Bertschinger H, Meltzer DG, van Dijk A, Coubrough RI, Solely JT, Collett FA. Cheetah lifeline.

Keywords: Acinonyx jubatus/breeding/captivity/cheetah/fertility/king cheetah/oestrus/release/research/testosterone

Abstract: A report on the breeding program and some information of the research that has lead to a better understanding of the reproductive physiology of cheetahs.
Different chapter about releasing captive-bred cheetahs in the wild and their survival success; first improvements to breeding management in separating males and females; reproductive data of the female oestrus cycle and hormone patterns and the males fertility and influence of testosterone; king cheetah has to be found out not to be a separate species but merely a colour variation.

Cheetah life-line

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Modern man's encroachment into their habitat, his decimation of their prey species and his direct impact on their numbers has driven wild felids into relatively small tracts of land. Cheetah populations are thinly spread in a natural environment. At the same time the confinement of an animal used to large open spaces, in zoos, has an impact on its breeding potential. An investigation in 1969, by the International Union for the Conservation of Nature, showed that cheetah numbers were declining in the wild and it was classified as an endangered species. The poor breeding performance of captive populations gave concern for its survival. The Cheetah Research Centre (National Zoological Gardens of South Africa) was conceived under these circumstances. The aim was to breed cheetah in sufficient numbers so that animals could be made available to zoos, and to establish whether such captive-bred animals could be released back into a natural environment.

Sceptics claimed that captive-bred cheetah would not survive if released into the wild before having been taught to hunt and fend for themselves. In order to disprove this theory Pettifer and co-workers [1,2] carried out an experiment in a game park near Hoedspruit, belonging to the South African Defence Force. Three 2 year old male litter mates, bred in captivity at the Cheetah Research Centre were fitted with radio-collars and released in the game park. Researchers monitored the movements of the animals 24 hours a day without disturbing or assisting them. The cheetah killed a giraffe on the first day of their release and thereafter, killed very regularly. They were recaptured after a period of 4 weeks and released once again in a much larger game park (Timbivati Reserve) where they stayed for a further 2 months. During this period they covered a distance of 500 km and showed their ability to survive by making regular kills and defending themselves against hyena as well as other cheetah. One of the cheetah was lost due to a puffadder bite.

The Cheetah Research Centre at de Wildt was established in 1971 when Ann van Dijk and her brother Godfrey donated 50 ha of land on the northern foothills of the Magaliesberg range, 25 km east of Pretoria. The cost of the fencing and development of the station was borne largely by these two generous people. The dedication of Ann van Dijk and her personnel has made the de Wildt project a great success. The object of this article is to report on the breeding program and some of the research that has lead to a better understanding of the reproductive physiology of cheetah.

Centre lay-out; breeding management

The original wild-captured breeding animals (9 females and 20 males) came from the northern Transvaal and SWA/Namibia. In the beginning it was thought that breeding success could be achieved by running the males and females together in one large camp. Although a number of females were seen on heat and some matings were observed, no litters were produced during the first 3 years (1972-1974)[3,4]. We soon realised that if progress was to be made, more had to be learnt about the reproductive physiology of the cheetah.

One of the first steps to improve our knowledge was to examine the males for fertility. The tests revealed that 11 of the 19 males examined were either subfertile or even sterile. As some of the males with poor fertility were amongst the dominant males, the fertile males were seldom if ever allowed to cover a female on heat. In addition some males exhibited abnormal behaviour in that they were aggressive towards the females. Selection of fertile and non-aggressive males, before breeding, and separation of males and females for most of the year led to the first breeding successes.

Figure 1 is a plan of the lay-out of the enclosures at the Cheetah Research Centre. The perimeter fences are 3 m high and are equipped with an overhang to prevent cheetah from climbing over the top (Figure 2). The internal fences are 1,8 m in height. All fences are made of pig-wire mesh with the bottom end fixed into a concrete apron to prevent burrowing underneath.

As mentioned above, one of the first improvements to breeding management was to separate males and females. The non-pregnant breeding females are housed individually in breeding camps of ±0,5 ha each. During the breeding season the fertile males are allowed to 'patrol' the walk-way between the two rows of breeding camps. As soon as a female is on heat, a selected male is allowed to enter her camp, where he remains for 2–3 days. During the non-breeding season the males are kept in several holding camps, which are fairly far removed from the breeding camps.

