PUBLICATIONS

CHEETAH BREEDING

A REPORT ON THE PRETORIA NATIONAL ZOOLOGICAL GARDENS DE WILDT PROJECT.

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ACKNOWLEDGEMENTS

Dr. D.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.
Nan Wrogemann: Gave great assistance in supplying available literature

SUMMARY

During April to September 1975 6 Cheetah litters were born at de Wildt, a total of 23 Cheetah cubs. This report deals with the management aspects and fertility sudies undertaken, from January 1974 to September 1975, which led to this success. A description of Cheetah semen and spermiograms 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 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

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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	Males	Unsexed	Litters	Females
66	59	15	44	29

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 20/3/72 – 21/3/72 26/1/73 – 31/1/73 14/2/73 – 16/2/73 11/2/74 – 14/2/74	2 2 7 3 4	
51	2 years 8 m	21/12/72 – 22/12/72 8/ 1/73 – 13/ 1/73 25/12/73 – 28/12/73	2 6 4	
52	2	21/ 3/72 18/12/73 4/ 1/74 – 11/1/74 21/ 1/74 – 24/1/74	1 1 8 4	Pseudopregnancy developed
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 2×10^6/ml to 120×10^6/ml

Semen 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 spermiograms 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 spermiograms of dogs with high fertility, and was therefore considered in this light when the semen samples form 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 cullular 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 spermiogram encountered, illustrating the high incidence of primary spermatozoan abnormalities found.

PLATE 1

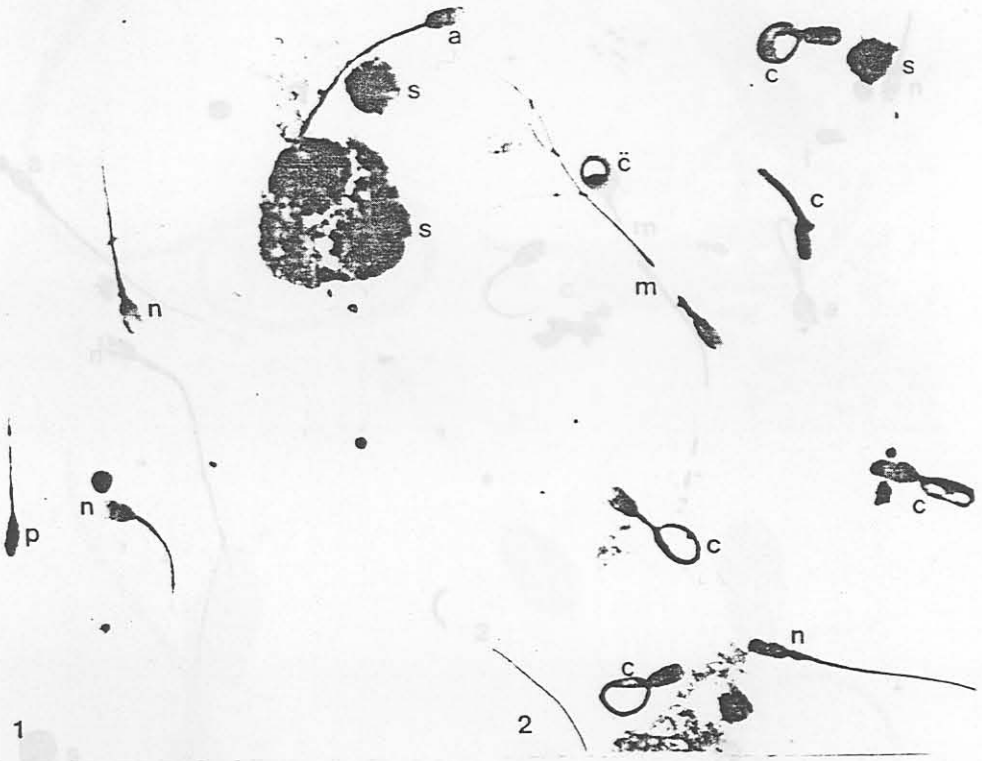
Washed cheetah spermatozoa

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; c̈ – coiled tail around microhead; s – spermatogenic cell.

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

Figure 4 c – varing degrees of coiled spermatozoa; s – spermatogenic cells.



