

REPRODUCTION IN THE MALE CHEETAH  
Acinonyx jubatus jubatus (SCHREBER, 1776)

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Acinonyx jubatus jubatus (SCHREBER, 1776)

by

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Submitted in partial fulfilment of the  
requirements for the degree of

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University of Pretoria  
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ABSTRACT

The objective of the study was the promotion of the breeding of cheetahs in captivity. Two means of achieving this end were selected. First a management approach was adopted to ensure the control of breeding activity in the captive population. Secondly cheetah males with the best semen quality were selected for use in the breeding program. Semen was collected from anaesthetized cheetah males by electroejaculation. Semen was evaluated immediately after collection and spermograms were counted on prepared semen smears. Sperm concentrations were determined in samples collected from cheetah males over a 24 h period, in animals that were anaesthetized, electroejaculated and after stimulation with GnRH. During the study period, 1975-1984, >240 cheetah cubs were born from 71 litters and a mean conception rate of 52% was achieved.

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## ACKNOWLEDGEMENTS

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The objective of the study was the promotion of the breeding of cheetahs in captivity. Two means of achieving this end were selected. First a management approach was adopted to ensure the control of breeding activity in the captive population. Secondly cheetah males with the best semen quality were selected for use in the breeding program. Semen was collected from anaesthetized cheetah males by electroejaculation. Semen was evaluated immediately after collection and spermograms were counted on prepared semen smears. Plasma testosterone concentrations were determined in samples collected from cheetah males over a 24 h period, in animals that were anaesthetized, electroejaculated and after stimulation with GnRH.

During the study period, 1975-1984, >240 cheetah cubs were born from 71 litters and a mean conception rate of 52% was achieved.



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The present study was undertaken with the aim of promoting the breeding of cheetahs at the De Wildt Centre. The population of cheetahs being kept at the time consisted of 29 animals (20/9). First the management of the population was investigated and changed with the view to stimulate breeding activity. Then the fertility of male cheetahs was examined with the objective of establishing a rational method of selecting males for the breeding program. After it was found that a large proportion of the males being kept at the Centre appeared to have poor semen quality the study was extended to include an investigation into aspects of reproductive endocrinology in the male cheetah. The



## INTRODUCTION

The De Wildt Cheetah Breeding Centre was started by Ann Van Dyk and her brother, Godfrey, in partnership with the National Zoological Gardens of South Africa in 1971. The objective was to breed cheetahs in large enough numbers to meet the requirements of zoological gardens for display animals and by so doing relieve the pressure on the wild populations from which animals were captured and sold. It also was hoped that, if the breeding of cheetahs was sufficiently successful, animals could be released into suitable conservation areas.

The cheetah was regarded as very difficult to breed in captivity (Hediger 1950, in Manton, 1970). Concern for the survival of the species was growing. Cheetahs were classified as endangered after it was reported that the population in the wild had decreased and appeared to be decreasing further (Myers, 1975, in Wrogemann, 1975).

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objective being to gain an understanding of possible factors in captivity that might influence male fertility through effects on testicular function.

#### STUDY AREA

The study was carried out at the De Wildt Cheetah Research Centre which is situated some 20 km west of Pretoria in the foothills of the Magaliesberg (25°40'S 27°52'E). For details of rainfall, climate and veld type vide Degenaar (1977).

#### MATERIAL & METHODS

##### Management

Cheetahs are fed on 6 days of the week and fast on Sundays. Each receives about 2 kg of meat per day to which a vitamin and mineral mixture is added. Beef or mutton supplied by the National Zoological Gardens and poultry from the neighbouring poultry farm are the staple diets. All meat is inspected prior to feeding and excess fat removed.

Cheetahs born on the farm are vaccinated at 3 months of age with a combined attenuated live vaccine against feline panleucopenia infectious rhinotracheitis and calici virus (Felocell CVR, Norden Labs., Lincoln). Adult cheetahs are vaccinated once a year with the same vaccine.

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#### STUDY AREA

The study was carried out at the De Wildt Cheetah Research Centre which is situated some 20 km west of Pretoria in the foothills of the Magaliesberg (25°40'S 27°52'E). For details of rainfall, climate and veld type vide Degenaar (1977).

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Cheetah cubs are numbered by ear notching using the Versfeld method as adopted by the South African Studbook Association.

Adult cheetahs are identified by Miss Van Dyk who recognizes and names them individually.

All animals are sprayed regularly with a 1:2000 solution of quintiofos (Bacdip, Bayer, Isando) for the control of ticks.

#### Breeding management

The cheetah enclosures were designed and erected taking cognisance of the reported reproductive behaviour of this species. Eaton (1974) indicated that the wild cheetah female spent most of her life as a solitary individual; made brief contact with males during oestrus and cared for her litter until the cubs reached approximately 15 months of age. It was suggested that a group of up to 5 cheetah males should be put with a captive female when she was in oestrus and that competition and aggression between males would stimulate sexual activity (Eaton & York 1970; Herdman 1972; Eaton 1974). Florio & Spinelli (1967, 1968) and Manton (1970, 1971) however reported the successful breeding of captive cheetahs using a single male. Cheetahs were bred successfully at Whipsnade Park where the sexes were kept apart for most of the year and the female came into oestrus shortly after the male was released into her enclosure (Manton, 1970, 1971).

Prior to the commencement of the present study a group of 5 cheetah males were released into a female's enclosure, at the Centre, when she was thought to be in oestrus. She was attacked, severely mauled and died as a result of her injuries.



A plan of the cheetah enclosure, about 45 ha in extent, is shown in figure 1. The perimeter is fenced with 50 mm diamond mesh to a height of 3 m with a 150 cm overhang at the top. Internal enclosures are fenced to a height of 2 m with the same diamond mesh.

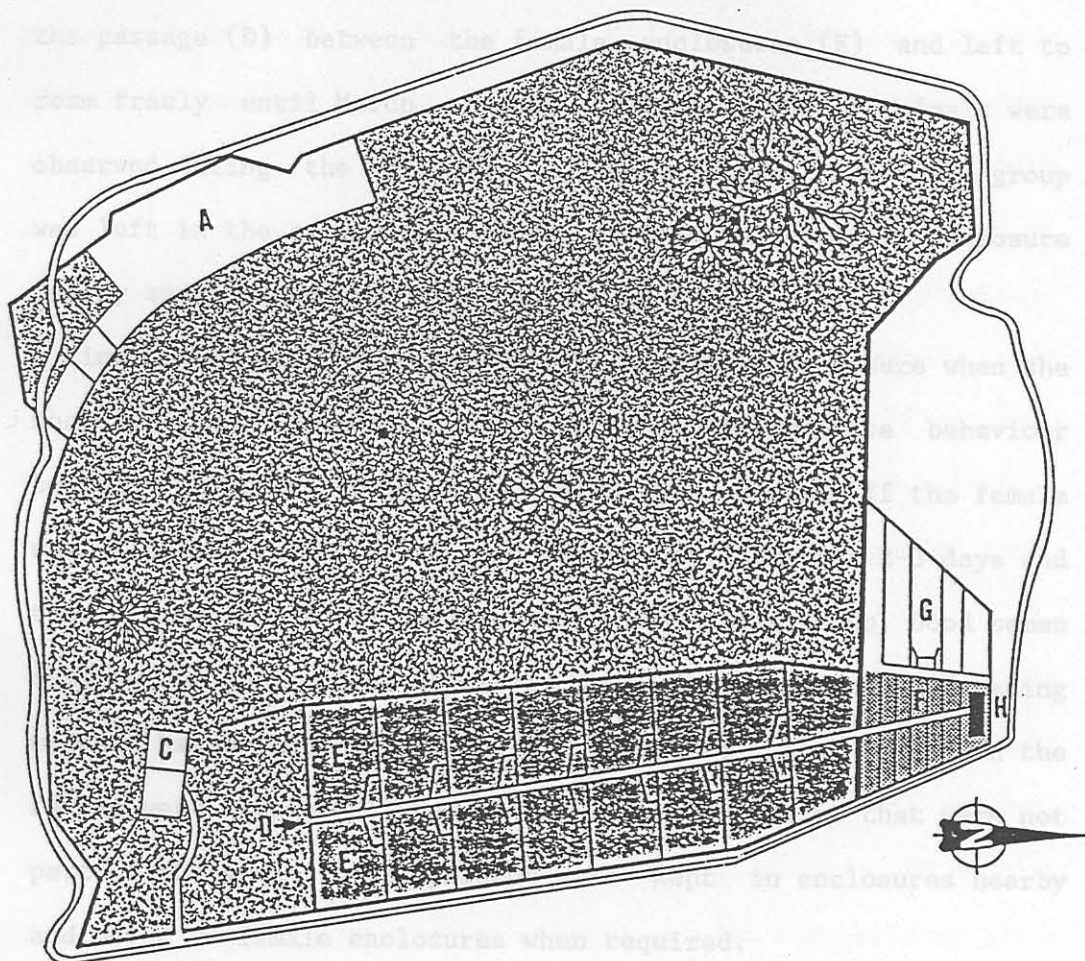


Figure 1: Plan of the cheetah enclosures at the De Wildt Cheetah Breeding and Research Centre of the National Zoological Gardens of South Africa.

at about 4 months of age. Thereafter cubs were removed to rearing

Female cheetahs were kept in individual enclosures (E) about 0,5 ha in size. Compatible males were kept in groups of 2 - 10 individuals in 0,5 - 5 ha enclosures situated out of sight and sound of the females for most of the year (A).

Between November and December a group of males was introduced to the passage (D) between the female enclosures (E) and left to roam freely until March of the following year. Animals were observed during the early morning and evenings. The male group was left in the area for 10 days, removed to a holding enclosure nearby and then brought back again a week later.

A single male was introduced into a female's enclosure when she showed signs of behavioural oestrus; interactive behaviour between the cheetah pair was observed and recorded. If the female was receptive the male was left in the enclosure for 2-3 days and then coaxed out into the passage to rejoin his group. Good semen quality and indications of libido were used in selecting breeding males. Males that did not show an immediate keen interest in the female were replaced after 30 min. Cheetah males that were not part of the group in the passage were kept in enclosures nearby and moved to female enclosures when required.

Females observed to copulate or which were believed to be pregnant, were transferred to maternity enclosures (F) where they were kept until parturition. Females that raised their cubs remained in the maternity enclosure until the litter was weaned

drawn through the mesh, a vein located and an intravenous injection given.



at about 4 months of age. Thereafter cubs were removed to rearing enclosures (G) in groups of a maximum of 10 animals and females were returned to their previous individual enclosures.

Immobilization effected by electrocathodal using an electro-stimulator described by Van Nieuwenburg & De Vos (1967) as modified

Males free in their enclosures were darted using a Palmer Capchur pistol to deliver a drug mixture of 6-8 mg/kg ketamine hydrochloride (Ketalar, Parke Davis, Isando) and 0,5 mg/kg xylazine hydrochloride (Rompun, Bayer, Isando), this dosage being sufficient to induce a state of hypnosis that made safe handling possible. Thereafter the level of anaesthesia was increased, when necessary, using intravenous thiopentone sodium (Intraval, Maybaker, Halfway House) or by the administration of halothane (Fluothane, ICI, Isando) after intubation with a cuffed endotracheal tube.

An alternative capture technique was later developed in which the animal was caught in a crush, pinned down with wooden poles, and given 2 mg/kg intravenous CT1341 (Saffan, Milvet, Pietermaritzburg). Crushes were constructed out of a 25 mm pipe frame 175 cm long, 75 cm wide and 120 cm high with sliding gates at each end. The sides of the crush were closed with 10 mm iron rods welded to form a square mesh with 150 mm X 150 mm openings. These made it possible for handlers to get their hands into and out of the crush freely. A limb of the captured animal could be drawn through the mesh, a vein located and an intravenous injection given.



applied the rectal probe was withdrawn in the rectum until the tip slipped behind caudal ridge of the pubic bone. It was then

#### Semen collection & evaluation

Ejaculates were collected in graduated glass or plastic test

Semen was collected by electroejaculation using an electroejaculator described by Van Rensburg & De Vos (1957) as modified by Van Tonder, Bolton, Roberson & Greeff (1973). The power source, a bicycle dynamo, is driven by a 120 mm rubber disc turned by hand. The dynamo used produced a sine wave AC current with a voltage that varied from 8 to 10 volts. X magnification.

A bipolar brass electrode consisting of a 250 mm brass rod, 150 mm in diameter, with an isolated 20 mm tip was used during the initial stages of the study. This was later replaced by a teflon probe of similar dimensions but with two 4 mm brass ring electrodes 10 mm apart 20 mm from the tip. In the latter stages of the study a probe with 3 ventral, 50 mm longitudinal electrodes was used (Ball & Furman 1972, in Ball 1976). The polarity of the central electrode being opposite to that of the two lateral ones. The probe was inserted 200 mm into the rectum and inclined ventrally onto the floor of the pelvis in the region of the symphysis.

The standard electrical stimulus applied consisted of a cycle of three to five 1 sec bursts of current each followed by a short rest period of similar interval. This was followed by a continuous stimulus for 6-8 sec and a rest period of two seconds. Thereafter the cycle was repeated. After 2-3 cycles had been

applied the rectal probe was withdrawn in the rectum until the tip slipped behind caudal ridge of the pubic bone. It was then inserted again.

Ejaculates were collected in graduated glass or plastic test tubes. The volume, colour and an estimate of density recorded and the pH measured using test tape (Spezial-Indikatorpapier, Merck, Darmstadt). Immediately after collection a fresh sample was placed on a glass slide that had been heated to a temperature of 32°C on a warm stage, covered with a coverslip and examined microscopically using phase contrast and a 400 X magnification. An estimate of sperm concentration, % live spermatozoa and % showing progressive motility was made. Forward progression of spermatozoa was scored on a scale of 0 - 5 (Carroll, Ball & Scott, 1963, Ball 1976). At the same time an estimate of the proportion of morphological abnormal spermatozoa present was made.

Fresh semen was classified being of good, fair, or poor quality after an assessment of the above parameters had been made. Examples of the characteristics of these categories of semen quality are presented in table 1.



Table 1.: Characteristics of fresh semen samples classified as good, fair and poor immediately after collection.

	Density x 10 <sup>6</sup> ml	% Live Sperm	% Motile Sperm	Forward progression
Good	> 30	> 70	> 70	4 - 5
Fair	20 - 30	50 - 70	40 - 60	3
Poor	< 20	< 50	< 40	3

Semen smears were prepared for microscopical examination using either phase contrast or after modified Karras staining (Bosman, 1975, in Van Tonder, 1977). Two hundred individual spermatozoa per smear were examined for morphological abnormalities using a 1000 X magnification. Morphological abnormalities were classified as either major or minor using criteria as suggested by Blom (1972). Spermatozoal concentration (spermatozoal numbers/ml of ejaculate) was calculated using a standard haemocytometer counting procedure.

Samples of the ejaculate were prepared for electron microscopic examination; 0,25-0,5 ml was immediately fixed in 2% glutaraldehyde in Millonig's phosphate buffer (pH 7,3) (Millonig, 1961) for 1 h at 40°C. Using a process of gentle centrifugation and resuspension, the sperm were washed once in phosphate buffer

and then post-fixed at room temperature for 1 h in 2% osmium tetroxide in the same buffer. After two more buffer rinses sperm were drawn into micro-capillary tubes and centrifuged in a MSE Minor centrifuge equipped with a micro haematocrit head assembly. The resultant pellets were removed from the capillary tubes using a fine glass piston, diced into small blocks dehydrated through a graded ethanol series, cleared in propylene oxide and embedded in Epon 812. Thin sections were cut with glass and diamond knives on a Reichert Om U4 ultramicrotome, stained for 5 min. each with a saturated aqueous solution of uranyl acetate (Watson, 1958) and 0,2% lead citrate (Reynolds, 1963) and examined with a Phillips EM301 transmission electron microscope operated at 80 kv.

Initially semen evaluations were conducted to establish a rational breeding approach at the centre. Males were selected for inclusion in the breeding program after an assessment had been made of their semen quality (Meltzer, Coubrough, Van Dyk & Brand 1974). Thereafter 16 cheetah males from two distinct groups were electroejaculated at regular intervals for a period of one year. The first group of seven animals consisted of cheetah males that had arrived at the centre as two pairs and a group of three and had been kept separately. The second group, nine animals, were selected from 12 cheetah males that had been captured as adults in South West Africa - Namibia - and kept together ever since. The remaining three animals were used for the purposes of a separate study (Degenaar, 1977). These animals arrived at the centre in 1973, in poor condition, having been confiscated by the



Transvaal Department of Nature Conservation. By the commencement of the present study, in 1975, they were in good condition and were being kept in an enclosure approximately 4 ha in extent. The conditions in which they were kept differed markedly from those of the first group in that all of these animals were subjected to hierarchical pressures within the group and fighting broke out from time to time. Animals kept as pairs or small groups were well adapted to one another and no such problems occurred in their case.

#### Blood sampling for plasma hormone determinations

Peripheral blood samples, for hormone assays, were collected from the saphenous or recurrent cephalic veins into 10 ml heparinized vacuum tubes (Venoject, Terumo, Tokyo) and centrifuged immediately thereafter. Plasma was harvested into three separate portions and stored at  $-20^{\circ}\text{C}$  until assayed.

#### Radioimmunoassays

##### Testosterone

Serial plasma samples from individual animals were assayed together, in duplicate. Pre-GnRH and maximum post-GnRH plasma testosterone samples were selected after the first round of assays and reassayed using a single assay kit. Data on plasma

testosterone levels analysed statistically were from this single radioimmunoassay performed at the end of the study. Control sera were included in all assays.

Plasma testosterone levels were measured using a RSL NOSOLVEX I<sup>125</sup> assay kit (Radioassay Systems Laboratories, Carson, California, USA.) which was later replaced by a COAT-A-COUNT I<sup>125</sup> kit (Diagnostics Products Corporation, Los Angeles, USA).

The latter assay was less time consuming and easier to perform. RSL NOSOLVEX I<sup>125</sup> testosterone kit is a non-extraction double antibody radioimmunoassay using testosterone I<sup>125</sup>, rabbit anti-testosterone and goat anti-rabbit gamma globulin. Cross reaction at 50% binding, as determined by the supplier, was 10,3% for dihydrotestosterone, 0,56% for androstenedione and less than 0,1% for progesterone, corticosterone, de-oxycorticosterone and oestradiol-17 $\beta$ . Recovery of exogenous testosterone added to female plasma was 96,8-98 %. Interassay and intra-assay coefficients of variation were 12,3% and 7% respectively. The mean sensitivity was 0,37 nmol/l (n=10).

The COAT-A-COUNT assay does not require extraction and the anti-body is bound to the wall of polypropylene tubes supplied by the manufacturer. One hundred microlitres of plasma was added to the tube together with I<sup>125</sup> testosterone and decanting of the supernatant after 3 h incubation terminated competitive binding. Standard solutions of testosterone ranged from 1,0 - 104 nmol/l. Maximum binding was approximately 50%. Cross reaction with dihydrotestosterone and androstenedione, as determined by the manufacturer, was less than 10% and 0,1% respectively. Recovery



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of exogenous testosterone ranged from 96-103%. Interassay and intra-assay coefficients of variation were 8,2% and 2,7% respectively. The mean sensitivity was 0,87 nmol/l (n = 8)

### Cortisol

The concentration of serum cortisol was measured using an  $I^{125}$  RIA kit (RIANEN<sup>TM</sup>, New England Nuclear, No. Billerica, MA. USA). This assay employed a cortisol antiserum complex solution containing rabbit cortisol antibody prereacted with an antiserum to rabbit gamma globulin in phosphate buffer. As determined by the manufacturer the rabbit cortisol antibody had the following crossreactions: 100% with cortisol; 38,9% with prednisolone; 26,4% with corticosterone; 7,4% with aldosterone; 5,9% with 11-deoxycortisol; 3,5% with  $17\alpha$ -hydroxyprogesterone and 2% or less with progesterone, testosterone, dihydrotestosterone and oestradiol- $17\beta$ . Interassay and intra-assay coefficients of variation were 6,2% (n = 6) and 2,7% (n = 10), respectively, and minimum assay sensitivity was 27,6 nmol/l.

Plasma testosterone concentrations in relation to time, the rate of testosterone production, were estimated from graphs drawn on standard metric graph paper. The rate of testosterone production was estimated by measuring the area under the curve using a Kontron Image intensifier and calculating the rate of production as follows:  $\text{nmol/l/h} = \frac{\text{Total area under the curve}}{\text{area of 1 hour at 1 nmol/l}}$

used ensuring that the crossreactivity of the antiserum for either steroid was negligible and nonsignificant.



### Luteinizing hormone

A heterologous antibody radioimmunoassay was used to measure serum LH. This assay originally developed by Chakraborty, Wildt & Seager (1979) for the domestic cat (Felis catus) was validated for use in cheetah (Wildt, Chakraborty, Meltzer & Bush 1983). The isotope was  $I^{125}$  ovine LH (LER-1056-C), the first antibody was a bovine LH antiserum (JJR-5: dilution, 1:80000) and the results were analysed on the basis of a canine pituitary standard (LER-1685-1). Interassay and intra-assay coefficients of variation were 11,7% (n=5) and 11,1% (n=9) respectively, with a minimum assay sensitivity of 0,3  $\mu\text{g}/\text{l}$ .

### Crossreactivity with CT1341

CT1341 consists of two pregnanedione derivatives,  $3\alpha$ -hydroxy- $5\alpha$ -pregnane-11, 20-dione (alphaxalone) and 21-acetoxy  $3\alpha$ -hydroxy- $5\alpha$ -pregnane-11, 20 dione (alphadolone acetate). Due to the steroidal nature and configuration of this drug, it was considered necessary to determine its potential crossreaction with the antisera used in the cortisol and testosterone assays. Five, 10, 20 and 50  $\mu\text{l}$  of a diluted CT1341 solution (1,2  $\mu\text{g}/\text{ml}$ ) were subjected to standard procedures used in each of the radioimmunoassays. Quantities of cortisol and testosterone measured were below the detectable limits of the assays used ensuring that the crossreactivity of the CT1341 with the antiserum for either steroid was negligible and nonsignificant.

the period by repeated intravenous injections of CT1341 as required.

## Diurnal variation in plasma testosterone

Diurnal variations in plasma testosterone concentration were determined in five cheetah males kept for 24h in crushes as described above. Blood samples were collected without drug restraint every two hours during this period.

## Plasma testosterone after increasing doses of GnRH

The effects of different intramuscular doses of gonadotrophin releasing hormone (GnRH) (Gonadorelin, Abbott Laboratories, Chicago, Illinois, USA) on plasma testosterone concentrations were measured in cheetah males anaesthetized with CT1341. Four males were selected at random from a group of animals aged between 4-5 years (m34, m45, m49 and Swa). On day 1 m34, the control, received an equivalent volume of saline, m45 was given 50  $\mu$ g, m49 100  $\mu$ g and Swa 250  $\mu$ g GnRH. At two weekly intervals thereafter animals were rotated each receiving a different dose of GnRH or acting as a control so that at the completion of the experiment each had received in turn 0  $\mu$ g, 50  $\mu$ g, 100  $\mu$ g or 250  $\mu$ g GnRH. Blood was collected prior to GnRH or saline injection, one hour later and thereafter at 30 min intervals until 300 min after GnRH injection. Animals were kept in a state of anaesthesia sufficient to allow safe handling throughout the period by repeated intravenous injections of CT1341 as required.



Plasma LH & testosterone after 50 µg GnRH Controls where animals were given an equivalent volume of saline in place of GnRH were

This study was conducted in January during the breeding period at the research centre. Fourteen sexually mature male cheetahs with a mean age of 3,7 ±0,4 years were used. Animals were caught and anaesthetized with CT1341. Immediately after the induction of anaesthesia a blood sample was collected (pre-stimulation plasma sample) the animals were divided at random into two groups, one of which was given the saline vehicle, the other 50 µg GnRH (Gonadorelin, Abbott Laboratories, Chicago, Illinois, USA) by intramuscular injection. Blood was collected at 15 min intervals for the first hour and thereafter every 30 min. Cheetah males given saline were bled for 120 min and the animals that received GnRH were bled until 180 min post injection.

Plasma testosterone after 50 µg GnRH in different age groups and divided into two groups. One group was anaesthetized with 2 mg/kg

Groups of six to eight cheetah males from different age groups were anaesthetized with CT1341 and gathered under a suitable shady tree. Blood was collected prior to the injection of a standard dose of 50 µg GnRH, one hour later and thereafter at 30 min intervals until 300 min after GnRH injection. This sampling regimen was followed until radioimmunoassays indicated that plasma testosterone concentrations reached a peak between 150 and 180 min after GnRH injection. Sampling was then limited to a then at 30 min intervals for two hours until 50 µg GnRH was

maximum post-injection period of 240 min. Controls where animals were given an equivalent volume of saline in place of GnRH were included, at random, from time to time.

#### Plasma testosterone in July & November

*Hormone studies in anaesthetized & electroejaculated males*

Five cheetah males were selected at random out of a group of 12 animals aged two years. These animals were examined as described above after 50 µg GnRH on two occasions during July and November of the same year using a regimented stimulation protocol and bi-directionally. Seven control animals were bled at similar time intervals to the electroejaculated group. The electroejaculator used (P-T Electronics, College Station, Texas, USA) permitted

The effect of anaesthesia on the plasma testosterone response to stimulations was given in three series consisting of 30, 30 and 80

Eight cheetah males of similar ages were selected at random and divided into two groups. One group was anaesthetized with 2 mg/kg CT1341, the other with thiopentone sodium. Thiopentone sodium was given as a single knock-down bolus of 400 mg by rapid intravenous injection. CT1341 was given in the standard manner. Intermittent intravenous injections of each of the drugs were given as required to maintain a state of hypnosis sufficient to allow safe handling. Animals caught first were maintained in this state until the entire group of eight males had been brought together at a central point. Blood was collected prior to anaesthesia and then at 30 min intervals for two hours until 50 µg GnRH was given 60 min after electroejaculation and 60 min later. Mean time inter-



given. Two control animals from each group were given an equivalent volume of saline. Thereafter blood was collected at intervals as above.

#### Hormone studies in anaesthetized & electroejaculated males

Two groups of cheetah males were examined. Both groups were captured and anaesthetized with CT1341. Fourteen males were electroejaculated using a regimented stimulation protocol and bled serially. Seven control animals were bled at similar time intervals to the electroejaculated group. The electroejaculator used (P-T Electronics, College Station, Texas, USA) permitted controlling and monitoring of voltage and amperage. A total of 80 stimulations was given in three series consisting of 30, 30 and 20 stimuli each. The stimulation in the first series consisted of 10 stimuli at 4 V followed by two sets of 10 stimuli at 5 and 6 volts respectively. The animal was rested for 2-3 min and then stimulated again. The first 10 stimuli of this series given at 5 V were followed by two sets of 10 stimuli each at 5 and 6 volts respectively. After a similar rest interval, two final sets of 10 stimulations each were given at 6 and 7 volts.

Blood samples were collected from both groups within 5 min of capture and prior to anaesthesia. Subsequent samples were collected from the electroejaculated group following anaesthesia, immediately after each series of electroejaculation stimuli, 30 min after electroejaculation and 60 min later. Mean time inter-

vals between blood collections were as follows: from pre-anaesthetic to post anaesthetic (pre-ejaculation), 25 min; post anaesthetic to the end of series 1, 12 min; from the end of series 1 to the end of series 2, 9 min and from the end of series 2 to the end of series 3, 9 min. A similar bleeding schedule was followed in the non-ejaculated anaesthetized control cheetah males. Blood samples were cooled immediately after collection, kept at 4 °C for 2-3 h, and serum collected after centrifugation. Sera were kept at -20°C and shipped to the USA packed in dry ice, where they were assayed by for cortisol, testosterone and luteinizing hormone in the Department of Obstetrics and Gynaecology, Uniformed Services University of the Health Sciences, Bethesda, Maryland. U.S.A.

#### Adrenal function tests.

Two adult cheetah males were anaesthetized with CT1341 in the usual way and bled serially. Following a zero time sample each male was given 25 I.U. ACTH by intramuscular injection. They were then bled at 15 min intervals for the next hour and two samples taken at 30 min intervals thereafter. Two cheetah males were caught, kept in crushes for 2 h, and treated similarly without drug restraint. Samples were assayed for cortisol, testosterone and luteinizing hormone.

#### Plasma testosterone after RCG injection

Four males, Harry, Gouws, Boytjie and m46, were selected at



Plasma testosterone and semen quality. The mean age of this group was 6,9 ± 1,6 years. Each of the animals was anaesthetized, and Eight cheetah males were immobilized, electroejaculated and then given 50 µg GnRH and serially bled for the next 4 h. Semen quality was assessed in fresh semen samples immediately after collection. The animals were classified into groups having good, fair and poor quality semen using criteria as summarized in Table 1. The pre-stimulation and maximum plasma testosterone concentrations were measured and the plasma testosterone response after GnRH was calculated. The means of each of these parameters measured in each of the groups were compared using an analysis of variance.

#### Plasma testosterone after intravenous & intramuscular GnRH

Six Cheetah males aged from 4-5 y that had previously been given 50 µg intramuscularly and serially bled were chosen at random and anaesthetized.

They were bled prior to being given 50 µg GnRH by intravenous injection and bled thereafter at 30 min intervals for 180 min. Plasma was harvested and stored until assayed as described above.

#### Plasma testosterone after HCG injection

Four males, Harry, Gouws, Boytjie and m46, were selected at

the male was introduced into the female's enclosure. In cases where a male was not available for introduction in the female he was random from a group of adult cheetahs. The mean age of this group was  $6,9 \pm 1,6$  years. Each of the animals was anaesthetized, and restrained for the 3 h period with CT1341. On day 1 of the trial Harry received the equivalent of 2,5 mg LH in the form of an intramuscular injection of HCG (APL, Ayerst Labs), m46 was given 5 mg, Gouws 10 mg and Boytjie 20 mg. At two weekly intervals thereafter animals were rotated each receiving a different dose of HCG so that at the completion of the experiment each had received at random the equivalent of 2,5 mg, 5 mg, 10 mg or 20 LH. Blood was collected prior to HCG injection, one hour later and thereafter at 30 min intervals until 300 min after HCG.

Conception rate (Ratio of number of pregnancies/total number of females available) of 32%.

The frequency distribution of cheetah births at the Centre during the period 1975 - 1980 is shown in Figure 2.

## RESULTS & DISCUSSION

### Management

Females came into oestrus soon after males were introduced into the passage between their enclosures for the second time. This became apparent when males congregated at a particular female's enclosure. The female was seen close by on the other side of the fence, chirping at the males, submissive and rolling. The males were excited, several developed an erection and short fights broke out amongst them. It was not uncommon to find two or three females in oestrus at the same time. When overt signs of oestrus were observed the animals were often seen to copulate soon after



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the male was introduced into the female's enclosure. In cases where the male showed little interest in the female he was replaced by another animal after 30 min.

Indications of oestrus were less overt in some females. Subtle changes in the female's behaviour were noticed by Miss van Dyk who would then introduce a male cheetah into the particular enclosure.

Cheetah males kept in large groups were often aggressive and attacked females despite the fact that they were in oestrus. These animals were of no use for breeding purposes but were kept as part of the group outside the female's enclosure.

Seventy one litters were born during the study period with a mean conception rate ( Ratio of number of pregnancies/total number of females available) of 52%.

The frequency distribution of cheetah births at the Centre during the period 1975 - 1984 is summarized in Figure 2.

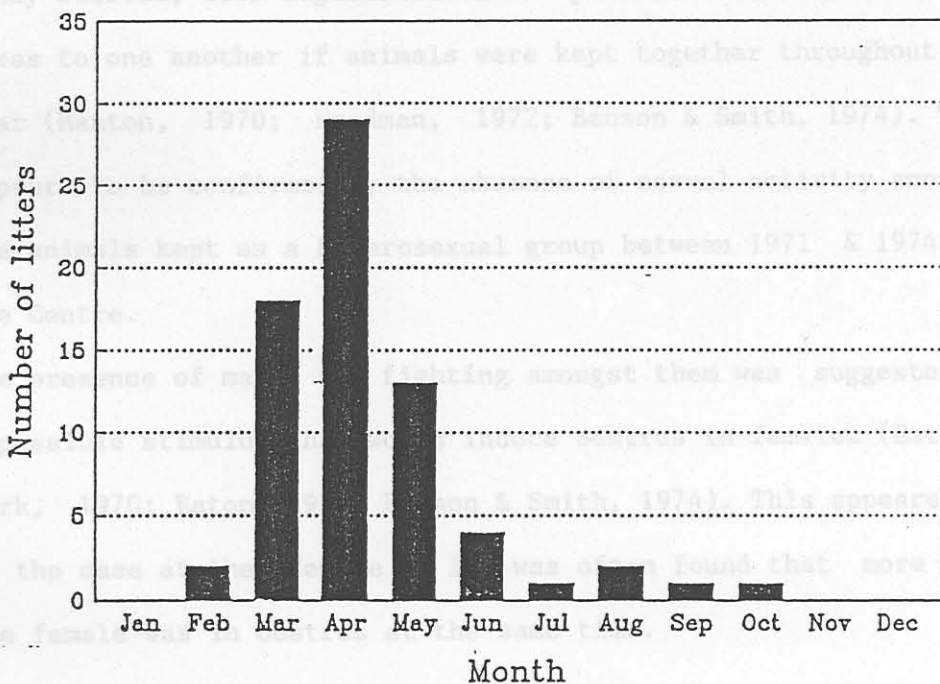


Figure 2 : Frequency distribution of cheetah births at the Centre during the period 1975 - 1984



### Immobilization

Males were allowed into the female area for the first time between November and December and left there until the end of March. ~~Animals required to enable capture.~~

This apparent seasonal distribution is a consequence of the management approach adopted at the centre. Births were planned for the late summer, the end of the rainy season. This also fitted in with other activities during the year. No conclusion as to the possible seasonality of breeding in cheetahs is therefore possible from these results. Eaton (1974) suggested that cheetahs were seasonal breeders. This was contradicted by birth records reported by Wrogemann (1975) and Wildt, O'Brien, Howard, Caro, Roelke, Brown & Bush (1986).

The breeding strategy adopted at the Centre, when the present study started, took cognizance of the possible habituation of the sexes to one another if animals were kept together throughout the year (Manton, 1970; Herdman, 1972; Benson & Smith, 1974). This appears to be confirmed by the absence of sexual activity amongst the animals kept as a heterosexual group between 1971 & 1974, at the Centre.

The presence of males and fighting amongst them was suggested as a possible stimulus that would induce oestrus in females (Eaton & York, 1970; Eaton, 1974; Benson & Smith, 1974). This appeared to be the case at the Centre as it was often found that more than one female was in oestrus at the same time.

Immobilization

Table 2. Induction, recovery times and drug dosages in cheetah

The dosage rate of the drugs used was intentionally reduced to the minimum required to enable capture. presented as mean (n = 23)

After two out of the first three animals captured with ketamine hydrochloride, at a dosage rate of 8 mg/kg, developed epilepti-

form convulsions xylazine hydrochloride was added to the drug mixture darted.

The results of 23 immobilizations of male cheetahs performed at the beginning of the study using a mixture of ketamine and xylazine are recorded in Table 2.

kg	mg/kg	mg/kg	(min)	(min)
4,75	10,22	10,20	14,3	101,4

Induction time is the time between darting and safe handling of the animal. The first signs of drug action, head shaking, were seen 3-5 min after darting. Ataxia developed followed by posterior paralysis, then the animal lay down on its side. The head was held erect, the eyes were open in a fixed stare and mydriasis was present. Palpebral reflexes were absent.

Animals approached at this stage reacted violently to sound or touch and attempted to get away. They were however unable to right themselves and their sight appeared to be impaired. As the effects of the drugs progressed cheetahs lay down on their side with their heads on the ground and could be handled. Muscular



Table 2. Induction, recovery times and drug dosages in cheetah males captured with a drug combination of ketamine hydrochloride and xylazine hydrochloride. Data are presented as mean (n = 23) and standard deviation ( $\pm$ )

MASS kg	KETAMINE mg/kg	XYLAZINE mg/kg	INDUCTION TIME (min)	RECOVERY TIME (min)
48,9	6,45	0,64	12,3	128,8
$\pm 4,75$	$\pm 0,82$	$\pm 0,20$	$\pm 4,9$	$\pm 61,4$

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hypertonicity was present with sporadic myoclonic contractions which developed into typical epileptiform convulsions in some animals particularly when higher doses of ketamine were given.

During this stage of anaesthesia a number of the animals showed a characteristic posture. The upper fore paw was placed on the side of the nose and spasmodic movements were made in which the animals rubbed their eye and the side of the face.

As consciousness returned the symptoms described above were reversed in chronological order. Animals were kept isolated, during the recovery period, to avoid the excitement following reaction to sounds or touch that resulted in an ataxic animal falling heavily when trying to escape. Recovery time was recorded when cheetahs were able to walk without signs of ataxia or incoordination.

Higher doses of either ketamine hydrochloride or xylazine hydrochloride resulted in increased recovery time and had little practical effect on the induction period.

Failure to immobilize cheetahs effectively following darting was recorded in over 15% of cases. Dart misfires, blocked needles, subcutaneous injection of the drugs and darts that bounced off the animal before the complete drug volume had been delivered caused these failures. After initial dosage studies had been completed a standard dart containing 300 mg Ketamine and 20 mg xylazine was used. Endotracheal intubation was possible in most animals at this dosage level. Larger males were on occasion given halothane with a facial mask to enable intubation to be performed. Hyperthermia (temperatures  $> 41^{\circ}\text{C}$ ) was seen in animals



that were chased for some time prior to darting or that developed convulsions. These animals were cooled by douching with cold water.

Convulsions were seen in approximately 5% of the cheetah males immobilized.

#### CT1341 Anaesthesia

Induction with intravenous CT1341 took place within 5-15 sec. Anaesthesia using 2 mg/kg was characterized by a relaxed state in which the animal could be handled safely but nevertheless responded to electroejaculation with muscular contractions of the hind quarters and occasional cries. Recovery was uneventful with the animal gradually regaining consciousness, attempting to stand and eventually able to walk away. Spasmodic pawing of the side of the face was also seen in these animals at the beginning of the recovery period.

Mean recovery time (n = 27) was  $139,4 \pm 48,7$  min which does not differ significantly from that of cheetahs anaesthetized with the ketamine/xylazine mixture (t = -0,42).

Repeated administration of CT1341 required to keep the animal down for long periods during hormone studies resulted in a prolonged recovery time (6-18 h), which was nevertheless uneventful.

The safety of CT1341 was tested when a cheetah intended for euthanasia because of chronic nephritis (Blood urea 24,6 mmol/l, plasma creatinine 323  $\mu$ mol/l) was given 40 ml of the drug, 16 mg/kg.

A state of deep surgical anaesthesia followed and the animal was still alive 2 h later when it was killed with pentobarbitone sodium (Euthabarb, Centaur Labs, Johannesburg).

One cheetah immobilized with ketamine/ xylazine was given an intravenous injection of 3,5 ml of CT1341, approximately 1 mg/kg, in an attempt to control epileptiform convulsions that had developed. Apnoea followed immediately. Intubation and artificial respiration were successful in keeping the animal alive until spontaneous respiration returned but the animal died 30 min later as a result of hyperthermia. This was the only fatality recorded as a result of drug immobilization during the study.

Thiopentone sodium was used in a small number of animals. The drug is known to cause apnoea in cats (Felis catus) (Booth 1982) and was not used on a routine basis as a result. Recovery after a single injection of 400 mg (10 mg/kg) is rapid and one animal escaped after recovering abruptly. An intravenous injection of 100 mg thiopentone sodium proved useful in controlling epileptiform convulsions in animals immobilized with ketamine and xylazine.

Campbell & Harthoorn (1963) stated the requirements for an effective drug for the capture and control of large wild felids as potency, rapid induction, wide therapeutic index and lack of excitatory effects.

A safe and effective method of chemical restraint with a short recovery period was needed to collect semen by electroejaculation from cheetah males. The animals were confined in enclosures and could not move far after darting. It was possible therefore to



reduce the quantity of capture drug used. As the study progressed it became important to be able to handle a greater number of animals in the time available and later it was necessary to keep cheetah males restrained for periods of up to five hours for serial blood collections. These changing requirements together with the erection of smaller enclosures and the construction of capture crushes resulted in changes in the drugs used for restraining cheetahs.

Phencyclidine hydrochloride has been used in combination with various neuroleptic drugs for the capture and anaesthesia of cheetahs (Griffiths, Haigh & Susan Harthorn 1968; Pienaar, le Riche & le Roux 1969; Ebedes 1970; Seal, Erickson & Mayo 1970; Holmes & Ngethe 1973; Smuts, Bryden, De Vos & Young 1973). There are disadvantages however that ruled out its use in these animals. Anaesthesia is prolonged (Griffiths et al. 1968; Ebedes 1970; Seal et al. 1970; Holmes & Ngethe 1973; Smuts et al. 1973). Severe epileptiform convulsions were described by Delfs (in Ebedes 1970), Ebedes (1970) and Seal et al. (1970) these were often complicated by hyperthermia (Ebedes 1970; Seal et al. 1970) and one animal died as a result of heat stroke (Seal et al. 1970). Smuts et al. (1973) compared the effects of phencyclidine and ketamine using these drugs, in combination with azaperone, in lions, leopards and cheetahs and concluded that ketamine had the following advantages over its more potent congener: muscular hypertonicity was less severe, no convulsions were seen and the recovery period was shorter. A temperature of 38,4 °C was recorded in an immobilized cheetah and it was

concluded that the drug combination used did not interfere with thermoregulation. Smuts et al. (1970) reported the death of a cheetah immobilized with phencyclidine from asphyxiation following regurgitation and aspiration. They suggested that ketamine would be a safer drug to use when animals had eaten as it did not suppress pharyngeal and laryngeal reflexes. The disadvantage in using ketamine as reported by Smuts et al. (1973) was the larger volume of drug required.

Both phencyclidine and ketamine disrupt central nervous system function by interfering with monoamine neurotransmission (Roberts 1968, in Booth 1982) as a result the thalamoneocortical system is depressed and the limbic system activated (Booth 1982). This produces a state characterized as dissociative anaesthesia by Corssen & Domino (1966, in Smuts et al. 1973). The catalepsy, muscular hypertonicity and epileptic seizures that result from these effects (Chen, Ensor, Russel & Bohner 1959) can be suppressed by the simultaneous administration of various neuroleptic drugs. Acetyl promazine was used by Young (1966) and Seager (1976), azaperone (Pienaar et al. 1969; Smuts et al. 1973) chlorpromazine (Campbell & Harthoorn 1963), diazepam (Reid & Frank, in Booth 1982), promazine (Chen et al. 1959; Seal et al. 1970), triflupromazine (Ebedes 1970) and xylazine (Amend, Klavano & Stone 1972; Sanford & Colby 1980; Clark, Martin & Short 1982; Bush, Custer, Smeller, Bush, Seal & Barton 1978; Watermann 1984).



Smuts et al. (1973) reported using 10,6 mg/kg ketamine hydrochloride together with 20 mg azaperone in a cheetah. The same animal was immobilized later with 6,3 mg/kg ketamine on its own. Induction times did not differ appreciably on each of the two occasions but the animal recovered in less time when the lower dosage was used (90 min compared to 270 min). The drug was effective for the capture and handling of cheetahs at this dosage. Higher dosages are used in cats (F.catus): Commons (1970) 20-40 mg/kg; Amend et al. (1972) 11-22 mg/kg (after premedication with 0,25-2,0 mg/kg xylazine); Haskins, Peiffer & Stowe (1975) 35 mg/kg; De Young et al. (in Booth, 1982) 11-33 mg/kg.

The three cheetahs immobilized at the beginning of this study went through all the stages of induction as described by Smuts et al. (1973) confirming their description of the changes that occur. However the muscular hypertonicity together with epileptic convulsions that took place in two of the animals were regarded as unacceptable and the inclusion of a tranquilizer was indicated. Insufficient evidence was available to show whether the hyperthermia reported in cheetahs by Ebedes (1970) and Seal et al. (1970) resulted as a direct effect of phencyclidine itself or as a result of the convulsions, which were severe. Both authors used neuroleptics derived from phenothiazine, triflupromazine (Ebedes 1970) and promazine (Seal et al., 1970) which have been reported to cause hyperthermia in other animals (Booth 1982). Seager (1976) blamed acetyl promazine for his failure to collect semen from a cheetah by electroejaculation.

Xylazine hydrochloride was included in the drug mixture darted. It was reported to reduce muscular hypertonicity when used in combination with ketamine hydrochloride in (F. catus) (Amend et al. 1972; Haskins et al. 1975; Sanford & Colby 1980; Clark et al. 1982), it lowers the dose of ketamine hydrochloride and is miscible with it in the same syringe (Amend et al. 1972).

The dosage of ketamine hydrochloride used by Smuts et al. (1973) was used as a guideline in this study. The availability of fluothane anaesthesia made it possible to reduce the dosages of both drugs used and thus the recovery time. 6-8 mg/kg of ketamine hydrochloride together with 0,5-0,8 xylazine hydrochloride was sufficient for the purpose. Increasing the quantity of either of the two drugs lengthened the time taken for the animals to recover. The capture of cheetah males free in their enclosures was time consuming. Animals soon became aware of the routine and were often difficult to dart. Despite the conclusion by Smuts et al. (1973) that ketamine could be used with relative safety in animals that had eaten, aspiration pneumonia following ketamine anaesthesia has been reported in man (Sears, 1971, Penrose 1972, and Sussman 1974, in Haskins et al. 1975). It was therefore considered necessary to starve the cheetahs for 12 h prior to anaesthesia. Small holding enclosures were erected adjoining the male enclosures. Animals were coaxed or chased into these where they could be starved and were darted with ease. The capture of cheetah males in crushes attached to these enclosures made it possible to use intravenous anaesthesia.



