, or slaughter for blood or tissue sampling purposes. More recently, Capture Collars (Delgiudice, Kunkel, Mech, & Seal, 1990; Mech, Kunkel, Chapman, & Kreeger, 1990) have been succesfully used. The former and latter procedures were ruled out because of the costs involved. Manual restraint of conscious tsessebe was also not considered for blood sampling because of the risk of injury to both animals and personnel. We were not able to acquire any remote controlled portable blood sampling devices (Stephan, & Cybic, 1989). A body clamp was, therefore, acquired to physically restrain the tsessebe cows for blood and faecal sampling. The design was based on the standard cattle body clamp, with certain modifications as follows;

1. The neck clamp was removed and replaced with a wire mesh gate.
2. The metal locking mechanism of the side walls was replaced with a rope and cleat, so as to diminish the noise created when operating the locking mechanism.
3. The upper half of the side walls was padded with strips of 1 cm thick polyurethane foam and the solid walled lower half of the side walls was extended upwards by an additional 10 cm using strips of wood (Plate. 1)

For two months before the initiation of the sampling procedures, the cows were conditioned to move between the different paddocks. This was achieved by placing their supplementary feed and water in paddock D (Plate 2) and keeping all the gates leading to the boma open, thus allowing the cows to explore the paddock system on their own volition.

For the first five months of the study, the cows were maintained in paddock B and the bull in paddock C. For the following eight months the cows were maintained in paddock A and the bull in paddock B. After this period the cows and the bull were maintained together in paddock A.

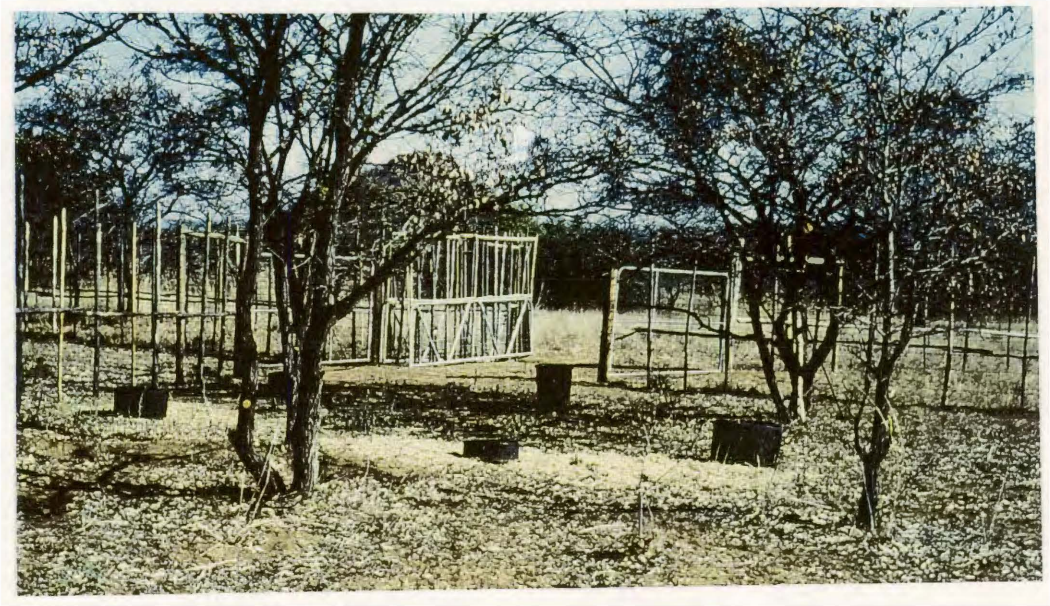

Plate 2. Feed and water bowls placed so as to attract the tsessebe cows into paddock

The herding, restraining and bleeding procedures were performed in a consistent and controlled manner. The day before blood sampling, the cows were calmly herded into paddock D by two persons. The next morning, the cows were again calmly herded by at least four persons into paddock E and the gate between paddocks D and E was then closed. After the cows were herded past the dividing fences in paddock E, a person in a hide constructed in paddock E closed the gate of the first dividing fence (Plate 3) and then closed the curtain of the second dividing fence (Plate 4) using cables and pulleys. The cows entered the passage leading to the boma (Plate 5), then a person stationed at the boma closed the two sliding doors of the passage and the gate of the sorting pen.

Two cows were allowed to enter the first section of the game pen and then one was coaxed into the furthest section of the game

pen. In this way the six cows were separated and ready to be individually coaxed into the body clamp. Two persons were used to hold the head of the cow while a third person operated the body clamp. A fourth person collected the blood and faecal samples (Plate 1).

Plate 3. Gate used with the first dividing fence of paddock E.

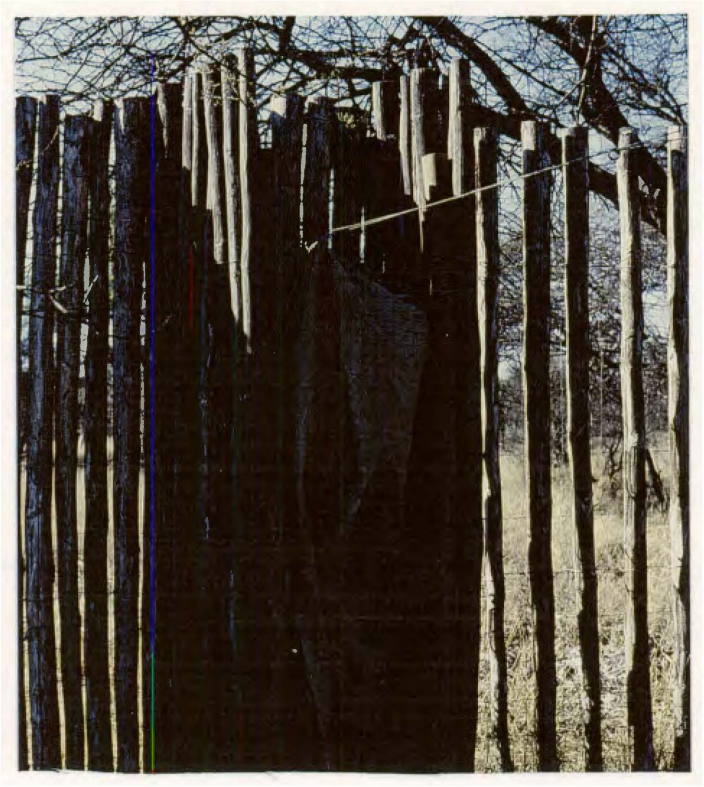

Plate 4. Curtain used with the second dividing fence of paddock E.

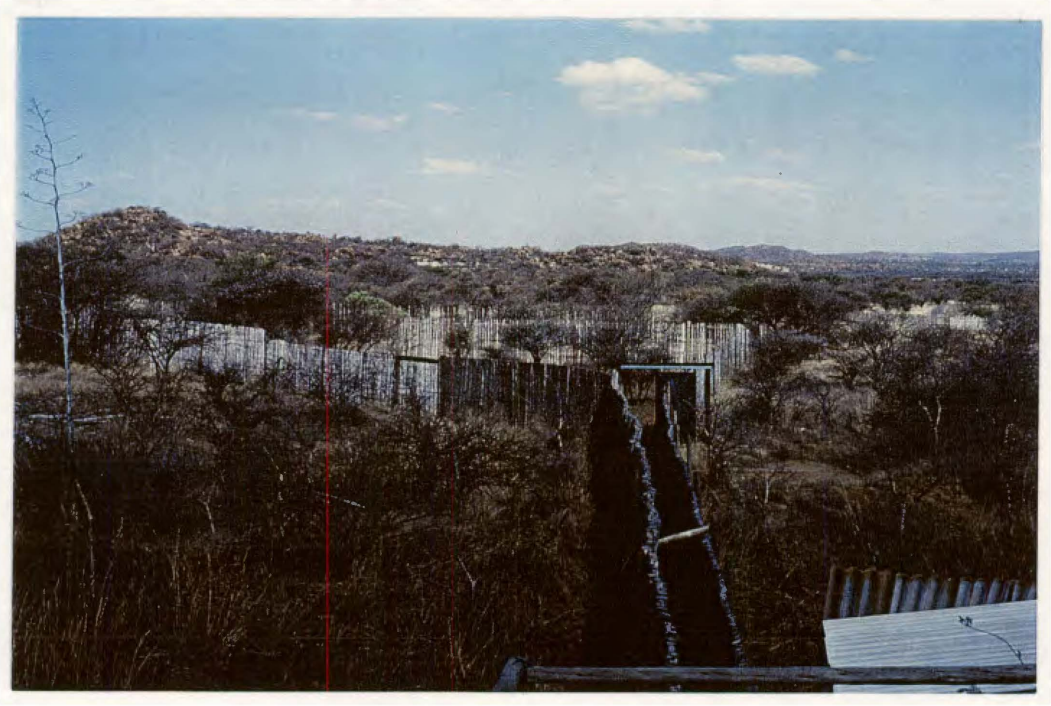

Plate 5. Passage and paddock E as viewed from the boma.

Venous blood was procured at least twice a week from March until the end of May. In January, February, and in the period from June to December of that year, blood was collected at two-week intervals. In the following year, blood was collected once a month until the cows were observed to be in the latter stages of pregnancy. The bull was allowed direct access to the cows only at the end of January of the second year. Table 1. summarises the blood sampling regimes used in the present study.