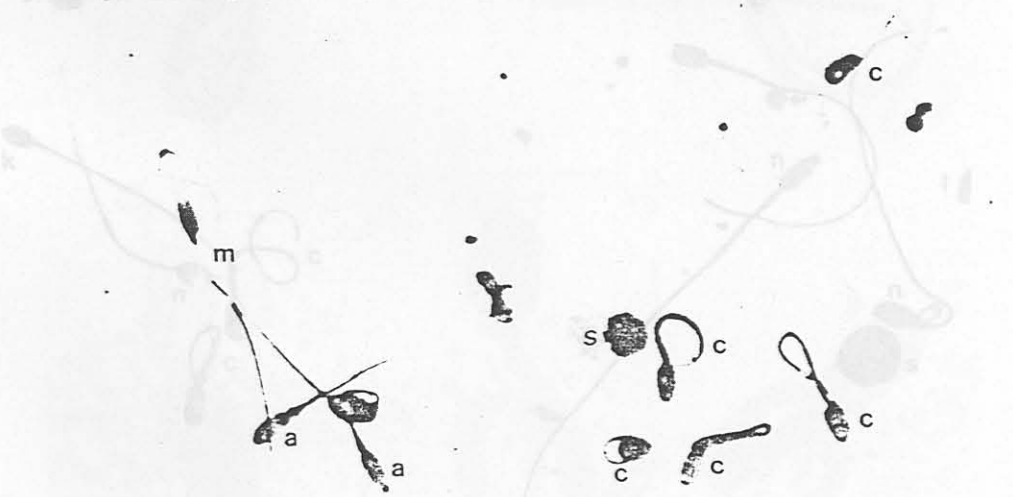


PLATE 2

Washed cheetah spermatozoa

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

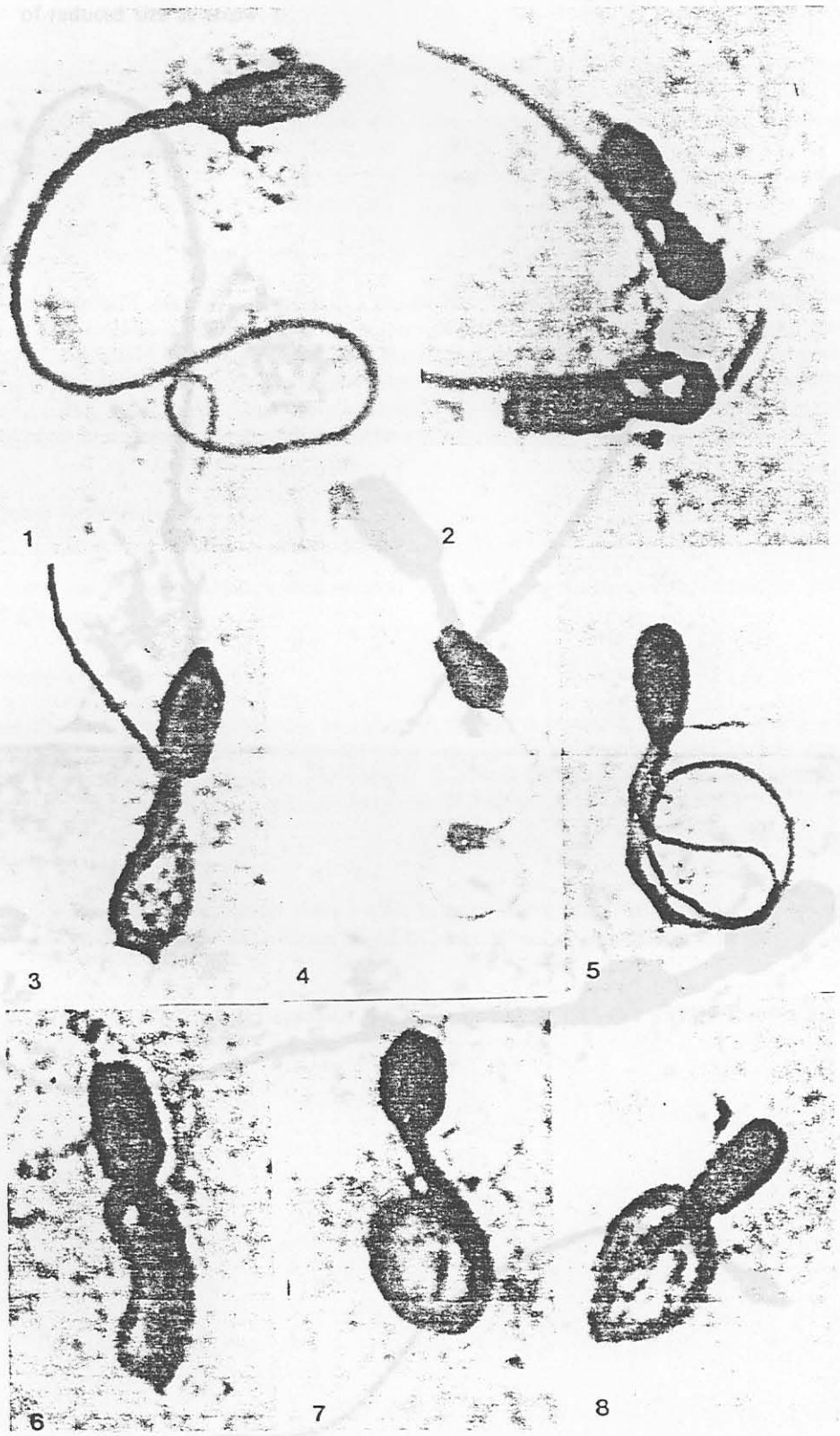


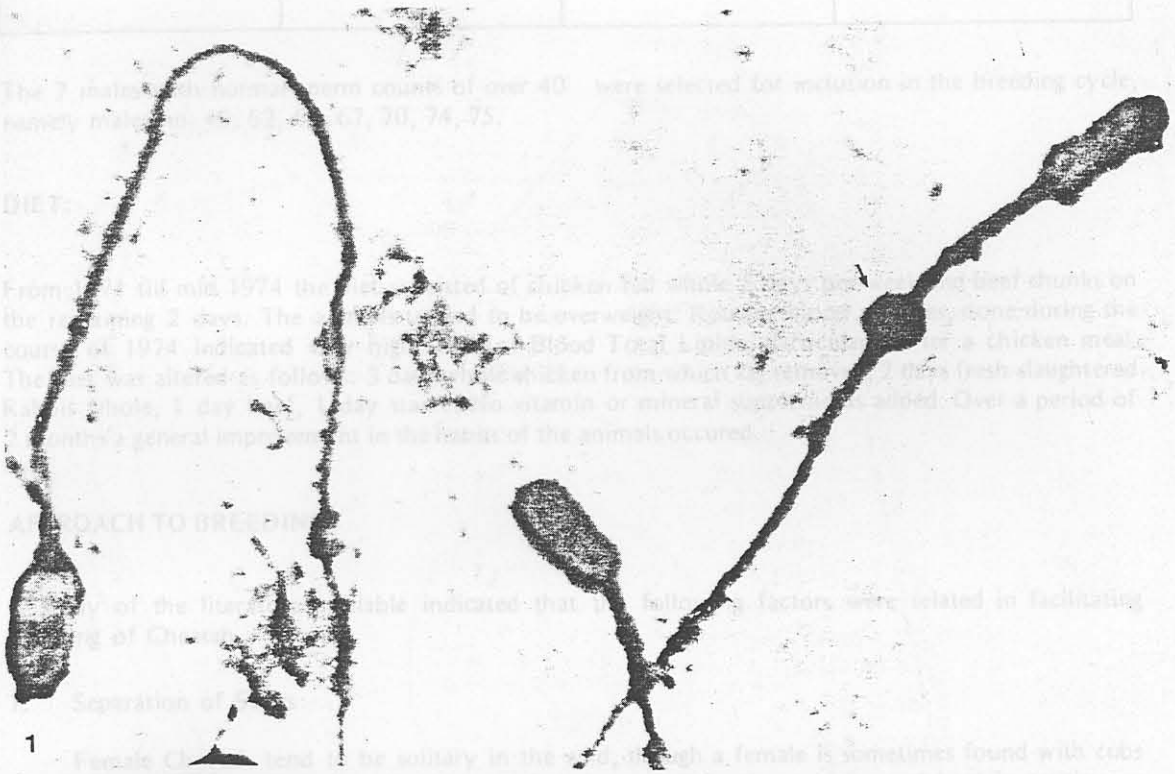
PLATE 4

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.



1

2

3

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 occured.

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 and mating to take place. This has been found to be incorrect as will be seen later.

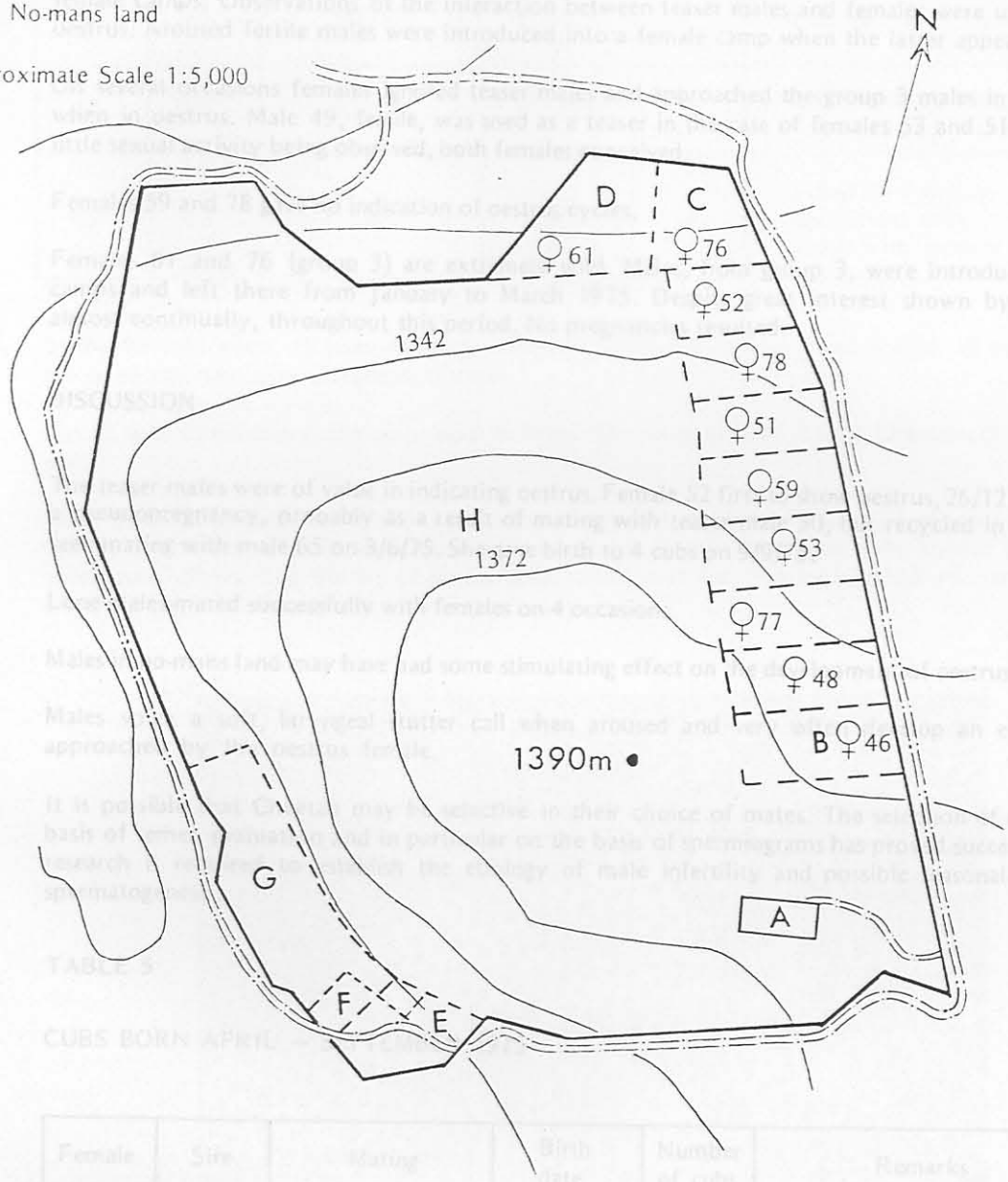
From the above, and from observations into behavioural patterns on the farm, camps were erected within the fenced area as in figure 1.