Initially, once breeding success was established, the pregnant females were allowed to cub in the breeding camps. The area of these camps, being fairly large, made control of partus and the newly born cubs difficult. One of the problems in captive-bred cheetah, which may also occur in the wild, is abandoning or killing of the young especially by inexperienced young females. A large percentage of the cubs lost in the first successful breeding year was due to these two factors. A probable contributing factor for this behaviour was the lack of privacy offered in the large camps.

Accordingly it was decided to build maternity camps. These camps are much smaller (30 x 20 m) and the perimeter fences are lined with thatching grass to offer greater privacy. In addition each camp has a den made of wire and thatching grass which the mothers use spontaneously for cubbing and nesting the cubs. The camps enable observation of the mother and litter without disturbing them. If the cubs are abandoned it will be noticed almost immediately, whereupon they can be removed and hand-reared. Hand-rearing and care of sick cubs is carried out in the hospital.

The cubs are weaned at 4-6 months of age when they are moved to the juvenile holding camps.

Reproductive data of the female cheetah

1 Seasonal incidence of breeding
The cheetah is a seasonally polyoestrus animal and at de Wildt the main breeding season is in the summer months (November–February). In mid-winter there appears to be a shorter breeding season (June–July) which is of lesser importance[4]. In the wild small cubs have been seen throughout the year but again the main breeding season appears to be in mid-summer.

2 Signs of oestrus Puberty in the female cheetah occurs at 2½–3 years of age. The external signs of oestrus in the cheetah are difficult to observe, mainly because the vulva is difficult to

Figure 1 Plan of the camps at the Cheetah Research Centre. (a) male holding camps; (b) exercise area; (c) quarantine camps; (d) walk-way for breeding males; (e) breeding camps for females; (f) maternity camps; (g) holding camps for juveniles; (h) hospital.

Figure 2 Perimeter fences with overhang and concrete apron.

Figure 3 Catching crush showing method used to restrain cheetah for treatment.

Figure 4 Catching crush which can be operated by means of ropes.