Table 1. Blood sampling regimes used in the present study

Sampling	1991											
frequency	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
twice/week:			x	x	x							
twice/mth:	x	x				x	x	x	x	x	x	x
Sampling	1992											
frequency	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct		
once/mth:	x	x	x	x	x	x	x	x	x	x		

For the determination of progesterone concentrations, 10 ml of blood were collected from the jugular vein of each cow into venoject tubes containing 14,3 U.S.P. units/ml of lithium heparin (Radem Laboratory Equipment, Johannesburg) using 18 gauge venoject needles. For the haematological analyses, 5 ml of additional blood were collected on a once a month basis into K3 EDTA venoject tubes (Radem Laboratory Equipment, Johannesburg). The body condition of each cow was scored as described below. Before release, the cows were inspected for injuries or loss of ear tags and treated as necessary. Regular treatments for internal and external parasites continued throughout the study period.

The sampling procedures were carried out at 07h00 and each cow was released as soon as the necessary specimens were

collected and treatments applied. The time taken to complete the sampling procedure, from herding the tsessebe up from paddock D until the last cow was released back into the paddock system was about 45 min.

The blood specimens were immediately placed into a polystyrene holder containing ice. The heparinised blood was centrifuged as soon as possible and the plasma aspirated and stored at -20°C until analysed. Faecal samples were also stored at -20°C. Within six h of collection, the whole blood contained in EDTA was taken to the Department of Clinical Pathology, Faculty of Veterinary Science, University of Pretoria for the haematological analyses.

Radioimmunoassay for progesterone concentrations

The methods used followed van Aarde & van Wyk (1991). Progesterone was extracted from duplicate plasma aliquants (0,1 ml each) by the addition of 4,0 ml petroleum ether (distillation range 40°C - 60°C; Saarchem (Pty) Ltd, Krugersdorp, South Africa) into duplicate plasma aliquants (0,1 ml) in glass tubes and mixing for five min on a vortex mixer. After freezing at -20°C for one h, the organic phase was decanted into a series of glass tubes (12 x 5 mm) and evaporated to dryness in a 37°C water bath under a continuous stream of filterd nitrogen. The dry extracts were reconstituted in 0,1 ml phosphate-buffered saline (0,06 M disodium hydrogen phosphate, 0,04 M sodium dihydrogen phosphate, 0,15 M sodium chloride, 0,02 M sodium azide, 1,0 g gelatin in 1 litre distilled water; pH = 7).

A series of standards containing 15,6; 31,2; 62,5; 125; 250; 500; 1000 and 2000 pg progesterone (4-pregnene-3,20-dione; Sigma, Dorset, U.K.) per 0,1 ml phosphate-buffered saline were prepared in duplicate and included in each assay. Duplicate buffer blanks and ether were also included in each assay as controls. Antiserum (A/S 1529) raised in a rabbit against progesterone-21-bovine serum albumin and supplied by R.P.Millar (Department of

Chemical Pathology, University of Cape Town, South Africa) was added to the plasma extracts, buffer blanks, ether blanks and standards at a 1:1500 dilution in 0,1 ml phosphate-buffered saline.

The contents of each tube were incubated at room temperature for 10 min before the addition of 0,1 ml radio-labelled progesterone containing 20,000 counts per minute [1,2,6,7-3H] progesterone (Radiochemical Centre Amersham, Bucks, U.K.) in 0,1 ml phosphate-buffered saline. The contents of each tube were mixed for one min on a vortex mixer and then incubated at 4°C for at least 12 h (overnight).

Progesterone bound to the antibody was separated from the free progesterone by the addition of 0,75 ml of a 0,156% dextran-coated charcoal (0,625% activated charcoal, Sigma, Dorset, U.K.; and 0,0625% dextran T40, Pharmacia, Uppsala, Sweden) mixture at 4°C. The contents were mixed for 30 sec, incubated at 4°C for 15 min, and then centrifuged at 4°C at 1500 x g for 15 min. The supernatants were decanted into scintillation vials (Packard Instrument Company, Downers Grove, IL, USA) and 4 ml of scintillation cocktail (Ultima Gold XR; Packard BV Chemical Operations, The Netherlands) were added to each vial. The contents of the vials were mixed thoroughly and the radioactivity counted at least three h later using a Packard 1500 Tri-Carb liquid scintillation analyzer. After taking into account extraction efficiency and the volume of plasma extracted, the final concentrations were calculated using the programme "Securia 2200" (Packard Instrument Co., Downers Grove, IL, USA).

Tsessebe semen collection

It was important to examine the quality of the semen from the one bull available for this study to disclose any apparent signs of infertility that may have been associated with the quality of the semen.

Prior to releasing the tsessebe bull into the paddock system, he was chemically immobilised on three separate occasions for semen collection by electroejaculation to evaluate semen characteristics and quality. The electrostimulator used was a model developed for domestic cattle (El Toro II, Lion Bridge Feeds (Pty) Ltd, Pretoria). The electro-ejaculation procedure was as follows: After immobilization, the bull was placed in a dorsi-lateral recumbency position with his head held up-right and his nose pointing down. Faeces were removed from the rectum and the electrostimulator probe inserted. The electrical charge delivered to the bull via the electrostimulator probe was controlled by a dial on the control box. Using the dial, the charge was slowly (two to four sec) increased to a low setting, kept there for about 4 sec after which the charge was dropped to zero. This was followed 2 sec later by again increasing the charge, this time to a higher setting, again followed by keeping the level at that point for 4 sec before dropping the charge down to zero. This was repeated a number of times until the full charge was being delivered and the bull ejaculated The procedure was stopped after about 20 min if no ejaculation occurred. Semen was collected in a warmed 15 ml test tube and taken to the laboratory for analysis.

At the first two attempts at semen collection, the bull was chemically immobilised using a combination of 3,2 mg etorphine hydrochloride and 40 mg azaperone tartrate (Kyron Laboratories, Johannesburg). At the third attempt, the bull was immobilised using a combination of 3,2 mg etorphine hydrochloride and 8,0 mg xylazine. A blowpipe (Telinject S.A., Johannesburg) was used to project the darts containing the immobilisation drugs. After each electroejaculation procedure, 7,0 mg diprenorphine hydrochloride was injected intravenously to reverse the effect of the etorphine hydrochloride.

The ejaculate was assessed for volume, colour, consistency, mass sperm motility (hanging drop), progressive motility status and sperm morphology for major and minor defects.

In ruminants semen consistency can be used as a subjective means of determining sperm concentration. The consistency was evaluated according to the following guideline:

Watery:	0,2 x 10 /ml
Milky:	0,5 x 10 /ml
Thick milky:	0,75 x 10 /ml
Creamy:	1,5 x 10 /ml
Thick creamy:	2,5 x 10 /ml

Mass motility is the product of the percentage linear motility and sperm concentration. The mass sperm motility of a 2 - 3 mm diameter sperm drop was evaluated on a warmed glass slide using 10X ocular and objective phase-contrast lenses of a microscope and graded according to the following guidelines:

0: No motility at all.
1: Individual sperm motility observed.
2: Approximately 10% alive, no wave movements.
3: Slow wave movements observed.
4: Well defined, strong waves with rounded turns, reaching the periphery.
5: Waves well defined, with sharp crisp turns giving a whiplash effect, extended to the periphery.

A 20 μl sperm sample under a warmed cover slip was used to determine progressive motility using the 10X ocular and 40X phase-contrast lenses of the microscope. The individual motility was rated as follows:

Sperm progressively (linear) motile: - %
Non-motile and abnormal (tight circular, reverse and oscillatory) motility: - %

A Nigrosin/Eosin (N/E) stained sperm slide was used to assess sperm morphology for major and minor defects. A drop of sperm and a drop of N/E stain was mixed on a glass slide. A drop

of the sperm/stain mixture was then transferred to a clean glass slide and a thin smear made for determining sperm morphology.

Radioimmunoassay for cortisol concentrations

Plasma cortisol (hydrocortisone, compound F) concentrations were measured by radioimmunoassay using a kit available commercially (Gamma Coat [125I]). Although these kits were designed for use in human patients, they have been used effectively in a number of other species including black-backed jackals (Canis mesomelas) and domestic dogs (Canis familiaris) (Van Heerden & Bertchinger, 1982) as well as impala (Knox, 1992).

The procedure is based on the competitive binding principle of radioimmunoassay. Standards and duplicate tsessebe plasma samples were incubated with radio-labelled cortisol (tracer) in tubes coated with antibody. After incubation, the contents of the tubes were decanted and counted using a gamma counter (Type 6,20; Wiel Organisation, Johannesburg). A standard curve was included in each assay with known duplicate tracer concentrations (10 - 600 ng/ml), buffer blanks and total tracer counts. Unknown values were interpolated from the standard curve.

Analyses of plasma biochemical constituents

Plasma concentrations of sodium, potassium, chloride, calcium, phosphate, creatinine, total protein, cholesterol and glucose were measured directly using a SMAC-3, Technicon autoanalyser. Magnesium concentrations were measured using a Perkin-Elmer 3030 atomic absorption spectrophotometer.