FIGURE 1

A – Quarantine area
B,C,D – Female camps
E – Male camp Group 1
F – Male camp Group 2
G – Male camp Group 3
H – No-mans land

Access Road
Contour lines 15 m interval
Internal 2,5 m fences
Outer 3,5 m fence

Approximate Scale 1:5,000



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 seperate 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 26/12/75. 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 spermiograms 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 contriction 1 reared
51	49	Not observed	28/4/75	4	abandoned at 2 weeks of age 3 died of starvation 1 hand reared

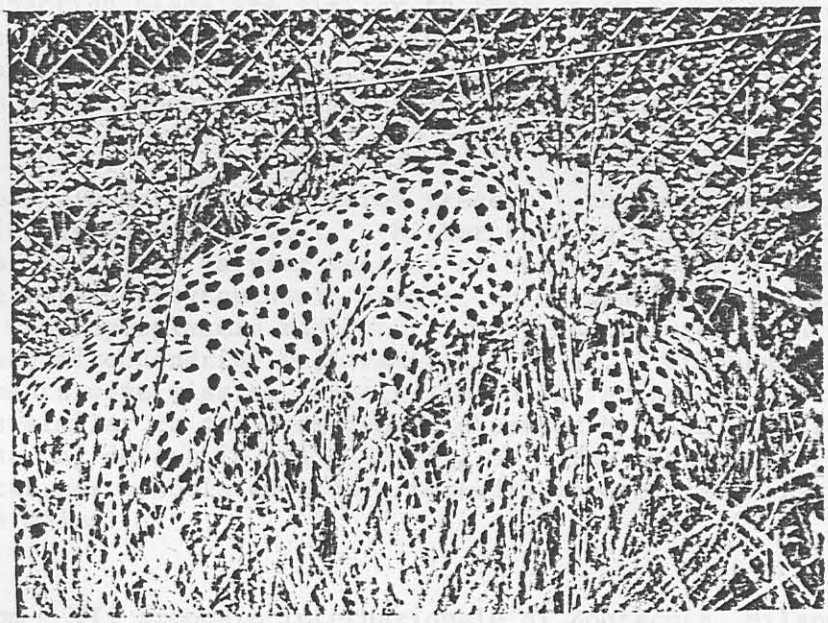
CUBS BORN APRIL – SEPTEMBER 1975 Continued

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 notoriusly 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[4]. Spermatozoon abnormalities have recently been reclassified by Blom[1] 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[6,7] 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[5]

The head of cheetah spermatozoa in planar view has a compressed elliptical outline while in sagital 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. Capitellum 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[3] (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[2] 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[6].

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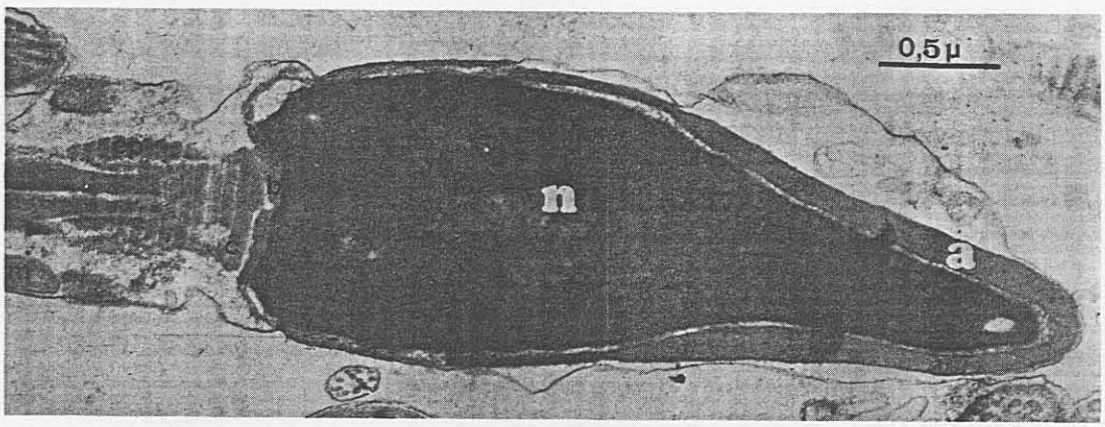


Fig 1
Sagittal section of a cheetah sperm

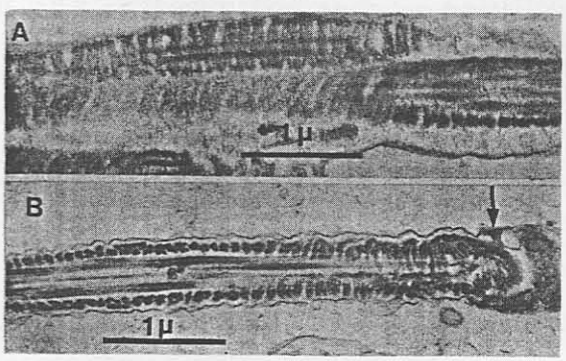


Fig 2
A & B The delicate sheath of the principal piece. An ill-defined annulus is seen at the arrow

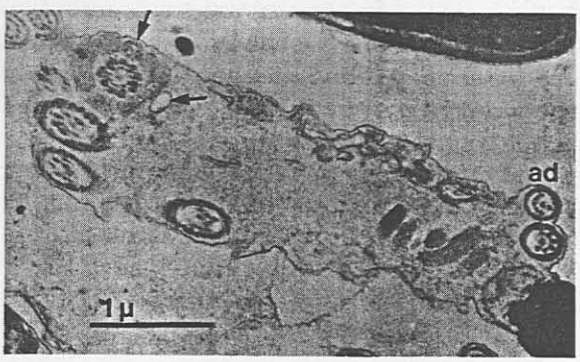


Fig 3
Axial filament derangement in a looped tail. Arrows denote mitochondrial changes

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 aankomelinge lyk nie anders as hulle voorgangers nie maar is wel besondere diere; besonders in dié sin dat hulle almal in gevangeskap 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