Young (1966) used pentobarbitone sodium for anaesthesia in cheetahs but reported recovery periods in excess of 12 h. Thiopentone sodium has a shorter acting anaesthetic effect (Booth 1982) but has to a large extent been replaced by CT1341 for the anaesthesia of cats (F. catus). It is safer, does not cause perivenous tissue damage when injected subcutaneously and apnoea, commonly seen when thiopentone is used in cats, does not occur (Child, Currie, Davis, Dodds, Pearce & Twissell, 1971; Davis & Pearce 1972; Evans, Aspinall & Hendy 1972). CT1341 consists of two pregnanedione steroids: alphaxalone and alphadolone in solution in polyethylated castor oil (Child et al. 1971). The concentration of alphaxalone is 9 mg/ml and that of alphadolone 3 mg/ml in the product supplied. Alphadolone a 21-acetoxy derivative of alphaxalone:  $3\alpha$ -hydroxy- $5\alpha$ -pregnane-11,20-dione, has half the activity of alphaxalone but is included in the mixture as it increases the solubility of the latter in the vehicle used (Davis & Pearce, 1972). Dosages reported refer to the two drugs which together make up 12 mg/ml in the anaesthetic solution. An intravenous injection of 1,2 mg/kg CT1341 results in the immediate loss of consciousness in a cat (Child et al. 1971) The drug has no analgesic effect but anaesthesia results from a general depression of the central nervous system (Haskins et al. 1975; Booth 1982). Recovery is rapid. Animals given 1,2 mg/kg were able to right themselves after 6-9 min, after 19,2 mg/kg recovery took two hours (Child et al. 1971). Evans, Krahwinkel & Sawyer (1972, in Booth 1982) recommend 7,5-9 mg/kg for minor  
ation, and the eyelids were closed.

surgical procedures. Additional injections of CT1341 may be given as it does not have a cumulative effect (Child et al. 1971; Davis & Pearce 1972; Hall 1976).

CT1341 has a high therapeutic index calculated in mice to be 30,6 by Child et al. (1971) who report indices for thiopentone and ketamine of 6,9 and 8,5 respectively in mice. These indices were however not regarded by Davis & Pearce 1972 as applicable to larger animals. Dodds (1972 in Gordh, 1972) stated that five 'doubling doses' of the minimum anaesthetic dose of both CT1341 and ketamine were needed to cause fatalities in cats (F.catus).

CT1341 is compatible with neuroleptic drugs and gas anaesthetic agents. It should not however be used in conjunction with barbiturates (Tavernor 1977, in Booth 1982). The polyethylated castor oil vehicle causes histamine release in dogs (Canis familiaris) (Child et al. 1971, Stocks 1973) and the drug is not recommended for use in these animals. Child et al. (1972) and Haskins et al. (1975) describe sneezing, pawing at the face and ears and hyperaemia of the skin of the nose in some cats anaesthetized with CT1341. They ascribe these reactions to histamine release. Marshall (1972) and Edmonds (1973) reported finding atrial and coronary artery thrombi in two cats that died while anaesthetized with CT1341.

Haskins et al. (1975) reviewed the literature dealing with the use of ketamine, xylazine and CT1341 and compared the effects of these drugs in cats. They reported that CT1341 anaesthetized animals made a faster recovery, had better muscular relaxation, and the eyelids were closed.



Both methods of immobilization proved effective and safe in cheetahs. Anaesthesia was not produced at the dosage levels used but animals could be handled and electroejaculated. Recovery time was relatively short.

CT1341 was used in preference to the ketamine xylazine mixture. The induction period was shorter, immobilized animals were relaxed, the eyes were closed and less excitement was seen during recovery as reported by Haskins et al. (1975) in cats. The danger of eye injury was high in animals immobilized with ketamine and xylazine because of the loss of palpebral reflexes and the open mydriatic eyes could conceivably be injured by direct sunlight. It was not possible to protect the eyes effectively as the animals shook off any shielding that was applied during the recovery stage. The rapid induction, when CT1341 was administered, reduced the time taken to examine each animal and, as a result, it was possible to immobilize and electroejaculate a male cheetah within 15 minutes. In contrast induction after the intramuscular administration of ketamine and xylazine was prolonged.

Greater quantities of either of the two drug combinations produce anaesthesia in cheetahs. The inclusion of xylazine hydrochloride together with ketamine reduced muscular hypertonicity and the incidence of epileptiform convulsions but the recovery time was increased as a result of its inclusion in the drug mixture administered. Thiopentone sodium in small quantities is more effective for this purpose and does not have effects on the recovery period when used in this way. The results of this study

indicate that recovery following the use of CT1341 is longer in cheetahs than times recorded in cats ( Child et al. 1971; Davis & Pearce 1972). The repeated administration of small quantities of the drug needed to prolong immobilization during hormone studies resulted in a protracted recovery which was characterized by a state akin to a relaxed sleep. No deleterious side effects were seen using CT1341 in cheetahs. Pawing of the face described in cats by Child et al. (1972) and Haskins et al. (1975) was seen when either ketamine/xylazine or CT1341 was used. Hyperaemia of the skin as described by these authors did not occur. This response seen during the recovery stage in cheetahs appears to originate in the central nervous system and no evidence of a histamine release was present. No fatalities were recorded after an estimated > 750 immobilizations performed using CT1341 alone. This drug should not however be used together with ketamine hydrochloride.

#### Semen collection & evaluation

The voltage, current form and frequency delivered by the electroejaculator was measured galvanometrically during the electroejaculation of three cheetah males and is illustrated in Figure 3.



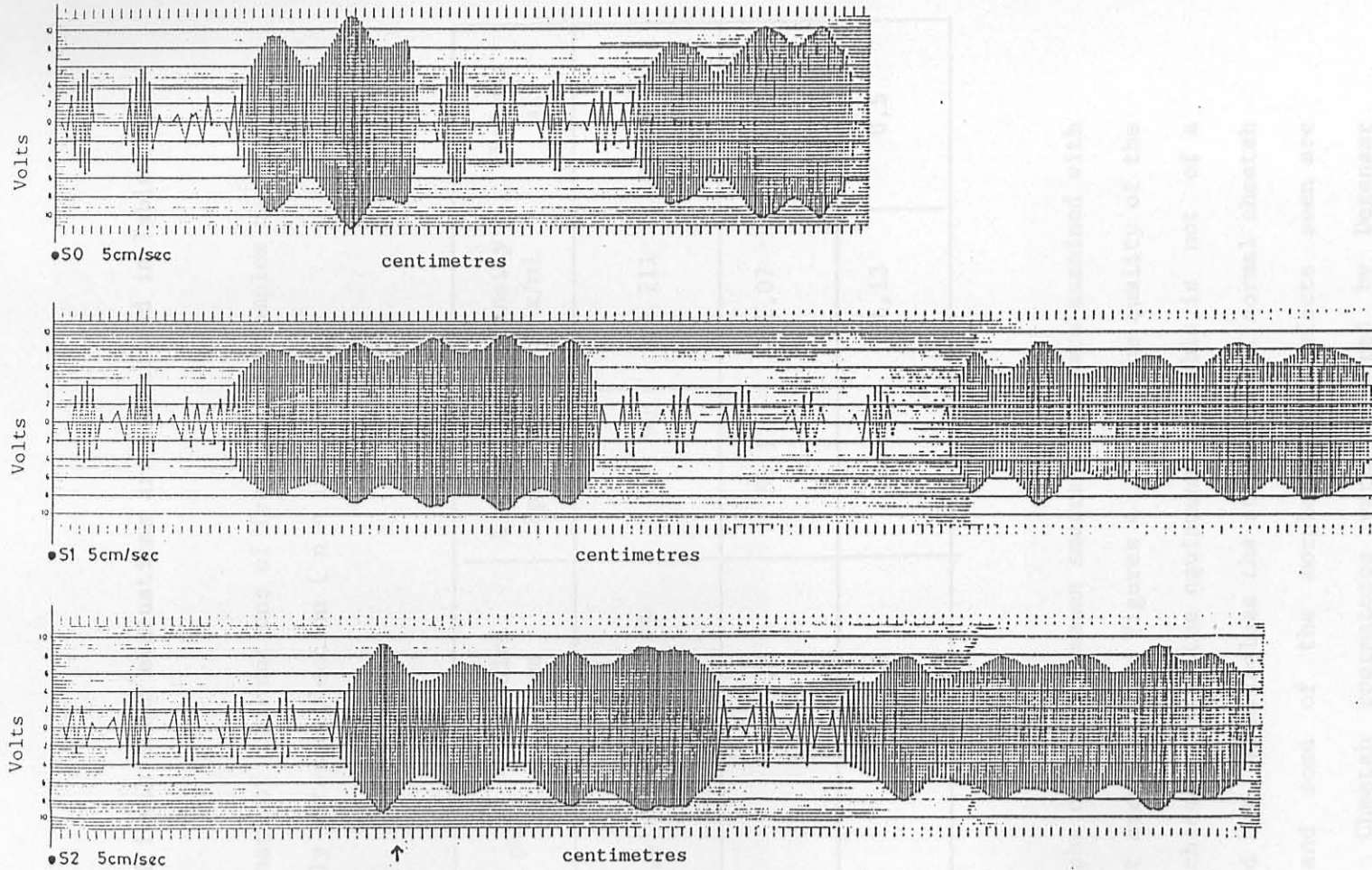


Figure 3: Ejaculatory stimulus.

A series of three stimuli are shown (S0, S1 & S2). Ejaculation at (↑)

The current measured at the output electrodes of the dynamo was 1 - 1,5 amps. Current was not recorded during electroejaculation as it varied considerably within and between animals.

The results of fresh semen evaluations are summarized in Table 3.

Table 3: Summary of examinations of fresh semen samples carried out immediately after collection ( n = 160).

	pH	% Live Sperm	% Motile Sperm	Density $10^6$ x/ml	Volume ml
Range	6,4 - 8	10 - 95	8 - 90	1 - 211	0,3 - 2,1
Mean		65,2	58,1	32,07	0,7
Std		18,5	18,6	36,13	0,5

Photomicrographs of stained semen smears and smears examined with phase contrast are shown in Figures 4 to 9. The quality of the photographs achieved with the equipment available is not of a high standard but nevertheless the structure of normal cheetah spermatozoa and some of the morphological defects seen are demonstrated. Cheetah spermatozoa were measured by Degenaar (1977).

Figure 6. 'Bag' defects with an abnormal head (a) and a normal head (b).



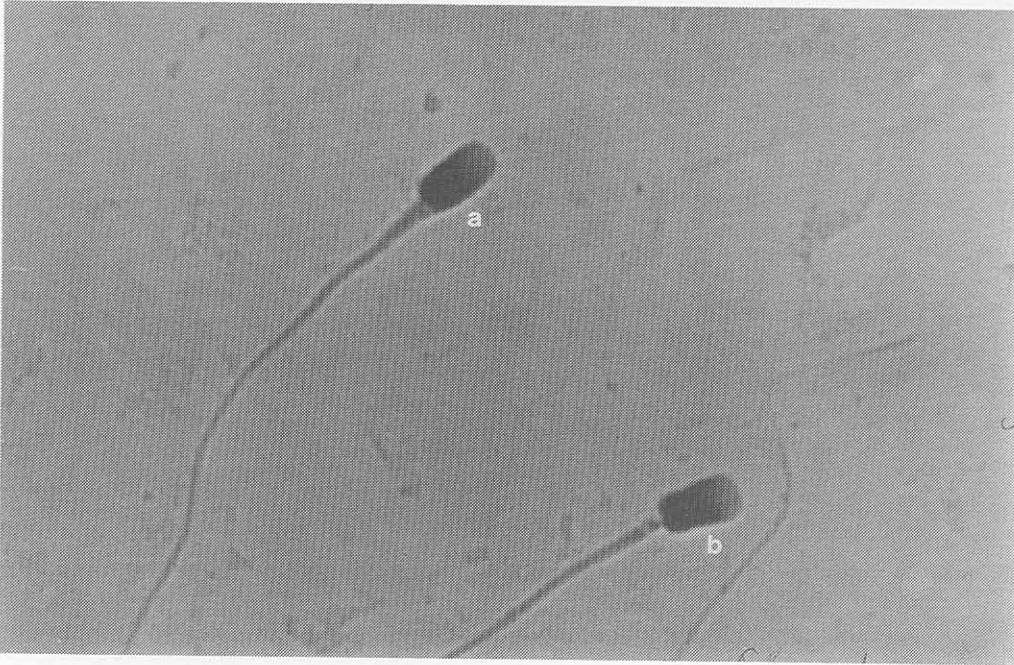


Figure 4. Normal spermatozoa (a); abnormal head and midpiece (B).

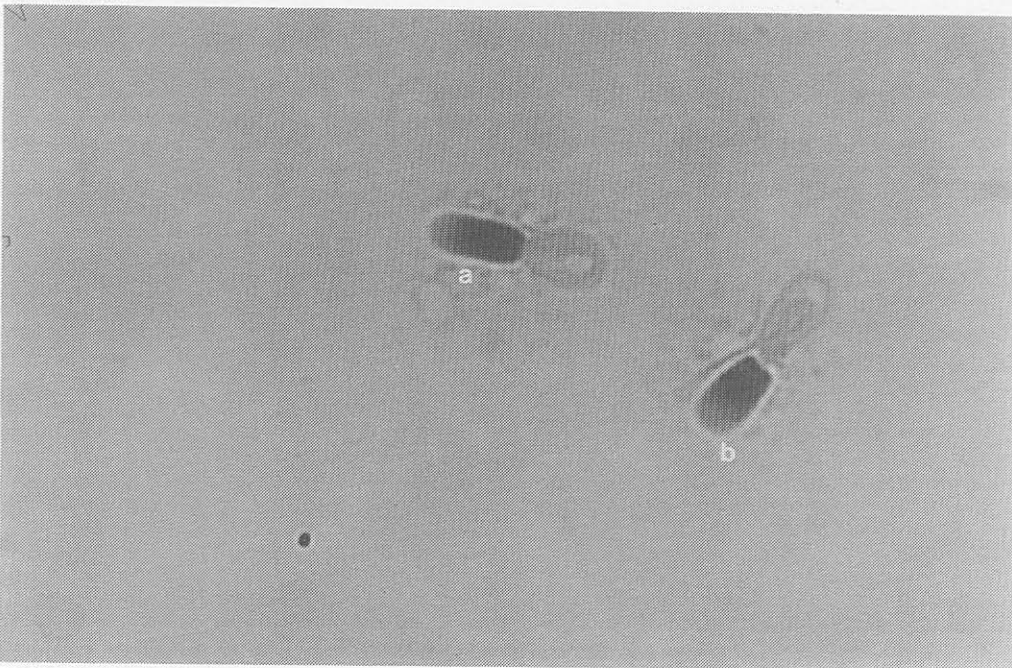


Figure 5. 'Dag' defects with an abnormal head (a) and a normal head (b).





Figure 8

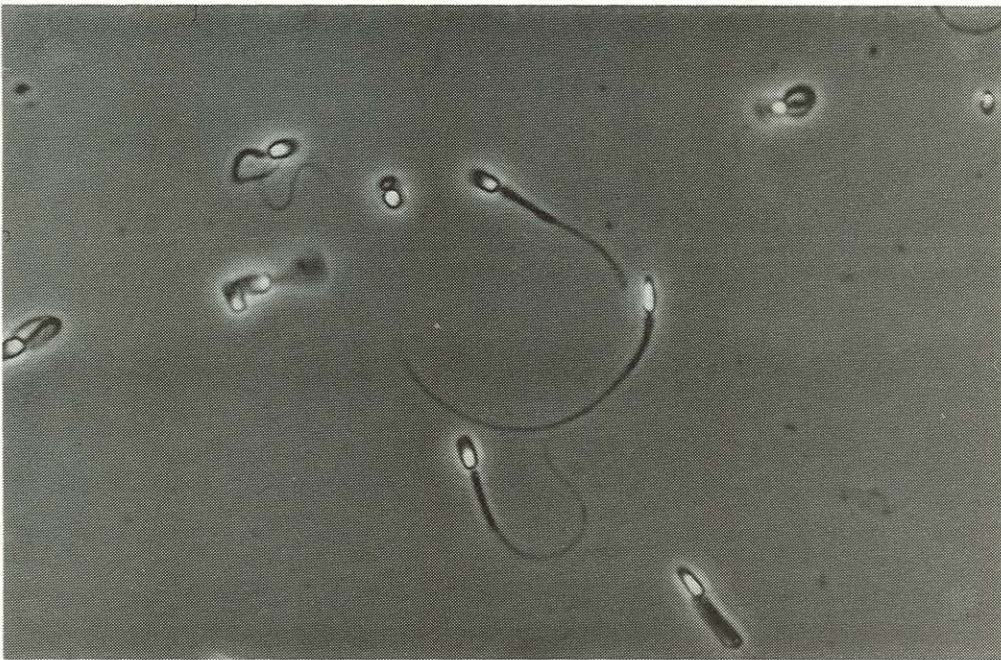


Figure 9

Figures 8/9. Phase contrasts showing 'Dag' defects and coiled tails.



The results of spermograms performed on stained smears or smears examined using phase contrast are summarized in Table 4.

Table 4: Summary of spermogram results ( n = 160)

	% Normal Spermatozoa	% Major Abnormalities
Range	0 - 86	4 - 90
Mean	40,3	26,2
Std	17,5	18,0

A summary of spermogram results from males that sired cubs is presented in Table 5 and shown in Figure 10.

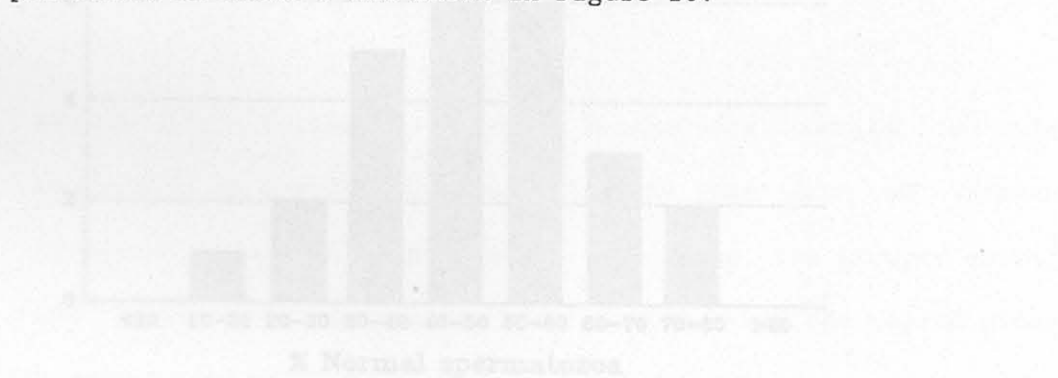


Figure 10. Frequency distribution of the percentage normal sperm counts from seven males that sired 28 litters.

Table 5: Summary of spermograms from seven cheetah males that sired 28 litters between 1975 and 1980. ( n = 63)

	% Normal Spermatozoa	% Major Abnormalities
Range	18 - 80	8 - 42
Mean	47,6	23,6
Std	16,9	8,8

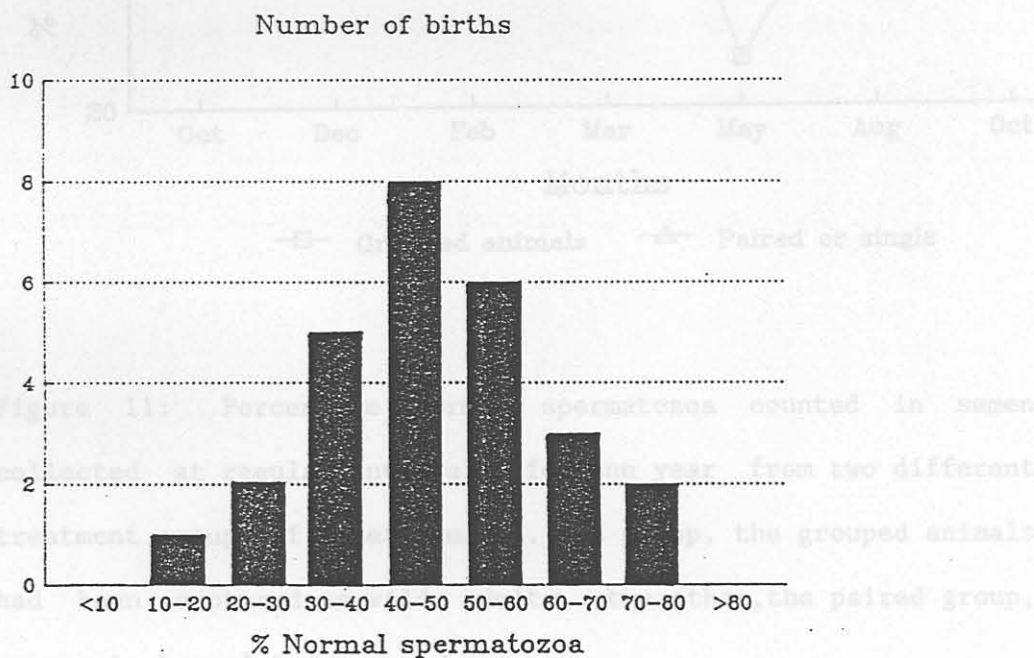


Figure 10. Frequency distribution of the percentage normal sperm counts from seven males that sired 28 litters.

(1976). The head of cheetah spermatozoa in planar view has a compressed elliptical outline while in sagittal section the head is distinctly pear-shaped being considerably broader at the basal



The percentage normal spermatozoa counted in semen samples from 16 cheetah males at regular intervals for one year are shown in Figure 11.

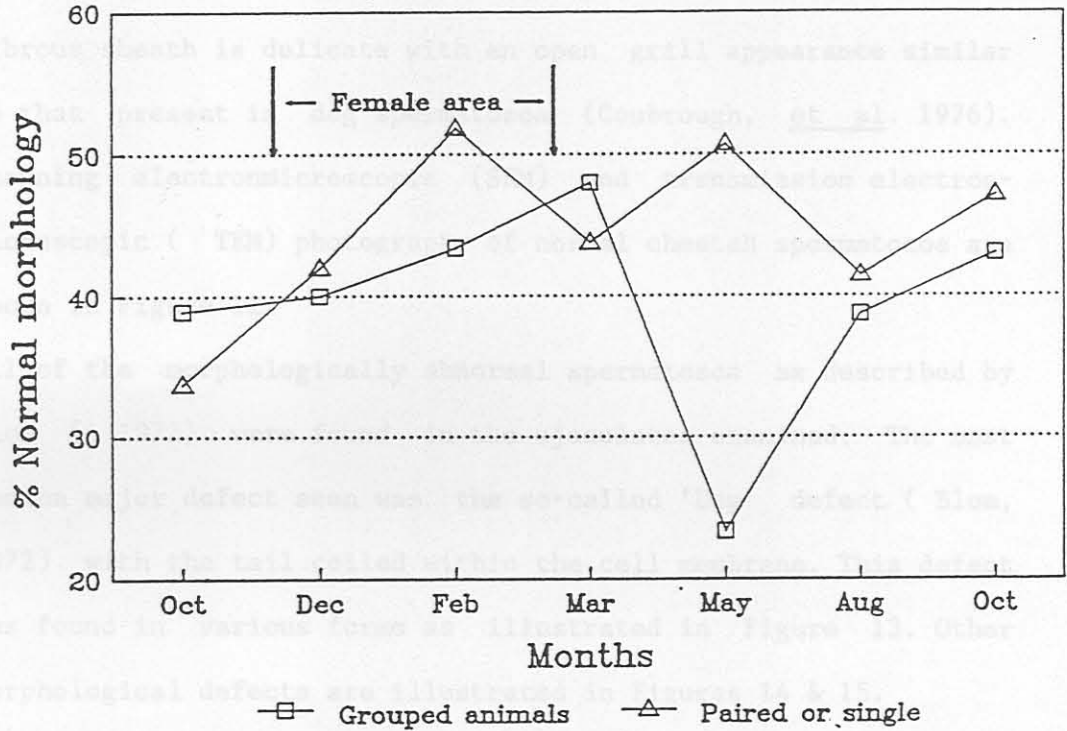


Figure 11: Percentage normal spermatozoa counted in semen collected at regular intervals for one year from two different treatment groups of cheetah males. One group, the grouped animals had been captured as wild adults, the other, the paired group, appear to be related.

The ultrastructural characteristics of normal cheetah spermatozoa were described by Coubrough, Bertschinger, Soley & Meltzer (1976). The head of cheetah spermatozoa in planar view has a compressed elliptical outline while in sagittal section the head is distinctly pear-shaped being considerably broader at the basal

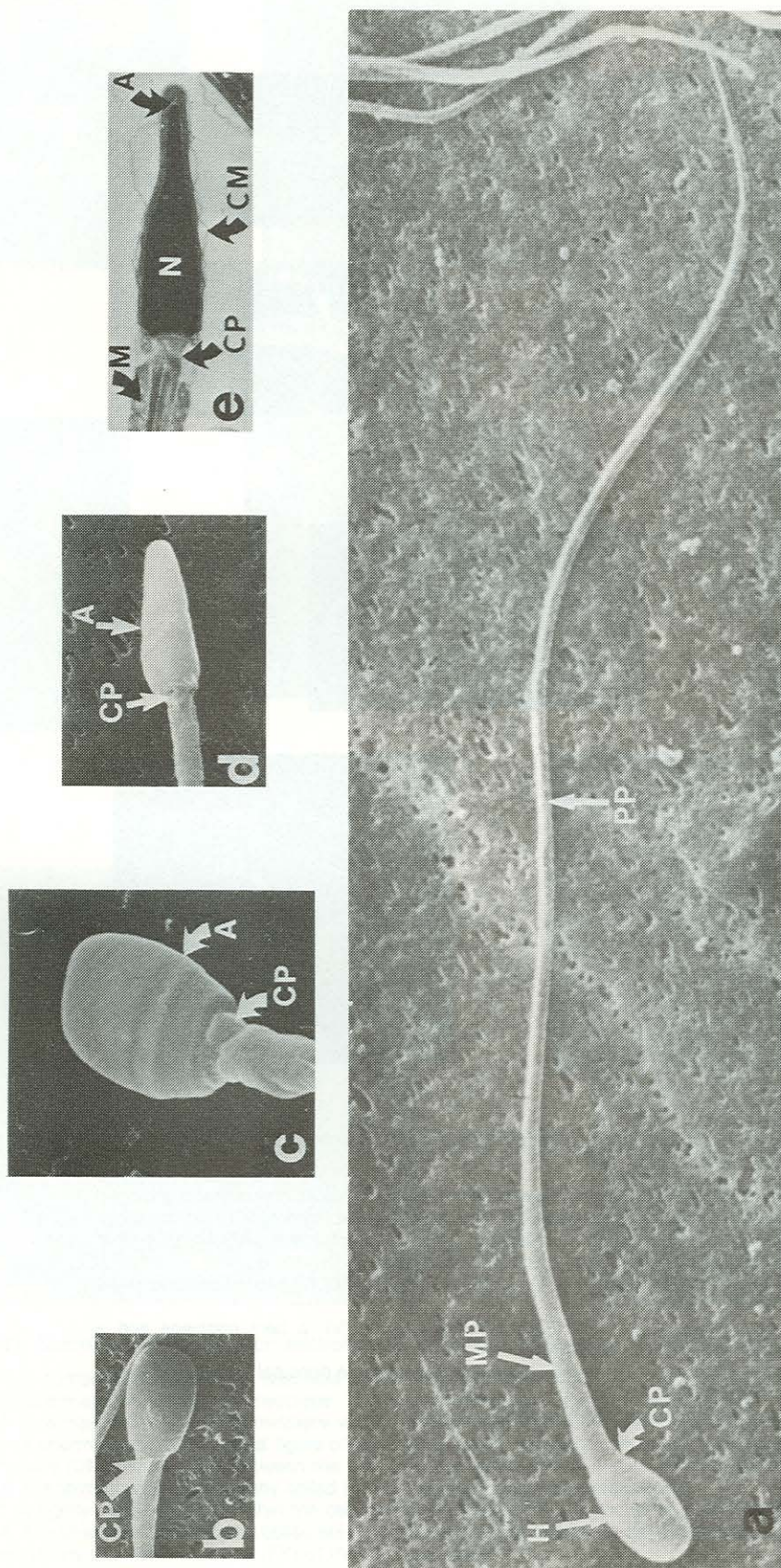
plate. The mitochondrial sheath has a pars spiralis as well as a pars ascendens which almost extends up to the redundant nuclear membrane folds. Capetulum and connecting piece are similar to those seen in other species. No distinct annulus was seen. The fibrous sheath is delicate with an open grill appearance similar to that present in dog spermatozoa (Coubrough, et al. 1976). Scanning electronmicroscopic (SEM) and transmission electronmicroscopic (TEM) photographs of normal cheetah spermatozoa are shown in Figure 12.

All of the morphologically abnormal spermatozoa as described by Blom (1972) were found in the ejaculates examined. The most common major defect seen was the so-called 'Dag' defect (Blom, 1972) with the tail coiled within the cell membrane. This defect was found in various forms as illustrated in Figure 13. Other morphological defects are illustrated in Figures 14 & 15.



(a) SEM. A frontal view of the spermatozoon showing the capetulum (CA) and the connecting piece (CP).  
(b) SEM. A side view of a spermatozoon showing the capetulum (CA) and the connecting piece (CP).  
(c) TEM. A longitudinal section of a spermatozoon showing the nucleus (N), mitochondria (M), and the connecting piece (CP).

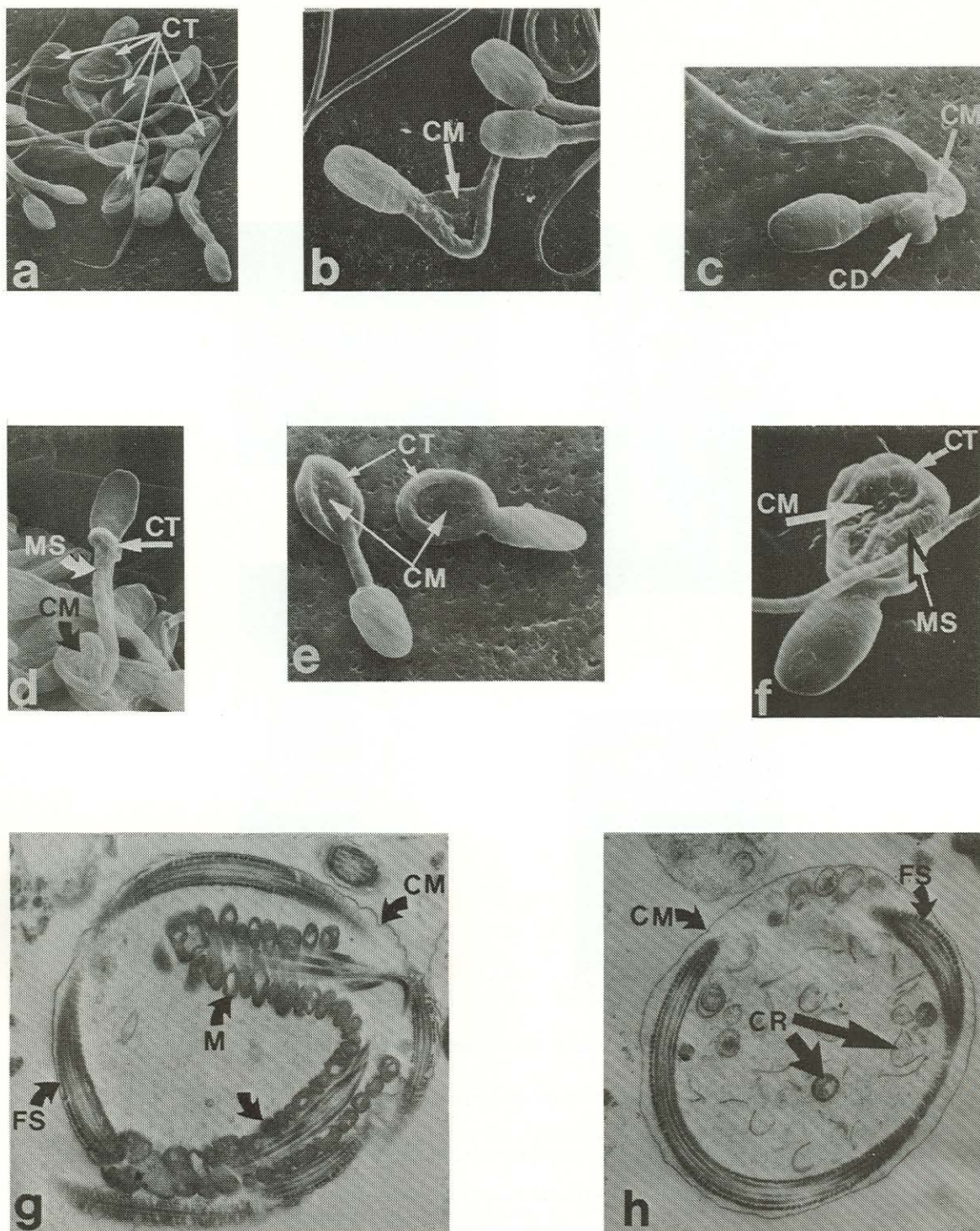




**Figure 12.** Scanning and transmission electronmicroscopy of a normal spermatozoa.

- (a) SEM. A normal spermatozoa showing the head (H), mid-piece (MP) and principal piece (PP).
- (b) SEM. A planar view of a normal head. The connecting piece (CP) is seen.
- (c) SEM. A planar view of the head with the posterior border of the acrosome (A) and the connecting piece (CP) identified.
- (d) SEM. A side view of a sperm head with the posterior border of the acrosome (A) and the connecting piece (CP).
- (e) TEM. A sagittal section of a spermatozoa showing the nucleus (N), acrosome (A), cell membrane (CM), connecting piece (CP) and mitochondria (M).

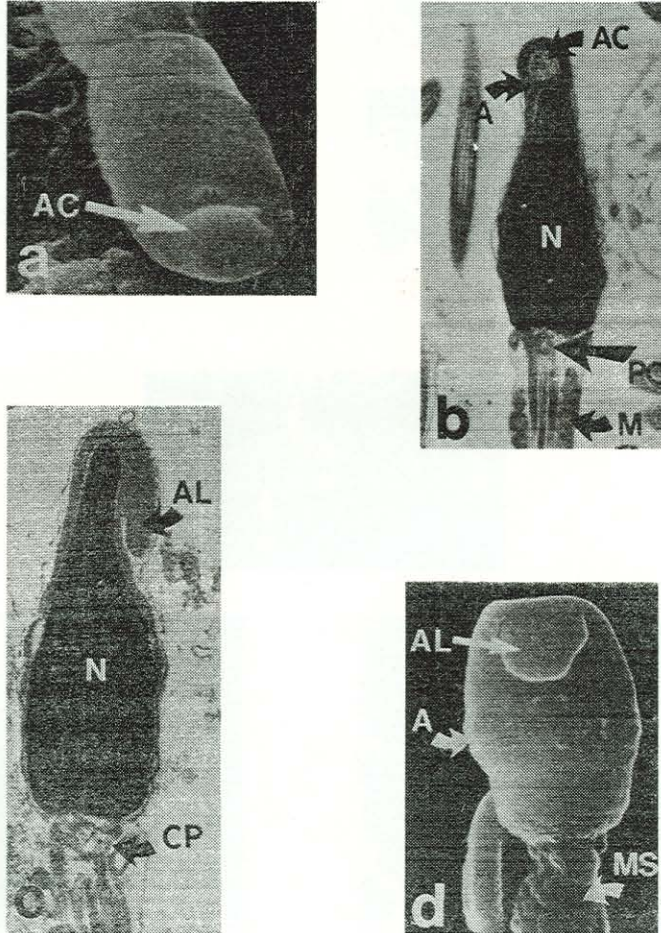




**Figure 13.** Scanning and transmission electromicroscopy of various forms of the 'DAG' defect.

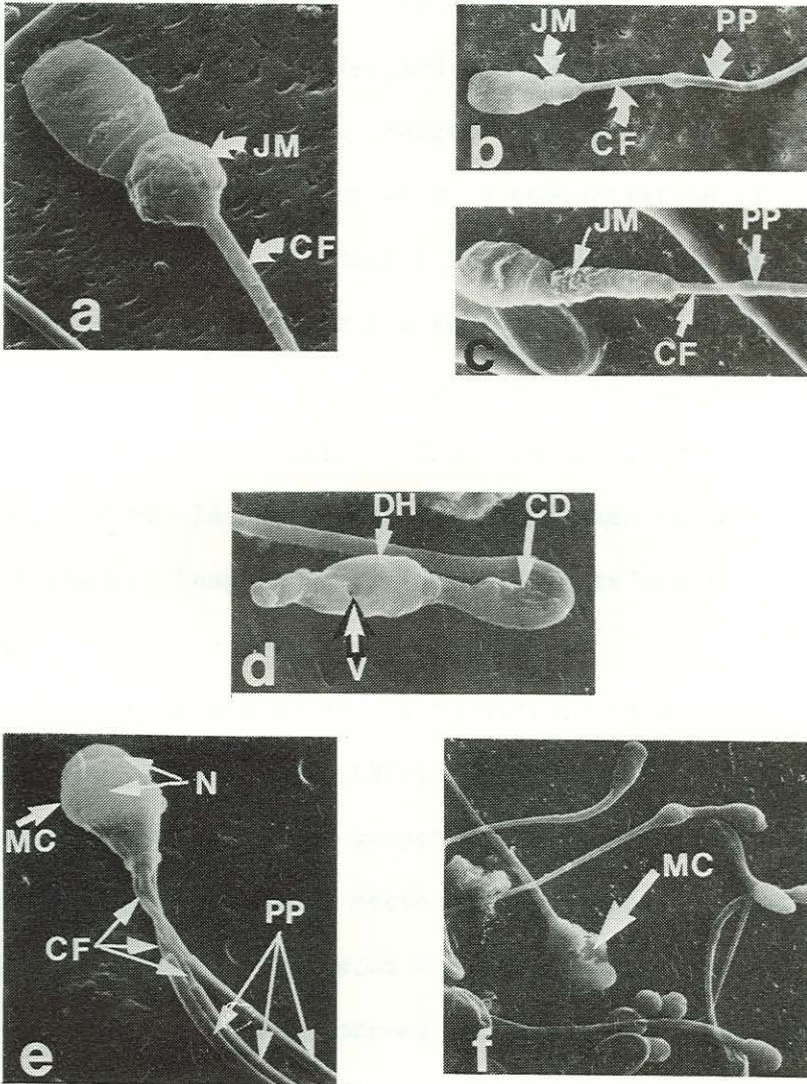
- (a) SEM. A number of spermatozoa with tails coiled within the cell membrane.
- (b) SEM. Spermatozoa with a bent midpiece. The cell membrane is visible in the bend (CM).
- (c) SEM. Spermatozoa with a bent midpiece within the cell membrane (CM) and a persistent cytoplasmic droplet (CB).
- (d) SEM. A spermatozoa with a typical figure of eight 'DAG' defect. Coiling of the tail (CT) around the connecting piece, the cell membrane (CM) can be seen between the coils and the mitochondrial sheath (MS) is visible.
- (e) SEM. A spermatozoa with a tightly coiled tail (CT) within the cell membrane (CM).
- (f) SEM. A tightly coiled tail (CT) within the cell membrane (CM). The mitochondrial sheath (MS) is visible.
- (g) TEM. A cranial section through a coiled tail (CT) showing coiling within the cell membrane (CM), mitochondria of the mid-piece (M) and the fibrous sheath (FS) of the principal piece.
- (h) TEM. Caudal section through a coiled tail showing the cell membrane (CM), fibrous sheath of the principal piece (FS) and cytoplasmic remnants (CR).





**Figure 14.** Scanning and transmission electromicroscopy of acrosomal defects.

- (a) SEM. An acrosomal cyst (AC) also referred to as 'Knobbed sperm'.
- (b) TEM. A sagittal view of a spermatozoa with an acrosomal cyst (AC). The nucleus (N), proximal centriole (PC) and mitochondria (M) are identified.
- (c) TEM. Acrosomal lipping (AL), with the nucleus (N) and the connecting piece (CP) identified.
- (d) SEM. Acrosomal lipping (AL), the acrosome (A) and the mitochondrial sheath (MS).



**Figure 15.** Scanning electronmicroscopy various major defects seen in cheetah spermatozoa.

- (a) Midpiece abnormality in which the mitochondria are displaced cranially and arranged in a jumbled manner at the neck (JM). The bared central filament (CF) can be seen.
- (b) A defect similar to that shown in (a) with jumbled mitochondria (JM), bared central filament (CF) and the principal piece (PP) identified.
- (c) Partially bared central filament (CF), jumbled mitochondria (JM) and the principal piece (PP).
- (d) View of a degenerated head (DH) with vacuolation (V), a bent midpiece and a persistent cytoplasmic droplet (CD).
- (e) Macrocephalic multinucleate (N) spermatozoa with three principal pieces (PP) and a bared midpiece (CF).
- (f) Macrocephalic multinucleate head (MC).



Sadleir (1966) attempted semen collection using electroejaculation in a male leopard (Panthera pardus) which was later found to be infertile due to epididymal occlusion. A semen sample was obtained from a snow leopard (Panthera uncia) by Mayo (1967) using electroejaculation. Seager (1976) electroejaculated two cheetahs and obtained semen with a concentration of < 1 million spermatozoa per ml. Degenaar (1977) electroejaculated 3 cheetah males on several occasions and reported obtaining semen volumes of 0 - 1 ml with spermatozoal concentrations too low to be accurately counted. Ball (1976) reviewed the history and the development of electroejaculation as a means of collecting semen from animals. Seager (1976) described its use in cats and wild felids.

The process of ejaculation can be divided into an emission and an ejaculation phase (Ball, 1976; Benson & McConnell, 1983). The former as the result of sympathetic nerve stimuli reaching the tract via the hypogastric nerve and the latter under the control of the parasympathetic system through innervation from the pelvic and internal pudendal nerves (Gomes, 1978). Emission, the deposition of seminal fluid in the posterior urethra, is accompanied by bladder neck closure which is also a response to adrenergic stimuli. Ejaculation takes place as a result of rhythmic contractions of the periurethral and anal sphincter muscles (Benson & McConnell, 1983).

Adrenergic drugs facilitate emission and bladder neck closure (Benson & McConnell, 1983). Seager (1976) found that cats anaesthetized with a drug combination of ketamine and acetyl promazine

urinated when electroejaculation was attempted. Phenothiazine derivatives used as neuroleptics have been reported to result in a decreased volume of ejaculate or to suppress ejaculation (Martin, 1978). Meltzer, Van Vuuren & Bornman (1987) were unable to obtain semen from chacma baboons (Papio ursinus) immobilized with ketamine and azaperone. Derivatives of both phenothiazine (acetyl promazine) and butyrophenone (azaperone), produce their effects through an adrenergic blockade (Booth, 1982) and thus appear to interfere with emission and bladder neck closure. The initial use of ketamine and xylazine for the immobilization of cheetah males in this study was fortuitous. Xylazine is an alpha-adrenergic agonist (Booth, 1982) and is thus more likely to facilitate effective ejaculation than to hinder it. CT1341 did not appear to influence the ejaculatory response of cheetah males to electrical stimulation in any way.

The characteristics of the electrical stimulus that results in ejaculation have been described (Furman, Ball & Seidel, 1975; Ball, 1976). A sine wave alternating current is the most effective. While the current flow produced by the equipment used is the critical factor in producing ejaculation, sufficient voltage is needed to ensure that tissue resistance is overcome and current flow occurs (Dalziel & Phillips, 1948 in Ball 1976). Furman et al. (1975) found that there was a considerable variation in the resistance to current flow within and amongst bulls (Bos taurus) that they examined and therefore did not measure the current required to elicit electroejaculation. The frequency of the current applied influences the degree of



muscular contraction and discomfort that follow as a side effect of the electrical stimulation. Frequencies of 50-80 Hz caused less unwanted responses than those evinced by lower frequencies (Furman et al. 1975). Muscular contractions are less severe when longitudinal electrodes are used (Ball & Furman 1972, in Ball 1976).

Most investigators use equipment that produces a voltage of up to 40 V for the electroejaculation of domestic animals (Furman et al. 1975). Mayo (1967) used equipment that could produce a voltage of up to 12 V, but did not measure the voltage used to obtain semen from the snow leopard (P. uncia) he examined. Domestic cats (F. catus) were electroejaculated by Platz & Seager (1978) using 2-8 V.

Standard equipment used for the electroejaculation of rams (Ovis aries) (Van Tonder et al. 1973) was used during this study as no external power source was required. The voltage produced was within the range used by Mayo (1967), Platz & Seager (1978) and, in a concurrent study, by Wildt, Bush, Howard, O'Brien, Meltzer, Van Dyk, Ebedes & Brand (1983). Changes in the type of electrode resulted in less muscular stimulation. The use of ring electrodes resulted in seminal fractions being ejaculated at different times as the electrode was moved within the rectum. This observation is similar to that of Furman et al. (1975) who reported obtaining different seminal fractions from bulls when ring electrodes were used. As these fractions were not of consequence to the

purpose of the study they were not evaluated separately. However fractionation of semen could influence the density of semen samples obtained from individual animals.

The quality of semen collected by electroejaculation has been found to be variable (Lawson, Krise & Sorenson 1967, in Watson, 1978; Roth & Smidt 1970, in Watson 1978). The concentration of spermatozoa in bull semen collected by electroejaculation has been shown to be lower than that of samples obtained using an artificial vagina (Dziuk, Graham, Donker, Marion & Peterson 1954; Austin, Hupp & Murphee, 1961). The volume collected by electroejaculation, however, was greater and thus no significant difference in the total number of sperm per ejaculate was obtained when the two methods were compared (Austin et al. 1961). Platz & Seager (1978) reported similar observations in cats.

Fresh semen samples evaluated immediately after collection were classified as good, fair or poor after assessing the sperm concentration, % live, % motile, the rate of forward progression and spermatozoal morphology. Accurate counts to determine sperm concentration and the percentage morphologically normal spermatozoa were performed later in the laboratory. All of these parameters were taken into account in arriving at the assessed quality of the semen sample. However, because of the possible of variability in sperm concentration in semen collected by electroejaculation semen motility and spermatozoal morphology were weighted higher in the final assessment of the quality of semen obtained.



There is lack of agreement in the literature as to the correlation between semen evaluation methods and fertility (Watson, 1978; Bartoov, Eltes, Weissenberg & Lunenfeld, 1980; Wildt et al. 1983). Blom (1972) reported six major sperm defects that had been shown to be associated with either decreased fertility or sterility in bulls.

Conception rates after artificial insemination, in cattle, were found to decrease as the proportion of spermatozoal abnormalities rose or when the percentage of live sperm fell below 70 % in the semen used (Munro, 1961). The motility score, a measure of individual sperm activity, was reported as a more reliable estimate of fertility by some (Clarke, O'Neill, Hewetson & Thompson 1973; Linford, Glover, Bishop & Stewart 1976). Carroll, Ball & Scott (1973) rated sperm motility higher than morphology in assessing the fertility of over ten thousand bulls. The proportion of abnormal sperm in ejaculates has been related to infertility in man (Chandley, Edmond, Christie, Gowans, Fletcher, Frankiewicz & Newton, 1975), the bull (B. taurus) (Blom, 1972; Chenoweth & Ball, 1980), the ram (O. aries) (Rhodes, 1980), the boar (Sus scrofa) (Gibson & Johnson, 1980), and the dog (Canis familiaris) (Larson, 1980).

