2. High reproductive activity and high breeding success in free-ranging Namibian female cheetahs

2.1 Introduction

Cheetah numbers in the wild have decreased dramatically in the last century, with a decline from an estimated 100.000 individuals in 1900 (Myers 1975) to fewer than 15.000 in 1998 (Marker 1998). The main reasons for this decline are the loss and fragmentation of habitat due to the growth of human populations, the illegal removal of cheetahs, i.e. poaching, and the legal removal of cheetahs, i.e. the elimination of 'problem animals' (Myers 1975; Wrogemann 1975; Morsbach 1987; Marker-Kraus et al. 1996). In addition, the captive cheetah populations in zoos and breeding facilities are also causing concerns. In the 1980s and 1990s the captive cheetah population in North America was not self-sustaining because of low reproductive success and high juvenile mortality, and thus was repeatedly re-stocked with cheetah imports from southern and eastern Africa (Marker-Kraus & Grisham 1993). Although breeding success in the captive cheetah population improved in the following years with the consequence that it was recognised to be self-sustaining (Marker-Kraus & Grisham 1993; Grisham & Marker-Kraus 1994), this population was still found to suffer from a variety of reproductive disorders and limited cub survival (Munson 1993; Wildt et al. 1993; Wielebnowski 1996).

2.1.1 Reproductive activity

A large survey on the reproductive status of cheetahs in North American zoos revealed that of 36 males and 45 females that had access to mating partners only 33% of the males and 31% of the females had ever reproduced (Wildt et al. 1993). Concerning all 68 females with and without mating opportunities laparoscopic investigation revealed that 22 (32%) of the females demonstrated minimal, if any, active ovarian cyclicity (Wildt et al. 1993).

Several years earlier, a lack of genetic diversity in free-ranging and captive South and East African cheetahs (*Acinonyx jubatus jubatus* and *A. jubatus raineyi*) was found (O'Brien et al. 1983; O'Brien et al. 1985; O'Brien et al. 1987). It was suggested that the present cheetah population originates from a small population that survived a demographic bottleneck about 10.000 years ago (O'Brien et al. 1987; Menotti-Raymond & O'Brien 1993; Menotti-Raymond & O'Brien 1995). It was further suggested that the more recent population decline during the last hundred years led to inbreeding between related individuals in small isolated populations (O'Brien et al. 1987). One of the possible consequences of the suggested inbreeding was thought to be reduced fertility in females (Wildt et al. 1993). Because the study of Wildt et al.

(1993) was conducted in captivity, it was, however, not possible to differentiate whether the observed low fertility in females was mainly due to consequences of the genetic monomorphism or to inappropriate husbandry and management conditions. Subsequent studies on free-ranging female cheetahs in the Serengeti National Park, Tanzania, supported the latter since all adult females (N = 14) reproduced and females that have lost a litter reconceived within an average of 18.7 days, which indicates a high reproductive potential (Laurenson et al. 1992).

To study a new aspect of the reproductive activity of cheetah females, the reproductive tract of free-ranging and captive female cheetahs on Namibian farmland was investigated. By studying these two populations living under similar environmental conditions it was tested whether the fertility in female cheetahs was influenced by genetic monomorphism or rather by captivity itself. If genetic monomorphism has an impact on the reproductive performance of female cheetahs, then low fertility would be expected in both populations, since it will be prevalent in both captive and free-ranging cheetahs. If, however, captivity as such negatively affects the reproductive activity of females, free-ranging animals would be expected to show a better reproductive performance than captive animals.

To investigate reproductive activity of free-ranging and captive cheetah females the minimally invasive technique of ultrasonography was applied (Hildebrandt et al. 2000). This technique provides information on soft tissue organs, specifically the genital organs, and poses no health risk to either the animal or the examining persons even in case of frequent use (Fritsch & Gerwing 1993). Cross-sectional images derived by ultrasonography characterise the physiology and morphology of the reproductive organs and their cyclic changes (Mattoon 1995). Also, pathologies of the reproductive organs can be identified and monitored (Mattoon 1995) (see Chapter 3), which other methods such as laparoscopy do not offer. Due to these advantages, ultrasonography has been frequently used in a wide range of wildlife species (Hildebrandt & Göritz 1998; Hildebrandt et al. 2000), and this is the first study of using this direct approach of reproductive activity assessment in free-ranging cheetahs. For this study, an adaptation of commercial ultrasound systems was essential to permit the transrectal screening of the genital tract of cheetahs. This adaptation has previously been applied to captive cheetahs and other medium sized mammals such as African wild dogs, roe deer (*Capreolus capreolus*) and bears (Hermes et al. 1997; Hildebrandt & Göritz 1998; Hermes et al. 2001). Transrectal ultrasonography of medium sized mammals has the advantage that it meets their anatomical specifications better than transabdominal ultrasonography and does not require the clipping of large areas of fur. Due to the portability of the equipment this technique can be used under field conditions. Since the visualised ultrasonographical morphology of the reproductive organs reflects

the endocrinological states during the reproductive cycle of females, results from this study can be related to results found in previous studies. Previous studies have investigated reproductive status by applying laparoscopy on immobilised animals (Wildt et al. 1993), analysing faeces endocrinologically (Brown et al. 1996; Terio et al. 2003) and conducting necropsies (Munson 1993).

2.1.2 Breeding success

Reproductive success of a female is not only described by her reproductive potential but also by her ability to raise offspring to independence. Studies on the North American captive populations and the De Wildt breeding centre in South Africa both reported a cub mortality of 37% by the age of 6 months (O'Brien et al. 1985; Marker & O'Brien 1989) and one of 55% by the age of 12 months (Bertschinger et al. 1984). These relatively high cub mortalities compared to other exotic captive animal species were explained, similarly to the low fecundity in females, as a consequence of the genetic monomorphism of the cheetah (O'Brien et al. 1985). However, when Wielebnowski (1996) reanalysed the North American studbooks she found that inbred offspring, i.e. offspring from related parents, showed higher cub mortality (62 %) at the age of 6 months than non-inbred offspring, i.e. offspring from unrelated parents (26 %) (see also Caughley 1994), and that inbred offspring were more likely to die from intrinsic factors such as stillbirths and congenital defects than non-inbred offspring. Thus, cheetahs appear to have sufficient variation at the loci affecting cub survival to cause differences in cub mortality rates of inbred and non-inbred offspring (Wielebnowski 1996). Furthermore, Wielebnowski (1996) showed that breeding success varied highly among breeding facilities, indicating that husbandry conditions and differences in breeding protocols had an influence on cub mortality rates. Kriek et al. (1998) found that a large proportion of cubs died because of complex nutritional deficiencies and that the problem could at least partly be solved by adapting the diet to the physiological needs of cheetahs.

If husbandry conditions are the main factor for cub mortality in captivity, then cubs in free-ranging populations should have a lower mortality. However, in the wild additional mortality factors occur that might distort a direct comparison of cub mortality between free-ranging and captive cheetahs. In the free-ranging Serengeti population, cub mortality was very high with 71 % of the cubs not surviving the first 2 months of their life when they were still supposed to be hidden in a lair (Laurenson 1994). After the first 4 months, 91% of cubs were dead and only 5% of the cubs survived to independence at the age of 14-18 months (Laurenson 1994). The large majority of cubs (78%) died due to predation by lions and spotted hyaenas (Laurenson 1994). Thus,

the Serengeti study did not provide evidence whether genetic monomorphism increases cub mortality.