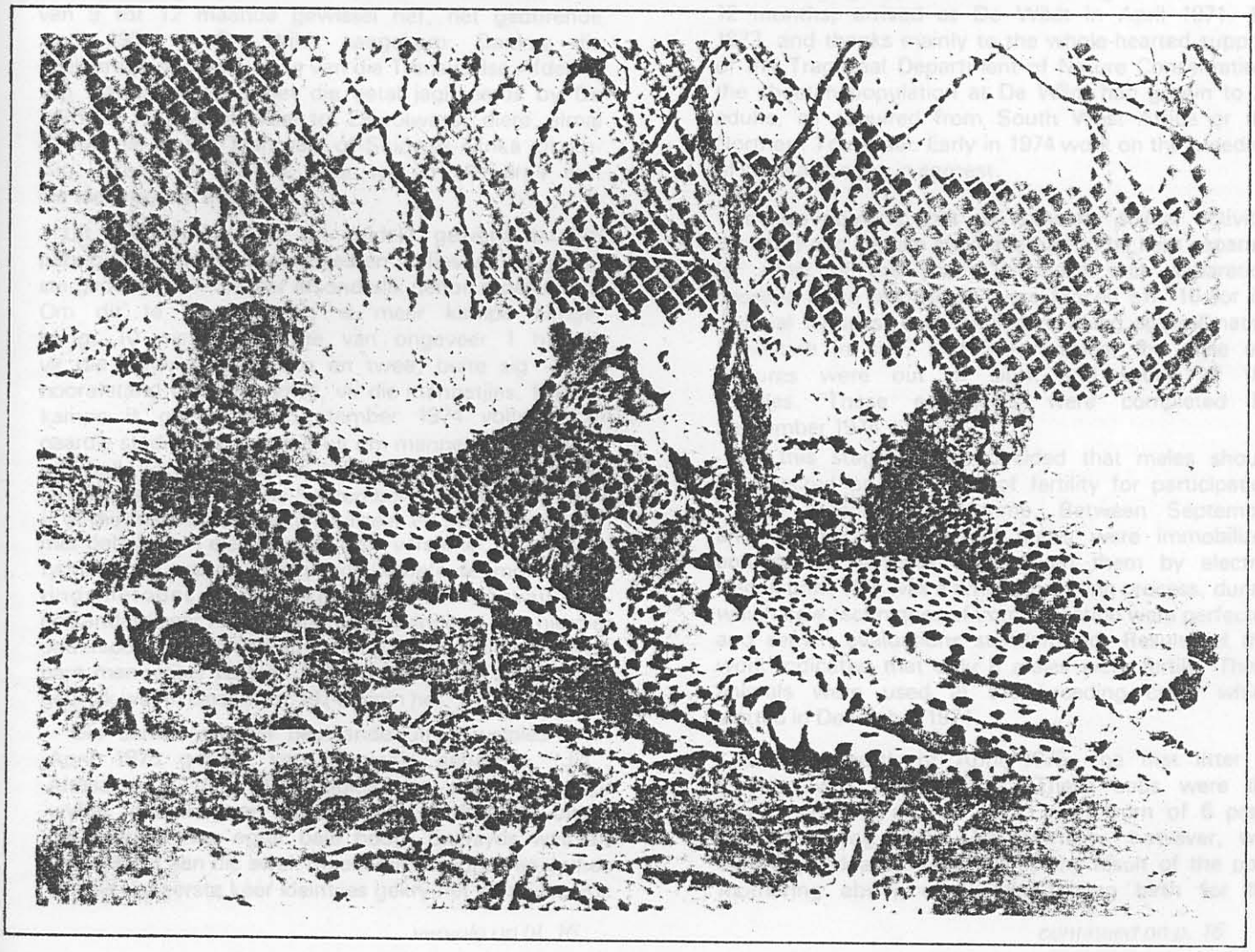
On the 1st November 1978, cheetahs returned to the Western Transvaal from where they had disappeared many years ago. The new arrivals appear to be no different from their predecessors. They are, however, unique in being captive-bred animals whose parents came from the Northern Transvaal and South West 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 navorsing 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 gevangeskap 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 gevangeskap 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 gevangeskap 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.

In 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 gestandardiseer moes word. Die bevindings van hierdie ondersoeke het aan die lig gebring dat daar slegs 8 vrugbare mannetjies was en hulle is toe in die teelseisoen 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

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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

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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 veranderings '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.

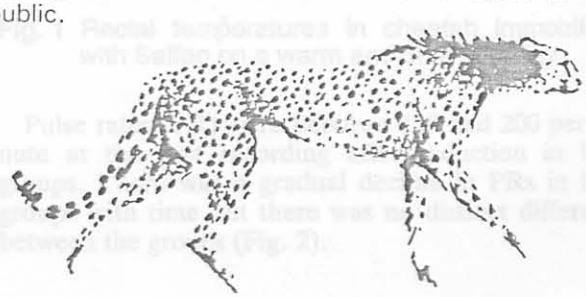
The following is a summary of the total number of cubs born to date:

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[1-3]. The injectable saline solution comprizes 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 measurements 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.

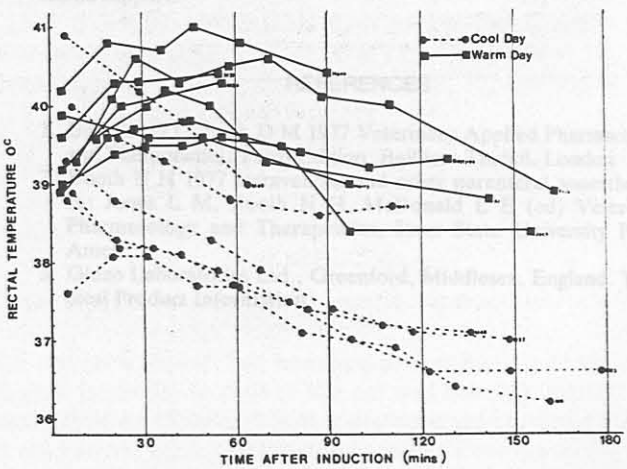


Fig. 1 Rectal temperatures in cheetah immobilized with Saffan on a warm and on a cool day

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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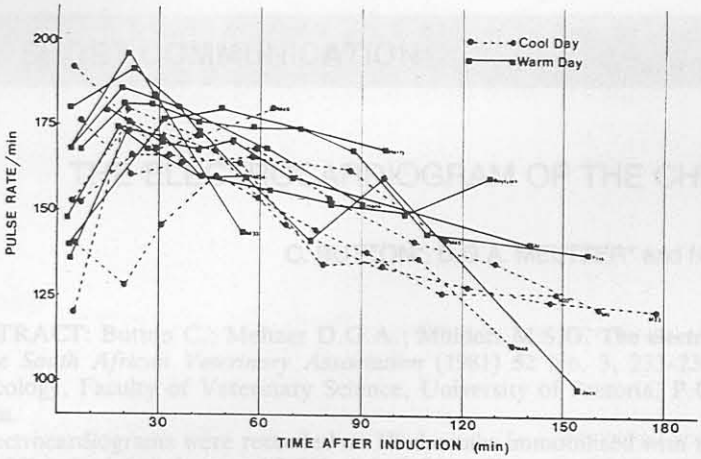

Fig. 2 Pulse rates in cheetah immobilized with Saffan on a warm and on a cool day

Respiratory rates (RR) (Fig. 3), not surprizingly followed RT closely. Animals with RTs greater than 39,5° 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° 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° C).

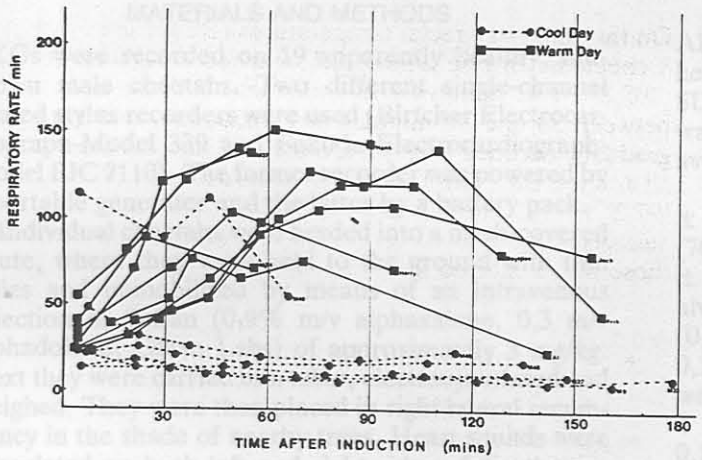

Fig. 3 Respiratory rates in cheetah immobilized with Saffan on a warm and on a cool day

DISCUSSION

The poikilothermic effects of Saffan on cheetah are presumed to be the result of supression 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 (19,5–20° C and 26–29° C) in this report are mild in comparison to the 37 to 43° 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° 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 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 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 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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SHORT COMMUNICATION KORT BERIG

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 ± SD 18 beats per minute. The average of mean electrical axes in the frontal plane was + 76° ± SD 13°. Mean ± SD durations in milliseconds on lead II were: P 47 ± 6,5; PR 93 ± 11,5; QRS 53 ± 7,5; QT 193 ± 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 ausculated 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 (CV_6LL) and on the dorsal midline just behind the scapulae (V_{10}).

Standard bipolar limb leads I, II and III; augmented unipolar limb leads aVR, aVL, and aVF; and unipolar leads CV_6LL and 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[1,2]. 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 ± 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 ± SD 13° with a range of 48–94°.