Haematological analyses

Whole blood samples were examined for total erythrocyte counts (RCC), haematocrits (Hct), haemoglobin contents (Hb), mean corpuscular volumes (MCV), mean corpuscular Hb concentrations (MCHV) and total leucocyte counts (WCC) using a System 9000

differential model analyser (Serono-Baker Diagnostics, Inc. Allentown, PA, U.S.A.).

Assessments of body condition

Table 2 summarises the method used to assess body condition.

Table 2. The method used to score body condition of tsessebe in this study.

Description of appearance	Class	Score
Hindquarters well rounded no ribs visible; general appearance with reference to stature: excellent; skin appearance: shiny.	Excellent	5
Hindquarters rounded; ribs show slightly.	Good	4
Hindquarters angular; ribs clearly visible.	Fair	3
Pelvic bones prominent; ribs protrude.	Poor	2
Skeletal bones clearly visible; rump concave; general appearance with reference to stature: very poor skin appearance: very dull.	Very poor	1

Prophylaxis and Medical Treatment

The tsessebe were placed on a prophylactic external and internal parasite control, as well as a vitamin and mineral supplementation programme. Moreover, a number of diseases were suspected or diagnosed in the tsessebe during the course of the

present study and the appropriate treatments applied.

Statistical Analyses

Statistically significant differences between summer and winter values were determined using the Student's t test, at a 5% probability level (Sokal & Rohlf 1987).

CHAPTER 3

Results

Plasma Progesterone Measurements

Validation and efficiency of the radioimmunoassay

The cross-reactivities of the antibody used in this study with steroids other than progesterone were: pregnenolone, 3,1%; 17α-hydroxyprogesterone, 1,9%; 11α-hydroxyprogesterone, 25,8%; 11β-hydroxyprogesterone, 47,1%; 5α-pregnane-3,20-dione, 24,8%; 20α-hydroxy-pregnane-3-one, 0,4%; 11-deoxycorticosterone, 2,2%; 3α-hydroxy-5-pregnane-20-one, 0,4%; 11-deoxycortisol, 1,5%; cortisol, 0,2%; testosterone, 4-androstenedione, 17β-oestradiol and oestrone, 0,01%.

Parallelism was ascertained by adding serially diluted plasma samples to the standard assay. These samples exhibited curves parallel to that of the standard curve, indicating that the substance being measured in the tsessebe plasma samples was indeed progesterone. Estimates of labelled progesterone recovered from a plasma sample compared to that recovered from the scintillation cocktail varied between 83,5% and 97% (mean 92,0% ± 4,4; n=8). Non-specific binding averaged 3,9% (n = 8), while specific binding of the diluted antiserum averaged 24,1% (n = 8). The inter-assay coefficient of variation for two samples, one containing 100 ng and the other 200 ng progesterone / 0,1 ml buffer (low and high quality control pools, respectively) and included in each assay was 15,6%. The intra-assay coefficient of variation was 11,8%. The sensativity of the assay, defined as twice the S.D. of the buffered blanks was 0,009 ng/ml.

Progesterone profiles of tsessebe cows

The baseline values for circulating progesterone were in the

region of 0,05 ng/ml. Values reported represent means ± S.E. For the purpose of this study, the rutting season was accepted as being from March until the end of May 1991 (Fig. 3). During this period, the mean circulating progesterone values rose, from those of the non-rutting season level of 0,16 ± 0,01 ng/ml, (range: 0,04 - 1,11 ng/ml, n = 119) to 0,26 ± 0,02 ng/ml, (range: 0,06 - 1,29 ng/ml, n = 154). The nadir for circulating progesterone concentrations associated with the oestrous cycles in the rutting season was 0,17 ± 0,02 ng/ml (range: 0,080 - 0,240 ng/ml, n = 14) while the mean for peak values was 0,74 ± 0,06 ng/ml (range 0,429 - 1,289 ng/ml, n = 13). From February 1992, after the bull was allowed direct access to the cows, the circulating progesterone values again rose, this time to 0,50 ± 0,03 ng/ml, (range: 0,15 - 0,78 ng/ml, n = 25) for the pregnant cows (Fig. 5). The first and second semester of the pregnant cows circulating progesterone concentrations was 0,46 ± 0,05 (range: 0,149 - 0,742, n = 11) and 0,59 ± 0,03 (range: 0,445 - 0,742, n = 11) respectively.

Cows 2 & 10 exhibited cyclic activity throughout the year as is evident by the fluctuations in circulating progesterone levels measured during 1991 (Fig. 3 & 4). Cows 3, 7 and 11 showed some fluctuations during certain times of the year while cow 1 showed the least obvious fluctuations in circulating progesterone levels. Cow 1's circulating progesterone level never rose above 0,2 ng/ml during 1991.

The circulating levels of progesterone during March to May 1991 indicated clear cycle periods for cows 2, 3, 7 and 10, as is evidenced by the curves of Fig. 3. The period between the minimal levels of eight of these' represented curves were used to calculate the modal duration of the oestrous cycle length of the cows and was found to be 21 days (range; 18 - 23 days, n = 8).

Cow 11 died in May 1992 and at post mortem was found to be barren. The other cows each conceived and all except cow 1 calved in late October of the same year. Cow 1 died at the end of

September and was found to have a near term fetus in her uterus.

Tsessebe Semen Evaluations

With the first two attempts at electro-ejaculation and semen collection, no ejaculation occurred and no semen was collected. With the third attempt, an ejaculate was collected. The semen was examined and the results are summarised in Table 3.

Table 3. Macroscopic and microscopic evaluation of the tsessebe bulls ejaculate.

Volume:	0,8 ml.
Colour:	reddish pink.
Consistancy:	thick milk.
Hanging drop:	4+ with wave movements.
Progressive motility of sperm:	80% plus.
Sperm morphology:	< 10% major & minor defects.

Haematological and Biochemical Measurements

Validation and efficiency of the radioimmunoassay

The cross-reactivity of the cortisol antibody with other steroids were: prednisolone, 77%; 6-methylprednisolone, 43%; 11-deoxycortisol, 6,3%; 17-hydroxyprogesterone, 1,2%; corticosterone, 0,4%; dexamethasone, 0,2%; prednisone, 0,2%; deoxycorticosterone, 0,1%; tetrahydrocortisone, 0,1%; aldosterone, 0,1%; β-cortol, 0,1%; β-cortolone, 0,1%; cortisone, 0,1%; dihydrocortisone, 0,1%; progesterone, 0,1%; spironolactone, 0,1%; tetrahydrocortisol 0,1% and 6-β-hydrocortisone, 0,1%.

Haematological and Biochemical Profiles

Haematological and plasma biochemical results (mean ± S.D.)

obtained during the winter and summer periods are summarized in Table 4. Significant differences ($P < 0,05$) in variables between summer and winter periods were not observed.

Table 4. Haematological and serum biochemical values (mean ± S.D.) in physically restrained tsessebe during summer and winter periods.

Variable	Summer	Winter
RCC (x 10 /mm³)	15,5 ± 0,6	14,6 ± 1,2
Hct (%)	36,8 ± 3,7	40,7 ± 3,8
Hb (g/dL)	15,9 ± 1,5	14,7 ± 1,5
MCV (μm³)	31,5 ± 2,2	30,2 ± 2,2
MCHC (%)	36,0 ± 1,0	36,0 ± 1,0
WCC (x 10 /mm³)	4,8 ± 0,3	5,1 ± 0,7
Creatinine (μmol/L)	118,2 ± 5,6	137,3 ± 5,4
LDH (IU)	364,0 ± 39,0	452,0 ± 65,0
CPK (IU)	73,0 ± 28,0	112,0 ± 30,0
SGOT (IU)	96,0 ± 13,0	100,0 ± 11,0
Na (mmol/L)	145,0 ± 2,0	143,0 ± 2,0
K (mmol/L)	4,0 ± 0,3	4,0 ± 0,2
Cl (mmol/L)	103,0 ± 1,0	98,0 ± 3,0
Ca (mmol/L)	2,8 ± 0,1	2,7 ± 0,0
Mg (mmol/L)	0,8 ± 0,1	0,9 ± 0,1
P (mmol/L)	0,5 ± 0,1	0,7 ± 0,2
Total protein (g/L)	68,0 ± 3,0	74,0 ± 4,0
Cholesterol (mmol/L)	0,9 ± 0,1	1,2 ± 0,2
Glucose (mmol/L)	6,5 ± 0,6	7,0 ± 0,5
Cortisol (nmol/L)	112,0 ± 19,0	80,0 ± 19,0

Body Condition Assessments

On arrival at the study facility, body condition scores of all the tsessebe were recorded as a four. At the start of the

study (about two and a half years later), the body condition scores were three; four; four; four; four and three for animals 1; 2; 3; 7; 10 and 11, respectively.

Over the study period, the trend in body condition was recorded as follows: the mean score decreased after the first two months of 1991, reaching a minimum between the third and twelth months, then increased over the remainder of the study period (Fig. 6). Cow 1's body condition remained low (˜2) for most of the study period, only increasing to a score of ˜4 towards the end of the study. Cow 11 also had a relatively low score which fluctuated somewhat erratically between a score of 2 and 3 and at her death had a recorded score of ˜2.