On Namibian farmland no lions and hyaenas are present, since they have been eliminated during the past century. This situation is an ideal opportunity to study cheetah cub survival in the absence of their main predators. Assuming that free-ranging females in the Serengeti and Namibia both mate with unrelated males, a higher cub survival and larger litter sizes on Namibian farmland than in the Serengeti National Park were expected.

2.2 Materials and Methods

2.2.1 Study areas and study periods

The study was conducted on four study sites (A, K, P and W) located on commercially used farmland in central Namibia (Figure 2.1).

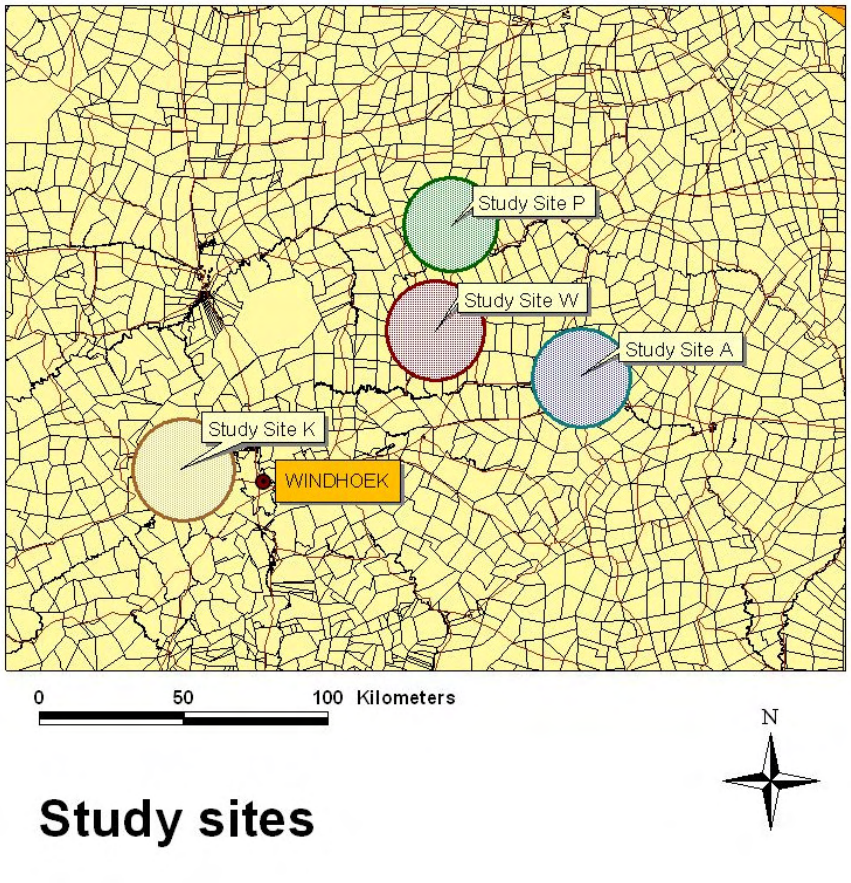


Figure 2.1 Location of study sites.

All study sites were situated in the Khomas Hochland Plateau at heights between 1400 m and 1700 m above sea level. The vegetation was classified

as Highland Shrubland, i.e. part of the acacia tree-and-shrub savannah biome, which is Namibia's largest biome (Mendelsohn et al. 2002). Study periods in the A- and P-sites lasted 26 months, in the W-site 18 months and in the K-site 6 months (Table 2.1).

Table 2.1 Study periods in the four study sites.

Study site	2002	2003	2004	2005	Total
A	Jun – Oct	Mar – Nov	Feb – Oct	Aug – Oct	26 months
K	Jun – Oct	Feb	-	-	6 months
P	Jun – Oct	Mar – Nov	Feb – Oct	Aug – Oct	26 months
W	-	May – Nov	Feb – Sep	Aug – Oct	18 months

2.2.2 Study animals

Identification and age assessment

Each animal was given an identification code composed of a letter and three numbers (e.g. A001). For free-ranging animals, the letter referred to the study sites A, K, P or W, the captive animals were denominated with Z.

The age of cheetahs was estimated using the key for body size established by Caro (1994) for East African cheetahs. Age classes were adopted from Caro (1994) with slight modifications to eliminate age gaps between the classes (Table 2.2). To improve identification of cheetahs in the two oldest age classes 7 and 8, the general physical appearance, condition of fur and size and condition of teeth were also used.

Table 2.2 Definition of age classes in cheetahs (modified from Caro 1994). The age range covers the period from the first day of the respective months/year until the last day of the respective month/year.

Number	Age class	Age	
1	Very young Cubs	0-1 months	In the lair, less than a fifth of their mother's shoulder height.
2	Young cubs	2-4 months	Black manes and less than one quarter of their mother's shoulder height (2 months) to a third of her shoulder height and with shoulders still fluffy (4 months). Cubs stay in the lair up to 8 weeks.
3	Middle-aged Cubs	5-7 months	Just about half the mother's shoulder height (5 months) to two-thirds of her height for sons and five-eights of her height for daughters (7 months).
4	Old cubs	8-12 months	Sons: three-quarters of the mother's shoulder height (8 months) to as large or larger as the mother (12 months); Daughters: two-third of the mother's shoulder height (8 months) to seven-eights of the mother's height or as large as the mother (12 months). They can be distinguished from their mothers by their lighter build, fluffier manes and round cub like faces.
5	Juveniles	13-23 months	Slight, gracile and rounded cub like faces when viewed from the front. Mane consisting of long light hairs that partially obscured the black spots on the nape. Littermates stay together for several months after they stopped accompanying their mother.
6	Young adults	24-41 months	Full size but cleaner appearance than adults, retained some of their manes. Females have rather circular faces. Males are not together with female littermates and females not together with any littermates.
7	Adults	3.5-7.0 years	No mane, black and light nape hairs about equal length. Often callused elbows where the fur is worn away.
8	Old individuals	> 7.0 years	Prominent black hairs on the mane and tail appear thin because the light hairs became shortened. Males heavily scarred.

Once an animal was anaesthetised (see below), identification photographs were taken of both sides of the body and the tail and the face. All free-ranging cheetahs were ear-tagged (SWAVET, Windhoek, Namibia) with a metal tag in which a code was impregnated, and a microchip (PET-ID, West Sussex, UK) was implanted subcutaneously in the middle of the back in close proximity to the root of the tail.

Free-ranging cheetahs

The study sites were equipped with a total of 24 box traps of 2.40 m length x 0.85 m height x 0.95 m width (Golz Maschinenbau, Okahandja, Namibia) to catch free-ranging cheetahs. Most traps were placed at so called 'marking trees' that are frequented by cheetahs. These trees can be identified by the presence of cheetah faeces on and around the trees, scratches on the bark and cheetah tracks around the trees. Layers of thorny bush of about 1.20 m height were set around the trees in a circle so that animals had to walk through the trap to reach the tree. The weight of the animal in the trap released a mechanism that immediately closed the front and back door of the trap.