On lead II for 12 cheetahs durations were: P wave 47 ± SD 6,5 ms (40–60); PR interval 93 ± SD 11,5 ms (70–110); QRS 53 ± SD 7,5 ms (40–60) and QT was 193 ± SD 19,7 ms (160–230). The P wave on lead II was always positive averaging 0,18 ± SD 0,05 mV (0,10–0,25) N = 19. Q and q waves on lead II averaged 0,13 ± SD 0,09 mV (0,05–0,4) N = 14; and R and r waves averaged 0,81 ± SD 0,24 mV (0,45–1,3) N = 19. T waves were usually negative on lead II, averaging 0,11 ± 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 CV_6LL), backward (positive P waves on II, III and aVF, negative P waves on aVR and aVL) and downward (negative P waves on V_{10}) direction. The major forces of ventricular depolarization (Table 2) are likewise leftward (predominance of r waves on I and R waves on CV_6LL), 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 V_{10}). The leftward and backward orientation of ventricular depolarization is

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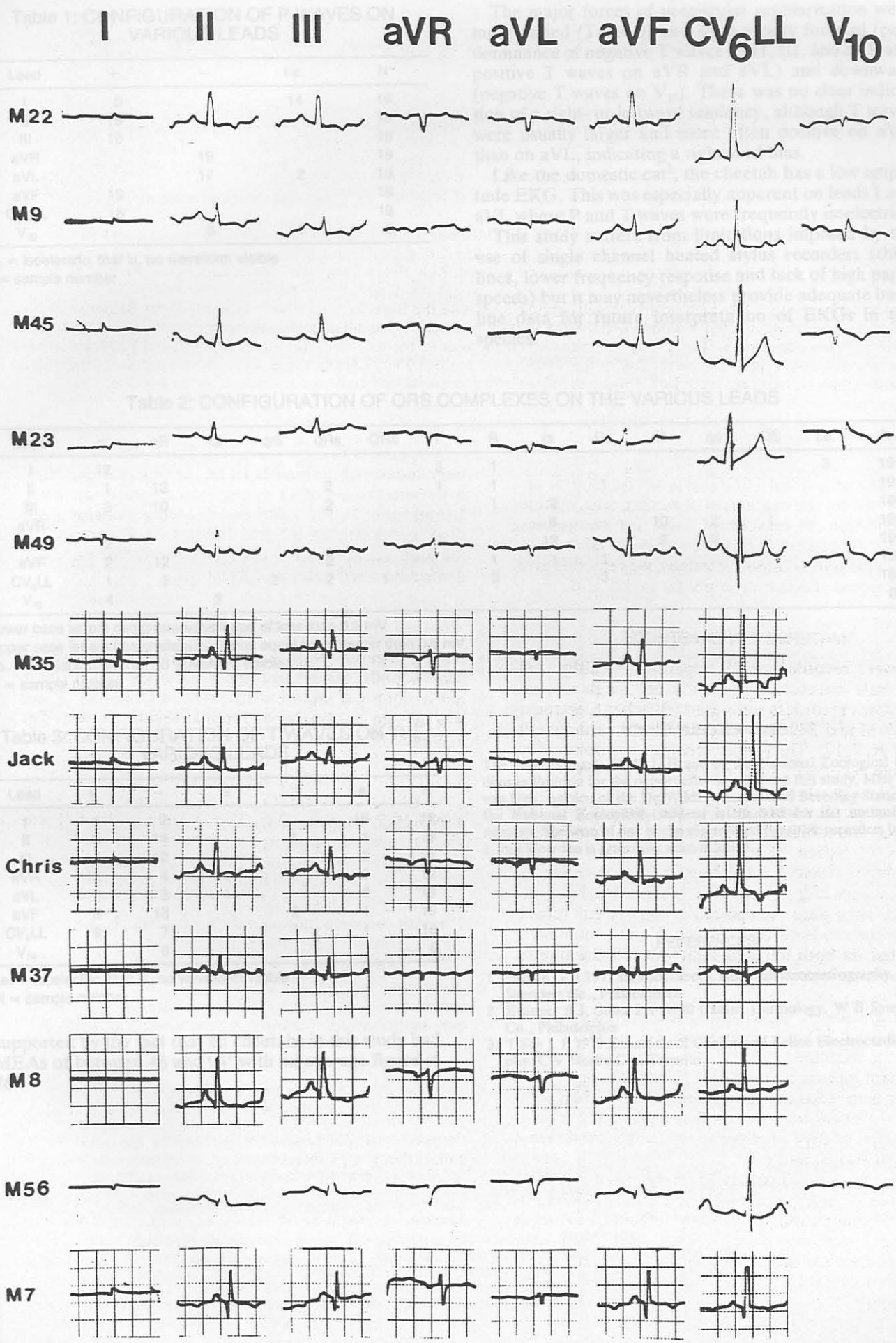

Fig. 1 Representative electrocardiographic complexes for various leads recorded at 50 mm/s for 12 cheetah. 1 cm = 1mV

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_6LL	18			18
V_{10}		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_{10}). 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[3], 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_6LL	1	6		2	2	1		3		3					18
V_{10}	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_6LL	9	7		1	1	18
V_{10}		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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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,[1] Mitchell Bush,[1] David Wildt,[2] D. J. Brand,[3] H. Ebedes,[3] A. van Dyk,[4] and D. Meltzer[5]

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.
2. Veterinary Resources Branch, National Institutes of Health, Bethesda, MD.
3. National Zoological Gardens of South Africa, Pretoria, South Africa.
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)	3.1 ± 0.2
Sperm count/ml ($\times 10^6$)	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^3)	17.8 ± 0.6
% morphological sperm abnormalities	71.0 ± 0.9

Table 2. Cheetah Sperm Morphology[6]

	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	1.4
Microcephalic sperm	1.2
Macrocephalic sperm	0.4

[6]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*

S.J. O'Brien,[1] D.E. Wildt,[1] J.M. Simonson,[1] D.J. Brand,[2] H. Ebedes,[2] A. van Dyk,[2] D. Meltzer,[3] L.G. Simmons,[4] and M. Bush[5]

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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Table 3. Cheetah Sperm Morphology.[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 zoo-geographic 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_2 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-Weinburg) equilibrium ($X^2 = 0.46$). 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 or 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=.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[1]	No. cats	Cluster P or H[2]
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
DIAL	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	α-L-Fucosidase	3.2.1.51	Lym.	7	P
GALA	β-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	β-Glucuronidase	3.2.1.31	Lym.	55	M
HBα	Hemoglobin-α		RBC	44	M
HBB	Hemoglobin-β		RBC	55	P
HEXA	Hexosaminidase-A	3.2.1.30	Lym.	55	M
HK1	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
PP	Pyrophosphatase (inorganic)	3.6.1.1	RBC	55	M
SOD1	Superoxide dismutase-1	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	16
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. [1]S.J. O'Brien, [6]M. Roelke, [6]L. Marker, [6]F. Hart, [1]D.G. Goldman, [1]C. Merrill, [5]J.G. Howard, [3]D. Meltzer, [2]A. van Dyk, [2]H. Ebedes, [2]D.J. Brand, [1]J.M. Simonson, [4]L.G. Simmons, [5]M. Bush, [1]D.E. Wildt. [1]National Institutes of Health; [2]National Zoological Gardens of South Africa, Pretoria; [3]Department of Veterinary Science, Onderstepoort, RSA; [4]Henry Doorley Zoo, Omaha; [5]National Zoological Park, Washington, D.C.; [6]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 sparce, 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 (14.5×10^6 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 consistantly 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.

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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 X South West) males. However, mean spermatozoal concentration (14.5×10^6 spermatozoa/ml of ejaculate) and percent motility (54.0%) were less in cheetahs than in domestic cats (147.0×10^6 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 (± SEM) 56.0 ± 0.2 kg in weight and 5.3 ± 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 ± 1.5 years; South West, 6.0 ± 1.9 years; hybrid 3.6 ± 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 400×. 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 μl) 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 (1000×) 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 ± 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 ± 5.5 × 10^6 spermatozoa/ejaculate) and second (29.4 ± 5.7 × 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 concentration, 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 ± 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 ± 1.8 × 10^6 (10 times less than domestic cats), and the percent motility rating of cheetah spermatozoa was 54.0 ± 3.0%, about 70% of that observed in cat ejaculates (Table 3). An average of 71.0 ± 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 ± 2.5, 75.7 ± 2.3 and 67.3 ± 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[a]	Ejaculate volume (ml)	Spermatozoal		
			Concentration/ml of ejaculate (×10^6)	Concentration/ ejaculate (×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

[a]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 ± 1.9%) was not different (P>0.05) from the first (69.4 ± 5.9%). An average of 29.1 ± 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.[a]

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

[a]Values are means ± SEM.