Mortalities and Necropsy Findings

Three of the tsessebe cows destined to be used in the present study died: two (cow 4 from the NZG and cow 6 from PFNR) within two months of being captured and translocated to the study facility, and one (cow 5 from NZG) a year after arrival. Cows 4 and 5 died within three days of the first noticable signs of disease (anorexia and depressed habitus), whereas the body condition of cow 6 deteriorated over six weeks before she died. Cows 1 and 11 died towards the end of the study period while being maintained in the paddock system.

Cow 5 died of an unknown cause, however on post mortem a compact ball (15 x 8 cm) of twine with a 20 cm extension was found in the rumen. The twine was of the type commonly used for baling lucerne. It is not known if this gastrolith had anything to do with the death of the cow.

A post-mortem examination of cow 4 indicated that she died of septicaemia caused by severe peritonitis. The peritonitis was caused by the penetration of a piece of wire (eight cm long) through the wall of the reticulum and into the spleen (Plate 6). The wire was also similar to the type used to bind lucerne bales.

Cow 6 appeared to adapt well to captivity, as reflected by her calm disposition and appetite, except for the gradual deterioration of body condition over a period of several weeks. She was dewormed (Ripercol Systemic pour-on solution, Levamisole HCl, Janssen Animal Health, Johannesburg) and put on a course of antibiotics (Compropen, Procaine penecillin G and benethamine penicillin, Centaur, Lion Bridge Feeds (Pty) Ltd. Pretoria), vitamin B complex and hepatic extractum (Phenix S.A. (Pty) Ltd, Randburg) and probiotics (Biorem, Janssen Animal Health, Johannesburg) without any noticeable improvements. She finally became so weak as to be unable to stand and, subsequently died. A post-mortem examination did not reveal any obvious cause of death.

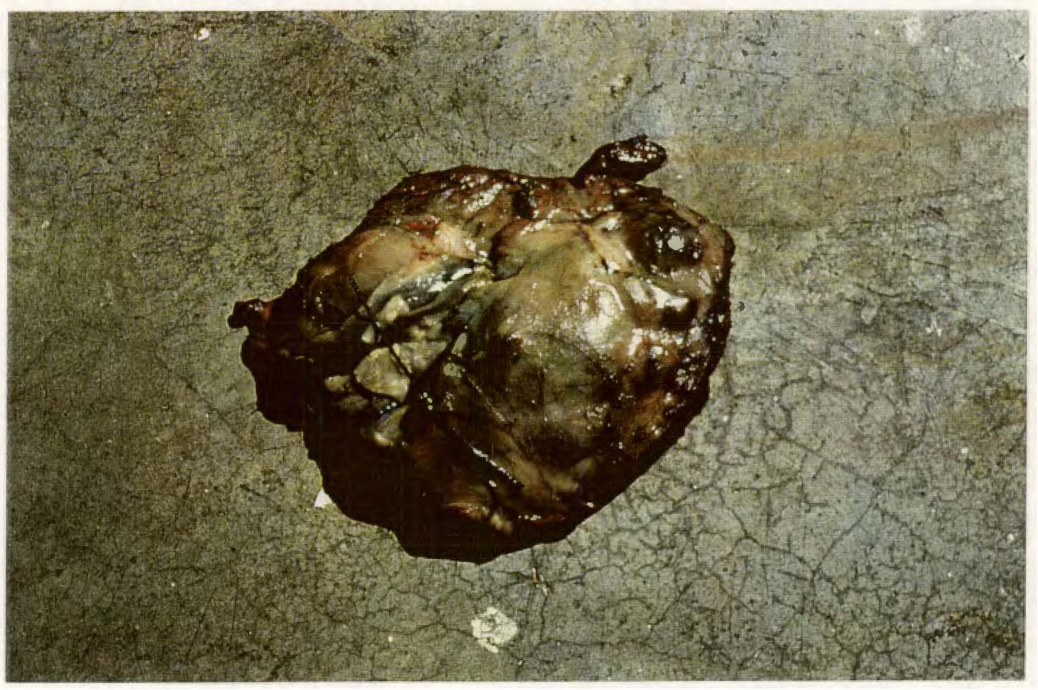

Plate 6. Spleen with abscess and section of wire removed from cow four.

The results of the post-mortem examinations of cow 1 revealed only that she had died about four days before being found. A fully-developed fetus was found in her uterus (Plate 7).

Cow 11 had her left foreleg broken by the bull while the

herd was being coaxed up to the boma for specimen collection. An amputation was performed but she died three days later. On postmortem, she was found to be barren.

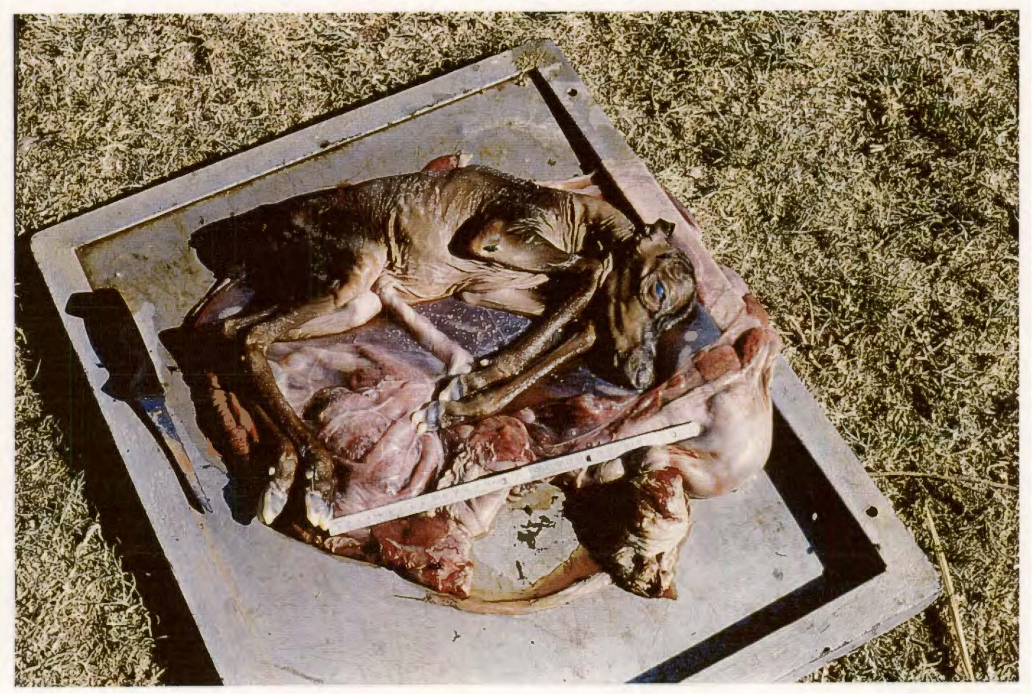

Plate 7. Fetus removed from the uterus of cow one.

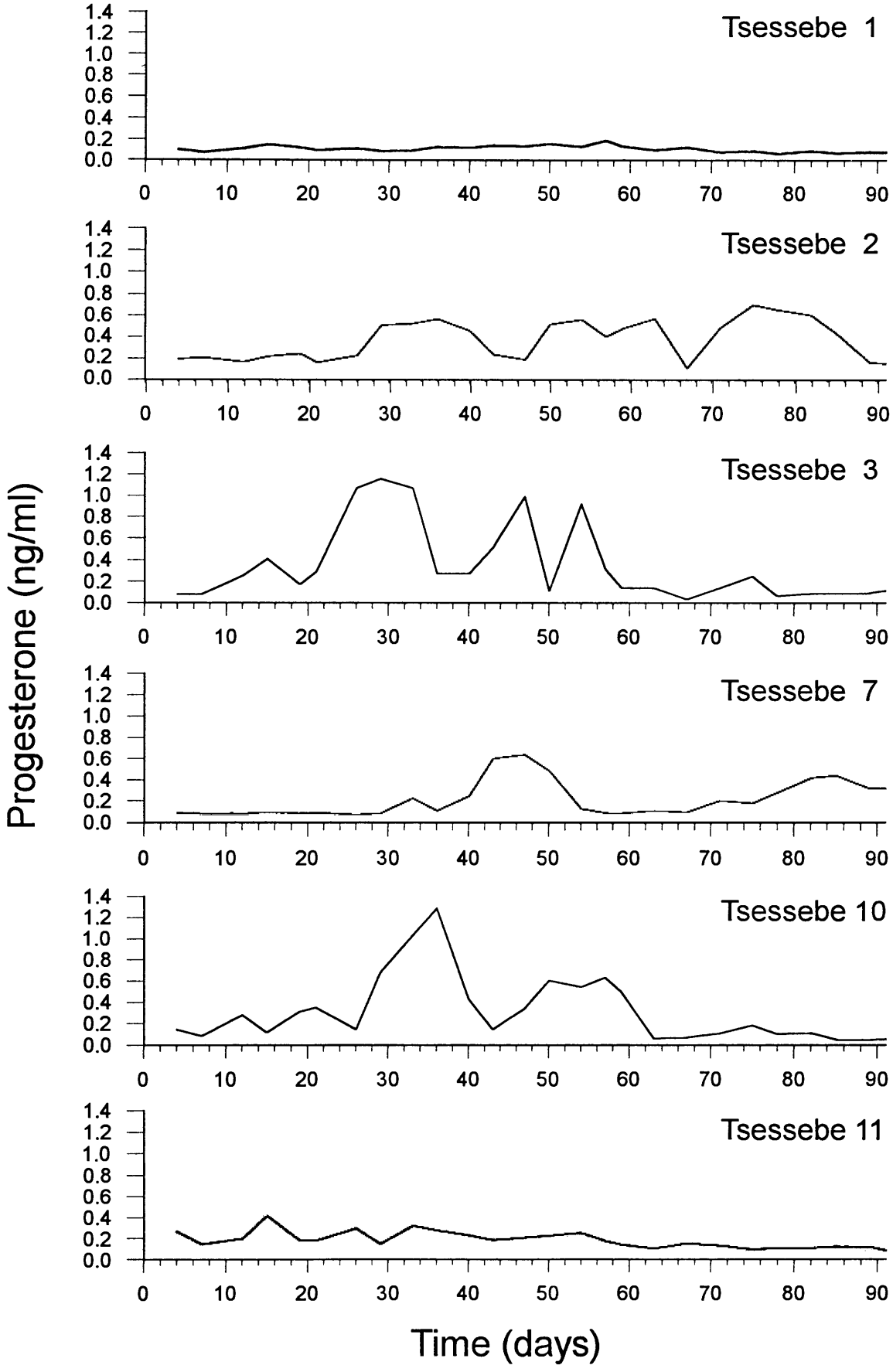

Fig 3: Circulating concentrations of progesterone in tsessebe cows from March to May 1991