Box traps were checked early every morning. Non-target animals were released immediately. Cheetahs were given shade and water until immobilisation took place. If spoors of one or more additional cheetah(s) were identified in close vicinity of the trap, a cage (1.35 m x 0.85 m x 0.95 m) was placed opposite to the box trap and the captured cheetah encouraged to move into the cage. The animal was given shade and water, while one to three additional box traps were positioned next to the tree. After the conspecifics were caught, the investigation started.

During the entire study period 74 cheetahs were caught, consisting of 39 adult males, 10 adult females, 6 sub-adult males, 6 sub-adult females, 6 male cubs and 7 female cubs. For the study presented in this chapter, all 10 adult females and the 5 of the 6 sub-adult females that were investigated by ultrasound techniques were used.

Table 2.3 Free-ranging females investigated in this study. For definition of age classes see Table 2.2

ID	Study site	Age class	Comments
A011	A	7	Accompanied by three cubs of age class 2
A028	A	7	Accompanied by three cubs of age class 3
K002	K	5	Single
P002	P	5	Juvenile offspring of P004
P003	P	5	Juvenile offspring of P004
P004	P	7	Accompanied by four sub-adult offspring
P005	P	5	Juvenile offspring of P004
P012	P	6	Single
P019	P	7	Accompanied by two cubs of age class 2
P028	P	7	Accompanied by three cubs of age class 4
P036	P	5	Together with three sub-adult males, probably her brothers
W006	W	8	Single
W007	W	6	Single
W014	W	7	Accompanied by two cubs of age class 4
W028	W	6	Accompanied by three cubs of age class 4

Captive cheetahs:

In June 2002, nine captive females in central, southern and northern Namibia were examined (Table 2.4). The females were kept in social groups of different compositions (Table 2.4), in enclosures of various sizes and varying contact rates to people (for details see Appendix 1), and were fed with meat of natural cheetah prey species. All females were originally wild-caught and the seven females (Z003, Z004, Z007, Z014, Z028, Z029, Z030), for which the duration of their stay in captivity was known by the farmers, were kept for at least 2 years in the enclosures. The animal's age was either reported by the owner or estimated by the project members (Z002). Two females (Z028, Z029) were examined twice and one female (Z030) three times (Table 2.4).

Table 2.4 Captive female cheetahs investigated in this study. For females investigated more than once, the age at the first investigation is given. For definition of age classes see Table 2.2.

ID	Age (years)	Age class	Comments	Region
Z002	3.5 -7.0	7	In same enclosure with one adult male	Central
Z003	2.0	6	Visual contact to one adult male and one adult female (Z004) in an adjacent enclosure	Southern
Z004	4.0	7	In same enclosure with an adult male sibling and in visual contact to one adult female (Z003)	Southern
Z007	6.0 – 7.0	7	In same enclosure with two sons and one daughter, all young adults	Southern
Z014 *	4.5	7	In same enclosure with one adult male	Central
Z025	2.5	6	In same enclosure with 18 other cheetahs of both sex and different age classes	Northern
Z028 a)*°	5.0	7	In same enclosure with two females (Z029, Z030), visual and olfactory contact to three adult males in an adjacent enclosure	Central
Z029 a)*°	5.0	7	In same enclosure with two females (Z028, Z030), visual and olfactory contact to three adult males in an adjacent enclosure	Central
Z030 b)*°	5.0	7	In same enclosure with two females (Z028, Z029), visual and olfactory contact to three adult males in an adjacent enclosure	Central

a) examined in June 2002 and October 2004; b) examined in June 2002, October 2003 and October 2004

* leopard in adjacent enclosure, ° spotted hyena in adjacent enclosure

2.2.3 Immobilisation and anaesthesia

Immobilisation of captured free-ranging cheetahs was done via blow dart injection (Telinject GmbH, Römerberg, Germany), whereas for captive ones a dart gun (Dan-inject®, Barkop, Denmark) was used. Animals were immobilised by intramuscular injection. All 9 captive and 12 of the 15 free-ranging females were immobilised with Hellabrunn mixture made of 100 mg/ml Ketamine (Ketavet®, Kyron Laboratories, Benrose, RSA) and 125 mg/ml Xylazine (Rompun®, Bayer, Isando, RSA) with a dosage of 0.04 ml per kg body mass. For the remaining 3 females (A011, P019, W006) and for the repeated investigation of Z028, Z028 and Z030 in October 2004 a mixture of Ketamine (Ketavet®, Kyron Laboratories, Benrose, RSA) at 4.5 mg/kg body mass) and Medetomidine (Domitor®, Novartis, Spartan, RSA, at 0.08 mg/kg body mass) was used.

To prevent dehydration of the corneas, a few drops of artificial eye liquid (Gemini®, Adcock Ingram, Bryanston, RSA) were given into both eyes. Rectal temperature was measured as soon as the animal was sedated, and the respiration rate was taken every 5 minutes. Heart frequency and oxygen partial pressure were checked continuously using an oxymeter (Nellcor N-20PA®, Nellcor Puritan Bennett Inc., Pleasanton, USA) and values were recorded every 5 minutes. On average anaesthesia lasted 80 ± 24 min.

Animals that were immobilised with Hellabrunn mixture were reversed with an i.m. or i.v. injection of Yohimbine (Yohimbine®, Kyron Laboratories, Benrose, RSA, at 0.1 mg/kg body mass), whereas animals that were immobilised with Ketamine/Medetomidine were reversed with Atipamezole (Antisedan®, Novartis, Spartan, RSA, at 0.25 mg/kg body mass). All cheetahs were observed until they recovered, stood up and walked away.

2.2.4 Ultrasonography

Animals were examined in lateral recumbency. Before transrectal ultrasonography, the animals were given an enema to empty the caudal parts of the larger intestines and to ensure sufficient acoustic coupling between the ultrasound probe and the intestinal wall. The ultrasound probe was fixed to a rigid extension (Schnorrenberg, Woltersdorf, Germany) specifically designed for transrectal examination of medium sized mammals to easily introduce and precisely move the probe in vertical direction and around the longitudinal axis (Hildebrandt & Göritz 1998; Hermes et al. 2001). The probe and rigid extension were lubricated (Aquasonic 100®, Parker Laboratories, New Jersey, USA) to prevent damage to the intestinal wall. Ultrasonography was performed with a CS 9100 Picker apparatus (Hitachi, Tokyo, Japan) using a 7.5 MHz fingertip transducer as a probe (Hitachi, Tokyo, Japan).

The inner reproductive tract, i.e. the vagina, cervix, uterus and ovaries were examined and visualized. The organs were screened in caudo-cranial direction and imaged longitudinally. They were evaluated by their echogenicity, sonomorphology and size. In general, high echogenicity reflects tissue of high material density, thick mucus and/or low inter- or intracellular fluid content and appears light in the image, whereas low echogenicity indicates low material density, thin mucus and/or high fluid content and appears darker in the image (Fritsch & Gerwing 1993).