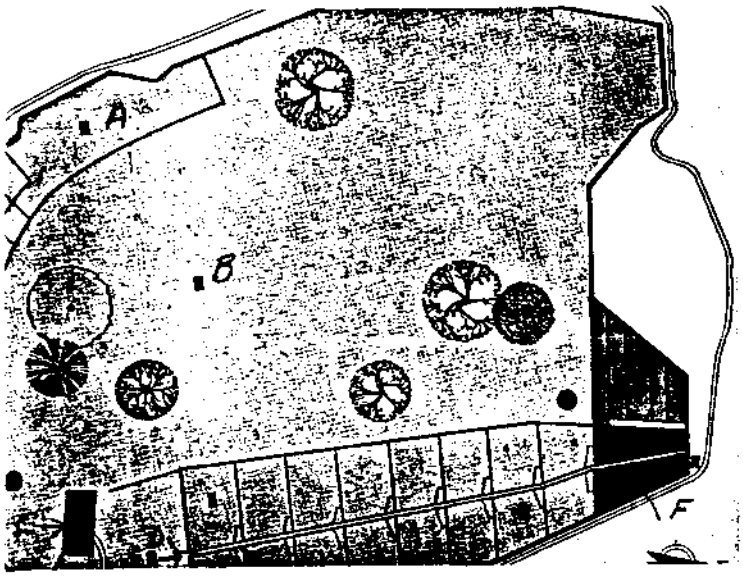

Figure 1

Figure 2

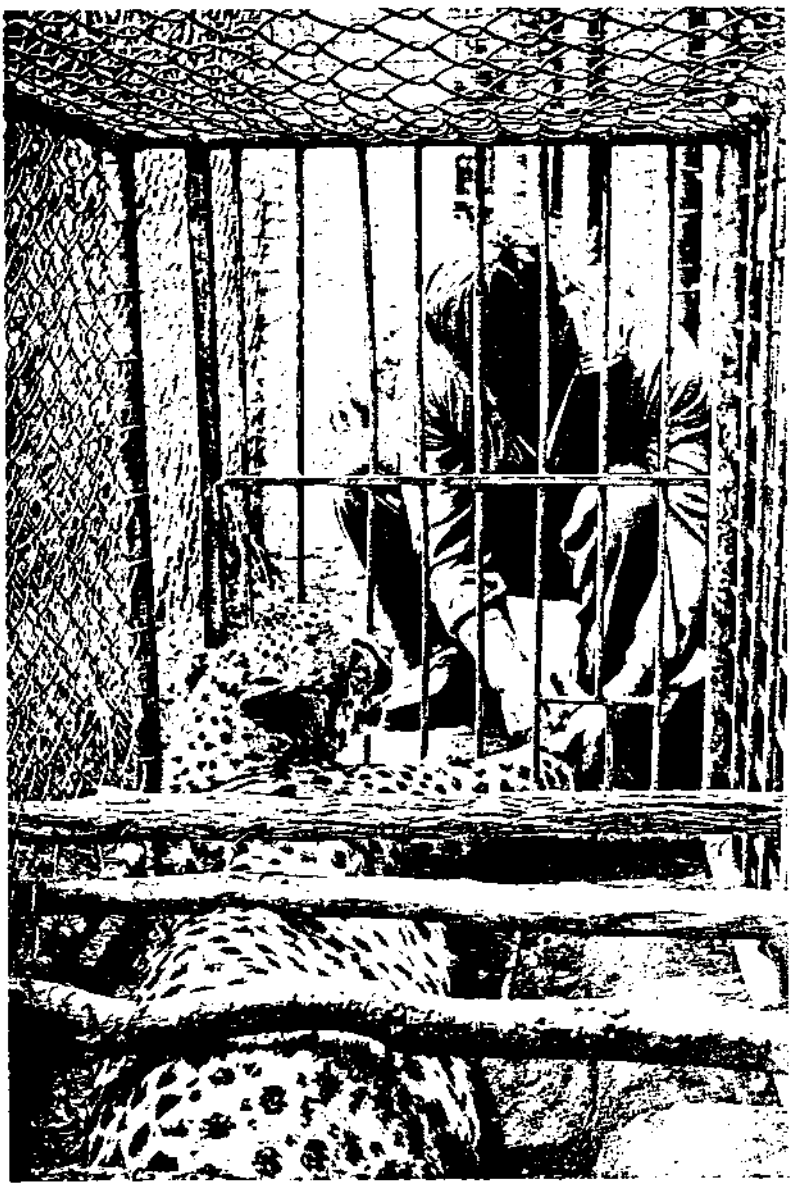

Figure 3

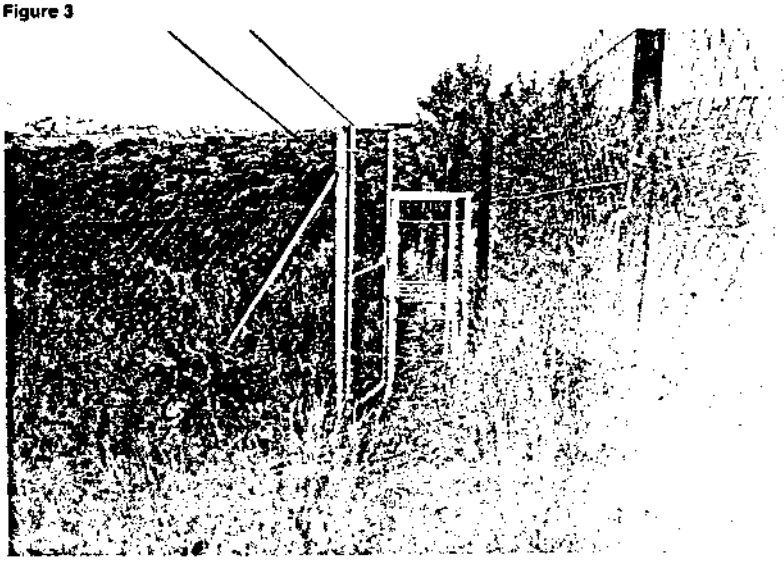

Figure 4

see unless the animal is caught and restrained. Even then, the mild swelling and increase in mucus secretion is not easily seen. Vaginal cytology can be used to diagnose oestrus but this is impractical on a regular basis. The most reliable means of spotting oestrus is to observe the females for behavioural changes, which is best carried out in the presence of the male. For this purpose, our breeding males are allowed into the walk-way on a daily basis in the presence of an experienced observer. Females coming into heat tend to withdraw themselves a day or so before heat, in that they stay away at feeding time. The most consistent sign of proper heat is rolling and arching of the back in the presence of the male. Males in the walk-way will also take more interest in the female on heat and gather on the other side of the fence. The mating position which is adopted by the male and female during copulation is the same as in other cats.

3 Oestrus cycle and hormone patterns While observations of the cheetah at the Cheetah Research Centre over the period 1972–1979 had provided a wealth of data on behaviour, gestation period, average litter size and so on, we still lacked accurate data on the length of oestrus cycle, hormone patterns during the cycle and whether or not cheetah are induced ovulators like other cats. The main reason for this lack of knowledge is that the females were inevitably mated as soon as they came on heat. In an effort to gain more data we decided to conduct a controlled experiment during the breeding season on 7 females. These animals were observed daily for signs of oestrus. Instead of allowing mating to take place at the first observed heat, she would be allowed to cycle once or twice.

In order to study some of the hormonal changes during the oestrus cycle and following mating, blood was collected from each female 2–3 times per week. More frequent bleeding was avoided so as not to stress the animals unduly, which could have affected the oestrus cycle. Cheetahs can be bled quite easily from the cephalic or saphenous veins without anaesthesia or tranquilisation provided they can be restrained properly. Catching crushes have been developed at the Cheetah Research Centre as they