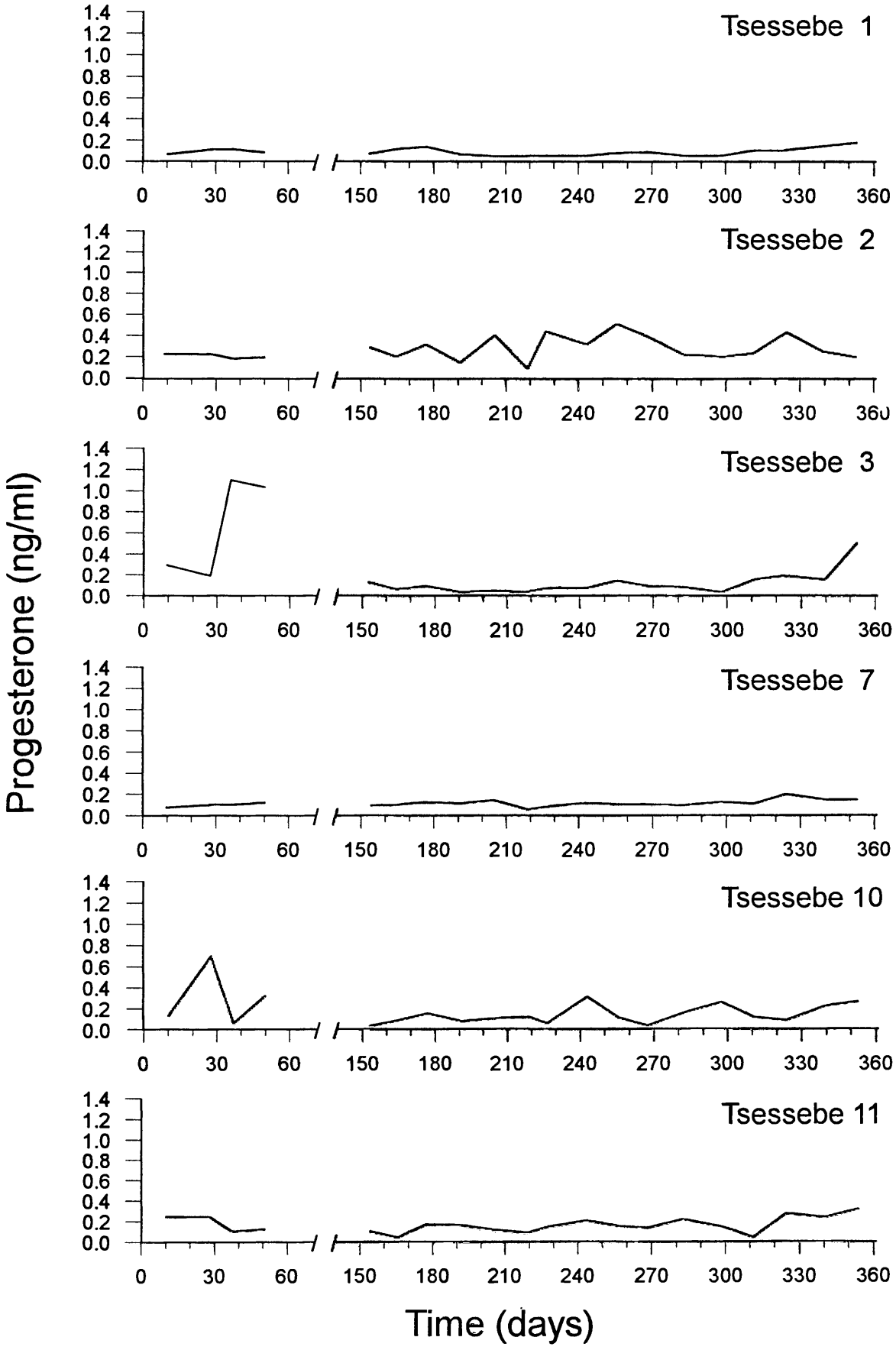

Fig 4: Circulating concentrations of progesterone in tsessebe cows in January and February and from June to December 1991

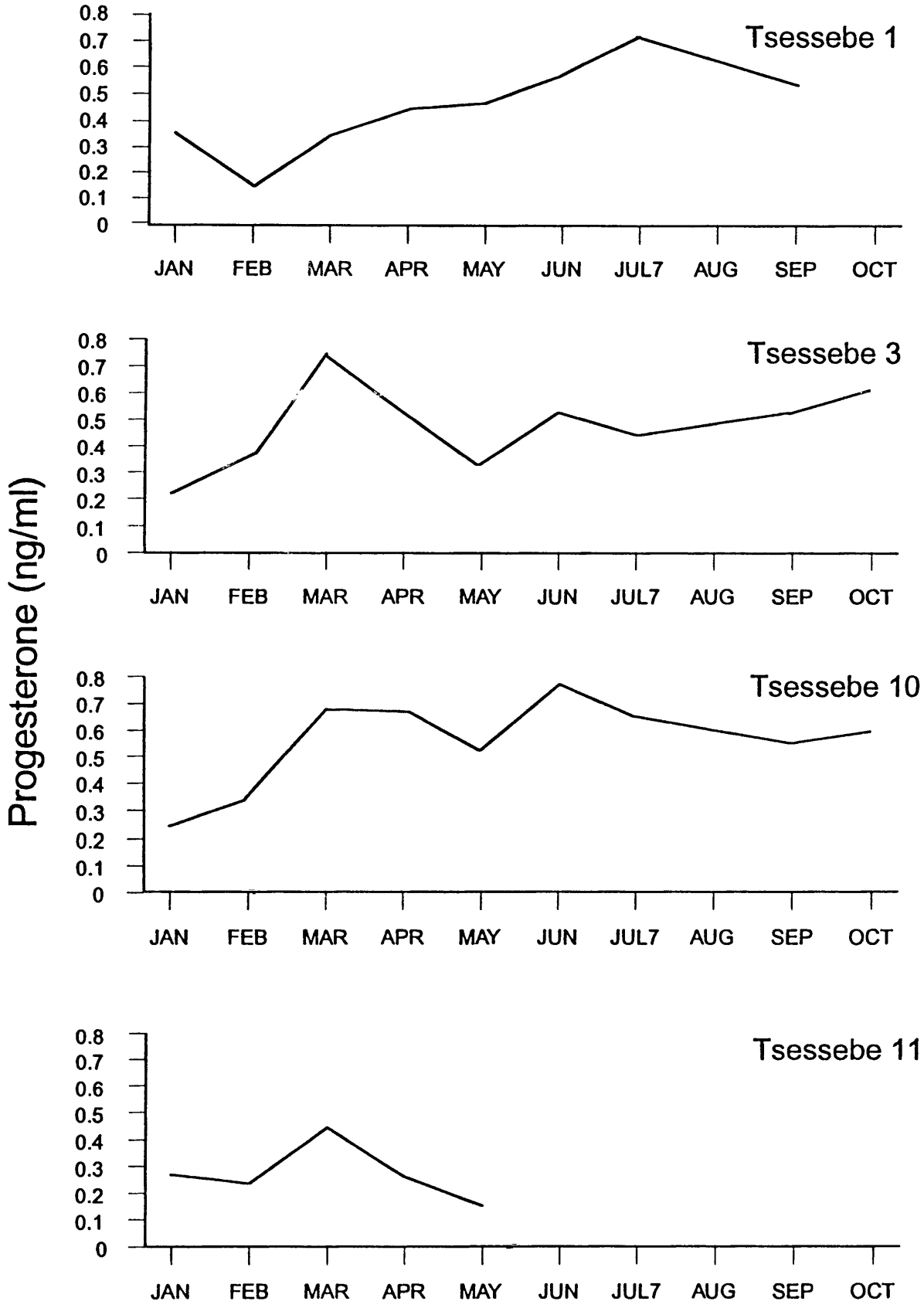

Fig 5: Circulating concentrations of progesterone after allowing a tsessebe bull direct acces to the cows