Wildt et al. (1983) proposed chronic stress or the markedly low genetic variation found in cheetahs (O'Brien, Wildt, Goldman, Merrill & Bush, 1983) as possible causes for the large proportion of abnormal spermatozoa found in semen from cheetah males and cited several authors who had shown that inbreeding adversely affected semen morphology. Wildt et al. (1986) found no difference

in the proportion of abnormal spermatozoa in semen from captive North American cheetahs and semen collected from wild cheetahs in the Serengeti National Park. The captive population, comprising 23 animals in zoos had a mean of 70,6 % morphologically abnormal spermatozoa as opposed to the 75,9 % found in 8 wild cheetahs. The percentage abnormal spermatozoa reported by Wildt et al. (1983) and Wildt et al. (1986) cannot be compared with those of the present study. Wildt et al. (1983 & 1986) classified spermatozoal abnormalities as primary and secondary. The former as the result of defective spermatogenesis and the latter as a result of defective maturation of spermatozoa in the duct system. The justification for this approach was questioned by Blom (1972) who had originally proposed it (Blom, 1948, in Blom 1972). He pointed out that new evidence indicated that some so-called secondary abnormalities in fact originated during spermatogenesis and also that secondary abnormalities could be of significance in their influence on fertility when their incidence was in excess of 10 - 15 %. He proposed a new approach in which morphological abnormalities that had been shown to affect fertility were classified as major and were counted together. Minor abnormalities were counted separately and only considered as significant when they exceeded 10-15 % of the total count. The abnormality counts of Wildt et al. (1983, 1986) tend to be misleading as all abnormalities are considered together in their conclusions. This practice has resulted in high abnormality counts even in fertile bulls ( Blom, 1972). Further confusion results from their classification of 'coiled flagellum' as a



primary defect. This defect has been shown to develop during passage through the caput epididymis (Blom, 1972).

The breeding results achieved at the De Wildt centre and the cheetah population explosion that was seen at the Suikerbosrand Nature Reserve (Pettifer, De Wet & Muller, 1979 & 1980) appear to belie suggestions, made by Wildt et al. (1983, 1986), that the cheetah is endangered as a result of poor reproductive ability. Low proportions of normal spermatozoa and relatively high numbers of morphologically abnormal spermatozoal were found in some of the nevertheless successful cheetah males at the Centre.

Priority was given to the production of cheetahs in large numbers at the Centre and only males with the best semen quality were used for breeding purposes. This precluded the use of males regarded as sub-fertile in the breeding program as a possible further means of assessing the relevance of the methods applied in evaluating fertility.

The temporal characteristics of spermograms from cheetah males that had either been kept as small groups from 4-6 months of age or had been captured as pairs differed from those of the group of 12 animals. The latter had been removed from the enclosure, where they spent most of the year, and used in the passage between cheetah female enclosures from November to March 1975 - 1976. Fighting amongst them was not uncommon during that time and after their return to their holding enclosure. The former animals were kept, relatively undisturbed, out of the area until required for breeding, and being habituated to one another they did not compete with their companions.

There is evidence that indicates that adult male cheetahs found in their natural environment, in small groups, are related (Eaton, 1974). The presence of females has been shown to affect semen quality (Furman et al. 1975). A seasonal variation in semen quality has been described in many species (Lincoln, 1981). It is possible that stress, as a result of the changed environment and aggression (Wildt et al. 1983), together with the fact that they were removed from the presence of the females, could have influenced spermatogenesis in the grouped cheetah males. The duration of spermatogenesis has been estimated at 54 - 63 days in bulls (B. taurus) and 40-49 days in rams (O. aries) (Courot, Houchereau-de & Ortravant, in Gomes 1978). Evidence as to the duration of spermatogenesis in carnivores is not available but likely to be of a similar order. The decline in semen quality seen in these animals 40 - 60 days after their removal to their enclosure away from the females appears to indicate that spermatogenesis may have been affected by this change. This observation is however not unequivocal and the influence of stress and the presence of the females should be investigated in controlled manner before conclusions in this regard can be made. Cheetahs are not seasonal breeders (Wrogemann, 1975 ; Wildt et al. 1986) and the fact that semen quality did not vary beyond the usual limits amongst the animals kept relatively undisturbed appears to indicate that semen quality in cheetah males does not change with the season. All of the samples collected over the 24 h period.



Diurnal variation in plasma testosterone.

The plasma testosterone concentrations measured in samples taken at two hourly intervals for a period of 24 h in five animals are shown in Figure 16.

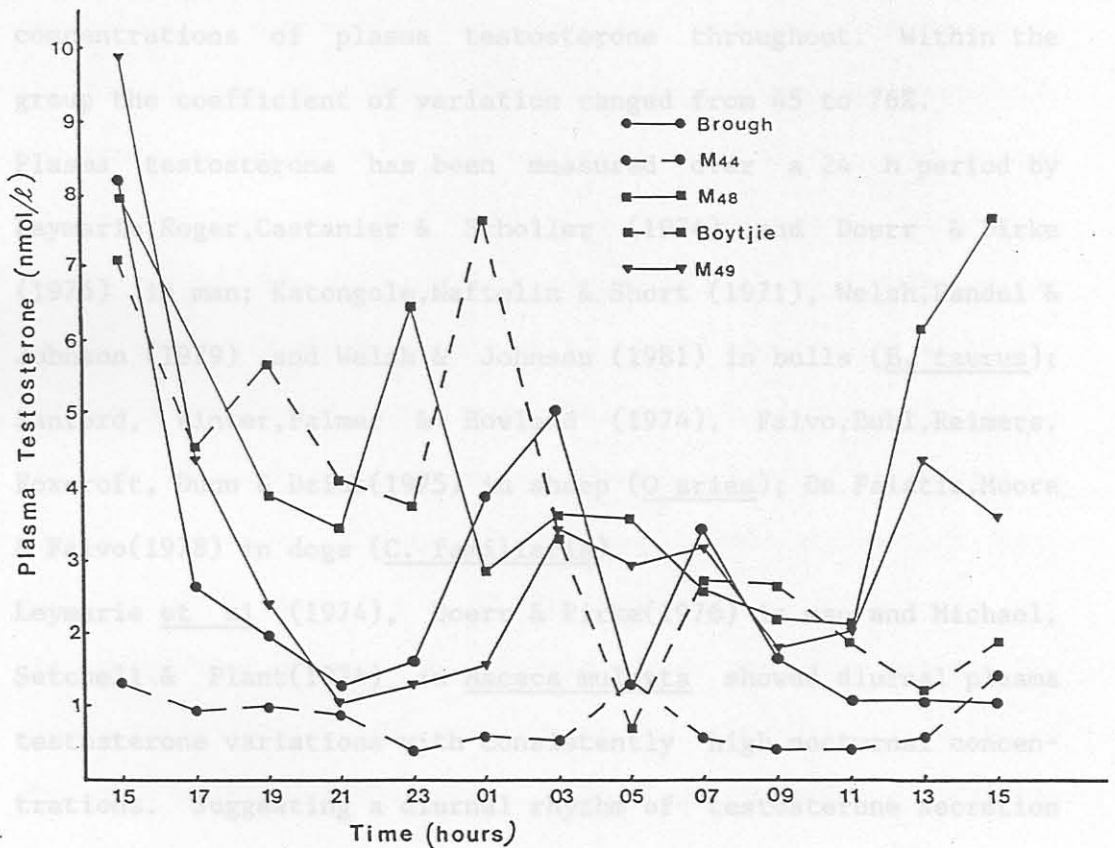


Figure 16: The plasma testosterone concentrations measured in samples taken at two hourly intervals for a period of 24 h. (n = 5).

Testosterone concentrations ranged from 0,4-9,9 nmol/l with a mean  $2,92 \pm 2,23$  in all of the samples collected over the 24 h period.

Plasma testosterone peaked 4 to 5 times in each of the animals during the 24 h period. Peaks and troughs in plasma testosterone differed in magnitude, duration and rate of change within and between individual animals. A coefficient of variation of 45% was measured in the plasma testosterone of m44 the animal that showed the lowest peak during the period and had consistently lower concentrations of plasma testosterone throughout. Within the group the coefficient of variation ranged from 45 to 76%.

Plasma testosterone has been measured over a 24 h period by Leymarie, Roger, Castanier & Scholler (1974) and Doerr & Pirke (1976) in man; Katongole, Naftolin & Short (1971), Welsh, Randel & Johnson (1979) and Welsh & Johnson (1981) in bulls (B. taurus); Sanford, Winter, Palmer & Howland (1974), Falvo, Buhl, Reimers, Foxcroft, Dunn & Dziuk (1975) in sheep (O aries); De Palatis, Moore & Falvo (1978) in dogs (C. familiaris).

Leymarie et al (1974), Doerr & Pirke (1976) in man and Michael, Setchell & Plant (1974) in Macaca mulatta showed diurnal plasma testosterone variations with consistently high nocturnal concentrations. Suggesting a diurnal rhythm of testosterone secretion which was not found in other animals. All confirm the unequivocal conclusion of Thomas, Gordon & Smid (1974) and Bartke, Steele, Musto & Caldwell (1973) that a single plasma or serum sample did not have any value in the assessment of plasma testosterone concentration and its functional significance in the animals or men that were examined.



The results of the present study on cheetahs are similar in that plasma testosterone varied considerably over the 24 h period. The cheetah males responded to the two hourly sampling with some distress and it was not possible to bleed the animals more frequently. The use of indwelling catheters proved to be unsuccessful. Animals kept in cages during the study period were nevertheless very mobile and aggressive and therefore catheterization of the relatively immobile jugular was not practical. Catheters in the cephalic and saphenous veins, which could be used by immobilizing the leg, were soon blocked due to kinking or pulled out of the vein. Falvo et al.(1975) who sampled at two hourly intervals and Katongole et al.(1971) who sampled at hourly intervals indicated that plasma testosterone fluctuated rapidly and more frequent sampling was required to show the rapid changes possible. Horton, Shinsako & Forsham (1965) showed that testosterone had a half-life of 34 min in man.

The animal that showed an uncharacteristic and consistently low plasma testosterone, m44, was 3 years old, the same age as two of the other animals in the trial, m48 & m49. He was not available during the later stages of the study when GnRH injections were used. The lower concentrations of plasma testosterone in this animal cannot be explained in the absence of an indication as to LH concentrations that might have influenced the rate of testicular testosterone production.

Table 6: Plasma luteinizing hormone and testosterone concentrations of different species.

ANIMAL	LH $\mu\text{g}/\ell$	PLASMA T nmol/ $\ell$	REFERENCE
Boar ( <u>Sus scrofa</u> )	0,8 - 1,8	4,35 - 6,9	Juniewicz & Johnson (1981)
Bull ( <u>Bos taurus</u> )	0,1 - 4,4	14,64 - 20,7	Chantaraprteep & Thibier (1978)
	0,5 - 2,5	13,46 - 33,6	Johnson, Welsh & Juniewicz (1982)
	5,0 - 50,0	6,92 - 69,2	Katongole, Naftolin & Short (1971)
	0,3 - 2,4	1,73 - 48,8	Thibier & Rolland (1976)
Sheep ( <u>Ovis aries</u> )		5,54 - 90,9	Falvo, Buhl, Reimers, Fox- croft, Dunn & Dziuk(1975)
	0,1 - 1,4		Galloway, Cotta, Pelletier & Terqui (1974)
		2,38 - 14,7	Sanford, Winter, Palmer & Howland (1974)
		3,46	Schanbacher & Echternkamp (1978)
Mice ( <u>Mus musculus</u> )		3,1 - 132,5	Bartke, Steele, Musto & Caldwell (1973)
Rabbit ( <u>Oryctolagus cuni- culus</u> )	1,6 - 3,0		Blake, Blake, Thorneycroft & Thorneycroft (1978)
Rat ( <u>Rattus norvegicus</u> )		6,23 - 52,9	Bartke, Steele, Musto & Caldwell (1973)
Dog ( <u>Canis familiaris</u> )	0,2 - 12,0	1,6 - 21,5	De Palatis, Moore & Falvo (1978)
		3,1 - 14,0	Folman, Haltmeyer & Eik- Nes (1972)
Ferret ( <u>Mustela putorius</u> )	0,5 - 2,0	0,4 - 29,4	Donovan & Ter Haar (1977)
Cheetah ( <u>Acinonyx Jubatus</u> )	0,45 - 3	0,35 - 7,27	Degenaar (1977)
	2,3 - 5,2	0,40 - 9,9	Present study
Rheses monkey ( <u>M mulatta</u> )		27,1 - 61,4	Michael, Setchell & Plant
Man		10,0 - 44,9	Beitins, Bayard, Kowarski & Migeon (1973)
		9,6 - 44,9	Leymarie, Roger, Castanier & Scholler (1974)
		13,6 - 41,5	Thomas, Gordon & Smid (1974)



Plasma testosterone after increasing doses of GnRH

Table 7. Plasma testosterone in cheetah males after increasing Pre-GnRH stimulation (0-time), maximum plasma testosterone (max. plasma T) and the maximal plasma testosterone response (max. plasma testosterone resp. = maximum plasma testosterone minus 0-time plasma testosterone) were measured together with the mean time taken for the maximum plasma testosterone to be reached (peak time). These results are shown in Figure 17 and summarized in Table 7.

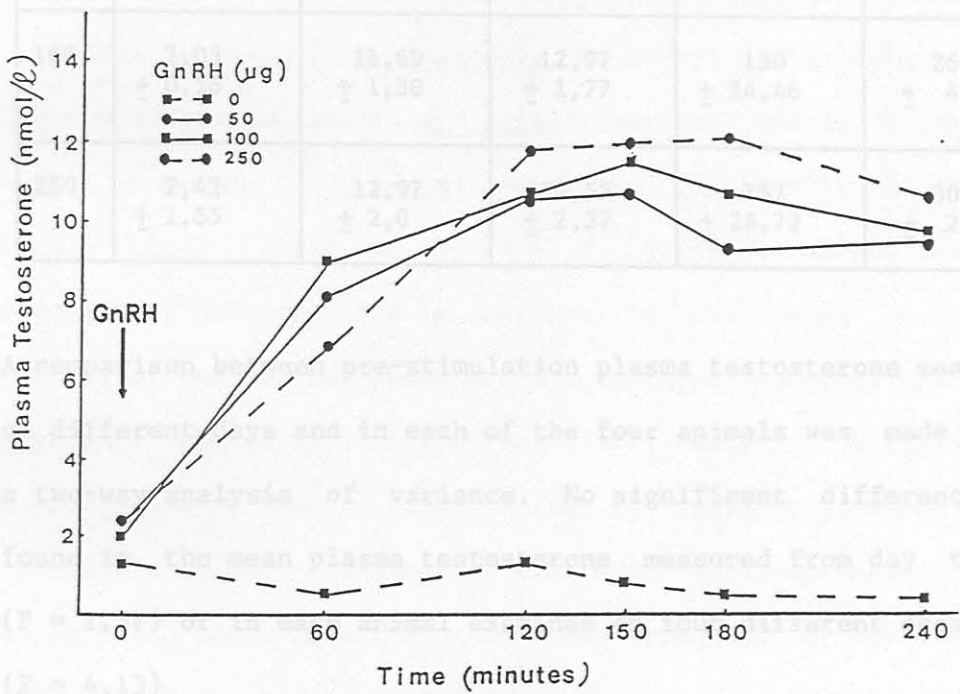


Figure 17. Plasma testosterone after increasing doses of GnRH ( n = 4) the testosterone response in nmol/l and the rate of testosterone production expressed as nmol/l/h indicated that there was no significant difference of the means of each of these parameters as the dose of GnRH was increased (F = 1.14, F = 0.59 and F = 1.03 respectively)

Table 7. Plasma testosterone in cheetah males after increasing intramuscular doses of GnRH. Data are presented as mean and standard deviation ( $\pm$ ) (n = 4).

GnRH ug	Prestimulation nmol/l	Maximum nmol/l	Maximum response	mean peak minimum	Rate of Production nmol/l/h
0	1,29 $\pm$ 0,72				
50	2,31 $\pm$ 1,02	10,81 $\pm$ 2,60	8,50 $\pm$ 3,58	165 $\pm$ 51,96	23,07 $\pm$ 10,59
100	2,03 $\pm$ 0,56	11,69 $\pm$ 1,30	12,97 $\pm$ 1,77	150 $\pm$ 24,46	26,39 $\pm$ 4,87
250	2,42 $\pm$ 1,55	12,97 $\pm$ 2,0	10,55 $\pm$ 2,37	157 $\pm$ 28,72	30,08 $\pm$ 2,77

A comparison between pre-stimulation plasma testosterone measured on different days and in each of the four animals was made using a two-way analysis of variance. No significant difference was found in the mean plasma testosterone measured from day to day ( $F = 1,57$ ) or in each animal examined on four different occasions ( $F = 4,13$ )

An analysis of variance of the maximum plasma testosterone, the plasma testosterone response in nmol/l and the rate of testosterone production expressed as nmol/l/h indicated that there was no significant difference of the means of each of these parameters as the dose of GnRH was increased ( $F = 1,14$ ,  $F = 0,59$  and  $F = 1,03$  respectively)



The study of the effect of increasing doses of GnRH on plasma testosterone levels was undertaken to estimate the optimal standard intramuscular dose of the releasing hormone in cheetahs. The amino acid sequence of porcine gonadotrophin releasing hormone (GnRH) was described by Matsuo, Baba, Nair, Arimura & Schally, 1971 in Golter, Reeves, O'Mary, Arimura, & Schally 1973) and synthesized by them (Matsuo et al. 1971b in Golter et al. 1973). Arimura, Matsuo, Baba, Debeljuk, Sandow & Schally (1972) showed that the biological activity of the natural and synthetic hormones were similar in rats (Rattus norvegicus). Golter et al.(1973) in bulls ( B. taurus); Galloway, Cotta, Pelletier & Terqui (1974) in rams ( O. aries) Hanrahan, Quirke, & Gosling (1981) in lambs ( O. aries); Pomerantz, Ellendorff, Elsaesser, König,& Smidt (1974) in boars ( S. scrofa); showed that an injection of GnRH resulted in increases in the plasma levels of luteinizing hormone and that the LH response to GnRH was dose dependent. Chakraborty & Fletcher (1977) used a 50 µg intramuscular dose of GnRH in Labrador dogs (C. familiaris) which have a similar mass to cheetahs. The standard intramuscular dose of 50 µg GnRH used later in the study produced a similar and probably maximal plasma testosterone response as did the 100 µg and 250 µg used. These results are similar to those of Galloway et al.(1974) who found that plasma testosterone concentrations did not increase with increasing doses of GnRH in rams. ~~because the~~  
secretion of testosterone is considered to be controlled by LH

Plasma LH & Testosterone after 50 µg GnRH states that the substances detected immunologically with the LH antiserum possessed LH-like activity. The ability of the LH assay used to measure this hormone in cheetahs was supported by several observations. Parallelism was observed between the dose response curves of the canine pituitary standard and pooled plasma from GnRH treated cheetahs ( Fig 18.).

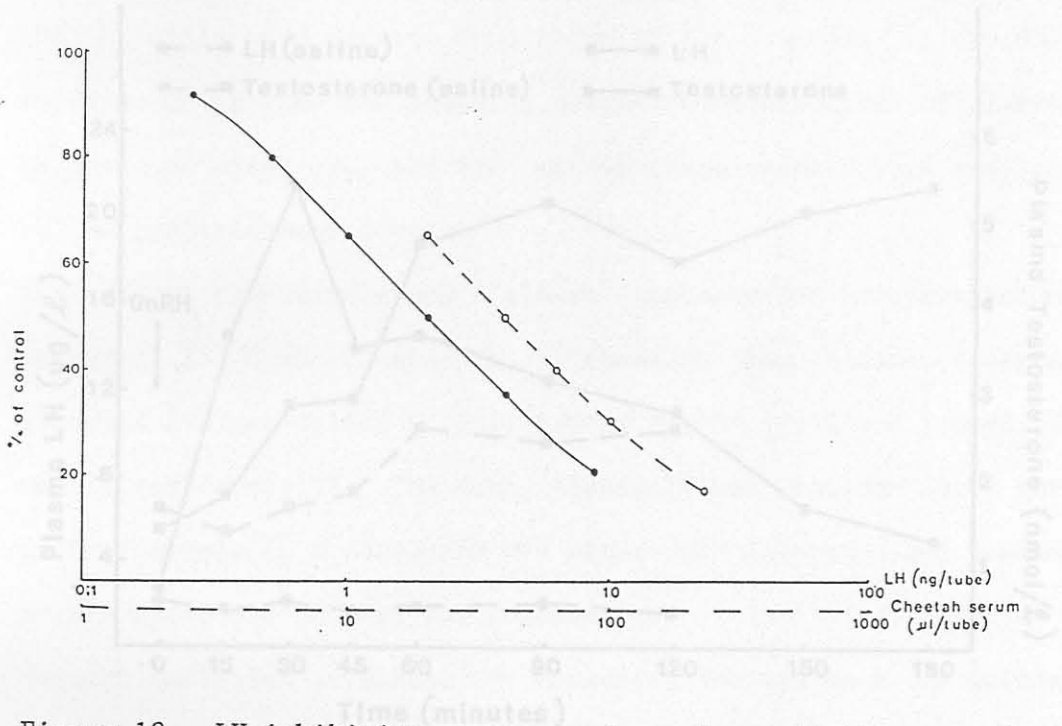


Figure 18. LH inhibition curves of canine pituitary standard ( LER-165-1) and pooled cheetah serum

Figure 19. Mean response in plasma LH and testosterone following GnRH injection. Untreated animals had significantly less detectable LH-like activity in 200 µl samples compared to activity levels measured in 100 - 200 µl of plasma from treated animals. Increased plasma testosterone was measured in GnRH stimulated animals. Because the secretion of testosterone is considered to be controlled by LH from 2.3 to 5.2 µg/l. GnRH injection stimulated an LH response in all of the cheetahs with considerable variation seen among



(Smith & Hafs, 1973), this observation indicates that the substance detected immunologically with the LH antiserum possessed LH-like biological activity.

The mean response in plasma LH and testosterone following GnRH is shown in Figure 19.

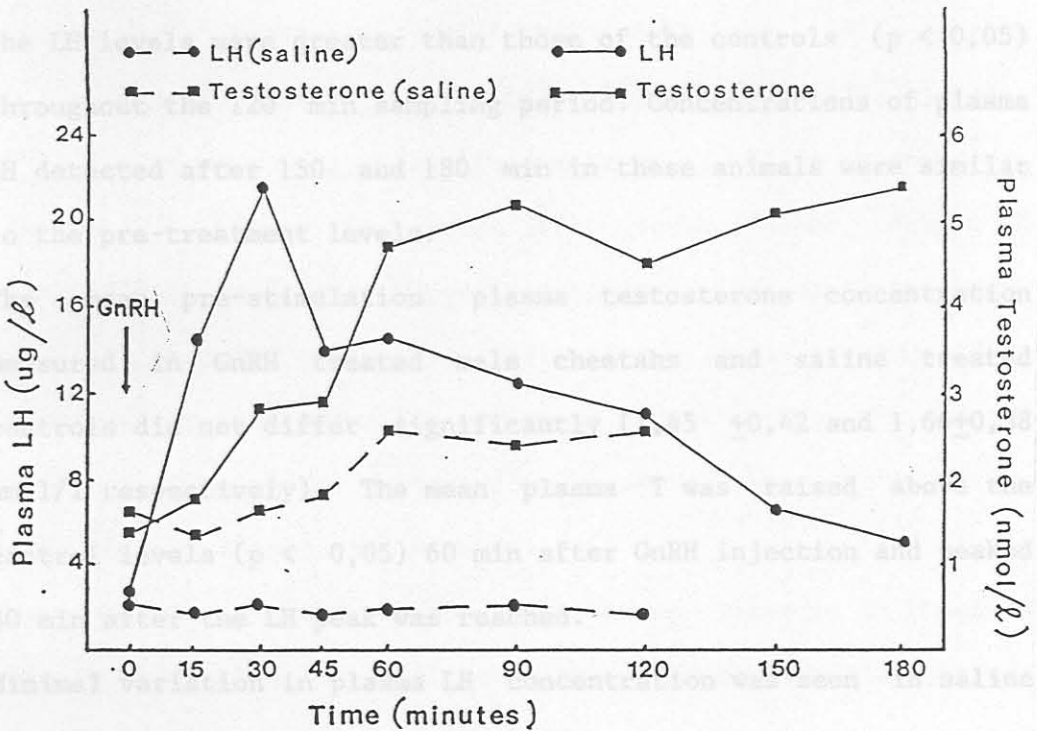


Figure 19. Mean response in plasma LH and testosterone following the administration of 50 µg GnRH.

Neither mean LH nor individual LH patterns fluctuated significantly over the 120 min sampling period in saline treated cheetah males. Mean basal LH concentrations in this group ranged from 2,3 to 5,2 µg/l. GnRH injection stimulated an LH response in all of the cheetahs with considerable variation seen among

individual animals. LH peaks ranged from 4,9 to 50  $\mu\text{g}/\text{l}$  and occurred at 15,30,45 and 60 min after the GnRH injection in one,three,two and one animals respectively. After GnRH the mean LH concentration was raised above control levels ( $p < 0,05$ ) at the 15 min sampling interval(  $14,7 \pm 4,7 \mu\text{g}/\text{l}$ ). The mean maximal LH concentration was reached at 30 min after GnRH injection and the LH levels were greater than those of the controls ( $p < 0,05$ ) throughout the 120 min sampling period. Concentrations of plasma LH detected after 150 and 180 min in these animals were similar to the pre-treatment levels.

The mean pre-stimulation plasma testosterone concentration measured in GnRH treated male cheetahs and saline treated controls did not differ significantly ( $1,45 \pm 0,42$  and  $1,66 \pm 0,38$  nmol/l respectively). The mean plasma T was raised above the control levels ( $p < 0,05$ ) 60 min after GnRH injection and peaked 30 min after the LH peak was reached.

Minimal variation in plasma LH concentration was seen in saline treated cheetahs over the 120 min period. Indicating that no episodic release of LH occurred during that time. An injection of 5  $\mu\text{g}$  intramuscular GnRH to anoestrous domestic cats (F. catus) stimulated peak LH concentrations after 30 min (Chakraborty, Wildt & Seager,1979). In cheetahs the response was similar, appearing within 10 min of the GnRH injection with the greatest mean LH concentration being reached 30 min later. However the quantitative LH response in cheetahs ( $21,6 \pm 7,2 \mu\text{g}/\text{l}$ ) was



considerably less than that measured in domestic cats (Chakraborty et al. 1979) (114 µg/l) and Labrador dogs (Chakraborty & Fletcher, 1977) (40 µg/l).

Studies in man (Franchimont, Chari & Demoulin, 1975); in bulls (B. taurus) (Mongkonpunya, Hafs, Convey & Tucker, 1975; Thibier, 1976; Kesler & Garverick, 1976; Chantaraprteep & Thibier, 1978; Schanbacher & Echternkamp, 1978; Malak & Thibier, 1982); in rams (O. aries) (Galloway et al., 1974; Falvo et al. 1975; Lincoln, 1979); in boars (S. scrofa) (Pomerantz et al. 1974); in dogs (C. familiaris) (Jones & Boyns, 1974; Jones, Baker, Fahmy, & Boyns, 1976); in ferrets (Mustela putorius) (Donovan & ter Haar, 1977) all indicate that plasma testosterone levels increase 10 to 15 min after GnRH injection and reach a peak between 90 and 180 min later. The plasma testosterone peak following 40 to 60 min after that of luteinizing hormone.

The temporal aspects of the testosterone response in cheetahs were within this range although the mean maximum plasma testosterone reached after GnRH 5,6 nmol/l is lower than that measured in most of the animals reported on.

Galloway et al. (1974)	Sheep ( <u>Ovis aries</u> )	27,0 - 43,9
Jones et al. (1976)	Dogs ( <u>Canis familiaris</u> )	18,8
Donovan & Ter Haar (1977)	Ferrets ( <u>Mustela putorius</u> )	27 - 50
Present study	Cheetah ( <u>Acinonyx jubatus</u> )	7,86

Table 8: Plasma testosterone after GnRH injection in different species.

REFERENCE	ANIMAL	PLASMA TESTOSTERONE nmol/l
Mongkonpunya et al. (1974)	Bulls ( <u>Bos taurus</u> )	34,25
Mongkonpunya et al. (1975)	Bull calves ( <u>Bos taurus</u> ) 2 months	2,42
	4 months	6,92
	6 months	20,1
Thibier (1976)	Bulls ( <u>Bos taurus</u> )	39,5
Tannen & Convey (1977)	Bull calves ( <u>Bos taurus</u> ) 1 month	24,2
Schanbacher & Echternkamp (1978)	Bulls ( <u>Bos taurus</u> )	24,2
Falvo et al. (1975)	Sheep ( <u>Ovis aries</u> )	62,3 - 103,8
Galloway et al. (1974)	Sheep ( <u>Ovis aries</u> )	27,0 - 43,9
Jones et al. (1976)	Dogs ( <u>Canis familiaris</u> )	18,8
Donovan & Ter Haar (1977)	Ferrets ( <u>Mustela putorius</u> )	27 - 50
Present study	Cheetah ( <u>Acinonyx jubatus</u> )	7,86



Plasma testosterone in different age groups after 50 µg GnRH

Table 9: Plasma testosterone following a standard dose of 50 µg

The effects of a standard dose of 50 µg GnRH by intramuscular injection on the plasma testosterone of animals from different age groups are shown in Figure 20 and summarized in Table 9.

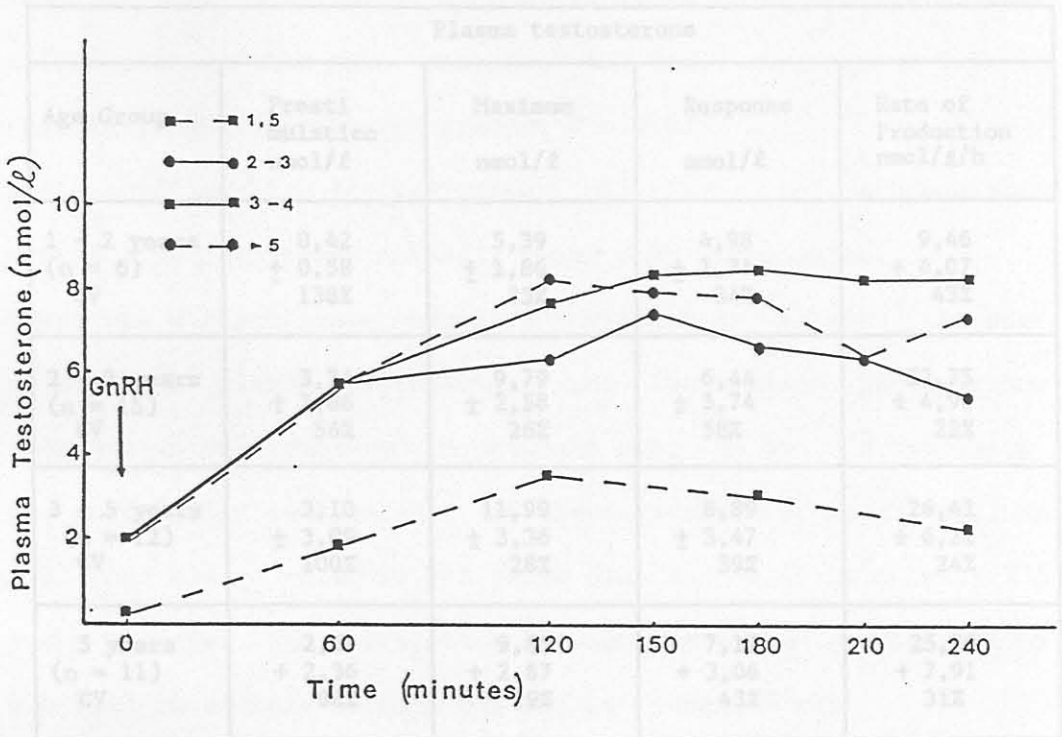


Figure 20. Plasma testosterone following 50 µg GnRH in animals from different age groups

Table 9: Plasma testosterone following a standard dose of 50 µg GnRH in cheetahs of different ages.

Data are presented as mean, standard deviation ( $\pm$ ) and coefficient of variation(%).

Plasma testosterone				
Age Group	Prestimulation nmol/l	Maximum nmol/l	Response nmol/l	Rate of Production nmol/l/h
1 - 2 years (n = 6) CV	0,42 $\pm$ 0,58 138%	5,39 $\pm$ 1,86 35%	4,98 $\pm$ 1,71 34%	9,46 $\pm$ 4,07 43%
2 - 3 years (n = 15) CV	3,34 $\pm$ 1,86 56%	9,79 $\pm$ 2,58 26%	6,44 $\pm$ 3,74 58%	22,75 $\pm$ 4,90 22%
3 - 5 years (n = 12) CV	3,10 $\pm$ 3,09 100%	11,99 $\pm$ 3,36 28%	8,89 $\pm$ 3,47 39%	26,41 $\pm$ 6,21 24%
5 years (n = 11) CV	2,69 + 2,36 88%	9,85 + 2,87 29%	7,17 + 3,06 43%	25,79 + 7,91 31%

The pre-stimulation plasma testosterone concentration in all of the animals over the age of two years ranged from 0,03 - 6,81 with a mean of 1,765  $\pm$  1,63 nmol/l (n = 44). This was significantly lower than that of the mean plasma testosterone measured in the animals sampled over 24 h (2,924  $\pm$  2,23 n = 64 )

The mean time taken to reach maximum plasma testosterone in animals from each of the age groups is summarized in Table 10.



The mean pre-stimulation and maximum plasma testosterone concentrations of animals less than 2 years of age were significantly lower than those of the older animals (  $F = 4,05$ ,  $p < 0,05$  and  $F = 8,40$   $p < 0,01$  respectively) as was their mean rate of production of testosterone expressed as plasma testosterone nmol/l/h (  $F = 11,76$   $p < 0,01$ ). There was no significant difference in the mean response to GnRH stimulation between animals from different age groups (  $F = 2,07$ ).

The coefficient of variation of plasma testosterone concentrations decreased markedly after GnRH stimulation.

There was a significant negative correlation between the pre-stimulation plasma testosterone and the plasma testosterone response to GnRH measured in cheetahs over the age of two years, the correlation coefficients of these parameters being  $-0,72$  in 2-3 year olds,  $-0,48$  in 3-5 years olds and  $-0,46$  in animals  $> 5$  years of age. The correlation between these two concentrations was  $0,13$  in animals between one and two years of age.

The pre-stimulation plasma testosterone concentration in all of the animals over the age of two years ranged from  $0,03 - 6,81$  with a mean of  $1,765 \pm 1,63$  nmol/l (  $n = 44$ ). This was significantly lower than that of the mean plasma testosterone measured in the animals sampled over 24 h (  $2,924 \pm 2,23$   $n = 64$  )

The mean time taken to reach maximum plasma testosterone in animals from each of the age groups is summarized in Table 10.

Table 10. Mean time taken to reach maximum plasma testosterone after GnRH stimulation.

Age group	Mean peak time (minutes)	Standard deviation
1 - 2 years	130	24,49
2 - 3 years	148	38,40
3 - 5 years	185	51,65
5 years	167	41,37

The maximum plasma testosterone after GnRH of cheetah males aged between 1- 3 years was reached significantly sooner than that of older animals ( $F = 2,96, p < 0,05$ )

The significant negative correlation between the pre-stimulation testosterone concentrations and the time taken to reach maximum testosterone concentrations after GnRH stimulation in cheetah males from different age groups indicated that the response to GnRH stimulation was attenuated in the younger groups of animals. Mongkonpunya et al.(1975) found that the plasma testosterone concentration of bulls (B. taurus) aged between 2 - 4 months was lower than that of animals aged six months and that the testicular response to GnRH injection was insignificant in the



younger animals. Kesler & Garverick(1976) measured both the LH and testosterone response to GnRH in two groups of calves aged 3 and 17 days younger animals showed a similar LH response to GnRH but their T response was insignificant ( $1,5 \pm 0,4$  nmol/l peak). The 17 day old calves showed a plasma testosterone response to GnRH with a mean peak of  $3,73 \pm 0,9$  nmol/l. Monkongpunya et al. (1975) measured peaks of 2,1 & 4,2 nmol/l in bull calves two and four months of age respectively and a peak of 18,3 nmol/l in calves aged six months after GnRH. Jacacki, Kelch, Sauder ,Lloyd, Hopwood & Marshall (1982) reported plasma testosterone concentrations that ranged from 0,2 to 1,5 nmol/l in pre-pubertal boys. This being considerably lower than the reported range of 9,6 to 13,9 nmol/l in adult men ( Leymarie et al. 1974). The onset of episodic secretion of LH has been proposed as a biological marker for the onset of puberty in man (Boyar, Finkelstein, Roffwarg, Kapen, Weitzman & Hellman,1972, in Jacacki et al. 1982). However this was contradicted by Jacacki et al.(1982) who, with others, showed that pulsatile LH occurred in pre-pubertal children as well.

The significant negative correlation between the pre-stimulation plasma testosterone and the plasma testosterone response to GnRH measured in cheetahs over the age of two years indicates that the 50 µg dose of GnRH was followed by maximal plasma testosterone concentrations. This was not the case in the group of cheetah males under the age of two years.

The plasma testosterone levels measured in cheetah males examined in July and November were not significantly different.

The maximum plasma testosterone after GnRH of cheetah males aged between 1 - 3 years was reached significantly sooner than that of older animals ( $F = 2,96, p < 0,05$ ).

Plasma testosterone levels in rams (*O. aries*) in the northern hemisphere following GnRH were significantly higher in January Plasma testosterone in July & November (et al, 1974). Indicating clearly the influence of seasonality on testicular function in Plasma testosterone (Plasma T) measured after 50 µg in July and November 1982 in 5 two year old cheetah males are summarized in Table 11. (*B. taurus*). The testosterone response however showed no Table 11: Plasma testosterone measured in cheetah males after 50 µg GnRH in July and in November. Data are presented as mean and standard deviation ( $\pm$ ).

Plasma testosterone				
Month	Prestimulation nmol/l	Maximum nmol/l	Response nmol/l	Rate of Production nmol/l/h
July	2,85 $\pm 2,40$	9,28 $\pm 1,44$	6,43 $\pm 2,94$	12,30 $\pm 5,84$
November	3,60 $\pm 2,05$	9,88 $\pm 1,99$	6,28 $\pm 3,53$	17,34 $\pm 5,16$

The plasma testosterone levels measured in cheetah males examined in July and November were not significantly different.



Results of paired t tests were  $t = 0,86$  for pre-stimulation plasma T,  $t = 2,36$  Maximum plasma T,  $t = 0,14$  for response plasma T and  $t = 1,79$  for plasma T nmol/l/h.

Plasma testosterone levels in rams (O. aries) in, the northern hemisphere, following GnRH were significantly higher in January than in May and September (Sanford et al, 1974). Indicating clearly the influence of seasonality on testicular function in these animals. Malak & Thibier (1982) found that there were significant individual differences in the LH response to GnRH in bulls (B. taurus). The testosterone response however showed no significant differences and repeatable results were obtained in the same animals examined one month apart.

The response after GnRH was remarkably similar in each of the two groups. plasma testosterone concentrations declined to undetectable levels after 60 min and remained depressed throughout.

The effect of anaesthesia on plasma testosterone response. The plasma T levels after CT1341 were not as low but nevertheless

Plasma testosterone concentrations after intravenous GnRH in animals anaesthetized with thiopentone sodium or CT1341 are compared in Figure 21.

all males over the age of two years, anaesthetized with CT1341, ranged from 0,03 - 6,81 with a mean of  $1,765 \pm 1,63$  nmol/l ( $n = 44$ ). This was significantly lower than the mean plasma testosterone,  $2,924 \pm 2,22$  nmol/l ( $n = 60$ ), measured in the animals sampled over 24 h.

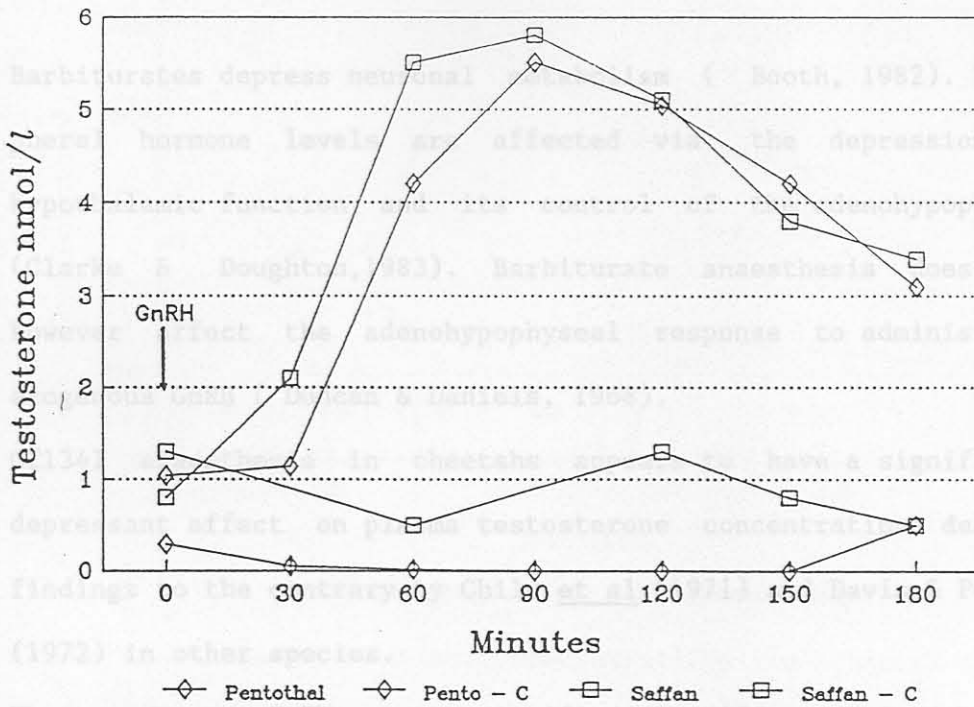


Figure 21. A comparison between the response to GnRH in cheetah males anaesthetized with thiopentone sodium or CT1341.

The response after GnRH was remarkably similar in each of the two groups. plasma testosterone concentrations declined to undetectable levels after 60 min and remained depressed throughout in two control animals anaesthetized with thiopentone sodium. The plasma T levels after CT1341 were not as low but nevertheless appear to be depressed below levels in un-anaesthetized animals. Pre-stimulation plasma testosterone concentrations measured in the present study in all males over the age of two years, anaesthetized with CT1341, ranged from 0,03 - 6,81 with a mean of 1,765 +1,63 nmol/l (n = 44). This was significantly lower than the mean plasma testosterone, 2,924 +2,22 nmol/l (n = 60), measured in the animals sampled over 24 h.



Barbiturates depress neuronal metabolism (Booth, 1982). Peripheral hormone levels are affected via the depression of hypothalamic function and its control of the adenohipophysis (Clarke & Doughton, 1983). Barbiturate anaesthesia does not however affect the adenohipophyseal response to administered exogenous GnRH (Duncan & Daniels, 1968).

CT1341 anaesthesia in cheetahs appears to have a significant depressant effect on plasma testosterone concentrations despite findings to the contrary by Child et al. (1971) and Davis & Pearce (1972) in other species.

The response to GnRH was not significantly different in the two groups of cheetahs anaesthetized with thiopentone sodium and CT1341 respectively.

Hormone studies in anaesthetized & electroejaculated males

Profiles of mean plasma concentrations of cortisol, testosterone and LH before and during CT1341 anaesthesia and before and during electroejaculation are presented in Fig 22, 23 & 24.

Figure 23: Plasma testosterone concentrations in cheetah males subjected to serial bleeding and regimented electroejaculation under CT1341 anaesthesia.

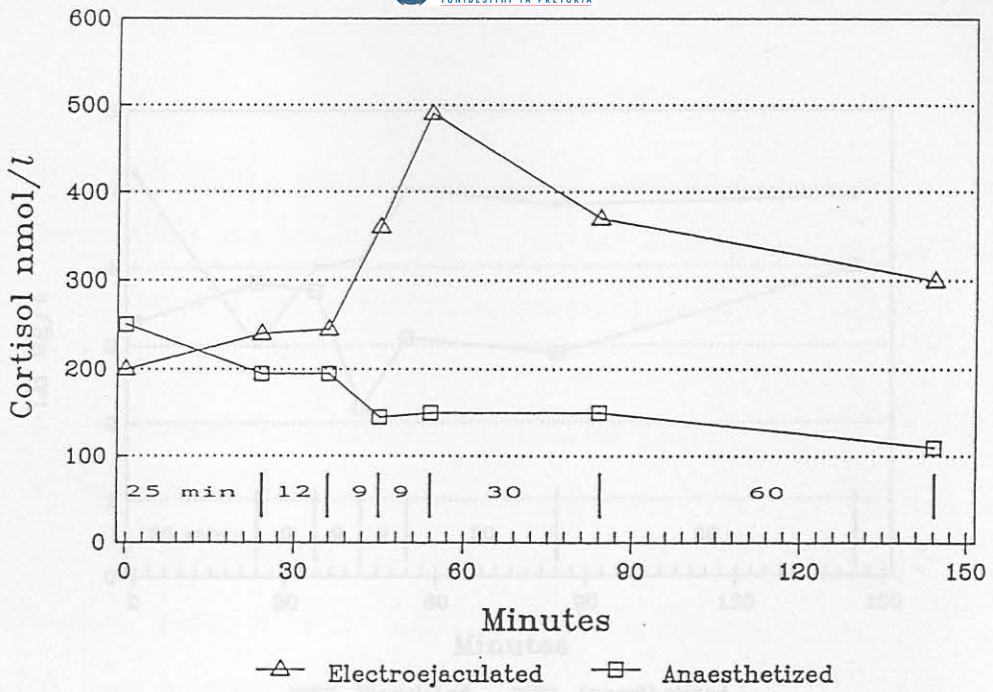


Figure 22: Plasma cortisol concentrations in cheetah males subjected to serial bleeding and regimented electroejaculation under CT1341 anaesthesia.