are often required for the handling, examination and treatment of cheetah. An example of such a crush is shown in Figure 3. The crush is equipped with a sliding door (horizontal or vertical) at each end. Vertical doors have the advantage that they can be operated by remote control by means of ropes (Figure 4). The crushes are either positioned in the middle of a shute (Figure 3) or at the exit of a funnel-shaped holding camp (Figure 4), as in the case of the breeding camps. With the exit door of the crush closed, the cheetah is herded into the crush and the door is closed. Once inside the cheetah is restrained by placing poles across its body (Figure 3), a fore- or hind-limb is retrieved and the blood sample is taken. The whole procedure seldom takes longer than 5 minutes after which the animal is released.

In order to correlate the oestrus observations and the hormonal findings with ovarian changes, the animals were examined from time to time with a laparoscope (Wolf, Type 4939.31) to visualise the internal reproductive organs. Laparoscopy was carried out under general anaesthesia using either thiopentone sodium (Intraval Sodium, May Baker) or alphaxalone/alphadolone (Saffan, Glaxo Labs) intravenously[5].

The blood samples (heparinized plasma) were assayed for progesterone and 17β-oestradiol. Progesterone was assayed by means of a direct ^{125}I radioimmunoassay kit (Coat-A-Count Progesterone, Diagnostic Products Corporation). For the 17β-oestradiol determinations the plasma samples were first extracted with ether. The ether extract was then assayed using an ^{125}I radioimmunoassay kit (Estradiol-17β Kit, Radioassay Systems Laboratories, Inc.).

Insufficient oestrus periods were observed to determine the average length of cheetah oestrus cycle. The oestrus cycle in 6 of the 7 cheetah that cycled, ranged from 10 to 21 days. Though difficult to determine other than by the period of receptivity, oestrus varied from 1 to 3 days. When mating occurred oestrus was shorter. These figures agree favourably with those reported for the domestic cat[6,7]. Circulating 17β-oestradiol plasma concentrations showed cyclical peaks which lasted ±12 days. Progesterone plasma concentrations only rose subsequent to mating. The lack of a rise in the basal plasma progesterone concentration in the absence of mating shows that the cheetah, like the domestic cat and some of the