The wavelength (λ) of the ultrasound - and thus the minimal size of detectable structures - of a probe is equivalent to the velocity (c) of the ultrasound divided by the frequency (v) of the probe (λ = c/ v). The velocity of ultrasound in a liquid medium i.e. in the body is approximately 1'500 m/sec, and with a probe frequency of 7'500'000 Hz/sec the wavelength is 0.0002 m. Thus, with the probe used in this study, structures with a minimal size of 0.2 mm could be identified when they were distinguishable from the surrounding tissue by changes in echogenicity.

For most cheetahs all organs were visualized. The organs and ovarian functional structures such as follicles and corpora lutea were measured at their maxima and to an accuracy of 0.1 mm using an integrated calliper system. Also, liquid filled structures in close proximity to the ovaries were measured at their maxima and other pathologies of the reproductive organs were visualised (see Chapter 3). The sizes of the vagina, uterus and ovarian functional structures were determined by their cross-sectional diameters, whereas the sizes of the cervix and ovaries were determined by their cross-sectional area, calculated by the formula:

$$\text{Ellipse} = (\frac{pi}{4}) * a * b$$

with a = maximum length and b = maximum cross-sectional diameter.

Most ultrasonographical examinations were recorded on digital video tapes (LP:90 premium dv, Sony Inc., Japan) using a digital video camera (MV 400i, Canon Inc., Japan) connected to the ultrasound scanning system. All recordings were viewed at a later state for verification of findings and measurements.

2.2.5 Radio- collaring

Nine adult free-ranging females were fitted with a VHF collar (Advanced Telemetry Systems (ATS), Minnesota, USA) or a GPS collar (Vectronics Aerospace GmbH, Berlin, Germany). The VHF collars emitted three types of signals: a) activity (when collar was moved, i.e. the animal was walking,

running, feeding), b) immobility (when collar was not moved for less than 24 hours, i.e. the animal was resting or sleeping) and c) mortality (when the collar was not moved for more than 24 hours, i.e. the animal was dead). Collar batteries had a longevity of about 36 months. The GPS collars emitted two types of signals: a) activity and immobility (when collar was moved with more than 8 vibrations per 5 minutes) and b) mortality (when collar was moved with less than 8 vibrations per 5 minutes). These collars had a battery life of about 17 months.

2.2.6 Ground follows and aerial tracking

During the study period collared animals were searched on the ground using a receiver (ATS, Minnesota, USA) to which an antenna and a headset were connected. Ground follows were conducted to look for females with known cubs or females that were expected to have cubs based on previous ultrasonographical examination.

Between August 2002 and January 2006 69 aerial tracking flights were conducted. Tracking flights were usually conducted twice a month in a two-seater Piper Super Cub. Both wings of the airplane were equipped with Yagi bidirectional antennas that received signals from a maximum distance of 20 km. While flying at an altitude of about 1 km over the study sites, the radio frequencies of all collared study animals were permanently scanned for signals. When a signal was received, the pilot was directed towards the signal and then decreased in circles to 30 – 80 m above ground. When cheetahs were sighted, the number of additional cheetahs and their estimated age classes were recorded. When yet unknown cubs were detected with a female, special effort was made to estimate their age. This was done by flying low and by reassessing the age during consecutive flights by different observers.

2.2.7 Determination of cub survival and litter size at different ages

Litter sizes and age of cubs were determined either at the place of capture or when sighted during aerial tracking of their collared mothers. During all subsequent aerial tracking flights, an effort was made to sight mothers with all their cubs. The first 2 months the cubs are hidden in a lair and then emerge and start to follow the mother. They stay close to the mother until they are weaned at about 4 months and then stay associated with her until about 14 months when they reach independence. Therefore, cub survival and litter sizes were determined at the ages of 2, 4 and 14 months of age.

To determine cub survival, only those cubs that were seen first in the lair or at 2 months of age were included in the analysis. It then was checked whether and how many of these cubs were still alive at 4 and 14 months of age. These

data were compared with respective data of the Serengeti cheetah study in Tanzania (Laurenson 1994). To provide a conservative test of the prediction that cub survival in Namibia should be higher than in the Serengeti, cub numbers at 4 and 14 months of age were assessed in a more conservative way in this study than in the Serengeti study. When a cub was seen last at the age of e.g. 3 months and its death was confirmed at the age of 5 months, this cub was assumed to have been dead already at the age of 4 months for the Namibian study. For the Serengeti study, however, the same cub was assumed to have been still alive at the age of 4 months.

To compare litter sizes at 2, 4 and 14 months of age between Namibian farmland and the Serengeti all litters and cubs seen at these ages were included in the analysis. This sample size was larger than the one to determine cub survival because it also includes cubs seen for the first time after 2 months of age.

2.2.8 Statistics

Statistical analysis was performed using SYSTAT 11.0 following the procedures outlined in Engel (1997). All means are given with standard deviations and p-values are for two-tailed tests.

2.3 Results

2.3.1 Sonomorphology and reproductive activity

Females were classified into different reproductive categories consisting of juvenile females, reproductively active and reproductively inactive females. Reproductively active females were further determined as being in pro-oestrous, oestrous, di-oestrous or in the luteal phase. The allocation of females into these six reproductive stages was based on the presence, size and echogenicity of functional structures on the ovaries, on the echogenicity and structure of the vagina, cervix and uterus, and by considering their approximate age. A comparative set of organ sizes and images of the ovary, uterus and cervix of female cheetahs categorized as juvenile, in oestrous, during a luteal phase and as reproductively inactive is shown on pages 27 and 28 (Figure 2.3, Table 2.7).

Juvenile females

All five free-ranging juvenile females examined (K002, P002, P003, P005, P036) had a homogenous vagina and cervix tissue of low echogenicity. In some cases, dense mucous was found in the lumen of these reproductive organs. The plicae of the cervix were not visible. In the uterine walls the

endometrium was distinguishable as a thin inner layer less echogenic than the myometrium. No fluid or mucous was observed in the uterine lumen of any of the juvenile females. On the ovaries the medulla (*zona parenchymatosa*), i.e. the denser and thus more echogenic inner part, was clearly distinguishable from the cortex (*zona vasculosa*), the less echogenic outer part. Small premature follicles with diameters between 1.1 - 1.4 mm were visible on the ovaries of one sub-adult female (P036). On the ovaries of the other four females no visible functional structures were found.

Reproductively active females

Pro-oestrous. In one free-ranging female (W028) vagina, cervix and uterus were echo low and contained some mucous of medium echogenicity. On one ovary several follicles with diameters between 1.5 and 2.8 mm were identified. In general, follicles were clearly distinguishable from the ovarian tissue by a markedly echo lower appearance and round shapes. This female was determined to be in a pro-oestrous period.

The pro-oestrous is endocrinologically characterised by an onset of fluid retention in the tissue caused by circulating serum oestrogens and growing follicles on the ovaries under the effect of FSH (follicle stimulating hormone). The echo low appearance of vagina, cervix and uterus reflected the beginning of oedematisation of the tissue. The female was accompanied by three cubs of about 9 months of age.