TABLE 3. Seminal traits in the South African cheetah compared to the domestic cat[a].

	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 ± 1.8	147.0 ± 39.5[b]
Spermatozoal motility (%)	54.0 ± 3.0	77.0 ± 3.0[b]
Morphological abnormalities of spermatozoa (%)	71.0 ± 0.9[b]	29.1 ± 3.7
Primary		
Coiled flagellum	25.8 ± 2.3[b]	5.5 ± 0.8
Microcephalic defect	1.2 ± 0.3[b]	0.2 ± 0.1
Macrocephalic defect	0.4 ± 0.2	0.1 ± 0.04
Secondary		
Bent midpiece	23.3 ± 1.1[b]	6.4 ± 0.8
Bent flagellum	16.2 ± 1.3[b]	5.1 ± 0.7
Bent flagellum tip	2.8 ± 0.6[b]	0.02 ± 0.01
Protoplasmic droplet	1.3 ± 0.3	11.8 ± 1.7[b]

[a]Values are means ± SEM.

[b]Significantly greater (P<0.05) than counterpart value.

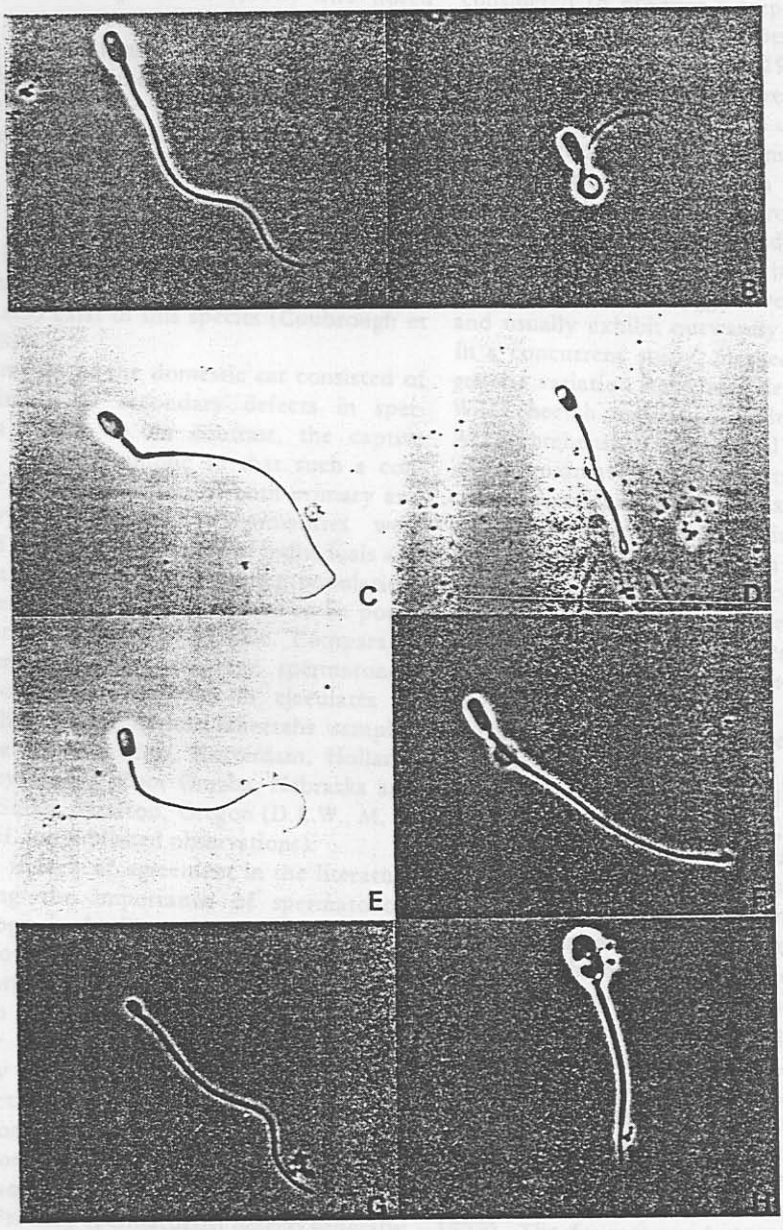

FIG. 1. Spermatozoal forms detected in the ejaculate of electroejaculated cheetahs: *A*) normal; *B*) coiled flagellum; *C*) bent midpiece; *D*) bent flagellum; *E*) bent flagellum tip; *F*) protoplasmic droplet; *G*) microcephalic defect; *H*) macrocephalic defect.

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 abnormals in inbred mice returns to normalcy (circa 2% abnormals) 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 ($P<0{\cdot}05$) 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 ($P<0{\cdot}05$) 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,

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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 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 µl samples compared with activity levels measured in 100–200 µl 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.

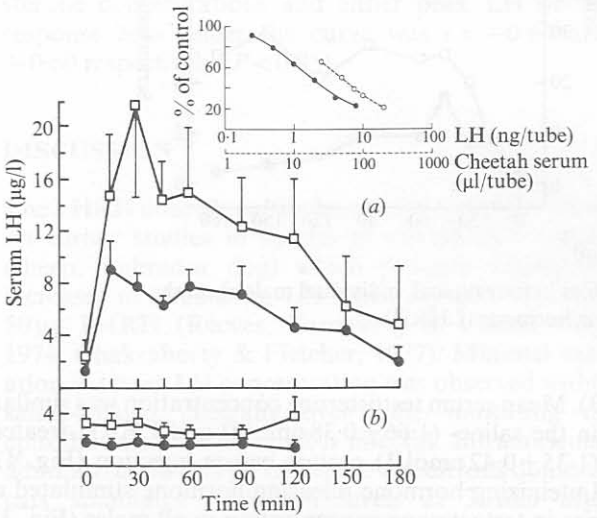

FIGURE 1. Serum LH concentrations in female (●) and male (□) cheetahs treated with (*a*) 50 µg LH releasing hormone or (*b*) saline. Each datum point is the average of seven individual values. Inset depicts LH inhibition curves of canine pituitary standard (LER-1685-1) (●) and pooled cheetah serum (○).

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 µg/l and from 2·3 to 3·3 µg/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 ($1143 \pm 260\ mm^2$/120 min) was greater ($P < 0{\cdot}05$) than that of females ($623 \pm 164\ mm^2$/120 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 µg/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 µg/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 ($P < 0{\cdot}05$) at the 15-min sampling period in both the female ($9{\cdot}0 \pm 2{\cdot}2$ µg/l) and male ($14{\cdot}7 \pm 4{\cdot}7$ µg/l) groups (Fig. 1). Mean serum LH in females was sustained for 90 min between 6·2 and 7 µg/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 ($21{\cdot}6 \pm 7{\cdot}2$ µg/l) occurred at 30 min after LHRH injection and LH levels were greater ($P < 0{\cdot}05$ or $P < 0{\cdot}01$) 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 ($5755 \pm 1589\ mm^2$/120 min) was greater ($P < 0{\cdot}05$) than in females ($2542 \pm 399\ mm^2$/120 min).