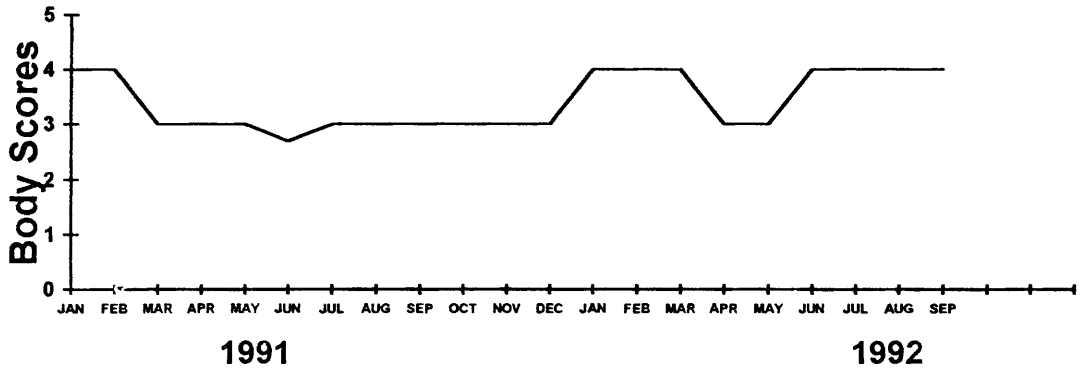

Fig 6: Mean body score of the tsessebe cows throughout the study period

CHAPTER 4

Discussion

Reproductive Physiology of Tsessebe

Progesterone concentrations in peripheral plasma of tsessebe cows provide valuable information about reproductive status. Regular examination of the tsessebe cows at close quarters did not reveal any signs of oestrus, however the fluctuations in plasma progesterone concentrations of some of the cows gave clear evidence of cyclicity in ovarian activity.

Some of the tsessebe cows exhibited cyclic activity throughout the year, as is evident by the fluctuations in progesterone levels throughout 1991. This is similar to what Marais (1988) found for blesbok ewes kept in the presence of a ram and given supplementary feed. Cyclic activity was apparently not affected by the physical procedures, but may have had an effect on the absolute progesterone concentrations. As seen in other domestic ungulates, plasma progesterone is low at oestrous and indistinguishable from anoestrus levels, rises to a peak in the luteal phase and thereafter declines to reach a basal level at the next oestrus.

The cyclic secretion of progesterone, as measured in the circulating progesterone levels, indicates an oestrous cycle length of about 21 days which is similar to that of the domestic cow (Yadev, Walton, & Leslie 1988), red deer (Adam, Moir & Atkinson, 1985), suni (Loskutoff et al 1990) and the scimitar-horned oryx (Loskutoff et al 1983), however, different to that of a close relative, the blesbok (28 - 32 days) as reported by Marais (1988). It is assumed that the progesterone resulted from corpus luteum activity directly after ovulation, which if correct, means that some tsessebe cows, as with some blesbok, are capable of breeding throughout the year when maintained under conditions as specified in the present study.

After allowing the bull direct access to the cows, three of the cows conceived at their subsequent oestrus. This was determined first, by observing the almost immediate rise in circulating progesterone levels and the maintenance of these high levels up until the last specimens were collected on 9 September (cow 1) and the 14 October (cows 3 and 10) 1992. Secondly, by finding at necropsy, a near-term fetus in cow one and noting the arrival of two calves, one each for cows three and ten. The plasma progesterone concentrations, as measured during pregnancy are indistinguishable from peaks measured during the luteal phase of the oestrous cycle.

Cow 1 did not show obvious reproductive cycles during 1991, however she did cycle and conceived when the bull was allowed direct access to the herd. This could have been due to a number of factors, such as, the establishment of a more acceptable social structure for her by the removal of the dominant cow (7) and cow 2; the decrease in the total herd size as related to the size of the area in which they were being maintained; a decrease in stress as a result of specimens only being collected once a month; or as a result of her foot injury (footrot) healing.

It is not known why cow 11 did not conceive after she was allowed direct access to the bull. Her circulating progesterone levels showed increased activity in the second half of the study, reaching a peak of just more than 0,4 ng/ml in March 1992. In May 1992 the bull broke her leg while engaged in herding behaviour during the coaxing of the herd up to the boma for specimen collection. The bulls herding behaviour was seen to disrupt the usually orderly movement of the cows up to the boma.

Electro-ejaculation of the tsessebe bull was only successful when xylazine instead of azaperone was used in the chemical immobilization drug regime. Similar observations have been made

by other investigators ([1]Meltzer; [2]de la Rey) on other species. Dott & Skinner, (1989) were able to recover viable sperm by electro-ejaculating tsessebe, however, they do report that with some bulls, no sperm was recovered. Some of the bulls were physically restrained for the procedures while others were chemically immobilized using azaperone in the chemical immobilization drug regime.

Although the present study showed that tsessebe can be physically restrained to collect blood samples for reproductive hormone determinations, it still remains a fairly tricky and stressful procedure for the antelope. A possible way of eliminating stress caused by physical restraint for specimen collection would be the collection and determination of hormone profiles from faeces or urine. Positive correlation between plasma progesterone and faecal progesterone metabolites has been shown to occur in cattle and other species (Desaulniers et al. 1989) and similarly, estrogens in faeces, which has proved useful in determining pregnancy in a variety of species (Bamberg, Mostl, Patzl & King, 1991; Safar-Hermann, Ismail, Choi, Mostl & Bamberg, 1987) should be determined so as to validate its applicability in determining pregnancy in tsessebe

Haematology and Plasma Biochemistry of Tsessebe

Although various aspects of the behaviour and biology of the genus Damaliscus have been described, there is a paucity of information regarding blood composition, especially serum / plasma biochemistry, which makes the haematology and serum / plasma biochemistry results difficult to interpret. For this reason, the range of basic haematological values established

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[1]Meltzer, D.G.A. Price Forbes Chair of Wildlife Diseases, Faculty of Veterinary Science, University of Pretoria, Pretoria.
[2]de la Rey, R. Private practitioner, Krokodildrift Oos, Brits.

by Pospisil *et al*. (1984d) in 25 antelope species were used to compare the results obtained for tsessebe in the present study.

The observation that no significant differences between summer and winter values were obtained in the present study is not unexpected in view of the fact that the animals were reasonably adapted to the existing conditions, and that the diet provided was constant throughout the year.

The mean erythrocyte count for tsessebe fell within the higher range of values established for antelope species, and exceeded those reported for blesbok (*D. dorcas phillipsi*), bontebok (*D. dorcas dorcas*) (Pospisil *et al*., 1984d) and topi (*D. lunatus topi*) (Drevemo *et al*., 1974). The mean corpuscular volume in tsessebe fell within the lower range of the scale reported for antelope. This is similar to the inverse relationship between this variable and the erythrocyte count which has been demonstrated in impala (Pospisil *et al*., 1984d). However, the possibility also exists that the relatively high erythrocyte count reflects splenic contraction and the release of red blood cells into the circulation as a consequence of the excitement induced by physical restraint. Haemoglobin content and haematocrit measurements were within the range of values obtained by Pospisil *et al*., (1984d) and were also similar to values reported for topi in Kenya (Drevemo *et al*., 1974). The mean leucocyte count for tsessebe was found to lie in the middle of the range for antelope in general, and was similar to that obtained for topi by Drevemo *et al*. (1974). Of interest was the fact that one particular individual which exhibited extreme excitement during restraint procedures was found to have elevated leucocyte counts of 15,7 and 11,9 x 10 /L during the summer and winter, respectively, with no apparent signs of pathology.

A number of serum biochemical values have been reported for various African antelope species (eg. Drevemo *et al*., 1974; Bush *et al*. 1981; Peinado *et al*. 1990) although the majority of variables determined in the present study have not been measured

in Damaliscus spp. Levels of inorganic phosphorus in tsessebe were low when compared to those of other antelope species, an observation which has also been made in the topi (Drevemo et al. 1974). Unfortunately, no data concerning serum enzyme levels in Damaliscus spp. are available with which to compare the values reported here.

Pospisil et al. (1984d) indicated some biological factors influencing blood composition which should be considered when interpreting results. Of importance are the effects of health status, age and diet which, not only are difficult to access or control, but may affect blood composition differently in captive and free-ranging populations. The method of blood collection, involving either immobilisation or physical restraint (as was the case in the present study), is also a cause for concern and numerous studies have observed changes in blood constituents as a result of varying treatments (eg. Franzman & Thorne, 1970; Franzman, 1972; Seal et al. 1972; Presidente, 1973; Drevemo & Karstad, 1974; Franzman et al. 1975; Wesson et al. 1979a,b; Mautz et al. 1980; Cheney & Hattingh, 1987; Knox et al. 1992). Manual restraint in particular is a potent stressor eliciting alarm reactions in the animals with resultant catecholamine and adrenocorticotrophic hormone release. These hormones then profoundly influence blood composition by increasing erythrocyte counts, haemoglobin levels, haematocrit and glucose concentration. It is therefore unlikely that these values represent baseline values.

If haematological and serum/plasma biochemical changes are to be used to accurately assess health and nutritional status in wild animals, it is essential that base-line values for the different variables be established in different species. In addition, the conditions under which blood samples are collected need to be adequately described and standardized (where possible) in order to make results comparable both across and within species.