Oestrous. At the time of examination four free-ranging females (P004, P012, P028, W006) had large reproductive organs (Table 2.7, page 27) of low echogenicity. The mean vagina diameter was 16 % to 63 % larger than the ones of the other reproductive states of adult females; the mean cross sectional area of the cervix was 36 % to 83 % larger and the mean uterus diameter was 48 % to 98 % larger (Table 2.7). The vagina showed a medium to low echogenicity with medium to thick mucus between the mucosal folds. The anatomic structure of the enlarged cervix was clearly visible. In the uterus wall an enlarged endometrium of low echogenicity was documented. The uterus of the female with the largest functional structures on the ovaries (P028, Table 2.5) showed a thin white line indicating adjacent endometrial folds.

On each ovary of the three females for which these organs were visualised (P012, P028, W006), two or three mature follicles were measured (Table 2.5). In P012 and W006 all follicles were well rounded, clearly shaped and of a very low echogenicity due to their high fluidal content. The functional structures of P028 were filled with echo low liquid and were internally septed, indicating that they were *corpora hemorrhagica*. *Corpora hemorrhagica* develop from an ovulated follicle and are a physiological finding *post ovulationem*. The

structures of P028 were significantly larger than the mature follicles of the other two females (Mann-Whitney-U-Test, U=0, N_1=11, N_2=5, p=0.0018, Table 2.5).

Table 2.5 Number and maximum diameter (in mm) of functional structure on right and left ovaries of females in oestrous. Functional structures on the ovaries of P012 and W006 were identified as mature follicles, the ones on the ovaries of P028 as *corpora hemorrhagica*.

ID	Functional structures on right ovary				Functional structures on left ovary			
	Number	Diameter			Number	Diameter		
P012	3	2.9	3.7	4.6	3	3.5	3.8	4.4
W006	2	3.9	4.0		3	4.2	4.4	4.9
P028	2	5.0	6.0		3	5.5	5.8	6.7

The enlargement and low echogenic appearance of the tissue reflected the oedematisation of the reproductive organs, which begins during pro-oestrous and peaks during oestrous. The oestrous is endocrinologically characterised by circulating oestrogens, which lead to the oedematisation of the reproductive organs. In one female (P004) the ovaries were not imaged, but the morphology of the other reproductive organs (vagina, cervix, uterus) allowed its classification as an oestrous female. P004 and P028 were accompanied by their offspring of about 15 months and 12 months respectively.

Luteal period. In four free-ranging (A011, P019, W007, W014) and three captive females (Z003, Z004, Z025) the vagina and cervix appeared homogenous and had a medium to low echogenicity. A common feature of all females was the presence of corpora lutea on their ovaries. During the female reproductive cycle, corpora lutea develop during the luteal phase, which is the period when the reproductive tract is under the influence of progesterone. Circulating levels of progesterone differ during the luteal phase and different states could be differentiated by the ultrasonographical images. They are now described in detail.

The folds of the cervix in the three captive females were still visible, almost as clearly as during oestrous. Their endometrium was distinguishable from the myometrium by its lower echogenicity and it appeared thicker due to the process of the beginning proliferation. These three females had corpora lutea of distinct, almost round shape and medium echogenicity with diameters between 3.8 mm and 6.5 mm (Table 2.6). Since these females were not

pregnant and did not have cubs, it was concluded that they were examined during an early luteal phase and that their corpora lutea were fresh.

One free-ranging female (W007) was examined while being pregnant at an early stage. Her vagina and cervix had a very echo low image and the endometrium did not appear as a distinct layer. The uterine walls had an undulating appearance and were hypertrophic with a uterus diameter of 7.5 mm, larger than the ones of the other six females determined to be in a luteal period (mean ± sd: 6.1 ± 0.6 mm, range: 5.4 mm – 7.0 mm). The low echogenicity of the uterus reflected the hyperaemia of the uterine tissue. In each uterine horn two embryonic vesicles were found. They had a distinct outer echo dense capsule being embedded in the endometrium. Each vesicle contained a clear, very echo low liquid. In every vesicle one embryo being attached to the vesicle wall was seen. The diameter of the echo low inner part of the vesicles ranged from 9.0 mm to 11.0 mm; the walls of the vesicles were 2.5 mm thick. The four embryos measured 2.9 mm, 3.2 mm, 3.5 mm and 3.9 mm in length. On each ovary two fresh corpora lutea of medium echogenicity with diameters between 6.6 mm and 8.2 mm were present (Table 2.6).

Vagina and cervix of one free-ranging female (W014) were of low echogenicity and contained some thick echo dense mucous. In the uterus and both uterine horns the endometrium appeared as a thin echo low layer. In the endometrium of the right uterine horn a round structure of medium dense echogenicity was seen, which was integrated in the endometrium and had no marked outer line. Unlike an embryonic vesicle with a distinct margin, embryonic structures attached to the vesicle's wall and low echogenic fluid inside the vesicle the structure found here was characterized by an irregular shape, a poorly defined margin, no embryonic structure and medium echogenic content. On the right ovary two fresh corpora lutea of medium echogenicity and 6.4 mm and 7.5 mm in diameter were found (Table 2.6). The characteristics of the described structure and the presence of corpora lutea led to the diagnosis of the embedded structure being an embryonic resorption (Figure 2.2). This female was accompanied by two cubs about 9 months old at the time of capture.

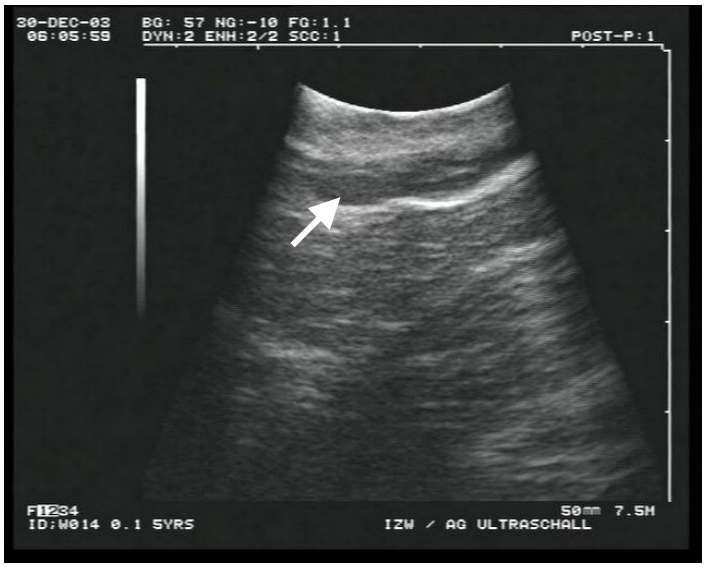


Figure 2.2 Right uterine horn with an embedded structure (white arrow) diagnosed as an embryonic resorption.

Vagina, cervix and uterus of one free-ranging female (A011) were of medium echogenicity, the three layers of the uterus wall were visible and the uterus contained some echogenic mucous. The organs of another free-ranging female (P019) were of medium to low echodensity and the uterus contained some mucous. Both females had round to oval shaped corpora lutea on the ovaries with diameters ranging between 4.0 mm and 6.0 mm (Table 2.6). The corpora lutea had almost the same echodensity as the surrounding ovarian tissue. Both females also had two about three months old cubs. Since these females were at the end of their lactation period, it was concluded that they were examined towards the end of the luteal phase and that their corpora lutea consequently were old. Their corpora lutea differed from the fresh ones described above in shape and echogenicity and therefore in the distinction to the surrounding ovarian tissue. The overall mean of the averaged corpora lutea diameter per female was 5.8 ± 1.2 mm (N = 7), with a range of 3.8 mm to 8.2 mm (Table 2.6).