Serum oestradiol-17β and testosterone response to LHRH

Gonadal sensitivity to treatment was examined by measuring serum oestradiol-17β in female and testosterone in male cheetahs. In individual saline-treated females serum oestradiol-17β 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 ($P < 0{\cdot}05$) in the LHRH group at only the 90-min sampling period (saline, 37 ± 15 pmol/l; LHRH, 147 ± 56 pmol/l). The

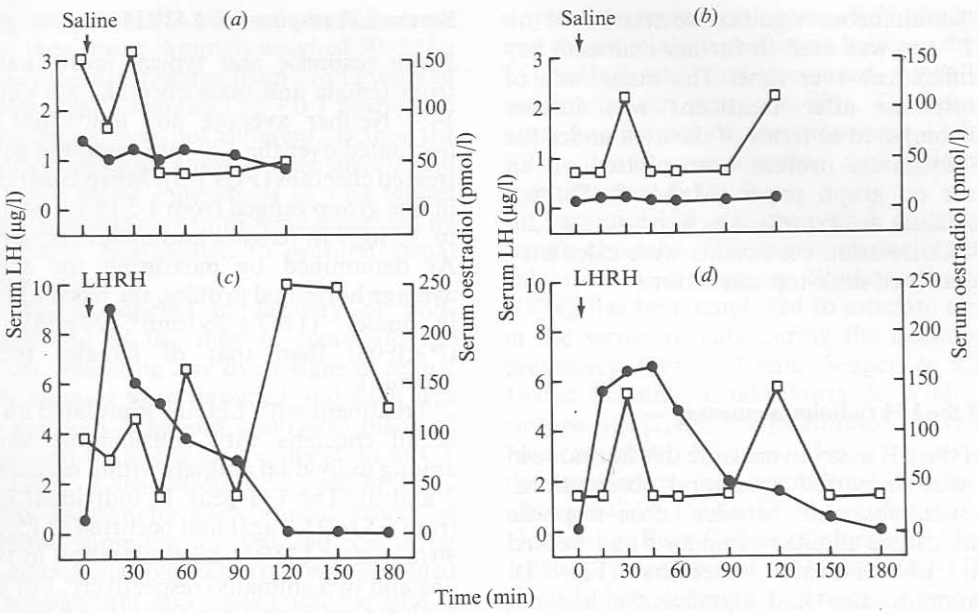

FIGURE 2. Serum LH (●) and oestradiol-17β (□) profiles in four typical, individual female cheetahs treated with (*a*, *b*) saline or (*c*, *d*) 50 μg LH releasing hormone (LHRH).

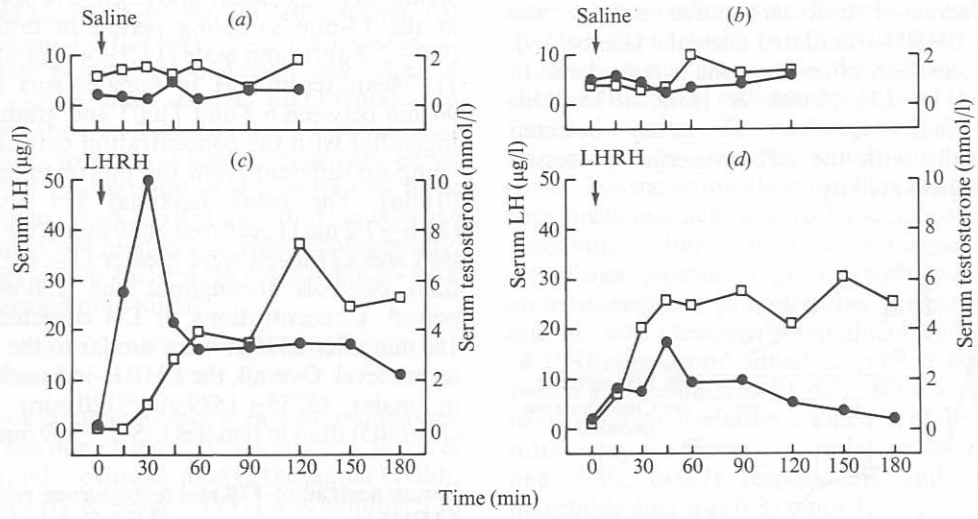

FIGURE 3. Serum LH (●) and testosterone (□) profiles in four typical, individual male cheetahs treated with (*a*, *b*) saline or (*c*, *d*) 50 μg LH releasing hormone (LHRH).

areas under the profiles were not different (saline, $1481 \pm 190\,mm^2/120\,min$; LHRH, $2562 \pm 621\,mm^2/120\,min$), 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 ($r = -0{\cdot}23$ and $-0{\cdot}13$ 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- ($1{\cdot}66 \pm 0{\cdot}38$ nmol/l) and LHRH-treated ($1{\cdot}35 \pm 0{\cdot}42$ 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 ($P < 0{\cdot}05$) above control levels by 60 min (Fig. 4). Overall the area under the testosterone curve in LHRH-stimulated males

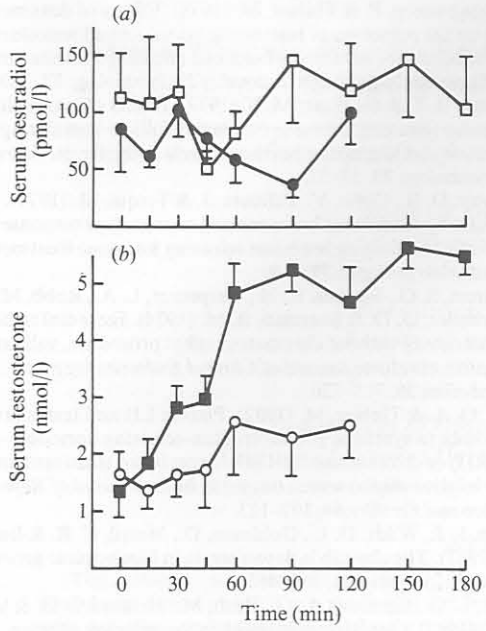

FIGURE 4. Serum (*a*) oestradiol-17β concentrations in female cheetahs and (*b*) testosterone levels in male cheetahs treated with saline (circles) or 50 μg LH releasing hormone (squares). Each datum point is the average of seven individual values.

($4471 \pm 364\,mm^2/120\,min$) was greater ($P<0{\cdot}05$) than in control males ($2611 \pm 631\,mm^2/120\,min$). The correlation coefficient between pre-LHRH testosterone concentrations and either peak LH or the response area under the curve was $r=-0{\cdot}64$ and $-0{\cdot}60$ respectively ($P<0{\cdot}05$).

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 μg 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 μg 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 radioimmunoassay system. Whereas the greatest average LH level in the female cheetah was 9 μg/l, as little as 5 μg LHRH injected i.m. into the anoestrous domestic cat results in a mean LH peak of 114 μg/l 30 min later (Chakraborty *et al.* 1979). Similarly, 50 μg LHRH administered by the same route to the Labrador bitch produce an LH peak of 40 μg/l 1 h after injection (Chakraborty & Fletcher, 1977). Interestingly, the quantitative and temporal profile of the bitch treated with 5 μg LHRH almost exactly mimicked the LH response of the female cheetah.

Oestradiol-17β was also measured in females as an indirect determinant of whether presumed LHRH-induced gonadotrophin release stimulated oestradiol-17β synthesis and release via modified or increased follicular activity. Oestradiol-17β 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-17β 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-17β levels, although it was possible that the 180-min sampling interval was insufficient to affect ovarian oestradiol-17β 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 ± 0·7 years) and males (3·7 ± 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 °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-Cl; 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 ± S.E.M.) from assay of nanogram amounts of LH (0·28, 1·2, 3·2 and 5·6) were: 0·3 ± 0·02, 1·2 ± 0·08, 3·0 ± 0·06 and 5·6 ± 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, 1981*a*; Schmidt, Chakraborty & Wildt, 1983). The isotope was [2,4,6,7-^{3}H]oestradiol-17β (New England Nuclear, North Billerica, Massachusetts, U.S.A) and the antiserum anti-oestradiol-17β-6–bovine serum albumin (244; Dr G. D. Niswender, Colorado State University, Fort Collins, Colorado, U.S.A.; dilution 1:7500). Recovery of [^{3}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-^{3}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, androstanedione, progesterone, pregnenolone, cholesterol and testosterone-β-glucuronide. Recovery of [^{3}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 ± 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 ± 0.2 kg BW, ranged from 2–12 yr of age (mean, 5.3 ± 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 ± 0.6 yr) and electroejaculated (5.7 ± 0.9 yr) group was not different ($P>0.05$).