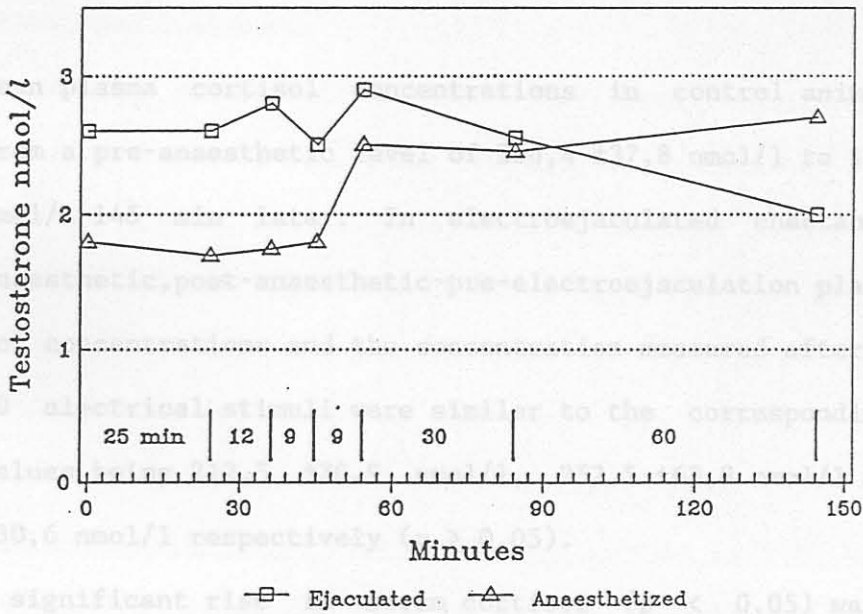


Figure 23: Plasma testosterone concentrations in cheetah males subjected to serial bleeding and regimented electroejaculation under CT1341 anaesthesia.



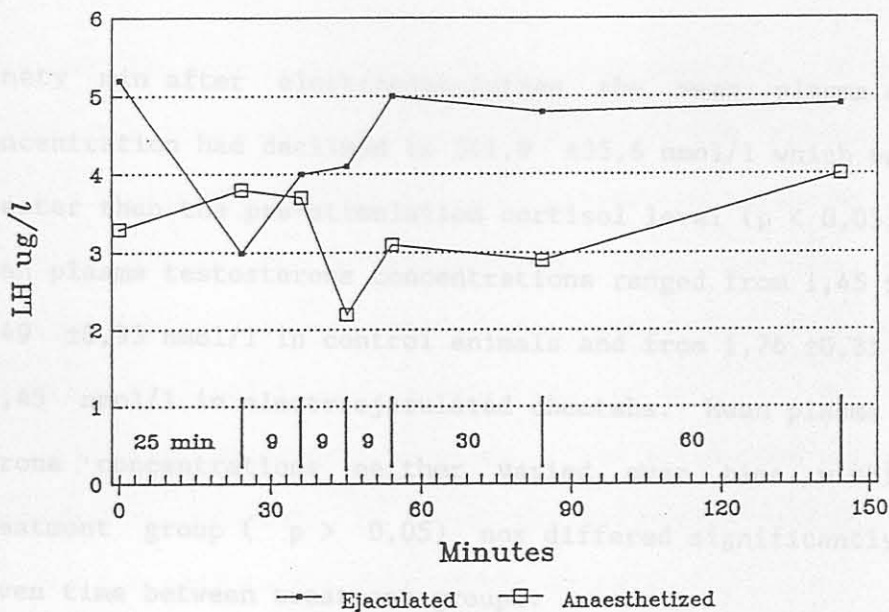


Figure 24: Plasma luteinizing hormone concentrations in cheetah males subjected to serial bleeding and regimented electroejaculation under CT1341 anaesthesia.

Mean plasma cortisol concentrations in control animals ranged from a pre-anaesthetic level of  $256,4 \pm 37,8$  nmol/l to  $121,7 \pm 21,3$  nmol/l 145 min later. In electroejaculated cheetahs the pre-anaesthetic, post-anaesthetic-pre-electroejaculation plasma cortisol concentrations and the concentration measured after the first 30 electrical stimuli were similar to the corresponding control values being  $212,5 \pm 30,9$  nmol/l,  $252,5 \pm 63,8$  nmol/l and  $256,1 \pm 30,6$  nmol/l respectively ( $p > 0,05$ ).

A significant rise in serum cortisol ( $p < 0,05$ ) was measured following the second series of electroejaculatory stimuli ( $374,5 \pm 26,5$  nmol/l) with a further rise to a peak of  $508 \pm 26,5$  nmol/l detected immediately after the end of the ejaculating stimuli.

Ninety min after electroejaculation the mean plasma cortisol concentration had declined to  $311,9 \pm 35,6$  nmol/l which was still greater than the pre-stimulation cortisol level ( $p < 0,05$ )

Mean plasma testosterone concentrations ranged from  $1,45 \pm 0,42$  to  $2,49 \pm 0,93$  nmol/l in control animals and from  $1,76 \pm 0,35$  to  $2,73 \pm 0,45$  nmol/l in electroejaculated cheetahs. Mean plasma testosterone concentrations neither varied over time within each treatment group ( $p > 0,05$ ) nor differed significantly at any given time between treatment groups.

LH titres ranged from  $2,3 \pm 0,4$  to  $3,9 \pm 1,3$   $\mu\text{g/l}$  in the control group of cheetahs and from  $3,2 \pm 0,4$  to  $5,2 \pm 1,1$   $\mu\text{g/l}$  in the electroejaculated animals. Not significantly different over time or between treatments.

Plasma LH concentrations within individual animals appeared to fluctuate randomly over time, varying by as much as  $9,0$   $\mu\text{g/l}$  in both the control and electroejaculated animals. Within individual cheetahs plasma LH concentrations appeared to be related to plasma testosterone concentrations. The correlation coefficient between these two hormones for both the control and electroejaculated groups combined was  $0,77$  ( $p < 0,01$ ).

#### Cortisol-testosterone relationships following an ACTH injection

Immediately prior to ACTH injection, plasma cortisol concentrations were  $168,3$  and  $179,4$  nmol/l respectively, in the two anaesthetized cheetahs and  $309,0$  and  $383,6$  nmol/l in the two un-anaesthetized animals. ACTH caused a rapid rise in the plasma cortisol concentration which peaked at  $602,0$  and  $1049$  nmol/l within 30 to 60 min after the injection and then gradually



declined. The use of anaesthesia had no discernible effect on the magnitude or the temporal characteristics of the plasma cortisol profile following ACTH. Neither plasma testosterone nor LH profiles appear to be affected by ACTH injection and the subsequent rise in cortisol concentration. Testosterone levels gradually declined in one awake animal and increased and declined in one of the anaesthetized males, levels remained unchanged in the other two cheetahs. LH concentrations varied randomly between 2,0 and 8,0  $\mu\text{g}/\text{l}$  in all four of the animals.

Electroejaculation under general anaesthesia was followed by an acute rise in plasma cortisol levels which declined immediately following the termination of the electrical stimulus. While it was evident that anaesthesia did not prevent an adrenal response to the electrical stimulus the plasma cortisol concentrations measured in these animals were lower than those in ACTH-treated cheetahs. There was no evidence that cortisol impaired or modulated tonic release of LH or testosterone.

Comparative data on the effects of electroejaculation in domestic animals are limited. In un-anaesthetized bulls (*B. taurus*), corticosteroids rise within 5 min of the beginning of electroejaculation and reach a peak 15 min thereafter. The hormone levels remain significantly greater than pre-electroejaculation values until 2 h post-stimulation (Welsh & Johnson, 1981). The adrenocortical response in anaesthetized cheetahs was similar to that of bulls (Welsh & Johnson, 1981). Plasma cortisol concentrations rose gradually with peak levels measured after electroejaculation. A marked variation in basal and

post-ejaculation, peak, cortisol concentrations was measured in individual cheetahs. Indicating that individual animals varied in the degree of susceptibility and combativeness to stress. Information on testosterone levels in carnivores is uncommon. In the domestic dog the circulating concentrations range from 7-20 nmol/l ( Jones et al. 1976; De Palatis et al. 1979). No comparative data are available on values in male Felidae, including the domestic cat (F. catus). In the bull electroejaculation induced elevations in the plasma cortisol concentrations which were correlated with decreasing levels of circulating testosterone and LH ( Welsh & Johnson, 1981). Data from a variety of species suggest that corticosteroid hormones can alter testicular function by affecting either the hypothalamus ( Collu, Tache & Ducharme, 1979) the adenohypophysis ( Chantaratreep & Thibier, 1978) or gonads (Beitins et al. 1973; Doerr & Pirke, 1976; Saez, Morera, Haour & Evain, 1977; Bambino & Hseuh, 1981). The administration of ACTH is negatively correlated to subsequent plasma testosterone levels in man ( Doerr & Pirke, 1976) and bulls (B. taurus) (Welsh & Johnson, 1981; Johnson et al. 1982; Barnes, Kazmer, Birrenkott & Grimes, 1983). The administration of cortisol or dexamethasone also eliminates the nocturnal rise of plasma testosterone in man (Doerr & Pirke, 1976) and decreases LH synthesis in bulls (B. taurus) (Thibier & Rolland, 1976; Chantaratreep & Thibier, 1978). However it is also evident that the stress associated adrenal-testicular-adenohypophyseal relationship is species-specific, not always directly linked to cortisol and not always easily explained.



Although dexamethasone or elevated cortisol decrease plasma testosterone concentrations in men, LH concentrations are not generally affected ( Schaison, Durand & Mowszowicz,1978; Rose & Sachar,1981). ACTH induces cortisol elevations in dogs (C. familiaris) and boars (S. scrofa), however, testosterone levels are unaffected in the former ( Eik- Nes,1962; Hagan & Andersen, 1981) and even increased in the latter species (Juniewicz & Johnson, 1981). The increased plasma testosterone concentrations in boars were seen in the absence of any detectable rise in LH (Juniewicz & Johnson, 1981). ACTH also has no influence on testosterone levels in rabbits (Oryctolagus cuniculus) (Haltmeyer & Eik-Nes, 1969) or the rhesus monkey ( M. mulatta) (Michael et al. 1974). The cheetah can be classified with these species as there was no evidence that the elevations in plasma cortisol, after ACTH or as a result of electroejaculation, modulated acute secretory patterns of either testosterone or LH.

Male (n = 6)	3,02 ± 4,03	11,31 ± 3,76	8,58 ± 3,14	23,87 ± 4,31
Peer (n = 7)	3,40 ± 2,72	10,21 ± 2,91	6,80 ± 3,35	23,89 ± 6,41

An analysis of variance of the data shows that mean plasma testosterone measured did not differ significantly in each of the three groups of cheetah males examined. ( F = 0,91 for pre-stimulation, F = 0,20 for maximum, F = 0,72 for response and F = 0,58 for plasma T in nmol/l/h).

Plasma testosterone and semen quality

Plasma testosterone concentrations measured in these animals are summarized in Table 12.

Table 12. The relationship of plasma testosterone concentrations and semen quality in 18 cheetah males. Data are presented as mean ( $\pm$ ) standard deviation.

Semen quality	Plasma testosterone			
	Pre-stimulation nmol/l	Maximum nmol/l	Response nmol/l	Rate of Production nmol/l/h
Good (n = 5)	2,17 $\pm$ 2,15	11,31 $\pm$ 5,00	9,14 $\pm$ 3,92	20,59 $\pm$ 9,27
Fair (n = 6)	3,02 $\pm$ 4,03	11,60 $\pm$ 3,76	8,58 $\pm$ 3,14	23,87 $\pm$ 4,31
Poor (n = 7)	3,40 $\pm$ 2,72	10,21 $\pm$ 2,91	6,80 $\pm$ 3,35	23,89 $\pm$ 6,41

An analysis of variance of the data shows that mean plasma testosterone measured did not differ significantly in each of the three groups of cheetah males examined. (  $F = 0,91$  for Pre-stimulation,  $F = 0,20$  for maximum,  $F = 0,72$  for response and  $F = 0,44$  for plasma T in nmol/l/h).



Plasma testosterone concentrations are influenced by the age of the animal ( Winters & Troen, 1982; Malak & Thibier, 1982), the season( Haigh,Cates & Glover 1982) and by testicular pathology ( Christiansen 1975, Franchimont, Chari & Demoulin 1975; Fossati Asfour, Blacker, Boutemy & Hermand, 1979)

Increased plasma gonadotrophins have been reported in men with azoospermia and oligozoospermia (Christiansen 1975; Franchimont et al. 1975; Fossati et al. 1979; Batrinos et al. 1982). While Batrinos et al. (1982) reported lower plasma testosterone concentrations in men with azoospermia, the others found plasma testosterone levels no different from those of fertile males.

The measurement of plasma testosterone was undertaken to establish an understanding of the hormonal relationships in male cheetah which might be of value in the assessment of an animals breeding potential. Measurement of gonadotrophins was not always possible during this study.

Plasma testosterone did not differ significantly in the groups of animals examined despite differences in semen quality.

diZerega & Sherins(1981) reviewed the endocrine control of testicular function.

Steinberger (1971, in diZerega & Sherins,1981) and Steinberger, Root, Ficher & Smith (1973, in diZerega & Sherins,1981) have shown that, in immature hypophysectomized rats, testosterone alone could account for the initiation of spermatogenesis and that FSH was required only for its completion. The administration of testosterone in hypogonadotropic men, however, does not induce spermatogenesis (MacLeod 1970, in diZerega & Sherins,

1981). HCG injection stimulates increased intratesticular testosterone concentrations and promptly initiates spermatogenesis (Sherins, Winters & Wachslicht 1970, in diZerega & Sherins, 1981).

Plasma testosterone			
Route of injection	Stimulation (nmol/l)	Maximum (nmol/l)	Rate of production (nmol/l/h)
IV	0,82 ± 0,46	5,05 ± 1,74	11,32 ± 4,79
IM	2,53 ± 0,83	11,39 ± 3,85	17,8 ± 5,85

Plasma testosterone after intravenous & intramuscular GnRH

The effect of 50 µg GnRH given intravenously and intramuscularly in four cheetah males is compared in Fig. 25 and the results are summarized in Table 13.

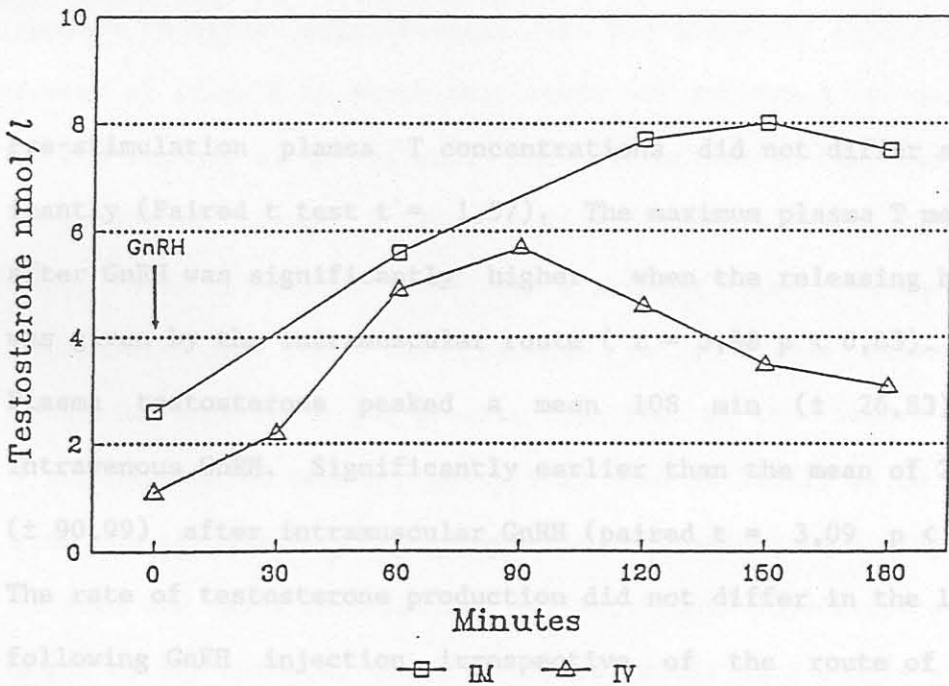


Figure 25: Plasma testosterone after GnRH by intravenous and intramuscular injection (n = 4).



Table 13: Plasma testosterone after GnRH by intravenous and intramuscular injection. Data are presented as mean and standard deviation ( $\pm$ ), (n = 4).

Plasma testosterone			
Route of injection	Prestimulation nmol/l	Maximum nmol/l	Rate of production nmol/l/h
IV	0,92 $\pm$ 0,46	5,66 $\pm$ 1,79	11,32 $\pm$ 4,79
IM	2,45 $\pm$ 2,23	11,39 $\pm$ 4,66	17,91 $\pm$ 7,65

The rate of response and decline in the plasma testosterone appears to differ significantly with the route of injection. The number of animals on which this study was performed is small and, Pre-stimulation plasma T concentrations did not differ significantly (Paired t test  $t = 1,57$ ). The maximum plasma T measured after GnRH was significantly higher when the releasing hormone was given by the intramuscular route ( $t = 3,88$   $p < 0,05$ ).

Plasma testosterone peaked a mean 108 min ( $\pm 26,83$ ) after intravenous GnRH. Significantly earlier than the mean of 216 min ( $\pm 90,99$ ) after intramuscular GnRH (paired  $t = 3,09$   $p < 0,05$ ).

The rate of testosterone production did not differ in the 180 min following GnRH injection irrespective of the route of administration ( $t = 0,79$ ).

Gonadotrophin releasing hormone has been administered intravenously by Schanbacher & Echterkamp (1978) and Johnson, Welsh, & Juniewicz (1982) in cattle (B. taurus); Bremner, Findlay, Lee,

de Kretser & Cumming (1980) in sheep (O. aries) Pomerantz et al.(1974) in boars (S. scrofa); Jones & Boyns (1974) and Jones, Baker, Fahmy & Boyns (1976) in dogs (C. familiaris) Haltmeyer & Eik-Nes (1969) in rabbits (Oryctolagus cuniculus). Comparisons were not made between the intravenous and intramuscular routes of administration.

The intravenous administration of GnRH as a standardised stimulus has advantages in that the peak of plasma testosterone is reached earlier. Serial blood collection takes place over a shorter period of time and the number of hormone assays required is decreased.

The rate of response and decline in the plasma testosterone appears to differ significantly with the route of injection. The number of animals on which this study was performed is small and, in the absence of LH values, this difference in response cannot be explained. Aiyer, Chiappa, & Fink (1973, in Fink & Pickering, 1980) have shown that GnRH has a priming effect on the adenohypophysis and that the response to a second exposure to releasing hormone is far greater than that of the first. GnRH is a decapeptide (Matsuo et al. 1971) and is likely to be removed from the circulation, by glomerular filtration, in a single pass through the renal circulation. An intravenous injection, a single bolus of the hormone, would therefore result in a single stimulus to the adenohypophysis. However intramuscularly injected hormone, with a slower rate of absorption into the circulation, could conceivably have a more prolonged effect. Added to this is the



Plasma testosterone after HCG injection  
 possibility of a partial subcutaneous or intraseptal placement of the hormone which would reduce its rate of absorption and extend The effect of increasing doses of HCG on plasma testosterone was its effect.

measured in four cheetah males and the results are summarized in Table 14.

Table 14: The effect of increasing doses of HCG on plasma testosterone in cheetah males. Data are presented as mean and standard deviation( $\pm$ ), n = 4.

Plasma Testosterone			
HCG mg	Pre-stimulation nmol/l	Maximum nmol/l	Rate of Production nmol/l/h
2,5	2,0 $\pm$ 1,21	10,84 $\pm$ 0,70	28,49 $\pm$ 2,33
5	1,15 $\pm$ 1,09	15,40 $\pm$ 5,33	35,24 $\pm$ 10,58
10	2,43 $\pm$ 1,54	11,84 $\pm$ 3,36	31,72 $\pm$ 8,41
20	0,71 $\pm$ 0,25	12,75 $\pm$ 3,01	34,20 $\pm$ 4,45

An analysis of variance of the above data shows that there was no significant difference of the mean pre-stimulation, mean maximum or the rate of testosterone production in nmol/l/h ( $F = 1,14$ ,  $F = 0,91$  and  $F = 0,52$  respectively). Plasma testosterone response and peak time were not included as Figure 26 shows that increases in plasma testosterone in animals after HCG were more sustained

Plasma testosterone after HCG injection concentrations were probably reached after the sampling period. Plasma testosterone The effect of increasing doses of HCG on plasma testosterone was measured in four cheetah males and the results are summarized in Table 14.

Table 14: The effect of increasing doses of HCG on plasma testosterone in cheetah males. Data are presented as mean and standard deviation( $\pm$ ), n = 4.

Plasma testosterone			
HCG mg	Prestimulation nmol/l	Maximum nmol/l	Rate of Production nmol/l/h
2,5	2,0 $\pm$ 1,21	10,84 $\pm$ 0,70	28,49 $\pm$ 2,33
5	1,16 $\pm$ 1,69	15,40 $\pm$ 5,33	35,24 $\pm$ 10,58
10	2,48 $\pm$ 1,54	11,84 $\pm$ 3,56	31,72 $\pm$ 8,41
20	0,71 $\pm$ 0,26	12,75 $\pm$ 3,01	34,20 $\pm$ 4,45

An analysis of variance of the above data shows that there was no significant difference of the mean pre-stimulation, mean maximum or the rate of testosterone production in nmol/l/h (F = 1,14, F = 0,91 and F = 0,52 respectively). Plasma testosterone response and peak time were not included as Figure 26 shows that increases in plasma testosterone in animals after HCG were more sustained



and that the maximum plasma testosterone concentrations were probably reached after the sampling period. Plasma testosterone measured in the same animals after 50 µg GnRH are included in Figure 27.

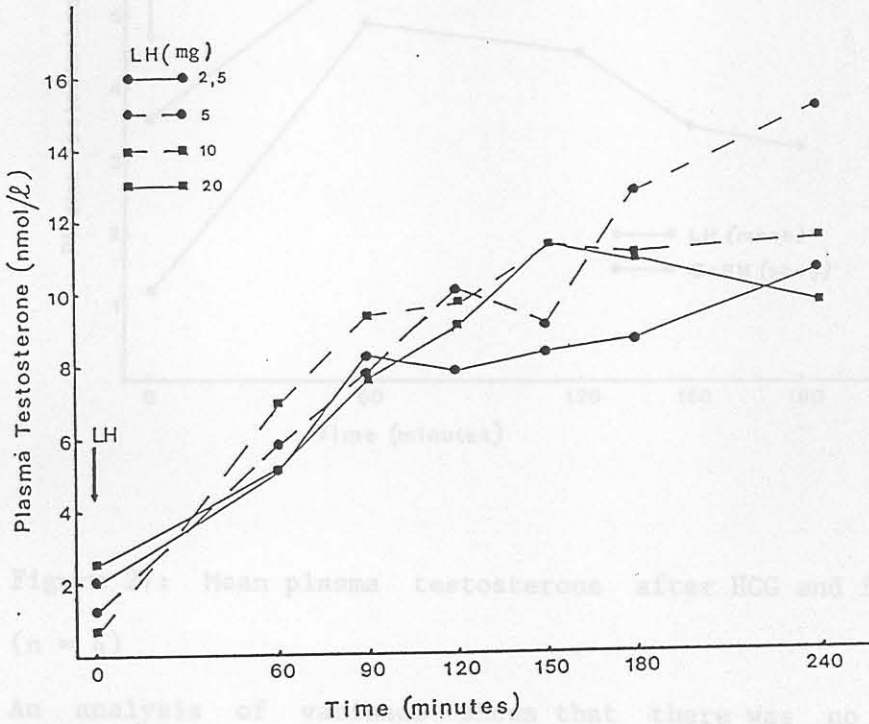


Figure 26: Plasma testosterone after increasing doses of HCG. (Dosage is presented as the equivalent dose of LH in mg).

240 minutes.

The plasma T response following HCG injection is used in the clinical assessment of testicular interstitial function in man (Anderson, Marshall, Young & Russell Fraser, 1974 in Anderson, 1984) and has been used by Falvo et al. (1975) in rams (*O. aries*); Halmeyer & Kik-Nas (1969) in rabbits (*O. cuniculus*); Bambino & Naush (1981) in rats (*R. norvegicus*). The response measured in cheetahs was prolonged in comparison to that seen after GnRH

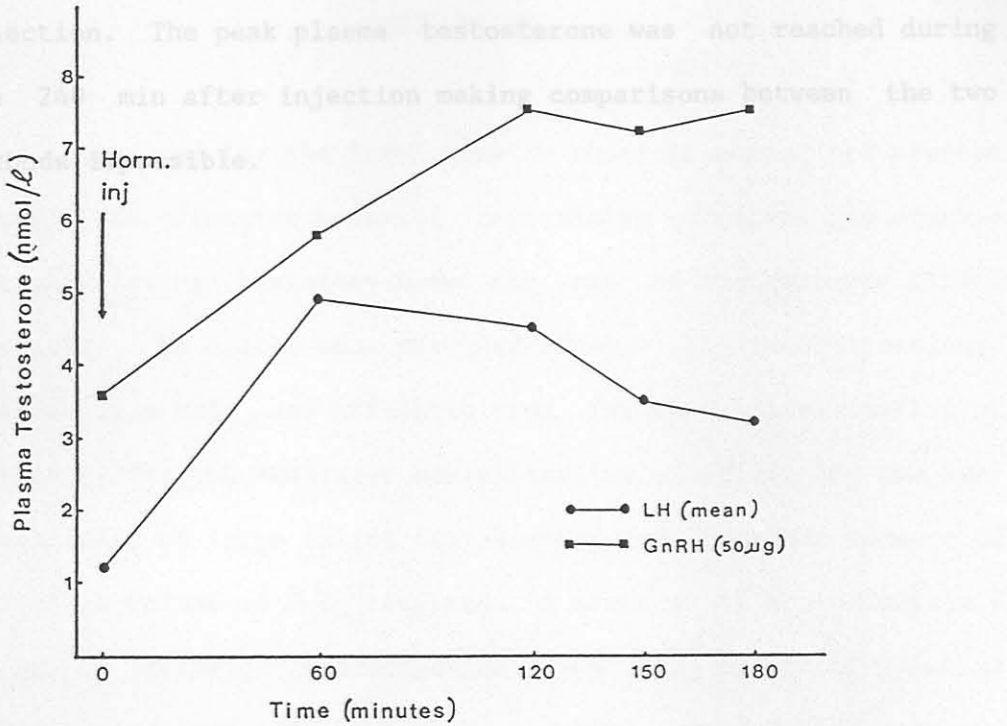


Figure 27: Mean plasma testosterone after HCG and 50 µg GnRH. (n = 4)

An analysis of variance shows that there was no significant difference in the mean rate of testosterone production, measured in nmol/l/h, after HCG and GnRH when measured over a period of 240 minutes.

The plasma T response following HCG injection is used in the clinical assessment of testicular interstitial function in man (Anderson, Marshall, Young & Russell Fraser, 1974 in Anderson, 1984) and has been used by Falvo *et al.* (1975) in rams (*O. aries*); Haltmeyer & Eik-Nes (1969) in rabbits (*O. cuniculus*); Bambino & Hsueh (1981) in rats (*R. norvegicus*). The response measured in cheetahs was prolonged in comparison to that seen after GnRH



The study of the effects of ACTH on plasma hormones and their relationship to one another reported on below shows that stress responses with raised plasma cortisol concentrations which undoubtedly were present in this group of animals probably did not influence testosterone concentrations significantly.

Plasma testosterone concentrations measured in other animals are summarized in Table 6.

	3,0 - 30,0	6,92 - 45,1	Katongole, Nafolin & Sharp (1971)
	0,3 - 2,4	1,73 - 48,5	Thibier & Bolland (1976)
Sheep ( <i>Ovis aries</i> )		5,54 - 90,9	Salvo, Juhl, Weiners, Forwood, Dunn & DeWitt (1975)
	0,1 - 1,4		Galloway, Gotta, Bellecier & Tardot (1974)
		2,38 - 14,7	Jenford, Winter, Palmer & Howland (1974)
		3,45	Schamberger & Schornberg (1975)
Horse ( <i>Equus caballus</i> )		3,1 - 132,5	Bertha, Steele, Haste & Caldwell (1972)
Rabbit ( <i>Cyrtolagus ssp. capus</i> )	1,4 - 1,0		Slake, Slake, Thorneycroft & Thorneycroft (1972)
Rat ( <i>Rattus norvegicus</i> )		0,23 - 32,9	Bertha, Steele, Haste & Caldwell (1972)
Dog ( <i>Canis familiaris</i> )	0,3 - 12,0	1,6 - 21,5	De Falatis, Moore & Salvo (1972)
		3,1 - 14,8	Felton, Waltmeyer & Mik-Max (1972)
Ferret ( <i>Mustela putorius</i> )	0,1 - 2,0	0,4 - 29,4	Donovan & Van Haar (1977)
Cheekah ( <i>Arimurus jubatus</i> )	0,45 - 3	0,35 - 7,27	Degenner (1977)
	2,5 - 5,2	0,45 - 0,6	Present study
Spout monkey ( <i>S. muletta</i> )		27,1 - 61,4	Michael, Setchell & Plant
Man		10,0 - 44,9	Heltzer, Bayard, Kowacki & Nixon (1973)
		9,6 - 44,5	Leymarie, Roger, Castanier & Scholler (1974)
		13,0 - 41,5	Thomas, Gordon & Reid (1974)

injection. The peak plasma testosterone was not reached during the 240 min after injection making comparisons between the two methods impossible.

the first time in cheetahs during the present study. The effective means of restraining cheetahs in crushes designed during the study made the use of intravenous CT1341 possible. No deaths were recorded after > 750 immobilizations. CT1341 is a safe and effective drug for the immobilization of cheetahs. The intramuscular administration of CT1341 for the immobilization of large felids is, however, not feasible because of the large volume of drug required. A mixture of approximately 8 mg/kg of ketamine hydrochloride with 0,5 mg/kg of xylazine hydrochloride was effective when cheetahs were darted. Anaesthesia with this drug combination was however characterized by muscular hypertonicity and occasional epileptiform convulsions. The possibility of eye damage as a result of the suppression of palpebral reflexes and mydriasis was ever present.

When the present study was embarked upon the fertility of the male cheetahs at the Centre was questioned. Standard methods of evaluating semen quality in domestic species were adopted in assessing the fertility of cheetah males. Males with what appeared to be good quality semen were used for breeding. This approach was successful. Over 240 cheetah cubs were born from 71 litters at a conception rate of 52% during the period 1975 - 1984.

Initially it was postulated that poor semen quality, as assessed on the basis of spermatozoal morphology, seen in the majority of the captive cheetah males examined was the most important factor affecting the fertility of this population. Spermiograms from



## CONCLUSIONS

CT1341 was used for the first time in cheetahs during the present study. The effective means of restraining cheetahs in crushes designed during the study made the use of intravenous CT1341 possible. No deaths were recorded after > 750 immobilizations. CT1341 is a safe and effective drug for the immobilization of cheetahs. The intramuscular administration of CT1341 for the immobilization of large felids is, however, not feasible because of the large volume of drug required. A mixture of approximately 8 mg/kg of ketamine hydrochloride with 0,5 mg/kg of xylazine hydrochloride was effective when cheetahs were darted. Anaesthesia with this drug combination was however characterized by muscular hypertonicity and occasional epileptiform convulsions. The possibility of eye damage as a result of the suppression of palpebral reflexes and mydriasis was ever present.

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Initially it was postulated that poor semen quality, as assessed on the basis of spermatozoal morphology, seen in the majority of the captive cheetah males examined was the most important factor affecting the fertility of this population. Spermograms from

successful sires at the Centre, however, indicate that spermatozoal morphology alone does not accurately reflect the fertility of cheetah males.

The percentage of normal spermatozoa counted in successful sires ranged from 18-80 % with half the recorded births resulting from matings when males with normal sperm counts below the population mean of 47,6 % were used. Other semen characteristics including sperm motility, percentage motile spermatozoa and concentration of spermatozoa per ml appear to be more important as measures of fertility in cheetahs as has been reported in other species (Austin et al. 1961; Clarke et al. 1973; Carroll et al. 1973). The subjective assessment of these factors made immediately after semen was collected, by a person experienced in the evaluation of semen quality, was used in the choice of males for breeding purposes during the last few years of the study and with good results.

O'brien et al. (1983) reported that the cheetah has a distinct monomorphic genome. The physiological relevance of this finding is open to question. Quantification of the proteins identified is not possible and the fact that a specific enzyme protein is present need not be a measure of its functional activity. Wildt et al. (1983 & 1986) reported on the high percentage of morphologically abnormal spermatozoa seen in both captive and free-ranging cheetahs examined by them. The conclusions of O'brien et al. (1983) and Wildt et al. (1983 & 1986) in which the

(Chandley et al. 1975; Afzelius, 1981 in Wildt et al. 1983).  
Borman, Van Vuuren, Maltzer, Van Der Merwe & Van Rensburg



species' lack of genetic variation is linked to the belief that the cheetah is endangered because of poor fertility and fecundity are however contradicted by the evidence available.

Captive cheetahs at the De Wildt Centre have reproduced in such numbers that at times the breeding program has been halted because the dramatic increase in the population has exceeded the demand for animals from world zoos. The release of eight cheetahs, five males and three females, into the Suikerbosrand Nature Reserve in 1975 was followed by a rapid increase of that population. Within five years and after 16 cheetahs from the reserve were relocated to other areas 30 cheetah were still in the reserve and the prey populations of blesbok (Damalisca dorcas phillipsi) and springbok (Antidorcas marsupialis) were in decline (Pettifer, 1981). Cheetahs are regarded as problem animals in the farming areas of the Northern Transvaal and relatively large numbers are shot or captured, yearly, by farmers in South West Africa - Namibia (Morsbach, pers. comm.). The cheetah was reported to be out of danger from extinction by Smithers (1986).

The spermiogram results obtained during the present study are not directly comparable with those of Wildt et al. (1983 & 1986) because of the different morphological classifications used. Nevertheless both studies have shown that cheetah semen is characterized by a high percentage of pleomorphic spermatozoa. Similar spermatozoal pleomorphism has been reported in man (Chandley et al. 1975; Afzelius, 1981 in Wildt et al. 1983). Bornman, Van Vuuren, Meltzer, Van Der Merwe & Van Rensburg

(1987) reported a 50 % mean normal spermatozoa count in 680 spermograms from chacma baboons (Papio ursinus). A result of the same order as the 47,6 % mean normal count in cheetahs during the present study.

The marked drop in semen quality seen in the grouped cheetah males after the breeding season may be an indication of the effects of stress or the presence of females on the quality of the semen of cheetah males. Cheetahs are not seasonal breeders (Wrogemann, 1975; Wildt et al. 1986). It appears that the conditions under which these animals were kept and the fact that they had been removed from the area around the female enclosures a month prior to the evaluation of their semen may have affected semen quality. Until these possible influences on semen quality have been investigated in a controlled manner it is not possible to reach a conclusion regarding the relevance of the poor spermatozoal morphology reported on both by Wildt et al. (1983 & 1986) and the present study.

Plasma testosterone studies were undertaken to obtain an understanding of the hormonal status of cheetah males considered to be either fertile or sub-fertile after the evaluation of their semen quality. The marked diurnal variation in plasma testosterone concentrations in the group of five cheetah males sampled over a 24 h period indicated that a single blood sample collected from an animal would be valueless. A controlled stimulus for testosterone production, the administration of 50 µg GnRH, resulted in a

libido. *Miss van Dyk's thorough understanding of individual be-*



maximal plasma testosterone response. However no significant difference in this response was found in mature cheetah males despite differences in semen quality.

The intravenous administration of GnRH appeared to be advantageous. Maximum testosterone concentrations were reached sooner. Prolonged anaesthesia with thiopentone sodium resulted in a significant decline in plasma testosterone concentrations in untreated control animals. A comparison of the variation in plasma testosterone concentrations measured in cheetahs over a 24 h period with those of cheetah males anaesthetized with CT1341 for up to 6 h appears to indicate that CT1341 does depress plasma testosterone concentrations, albeit to a lesser degree. The plasma testosterone response after GnRH administration did not differ when either of these two anaesthetics was used.

The hormone studies performed during this study indicate that the assessment of these animals' hormonal status was of little or no value in the pursuance of the objective of promoting optimal reproduction.

As suggested at the beginning of the present study effective management of the cheetah population made an important contribution to the breeding success achieved at the De Wildt Centre. The fact that males were kept separated from the females for most of the year prevented habituation of the sexes to one another. The presence of males appeared to stimulate females to come into oestrus and the interaction between males in a group stimulated libido. Miss van Dyk's thorough understanding of individual be-

havioural characteristics of the animals made it possible to introduce males into female enclosures when oestrus was not apparent.

The use of selected males with the best semen quality and the meticulous attention to the management of the animals at the Centre were the most important factors that resulted in the successful breeding of cheetahs in large numbers.

Comparative data for conception rates in non-domestic species are not available, nevertheless the mean conception rate of 52% achieved during a 10 year period appears to indicate that the captive cheetah, like their wild counterparts, do not have a fertility problem.

The appropriate management of the captive population was essential for the stimulation of sexual activity. The fertility of the male cheetahs was examined and males were selected on the basis of their semen quality for inclusion in the breeding program. Semen was collected by electroejaculation from cheetah males immobilised with a combination of ketamine and xylazine or with GT1361. Semen quality was evaluated using standard methods. Fresh semen samples were examined for motility, percentage live and density. Spermatozoal morphology was examined using stained semen smears.

Plasma testosterone was determined in cheetah males bled at 2 hourly intervals for 24 h. Thereafter an intramuscular injection of 50 µg GnRH was used as a standard stimulus. Plasma testosterone concentrations, after GnRH, were measured in cheetah males from different age groups, at different times of the year, in electroejaculated animals and animals with differences in



SUMMARY

The De Wildt Cheetah Breeding & Research Centre of the National Zoological Gardens of South Africa was established in 1971 with the aim of breeding enough cheetahs (Acinonyx jubatus) for display purposes in Zoological Gardens and to make animals available, where possible, for release into suitable natural areas. At the commencement of this undertaking cheetahs were regarded as being very difficult to breed in captivity. The present study investigated two postulates; First that captivity had a deleterious effect on the fertility of the male cheetahs; and secondly, that the appropriate management of the captive population was essential for the stimulation of sexual activity. The fertility of the male cheetahs was examined and males were selected on the basis of their semen quality for inclusion in the breeding program. Semen was collected by electroejaculation from cheetah males immobilized with a combination of ketamine and xylazine or with CT1341. Semen quality was evaluated using standard methods. Fresh semen samples were examined for motility, percentage live and density. Spermatozoal morphology was examined using stained semen smears.

Plasma testosterone was determined in cheetah males bled at 2 hourly intervals for 24 h. Thereafter an intramuscular injection of 50 µg GnRH was used as a standard stimulus. Plasma testosterone concentrations, after GnRH, were measured in cheetah males from different age groups, at different times of the year, in electroejaculated animals and animals with differences in

assessed semen quality. The effect of a rise in cortisol concentration, measured during and after electroejaculation, on plasma LH and testosterone was also examined.

The management of the cheetah population was changed from one in which cheetahs were kept as a heterosexual group. Cheetah females were kept singly in individual enclosures and the males were kept in groups far removed from the female area for most of the year. During November and for the following 2 to 3 months male groups were introduced into the passage between the female enclosures and the animals were observed for signs of breeding activity. When behavioural oestrus was observed a single male, selected because of good semen quality, was introduced into the female's enclosure and left there for 2-3 days.

Sperm motility, percentage live and semen density were found to be more reliable than spermatozoal morphology as parameters of fertility in cheetah males. Litters were born to females that had been mated by males with normal spermatozoal counts of 18 - 80 %. Fifty percent of the litters produced between 1975-1984 were sired by males with normal sperm counts of less than the population mean of 47,6%.

A marked variation in plasma testosterone concentration was measured in cheetah males bled for 24 h. An intramuscular injection of 50 µg GnRH was followed by a maximal testosterone concentration in the plasma 120 - 200 min later. The maximum plasma testosterone concentrations measured after 50 µg GnRH in cheetah males aged eighteen months were significantly lower than those of older animals. No differences were seen in the response



to GnRH during July and November. The testosterone response was not affected by anaesthesia or a rise in plasma cortisol concentration. There was no correlation between semen quality and the plasma testosterone response to GnRH.

The management of the captive cheetah population appears to have been the most important factor in the success achieved. The introduction of cheetah males into the female area was followed within a few weeks by oestrus in the females. Interaction between males in the group appeared to stimulate libido and cheetahs were often seen to mate soon after a male was released into the female's enclosure. Between 1975 & 1984 > 240 cheetah cubs were born from 71 litters.

## OPSOMMING

Die De Wildt Jagluiperd Teel- en Navorsingsentrum van die Nasionale Dieretuin van Suid-Afrika is gestig in 1971 met die doel om genoeg jagluiperds (Acinonyx jubatus) te produseer vir vertoning in dieretuine en ook om, indien moontlik, diere beskikbaar te stel vir vrystelling in geskikte natuurlike gebiede. Met die begin van hierdie onderneming is die teel van jagluiperds in gevangenskap as baie moeilik beskou. Die huidige studie het twee stellings ondersoek; eerstens dat gevangenskap die vrugbaarheid van jagluiperds nadelig beïnvloed en tweedens dat die geskikte bestuur van die jagluiperdpopulasie noodsaaklik sou wees om seksuele aktiwiteit te stimuleer.

Die vrugbaarheid van jagluiperdmannetjies is ondersoek en hulle is op grond van hulle semenkwaliteit gekeur vir gebruik in die teelprogram. Jagluiperdmannetjies is met 'n mengsel van ketamien en xylazien of met CT1341 geïmmobiliseer en semen is deur elektroejakulasies versamel. Semenkwaliteit is volgens standaardmetodes ge-evalueer. Vars semen is ondersoek vir motilitiet, persentasie lewendig en digtheid. Hierna is gekleurde semensmere gebruik vir die morfologiese ondersoek van spermatozoa.

Plasma-testosteroonkonsentrasies is bepaal in plasma van jagluiperdmannetjies wat elke 2 uur vir 24 h gebloei is. Hierna is die binnespiersse toediening van 50 µg van LH-vrystellingshormoon (GnRH) as 'n standaardstimulus gebruik. Plasma-testosteroonkonsentrasies, na GnRH, is bepaal in diere van



verskillende ouderdomsgroepe, gedurende verskillende tye van die jaar, in mannetjies waarvan semen deur elektro-ejakulasie versamel is en in diere met verskille in semenkwaliteit. Die invloed van 'n styging in plasma-cortisolkonsentrasies, gemeet gedurende en na elektro-ejakulasie, op plasma-LH en -testosteroon vlakke is ook bepaal.

Die bestuur van die jagluiperdpopulasie is verander deurdat heteroseksuele groepe verdeel is. Jagluiperdwyfies is alleen in afsonderlike kampe aangehou is en die mannetjies is in groepe so ver moontlik van die wyfies weggehou. Gedurende November, en 2 tot 3 maande daarna, is jagluiperdmanntjies na die gang tussen die wyfiekampe gebring. Die gedrag van die diere is dopgehou. Sodra 'n wyfie estrus getoon het is 'n mannetjie, geselekteer vir goeie semenkwaliteit, in haar kamp toegelaat.

Spermmotiliteit, persentasie lewendig en digtheid is blykbaar meer betroubaar as spermmorfologie as parameters van vrugbaarheid in jagluiperdmanntjies.

Werpsele is gebore nadat wyfies gepaar het met jagluiperd manntjies met normale spermtellings van 18 - 80%. Vyftig persent van die werpsele wat tussen 1975 en 1984 geproduseer is, is deur manntjies met normale spermtellings minder as die populasie-gemiddeld van 47,6% verwek.

Aansienlike variasies in plasma-testosteroonkonsentrasies is gemeet in die jagluiperdmanntjies wat oor 'n tydperk van 24 h gebloei is. Die binnespiersse toediening van 50 µg GnRH is gevolg deur die bereiking van die maksimum testosteroonkonsentrasie na 120 - 200 min. Maksimum plasma-testosteroonkonsentrasies na 50 µg

GnRH in 18 maand oue jagluiperdmannetjies was betekenisvol laer as die van ouer diere. Geen verskille in plasma-testosteroonkonsentrasies is getoon na GnRH in Julie en November van dieselfde jaar nie. Die plasma-testosteroonrespons is nie deur narkosemiddels beïnvloed nie. Verhogings in plasma-cortisolkonsentrasies het nie plasma-testosteroonkonsentrasies asook die plasma-testosteroonrespons op GnRH beïnvloed nie. Geen korrelasie is gevind tussen semenkwaliteit en plasma-testosteroonkonsentrasies nie.

Die bestuur van die jagluiperdpopulasie is skynbaar die deurslaggewende faktor in die teelsukses wat behaal is. Jagluiperdwyfies het estrus getoon binne 'n paar weke nadat die mannetjies in die gang tussen hulle kampe losgelaat is. Interaksie tussen mannetjies in die groep het oënskynlik libido gestimuleer en paring is telkemale gesien kort nadat mannetjies in die wyfiekampe vrygestel is. In die tydperk 1975 - 1984 is > 240 jagluiperd welpies gebore van 71 werpsels.

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# CHEETAH BREEDING

A REPORT ON THE PRETORIA NATIONAL ZOOLOGICAL GARDENS DE WILDT PROJECT

D.G.A. Mudge\*, E.J. Coetzee\*\* and A. van Dyk\*\*\*

## ACKNOWLEDGEMENTS

Dr. H.J. Brand, conceived the idea of the Cheetah breeding project, made it a reality and gave it his support and guidance. We thank him for permission to publish this article.  
Neil Wiegmann: Gave great assistance in supplying suitable literature.

## PUBLICATIONS

## SUMMARY

During April to September 1976 6 Cheetah litters were born at de Wildt, a total of 25 Cheetah cubs. This report deals with the management aspects and breeding values undertaken, from January 1974 to September 1975, which led to this success. A description of Cheetah semen and spermograms is included.

## INTRODUCTION

This project was started in 1971, on a farm in the northern foothills of the Magaliesberg range, some 20 km west of Pretoria near de Wildt, with the donation of 45 ha of Tented land by Godfrey and Ann van Dyk. The Cheetah camp consists mainly of sour Bushveld (Arack type 20 Veld types of S.A.) with tall grass and areas of dense bush, with a warm climate and summer rainfall of 100-150 mm per annum. The Cheetah population is made up of 25 adults, divided into 4 groups as in Table 1.

TABLE 1

	MALES (numbered)	FEMALES (numbered)	ARRIVAL DATES
Group 1	49-50-54	46-48-51-52-53	Original group on farm 1971 - Age 9 - 15 months
Group 2	56-57	59	Acquired Messins 56 - 1972 57 - 59 - 1973 kept at Pretoria Zoo brought to de Wildt - 1974
Group 3	64 (62-75)	61-76	S.W.A. May - 1973
Group 4		77-78	S.W.A. 9 October 1974

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D.G.A. Meltzer\*, R.I. Coubrough\*\* and A. van Dyk\*\*\*

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This project was started in 1971 on a farm in the northern foothills of the Magaliesberg range, some 20 km west of Pretoria near de Wildt, with the donation of 45 ha of fenced land by Godfrey and Ann van Dyk. The Cheetah camp consists, mainly of sour Bushveld (Acock type 20 Veld types of S.A.) with tall grass and areas of dense bush, with a warm climate and summer rainfall of 100-150 mm per annum. The Cheetah population is made up of 29 adults, divided into 4 groups as in Table 1.