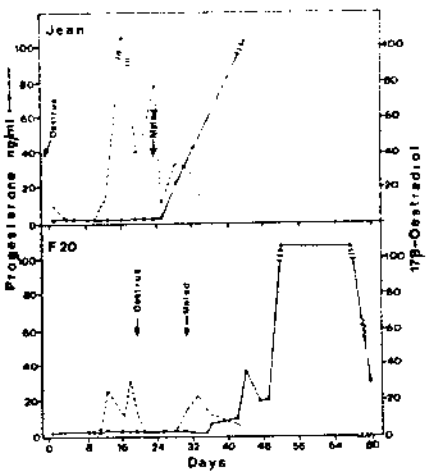

Figure 5

other members of the cat family[6,7,8,9] is an induced ovulator. In other words luteinizing hormone surges required for ovulation to take place only follows mating. Two typical endocrine cycles are shown in Figure 5. Plasma progesterone concentrations are now used routinely to confirm that mating, which is not always seen, has taken place.

The results of the laparoscopic examinations were able to substantiate the findings of the oestrus observations as well as the hormonal patterns. Figure 6 shows a Graafian follicle in an ovary of a cheetah in oestrus and Figure 7 shows an ovary of a female with 2 distinct developing corpora lutea, 5 days after mating. The average length of gestation in the cheetah is 93 days.

Examinations carried out on male cheetah

1. Examination of males for fertility Since the original semen examinations performed in 1974 all the adult males have been examined for fertility prior to each breeding season. Before the examination can be carried out the males are caught one by one in a crush and anaesthetised. The anaesthetised male is then placed on a table, his external genitalia are examined and semen is collected by electro-stimulation using a ram ejaculator. A complete spermiogram is carried out on the semen sample. The information obtained from the examination of the genitalia and the semen sample is used to decide whether the animal is suitable for breeding.

A range of interesting morphologinal defects, which have been studied

Figure 8 Scanning electron micrograph of a normal cheetah sperm

Figure 9 Scanning electron micrograph of cheetah sperms showing the 'Dag-defect'.

Figure 10 Plasma testosterone concentrations in 4 male cheetah bled every 2 hours for a 24-hour period.

Figure 11 Plasma testosterone concentrations in 4 males following a GnRH stimulation of 50 µg. The bottom curve is that of an unstimulated control animal.

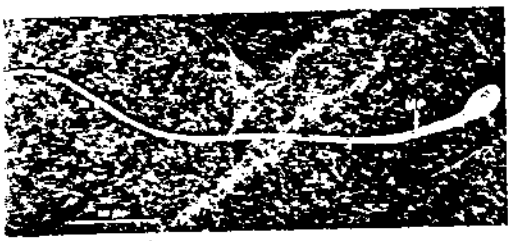

Figure 8

Figure 9

using light microscopy, transmission and scanning electromicroscopy, occur in cheetah sperm[10,11,12,13]. A serious defect, seen commonly in cheetah sperm, is the so-called 'Dag-defect'[10,13], which if seen in a high percentage of the sperm, indicates infertility. Figures 8 and 9 show scanning electron micrographs of a normal sperm and 'Dag-defect' sperms with coiled tails, respectively.

Semen examinations carried out at various times throughout the year in our cheetah revealed a seasonal variation in semen quality. The best quality was observed during the breeding season, whereas a trough in semen quality was seen in August to September. Puberty in the male occurs at approximately 2 years of age with the maximum breeding potential occurring at 3 to 4 years of age.

2. Circulating plasma testosterone concentrations and fertility[14] In the male testosterone is the most important androgen. It is involved in the maintenance of normal spermatogenesis, the support of the secondary genital organs and the expression of libido. Our interest in testosterone of male cheetah lay in the connection of this hormone with semen quality (spermatogenesis and maturation of sperm).

One of the problems associated with the interpretation of circulating plasma testosterone concentrations is the fact that these concentrations are subject to tremendous diurnal fluctuations and therefore, a tremendous normal range. Figure 10 demonstrates the fluctuations observed in 4 adult male cheetah bled every 2 hours over a 24-hour period. Each male shows approximately 3 peaks per 24-hour period. Plasma testosterone concentrations were determined by means of direct ^{125}I radioimmunoassay (Coat-A-Count Testosterone, Diagnostic Products Corporation).