Radioimmunoassays

Serum was collected and stored at −20°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}I RIA kit (RIANEN^{TM}, New England Nuclear, No. Billerica, MA). This assay employed a cortisol antiserum complex solution containing rabbit cortisol antibody prereacted 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 17α-hydroxyprogesterone; 2% or less with progesterone, testosterone, dihydrotesterone and estradiol-17β. 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}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, androstanedione, progesterone, pregnenolone, cholesterol and testosterone-β-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 μl) 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 [^{3}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 ^{125}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, 3α-hydroxy-5α-pregnane-11, 20-dione (alphaxalone) and 21-acetoxy-3α-hydroxy-5α-pregnane-11, 20-dione (alphadolone acetate). Each ml of solubilized CT-1341 contained 12 mg of total steroids composed of 9 mg alphaxolone 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 μl) of a diluted solution of CT-1341 (1.2 μg/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 ± SEM. An SPSS computer program employing a general analysis of repeated measures and a Greenhouse-Geisser correction was used to satistically analyze hormonal results (Greenhouse and Geisser, 1959). A simultaneous significance test based on Hotellings' T^2 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 ± 13.7 ng/ml (preanesthesia) to 44.1 ± 7.7 ng/ml (145 min later) and gradually declined over time ($P<0.01$). In electroejacula-

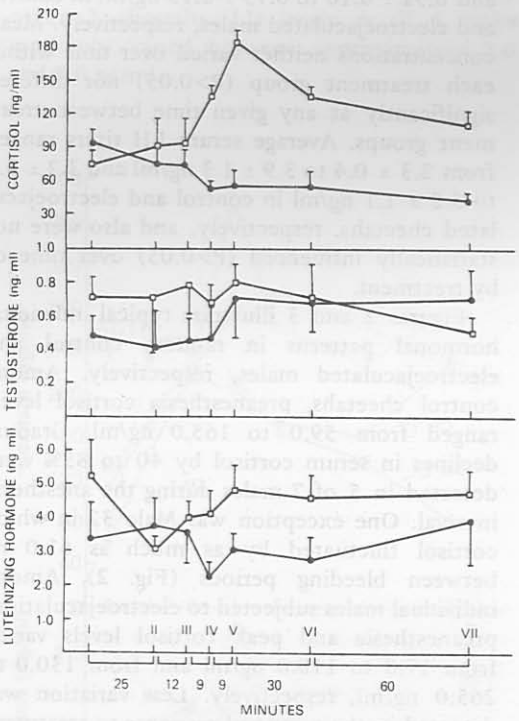

FIG. 1. Serum cortisol, testosterone and LH concentrations in male cheetahs serially bled *1*) before and during CT-1341 anesthesia (n=7, ●) or *2*) before, during and after electroejaculation under CT-1341 anesthesia (n=14, □). *Roman numerals* indicate time of blood sampling: *I*=preanesthesia, preelectroejaculation; *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 ± SEM.

ted cheetahs, mean cortisol concentrations preanesthesia (77.0 ± 11.2 ng/ml), postanesthesia, preelectroejaculation (91.5 ± 23.1 ng/ml) and following the first 30 electrical stimulations (92.8 ± 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 ± 9.9 ng/ml) with a further rise and peak of 184.0 ± 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 ± 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 ± 0.12 to 0.72 ± 0.27 ng/ml and 0.51 ± 0.10 to 0.79 ± 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 ± 0.4 to 3.9 ± 1.3 ng/ml and 3.2 ± 0.4 to 5.2 ± 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

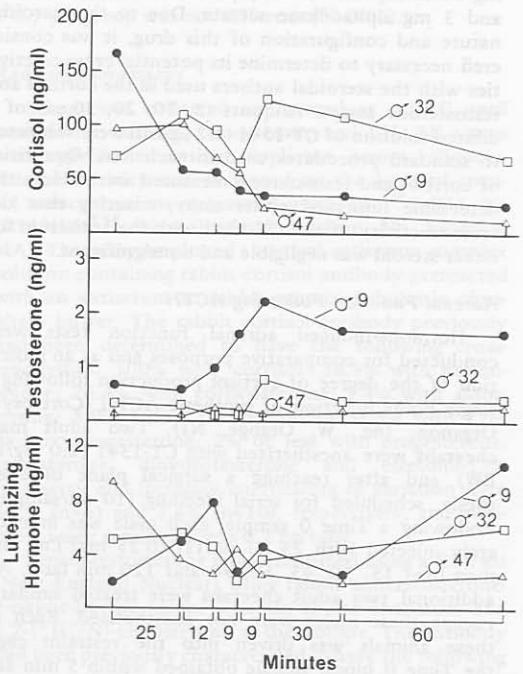

FIG. 2. Serum cortisol, testosterone and LH concentrations in male cheetahs subjected to serial bleeding under CT-1341 anesthesia only. See Fig. 1 for explanation of bleeding times.

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-Gondal 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.

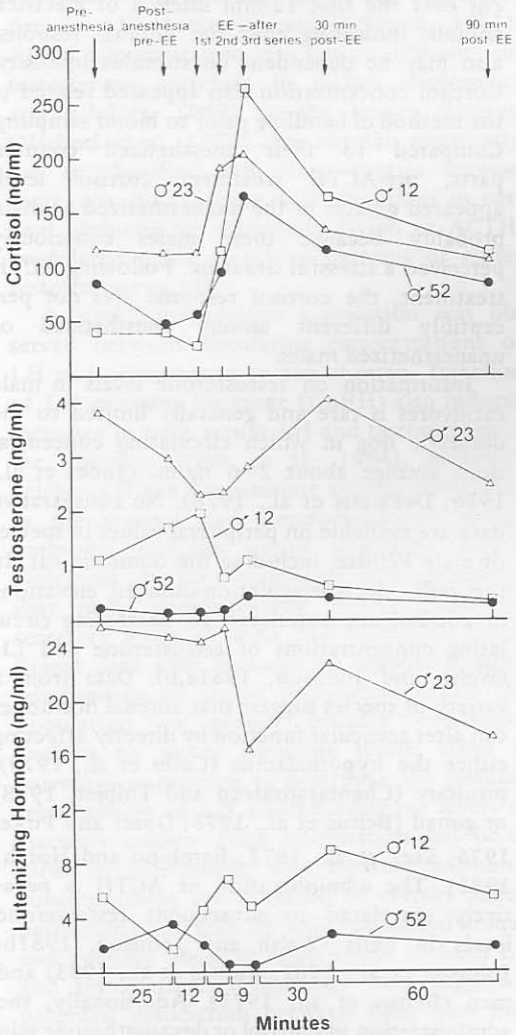

FIG. 3. Serum cortisol, testosterone and LH concentrations in male cheetahs subjected to serial bleeding and regimented electroejaculation under CT-1341 anesthesia. See Fig. 1 for explanation of bleeding times.

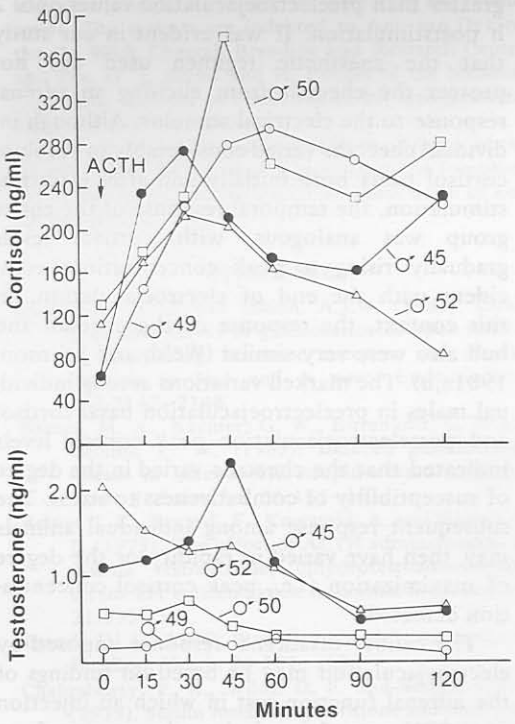

FIG. 4. Serum cortisol and testosterone concentrations in two unanesthetized (Males *50* and *52*) and two CT-1341 anesthetized (Males *45* and *49*) cheetahs injected with 25 i.u. ACTH immediately after collection of a 0-min blood sample.

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 (Chantaraprateep 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 aslo eliminates the nocturnal rise of testosterone in man (Doerr and Pirke, 1976) and decreases LH synthesis in bulls (Thibier and Rollard, 1976; Chantaraprateep 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 interrelationships 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 ± 0.4 and 9.7 ± 3.3 ng/ml, respectively, which were greater ($P<0.05$) than the levels of 0.51 ± 0.1 and 4.5 ± 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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