TABLE 1

	MALES (numbered)	FEMALES (numbered)	ARRIVAL DATES
Group 1	49-50-54	46-48-51-52-53	Original group on farm 1971 – Age 9 – 15 months
Group 2	56-57	59	Acquired Messina 56 – 1972 57 – 59 – 1973 kept at Pretoria Zoo brought to de Wildt – 1974
Group 3	14 (62-75)	61-76	S.W.A. May - 1973
Group 4		77-78	S.W.A. 9 October 1974

\* Honorary Veterinary Surgeon, de Wildt, Transvaal

\*\* Faculty of Veterinary Science, Onderstepoort, Pretoria

\*\*\* Honorary Game Ranger, de Wildt, Transvaal

## INVESTIGATIONS INTO FERTILITY OF CHEETAH MALES

It is a well known fact that Cheetahs are poor breeders in captivity. During the period 1956 – 1974 only 140 cubs were recorded, born in captivity throughout the world.

TABLE 2 Summarises (compiled Nan Wrogeman)

TOTAL No's. RECORDED 1956 – 1974

Female 66	Males 59	Unsexed 15	Litters 44	Females 29
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Survival rates to 5 months – 57%

TABLE 3

Summary of reproductive activity observed from 1971 to March 1974

Female	Age 1st Oestrus	Dates of Oestrus	Days	Remarks
46	2	5/2/72 – 6/2/72	2	
		20/3/72 – 21/3/72	2	
		26/1/73 – 31/1/73	7	
		14/2/73 – 16/2/73	3	
		11/2/74 – 14/2/74	4	
51	2 years 8 m	21/12/72 – 22/12/72	2	
		8/ 1/73 – 13/ 1/73	6	
		25/12/73 – 28/12/73	4	
52	2	21/ 3/72	1	Pseudopregnancy developed
		18/12/73	1	
		4/ 1/74 – 11/1/74	8	
		21/ 1/74 – 24/1/74	4	
61	?	26/1/74		Males showed interest at fence Male 62 mated immediately when admitted Pseudopregnancy

The extent of the area, rough terrain and density of bush mitigated against close observation of the animals during this time. Oestrus observed due to interest shown by males and slight mucus discharge.

During February and March 1974, three (3) females (52-61-76) appeared to be pregnant. These, however, proved to be pseudopregnancies.

It was decided, at the commencement of this study that a detailed examination of the wild female and her reproduction was likely to interfere with the normal breeding cycle. The males were thus examined to determine their fertility status.



## INVESTIGATIONS INTO FERTILITY OF CHEETAH MALES:

### CLINICAL EXAMINATION

All males were examined clinically, without sedation, in a crush. Penis and testicles were examined for any visual or palpable defects followed by measurement of the testicles with a Hauptner dermal calipers.

### SUMMARY OF RESULTS

PENIS .....: CONICAL  
PENIS RETROFLEXUS 4 – 4,5 cm  
PENILE SPINES

TESTES .....: ALMOST ROUND  
EPIDIDYMIS – INDISTINCT  
17X17X17,5 mm

AVERAGE WEIGHT: 48,5 Kg (40-54,3)

### SEMEN COLLECTION AND EVALUATION

Semen was collected by drug immobilization and then electroejaculation. Immobilization using a minimum of parenteral tranquilization, sufficient only to enable capture, was followed by Halothane gas anaesthesia in open circuit. Tranquilizer, delivered either by injection or by dart using Palmer Capchur Pistol, consisted of a combination of Ketamine hydrochloride (Ketalar – Parke Davis) Xylazine (Rompun-Bayer) with the addition of Atropine Sulphate to prevent excessive salivation and emesis.

### DOSAGE RANGE

KETAMINE: 5 – 7 mg/kg  
ROMPUN: 0,7 – 1 mg/kg  
ATROPINE: 0,6 mg per Animal  
ENDOTRACHEAL TUBE: diameter 10 mm  
MEAN IMMOBILISATION TIME: 12 mins  
RECOVERY TIME: 1/2 – 3 hours.  
3 Animals developed epileptiform fits while immobilised.

### ELECTROEJACULATION

Apparatus: Rectal probe 15 cm Diameter 1 cm  
Power source: 6 volt, hand driven dynamo.  
Probe inserted into the rectum 8 – 10 cm and inclined towards the pelvic floor. Stimulation in a pattern of 3 – 5 short bursts followed by a continuous burst of 3 – 5 seconds duration, and then 3 – 5 short bursts, usually resulted in ejaculation.

Densities obtained varied from  $2 \times 10^6$ /ml to  $120 \times 10^6$ /ml

Seven samples were examined on the spot for progressive motility, an estimate of % live sperm made and pH measured using test tape. pH below 7 taken to indicate possible urine contamination.

## SPERMIOGRAMS

The spermograms of most males examined showed a very high abnormality count, despite a good motility rating in most cases. The abnormalities encountered were mostly primary, and therefore major defects generally associated with lowered fertility in other species. A common feature encountered in spermatozoa considered normal, was a marked pleomorphism in head shape. This phenomenon is often encountered in the spermograms of dogs with high fertility, and was therefore considered in this light when the semen samples from the cheetah were evaluated. Cases showing an exceptionally high abnormality count also had numerous spermatogenic cells in the ejaculate, indicating an active degeneration of the spermatogenic epithelium. In these cases cellular debris was always present in the ejaculates. Although some males showed an improved semen picture in serial collections over a few months, other did not. Thus any speculation on a seasonal influence on spermatogenesis must at this stage be reserved.

Plates 1 – 4 show the typical spermogram encountered, illustrating the high incidence of primary spermatozoan abnormalities found.





PLATE 1

Washed cheetah spermatozoa

Figure 1 n — normal; a — normal head shape with acrosome defect; p — narrow head with pyriform base; s — spermatogenic cells.

Figure 2 n — normal sperm with high protoplasmic droplet; c — various degrees of tail coiling; m — midpiece showing mitochondrial absence; c̄ — coiled tail around microhead; s — spermatogenic cell.

Figure 3 a — acrosome defect; m — midpiece with mitochondrial absence.

Figure 4 c — varying degrees of coiled spermatozoa; s — spermatogenic cells.

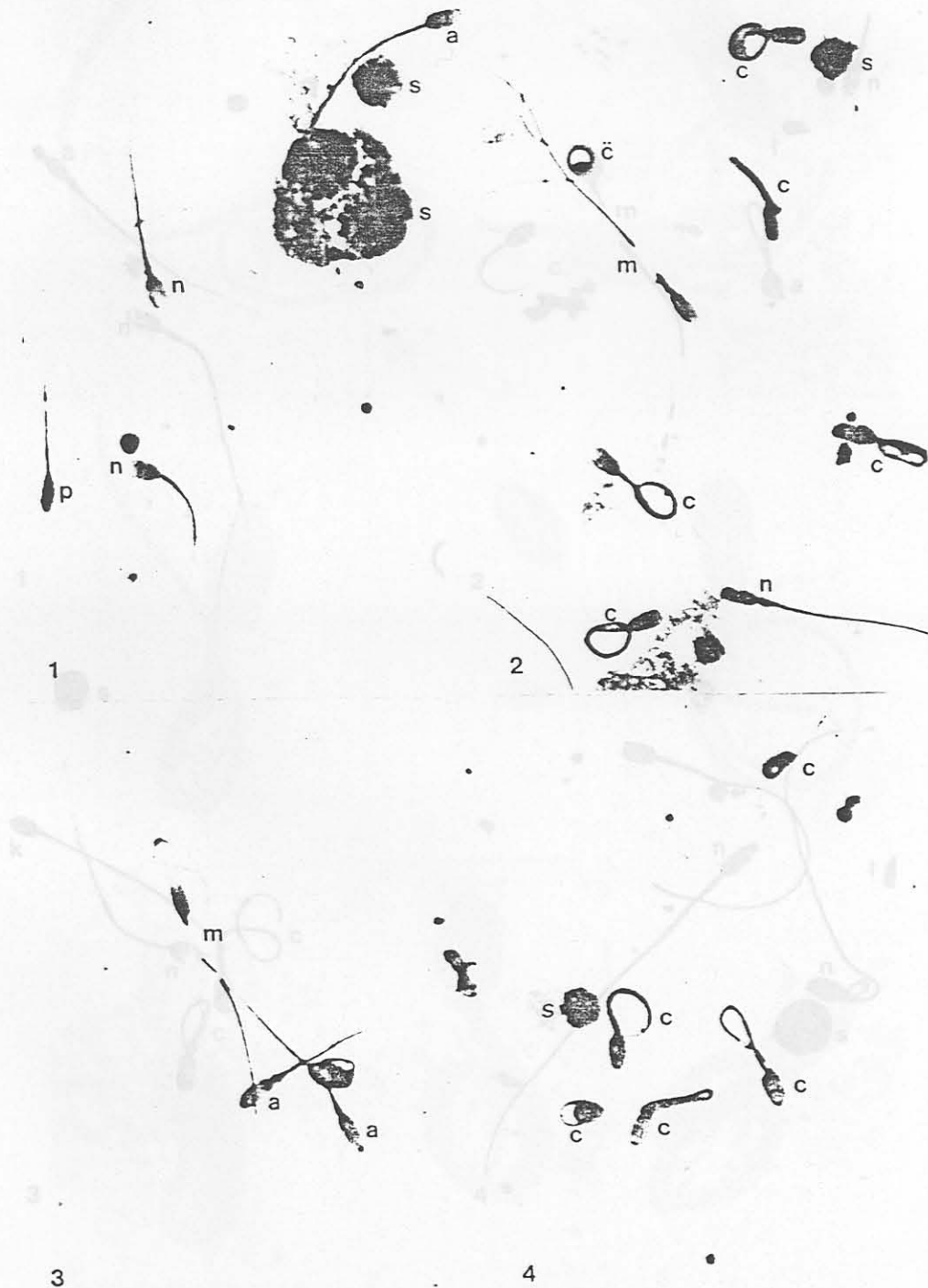


PLATE 2

Washed cheetah spermatozoa. Note mottled background due to presence of metacarpin in seminal

- Figure 1 n – normal; a – acrosome defect; m – small head with mitochondrial absence on midpiece.  
 Figure 2 n – normal; a – acrosome defect; c – coiled tail; m – mitochondrial absence on midpiece of incompletely separated small heads; l – loose head.  
 Figure 3 n – normal; c – coiled tails; k – small head; s – spermatogenic cell.  
 Figure 4 n – normal; s – spermatogenic cell; l – loose head with midpiece stump.





PLATE 3

Unwashed cheetah spermatozoa. Note mottled background due to presence of mucoprotein in seminal plasma.

Figure 1 – 8 show various degrees of midpiece and tail coiling, in many cases associated with head abnormalities. Note head pleomorphism shown

Figure 3 Microhead, absence of mitochondrial sheath around midpiece and a protoplasmic droplet of reduced volume

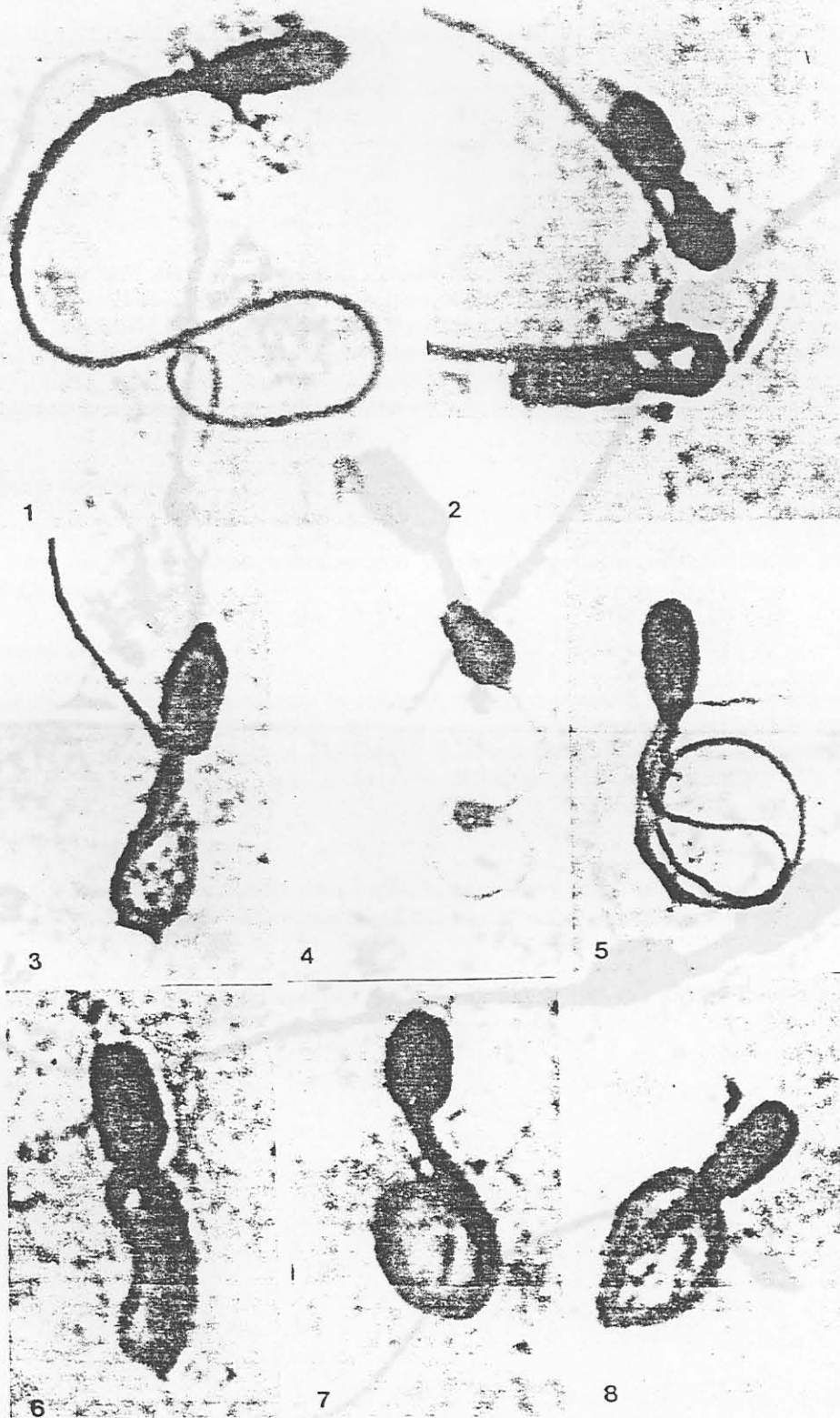


PLATE 4

Unwashed cheetah spermatozoa.

Figure 1 Note head pleomorphism. Protoplasmic droplet shown at arrow.

Figure 2 Normal sperm with degenerating protoplasmic droplet at arrow.

Figure 3 Microhead, absence of mitochondrial sheath around midpiece and a protoplasmic droplet of reduced size at arrow.

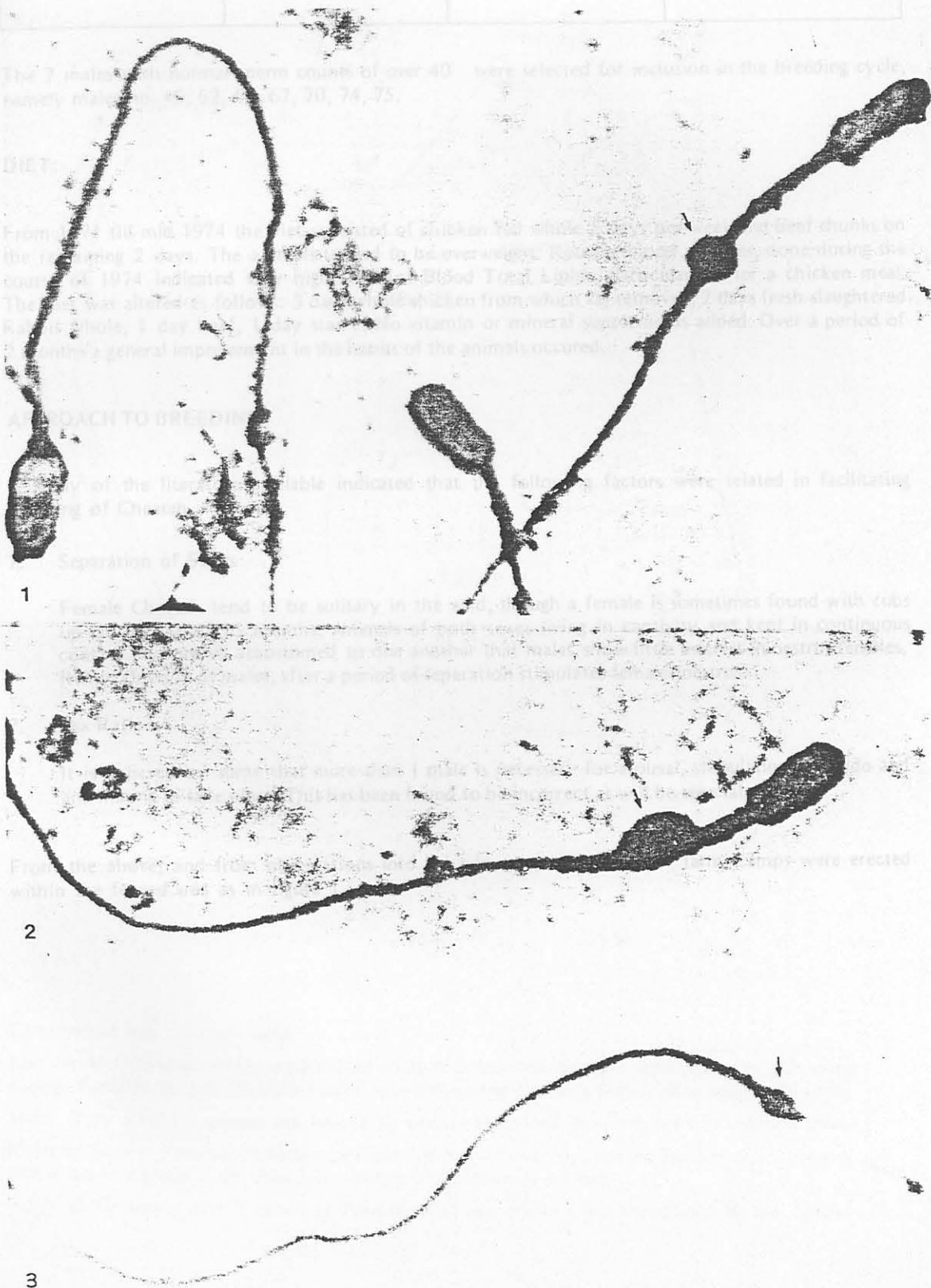




TABLE 4

Grouping of males on the basis of % normal sperm morphology

% normal sperm	Greater than 40 %	20 – 40 %	Less than 20 %
Total No. animals	7	3	9

The 7 males with normal sperm counts of over 40 % were selected for inclusion in the breeding cycle, namely males no. 49, 62, 65, 67, 70, 74, 75.

#### DIET:

From 1971 till mid 1974 the diet consisted of chicken fed whole 5 days per week and beef chunks on the remaining 2 days. The animals tended to be overweight. Routine blood analyses, done during the course of 1974 indicated very high levels of Blood Total Lipids, particularly after a chicken meal. The diet was altered as follows: 3 days whole chicken from which fat removed, 2 days fresh slaughtered Rabbit whole, 1 day beef, 1 day starve. No vitamin or mineral supplements added. Over a period of 2 months a general improvement in the habits of the animals occurred.

#### APPROACH TO BREEDING

A study of the literature available indicated that the following factors were related in facilitating breeding of Cheetah.

##### 1. Separation of Sexes

Female Cheetah tend to be solitary in the wild, though a female is sometimes found with cubs up to the age of 15 months. Animals of both sexes living in captivity and kept in continuous contact become so accustomed to one another that males show little interest in oestrus females. Reintroduction of males, after a period of separation stimulates females oestrus.

##### 2. Sex Ratio

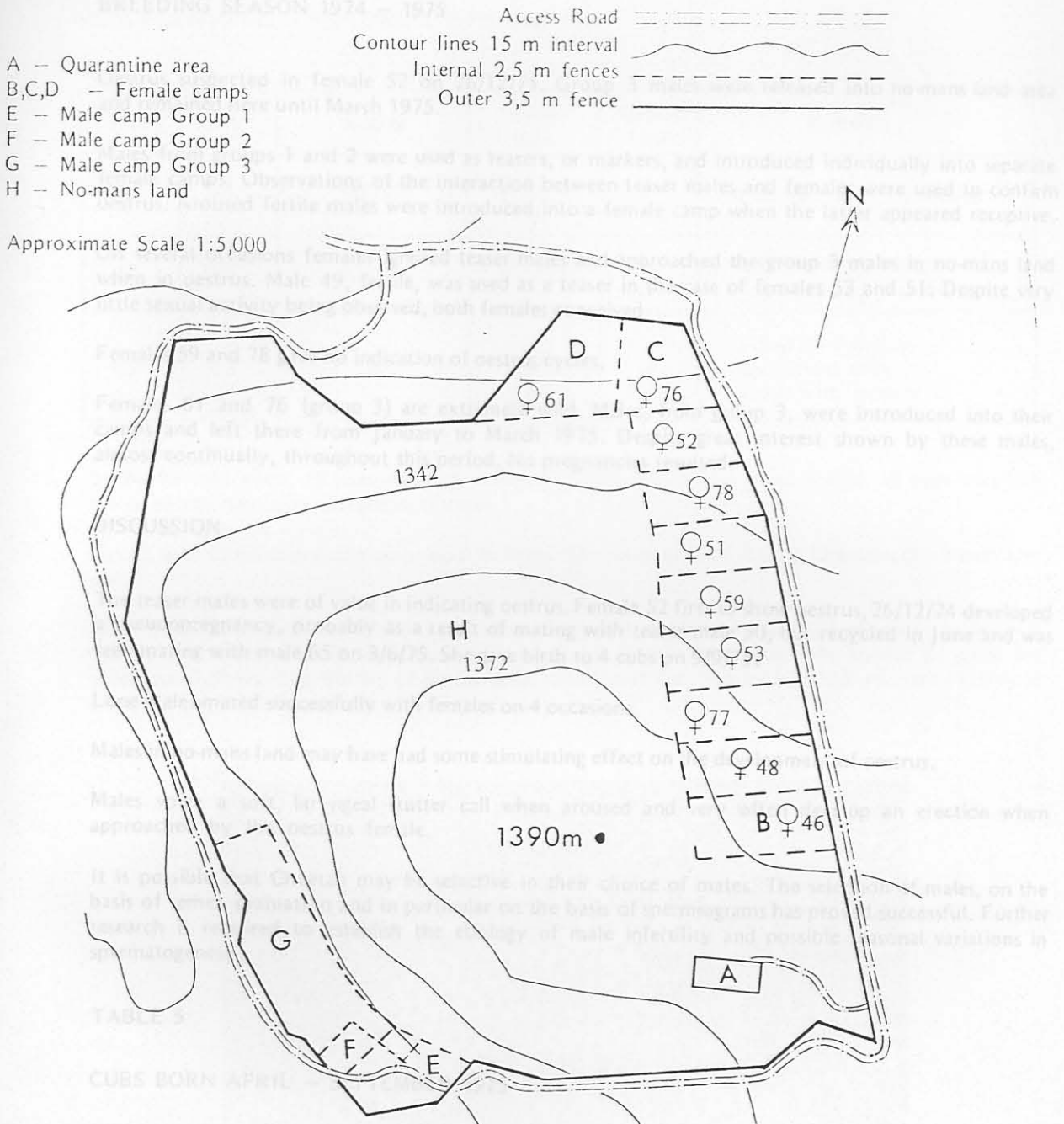
It is believed by some that more than 1 male is necessary for arousal, stimulation of libido and mating to take place. This has been found to be incorrect as will be seen later.

From the above, and from observations into behavioural patterns on the farm, camps were erected within the fenced area as in figure 1.

#### NOTE

1. Each female has her own camp.
2. Females in individual camps were placed so that females of Group 1 were not in neighbouring camps. Females 48 and 46 were placed in neighbouring camps as female 48 is extremely timid.
3. Males from different groups are hostile to one another and thus are kept in separate areas.
4. Males of Group 3 hostile to females, except females 67 and 76, attacked females not in oestrus and when in a group larger than 3 in number irrespective of oestrus.
5. Males of Groups 1 and 2 raised at Pretoria Zoo are docile when introduced to any female.

FIGURE 1



Female	Site	Mating	Birth date	Number of cubs	Remarks
53	48	Not observed	4/4/75	3	Abandoned at birth Hand reared 3
					1 eaten at birth head found
					3 died of starvation

NOTE

1. Each female has her own camp.
2. Females in individual camps were placed so that females of Group 1 were not in neighbouring camps. Females 48 and 46 were placed in neighbouring camps as female 48 is extremely timid.
3. Males from different groups are hostile to one another and thus are kept in separate areas.
4. Males of Group 3 hostile to females, except females 61 and 76, attacked females not in oestrus and when in a group larger than 3 in number irrespective of oestrus.
5. Males of Groups 1 and 2 raised at Pretoria Zoo are docile when introduced to any female.



## BREEDING SEASON 1974 – 1975

Oestrus suspected in female 52 on 26/12/74. Group 3 males were released into no-mans land area and remained here until March 1975.

Males from groups 1 and 2 were used as teasers, or markers, and introduced individually into separate female camps. Observations of the interaction between teaser males and females were used to confirm oestrus. Aroused fertile males were introduced into a female camp when the latter appeared receptive.

On several occasions females ignored teaser males and approached the group 3 males in no-mans land when in oestrus. Male 49, fertile, was used as a teaser in the case of females 53 and 51. Despite very little sexual activity being observed, both females conceived.

Females 59 and 78 gave no indication of oestrus cycles.

Females 61 and 76 (group 3) are extremely wild. Males, from group 3, were introduced into their camps and left there from January to March 1975. Despite great interest shown by these males, almost continually, throughout this period. No pregnancies resulted.

## DISCUSSION

The teaser males were of value in indicating oestrus. Female 52 first to show oestrus, 26/12/74 developed a pseudopregnancy, probably as a result of mating with teaser male 50, but recycled in June and was seen mating with male 65 on 3/6/75. She gave birth to 4 cubs on 9/9/75.

Lone males mated successfully with females on 4 occasions.

Males in no-mans land may have had some stimulating effect on the development of oestrus.

Males voice a soft, laryngeal stutter call when aroused and very often develop an erection when approached by the oestrus female.

It is possible that Cheetah may be selective in their choice of mates. The selection of males, on the basis of semen evaluation and in particular on the basis of spermograms has proved successful. Further research is required to establish the etiology of male infertility and possible seasonal variations in spermatogenesis.

TABLE 5

## CUBS BORN APRIL – SEPTEMBER 1975

Female	Sire	Mating	Birth date	Number of cubs	Remarks
53	49	Not observed	4/4/75	3	Abandoned at birth Hand reared 3
77	62	Not observed	27/4/75	3	1 eaten at birth head found 1 died at 2 weeks of aortic constriction 1 reared
51	49	Not observed	28/4/75	4	abandoned at 2 weeks of age 3 died of starvation 1 hand reared

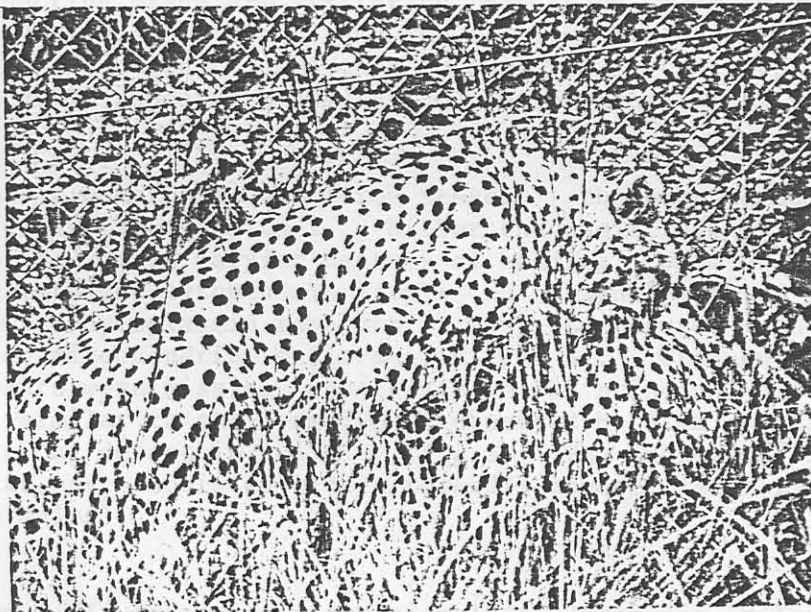
CUBS BORN APRIL – SEPTEMBER 1975 Continued

Female	Sire	Mating	Birth date	Number of cubs	Remarks
46	65	18/2/75	21/5/75	4	4 all well raised by mother
48	65	22/2/75	26/5/75	5	4 stillborn, uncleaned 1 rescued and reared
52	65	3/6/75	9/9/75	4	2 died on 2nd day insufficient milk 2 still with mother

Of the 23 cubs born, 12 have survived to date of which 6 have been hand reared, 10 cubs were lost due to abandonment, or starvation, 1 eaten.

Captive wild Cheetah are notoriously poor mothers. The value of hand-rearing Cheetah cubs is questionable.

After the first success on 4/4/75 it was felt these cubs should be reared at all costs, however if females are to be of value in a breeding programme of this magnitude they should be given the opportunity of learning the art of motherhood, albeit that the resulting high cub mortality rate is so disappointing to all concerned.



*Cheetah mating male 65 female 46*

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SOME ASPECTS OF NORMAL AND ABNORMAL SPERMATOZOA IN CHEETAH  
(ACINONYX JUBATUS)

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The ultrastructure of the mammalian spermatozoon has been well described by Fawcett<sup>4</sup>. Spermatozoon abnormalities have recently been reclassified by Blom<sup>1</sup> into major and minor categories. This paper outlines some ultrastructural characteristics of normal cheetah spermatozoa as well as some major defects encountered in semen samples collected from a group of male cheetah examined for fertility

Semen was collected by electro-ejaculation<sup>6,7</sup> from sedated or anaesthetised animals. Material was prepared for both light microscopic and ultrastructural investigations immediately after collection. Sections for electron microscopy were prepared according to the method described by Jones<sup>5</sup>

The head of cheetah spermatozoa in planar view has a compressed elliptical outline while in sagittal section the head is distinctly pear-shaped being considerably broader at the basal plate. (Fig 1) The head is thus more globular than that encountered in most other mammals. The mitochondrial sheath has a pars spiralis as well as a pars ascendens which almost extends up to the redundant nuclear membrane folds. Capitulum and connecting piece are similar to those seen in other species. No distinct annulus has been found and the fibrous sheath of the principal piece is far more delicate than that encountered in other species. It has an open grill appearance similar to that present in dog spermatozoa<sup>3</sup> (Fig 2)

Although head pleomorphism was often present the most commonly seen abnormalities involved the tail. Either the whole tail or only the principal piece showed varying degrees of bending from a simple loop through the retained cytoplasmic droplet to a tightly coiled tail again associated with cytoplasmic remnants. Many spermatozoa with this defect also had mitochondrial malformation (Fig 3). Frequently axial filament elements were missing especially in the principal piece. This defect was similar to the derangement observed by Blom and Birch-Andersen<sup>2</sup> in the Dag defect in bulls. (Fig 3) The incidence of these major defects was associated with a markedly lowered fertility in otherwise virile males<sup>6</sup>.

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3 R I Coubrough Unpublished data

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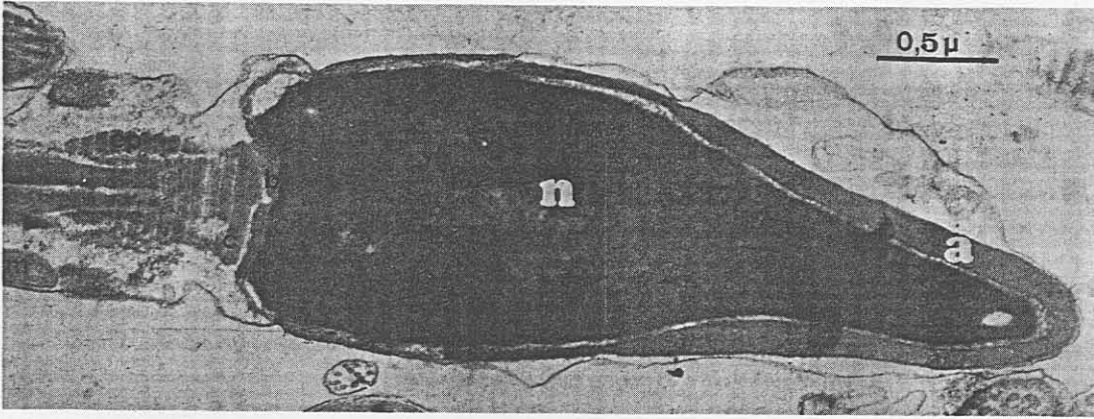


Fig 1  
Sagittal section of a cheetah sperm

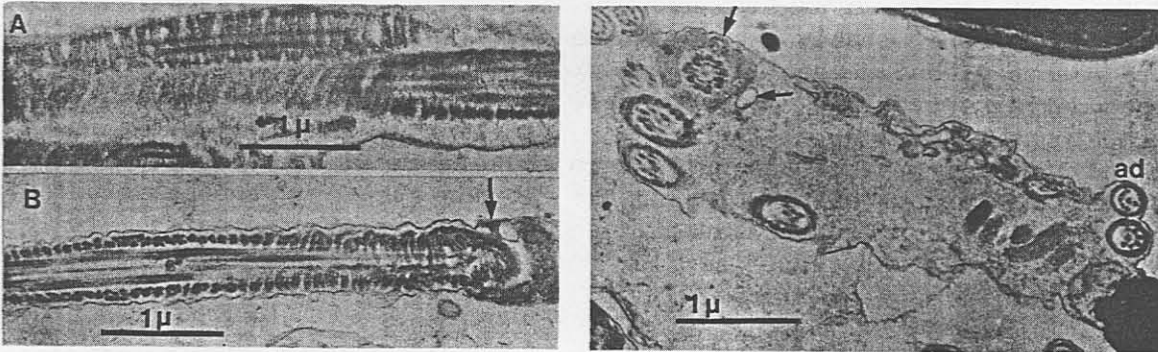


Fig 2

A & B The delicate sheath of the principal piece. An ill-defined annulus is seen at the arrow

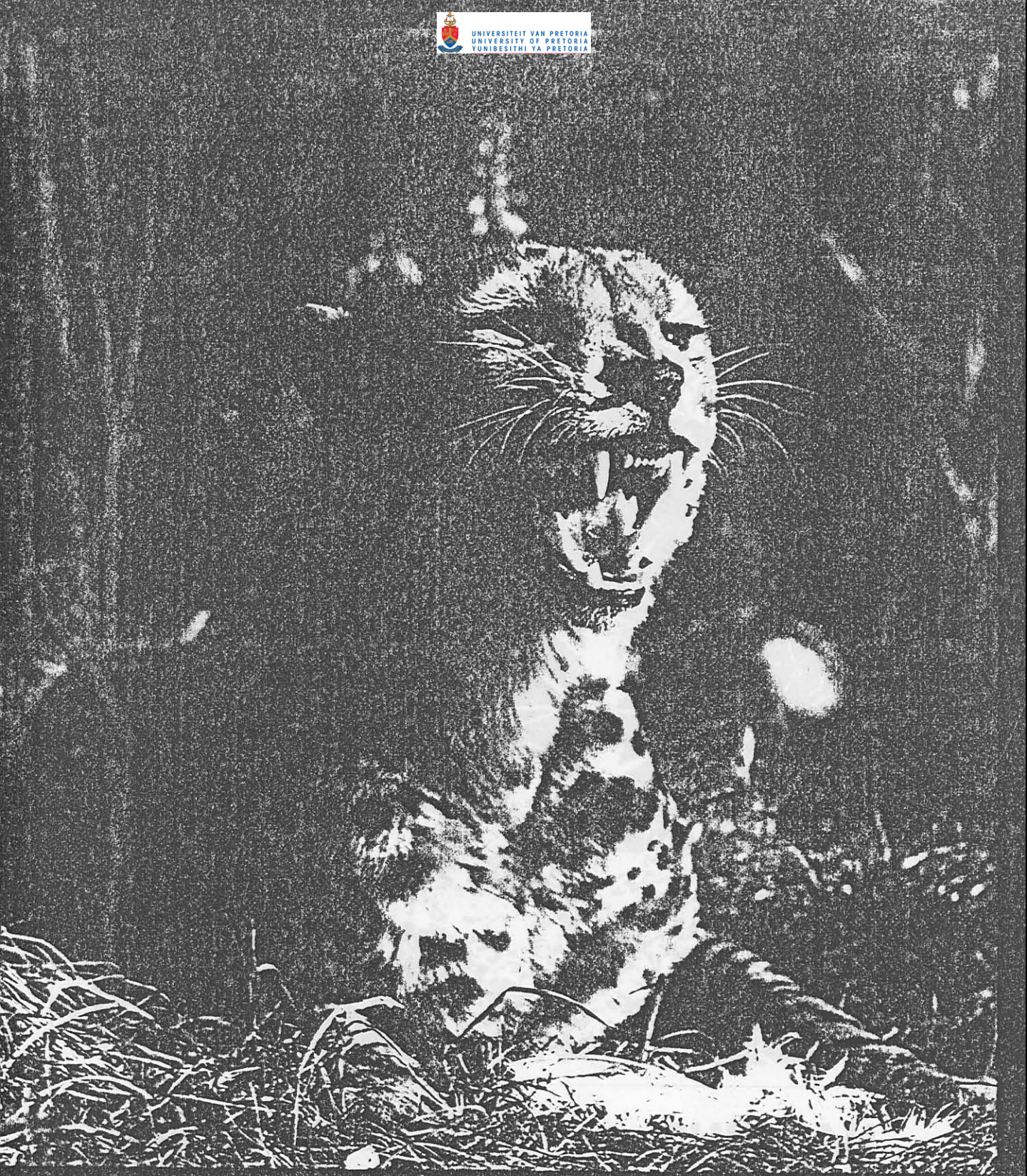
Fig 3

Axial filament derangement in a looped tail. Arrows denote mitochondrial changes

- a acrosome
- b basal plate
- c capitellum
- m mitochondria

- n nucleus
- ad axial filament derangement
- cp connecting piece





# ZOÖN

1979-1





## JAGLUIPERD-NAVORSING

Dr. D. G. A. Meltzer

Op die 1ste November 1978 is 'n groep jagluiperds in die Wes-Transvaal hervestig waar hulle alreeds vir baie jare verdwyn het. Die nuwe aankomlinge lyk nie anders as hulle voorgangers nie maar is wel besondere diere; besonders in dié sin dat hulle almal in gevangenskap geteel is van ouers wat uit die Noord-Transvaal en Suidwes-Afrika afkomstig is. Die verhaal van hierdie jagluiperds is nou gekoppel aan die stigting en latere ontwikkeling van die Nasionale Dieretuin van Suid-Afrika se Jagluiperd Navorsing- en Teelsentrum te De Wildt.

## CHEETAH RESEARCH

Dr. D. G. A. Meltzer

On the 1st November 1978, cheetahs returned to the Western Transvaal from where they had disappeared many years ago. The new arrivals appear to be no different from their predecessors. They are, however, unique in being captive-bred animals whose parents came from the Northern Transvaal and South West Africa. Their story is also the story of the establishment and development of the De Wildt Cheetah-breeding and Research Station of the National Zoological Gardens of South Africa.

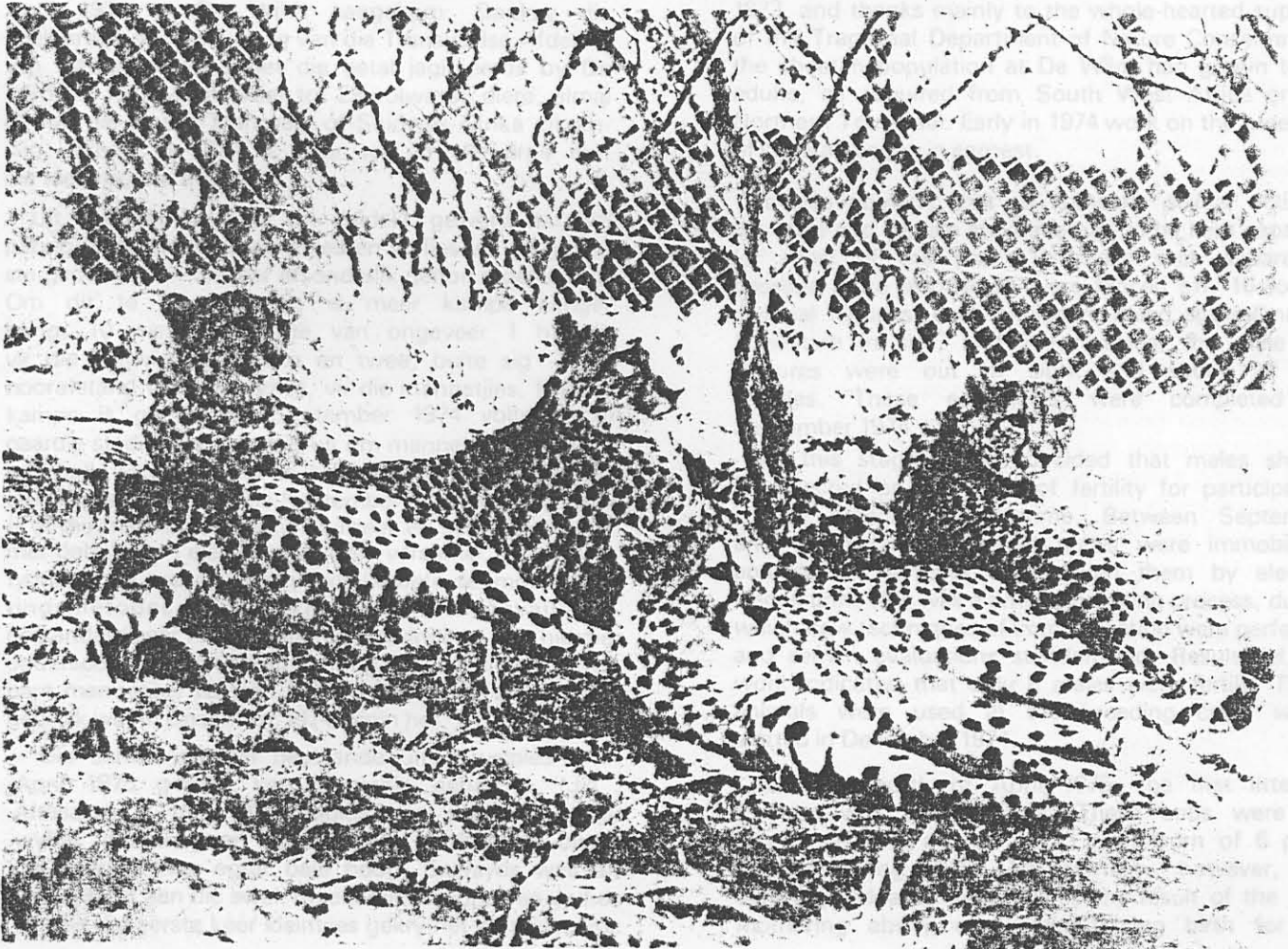


Gedurende die afgelope aantal jare het navorser op ekologiese en natuurbewaringsgebied die benarde posisie van die jagluiperd in die natuur beklemtoon. Oor die afgelope tien jaar het die aantal jagluiperds in die wêreld drasties verminder. Die faktore wat hiertoe bygedra het, is die besetting deur die mens van die gebiede waar jagluiperds nog voorkom, die vang en verkoop van hierdie diere en die aanvraag vir hulle pelse in die handel. Die hervestiging in 'n omgewing waar hulle reeds uitgesterf het, van 'n groep jagluiperds wat in gevangenskap geteel is, is dus 'n besondere prestasie van die Nasionale Dieretuin van Suid-Afrika in sy pogings om 'n skaars diersoort van uitsterwing te red.