To enable comparison of males, despite these variations, they can be subjected to a standardised gonadoliberin (GnRH) stimulation test. The animals are anaesthetised with Saffan for the duration of the test. A standard dose of GnRH (50 µg) is injected intramuscularly and blood samples are taken at half-hourly intervals for 4 hours. Figure 11 shows the response of 4 males to GnRH stimulation. One animal was not injected with GnRH to serve as a control.

By using the above described standardised stimulation test we were able to compare the testosterone response of cheetah with different semen qualities. They were divided into 3 groups namely: males with good semen quality, males with fair semen quality and males with poor semen quality. The results are tabulated in Table 1. No statistically significant differences in testosterone concentrations could be seen either at zero time (before injection of GnRH) or at maximum response between any of the groups.

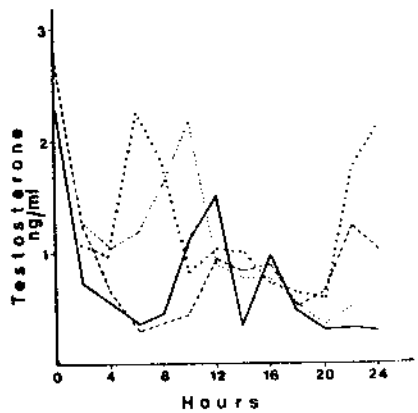

Figure 10

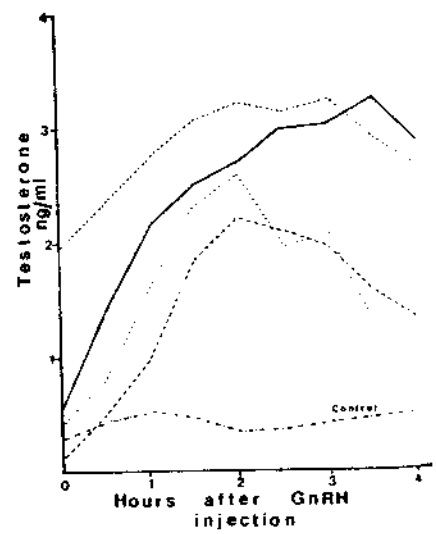

Figure 11

As an indication of sexual maturity, we also tested the testosterone response in various age groups of cheetah males. Table 2 gives the maximum response to GnRH-stimulation under Saffan anaesthesia. There is a statistically significant difference ($P < 0,01$) between the 20–27 and the 42–50 month old groups. The

Table 1 Mean zero time (before GnRH) and mean maximum (after GnRH) plasma testosterone concentrations in ng/ml in male cheetah of good, fair and poor fertility.

Fertility group	n	Zero time Testosterone ng/ml mean	SD	n	Maximum concentration Testosterone ng/ml Mean	SD
Good	5	0.40	0.23	5	2.52	0.36
Fair	7	0.57	0.49	7	2.28	0.60
Poor	3	2.76	0.77	3	2.63	1.02

Table 2 The mean rise in plasma testosterone concentration (ng/ml) following GnRH stimulation in male cheetah of 3 age groups

Age group (months)	n	Testosterone ng/ml Mean	SD
20–27	10	1.21*	0.63
42–50	*2	3.98*	0.41
60 and over	9	3.65	0.4