Table 2.6 Number and maximum diameter (in mm) of corpora lutea on right and left ovaries, their average diameter, the echogenicity of corpora lutea and endometrium and the concluded luteal status of the females. In the uterine horns of W007 four embryonic vesicles were detected and in the endometrium of W014 an embedded structure diagnosed as an embryonic resorption was found (see text for details).

ID	Corp. lutea on right ovary			Corp. lutea on left ovary			Average	Echogenicity		Status
	Number	Diameter		Number	Diameter		Diameter	Corp. lutea	Endometrium	Endometrium
Z003	1	6.5	-	0	-	-	6.5	medium	low	early luteal
Z004	2	4.0	4.2	2	3.8	4.8	4.2	medium	low	early luteal
Z025	1	5.8	-	0	-	-	5.8	medium	low	early luteal
W007	2	8.2	6.6	2	6.7	7.3	7.2	medium	low	pregnant
W014	2	6.4	7.5	0	-	-	7.0	medium	low	embryonic resorption
A011	2	5.0	4.0	2	4.5	5.0	4.6	high	medium	late luteal
P019	0	-	-	2	5.0	6.0	5.5	high	medium-low	late luteal

Di-oestrous. Vagina, cervix and uterus of one free-ranging female (A028) were of medium to low echogenicity and firm mucous was screened in the vagina lumen, but not in the lumina of cervix and uterus. The ovaries were oval shaped and the echo lower cortex was clearly distinguishable from the more echo dense medulla. On both ovaries many small follicles of diameters between 0.5 mm and 1.0 mm were found.

This female was determined to be in a di-oestrous period, which is the time span between two consecutive oestrous. She was accompanied by three cubs of about 5 months of age.

Reproductively inactive females

Six captive females (Z002, Z007, Z014, Z028, Z029, Z030) showed reproductive tracts with homogenous, medium echogenic vaginas and cervices. In some cases very echo dense mucous in the vaginas and the cervix lumen appeared in a non-continuous line visible as bright spots on the ultrasonography image. In a few cases small, highly echogenic areas were seen in the ovaries and cervix tissue. In none of the cervices mucosal folds were visible. The highly echogenic uterus showed an endometrium barely distinguishable from the other components of the uterine wall. Ovaries were oval shaped and throughout of high echogenicity, i.e. the cortex was

indistinguishable from the medulla. Due to their homogenous consistency and the absence of larger functional structures such as tertiary follicles or corpora lutea the ovaries were hardly distinguishable from the surrounding tissues. Multiple small follicles with diameters between 1.0 mm and 1.5 mm were seen in Z004, Z007 and Z028. These six females were thus classified as reproductively inactive. Z028, Z029 were examined twice and Z030 three times and diagnosed as reproductively inactive on each occasion.

Table 2.7 summarises the sizes of different reproductive organs and Figure 2.3 shows ultrasonographic images in the different reproductive states.

Table 2.7 Diameters of vaginas and uteri in mm and cross-sectional surfaces of cervices, right and left ovaries in mm^2 all given as means ± SD for different reproductive categories. Sample sizes (number of females investigated) are given in brackets.

	juvenile	prooestrous	oestrous	luteal	di-oestrous	inactive
Vagina	4.1 ± 1.2 (5)	4.0 (1)	6.5 ± 1.1 (4)	5.6 ± 1.3 (7)	4.9 (1)	5.1 ± 1.9 (6)
Cervix	82.4 ± 27.8 (4)	100.9 (1)	172.9 ± 46.2 (4)	126.8 ± 37.2 (7)	94.6 (1)	114.4 ± 71.7 (6)
Uterus	4.1 ± 0.3 (5)	5.0 (1)	9.9 ± 1.2 (4)	6.3 ± 0.7 (7)	6.7 (1)	5.7 ± 1.5 (6)
Ovary right	71.4 ± 6.4 (3)	-	168.7 ± 88.6 (3)	97.5 ± 25.5 (7)	81.3 (1)	73.8 ± 16.5 (6)
Ovary left	59.6 ± 6.0 (5)	90.1 (1)	143.3 ± 34.4 (3)	102.3 ± 24.6 (6)	84.9 (1)	65.2 ± 13.5 (5)