Body score, Disease & Post Mortems

At the start of the presant study, all the cows had been at the study facility for more than a year and were considered acclimated to their environment and diet (social and physical). Specimen collection procedures commenced quite intensively at the initial part of the study (March 1991) and this period coincided first with the rutting season and secondly with Autumn where the nutritional value (quantity and quality) of the veld starts to decline. Although the tsessebe's diet was supplemented with concentrates and a grass/lucerne mixture, the mean body score decreased quite rapidly in March 1991 and remained low until the end of the same year. The sudden decline in body condition can probably be ascribed to the stress created by the frequent specimen collection procedures conducted at this time. Similar declines in mass associated with handling was also observed by Knox (1992) in impala. When the frequency of procedures decreased, winter had set in and this, combined with the declining quality of the nutrition, probably resulted in the tsessebe not being able to regain their body condition before the resumption of the rainy season with the concomitant establishment of better quality pasture in the grazing paddocks.

The mean body score improved in 1992 and this can probably be attributed to pregnancy in combination with one or more of the following, namely that the tsessebe were now completely adapted to the specimen collection procedures and or the nutritional value of the veld in the grazing paddocks was of a suitably high quality and quantity and or that less stress was being experienced by the cows as a result a decrease in the number of times that the cows were being bled and or that the social structure of the herd (bull joined herd and decrease in number of cows) became more acceptable and therefore less stressful considering the size of the paddock in which they were maintained.

The capture techniques used for both groups of tsessebe (NZG

and PFNR) were considered acceptable and no signs of capture myopathy were apparent at post mortem.

Reticulo-peritonitis and reticulo-pericarditis are well described diseases of cattle and often occur when a sharp and heavy object (usually metal) is ingested by the animal. The object often lodges in the reticulum and penetrates the reticular wall due to the mixing movements of the reticulo-rumen wall. The lucerne bales used by the NZG to feed the tsessebe (Potgietersrus Pens) were bound using wire while the bales used by the TSAC were bound using baling twine. The section of wire found in cow 4 was probably included in a bale of lucerne by the baler. Another possibility is that the piece of wire was included in a sack of chopped lucerne produced by a hammermill. The ingestion of wire probably took place while cow 4 was kept at the NZG's pen. The twine making up the gastrolith found in cow 5 probably came from the lucerne bales fed to the cows at the TSAC pens.

Tsessebe are therefore quite capable of taking in foreign bodies, such as metal objects and twine strands when fed baled fodder. It is therefore advisable to pay particular attention to the physical feeding of tsessebe, so as to prevent the possible ingestion of foreign bodies.

It is possible that cow 6 could not cope with the stress of confinement. The collection and determination of key physiological variables as suggested by Knox (1992) could well have led to her timely release and possible survival.

The reason for cow 1's death is unknown. She was due to calve within a week or two. Her body condition was good (Body score 4) and no clinical symptoms were observed before her death. A possible explanation is a chance snake bite as there are known to be large numbers of a variety of poisonous snakes in the study area.

CHAPTER 5

Conclusion

As with many other wild ungulate species, the tsessebe cows were never observed to be in oestrus. However, as is evidenced by the fluctuations in circulating plasma progesterone concentrations, some of the cows showed cyclic reproductive activity throughout the year. This could be significant for the breeding of endangered Damaliscus and other similar species faced with extinction and which need to be managed and bred more intensively to save them. This is of course subject to the tsessebe bull being able to produce a suitably viable ejaculate when employing a natural breeding programme (bull and cows maintained in a natural paddock or veld environment with supplementary nutrition). There appears to be a seasonal change in spermatogenic activity in the testes of tsessebe bulls (Penzhorn & van der Merwe, 1993) which might influence the breeding ability of tsessebe in the non-breeding season. If this is so then one would have to employ artificial insemination, using sperm collected in the breeding season to inseminate cows in their non-breeding season.

From circulating progesterone levels, it was deduced that the oestrous cycle length was about 21,5 days and the most regular cycles were observed in the rutting season. It was not possible to distinguish between the luteal phase of the oestrous cycle and pregnancy based on the plasma progesterone concentrations. Thus, in order to distinguish between these two conditions, circulating progesterone values will have to be determined from a series of specimens collected from the same individual, so as to include at least one or more follicular phase's of a possible oestrous cycle. Alternatively, a constant level of around 0,50 ng/ml progesterone over at least one full cycle length could possibly also indicate pregnancy. Indications are that it is the progesterone from the corpus luteum that maintains pregnancy.

Physical restraint did not appear to influence the reproductive cyclicity of the cows. It is probable that the husbandry procedures employed, in combination with the method used to collect specimens, did not pose a big enough insult to completely prevent the tsessebe cows from cycling. This is further supported by the fact that three of four cows became pregnant soon after being allowed direct access to the bull.

The majority of haematological and biochemical variables fell within the range of other antelope species. However, for these results to be of any benefit to other studies or cases, similar physical restraint and conditioning procedures will have to be followed. Other factors, such as sex, age, diet and health status will also have to be taken into account when interpreting the results.

The decline in body condition of the cows for the first part of the study was not unexpected, considering the relative stress of the procedure, the seasonal change in the nutritional status of the grazing and the over crowding of the paddocks.

Some factors that led to disease or mortality of tsessebe or which were suspected of playing a role in disease or mortality were: Ingestion of foreign objects (wire and baling twine); bull aggression (attacking cows during herding behaviour); inability to adapt to captive conditions (at the initial capture and processing period); suspected poisonous snake bite. Most of these factors can be avoided in any future similar study by taking the following precautions: Removal of foreign objects from feed; Dehorning of the bull; Timeous determination of selected blood parameters when preparing antelope for a project study and their immediate exclusion from the study if they show parameter changes indicative of inability to adapt to husbandry or specimen collection procedures

All the tsessebe that died, did not appear to do so as a result of the specimen collection procedures performed after the

initial adaptation period. The stress caused by the above procedures was of a short but intense nature which can be described as acute stress. Animals appear to be able to withstand such an insult, as long as it is of a short duration and the animals have enough time to recover from the insult before the next stressful insult takes place. The duration and frequency of specimen collection procedures should therefore also be considered when designing a similar study.

The present study indicated that, by taking certain factors into account, such as animal management and the behaviour of the particular species, medium to large-size antelope species can be managed and maintained in a paddock system and physically restrained to collect blood and faecal specimens. Such projects could also be instituted for the intensive breeding of endangered wild ungulate species. Assisted reproductive techniques for endangered wildlife species has the potential to contribute to the maintenance of genetic diversity within species and therefore to the conservation of that species.

CHAPTER 6

SUMMARY

Six adult tsessebe cows and a bull were acquired for the study from two sources. Before the study could start, the bull was separated from the cows. The tsessebe cows underwent an adaptation period during which time the pregnant cows were allowed to calve and wean their calves, they were allowed to interact with each other so as to work out a social structure for themselves, and they familiarised themselves with their new environment and feed. The bull was electro-ejaculated and his sperm examined for any obvious abnormalities.

A customised paddock and pen complex and a body clamp were built for the study. The cows were kept separate from the bull for the first part of the study. At least twice a week, from March until May, the cows were coaxed up from their paddock into the pen complex, where they were separated into single pens. From here, each cow was separately coaxed into the body clamp for physical restraint and specimen collection. The procedures were carried out at the same time each morning and the whole procedure, from coaxing the cows up to the pens until the last cow was released after specimen collection, lasted on average 45 min. In January and February and from June up until December of 1991, sampling procedures took place twice a month. From January 1992, after the bull was allowed direct access to the cows, sampling procedures were carried out once a month only.

The objectives of the present study were to: Elucidate the annual reproductive hormone patterns in tsessebe; provide information describing ranges for various blood constituents in tsessebe, thereby expanding the database available for African antelope species; formulate a model for reproductive research and assist in the development of a management protocol for medium-sized wild ungulates in semi-intensive management systems; form the basis for further reproductive research which may result in

the development of an *in situ* as well as an *ex situ* conservation plan for endangered medium to large size antelope.

During the rutting season, the cows exhibited increased ovarian activity, as represented by the increase in circulating progesterone concentrations. Some of the cows did exhibit ovarian activity throughout the year, as represented by the fluctuations in circulating progesterone concentrations. The cyclic secretion of progesterone indicated an oestrous cycle length of about 21 days. After conceiving, the mean circulating progesterone concentrations rose sharply and remained high during pregnancy.

Body score and various blood constituents were also measured in order to interpret the progesterone results more critically. Pooled winter versus summer blood constituents did not differ significantly. This is not unexpected in view of the fact that the animals were reasonably well adapted to the existing conditions and that the diet provided was consistent throughout the year. The mean body score decreased during winter and rose the following summer, something that has been observed with a number of ungulate species in the summer rainfall areas. However, the sudden decline in body condition at the start of this trial can probably be ascribed to the initiation of the specimen collection procedures. The maintenance of body condition during 1992 can be ascribed to the cows having conceived sometime in February or March. The herd size decreased and the number of specimen collection procedures decreased during this period, something that could also have had a positive outcome on body condition.

Before the commencement of the present study, two of the tsessebe that died were found to have foreign bodies in their rumen (baling rope) and spleen (baling wire). This indicates that tsessebe are quite capable of swallowing such objects and care should be taken in not leaving such objects in their feed bowls.