*Difference between these groups is significant

Figure 6

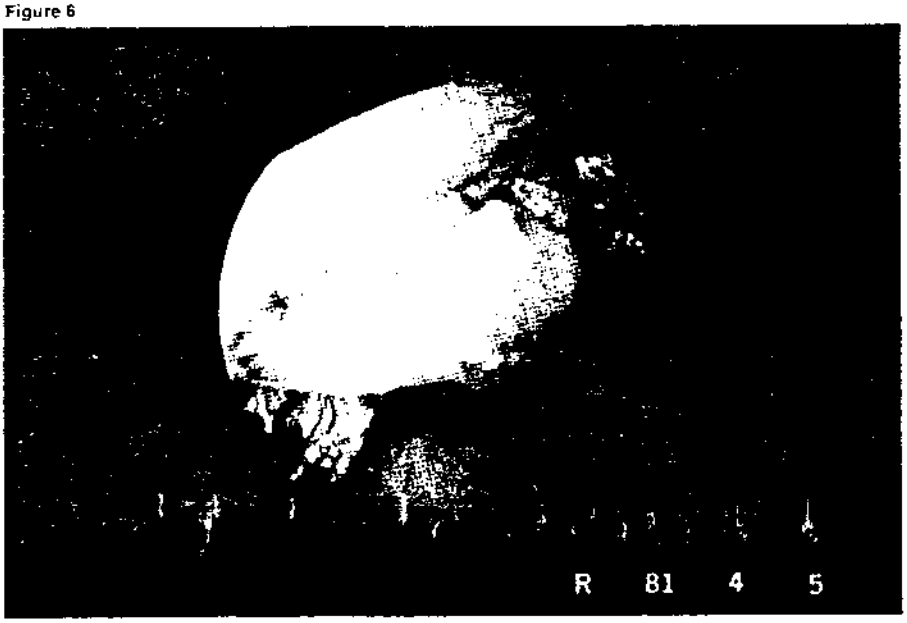

Figure 7

results indicate that sexual maturity is reached at approximately 3 to 4 years of age, which is in agreement with the findings of the semen analyses.

Breeding success

During the period 1956 – 1974 the total number of cubs born in captivity throughout the world was 140[3]. Since founding the Centre in 1971, 230 cubs have been born at de Wildt, notwithstanding the fact that during 1981 and 1982 few females were bred. The reason for this was that the production of cheetah was, by far, exceeding demand. Table 3 gives a summary of the cubs born and cubs surviving at the age of one year at the Centre. The overall survival rate of 55 % at one year indicates that a lot of work still needs to be carried out on the raising of cubs, especially during the first 3 weeks following birth.

Table 3 The number of litters and cubs born, and the number of cubs surviving at the age of one year.

Year	Number of litters	Number of cubs born	Number of cubs at 1 year
1975	6	23	7
1976	8	34	15
1977	6	19	17
1978	9	29	22
1979	7	25	21
1980	13	52	25
1981	3*	9*	8*
1982	3	5	0
1983	13	34	11
Total	68	230	126
Total survival rate at 1 year 55 %			

*One of these litters (3 cubs) was conceived at de Wildt, but born near Port Elizabeth. All 3 cubs were alive at one year.

Birth of King cheetah at the Cheetah Research Centre

During the past century isolated King cheetah have been seen in southern and East Africa. King cheetah have been so named because they differ in their colouring from normal cheetah and because they appear to be bigger. A few of these animals have been shot in the past and some records in the form of photographs and one or two skins are available.

Some believed that the King cheetah was a separate subspecies or even species, although sightings were made hundreds of kilometres apart, which made propagation of the animals a physical impossibility. The search for the King cheetah reached a climax with a hot-air balloon expedition by a young married couple over the Kruger National Park in 1979. It turned out to be unsuccessful.

The problem was solved in 1981 when purely by chance a male King cheetah (one of a litter of 5) was born at the Cheetah Research Centre. A second King cheetah (this time a female) was born to a female which was sold to a game farm near Port Elizabeth after she had been mated at de Wildt. The mother was a full sister to the mother of the King cheetah at the Centre. Both cubs had the same father.

The male King cheetah at the Centre is shown in Figures 12 and 13 alone and with his litter mates. Instead of the characteristic 15–30 mm/spots, which normal cheetah show, his black hair pattern varies from stripes on the back, to irregular large blotches and spots on the flanks and legs. His size, taking sex into account, does not differ from that of his litter mates or other cheetah of a similar age at de Wildt.

Unfortunately the sire of the original King cubs, who was 12 years old when the cubs were conceived, has died. However, in 1983 it was possible to breed 3 more litters with King cheetah cubs. One out of the 5 cubs born survived. Fortunately the parents are young and can be bred a number of times in the future, when particularly good care will be taken of the cubs.