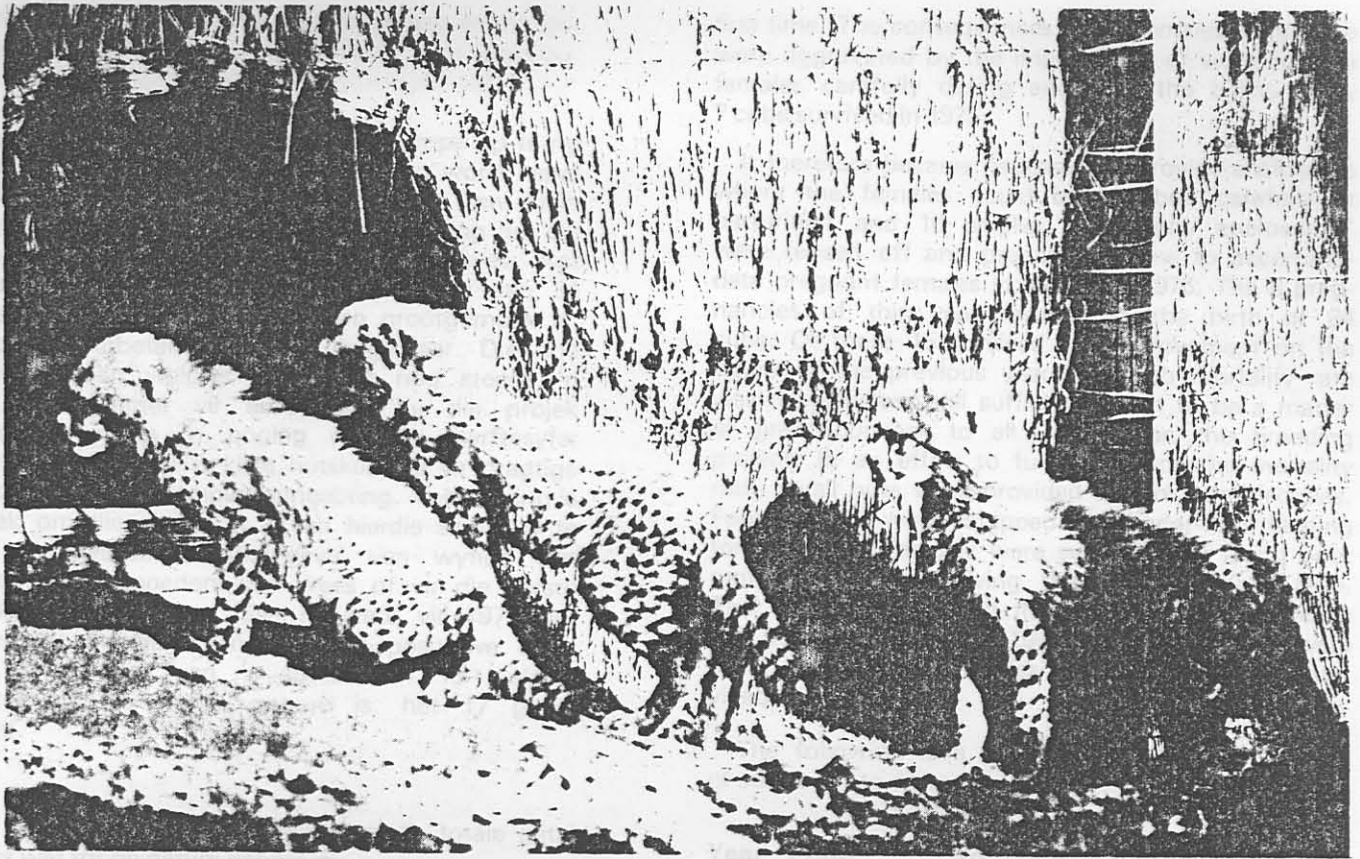
Die De Wildt Jagluiperd Navorsing- en Teelsentrum het in 1969 tot stand gekom nadat Ann en Godfrey van Dyk 50 ha van hulle plaas vir hierdie doel beskikbaar gestel het. In die laat sestiger jare was sommige persone van mening dat jagluiperds nie met sukses in gevangenskap geteel kan word nie. Dr D.J. Brand, Direkteur van die Nasionale Dieretuin, was nietemin gedetermineerd om die uitdaging te aanvaar en het besluit om voort te gaan met 'n projek om jagluiperds in gevangenskap te probeer teel. Die Van Dyks het 'n gedeelte van hulle plaas te De Wildt omhein en ook as ere-wildbewaarders opgetree. Ongelukkig sterf Godfrey in November 1976 maar Ann het voortgegaan in vermelde hoedanigheid. Haar onbaatsugtige toegewydheid, entoesiasme en aansienlike geldelike bystand was die belangrikste faktore wat tot die sukses van hierdie projek bygedra het.

recent years work in the fields of ecology and nature conservation has emphasized the plight of cheetah in the wild. During the past ten years the world population of cheetah has decreased dramatically. Man's encroachment on the cheetah's habitat, the capture and sale of live animals and the fur trade have all taken their toll of this species. The return of animals bred under artificial circumstances into an area where the species had previously occurred, is a measure of the success achieved by the National Zoological Gardens of South Africa in its efforts to save a species threatened by extinction.

The De Wildt Cheetah-breeding and Research Station was established in 1969 on 50 hectares of land offered to the National Zoological Gardens of South Africa by Ann and Godfrey van Dyk for this purpose. In the late sixties the cheetah was regarded, in some circles, as a non-breeder in captivity. Dr D.J. Brand, Director of the National Zoological Gardens, was nevertheless determined to accept the challenge afforded by the Van Dyks' offer and decided to go ahead with the project of attempting to breed cheetah in captivity. The Van Dyks fenced off a part of their farm at De Wildt and acted as honorary Game Rangers. Godfrey died in November 1976 but Ann has continued in this capacity and her dedication, energy and considerable financial support have been the principal factors in ensuring the success of the project to date.







Die eerste groep van 6 diere, waarvan die ouderdom van 9 tot 12 maande gewissel het, het gedurende April 1971 te De Wildt aangekom. Danksy die heelhartige ondersteuning van die Transvaalse Afdeling van Natuurbewaring het die getal jagluiperds by De Wildt in 1973 vermeerder tot 29 volwasse diere, almal óf van die Noord-Transvaal óf Suidwes-Afrika afkomstig. Vroeg in 1974 is daar toe in alle erns met die teelprogram begin.

Uit waarnemings het dit duidelik geword dat om paring te bevorder, mannetjies en wyfies vir die grootste gedeelte van die jaar afsonderlik gehou moes word. Om dit te bewerkstellig is meer kampe aangebring; 10 met 'n grootte van ongeveer 1 ha elk vir die individuele wyfies en twee, buite sig – en hoorafstand van die wyfies, vir die mannetjies. Hierdie kampe is gedurende September 1974 voltooi. Op daardie stadium is daar besluit om mannetjies op grond van hulle vrugbaarheid te selekteer vir gebruik in die teelprogram. Gedurende September en November 1974 is al die mannetjies geïmmobiliseer en semenmonsters met behulp van elektro-ejakulasie versamel. Dit was 'n tydrowende proses waartydens verbeterde immobiliseringsmetodes ontwikkel en semenevaluasie gestandaardiseer moes word. Die bevindings van hierdie ondersoek het aan die lig gebring dat daar slegs 8 vrugbare mannetjies was en hulle is toe in die teelsoon gebruik wat in Desember 1974 begin het.

Die eerste werpsel bestaande uit 3 welpies is in April 1975 gebore van 'n wyfie genaamd "Jill". Altesaam is daar 23 welpies van die 6 dragtige wyfies daardie jaar gebore. Die sterftesyfer onder die welpies was egter baie hoog. Enersyds was dit toe te skryf aan die swak moedereienskappe van wyfies wat vir die eerste keer kleintjies gekry het en andersyds

The first group of 6 animals, aged about 9 to 12 months, arrived at De Wildt in April 1971. By 1973, and thanks mainly to the whole-hearted support of the Transvaal Department of Nature Conservation, the cheetah population at De Wildt had grown to 29 adults, all acquired from South West Africa or the Northern Transvaal. Early in 1974 work on the breeding of cheetah began in earnest.

It was obvious that to stimulate sexual activity, the male and female cheetahs had to be kept separate for most of the year. To make such separation possible more enclosures were fenced off, 10 for individual females, each enclosure being approximately 1 hectare in size, and 2 for males; the male enclosures were out of sight and sound of the females. These enclosures were completed by September 1974.

At this stage it was decided that males should be selected on the basis of fertility for participation in the breeding programme. Between September and November, 1974, all males were immobilized and semen samples taken from them by electro-ejaculation. This was a time-consuming process, during which new techniques of immobilization were perfected and semen evaluations standardized. Results of this work indicated that only 8 males were fertile. These animals were used in the breeding cycle which started in December 1974.

On the fourth of April, 1975, the first litter of 3 cubs was born to Jill. These cubs were the fore-runners of a total of 23 cubs born of 6 pregnancies during 1975. Cub mortality, however, was very high, deaths were mainly the result of the poor mothering ability of females giving birth for the



was dit onmoontlik om die wyfies tydens en na geboorte van die welpies, behoorlik te kon dophou. Slegs 7 van die welpies het in 1975 groot geword.

Dit het gevolglik nodig geword om kampe op te rig waarin die wyfies gedurig dopgehou kon word, veral tydens die geboorte van die welpies. Tien klein kraamkampies is betyds in gereedheid gebring om die dragtige wyfies in Maart 1976 te kon huisves. Agt wyfies het in hierdie jaar geboorte gegee aan 34 welpies. Alhoewel slegs 15 hiervan grootgemaak is, was dit 'n verbetering op die vorige jaar. Die hoë sterftesyfer van welpies was egter nog steeds 'n bron van kommer vir almal wat by die projek betrokke was. In 'n poging om die sterftesyfer verder te verminder, is klein hutskuilings vir dragtige wyfies in die kraamkampies aangebring. Voorsiening is gemaak om die vloere in 4 van hierdie skuilings te verhit vir gebruik in die geval van wyfies wat moontlik swak moeders kon wees of vir die eerste keer kleintjies sou kry. Die resultate vir 1977 het getoon dat hierdie verandering 'n positiewe effek gehad het, want van 'n totaal van 19 welpies wat toe van 6 wyfies gebore is, het 17 groot geword.

Die volgende is 'n opsomming van die totale getal welpies wat tot op datum gebore is:

Jaar	Aantal dragtige wyfies	Aantal welpies gebore	Gem. werpsel-grootte	Oorlewing
1975	6	23	3,8	30%
1976	8	34	4,2	45%
1977	6	19	3,1	90%
1978	8	29	3,6	90%

'n Bemoedigende aspek van die teelprojek was die toename oor die jare in die getal wyfies wat self hulle kleintjies grootgemaak het. Gedurende 1975 is slegs 13% van die welpies deur hulle moeders grootgemaak. Die toestand het egter so verbeter dat nie minder nie as 90% van die welpies nou deur hulle moeders grootgemaak word.

Die Navorsingsentrum by De Wildt sien vandag heeltemal anders daar uit as toe die eerste kamp in 1971 daar opgerig is. Die kompleks bestaan nou uit 'n hospitaal sowel as 'n aantal kraamkampies asook kampe vir wyfies en mannetjies en die grootmaak van welpies. Verbeterde tegnieke stel die navorsingspan in staat om in een dag semen, van al die volwasse mannetjies by die sentrum te versamel. Navorsing betreffende variasies in vrugbaarheid, asook ander aspekte van die teel van hierdie bedreigde diersoort, word egter nog voortgesit.

Tot dusver is 20 jagluiperds in die Wes-Transvaal hervestig in die Lichtenburgse Natuurreservaat en Wildteelsentrum van die Nasionale Dieretuin. Hierdie Reservaat is oop vir die publiek.

first time. The consequences of the females' ineptitude were aggravated by the impossibility of watching the females carefully during and after the births. Only 7 cubs survived in 1975.

It therefore became necessary to provide enclosures where the females could be watched carefully at parturition and 10 smaller, "maternity enclosures" were fenced off and prepared in time to accommodate pregnant females in March of 1976. The 8 pregnancies of this year resulted in the birth of 34 cubs. Of these 15 survived, an improvement on the figure of the previous year. The cub mortality rate was nevertheless still sufficiently high to be a matter of great concern to all working on the breeding project. In an effort to further reduce the mortality rate, small huts were provided for pregnant females. Four of these were equipped with under-floor heating for use when females were suspected of being poor mothers or were giving birth for the first time. The 1977 results show that the new arrangements had a positive effect. In 1977 a total of 19 cubs were born from 6 pregnancies and of these 17 survived.

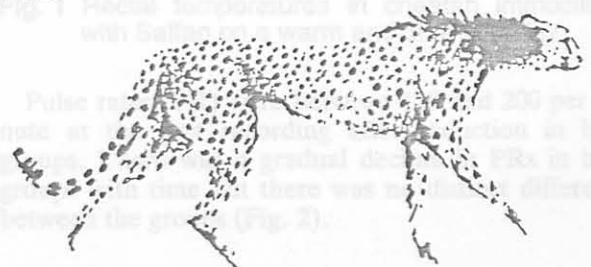
The following is a summary of the total number of cubs born to date:

Year	Pregnancies	No. cubs born	Average litter size	Survival rates
1975	6	23	3,8	30%
1976	8	34	4,2	45%
1977	6	19	3,1	90%
1978	8	29	3,6	90%

An encouraging feature of the breeding project has been the improvement, over the years, in maternal rearing. During 1975 only 13% of cubs born were reared by their mothers. The position has improved to the extent that at present 90% of cubs born are reared by their mothers.

Today the Station at De Wildt bears no resemblance to the first enclosure erected there in 1971. The complex now consists of a hospital as well as maternity, female, male and cub-rearing enclosures. New techniques enable the research team to collect semen in one day from all the mature males at the establishment. Research into fertility variations continues, as do other projects aimed at the propagation of this threatened species.

To date 20 cheetah have been re-established in the Western Transvaal at the Lichtenburg Nature Reserve and Game Breeding Centre of the National Zoological Gardens. This Reserve is open to the public.





SAFFAN INDUCED POIKILOThERMIA IN CHEETAH (*ACINONYX JUBATUS*)

C. BUTTON\*, D.G.A. MELTZER\* and MARIA S.G. MÜLDERS\*

ABSTRACT: Button C., Meltzer D.G.A., Mülders M.S.G. *Saffan induced poikilothermia in cheetah (Acinonyx jubatus)*. *Journal of the South African Veterinary Association* (1981) 52 No. 3 237-238 (En) Department of Physiology, Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa.

The steroidal anaesthetic agent Saffan (a 1,2% m/v mixture of alphaxalone and alphadolone) induced a state of poikilothermia in cheetahs. On a warm day (maximum temperature 29° C) rectal temperatures rose in 7 of 8 male cheetahs given Saffan. The highest rectal temperature recorded was 41° C. On a cool day (minimum temperature 19,5° C) rectal temperatures fell in 6 of 6 male cheetahs. The lowest rectal temperatures recorded was 36,2° C. Saffan at 3 mg/kg intravenously in cheetahs is an excellent and safe hypnotic but should be used with caution on both hot and cold days

INTRODUCTION

Saffan (CT 1341, Althesin, Glaxo Labs) is an injectable steroid sedative, hypnotic or anaesthetic drug recommended for use in domestic cats and monkeys<sup>1-3</sup>. The injectable saline solution comprises a 1,2% m/v mixture of 2 steroids, alphaxalone or steroid I (0,9% m/v) and alphadolone or steroid II (0,3% m/v). Polyoxyethylated castor oil (20% m/v), included in the solution as a solubilizer, is a potent histamine releasing agent in dogs and, occasionally, in the domestic cat.

One of us (DGAM) has been using Saffan for immobilizing cheetahs (*Acinonyx jubatus*) for 4 years. More than 250 hypnotic administrations of Saffan have been made with no associated deaths.

Recently we decided for general interest, to monitor rectal temperatures and pulse and respiratory rates (TPR) in Saffan hypnotised cheetahs. The results indicate that Saffan has a poikilothermic effect in this species.

MATERIALS AND METHODS

Two groups of male cheetahs, comprising 8 and 6 animals respectively, were given hypnotic doses of Saffan on 2 days, one week apart. On the first occasion the weather was warm and sunny (26° C at 10h15 rising to 29° C at 12h21) and on the second it was cool and overcast (19,5° C at 09h05 rising to 20° at 10h05).

Individual cheetahs were herded into mesh covered chutes where they were held to the ground by passing narrow poles through the chute mesh. A fore or rear leg was pulled through the mesh for an injection of Saffan into the cephalic or recurrent tarsal vein. Between 8 and 10 ml were injected over approximately 15 seconds. Retrospective calculations showed that between 2,2 and 3,3 mg/kg combined steroids had been injected into the 14 male cheetahs which averaged 43,3 ± 4,1 kg body mass.

After induction, they were moved to a table where initial TPR were recorded before anatomical measure-

ments were made and semen was collected by electroejaculation. The animals were then carried under the shade of nearby trees, laid in lateral recumbency and an electrocardiogram was recorded. TPRs were noted as often as possible, but practical difficulties prevented recordings being made at fixed and regular time intervals.

RESULTS

On the warm day rectal temperatures (RT) rose in 7 of 8 cheetahs. In one animal RT reached a high of 41° (105,8° F) 42 minutes post induction. RTs peaked at between 30 and 80 minutes post induction, and then gradually declined (Fig. 1). On the cool day, RTs fell in all 6 cheetahs. The lowest RT recorded was 36,2° C (97,2° F) 161 minutes post induction. On both days, animals which had to be chased had higher RTs than those which entered the chute more calmly.

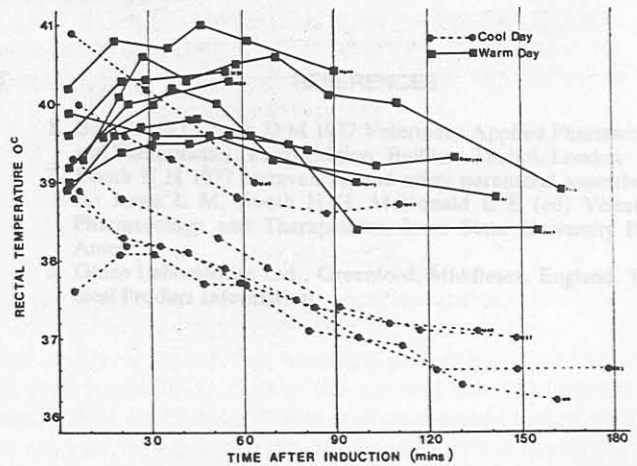
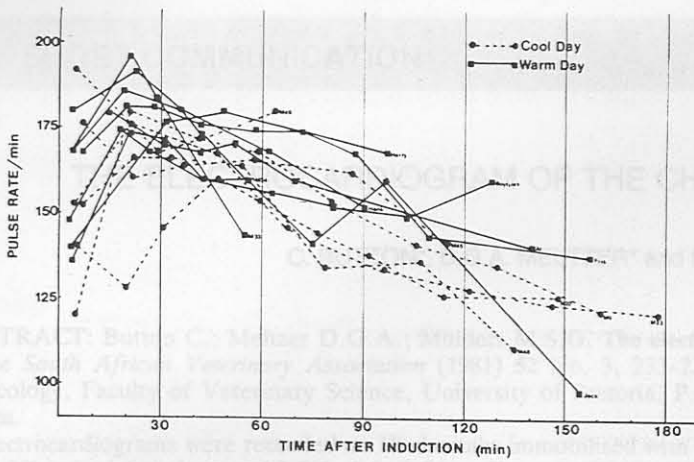


Fig. 1 Rectal temperatures in cheetah immobilized with Saffan on a warm and on a cool day

Pulse rates (PR) were between 120 and 200 per minute at the first recording after induction in both groups. There was a gradual decline in PRs in both groups with time but there was no distinct difference between the groups (Fig. 2).

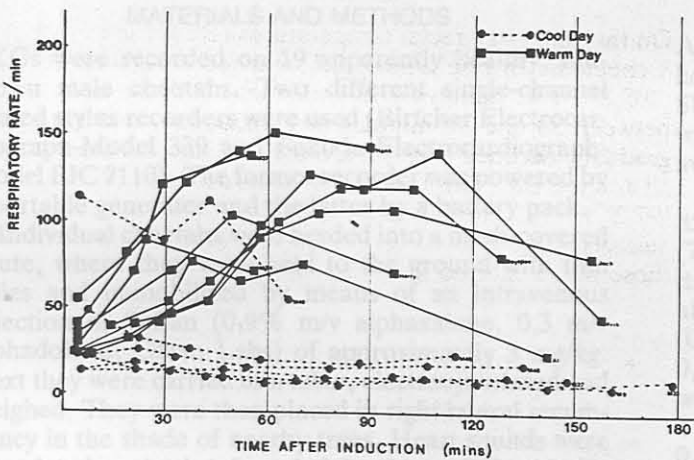
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**Fig. 2** Pulse rates in cheetah immobilized with Saffan on a warm and on a cool day

Respiratory rates (RR) (Fig. 3), not surprisingly followed RT closely. Animals with RTs greater than  $39,5^{\circ}\text{C}$  with few exceptions had RRs of more than 30 per minute. A maximum RR of 152 per minute was recorded on the warm day in a cheetah at 62 minutes post induction with a RT of  $40,8^{\circ}\text{C}$ . On the cool day, only 2 of 6 cheetahs had RRs of more than 30 per minute. Both of these had been very excited before induction, and both had high RTs immediately after induction ( $40,9$  and  $40^{\circ}\text{C}$ ).



**Fig. 3** Respiratory rates in cheetah immobilized with Saffan on a warm and on a cool day

#### DISCUSSION

The poikilothermic effects of Saffan on cheetah are

presumed to be the result of suppression of hypothalamic thermoregulatory areas. This effect of Saffan has not, to our knowledge, been responsible for the death of any cheetahs but obviously it has that potential. The environmental temperatures ( $19,5\text{--}20^{\circ}\text{C}$  and  $26\text{--}29^{\circ}\text{C}$ ) in this report are mild in comparison to the  $37$  to  $43^{\circ}$  experienced in some parts of the country during summer months. It would be advisable to anaesthetize cheetahs with Saffan during cooler parts of the day and to monitor RTs when environmental temperatures are above  $25^{\circ}\text{C}$ . Likewise it would be advisable to avoid anaesthetizing them in cold weather or, at least, to take steps to minimize heat loss.

The dose of Saffan in cheetahs which has been found safe and effective for minor procedures is approximately  $3\text{ mg/kg}$  by intravenous injection. At this dosage they show hypnosis (deep sleep) but can be partly roused by noxious stimuli, e.g. electroejaculation. When left alone they remain recumbent for between 1 and 3 hours. In contrast the recommended intravenous dose for a healthy domestic cat is  $9\text{ mg/kg}$  which gives approximately 10 minutes of surgical anaesthesia.

We conclude that Saffan is a safe and effective hypnotic when administered intravenously to cheetahs at approximately  $3\text{ mg/kg}$ , but caution that rectal temperature should be monitored when environmental temperatures are extreme. We have never observed the histamine-release phenomenon in cheetahs at the above dose range. Intramuscular administration of Saffan is less satisfactory because larger doses have to be given to produce adequate hypnosis and the larger volume is less easily administered.

#### ACKNOWLEDGEMENTS

The authors thank Dr D.J. Brand of the National Zoological Gardens in Pretoria for the opportunity to carry out this study. Miss Ann van Dyk, warden of the De Wildt Cheetah Research and Breeding Station of the National Zoological Gardens is thanked for her enthusiastic support.

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THE ELECTROCARDIOGRAM OF THE CHEETAH (*ACINONYX JUBATUS*)

C. BUTTON\*, D.G.A. MELTZER\* and MARIA S.G. MÜLDERS\*

**ABSTRACT:** Button C.; Meltzer D.G.A.; Mülders M.S.G. **The electrocardiogram of the cheetah (*Acinonyx jubatus*).** *Journal of the South African Veterinary Association* (1981) 52 No. 3, 233-235 (En) Department of Physiology, Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa.

Electrocardiograms were recorded on 19 cheetahs immobilized with the steroidal anaesthetic-hypnotic agent Saffan comprising 0,9% m/v alphaxalone and 0,3% alphadolone. Sinus rhythm was recorded in all animals and heart rate was rapid averaging  $173 \pm \text{SD } 18$  beats per minute. The average of mean electrical axes in the frontal plane was  $+76^\circ \pm \text{SD } 13^\circ$ . Mean  $\pm$  SD durations in milliseconds on lead II were: P  $47 \pm 6,5$ ; PR  $93 \pm 11,5$ ; QRS  $53 \pm 7,5$ ; QT  $193 \pm 19,7$ . The amplitude of limb lead electrocardiographic complexes were low, resembling those of the domestic cat more closely than those of the dog.

## INTRODUCTION

The electrocardiogram (EKG) is a useful clinical and research tool for the evaluation of cardiac rate, rhythm, and conduction. It may be of value for determining chamber enlargement (hypertrophy and/or dilatation).

To provide normal baseline values, we recorded electrocardiograms (EKGs) on cheetahs in the field.

## MATERIALS AND METHODS

EKGs were recorded on 19 apparently healthy, full-grown male cheetahs. Two different single-channel heated stylus recorders were used (Birtcher Electrocardiograph-Model 339 and Fukuda Electrocardiograph-Model FJC 7110). The former recorder was powered by a portable generator and the latter by a battery pack.

Individual cheetahs were herded into a mesh-covered chute, where they were held to the ground with thin poles and immobilized by means of an intravenous injection of Saffan (0,9% m/v alphaxalone, 0,3 m/v alphadolone, Glaxo Labs) of approximately 3 mg/kg. Next they were carried to a table, electroejaculated and weighed. They were then placed in right lateral recumbency in the shade of nearby trees. Heart sounds were auscultated on both left and right sides of the thorax. Alligator clip electrodes were placed on electrode-paste prepared skin just below the elbows and stifle joints, in the 6th left lower intercostal space over the cardiac apical impulse ( $\text{CV}_6\text{LL}$ ) and on the dorsal midline just behind the scapulae ( $\text{V}_{10}$ ).

Standard bipolar limb leads I, II and III; augmented unipolar limb leads aVR, aVL, and aVF; and unipolar leads  $\text{CV}_6\text{LL}$  and  $\text{V}_{10}$  were recorded at a calibration of 10mm equal to 1mV at paper speeds of 25 and (in most instances) also 50 mm/s.

Cardiac rate was calculated from the mean RR interval on lead II, and the mean electrical axis (MEA) was calculated using standard methods<sup>1,2</sup>. Configurations of wave forms were noted for all leads, using lower case letters q, r and s if the deflection was less than 0,5 mV and capital letters Q, R and S if the deflection was 0,5 mV or more.

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Amplitudes of the P wave, QRS complex and T wave were measured on lead II to the nearest 0,5 mm (0,05 mV) with the aid of an illuminated magnifying viewer. Durations of P, PR, QRS and QT were likewise measured to the nearest 0,5 mm (10 ms) on 50 mm/s strips for lead II. At least 5 complexes were measured to determine amplitudes and durations.

## RESULTS

All the cheetahs were in sinus rhythm and had normal heart sounds. Most had rapid heart rates: mean  $173 \pm \text{SD } 18$  beats per minute with a range of 124-195. There was no sign of sinus arrhythmia at this heart rate. The average MEA was  $76 \pm \text{SD } 13^\circ$  with a range of 48-94°.

On lead II for 12 cheetahs durations were: P wave  $47 \pm \text{SD } 6,5$  ms (40-60); PR interval  $93 \pm \text{SD } 11,5$  ms (70-110); QRS  $53 \pm \text{SD } 7,5$  ms (40-60) and QT was  $193 \pm \text{SD } 19,7$  ms (160-230). The P wave on lead II was always positive averaging  $0,18 \pm \text{SD } 0,05$  mV (0,10-0,25) N = 19. Q and q waves on lead II averaged  $0,13 \pm \text{SD } 0,09$  mV (0,05-0,4) N = 14; and R and r waves averaged  $0,81 \pm \text{SD } 0,24$  mV (0,45-1,3) N = 19. T waves were usually negative on lead II, averaging  $0,11 \pm \text{SD } 0,06$  mV (0,05-0,25) N = 14.

The configuration and polarity of P waves, QRS complexes and T waves are detailed in tables 1-3 respectively and representative electrocardiographic complexes for 12 cheetah are reproduced in figure 1.

## DISCUSSION

The results indicate that myocardial conduction of the cheetah is similar to that of the cat and the dog. Atrial depolarization (Table 1) is in a net leftward (positive P waves I and  $\text{CV}_6\text{LL}$ ), backward (positive P waves on II, III and aVF, negative P waves on aVR and aVL) and downward (negative P waves on  $\text{V}_{10}$ ) direction. The major forces of ventricular depolarization (Table 2) are likewise leftward (predominance of r waves on I and R waves on  $\text{CV}_6\text{LL}$ ), backward (predominance of R waves on II, III and aVF, predominance of S and s waves on aVR and aVL) and downward (predominance of Q and q waves on  $\text{V}_{10}$ ). The leftward and backward orientation of ventricular depolarization is



Table 1: CONFIGURATION OF P WAVES ON THE VARIOUS LEADS

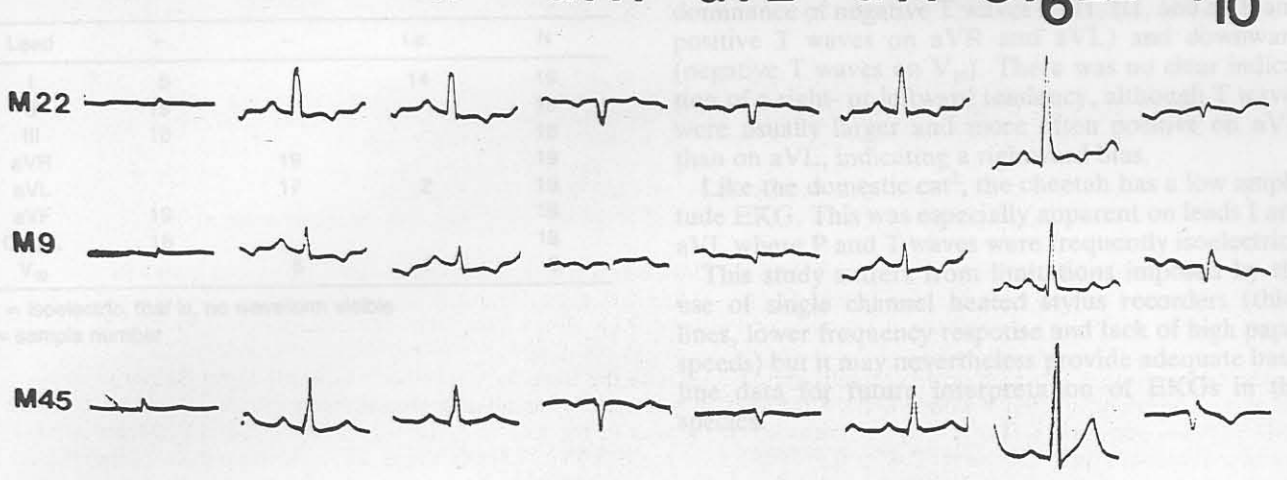


Table 2: CONFIGURATION OF QRS COMPLEXES ON THE VARIOUS LEADS

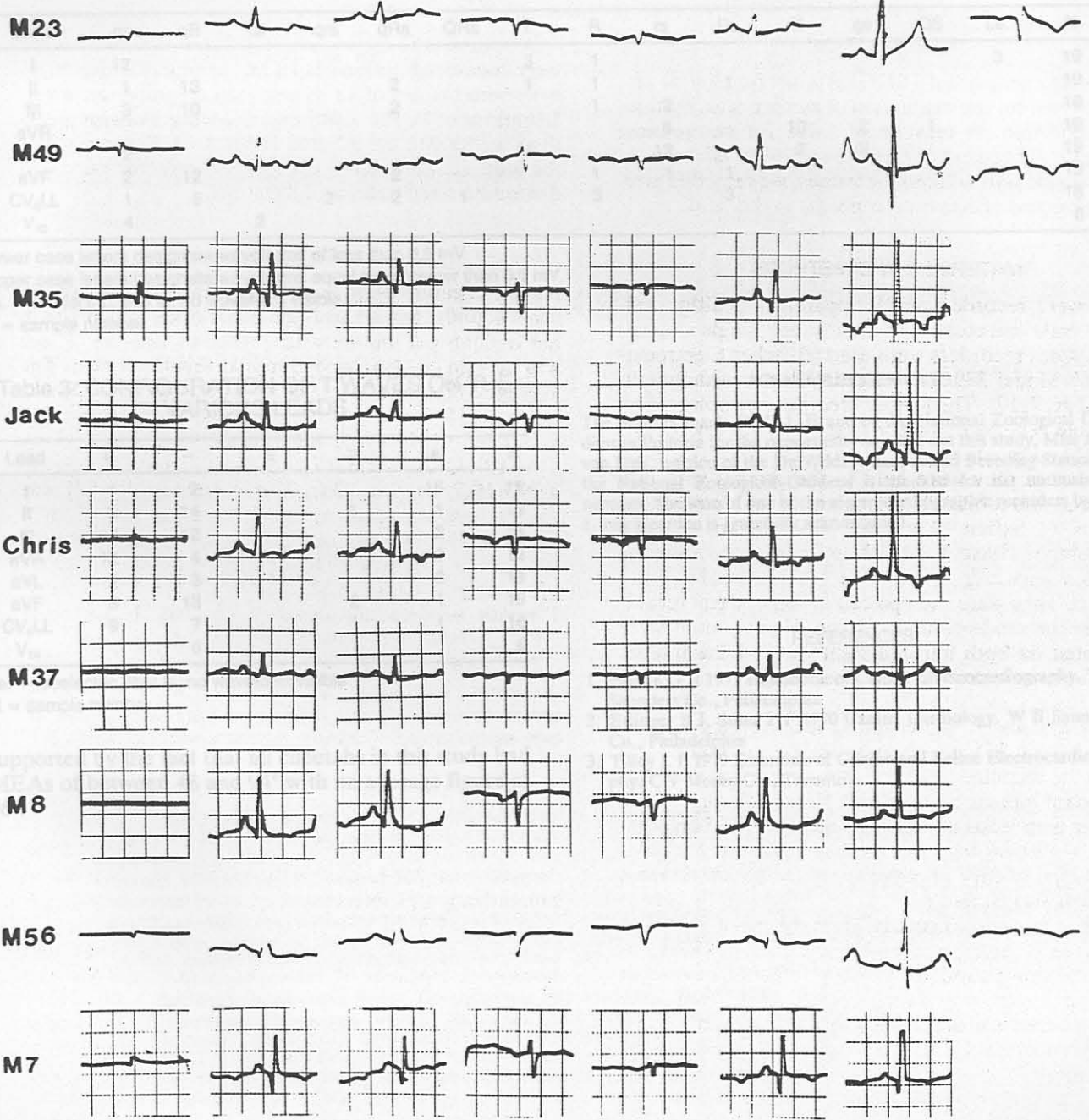


Fig. 1 Representative electrocardiographic complexes for various leads recorded at 50 mm/s for 12 cheetah. 1 cm = 1mV



Table 1: CONFIGURATION OF P WAVES ON VARIOUS LEADS

Lead	+	-	i.e.	N
I	5		14	19
II	19			19
III	18			18
aVR		19		19
aVL		17	2	19
aVF	19			19
CV <sub>6</sub> LL	18			18
V <sub>10</sub>		5	1	6

i.e. = isoelectric, that is, no waveform visible  
N = sample number

The major forces of ventricular repolarization were more varied (Table 3) and were mostly forward (predominance of negative T waves on II, III, and aVF and positive T waves on aVR and aVL) and downward (negative T waves on V<sub>10</sub>). There was no clear indication of a right- or leftward tendency, although T waves were usually larger and more often positive on aVR than on aVL, indicating a rightward bias.

Like the domestic cat<sup>3</sup>, the cheetah has a low amplitude EKG. This was especially apparent on leads I and aVL where P and T waves were frequently isoelectric.

This study suffers from limitations imposed by the use of single channel heated stylus recorders (thick lines, lower frequency response and lack of high paper speeds) but it may nevertheless provide adequate baseline data for future interpretation of EKGs in this species.

Table 2: CONFIGURATION OF QRS COMPLEXES ON THE VARIOUS LEADS

Lead	qr	qR	Qr	qrs	qRs	QRs	r	R	rs	Rs	rS	qs	QS	i.e.	N
I	12						3	1						3	19
II	1	13			2		1	1		1					19
III	3	10			2			1	2						18
aVR									6		10	2	1		19
aVL	2						1		12		2	2			19
aVF	2	12			2			1	1	1					19
CV <sub>6</sub> LL	1	6		2	2	1		3		3					18
V <sub>10</sub>	4		2												6

Lower case letters designate waveforms of less than 0,5 mV  
Upper case letters designate waveforms equal to or greater than 0,5 mV  
i.e. = isoelectric, that is, no waveform visible  
N = sample number

Table 3: CONFIGURATION OF T WAVES ON THE VARIOUS LEADS

Lead	+	-	±	∓	i.e.	N
I	1	2			16	19
II	3	14		1	1	19
III	4	12			2	18
aVR	12	4	1		3	19
aVL	7	3	1		8	19
aVF	3	13		2	1	19
CV <sub>6</sub> LL	9	7		1	1	18
V <sub>10</sub>		6				6

i.e. = isoelectric, that is, no waveform visible  
N = sample number

supported by the fact that all cheetahs in this study had MEAs of between 48 and 94° with an average figure of 76°.

ACKNOWLEDGEMENTS

The authors thank Dr D.J. Brand of the National Zoological Gardens in Pretoria for the opportunity to carry out this study. Miss Ann van Dyk, warden of the De Wildt Research and Breeding Station of the National Zoological Gardens is thanked for her enthusiastic support. The loan of one of the electrocardiographic recorders by Dr J. van Heerden is gratefully acknowledged.

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The initial microscopic examination of the fresh smears revealed a moderate to abundant sperm force. This feature was previously reported by earlier workers (Coulter, 1964; De Wildt Breeding Centre, 1971). Upon evaluation of the fixed mounts, an average of 30% of such ejaculate contained morphologically abnormal spermatozoa. In domestic animals, the presence of greater than 40% abnormalities is associated with reduced fertility (4). Recent unpublished data from our laboratory indicate that the high percentage of abnormal sperm observed in the cheetah is not present in domestic cat semen using the same classification procedure and fixation. The average abnormal sperm per ejaculate in the domestic cats collected was less than 20%.

1. National Zoological Park, Smithsonian Institution, Washington, D.C.
2. Veterinary Resources Branch, National Institutes of Health, Bethesda, MD.
3. National Zoological Gardens of South Africa, Pretoria, South Africa.
4. De Wildt Cheetah Breeding and Research Centre, De Wildt, South Africa.
5. College of Veterinary Medicine, Onderstepoort, South Africa.



in Calgary, New York or Chicago, and they tend to be more than a little damp in Portland and Seattle. But in the first year at the start of the project there were 34 people and at the end four months later, 33 were still on board.

There are side benefits, too. Keepers and staff who became involved in the research said it gave them a broader view of their work and elevated their job satisfaction. Also, keepers felt they could be of more assistance to the veterinarian because of their increased ability to pinpoint and notice early-on problems occurring in an individual cat or a pair.

So in many ways we are learning about snow leopards.

#### Preliminary Reproductive Physiology Studies on Cheetahs in South Africa

Jo Gayle Howard,<sup>1</sup> Mitchell Bush,<sup>1</sup> David Wildt,<sup>2</sup> D. J. Brand,<sup>3</sup>  
H. Ebedes,<sup>3</sup> A. van Dyk,<sup>4</sup> and D. Meltzer<sup>5</sup>

The cheetah (*Acinonyx jubatus*) is threatened with extinction and the survival of the species may depend on intensified natural and artificial breeding in captivity. Cheetah conservation and propagation are being studied at the National Zoological Gardens of South Africa. In an ongoing collaborative research project with the National Zoological Park (Washington, D.C.), data on the reproductive physiology of the cheetah were collected at two facilities in January, 1981: 1) DeWildt Cheetah Breeding and Research Centre, and 2) Lichtenburg Game Preserve, both located in the Transvaal Province of the Republic of South Africa. The project was designed to increase the scientific knowledge on various reproductive parameters in captive male and female cheetahs and to import frozen cheetah semen to the United States for use in captive propagation studies. This report only concerns the reproductive traits on the semen collected by electroejaculation. The relationship of semen quality was compared with the various cheetah subspecies and also with the available size of the animal enclosures at the two facilities.

The cheetah has evolved independently in the cat family, being the only member of the genus *Acinonyx*. There is controversy regarding the number of subspecies described for the cheetah. Ellerman lists only two subspecies: an African, *Acinonyx jubatus jubatus*; and an Asian, *A. jubatus venaticus* (1). Eaton describes additional subspecies that have been classified by various authors according to more specific geographical locations; however, he acknowledges the fact that much of the subspeciation may have been artificially classified due to distinct phenotypic characteristics (2). The subspecies under consideration at the two South African breeding facilities include three types: Transvaal, South West, and hybrids of Transvaal-South West ancestry.

#### Semen Collection and Evaluation

Adult cheetahs were anesthetized with intravenous Saffan (2.0 mg/kg body weight, Glaxo Laboratories England) and placed in lateral recumbency for ejaculation procedure. A lubricated Teflon rectal probe (1.6 cm diameter, 20 cm long) containing 3 raised longitudinal stainless electrodes (6.5 cm each in length, 0.3 cm in width) was positioned in the rectum with the electrodes placed ventrally. A standard protocol of 80 stimulations per animal, divided into 3 sets, was used so that ejaculate quality could be compared between males and also on the same cheetah at different collections. Some males were ejaculated up to 4 times with a 2- to 7-day interval between collections. The electroejaculator (PT Electronics, College Station, Texas) was capable of monitoring voltage and amperage and used AC current of 120 V, with a transformer producing a maximum of 60 V and 1 A. The voltage and amperage used during electroejaculation ranged from 4-7 volts and 50-200 mA.

A total of 22 males was used to provide 53 ejaculates. Semen was immediately examined for traditional reproductive traits and an aliquot was fixed in 1% glutaraldehyde for morphological evaluation in the United States. Twenty-seven ejaculates from 13 males were judged to be of a quality sufficient to survive cryopreservation and 25 of these were imported to the United States for future use in artificial breeding programs. The results of the reproductive traits are averaged in Table 1.

The initial microscopic examination of the fresh semen revealed an abnormal proportion of aberrant sperm forms. This feature was previously observed in earlier work performed on cheetahs at the DeWildt Breeding Centre (3). Upon evaluation of the fixed aliquot, an average of 71% of each ejaculate contained morphologically abnormal spermatozoa. In domestic animals, the presence of greater than 40% abnormalities is associated with reduced fertility (4). Recent unpublished data from our laboratory indicate that the high percentage of abnormal sperm observed in the cheetah is not present in domestic cat semen using the same ejaculation procedure and fixative. The average abnormal sperm per ejaculate in the domestic cats collected was less than 30%.

1. National Zoological Park, Smithsonian Institution, Washington, D.C.
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4. DeWildt Cheetah Breeding and Research Centre, DeWildt, South Africa.
5. College of Veterinary Medicine, Onderstepoort, South Africa.



The overall sperm forms detected in the South African cheetahs are listed in Table 2.

The average of 71% abnormal sperm per ejaculate included both primary and secondary abnormalities. The primary abnormalities (tightly coiled tails and abnormal head shapes), considered to be caused by faulty spermatogenesis, were observed in repeated ejaculates in the animals collected serially. Secondary abnormalities (bent midpieces and tails, and protoplasmic droplets) are less serious and occur during the passage of sperm through the excurrent duct system.

#### Effect of Cheetah Subspecies on Semen Quality

Of the 22 animals evaluated, there were 11 Transvaal, 3 South West, and 8 hybrids. The data were analyzed to determine if a correlation existed among the various reproductive traits and the subspecies. There were no significant differences in ejaculate volume, sperm count/ml, or testes volume among the Transvaal, South West, or hybrid cheetahs. Neither were there differences in the percent of sperm motility or morphological sperm abnormalities. The progressive forward status was slightly improved in the South West males, but only 3 were evaluated. These preliminary results would suggest that no difference in semen quality exists between the subspecies or hybrid types.

#### Effect of Enclosure Size on Semen Quality

In both wild and captive environments, cheetahs are primarily solitary, preferring large territorial ranges (5). Large-sized enclosures, offering some cover and elevated areas for scanning, have been recommended to optimize breeding (6). The enclosure size varied greatly between the DeWildt and Lichtenburg camps. At DeWildt, the cheetahs were maintained in groups of 3 to 8 in 1-hectare fenced enclosures, while at Lichtenburg, 7 males were grouped together with 7 females in a 400-hectare fenced enclosure. The data were evaluated to correlate the effects of enclosure size on semen quality. Of major significance was the proportion of cheetahs producing spermatozoa in the ejaculate. Four of 15 males maintained in the smaller camps at DeWildt produced ejaculates void of spermatozoa. In contrast, the ejaculates of all 7 males at Lichtenburg contained spermatozoa. Concerning the specific reproductive traits: the average ejaculate volume was not significantly different between DeWildt and Lichtenburg cheetahs; sperm count was slightly elevated in Lichtenburg males; and combined testes volume was greater in the DeWildt males. Progressive forward status and percent motility tended to be nonsignificantly greater in the Lichtenburg males. One of the interesting observations was that Lichtenburg cheetahs produced approximately 8% fewer abnormal spermatozoa.

#### Conclusions

The future application of artificial breeding techniques depends largely on accumulation of data which will aid in the development of semen evaluation and preservation. Normal values of semen characteristics need to be established for each species, and methods of fertility evaluation need to be developed. Many reproductive parameters in this study are still to be analyzed. However, the preliminary results suggest several points:

1. This study has characterized expected reproductive norms for the electroejaculated cheetah.
2. A great proportion (71%) of cheetah spermatozoa per ejaculate was morphologically abnormal.
3. There were no significant differences in reproductive traits among Transvaal, South West, or hybrid cheetahs.
4. Available enclosure size may have an influence on male reproduction, in that a greater proportion of males maintained in large enclosures produced ejaculates with greater sperm concentrations and fewer abnormal sperm.

The female cheetah in South Africa is considered seasonally polyestrous. Although estrous behavior and breedings occur throughout the year, 77% of the matings occur in December and January (3). In this study, semen was collected in January, eliminating possible seasonal variation in ejaculate quality. The cause of the abnormal proportion of aberrant sperm forms is unknown. However, this observation is unique in such a successful breeding population.

#### Acknowledgements

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Table 1. Mean Reproductive Traits of 22 Electroejaculated Cheetahs.

Ejaculate volume (ml)	2.1 ± 0.4
Sperm count/ml (x 10 <sup>6</sup> )	14.5 ± 1.8
% motility of sperm	54.6 ± 3.1
Progressive forward status [scale of 0 (low) to 5 (high)]	3.6 ± 0.4
Combined testes volume (cm <sup>3</sup> )	17.8 ± 0.6
% morphological sperm abnormalities	71.0 ± 0.9



Table 2. Cheetah Sperm Morphology.<sup>6</sup>

	Mean %/Ejaculate
Normal spermatozoa	29.0
Tightly coiled tails	25.8
Bent midpieces	23.3
Bent tails	16.2
Bent tail tips	2.8
Protoplasmic droplets	1.1
Microcephalic sperm	1.2
Macrocephalic sperm	0.4

<sup>6</sup>Aliquots of fresh semen were fixed in 1% glutaraldehyde for evaluation of morphologic forms.

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## On the Extent of Genetic Variation of the African Cheetah, *Acinonyx jubatus*

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 D. Meltzer,<sup>3</sup> L.G. Simmons,<sup>4</sup> and M. Bush<sup>5</sup>

The cheetah (*Acinonyx jubatus*) is the world's fastest mammal and probably one of the most specialized of felids (1,2). Unlike its feline relatives, it has nonretractable claws and a long slim skeleton which contribute to its awesome speed. The cheetah is the single member of the genus *Acinonyx* which is considered by most taxonomists and naturalists to be markedly divergent from the two other genera of the Felidae Family, *Panthera* (the great cats, 5 species) and *Felis* (small and middlesized cats, approximately 35 species). Because of its distinct position in felid phylogeny, the sleek and swift species has often been accused of being a cat anxiously trying to become (through evolution) a dog!

The cheetah is a relatively successful predator with no apparent natural enemies (1,2). Nonetheless, its numbers are sparse (possibly as few as 25,000 in Africa today), the species is endangered, and a tendency of population deceleration has been evident in recent years. Further, an accompanying study of the cheetah (5) has found that reproductive parameters of certain cheetah populations show evidence of inborn defects when compared to other felids (e.g. the domestic cat) or other mammals. For these reasons, we initiated a study of the extent and character of biochemical genetic variation in the cheetah. An estimation of the extent of genic variation when extrapolated to the entire genome would offer an opportunity to compare the genetic structure of this endangered species to other feral species which are in the process of population acceleration (domestic cats, house mice and man).