When the bull was allowed access to the cows, he was seen

to exhibit herding behaviour on them. This complicated the coaxing procedure as the bull would interfere with the progress of the cows by stopping them ever so often. This aggressive behaviour by the bull eventually resulted in one of the cows death due to a broken leg.

Some animals simply do not adapt to confinement and this is probably true for one of the cows that was destined for this study. One can also expect that, when keeping animals under similar conditions, some animals may die without having any indication as to what was actually the cause of death. This is certainly the case for cow one.

The specimen collection procedure from wild caught tsessebe maintained under semi-intensive conditions did not hinder attaining the objectives of this study in any significant way. Similar projects could be used to breed endangered antelope species more intensively, possibly incorporating assisted reproduction techniques as a conservation tool.

OPSOMMING EN GEVOLGTREKKING

Ses volwasse tsessebe koeie en 'n bul is vanaf twee bronne vir hierdie studie verkry. Voor aanvang van die studie is die bul en die koeie van mekaar geskei. Die tsessebe koeie het 'n aanpassingsperiode ondergaan waartydens dragtige koeie toegelaat is om te kalf en hulle kalwers te speen, asook met mekaar te meng en sodoende 'n sosiale struktuur vir hulself te bepaal en hulle vertroud geraak het met die nuwe omgewing en voeding. Sperm is deur middel van elektro-ejakulasie by die bul versamel en vir enige ooglopende abnormaliteite ondersoek.

'n kamp, 'n hok-kompleks en 'n lyfklem is vir hierdie studie gebou. Die koeie en bul is vir die eerste gedeelte van die studie van mekaar geskei. Die koeie is ten minste twee keer per week vanaf Maart tot Mei 1991, vanaf hulle kamp tot by die hok-kompleks aangemoedig waarna hulle dan in enkele hokke van mekaar geskei is. Vanaf hier is elke koei apart tot in die lyfklem aangemoedig vir fisiese inperking en die versameling van monsters. Hierdie prosedure is elke keer op dieselfde tyd in die oggend uitgevoer. Die hele proses vanaf aanmoediging tot in die hokke tot vrylating van die laaste koei na monsterversameling het gemiddeld 45 min geduur. In Januarie en Februarie en vanaf Junie tot Desember 1991 is monsterversameling twee keer per maand herhaal. Vanaf Januarie 1992, nadat die bul direkte toegang tot die koeie toegelaat is, is monsters slegs een maal per maand versamel.

Die doelwitte van hierdie huidige studie was om: Die jaarlikse patrone van sirkulerende progesteroon konsentrasie te verklaar; inligting te verskaf van die speling van verskeie bloedkomponente in die tsessebe, om sodoende die beskikbare data basis vir Afrikaanse wildboksoorte uit te brei; om 'n model te omskryf vir voortplantingsnavorsing en om te help in die ontwikkeling van 'n bestuursprotokol vir medium-grootte wilde hoefdiere aangehou in semi-intensiewe bestuursisteme; 'n basis te vorm vir verdere voorplantingnavorsing wat moontlik mag lei

tot die ontwikkeling van 'n *in situ* sowel as 'n *ex situ* bewaringsplan vir bedreigde medium tot groot wildsbokke.

Soos in die geval van baie ander wilde hoefdiere, is bronstigheid nooit by tsessebe koeie opgemerk nie. Fluktuasies in sirkulerende plasma progesteroon konsentrasies het egter bewys dat sommige van die koeie wel sikliese reproduktiewe aktiwiteit dwarsdeur die jaar getoon het. Hierdie verskynsel mag betekenisvol wees vir die teling van die bedreigde **Damaliscus** en ander soortgelyke spesies wat uitwissing in die oë staar en waar meer intensiewe bestuur en teling nodig is om hulle te red. Dit is natuurlik onderworpe aan die vermoë van 'n tsessebe bul om 'n gepaste, lewensvatbare ejukulaat te lewer in 'n natuurlike telingsprogram (waar die bul en koeie saam aangehou word in 'n natuurlike kamp of veld met bykomstige voeding.) Daar blyk 'n seisoenale verandering in die spermatogeniese aktiwiteit in die testes van tsessebe bulle te wees (Penzhorn & van der Merwe, 1993) wat die telingsvermoë van die tsessebe in die nie-teel seisoen mag beïnvloed. As dit so is, sal dit nodig wees om koeie tydens die nie-teel seisoen kunsmatig te insemineer met sperm wat tydens die teel seisoen versamel is.

Vanaf die sirkulerende progesteroon vlakke kon afgelei word dat die lengte van die oestrussiklus 21 dae was en die mees gereelde siklusse is gedurende die paartyd waargeneem. Dit was onmoontlik om tussen die luteale fase van die oestrussiklus en dragtigheid op grond van plasma progesteroon vlakke te onderskei. Om tussen hierdie twee toestande te onderskei sal dit dus nodig wees om sirkulerende progesteroon waardes te bepaal in 'n reeks monsters versamel vanaf dieselfde indiwidu, om sodoende ten minste een of meer follikulêre fases van 'n moontlike oestrussiklus in te sluit. Alternatiewelik mag 'n konstante progesteroon vlak van ongeveer 0,50 ng/ml oor ten minste een volle sikluslengte, ook moontlik dragtigheid aandui.

Dit bleik asof fisiese inperking nie die reproduktiewe sikliese aktiwiteit van die koeie beïnvloed het nie. Dit mag

wees dat die hanteringsprosedures en die metodes wat toegepas was om die monsters te versamel, die tsessebe koei nie so erg benadeel het om hulle sikliese aktiwiteit te voorkom nie. Dit word verder ondersteun deur die feit dat drie uit die vier koeie dragtig geraak het kort nadat hulle by die bul toegelaat is.

Daar is gekyk na liggaamsamestelling en verskeie bloedkomponente is gemeet om sodoende die progesteroonwaardes meer krities te vertolk. Gekombineerde winter versus somer bloed komponente het nie betekenisvol van mekaar verskil nie. Dit is nie onverwags nie, veral as in ag geneem word dat die diere redelik goed by die bestaande toestande aangepas was en dat die dieet wat voorsien is, bestendig deur die jaar gebly het. Vir hierdie resultate om van waarde te wees vir ander studies of gevalle, sal dit egter nodig wees om dieselfde fisiese inperkings - en kondisioneringsprosedures toe te pas. Ander faktore soos geslag, ouderdom, dieet en gesondheidstoestand sal ook tydens die vertolking van hierdie resultate in ag geneem moet word. Soos al voorheen by 'n aantal hoefdier spesies in somer reënvalgebiede opgemerk, het die gemiddelde liggaamsamestelling gedurende die winter afgeneem en in die volgende somer weer toegeneem. Die afname in liggaamskondisie aan die begin van hierdie ondersoek kan waarskynlik aan die aanvang van monsterversamelingsprosedures toegeskryf word. Die behoud van liggaamskondisie in 1992 kan daaraan toegeskryf word dat die koeie in Februarie of Maart dragtig geraak het. Die afname in kuddegrootte en die vermindering in die aantal monsterversamelingsprosedures gedurende hierdie periode kon ook 'n positiewe uitwerking op liggaamskondisie gehad het.

Die volgende faktore het gelei tot siekte of dood van die tsessebes of het vermoedelik 'n rol gespeel in die siekte of dood: Inname van vreemde voorwerpe (draad en balingstou), bul aggressie (aanval van koeie tydens tropgedrag), onvermoë om by die ingeperkte kondisies aan te pas (by die aanvanklike vangs en die proseseringsperiode) en vermoeddlike slangvergiftiging. Meeste van hierdie faktore kan egter in enige soortegelyke

toekomstige studies voorkom word deur die volgende voorsorg te tref: Verwydering van vreemde voorwerp uit die voer, onthoring van bulle, tydige bepaling van geselekteerde bloedparameters tydens die voorbereiding van die wildsbokke vir 'n projekstudie en hul onmiddellike uitsluiting as daar parameterveranderings is wat daarop dui dat hulle nie in staat is om by die hanterings - of monsterversamelingsprosedures aan te pas nie.

Dit bleik asof die dood van sommige van die tsessebes nie die gevolg was van die aanvanklike monsterversamelingsmetodes na die aanvanklike aanpassingsperiode nie. Die stres wat veroorsaak was deur die bogenoemde prosedures was van 'n kort, maar intensiewe aard wat as akute stres beskryf kan vord. Dit blyk asof die diere in staat is om so 'n ingryping te weerstaan, solank as dit van korte duur is en daar genoeg tyd is om van die ingryping te herstel voordat 'n volgende stresvolle ingryping plaasvind. Die durasie en frekwensie van die monsterversamelings-prosedure moet dus ook by die beplanning van 'n soortgelyke studie in ag geneem word.

Die huidige studie het aangetoon dat indien sekere faktore in ag geneem word soos dierebestuur en die gedrag van die spesies, dit moontlik is om medium tot groot wildboksoorte in 'n kampsisteem aan te hou en te bestuur en hulle fisies in te perk vir die versameling van bloed-en mismonsters. Sulke projekte kan ook vir die intensiewe teling van soortgelyke bedreigde wildsoorte ingestel word. Gerugsteunde voortplantingstegnieke vir bedreigde wildsoorte het die potensiaal om by te dra tot die genetiese verskeidenheid binne spesies en derhalwe ook tot die bewaring van daardie spesie.

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