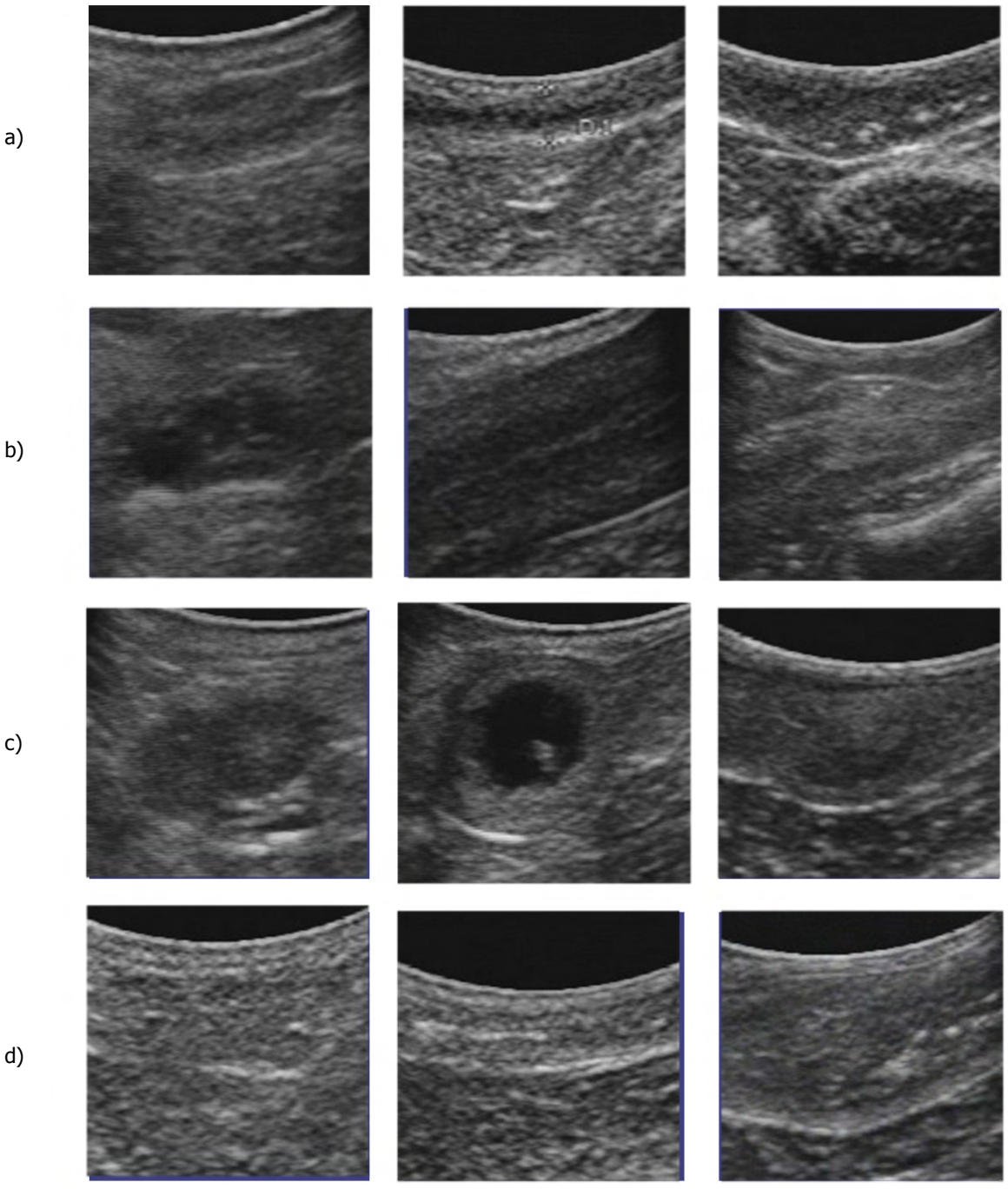


Figure 2.3 Typical sonograms of ovary, uterus and cervix of female cheetahs categorized as a) juvenile, b) in oestrous, c) during a luteal phase (in this case during early pregnancy) and d) as reproductively inactive.

2.3.2 Comparison of the reproductive state in free-ranging and captive females

According to the overall appearance of their reproductive organs as described in the previous section, captive and free-ranging adult females were classified as either reproductively active (pro-oestrous, oestrous, luteal phase, di-oestrous) or inactive. A comparison of the incidence of reproductive activity of free-ranging and captive adult females showed that, at the time of examination, adult free-ranging females were significantly more likely to be reproductively active than captive females (Fisher's exact test, $p = 0.0031$, $N = 19$, Table 2.8).

Table 2.8 Number of reproductively active and inactive free-ranging and captive female cheetahs.

	Reproductively inactive	Reproductively active
Free-ranging	0 (0%)	10 (100%)
Captive	6 (67%)	3 (33%)

This result was not a consequence of differing age structures. The ratio of females younger than 3.5 years (age class 6) to females at or older than 3.5 years (age classes 7 and 8) were similar for adult free-ranging and captive females (age class 6: $N = 3$ for free-ranging, $N = 2$ for captive; age classes 7 and 8: $N = 7$ for free-ranging, $N = 7$ for captive; Fisher's exact test, $p = 1.0$, $N = 19$).

2.3.3 Fecundity of free-ranging females

Seven of the ten investigated adult free-ranging females were associated with cubs at the time of examination, and two more females were seen with cubs a few months later. The remaining female was not collared and not seen again from either the air or on the ground after investigation, thus it was not confirmed whether she reproduced. Therefore, at least 90% of the free-ranging females in this study were fecund.

2.3.4 Breeding success

Cub survival

Three litters with three, five and six cubs, respectively, were seen at the age of 2 months (one litter in the lair and 2 litters shortly after emergence). During

the study period, two of these litters were followed up to 14 months of age and one litter up to 9 months. When considering all 14 cubs, 11 (79%) were still alive at 4 months (Table 2.9a). When considering only the cubs that were observed up to 14 months of age, 8 (89%) of the 9 cubs were still alive at 4 and at 14 months of age (Table 2.9b).

Table 2.9 Number of litters and cubs, and cub survival (in %) in Namibia and Tanzania for cubs observed up to 4 months (Table 2.9a) and up to 14 months respectively (Table 2.9b). Data from Tanzania were extracted from Laurenson (1994).

Table 2.9a

		2 months	4 months
Namibia	# of litter	3	3
	# of cubs	14	11 (79%)
Tanzania	# of litter	10	6
	# of cubs	36	12 (33%)

Table 2.9b

		2 months	4 months	14 months
Namibia	# of litter	2	2	2
	# of cubs	9	8 (89%)	8 (89%)
Tanzania	# of litter	10	6	4
	# of cubs	36	12 (33%)	7 (19%)

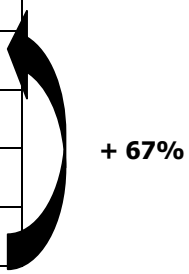
In the Serengeti, 36 cubs from 10 litters were seen at 2 months of age and followed up to 14 months of age. At 4 months of age, 12 cubs (33%) were still alive and at 14 months of age 7 cubs (19%) were alive (Laurenson 1994) (Table 2.9a, 2.9b). A comparison between Namibia and the Serengeti revealed that cubs on Namibian farmland have a 4.7 times higher chance to survive to independence once they have left the lair.

Litter sizes at different ages

During the study period, three, seven and five litters were seen at the age of 2, 4 and 14 months respectively. The mean litter sizes at these ages were 4.7, 3.3 and 3.0 (Table 2.10).

Table 2.10 Number of litters and cubs and mean litter size at different ages in Namibia and Tanzania. Data from Tanzania were extracted from Laurenson (1994).

		2 months	4 months	14 months
Namibia	# of litter	3	7	5
	# of cubs	14	23	15
	mean litter size	**4.7**	**3.3**	**3.0**
Tanzania	# of litter	10	6	4
	# of cubs	36	12	7
	mean litter size	**3.6**	**2.0**	**1.8**



+ 67%

In the Serengeti, the respective mean litter sizes were 3.6, 2.0 and 1.8 (Laurenson 1994) (Table 2.10). A comparison between the two studies revealed that mean litter size at 2 months of age was 31% higher in Namibia than in the Serengeti, at 4 months of age 65% higher and at the age of independence 67% higher (Table 2.10).

2.4 Discussion

2.4.1 Determination of reproductive stages by using ultrasonography

In this study the minimally invasive technique of ultrasonography was applied to investigate the reproductive tracts of free-ranging and captive Namibian cheetah females. This technique allowed a distinct classification in six different reproductive stages by including all information on the presence, size and echogenicity of functional structures on the ovaries, the echogenicity and structure of the vagina, cervix and uterus. Additional information on the approximate age of the females and the presence and age of their cubs were used to support the findings. Due to the portability of the equipment and the possibility of recording images for later verification of the findings, ultrasonography technique is ideal for such investigations under field conditions (Hildebrandt & Göritz 1998).

Reproductive stages

By using the comprehensive set of ultrasonography images of the reproductive organs, adult females were identified in pro-oestrous, oestrous, di-oestrous or in a luteal phase and distinguished from inactive reproductive

tracts of adult females or juvenile females. Oestrous females had enlarged organs due to fluid-retention caused by oestrogens and follicles between 2.9 mm and 4.9 mm on the ovaries. Using laparoscopy on six hormonally treated cheetah females in North American breeding facilities, Wildt et al. (1981) suggested follicles equal to or larger than 4.0 mm to be fully mature, i.e. sufficiently developed to ovulate. Smaller follicles of oestrous females found in this study were presumably still growing to the size of full maturity as suggested by Wildt et al. (1981).

One examined female had large functional structures on her ovaries determined to be *corpora hemorrhagica.* Since the reproductive tract of this female was determined to be in oestrous and cheetahs are mainly induced ovulators (Asa et al. 1992; Wildt et al. 1993), it might have been possible that this female mated recently, thus inducing follicle ovulation.