From the data available at the Centre one can clearly say that the King cheetah is not a separate species or

Figure 12 King cheetah cub showing typical markings

Figure 13 King cheetah (note typical markings on the flank) with two litter mates.

even subspecies. It is merely a colour variation. The heredity of the colour mutation is probably autosomal recessive, and only animals which are homozygous for the trait show the characteristic colour change. It remains to be seen whether the homozygous animals (King cheetah) are fertile or not.

Acknowledgements
The authors would like to thank the University of Pretoria and the Endangered Wild Life Trust for providing financial assistance for the project, and Dr Brand, Director of the National Zoological Gardens of South Africa, Pretoria, for allowing us to make use of the cheetah at the Cheetah Research Centre.

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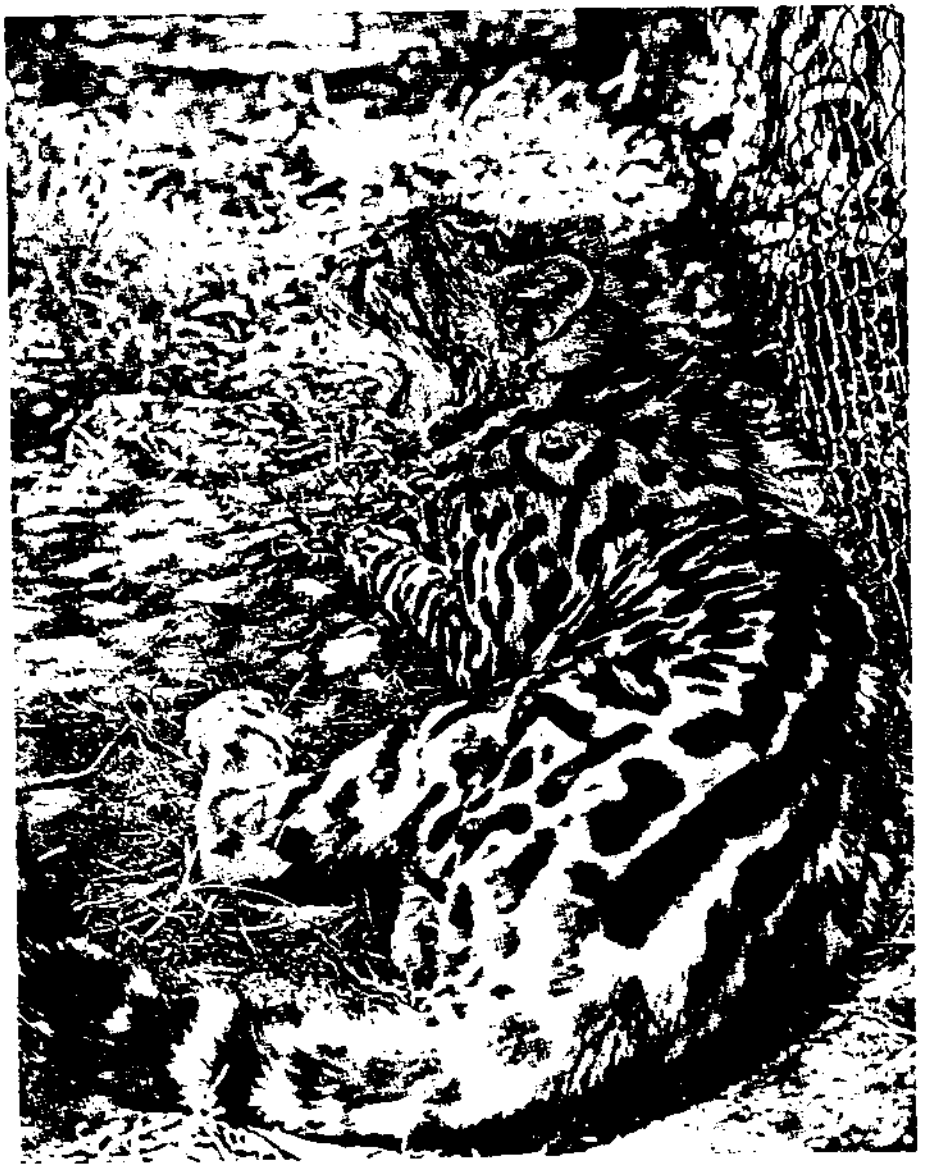

Figure 12

Figure 13