In the past 15 years, approximately 250 species have been examined for the extent and character of biochemical genetic variation in natural populations (6-8). The strategy involves collection of various tissues from a group of feral animals and preparation of soluble extracts of homogenized material. The extracts are applied to a gel matrix and subjected to an electric current (gel electrophoresis). The crude extracts contain virtually thousands of enzymes and soluble proteins which migrate to different positions on the gel as a function of their net charge. Individual enzymes are visualized on the gels by histochemical or protein stains which are specific to one or a few individual enzymes. Genetic variation is observed when different individuals show the same enzyme in different electrophoretic positions on the gels. The altered mobilities reflect single amino acid substitutions in the protein's sequence as a

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result of nucleotide base substitutions in the structural gene which encodes the enzyme and specifies its sequence. These "electrophoretic variant" proteins are seldom deleterious and usually do not affect the enzyme's catalytic activity. The biochemical variants are inherited in a Mendelian fashion and are analogous to morphological genetic variants of eye, hair or skin color.

## Materials and Methods

Cheetahs: Heparinized blood (2 cc) was collected from 50 cheetahs in South Africa (26 males and 24 females). The African cheetahs were derived from feral populations from Transvaal, from Southwest or hybrids between the two populations (1,2). There is presently some controversy as to whether these zoogeographic distinctions form the basis for definition of cheetah subspecies. One male cheetah blood sample was collected at the St. Louis Zoo by Dr. William Boever. The remaining four cheetahs were sampled at the Henry Doorly Zoo, Omaha, Nebraska.

Preparation of isozyme extracts: 2 cc of heparinized blood were washed extensively with phosphate buffered saline (PBS); generally, 3X15 ml washes with centrifugation. The supernatant was decanted and the red cell pellet was frozen on dry ice for shipment to NCI for analysis. Ten samples of cheetah lymphocytes were prepared from the American zoo cheetahs and from five South African cheetahs using plasmagel sedimentation as previously described (9). Isozyme extracts were prepared by freeze-thawing 2 volumes of hypotonic buffer (0.05 M Tris PH 7.1, 0.001 M Na<sub>2</sub> EDTA), and sonication as previously described (10,11).

Gel preparation and gel electrophoresis: Extracts were resolved in 12% Electrostarch using buffer systems and strain recipes which are published in detail (10-14).

## Results

Crude extracts of erythrocytes from 55 cheetahs and lymphocytes from 10 of the cheetahs were subjected to gel electrophoresis and histochemically developed for 40 gene-enzyme systems. Because certain enzyme strains detect more than one gene product (e.g. soluble and mitochondrial malate dehydrogenase) the sample of loci represents 47 distinct gene products. These genes were selected merely because the technical feasibility to resolve them electrophoretically exists in our laboratory. Table 1 presents a compilation of the enzyme systems studied in this analysis, their IUPAC-IUB identification number, the gene symbol for the structural gene in domestic cat and man (15), the tissue employed (lymphocytes or red blood cells) and the number of cheetahs screened.

Of the 47 loci, all were invariant with the single exception of purine nucleoside phosphorylase (NP) which had 2 allozymes (allelic isozymes), A and B. The frequency of the slower migrating allele (A) was 0.77 and the faster migrating allele (B) was 0.23. The phenotypic frequencies of the population conformed to genetic (Hardy-Weinberg) equilibrium ( $\chi^2 = 0.46$ ). Thus, over all loci the frequency of polymorphic loci ( $P = 1/47 = 0.02$ , and the average heterozygosity ( $H = 0.008$ ). Average heterozygosity is defined as the frequency of heterozygotes over all loci in all individuals in a population (16). Conversely, the average heterozygosity of a population is the probability that an organism will be heterozygous at a given locus.

Table 1 also indicates the loci which we have previously defined as "monomorphic cluster" genes and "polymorphic cluster" genes (17). Certain homologous gene enzyme loci have a tendency to be monomorphic in surveys of mammalian populations (about 60% of the standard markers examined) while others tend to be polymorphic in several different species (about 30% of the standard markers). This conservation of the tolerance of genetic polymorphism is apparently more characteristic of a particular locus than that of the vertebrate species or of the genome. None of the 18 polymorphic cluster markers tested were polymorphic in the cheetah. The single polymorphic cheetah locus, NP, had been designed as a monomorphic cluster locus in our original analysis (17).

## Discussion

The study of the extent and basis of gene-enzyme variation has long been a principle concern of population genetics. Numerous surveys have indicated considerable amounts of genetic variation detectable in natural populations with only a few exceptions. For comparison, we present in Table 2 a group of several species which have been tested for genic variation using isozyme techniques. Between 10 and 50 percent of loci samples in mice, humans, cats, wolves, fruit flies and various other species exhibit genic variation. This amount of genetic variation has thus been considered as normal and typical for panmictic (random-bred) biological species.

The cheetah populations tested here have a marked paucity of genetic variation compared to several other relatively successful panmictic (random-bred) mammalian populations. The frequency of polymorphic loci ( $P=0.02$ ) and heterozygosity ( $H=0.008$ ) approaches that were observed in a colony of inbred mice where 1-2% of isozyme loci may exhibit allelic variation due to mutation or genetic mechanisms which tend to delimit statistically probable homozygosity.

The cheetah is not unique in having low levels of variation (see Table 2). The northern elephant seal, moose, polar bear and elk have been reported to have diminished levels of variation as well with various suggested interpretations (18-21). A more extensive sample of the moose using 734 individuals for 23 loci (22) revealed a substantive amount of variation in a species previously thought to be relatively monomorphic. Thus, it seems that studies which include large numbers of individuals as well as numerous sample loci are required to adequately reflect the extent of genetic variation present in a species (8,17,22).



The evolutionary interpretation of the data presented here is not immediately apparent, although two possible models are being considered. The first model is that the cheetahs have gone through a recent bottleneck for several generations, causing inbreeding to contribute to the relative homozygosity. This would require a substantial amount of inbreeding (nearly 20 generations of sib mating) to achieve the level of monomorphism observed in the cheetahs (23,24). A single one generation bottleneck of 7-10 animals has nowhere near the effect observed here since less than 10% of the endemic variation is removed by such a bottleneck (23-25). A model of cheetah bottleneck would have to account for the apparent identity of the two subpopulations (Transvaal and Southwest) and the American zoo cheetah.

A second model, which may be more fanciful, has certain appealing aspects with respect to the cheetah. It is tempting to speculate that as species adapt in evolution, it may be possible to become ideally suited for a particular niche. In such a hypothetical state, the load of genetic variation, characteristic of rapidly evolving and environmentally more "plastic" species might be gradually lost as the pursuit of the "ideal" genotype is approached. Such a species would be devoid of closely-related species or subspecies. Such a situation might also render the species more vulnerable than its polymorphic neighbors and signal impending extinction!

A final aspect of these data worth considering is the apparent identity of the Transvaal and Southwest "subspecies." The similarities between these samples far exceed those of human or murine races or subspecies using similar technologies. For these reasons, we feel it unlikely that these populations are indeed subspecies or even that they have been reproductively isolated for an extended period of time, despite their geographic isolation presently.

Table 1  
Gene-enzyme systems studied in cheetahs

Gene Symbol	Enzyme	IUPAC-IUB No.	Tissue <sup>1</sup>	No. cats	Cluster, P or H <sup>2</sup>
ACP1	Acid phosphatase-1	3.1.3.2	RBC	55	P*
ACP2	Acid phosphatase-2	3.1.3.2	Lym.	10	-
ADA	Adenosine deaminase	3.5.4.4	Lym.	10	P
AK1	Adenylate kinase	2.7.4.3	RBC	55	M
ALB	Albumin		RBC	55	M
APRT	Adenine phosphoribosyl transferase	2.4.2.7	RBC	55	M
CPKB	Creatinine kinase-B	2.7.3.2	RBC	55	M
D1A1	Diaphorase 1	1.6.2.2	Lym.	10	M
ES-1	Esterase-1	3.1.1.1	RBC	55	P
ES-2	Esterase-2	3.1.1.1	RBC	55	P
ES-3	Esterase-2	3.1.1.1	RBC	55	-
ES-4	Esterase-4	3.1.1.1	RBC	55	-
FUCA	$\alpha$ -L-Fucosidase	3.2.1.51	Lym.	7	P
GALA	$\beta$ -Galactosidase	3.2.1.22	Lym.	9	M
GLO	Glyoxylase-1	4.4.1.5	RBC	55	P
GOT1	Glutamate oxaloacetate transaminase (soluble)	2.6.1.1	Lym.	10	P*
G6PD	Glucose-6-phosphate dehydrogenase	1.1.1.49	RBC	55	P*
GPT	Glutamate pyruvate transaminase	2.6.1.2	RBC	55	P
GPI	Glucose phosphate isomerase	5.3.1.9	RBC	55	P
GSR	Glutathione reductase	1.6.4.2	RBC	55	P
GUSB	$\beta$ -Glucuronidase	3.2.1.31	Lym.	55	M
HB $\alpha$	Hemoglobin- $\alpha$		RBC	44	M
HBB	Hemoglobin- $\beta$		RBC	55	P
HEXA	Hexosaminidase-A	3.2.1.30	Lym.	55	M
HKL	Hexokinase-1	2.7.1.1	Lym.	8	M
HPRT	Hypoxanthine guanine phosphoribosyl transferase		Lym.	10	
IDH1	Isocitrate dehydrogenase-1 (soluble)	1.1.1.42	Lym.	8	P*
LDHA	Lactate dehydrogenase-A	1.1.1.27	RBC	55	M
LDHB	Lactate dehydrogenase-B	1.1.1.27	RBC	55	M
MDH1	Malate dehydrogenase-1	1.1.1.37	RBC	55	M
MDH2	Malate dehydrogenase-2 (mitochondrial)	1.1.1.37	RBC	55	M
ME1	Malic enzyme-1 (soluble)	1.1.1.40	RBC	55	P
ME2	Malic enzyme-2 (mitochondrial)	1.1.1.40	RBC	55	-
MPI	Mannose phosphate isomerase	5.3.1.8	RBC	55	M
NP	Purine nucleoside phosphorylase	2.4.2.1	RBC	55	M
PEPB	Peptidase B	3.4.11	RBC	55	M
PEPD	Peptidase D	3.4.11	RBC	55	M
PFK	6-Phosphofructo-Kinase	2.7.1.11	RBC	55	P*
PGD	6-Phosphogluconate dehydrogenase	1.1.1.44	RBC	55	P
PGM1	Phosphoglucomutase-1	2.7.5.1	RBC	55	P
PGM2	Phosphoglucomutase-2	2.7.5.1	RBC	55	M
PGM3	Phosphoglucomutase-3	2.7.5.1	RBC	55	P
PF	Pyrophosphatase (inorganic)	3.6.1.1	RBC	55	M
SOD1	Superoxide dismutase-2	1.15.1.1	RBC	55	M
SOD2	Superoxide dismutase-2	1.15.1.1	RBC	55	M
TPF	Triosephosphate isomerase	5.3.1.1	RBC	55	M
XDH	Xanthine dehydrogenase	1.2.3.2	RBC	55	-



## Footnotes - Table 1:

1. Tissues used: RBC - washed red blood cells; Lym. - washed lymphocytes extracted as described in Materials and Methods.
2. Cluster - We have defined as monomorphic cluster (M) enzymes those systems which are invariant in natural populations of mouse, domestic cat and man (17). Polymorphic cluster enzymes (P) vary in 2 of the same 3 species. P\* indicate those systems which vary in one of the 3 species. - indicates that the system has not been assigned to P or M due to lack of information.

 Table 2  
 Estimated genetic variation of mammals

Species	Number of loci	Number of populations	Proportions of polymorphic loci (P)	Average heterozygosity (H)	Reference
House mouse	46	2	20	0.08	26
House cat	55	1	22	0.07	11
Man	100	many	28	0.07	27
White tailed deer	28	1	32	0.10	28
Yellowstone elk	24	1	8	0.012	20
Moose	22	1	4	0.0006	19
Northern elephant seal	24	1	0	0.0	18
Black bear	16	1	12	0.02	28
Cheetah	47	2	2	0.002	this report

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The South African Cheetah: A multidisciplinary approach reveals a provocative genetic status and natural history. <sup>1</sup>S.J. O'Brien, <sup>6</sup>M. Roelke, <sup>8</sup>L. Marker, <sup>8</sup>F. Hart, <sup>1</sup>D.G. Goldman, <sup>1</sup>C. Merrill, <sup>5</sup>J.G. Howard, <sup>3</sup>D. Meltzer, <sup>2</sup>A. van Dyk, <sup>2</sup>H. Ebedes, <sup>2</sup>D.J. Brand, <sup>1</sup>J.M. Simonson, <sup>4</sup>L.G. Simmons, <sup>5</sup>M. Bush, <sup>1</sup>D.E. Wildt. <sup>1</sup>National Institutes of Health; <sup>2</sup>National Zoological Gardens of South Africa, Pretoria; <sup>3</sup>Department of Veterinary Science, Onderstepoort, RSA; <sup>4</sup>Henry Doorley Zoo, Omaha; <sup>5</sup>National Zoological Park, Washington, D.C.; <sup>6</sup>Wildlife Safari, Winston, Oregon.

The cheetah (*Acinonyx jubatus*) is the world's fastest mammal and probably one of the most specialized of felids. The cheetah is a relatively successful predator with few apparent natural enemies, although certain scavenger species (hyaenas, lions, wild dogs) are considered as competitors. Nonetheless, its numbers are sparse, the species is endangered, and a tendency of population deceleration has been evident in recent years. An analysis of mortality data of captive bred animals over the past 70 years revealed a considerable infant mortality (30%). In addition, captive breeding has been only occasionally successful despite attempts in a variety of zoological and wildlife preserves. Pursuant to these observations, a comprehensive analysis of the South African cheetah was initiated in 1980. Analysis of 40 cheetah ejaculates collected from 18 different male cheetahs revealed a general paucity in sperm concentration ( $14.5 \times 10^6$  spermatozoa/ml of ejaculate) as well as a rather high frequency of abnormal sperm forms (71%) in each ejaculate (Wildt et al., *Biol. Reprod.*, in press). A biochemical genetic analysis of 55 South African cheetahs from two geographically isolated populations in South Africa showed the species to be genetically monomorphic at each of 47 allozyme (allelic isozyme) loci. Two-dimensional gel electrophoresis of 155 abundant soluble proteins from cheetah fibroblasts also revealed a low frequency of polymorphism (average heterozygosity, 0.013). Both estimates are dramatically lower than levels of variation reported in other cats and mammals in general (O'Brien et al., *Sicence* 221:459). Reciprocal skin grafts from each of 16 unrelated cheetahs were surgically performed to monitor genetic variation of the major histocompatibility complex (MHC) of the cheetah. The MHC is among the most polymorphic of all mammalian loci as a consequence of its proposed function as an immunoregulatory locus for T-cell mediated surveillance. Each of the 16 grafts were not rejected in an acute manner characteristic of differences at the MHC in other cats and mammals. We conclude that the cheetah is extraordinary among mammals in its apparent monomorphism at this consistently polymorphic genetic locus. The extreme monomorphism may be a consequence of a demographic contraction of the cheetah (a population bottleneck) in association with a reduced rate of increase in the recent natural history of this endangered species. Recommended breeding strategies, in light of these conclusions will be discussed.

distinct geographic regions: 1) the northern region of the Transvaal Province of the Republic of South Africa and 2) South West Africa (Namibia). Initial propagative attempts were made at the Dr Wildt Cheetah Breeding and



## Unique Seminal Quality in the South African Cheetah and a Comparative Evaluation in the Domestic Cat

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### ABSTRACT

Analysis of 40 semen samples collected by electroejaculation from 18 cheetahs revealed no major differences in seminal traits among Transvaal, South West (Namibia) or hybrid (Transvaal × South West) males. However, mean spermatozoal concentration ( $14.5 \times 10^6$  spermatozoa/ml of ejaculate) and percent motility (54.0%) were less in cheetahs than in domestic cats ( $147.0 \times 10^6$  spermatozoa/ml of ejaculate, 77.0% motility) subjected to the same electroejaculation regimen. On the average, cheetah ejaculates contained 71.0% morphologically abnormal spermatozoa compared to 29.1% aberrant spermatozoal forms in the domestic cat. These results indicate that seminal characteristics in the cheetah are markedly inferior compared to the domestic cat, particularly with respect to the incidence of pleiomorphic spermatozoa. Because a recent parallel study demonstrates that the cheetah lacks genetic variation, it appears likely that spermatozoal abnormalities are a genetic consequence of genomic homozygosity characteristic of this endangered species.

### INTRODUCTION

Reproductive-genetic studies in the cheetah are relevant due to this animal's endangered status and unique taxonomic classification as the only species (*jubatus*) in the felid genus *Acinonyx*. The physiological data base for this species is extremely limited (Eaton, 1974; Wrogemann, 1975). An abstract by Coubrough et al. (1978) suggests that cheetah spermatozoa exhibit a number of structural defects including coiled and bent flagella; however, no specific details were provided. O'Brien et al. (1983),

using allozyme and two-dimensional gel electrophoretic analyses, recently have demonstrated a strikingly reduced amount of biochemical genetic variation in the South African cheetah. This finding in conjunction with the notation of Coubrough et al. (1978) emphasizes the need to examine further the influence of the monomorphic genotype on reproductive function in this species.

In 1971, the National Zoological Gardens of South Africa initiated a comprehensive program for the captive propagation of cheetahs (Brand, 1980). The original wild-captured breeding stock consisted of males and females from two distinct geographic regions: 1) the northern region of the Transvaal Province of the Republic of South Africa and 2) South West Africa (Namibia). Initial propagative attempts were made at the De Wildt Cheetah Breeding and

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Research Center. Successful captive breeding at this facility allowed the transfer of adult offspring to the Lichtenburg Nature Preserve and Game Breeding Park in 1978. These conservation complexes were both located in the Transvaal Province and were separated by a distance of 220 km.

Sexual maturity in both the male and female cheetah is thought to occur between 13 and 16 months of age (Wrogemann, 1975). In southern Africa the female is considered seasonally polyestrous, exhibiting overt estrous cycles from December through February (Brand, 1980). During the breeding season at De Wildt, males are maintained approximately 300 m from the female enclosures. A group of males is released daily near the female camps to monitor the onset of sexual receptivity. Estrous females are then permitted to copulate ad libitum for 2 to 3 days with a designated male. Using such methods, a total of 181 offspring have been produced from 1975 through 1982.

The purposes of the present study were to determine ejaculate norms and compare reproductive traits in established populations of male Transvaal, South West (Namibia) or hybrid (Transvaal × South West) cheetahs. Because of the unusual seminal quality observed, a comparative study also was conducted in the domestic male cat.

## MATERIALS AND METHODS

### *Animals and Facilities*

Ejaculates were collected in January, 1981 (mid-breeding season) from 11 Transvaal, three South West and eight hybrid cheetahs of Transvaal × South West ancestry. All animals were untamed and averaged ( $\pm$  SEM) 56.0  $\pm$  0.2 kg in weight and 5.3  $\pm$  0.7 years in age. Although the population ranged in age from 2–12 years, the mean ages of the population subgroups were similar ( $P > 0.05$ ): Transvaal, 5.9  $\pm$  1.5 years; South West, 6.0  $\pm$  1.9 years; hybrid 3.6  $\pm$  0.4 years. A total of 15 adult males were collected at De Wildt (seven Transvaal, two South West, six hybrid) and seven males were sampled at Lichtenburg (four Transvaal, one South West, two hybrid). At De Wildt, male cheetahs were maintained in groups of three to six in 1-hectare (ha) fenced enclosures. At Lichtenburg and prior to the initiation of the study, males were grouped together with seven female cheetahs and accorded free range of a 400-ha fenced enclosure. All males were separated from females at least 4 weeks before the experiment and, during the 5-day interim of data collection at Lichtenburg, the cheetahs were restricted to a 1-ha fenced camp.

Domestic cat ejaculates were collected in May, 1982, from 16 random source, adult males (3.2–5.0 kg body weight) maintained indoors in a colony

conducive to year-round production of kittens (Wildt et al., 1978). Like the cheetahs, domestic cats were not used for breeding purposes during the electroejaculation experiment or during at least the 4-week interval preceding the experiment.

### *Semen Collection*

In both species, semen was collected by electroejaculation using similar techniques including anesthesia, voltage and number of electrical stimuli. Individual animals were physically restrained and general anesthesia induced by an i.v. injection of CT 1341 (2.0 mg/kg of body weight, Saffan, Glaxo Labs., Middlesex, England). Semen was collected from cheetahs on one to four occasions/animal and from each domestic cat one time using rectal probe electroejaculation equipment and procedures similar to those described earlier (Platz and Seager, 1978; Platz et al., 1983). To permit comparative analysis of seminal traits, the electroejaculation regimen was standardized so that each animal was allotted 80 electrical stimuli of similar voltage (4 to 7 V) and milliamperage (50–200 MA) given over a 30-min interval. The pattern of applied stimuli was consistent with a previous report (Howard et al., 1981). The ejaculate was collected in a prewarmed vial.

### *Seman Evaluation*

Ejaculate volume was recorded and all microscopic analyses performed at 37°C using undiluted seminal aliquots. Spermatozoal percent motility was evaluated immediately based on observations of four separate microscopic fields at 400X. Spermatozoal concentration (spermatozoal numbers/ml of ejaculate) was calculated using a standard hemocytometer counting procedure, evaluating all 64 squares of both counting chambers of the hemocytometer. Spermatozoal concentration/ejaculate was calculated for the cheetah but not the domestic cat. For the latter species, the very small ejaculate volume (150  $\mu$ l) and the potential loss of fluid during collection made such a measurement inaccurate.

An aliquot of semen from the first ejaculate containing spermatozoa was fixed in 1% glutaraldehyde according to the protocol of Pursel and Johnson (1974) and 300 spermatozoa/individual were microscopically examined (1000X) for morphological abnormalities. Structural evaluations of spermatozoa were performed in six of the cheetahs twice by fixing an aliquot during a second semen collection occurring 2 to 7 days following the first electroejaculation. Aberrant forms of spermatozoa were classified as primary (a coiled flagellum or a pleiomorphic head defect, which originates during spermatogenesis) or secondary (a bent midpiece or flagellum or a protoplasmic droplet, which originates in the excurrent duct system) deformities.

### *Data Evaluation*

Values reported are means  $\pm$  standard error of the mean (SEM). Average and SEM values of the subjective estimate trait of percent motility were rounded to the nearest whole percentage. Significant differences were determined by analysis of variance. Individual means were then compared by Student's *t* test.



## RESULTS

Eighteen of 22 cheetahs produced ejaculates containing spermatozoa, the four aspermic males (two Transvaal, two hybrids) all being located at De Wildt. Repeated collections of semen had no discernible influence on standard seminal traits. Fourteen of the males were electroejaculated twice within a 48-h interval. Compared to the first collection, volume of the second ejaculate was greater in seven, less in five and unchanged in two cheetahs. In the second sample, spermatozoal concentration/ml of ejaculate was greater in seven, less in six and the same in one male compared to the spermatozoal numbers from the initial semen sample. Compared to the first sample, spermatozoal concentration/ejaculate in the second sample was greater in nine and less in five cheetahs; however, total sperm numbers varied considerably among individuals and mean values between the first ( $23.9 \pm 5.5 \times 10^6$  spermatozoa/ejaculate) and second ( $29.4 \pm 5.7 \times 10^6$  spermatozoa/ejaculate) collection were not different ( $P > 0.05$ ). Data from five representative males electroejaculated three times over a 6-day interval are shown in Table 1. Ejaculate volume, spermatozoal con-

centration, and percent motility fluctuated in a random fashion.

No differences were observed in mean ejaculate volume or spermatozoal concentration and motility among Transvaal, South West and hybrid cheetah groups (Table 2). Combining all data and based on a total of 40 seminal collections containing spermatozoa, an average cheetah ejaculate consisted of  $2.1 \pm 0.2$  ml of fluid. Seminal traits in the cheetah were markedly less ( $P < 0.05$ ) than results from domestic cats subjected to the same quantitative and qualitative electroejaculation stimuli. Mean spermatozoal concentration (sperm numbers/ml of ejaculate) for the cheetah was  $14.5 \pm 1.8 \times 10^6$  (10 times less than domestic cats), and the percent motility rating of cheetah spermatozoa was  $54.0 \pm 3.0\%$ , about 70% of that observed in cat ejaculates (Table 3). An average of  $71.0 \pm 0.9\%$  (range, 44–87%) of the spermatozoa collected in each cheetah ejaculate consisted of abnormal pleiomorphic forms (Fig. 1). The mean percentage of structural deformities in the first ejaculate of males in the Transvaal, South West and hybrid subgroups was  $73.2 \pm 2.5$ ,  $75.7 \pm 2.3$  and  $67.3 \pm 3.0\%$ , respectively ( $P > 0.05$ ). Overall, of the total defective forms,

TABLE 1. Seminal traits in five representative cheetahs electroejaculated three times over a 6-day interval.

Male	Day <sup>a</sup>	Ejaculate volume (ml)	Spermatozoal		
			Concentration/ml of ejaculate ( $\times 10^6$ )	Concentration/ejaculate ( $\times 10^6$ )	Motility (%)
1	1	3.3	0.5	1.6	50
	3	1.4	7.0	9.8	35
	7	1.6	11.0	17.6	55
2	1	2.0	11.5	23.0	50
	3	1.9	40.0	76.0	60
	7	0.8	19.0	15.2	40
3	1	1.0	14.5	14.5	65
	3	2.8	17.5	49.0	80
	7	2.1	7.5	15.8	65
4	1	1.8	26.0	46.8	70
	3	3.6	13.0	46.8	55
	7	2.0	13.0	26.0	55
5	1	1.4	28.0	39.2	70
	3	1.2	10.5	12.6	45
	7	1.3	3.5	4.6	60

<sup>a</sup>Day 1-Day of first electroejaculation.

38.6% and 61.4% were in the primary and secondary classification, respectively (Table 3). In the cheetahs evaluated twice, the percent abnormal spermatozoal forms/ejaculate for the group during the second evaluation ( $73.9 \pm 1.9\%$ ) was not different ( $P > 0.05$ ) from the first ( $69.4 \pm 5.9\%$ ). An average of  $29.1 \pm 3.7\%$  aberrant forms of spermatozoa was noted in the domestic cat samples. Approximately 80% of these were attributable to secondary defects, usually a protoplasmic droplet (Table 3).

### DISCUSSION

Seminal traits studied did not vary among cheetahs with Transvaal, South West or hybrid genotypes while ejaculate characteristics in

domestic cats were comparable to values reported earlier (Platz and Seager, 1978; Platz et al., 1978). Based on collections from a relatively large population of cheetahs during peak breeding season, spermatozoa concentration, percent motility and normal morphology were less than that observed in domestic cats. It is unlikely that the elevated number of morphologically abnormal spermatozoa in the cheetah was the result of sexual abstinence or degenerative processes associated with elimination of aged spermatozoa. A similar number of defective forms of spermatozoa was observed in cheetahs evaluated twice over a relatively brief interval. Furthermore, both cheetah and domestic cat semen was handled similarly and all precautions were taken to avoid spermatozoal damage from cold shock.

TABLE 2. Seminal trait comparisons among Transvaal, South West and hybrid cheetahs.<sup>a</sup>

	Transvaal	South West	Hybrid
Number of males	9	3	6
Number of ejaculates	19	4	17
Ejaculate volume (ml)	$1.6 \pm 0.2$	$3.6 \pm 1.1$	$2.4 \pm 0.2$
Spermatozoal concentration			
Sperm numbers/ml of ejaculate ( $\times 10^6$ )	$15.6 \pm 3.0$	$13.2 \pm 5.0$	$13.5 \pm 2.1$
Sperm numbers/ejaculate ( $\times 10^6$ )	$22.1 \pm 4.6$	$39.6 \pm 12.9$	$30.6 \pm 4.2$
Spermatozoal motility (%)	$52.0 \pm 5.0$	$58.0 \pm 8.0$	$57.0 \pm 5.0$

<sup>a</sup>Values are means  $\pm$  SEM.

TABLE 3. Seminal traits in the South African cheetah compared to the domestic cat<sup>a</sup>.

	Cheetah	Domestic cat
Number of males	18	16
Number of ejaculates	40	16
Spermatozoal concentration (sperm numbers/ml of ejaculate) ( $\times 10^6$ )	$14.5 \pm 1.8$	$147.0 \pm 39.5^b$
Spermatozoal motility (%)	$54.0 \pm 3.0$	$77.0 \pm 3.0^b$
Morphological abnormalities of spermatozoa (%)	$71.0 \pm 0.9^b$	$29.1 \pm 3.7$
Primary		
Coiled flagellum	$25.8 \pm 2.3^b$	$5.5 \pm 0.8$
Microcephalic defect	$1.2 \pm 0.3^b$	$0.2 \pm 0.1$
Macrocephalic defect	$0.4 \pm 0.2$	$0.1 \pm 0.04$
Secondary		
Bent midpiece	$23.3 \pm 1.1^b$	$6.4 \pm 0.8$
Bent flagellum	$16.2 \pm 1.3^b$	$5.1 \pm 0.7$
Bent flagellum tip	$2.8 \pm 0.6^b$	$0.02 \pm 0.01$
Protoplasmic droplet	$1.3 \pm 0.3$	$11.8 \pm 1.7^b$

<sup>a</sup>Values are means  $\pm$  SEM.

<sup>b</sup>Significantly greater ( $P < 0.05$ ) than counterpart value.



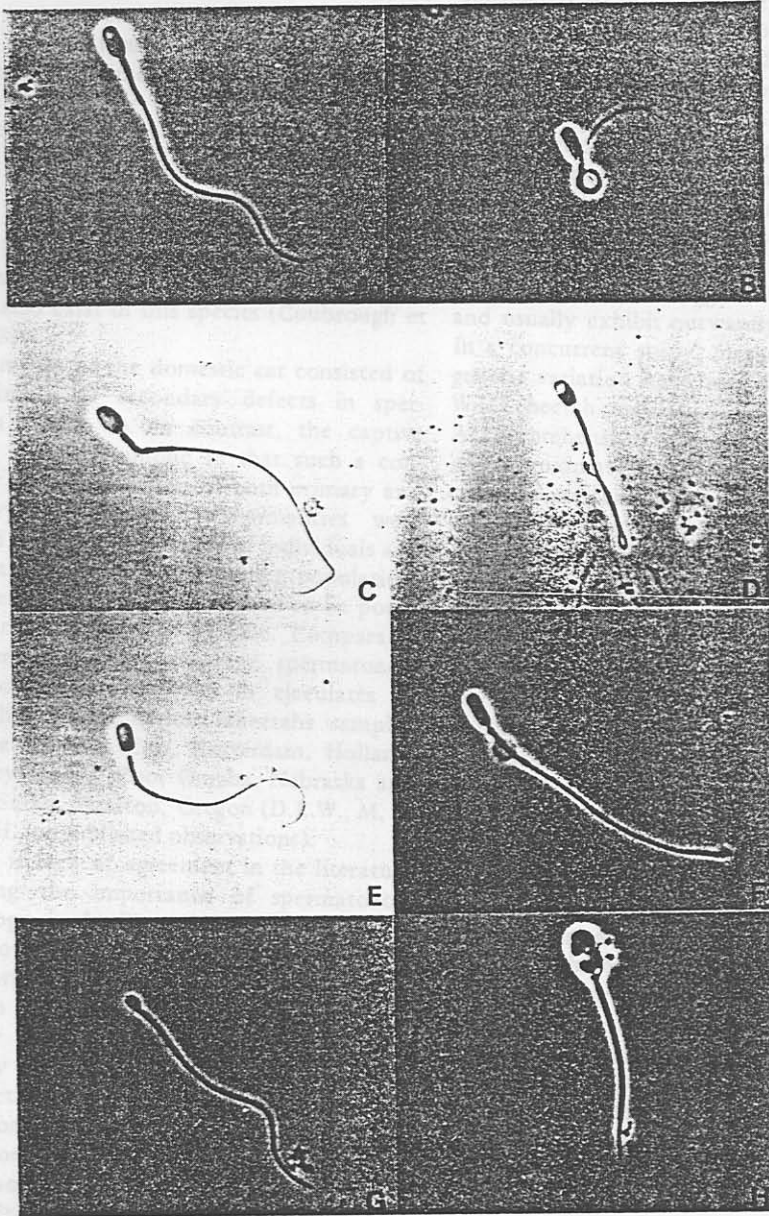


FIG. 1. Spermatozoal forms detected in the ejaculate of electroejaculated cheetahs: A) normal; B) coiled flagellum; C) bent midpiece; D) bent flagellum; E) bent flagellum tip; F) protoplasmic droplet; G) microcephalic defect; H) macrocephalic defect.

of the spermatozoal population, fertility dysfunction may be indicated in the bull (Chenoweth and Bell, 1980) and dog (Larson, 1960). Until this report, human (MacLeod, 1964) and gorilla (Suzanes et al., 1977) spermatozoa were considered to show far greater variation in structure than most genera from other species. Even in fertile men, 20 to 33% of spermatozoa have a

proportion of minor defects suggesting complementation of a variety of chromosomal genes which contribute to the integrity of mammalian spermatozoa. Possibly more subtle to the cheetah data described here is the recent examination of records of various species of captive zoo stock which reveals a high degree of inbreeding correlated with numerous deleterious effects, including increased juvenile mortality (Ralls et al., 1979). Whether the poor ejaculate



The results confirm and extend the preliminary data of Coubrough et al. (1978) who noted similar spermatozoal defects in cheetahs classified as either fertile or infertile. Other abnormalities of gametic substructure including acrosomal integrity could not be accurately evaluated using the microscopic methods of the present study. The acrosomal ridge of the cheetah spermatozoon is extremely narrow and fails to protrude beyond the apex of the head region. However, scanning electron microscopy provides preliminary evidence that acrosomal defects also exist in this species (Coubrough et al., 1978).

The semen of the domestic cat consisted of few primary or secondary defects in spermatozoa (Table 3). In contrast, the captive cheetah appeared unique in that such a consistently great proportion of both primary and secondary spermatozoal abnormalities were observed across a wide range of individuals and in a relatively successful breeding population. The latter finding does not appear to be population or geographically specific. Comparable high percentages of aberrant spermatozoal morphology were observed in ejaculates of eight other South African cheetahs sampled from the Blijdorp Zoo, Rotterdam, Holland, the Henry Doorly Zoo, Omaha, Nebraska and Wildlife Safari, Winston, Oregon (D.E.W., M. B. and J.G.H., unpublished observations).

There is lack of agreement in the literature concerning the importance of spermatozoal morphology in fertility (Salisbury and Baker, 1966). However, in general, the vast majority of the abnormalities detected in spermatozoa is found in mammals exhibiting pronounced infertility (Salisbury et al., 1977). In man (Chandley et al., 1975), as well as the bull (Chenoweth and Ball, 1980), ram (Rhodes, 1980), boar (Gibson and Johnson, 1980) and dog (Larson, 1980), the proportion of abnormal spermatozoa in the ejaculate has been related to fertility. Primary spermatozoal defects generally are considered more detrimental than secondary deformities (Chenoweth and Ball, 1980). When they exceed 20% of the spermatozoal population, fertility dysfunction may be indicated in the bull (Chenoweth and Ball, 1980) and dog (Larson, 1980). Until this report, human (MacLeod, 1964) and gorilla (Seuanez et al., 1977) spermatozoa were considered to show far greater variation in structure than male gametes from other species. Even in fertile men, 20 to 35% of spermatozoa have a

structural defect (Afzelius, 1981). The gorilla is considered to produce a preponderance (29 to 92.5%) of pleiomorphic spermatozoa in the ejaculate (Seuanez et al., 1977; Platz et al., 1980; Afzelius, 1981); however, the significance of this finding to fecundity is unknown.

The etiology of abnormal spermatozoal characteristics in the cheetah is unknown. The possibility exists that the chronic stress associated with captivity has adversely affected testicular function. However, in general, captive cheetahs are neither aggressive nor hyperactive and usually exhibit outwardly serene behavior. In a concurrent study, markedly low levels of genetic variation have been detected in the De Wildt cheetah population (O'Brien et al., 1983). A comprehensive biochemical genetic analysis of approximately 200 structural loci of 55 cheetahs indicates that less than 1% of the loci are polymorphic, a value 10 times less than the extent of variation detected in man (Harris and Hopkinson, 1972), feral mice (Rice et al., 1980) or domestic cats (O'Brien, 1980). The level of variation in the cheetah approaches that observed in inbred mouse strains after 10 or 20 generations of sib mating (Green, 1982).

Numerous studies have established that spermatozoal development and morphology are under rigorous genetic control (Beatty, 1970; Krzanowska, 1976; Wyrobek, 1979). Using variation between inbred mouse strains as a monitor, Wyrobek (1979) has suggested that the contribution from biological (nongenetic) factors to spermatozoal morphology is generally trivial. Furthermore, it is well established that seminal quality can be adversely affected in highly inbred homogenous populations of mammals (Salisbury and Baker, 1966; Rice et al., 1967; Johansson and Rendel, 1968; Wildt et al., 1982). For example, approximately 66% of spermatozoa from the BALB/c inbred mouse strain are abnormally shaped compared to <5% abnormal sperm in noninbred mice (Wyrobek, 1979). The frequency of abnormalities in inbred mice returns to normalcy (circa 2% abnormalities) in hybrid progeny of inbred parents, suggesting complementation of a variety of chromosomal genes which contribute to the integrity of mammalian spermatozoa. Possibly more salient to the cheetah data described here is the recent examination of records of various species of captive zoo stock which reveals a high degree of inbreeding correlated with numerous deleterious effects, including increased juvenile mortality (Ralls et al., 1979). Whether the poor ejaculate



quality of the cheetah is a genetic consequence or a unique species norm cannot be determined by the present study; however, it is indeed possible that both are the case.

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# Pituitary and gonadal response to LH releasing hormone administration in the female and male cheetah

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## ABSTRACT

Luteinizing hormone releasing hormone (LHRH, 50 µg) or saline was administered (i.m.) to adult female and male cheetahs under anaesthesia to evaluate pituitary and gonadal response. Serum LH levels did not fluctuate over a 120-min sampling period in saline-treated animals. Serum LH concentrations were raised ( $P < 0.05$ ) in both female and male cheetahs after LHRH injection, the temporal response being similar to previously reported results in unanaesthetized, domestic carnivores. The magnitude of the LHRH-induced LH response was sex-dependent. Over a 120-min post-injection period both saline control and

LHRH-induced LH levels were about twofold greater in males than females. Although LHRH had no acute influence on ovarian oestradiol-17β production in the female, serum testosterone levels were raised ( $P < 0.05$ ) in male cheetahs by 60 min after treatment. This study (1) provides introductory endocrine information on the cheetah, an endangered species, and (2) indicates that exogenous LHRH is effective in acutely altering pituitary (female) and pituitary/gonadal (male) function in an anaesthetized, non-domestic felid.

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## INTRODUCTION

The cheetah (*Acinonyx jubatus*) is considered ecologically endangered due to population deceleration in natural habitats and relatively poor reproductive performance under most zoological park management programmes. The cheetah in southern Africa achieves sexual maturity at 13–16 months of age (Wrogemann, 1975) with behavioural oestrous cycles of widely variant duration exhibited seasonally from December to February (Brand, 1980). Behavioural oestrus and repeated copulations with the male generally occur for 3 days (Wrogemann, 1975). Other than unique ejaculate/genetic interrelationships in the male (O'Brien, Wildt, Goldman *et al.* 1983; Wildt, Bush, Howard *et al.* 1983) and anecdotal information on gestation duration and litter size, the reproductive–endocrine data base for this species is extremely limited. Ovulation can be induced in the anoestrous female using exogenous gonadotrophins (Wildt, Platz, Seager & Bush, 1981*b*; Phillips, Simmons, Bush *et al.* 1982); however, whether the cheetah is a spontaneous or reflex ovulator is still unknown.

The present paper is concerned with pituitary and gonadal response in the adult female and male cheetah to luteinizing hormone releasing hormone (LHRH). This information is considered important not only for expanding the data base, but also for future studies of natural ovulatory luteinizing hormone (LH) release and the ancillary value of LHRH for timing ovulation and evaluating fertility potential or dysfunction. Furthermore, because of behavioural disposition, most non-domestic felids cannot be handled for research or artificial breeding purposes unless sedated or anaesthetized. Consequently, it was also important to determine if an exogenous LHRH challenge could elicit an LH response in an anaesthetized cheetah.

## MATERIALS AND METHODS

### Animals, hormonal challenge and blood sampling

Eleven female and 11 male cheetahs maintained at the De Wildt Cheetah Breeding and Research Centre in the Transvaal Province of the Republic of South Africa were used. Except for one female and one male,



and then a simultaneous significance test based on Hotelling's  $T^2$  test was used to further delineate any significant differences over time. The magnitude of hormonal response after treatment was further assessed and compared in terms of the area under the curve when hormonal profiles were plotted on an arbitrary scale on graph paper (Malak & Thibier, 1982). These values are expressed as  $\text{mm}^2$  over a 120-min interval. Correlation coefficients were calculated on a preprogrammed desk-top calculator.

## RESULTS

### Validation of the LH radioimmunoassay

The ability of the LH assay to measure this hormone in the cheetah was supported by several observations. Parallelism was observed between dose-response curves for the canine pituitary standard and pooled serum from LHRH-treated cheetahs (Fig. 1). Untreated animals also had significantly less detectable LH-like activity in 200  $\mu\text{l}$  samples compared with activity levels measured in 100–200  $\mu\text{l}$  samples from LHRH-treated animals (see below). Additionally, increased testosterone-like activity was measured in LHRH-stimulated cheetahs (see below). Because the secretion of testosterone is considered to be controlled by LH (Smith & Hafs, 1973), this observation indicated that the entity detected immunologically with the LH antiserum possessed LH-like biological activity.

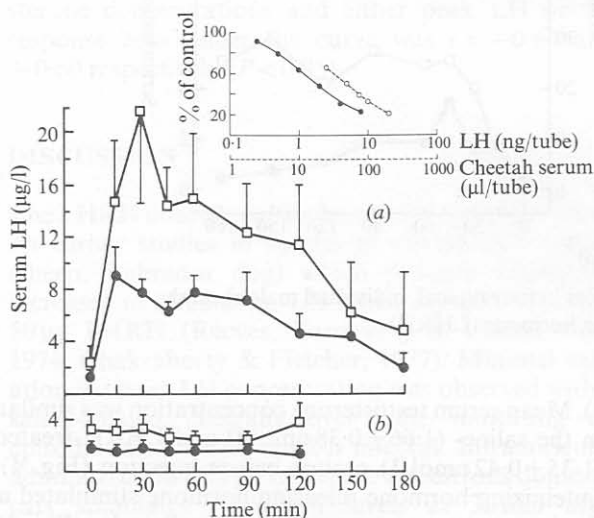


FIGURE 1. Serum LH concentrations in female (●) and male (□) cheetahs treated with (a) 50  $\mu\text{g}$  LH releasing hormone or (b) saline. Each datum point is the average of seven individual values. Inset depicts LH inhibition curves of canine pituitary standard (LER-1685-1) (●) and pooled cheetah serum (○).

### Serum LH response to LHRH

Mean response and typical individual LH profiles from female and male cheetahs are depicted in Figs 1–3. Neither average nor individual LH patterns fluctuated over the 120-min sampling period in saline-treated cheetahs (Figs 1–3). Mean basal concentrations in this group ranged from 1.5 to 1.9  $\mu\text{g}/\text{l}$  and from 2.3 to 3.3  $\mu\text{g}/\text{l}$  in females and males respectively (Fig. 1). As determined by measuring the area under the average hormonal profiles, the basal LH level detected in males ( $1143 \pm 260 \text{ mm}^2/120 \text{ min}$ ) was greater ( $P < 0.05$ ) than that of females ( $623 \pm 164 \text{ mm}^2/120 \text{ min}$ ).

Treatment with LHRH stimulated an LH response in all cheetahs with considerable variation noted among individual animals within each sex group (Figs 2 and 3). The LH peak in individual females varied from 5.5 to 21.8  $\mu\text{g}/\text{l}$  and occurred at 15, 30, 45, 60 and 90 min after LHRH administration in two, one, one, one and two animal(s) respectively. For males, the LH peak ranged from 4.9 to 50  $\mu\text{g}/\text{l}$  and occurred at 15, 30, 45 and 60 min after LHRH injection in one, three, two and one animals respectively. After treatment, mean serum LH was raised above control levels ( $P < 0.05$ ) at the 15-min sampling period in both the female ( $9.0 \pm 2.2 \mu\text{g}/\text{l}$ ) and male ( $14.7 \pm 4.7 \mu\text{g}/\text{l}$ ) groups (Fig. 1). Mean serum LH in females was sustained for 90 min between 6.2 and 7  $\mu\text{g}/\text{l}$  and gradually declined thereafter with the concentration detected at 180 min being no different from the pretreatment serum titre (0 min). The mean maximal LH level in males ( $21.6 \pm 7.2 \mu\text{g}/\text{l}$ ) occurred at 30 min after LHRH injection and LH levels were greater ( $P < 0.05$  or  $P < 0.01$ ) than controls throughout the 120-min sampling period. Concentrations of LH detected at 150 and 180 min after LHRH were similar to the pretreatment serum level. Overall, the LHRH-induced LH response in males ( $5755 \pm 1589 \text{ mm}^2/120 \text{ min}$ ) was greater ( $P < 0.05$ ) than in females ( $2542 \pm 399 \text{ mm}^2/120 \text{ min}$ ).

### Serum oestradiol-17 $\beta$ and testosterone response to LHRH

Gonadal sensitivity to treatment was examined by measuring serum oestradiol-17 $\beta$  in female and testosterone in male cheetahs. In individual saline-treated females serum oestradiol-17 $\beta$  concentrations varied markedly (Fig. 2) ranging from 37 to 312 pmol/l with as much as a 275 pmol/l concentration change during the 120-min sampling interval. The comparable range in LHRH-treated females was 37–422 pmol/l (Fig. 2) with serum titres fluctuating by as much as 349 pmol/l in individuals over the 180-min period. Mean concentrations (Fig. 4) were greater ( $P < 0.05$ ) in the LHRH group at only the 90-min sampling period (saline,  $37 \pm 15 \text{ pmol}/\text{l}$ ; LHRH,  $147 \pm 56 \text{ pmol}/\text{l}$ ). The

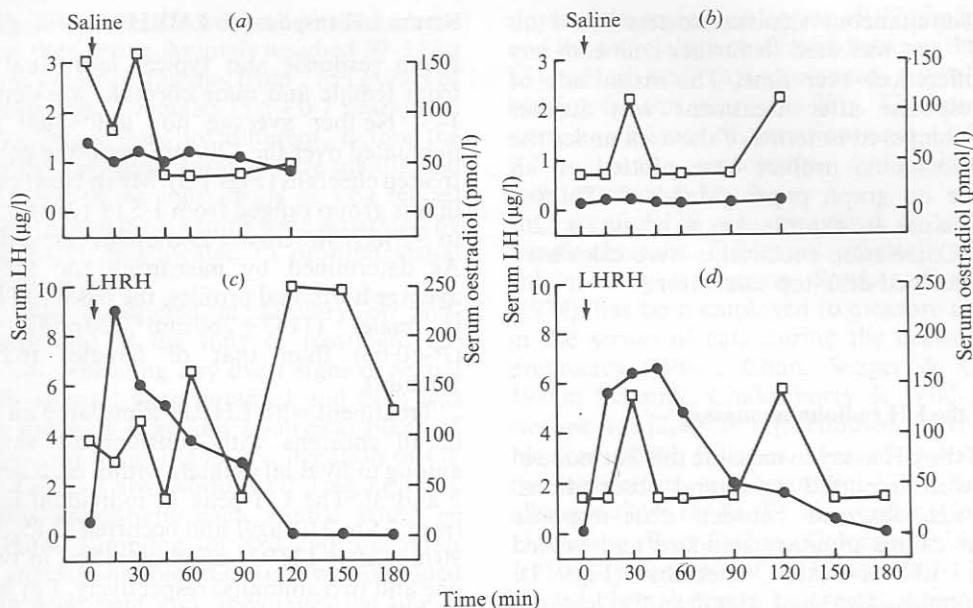


FIGURE 2. Serum LH (●) and oestradiol-17β (□) profiles in four typical, individual female cheetahs treated with (a, b) saline or (c, d) 50 μg LH releasing hormone (LHRH).

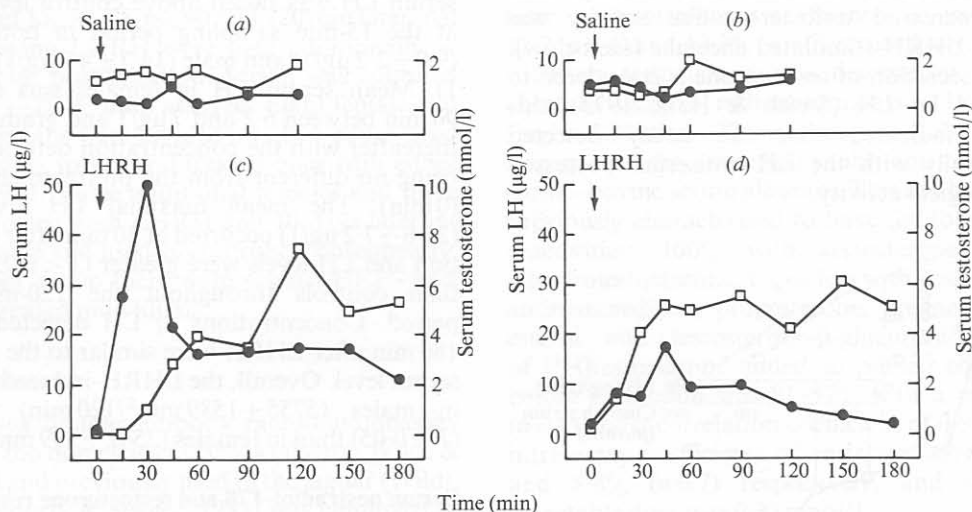


FIGURE 3. Serum LH (●) and testosterone (□) profiles in four typical, individual male cheetahs treated with (a, b) saline or (c, d) 50 μg LH releasing hormone (LHRH).

areas under the profiles were not different (saline,  $1481 \pm 190 \text{ mm}^2/120 \text{ min}$ ; LHRH,  $2562 \pm 621 \text{ mm}^2/120 \text{ min}$ ), and neither maximal serum LH concentration nor the overall LHRH-induced LH response as measured in area was correlated with the pre-LHRH treatment oestradiol-17β concentration ( $r = -0.23$  and  $-0.13$  respectively).

In individual saline-treated males, mean testosterone ranged from 1.35 to 2.50 nmol/l with no marked fluctuations during the sampling interval (Fig.

3). Mean serum testosterone concentration was similar in the saline- ( $1.66 \pm 0.38 \text{ nmol/l}$ ) and LHRH-treated ( $1.35 \pm 0.42 \text{ nmol/l}$ ) groups before injection (Fig. 4). Luteinizing hormone releasing hormone stimulated a rise in testosterone concentration in all males (Fig. 3) to levels greater than 3.5 nmol/l and as high as 7.45 nmol/l in one animal. The mean concentration for the LHRH group was raised ( $P < 0.05$ ) above control levels by 60 min (Fig. 4). Overall the area under the testosterone curve in LHRH-stimulated males



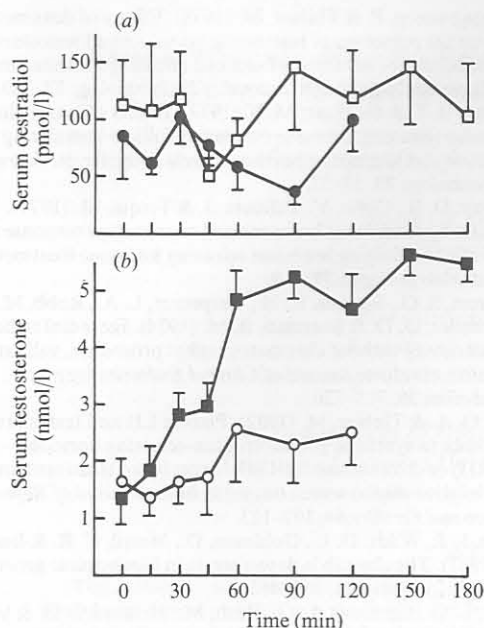


FIGURE 4. Serum (a) oestradiol-17 $\beta$  concentrations in female cheetahs and (b) testosterone levels in male cheetahs treated with saline (circles) or 50  $\mu$ g LH releasing hormone (squares). Each datum point is the average of seven individual values.

(4471  $\pm$  364 mm<sup>2</sup>/120 min) was greater ( $P < 0.05$ ) than in control males (2611  $\pm$  631 mm<sup>2</sup>/120 min). The correlation coefficient between pre-LHRH testosterone concentrations and either peak LH or the response area under the curve was  $r = -0.64$  and  $-0.60$  respectively ( $P < 0.05$ ).

## DISCUSSION

The LHRH dose chosen in the present study was based on earlier studies in species of comparable weight (sheep, Labrador dog) which produce substantial increases in circulating LH when injected i.m. with 50  $\mu$ g LHRH (Reeves, Tarnavsky & Chakraborty, 1974; Chakraborty & Fletcher, 1977). Minimal variation in serum LH concentration was observed within saline-treated cheetahs over time indicating no episodic release over the 2-h interval. Intramuscular administration of 5  $\mu$ g LHRH to anoestrous domestic cats stimulates peak LH levels at 30 min after treatment (Chakraborty *et al.* 1979). In the cheetah the LH response was similar, appearing almost immediately with the greatest average LH concentrations reached at either 15 (female) or 30 min (male) after treatment. Titres of LH in female cheetahs then declined to baseline by 180 min after injection

similar to the interval reported for female domestic cats (Chakraborty *et al.* 1979).

The highly individualistic LH responses to LHRH within either sex group were similar to findings in other species (Reeves *et al.* 1974; Malak & Thibier, 1982). This implies that within groups, females showing no sexual behaviour and even males vary considerably in actual endocrine status, which in turn can influence the LHRH-induced LH response. A striking observation was the quantitative differences in LH secretion between sex groups. Male cheetahs produced a two-fold increase over females in baseline and LHRH-induced LH release indicating that the magnitude of the response to LHRH is sex-dependent and variable in sensitivity. Comparative studies in other carnivores have not been conducted, with the exception of the ferret, in which males and females given the same LHRH dose produce similar LH responses (Donovan & ter Haar, 1977). The cheetah also differed quantitatively in LH response from previous observations made in the cat (Chakraborty *et al.* 1979) and dog (Chakraborty & Fletcher, 1977) using the same radio-immunoassay system. Whereas the greatest average LH level in the female cheetah was 9  $\mu$ g/l, as little as 5  $\mu$ g LHRH injected i.m. into the anoestrous domestic cat results in a mean LH peak of 114  $\mu$ g/l 30 min later (Chakraborty *et al.* 1979). Similarly, 50  $\mu$ g LHRH administered by the same route to the Labrador bitch produce an LH peak of 40  $\mu$ g/l 1 h after injection (Chakraborty & Fletcher, 1977). Interestingly, the quantitative and temporal profile of the bitch treated with 5  $\mu$ g LHRH almost exactly mimicked the LH response of the female cheetah.

Oestradiol-17 $\beta$  was also measured in females as an indirect determinant of whether presumed LHRH-induced gonadotrophin release stimulated oestradiol-17 $\beta$  synthesis and release via modified or increased follicular activity. Oestradiol-17 $\beta$  concentrations varied in both saline- and LHRH-treated females. This suggests that some of these cheetahs may have had ovarian follicular activity, although none was showing any overt sexual behaviour at the time of the study. Although some spiking in oestradiol-17 $\beta$  concentrations was observed in individual saline-treated cheetahs, the bleeding interval was too short to confirm positively a regular episodic release of this hormone. Luteinizing hormone releasing hormone also had no acute influence on serum oestradiol-17 $\beta$  levels, although it was possible that the 180-min sampling interval was insufficient to affect ovarian oestradiol-17 $\beta$  production.

Studies in the rabbit, ram and bull demonstrate that exogenous LHRH increases blood testosterone levels within 15–30 min of injection (Galloway, Cotta, Pelletier & Terqui, 1974; Blake, Blake, Thorneycroft & Thorneycroft, 1978; Chantaraprateep & Thibier, 1978;



both originally caught in the wild, all cheetahs were captive-born at the Centre. Animals weighed 50–55 kg and were sexually mature, ranging from 2 to 12 years of age. The average age of females ( $3.5 \pm 0.7$  years) and males ( $3.7 \pm 0.4$  years) was not different. Before the scheduled day of treatment, animals were maintained in groups of three to six individuals in 1 ha fenced enclosures. Male and female camps were separated by a distance of 300 m and located to prohibit visual contact between sexes.

The study was conducted in January or mid-breeding season and at the time of treatment the females were not exhibiting any overt signs of sexual behaviour. All animals were untamed and each was driven into a restraint cage and a surgical plane of anaesthesia induced by giving an i.v. injection of CT 1341 (Saffan; Glaxo Labs, Middlesex; 2 mg/kg body wt). Using this anaesthetic each animal could be handled for blood sampling for 180–240 min. After induction of anaesthesia, blood (10 ml) was obtained from the saphenous vein and designated the time 0 sample. Either saline vehicle or 50 µg aqueous LHRH (Gonadorelin; Abbott Laboratories, Chicago, Illinois, U.S.A.) was administered immediately (i.m.) and blood collected 15, 30, 45, 60, 90 and 120 min later. All cheetahs receiving LHRH were bled additionally at 150 and 180 min post-injection. Serum was collected and stored at  $-20^\circ\text{C}$  until assayed. Eight female and eight male cheetahs were divided into two equal-sized groups according to sex and treated once with either saline or LHRH. Three additional females and three males were treated with LHRH and 10 days later injected with saline and used as controls. Consequently, within each sex group there were seven LHRH- and seven saline-treated individuals.

### Radioimmunoassays

A heterologous double-antibody radioimmunoassay validated for the domestic cat (Chakraborty, Wildt & Seager, 1979) and previously used in the jaguar (Wildt, Platz, Chakraborty & Seager, 1979) was employed to measure serum LH. This assay used a bovine LH antibody (JJR 5; Dr J. J. Reeves, Washington State University, Pullman, Washington, U.S.A.; dilution 1:80 000), radioiodinated ovine LH (LER-1056-CI; Dr L. E. Reichert, Albany Medical School, Albany, New York, U.S.A.) and a canine pituitary standard (LER-1685-1; Dr L. E. Reichert). Recovery was determined by adding graded dosages of known amounts of pooled cheetah serum to tubes in quadruplicate, assaying and estimating recovery. Recovery estimates (mean  $\pm$  S.E.M.) from assay of nanogram amounts of LH (0.28, 1.2, 3.2 and 5.6) were:  $0.3 \pm 0.02$ ,  $1.2 \pm 0.08$ ,  $3.0 \pm 0.06$  and  $5.6 \pm 0.10$  respectively. Regression analysis indicated that the

slope of the regression line was 0.97, the intercept 0.01 and the correlation coefficient between the quantity of LH added and that recovered 0.99. The specific binding for this assay was 28%. The inter- and intra-assay coefficients of variation were 12.5 ( $n=5$ ) and 9.7% ( $n=6$ ) respectively, and minimum assay sensitivity was 0.3 µg/l.

Oestradiol-17β was measured in serum from all female cheetahs. This assay, originally described and validated by Korenman, Stevens, Carpenter *et al.* (1974), has been employed to measure oestradiol-17β in the serum of cats during the oestrous cycle and pregnancy (Wildt, Chan, Seager & Chakraborty, 1981a; Schmidt, Chakraborty & Wildt, 1983). The isotope was [2,4,6,7-<sup>3</sup>H]oestradiol-17β (New England Nuclear, North Billerica, Massachusetts, U.S.A.) and the antiserum anti-oestradiol-17β-bovine serum albumin (244; Dr G. D. Niswender, Colorado State University, Fort Collins, Colorado, U.S.A.; dilution 1:7500). Recovery of [<sup>3</sup>H]oestradiol-17β added to pooled cheetah serum before extraction with diethyl ether ranged between 79 and 95% with a mean of 91% ( $n=4$ ) and a correlation coefficient of 0.97. The minimum detectable level was 15 pmol/tube and the inter- and intra-assay coefficients of variation were 12.2 ( $n=4$ ) and 10.4% ( $n=7$ ) respectively.

Serum testosterone was determined in all male cheetahs using a radioimmunoassay kit from New England Nuclear. The isotope was [1,2-<sup>3</sup>H(N)] testosterone and the antiserum rabbit anti-testosterone-3-oxime-bovine serum albumin. This antibody had been previously characterized to have the following cross-reactivities: 100% with testosterone; 56% with dihydrotestosterone; 1% or less with androstenedione, androstenedione, progesterone, pregnenolone, cholesterol and testosterone-β-glucuronide. Recovery of [<sup>3</sup>H]testosterone added to pooled cheetah serum before extraction was 81–97% with a mean of 95% ( $n=4$ ) and a correlation coefficient of 0.98. Inter- and intra-assay coefficients of variation were 11.7 ( $n=4$ ) and 8.8% ( $n=7$ ) respectively, and the minimal detectable dose was 0.35 nmol/l.

For both steroid radioimmunoassays pooled cheetah serum extracted with the appropriate solvent was assayed in graded doses along with the oestradiol-17β and testosterone standards respectively. The results indicated parallelism of both extracted steroids with their respective standard curves.

### Data presentation and statistics

Values reported are means  $\pm$  S.E.M. Differences in mean hormonal values between control and LHRH treatment groups for any given time were statistically compared using Student's *t*-test. Differences within groups were first analysed by analysis of variance



Bremner, Findlay, Lee *et al.* 1980; Malak & Thibier, 1982). A similar response was observed in the male cheetah with testosterone rising significantly and achieving about a twofold increase by 60 min after LHRH injection. A relatively short lag time (30 min) existed between circulating peak concentrations of LH and testosterone. It did appear that pre-LHRH testosterone levels were related to subsequent LH response with the rise in serum LH being greatest in animals with initially low testosterone concentration. This finding suggests that in this wild carnivore the sensitivity of pituitary LH release to stimulation by synthetic LHRH is influenced by circulating testicular hormones.

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## Adrenal-Testicular-Pituitary Relationships in the Cheetah Subjected to Anesthesia/Electroejaculation

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### ABSTRACT

The influence of electroejaculation on the acute response in serum cortisol, testosterone and luteinizing hormone (LH) was studied in the South African cheetah. Males were either anesthetized with CT-1341 and 1) serially bled only (controls, n=7) or 2) serially bled during and following a regimented protocol of rectal probe electroejaculation (n=14). In the control cheetahs, mean cortisol concentrations declined over time ( $P<0.05$ ) and neither testosterone nor LH varied over the 145-min sampling interval. Serum cortisol rose immediately in electroejaculated cheetahs, peaked at the end of electroejaculation in 13 of 14 males and then declined during the next 90 min. Temporal profiles and serum levels of testosterone and LH were similar in the electroejaculated and control groups ( $P>0.05$ ). Within individual cheetahs, serum levels of LH and testosterone were highly correlated ( $r=0.77$ ,  $P<0.01$ ).

Awake (n=2) and CT-1341 anesthetized (n=2) cheetahs also were bled and then challenged with an i.m. injection of 25 IU adrenocorticotropic hormone (ACTH, Cortrosyn). Serial blood samples were collected during the next 2 h and assayed. Cortisol concentrations prior to ACTH administration were greater in awake than in anesthetized males. In all animals, cortisol rose immediately and peaked within 30-60 min of injection. Whereas all 4 ACTH-treated cheetahs produced cortisol titers in excess of 200 ng/ml, only 4 of 14 electroejaculated males produced cortisol levels comparable to this concentration range. Neither testosterone nor LH profiles were affected by ACTH-induced elevations in cortisol.

These data demonstrate that electroejaculation of an anesthetized wild felid, the cheetah, stimulates an acute adrenal response as indicated by elevated and peak serum cortisol levels immediately postelectrical stimulation. This response is 1) short-term as evidenced by rapidly falling serum cortisol levels following electroejaculation and 2) submaximal as indicated by greater cortisol concentrations in cheetahs treated with exogenous ACTH. Cortisol release in electroejaculated cheetahs did not appear to affect the tonic release of testosterone or LH. Consequently, there is no evidence that electroejaculation-induced cortisol secretion exerts a modulating influence on testosterone or LH secretion or adversely affects reproductive function.

### INTRODUCTION

Electroejaculation frequently is used to collect semen for fertility evaluation or research purposes in captive wildlife species. The effect of this procedure on endocrine function of most mammals is unknown and is of importance

because nondomestic species generally are perceived as being more excitable and thus, potentially more susceptible to stress. In the management of such animals it is important to determine if handling, anesthesia or other manipulatory procedures, including electroejaculation, acutely or chronically influence reproductive function or general physiological status.

Our laboratories recently have focused on reproductive-endocrine studies in the cheetah (*Acinonyx jubatus*). This endangered species is

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an interesting animal model because of unique traits associated with ejaculate quality (Wildt et al., 1983), pituitary and/or gonadal response to exogenous follicle-stimulating hormone (FSH) or LH releasing hormone (GnRH) (Wildt et al., 1981, 1984; Phillips et al., 1982) and population genetics (O'Brien et al., 1983). During evolution the cheetah has substituted strength characteristics for predatory speed, a distinct difference from other large felids. As a result, the disposition of the cheetah, although by nature wild, is considerably more attenuated and less aggressive than other Felidae species. Consequently, it was of interest to determine endocrine norms in the restrained and anesthetized cheetah as well as males experiencing a complementary stress of electroejaculation. The present study was made possible by having rare access to the animal population at the De Wildt Cheetah Breeding and Research Center (Republic of South Africa), one of few facilities naturally propagating cheetahs and with research stock available.

## MATERIALS AND METHODS

### Animals

A total of 18 adult male cheetahs maintained at the De Wildt Center were studied. Descriptions of genetic background and maintenance standards have been presented (O'Brien et al., 1983; Wildt et al., 1983). In brief, the males averaged  $56.0 \pm 0.2$  kg BW, ranged from 2–12 yr of age (mean,  $5.3 \pm 0.7$  yr) and were maintained in groups of 3 to 6 in 1-hectare (ha) fenced enclosures. All semen collections were conducted in January or midbreeding season for cheetahs in southern Africa (Brand, 1980). None of the animals were used for breeding purposes during the present study or during at least the 4-week interval preceding the study.

### Anesthesia, Electroejaculation and Blood Sampling

Cheetahs were anesthetized and serially bled only (controls) or anesthetized, electroejaculated and serially bled. In the latter group, rectal probe electroejaculation using a regimented stimulation protocol was performed in 14 males under CT-1341 (2 mg/kg BW, i.v.; Saffan, Glaxo Ltd., Middlesex, England) general anesthesia. A rectal probe (1.6 cm in diam and 23 cm in length) with three longitudinal stainless steel electrodes (3.3 mm in width and 6.8 cm in length) was lubricated and inserted approximately 15 cm into the rectum. The probe was positioned so that the electrodes were oriented ventrally. The electrostimulator used (P-T Electronics, College Station, TX) permitted controlling and monitoring voltage and amperage. A total of 80 stimulations was given in three series consisting of 30, 30 and 20 stimuli each. Initial voltage in series 1 consisted of applying 10 stimulations at 4 V, the next two sets of 10 stimulations being increased by 1 V each. After a total of 30 stimulations, the animal was rested for 2–3 min and then the

second series of 30 stimulations administered. During this series the first stimulations were given at 5 V and later increased in increments of 1 V for each additional 10 stimuli. After a similar rest interval, two final sets of 10 stimulations at 6 and 7 V, respectively, were administered and then electroejaculation discontinued. Semen was collected during each stimulation series and ejaculates were evaluated for standard traits, results of which have been presented (Wildt et al., 1983).

Both control and electroejaculated cheetahs were driven into restraint cages and blood samples (10 ml) drawn from the saphenous vein within 5 min of the initial disturbance. In the electroejaculated group subsequent samples were obtained following CT-1341 anesthesia induction and immediately after series 1, 2 and 3 of electroejaculation. Additional samples also were taken 30 and 90 min after the termination of electroejaculation. Mean time intervals for each handling or manipulatory procedure for the electroejaculated groups were calculated and found to be the following: from preanesthetic bleeding to postanesthetic/preelectroejaculation bleeding, 25 min; from preelectroejaculation to end of series 1, 12 min; from end of series 1 to end of series 2, 9 min; from end of series 2 to end of series 3, 9 min. These values were used to determine the appropriate intervals to permit a similar bleeding schedule in the nonelectroejaculated but anesthetized, control cheetahs. The latter group consisted of 7 males, 3 of which previously had been electroejaculated and serially bled. For the animals used twice, an interval of at least 7 days elapsed between the two sampling procedures. The mean age of the control ( $3.9 \pm 0.6$  yr) and electroejaculated ( $5.7 \pm 0.9$  yr) group was not different ( $P > 0.05$ ).

### Radioimmunoassays

Serum was collected and stored at  $-20^{\circ}\text{C}$  until assayed for cortisol, testosterone and LH. All assays were performed using duplicate serum aliquots. Adrenal activity was evaluated on the basis of cortisol concentrations measured using an  $^{125}\text{I}$  RIA kit (RIANEN<sup>TM</sup>, New England Nuclear, No. Billerica, MA). This assay employed a cortisol antiserum complex solution containing rabbit cortisol antibody pre reacted with an antiserum to rabbit gamma globulin in phosphate buffer. The rabbit cortisol antibody previously had been determined to have the following cross-reactivities: 100% with cortisol; 38.9% with prednisolone; 26.4% with corticosterone; 7.4% with aldosterone; 5.9% with 11-deoxycortisol; 3.5% with  $17\alpha$ -hydroxyprogesterone; 2% or less with progesterone, testosterone, dihydrotestosterone and estradiol- $17\beta$ . Interassay and intrassay coefficients of variation were 6.2% ( $n=6$ ) and 7.4% ( $n=10$ ), respectively, and minimum assay sensitivity was 0.2 ng/tube.

Serum testosterone was determined using a RIA kit (New England Nuclear) using rabbit antitestosterone-3-oxime-bovine serum albumin as the antiserum and 1,2- $^3\text{H}$  (N)-testosterone as the isotope. The antibody had been previously characterized to have the following cross-reactivities: 100% with testosterone; 56% with dihydrotestosterone; 1% or less with androstenedione, androstenedione, progesterone, pregnenolone, cholesterol and testosterone- $\beta$ -glucuronide. Standard solutions of testosterone ranged from 0.005 ng to 0.5



ng/tube. Aliquots (0.5 ml) of each serum sample were extracted with 5.0 ml of diethyl ether, the ether extract evaporated to dryness and redissolved in 0.5 ml of absolute ethanol. Aliquots (100  $\mu$ l) were dried in a 55°C water bath. Tracer and antiserum were added to all sample tubes, vortexed and incubated at 4°C for 2 h. A cold dextran-coated charcoal suspension (0.25% dextran; 0.025% charcoal) then was added and following a 5-min ice bath equilibration, all tubes were centrifuged and the supernatant fraction decanted into counting vials. Scintillator solution was added and each tube counted for tritium. Recovery rates of [<sup>3</sup>H] testosterone added to pooled cheetah serum prior to extraction ranged from 81 to 97% (mean, 95%, n=4). Interassay and intraassay coefficients of variation were 11.7% (n=4) and 8.8% (n=7), respectively, and the minimal detectable dose was 0.01 ng/tube.

A heterologous double antibody RIA was used to measure serum LH. This assay originally developed by Chakraborty et al. (1979) for the domestic cat recently has been described and validated in detail for use in the cheetah (Wildt et al., 1984). The isotope was <sup>125</sup>I ovine LH (LER-1056-C2), the first antibody was a bovine LH antiserum (JJR-5: dilution, 1:80,000) and the results were analyzed on the basis of a canine pituitary standard (LER-1685-1). The interassay and intraassay coefficients of variation were 11.7% (n=5) and 11.1% (n=9), respectively, with a minimum assay sensitivity of 0.25 ng/tube.

The anesthetic CT-1341 used in this study consisted of two pregnanedione derivatives, 3 $\alpha$ -hydroxy-5 $\alpha$ -pregnane-11, 20-dione (alphaxalone) and 21-acetoxy-3 $\alpha$ -hydroxy-5 $\alpha$ -pregnane-11, 20-dione (alphadolone acetate). Each ml of solubilized CT-1341 contained 12 mg of total steroids composed of 9 mg alphaxalone and 3 mg alphadolone acetate. Due to the steroidal nature and configuration of this drug, it was considered necessary to determine its potential cross-reactivities with the steroidal antisera used in the cortisol and testosterone assays. Aliquots (5, 10, 20, 50  $\mu$ l) of a diluted solution of CT-1341 (1.2  $\mu$ g/ml) were subjected to standard procedures used in each RIA. Quantities of cortisol and testosterone measured were below the detectable limits of either assay, ensuring that the cross-reactivity of the CT-1341 with the antiserum for either steroid was negligible and nonsignificant.

#### Adrenal Function Following ACTH

Hormone-induced adrenal function tests were conducted for comparative purposes and as an indication of the degree of cortisol production following a single bolus injection of synthetic ACTH (Cortrosyn, Organon, Inc., W. Orange, NJ). Two adult male cheetahs were anesthetized with CT-1341 (2.0 mg/kg BW) and after reaching a surgical plane of anesthesia, scheduled for serial bleeding (10 ml/sample). Following a Time 0 sample, each male was immediately injected with 25 IU ACTH (0.25 mg) i.m. and then bled 15, 30, 45, 60, 90 and 120 min later. An additional two adult cheetahs were treated similarly except these males were not anesthetized. Each of these animals was driven into the restraint cage, the Time 0 blood sample obtained within 5 min and then ACTH injected. These males then were maintained in the cage during the 2-h sampling interval. Sera were collected and assayed for cortisol, testosterone and LH.

#### Statistical Analysis

Values reported are means  $\pm$  SEM. An SPSS computer program employing a general analysis of repeated measures and a Greenhouse-Geisser correction was used to statistically analyze hormonal results (Greenhouse and Geisser, 1959). A simultaneous significance test based on Hotellings' T<sup>2</sup> test was used to further delineate any significant differences over time (Morrison, 1967). Correlation coefficients were calculated on a preprogrammed desk-top calculator.

#### RESULTS

Profiles of mean blood concentrations of cortisol, testosterone and LH before, during and after electroejaculation and in corresponding controls are illustrated in Fig. 1. Mean preanesthesia cortisol levels in control cheetahs ranged from 92.9  $\pm$  13.7 ng/ml (preanesthesia) to 44.1  $\pm$  7.7 ng/ml (145 min later) and gradually declined over time (P<0.01). In electroejacula-

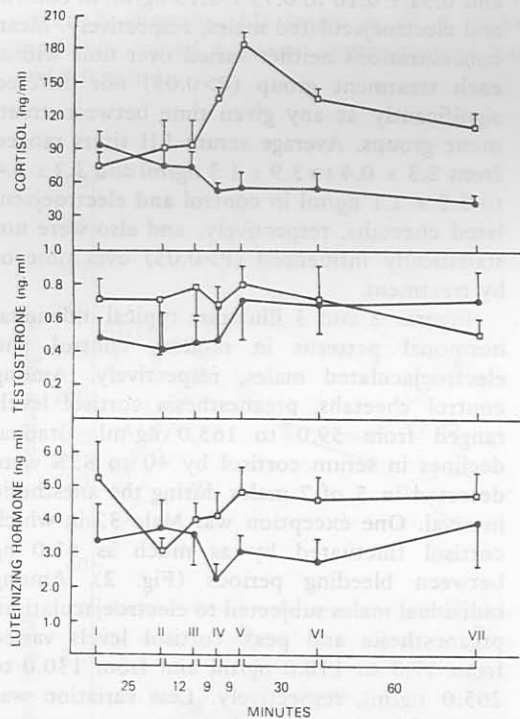


FIG. 1. Serum cortisol, testosterone and LH concentrations in male cheetahs serially bled 1) before and during CT-1341 anesthesia (n=7, ●) or 2) before, during and after electroejaculation under CT-1341 anesthesia (n=14, ◻). Roman numerals indicate time of blood sampling: I=preanesthesia, preelectroejaculation; II=postanesthesia, preelectroejaculation; III=end of electroejaculation series 1; IV=end of electroejaculation series 2; V=end of electroejaculation series 3; VI=30 min after electroejaculation; and VII=90 min after electroejaculation. Figure values are means  $\pm$  SEM.



ted cheetahs, mean cortisol concentrations preanesthesia ( $77.0 \pm 11.2$  ng/ml), postanesthesia, preelectroejaculation ( $91.5 \pm 23.1$  ng/ml) and following the first 30 electrical stimulations ( $92.8 \pm 11.1$  ng/ml) were similar to each other and to the corresponding control values ( $P > 0.05$ ). A significant rise ( $P < 0.05$ ) in serum cortisol was first observed in the electroejaculated group following the second series of stimuli ( $135.7 \pm 9.9$  ng/ml) with a further rise and peak of  $184.0 \pm 9.6$  ng/ml detected immediately following the end of electroejaculation. Mean serum cortisol then decreased and by 90 min after electroejaculation had returned to a level ( $113.6 \pm 12.9$  ng/ml) which was still greater ( $P < 0.05$ ) than the prestimulation concentration or the corresponding value in nonstimulated controls. Mean serum testosterone values ranged from  $0.42 \pm 0.12$  to  $0.72 \pm 0.27$  ng/ml and  $0.51 \pm 0.10$  to  $0.79 \pm 0.13$  ng/ml in control and electroejaculated males, respectively. Mean concentrations neither varied over time within each treatment group ( $P > 0.05$ ) nor differed significantly at any given time between treatment groups. Average serum LH titers ranged from  $2.3 \pm 0.4$  to  $3.9 \pm 1.3$  ng/ml and  $3.2 \pm 0.4$  to  $5.2 \pm 1.1$  ng/ml in control and electroejaculated cheetahs, respectively, and also were not statistically influenced ( $P > 0.05$ ) over time or by treatment.

Figures 2 and 3 illustrate typical individual hormonal patterns in random control and electroejaculated males, respectively. Among control cheetahs, preanesthesia cortisol levels ranged from 59.0 to 165.0 ng/ml. Gradual declines in serum cortisol by 40 to 85% were detected in 5 of 7 males during the anesthetic interval. One exception was Male 32 in which cortisol fluctuated by as much as 61.0 ng between bleeding periods (Fig. 2). Among individual males subjected to electroejaculation, preanesthesia and peak cortisol levels varied from 17.0 to 118.0 ng/ml and from 130.0 to 265.0 ng/ml, respectively. Less variation was detected in the temporal response to treatment as illustrated by the profiles of three typical males in Fig. 3. Thirteen of 14 cheetahs produced peak cortisol concentrations at the bleeding immediately after the third electroejaculation series. Peak cortisol concentrations greater than 200.0, from 165.0 to 200.0 and less than 165.0 ng/ml were detected in 4/14, 8/14 and 2/14 cheetahs, respectively.

Within individual cheetahs, testosterone fluctuated by as much as 1.79 ng/ml in the

control group (Fig. 2, Male 9) and 1.72 ng/ml in the electroejaculated group (Fig. 3, Male 23). Among individual cheetahs, considerable differences were observed in acute testosterone profiles with absolute concentrations sustained at different levels among various males. Testosterone titers in the majority of males (13 of 21 cheetahs sampled) were 1.0 ng/ml or less during the sampling interval. Two or more blood samples with concentrations between 1.0 and 2.0 ng/ml were detected in 7/21 males and 1 cheetah (male 23, Fig. 3) produced testosterone levels considerably greater (range 2.32 to 4.11 ng/ml) than its counterparts.

Serum LH concentrations within individual cheetahs appeared to fluctuate in random fashion over time, varying by as much as 9.0 ng/ml in both the control and electroejaculated groups (Figs. 2 and 3). However, similar to the testosterone patterns, serum LH levels appeared characteristically unique among various males. In general, serum LH values were less than 10.0 ng/ml. An exception was Cheetah 23, the male producing the greatest testosterone levels. In

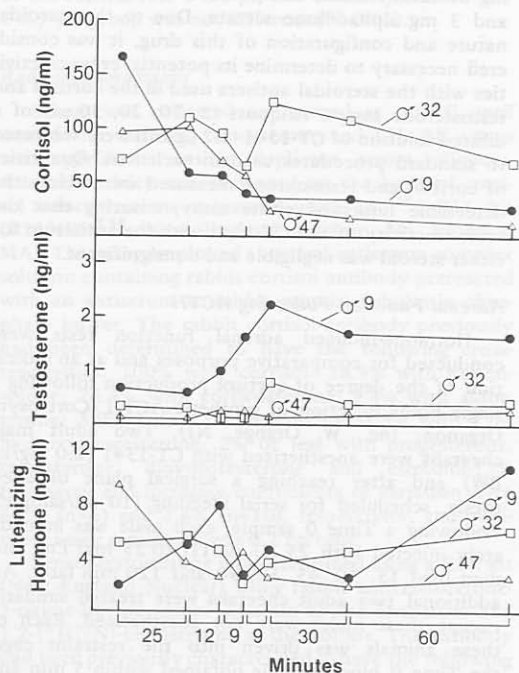


FIG. 2. Serum cortisol, testosterone and LH concentrations in male cheetahs subjected to serial bleeding under CT-1341 anesthesia only. See Fig. 1 for explanation of bleeding times.

this animal serum LH varied from 16.5 to 25.5 ng/ml (Fig. 3). Overall, serum LH concentrations within individual cheetahs appeared related to serum testosterone. The correlation coefficient between these two hormones for both the control and electroejaculated groups combined was 0.77 ( $P < 0.01$ ).

*Adrenal-Gonadal Function Following ACTH Injection*

Immediately prior to ACTH injection, serum cortisol levels were 61.0 and 65.0 ng/ml,

respectively, in the 2 anesthetized cheetahs and 112.0 and 130.0 ng/ml, respectively in the 2 unanesthetized counterparts (Fig. 4). Intramuscular administration of ACTH caused an immediate rise in cortisol which peaked in individual males (range, 218.0 to 380.0 ng/ml) within 30 to 60 min of injection. Cortisol levels gradually declined after this time, although rebounding values were detected in 1 anesthetized and 1 unanesthetized male at the 120-male sampling interval. The use of anesthesia had no discernible effect on the magnitude or temporal characteristics of the cortisol profile following ACTH injection.

Neither serum testosterone (Fig. 4) nor LH (data not shown) profiles appeared affected by ACTH administration. Testosterone levels gradually declined in 1 of the awake males and rose and fell in 1 of the anesthetized males. Testosterone concentrations were unchanged in the other 2 cheetahs. LH levels in all 4 males randomly varied between approximately 2.0 and 8.0 ng/ml.

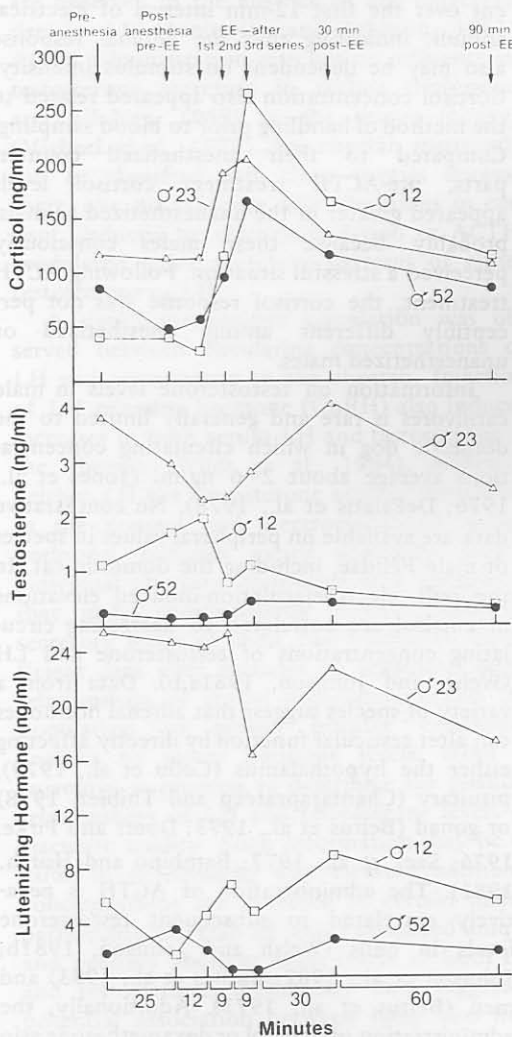


FIG. 3. Serum cortisol, testosterone and LH concentrations in male cheetahs subjected to serial bleeding and regimented electroejaculation under CT-1341 anesthesia. See Fig. 1 for explanation of bleeding times.

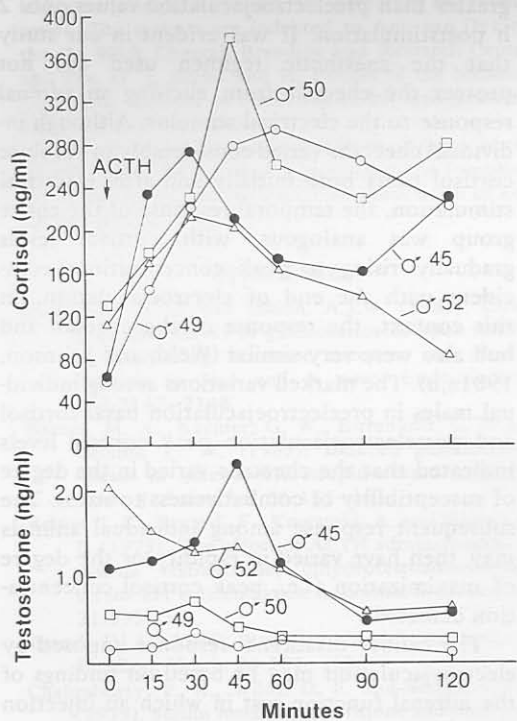


FIG. 4. Serum cortisol and testosterone concentrations in two unanesthetized (Males 50 and 52) and two CT-1341 anesthetized (Males 45 and 49) cheetahs injected with 25 i.u. ACTH immediately after collection of a 0-min blood sample.



### DISCUSSION

A prerequisite to the routine study of reproductive function in endangered species is the initial determination that manipulatory procedures such as anesthesia or electroejaculation do not compromise general physiological or reproductive status. The present study provides the first available data on adrenal-testicular-pituitary relationships in a non-domesticated felid species. Electroejaculation under general anesthesia stimulated an acute adrenal response based on significant increases in serum cortisol levels which then declined immediately following termination of the electrical stimulus. There was no evidence that elevated cortisol impaired or modulated tonic release of testosterone or LH, or adversely affected reproductive function. Comparative data on the effects of this stressor in domestic species is limited. In unanesthetized bulls, corticosteroids rise concomitantly within 5 min of the beginning of electroejaculation, with peak levels attained 15 min postelectroejaculation (Welsh and Johnson, 1981a,b). Adrenal hormone concentrations remain significantly greater than preelectroejaculation values until 2 h poststimulation. It was evident in our study that the anesthetic regimen used did not protect the cheetah from eliciting an adrenal response to the electrical stimulus. Although individual cheetahs varied considerably in absolute cortisol titers both initially and after electrical stimulation, the temporal response of the entire group was analogous, with cortisol levels gradually rising to peak concentrations coincident with the end of electroejaculation. In this context, the response of the cheetah and bull also were very similar (Welsh and Johnson, 1981a,b). The marked variations among individual males in preelectroejaculation basal cortisol and postelectroejaculation peak cortisol levels indicated that the cheetahs varied in the degree of susceptibility or combativeness to stress. The subsequent response among individual animals may then have varied in rapidity or the degree of maximization (i.e., peak cortisol concentration detected).

The caliber of adrenal response imposed by electroejaculation may be based on findings of the adrenal function test in which an injection of ACTH caused cortisol peaks ranging from 218 to 380 ng/ml. In comparison, electroejaculation did not induce the potential maximal response. Whereas all ACTH-treated cheetahs produced cortisol titers in excess of 200 ng/ml,

only 4 of 14 electrically stimulated males produced cortisol levels in this concentration range. The domestic cat responds to exogenous ACTH stimulation by a 4- to 10-fold increase in plasma cortisol concentrations within 1 h of injection (Johnston and Mather, 1979). The cheetah reacted similarly since the ACTH bolus used caused a 1.9- to 4.6-fold increase in serum cortisol levels. Additionally, the temporal cortisol response suggests that the ACTH injection may have simulated a more severe stress than electroejaculation. Serum cortisol was markedly increased in all ACTH-treated males within 15 min of administration. In contrast, levels of this hormone were no different over the first 12-min interval of electrical stimuli, indicating that the adrenal response also may be dependent on stimulus intensity. Cortisol concentration also appeared related to the method of handling prior to blood sampling. Compared to their anesthetized counterparts, pre-ACTH treatment cortisol levels appeared greater in the unanesthetized animals, probably because these males consciously perceived a stressful situation. Following ACTH treatment, the cortisol response was not perceptibly different among anesthetized or unanesthetized males.

Information on testosterone levels in male carnivores is rare and generally limited to the domestic dog in which circulating concentrations average about 2–6 ng/ml (Jones et al., 1976; DePalatis et al., 1978). No comparative data are available on peripheral values in species of male Felidae, including the domestic cat. In the bull, electroejaculation-induced elevations in cortisol are correlated to decreasing circulating concentrations of testosterone and LH (Welsh and Johnson, 1981a,b). Data from a variety of species suggest that adrenal hormones can alter testicular function by directly affecting either the hypothalamus (Collu et al., 1979), pituitary (Chantaraprteep and Thibier, 1978) or gonad (Beitus et al., 1973; Doerr and Pirke, 1976; Saez et al., 1977; Bambino and Hsueh, 1981). The administration of ACTH is negatively correlated to subsequent testosterone levels in bulls (Welsh and Johnson, 1981b; Johnson et al., 1982; Barnes et al., 1983) and men (Beitus et al., 1973). Additionally, the administration of cortisol or dexamethasone also eliminates the nocturnal rise of testosterone in man (Doerr and Pirke, 1976) and decreases LH synthesis in bulls (Thibier and Rollard, 1976; Chantaraprteep and Thibier, 1978). However,



it also is evident now that the stress associated adrenal-testicular-pituitary relationship is species-specific, not necessarily directly linked to cortisol and not always easily explained. Although dexamethasone or elevated cortisol decreases blood testosterone levels in men, LH concentrations generally are unaffected (Schaison et al., 1978; Rose and Sachar, 1981). ACTH induces cortisol elevations in dogs and boars, however, testosterone levels are unaffected in the former (Eik-Nes, 1962; Hagan and Andersen, 1981) and even elevated in the latter species (Juniewicz and Johnson, 1981). The increased testosterone concentrations in boars are observed in the absence of any detectable rise in LH (Juniewicz and Johnson, 1981). ACTH administration also has no influence on testosterone secretion in rabbits (Haltmeyer and Eik-Nes, 1969) or the rhesus monkey (Michael et al., 1974). The cheetah apparently can be classified with these species because there was no evidence that elevations in cortisol, induced by electroejaculation or ACTH, modulated acute secretory patterns of either testosterone or LH.

A significant positive correlation was observed between circulating concentrations of LH and testosterone in the cheetah. Injection of LH releasing hormone (GnRH) also induces increases in both serum LH and testosterone in the cheetah (Wildt et al., 1984). Although neither LH nor testosterone acutely fluctuated in the control and electroejaculated group, hormonal patterns varied markedly among individual cheetahs, consistent with the theory that males independently produce different secretory levels of these two hormones. Insufficient data were available on behavioral inter-relationships or sexual dominance within the population to permit possible correlative analysis to serum hormone levels. Work is currently in progress to determine the possible relationships of LH and testosterone levels to ejaculate quality. Such information may be of value in determining why elevated hormonal concentrations are observed in certain males, particularly Cheetah 23 which produced unique and possibly aberrant tonic levels of both LH and testosterone. Preliminary data suggest a potential association between these two hormones and the absence of spermatozoa in the cheetah ejaculate. Four of the 14 males, including Cheetah 23, failed to produce spermatozoa in the seminal fluid collected at electroejaculation. Mean testosterone and LH

concentrations in these males were  $1.58 \pm 0.4$  and  $9.7 \pm 3.3$  ng/ml, respectively, which were greater ( $P < 0.05$ ) than the levels of  $0.51 \pm 0.1$  and  $4.5 \pm 0.6$  ng/ml, respectively, measured in the remaining 10 cheetahs producing spermic ejaculates. Previous studies relating LH and testosterone levels to testicular exocrine function have been limited and often equivocal (Katongole et al., 1971; Sandford et al., 1974; Lincoln, 1979). Correlations between individual LH/testosterone response to seminal output characteristics following GnRH are not significant in bulls (Malak and Thibier, 1982). However, LH concentrations are reported as greater in men with oligo- or azoospermia compared to normospermic individuals (Christiansen, 1975; Hopkinson et al., 1977; Fossati et al., 1979), a finding which may be of particular interest in view of the observations made in our study.

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