Some females with inactive reproductive tracts in this study had follicles of 1.0 mm to 1.5 mm, consistent with earlier findings that follicles of such small size (i.e. < 2.0 mm) occur at any time of the oestrous cycle on ovaries of felids and do not demonstrate ovarian cyclicity (Goodrowe et al. 1989).

2.4.2 Free-ranging versus captive Namibian females

In this study, free-ranging females were significantly more likely to be reproductively active than captive female cheetahs. All ten examined adult free-ranging females were reproductively active, i.e. cycling, pregnant, lactating or raising young, whereas only a third of the females kept in enclosures on Namibian farmland were cycling. Furthermore, at least 90% of the adult free-ranging females had cubs at the time of examination or later and therefore did successfully reproduce. This result is consistent with the high reproductive activity shown by free-ranging females in the Serengeti where all study animals were observed to reproduce (Laurenson et al. 1992).

Since all free-ranging and captive females examined in this study originated from the same cheetah population from Namibian farmland, it was concluded that the lack of genetic diversity in this species did not lead to a reduction in the reproductive potential of females and that captivity itself somehow negatively affects reproductive activity of females. This study therefore supports the conclusion of Caro & Laurenson (1994) and Laurenson et al. (1992) from their study in the Serengeti.

Additional support for this conclusion is provided by a comparison of reproductive inactivity between North American zoos and enclosures on Namibian farmland. In North American zoos 32% of 68 females had inactive ovaries with follicles smaller than 2.0 mm in size (Wildt et al. 1993) whereas in this study 67% of nine captive females were identified to have an inactive reproductive tract with follicles smaller than 2.0 mm. Thus, females held under

more natural conditions do not show higher reproductive activity than females kept in zoos, further suggesting that captivity itself may lead to a reduction in reproductive inactivity.

2.4.3 Reproductive activity in free-ranging females

Cycling with dependent offspring

In this study, four females were found to be in oestrous and one in pro-oestrous. Three of these females were accompanied by their offspring of about 9, 12 and 15 months of age, demonstrating that they resumed their cycling activity before separating from their offspring. In the Serengeti, females gave birth shortly after separating from their offspring, which at this point were on average 17.1 months old, indicating that females reconceived while being accompanied by their offspring (Kelly et al. 1998).

Embryonic resorption

One free-ranging female (W014) with offspring 9 months old had two fresh corpora lutea on one ovary, an integrated structure in the endometrium and an irregularly shaped embryonic vesicle embedded in the endometrium but had no embryonic structure. This structure was diagnosed as an embryonic resorption and was thus indicative of a recent event of conception. During lactation, which lasts about 4 months in cheetahs (Caro 1994), the sucking reflex provokes prolactin secretion. Prolactin inhibits the secretion of FSH (follicle stimulating hormone) and LH (luteinising hormone) and therefore prevents the maturation of follicles and thus ovulation. After the end of lactation, females can resume their regular cycle. Since cheetah mothers continue to be associated with their weaned cubs for approximately another 13 months after lactation in the Serengeti (Kelly et al. 1998) and for another 20 months in Namibia (Marker et al. 2003), they presumably cycle and are thus receptive throughout this time. However, a female with dependent cubs does not raise a new litter before she has separated from the current one (Laurenson et al. 1992; Caro 1994; Marker et al. 2003). It therefore seems highly unlikely that such a female is likely to copulate only a few months after lactation was terminated and many months before the separation from the current litter.

A possible explanation for the pregnancy of W014 while still being accompanied by dependent cubs might be a violent encounter with one or more cheetah males. Sexual coercion from males towards females is a widespread phenomenon in mammalian species, ranging from harassment and intimidation to forced copulations (review in Clutton-Brock 1995). Copulations of cheetahs have been observed extremely rarely in the wild, but

other encounters between male and female cheetahs have been reported where males were highly aggressive towards females (Caro 1994). It thus might be possible that this female was involved in a coerced copulation that resulted in a pregnancy. Since her cubs were still dependent for another 5-6 months, W014 might have activated resorption of the embryo to prevent being burdened with a new litter before the current litter had separated from her. Although factors such as genetic incompatibility, stress or insufficient nutrition have been shown to lead to a substantial percentage of embryos being aborted or resorbed in large mammals (Immegart 1997), the resorption diagnosed in this case is more likely to have been activated by the female because of the presence of her dependent cubs rather than being the consequence of the above mentioned factors during a planned pregnancy.

2.4.4 Reproductive activity in captive females

Three captive females were assigned to a reproductively active state. Z003 was held alone in a relatively small enclosure adjacent to Z004, who was permanently kept with her brother, whereas Z025 stayed with 18 other cheetahs of both sexes in a large enclosure. All three females were found to be in an early luteal phase, indicating that they had ovulated before, i.e. were cycling. Since cheetahs are, like other felids, induced ovulators with few reported exceptions of spontaneous ovulations (Asa et al. 1992; Wildt et al. 1993) and Namibian law prohibits the breeding of cheetahs in captivity (Stander et al. 2003), these females either ovulated spontaneously or were not effectively prevented from mating. The respective owners of the females confirmed that they did not give birth. Thus, the early luteal phases either represented pseudo-pregnancies or the females conceived but their pregnancies ended in undetected embryonic or foetal resorption or abortion. Alternatively, the females might have been at such an early stage of pregnancy that embryonic vesicles at the time of examination could not be detected and resorbed or aborted the embryos unnoticed later on.

The other six captive females were classified as being reproductively inactive at the time of investigation. They were kept in social groups of different composition with varying numbers of potential mating partners. There were or were not other large predators in neighbouring enclosures and the intensity of their contact to people varied (Appendix 1). Due to the variety of husbandry condition in the different facilities and the limited sample size, it is not possible to describe particular management conditions as conducive for reproductive activity in captive females.

2.4.5 Breeding success of free-ranging cheetahs

On Namibian farmland, cub survival and litter sizes at 2, 4 and 14 months of age were substantially higher than in the Serengeti, resulting in Namibian cubs having a higher chance to reach independence. In the Serengeti, the main mortality factor for cubs are large predators such as lions and spotted hyenas (Laurenson 1994) which are absent from Namibian farmland. This study shows that in an ecosystem without such large predators, cheetah cubs survive well and are not impaired by their genetic make-up. Thus, in a free-ranging population where mating between unrelated partners is likely, intrinsic mortality factors as a consequence of the species' genetic monomorphism do not act as main mortality factors.

This result confirms the conclusion from the Serengeti study (Laurenson et al. 1992, 1995; Caro & Laurenson 1994) that in free-ranging populations other factors rather than intrinsic factors are important. They are also in accordance with the study of Wielebnowski (1996) on captive cheetahs which suggested that cubs of unrelated parents have sufficient variation at those loci affecting cub survival that genetic monomorphism as such is unlikely to be of much relevance. This study further suggests that for captive cheetahs management and husbandry conditions are crucial for good breeding success and that detailed investigations on management practices that might encourage reproduction are required.

References

Asa,C.S., Junge,R.E., Bircher,J.S., Noble,G.A., Sarri,K.J. & Plotka,E.D. 1992. Assessing reproductive cycles and pregnancy in cheetahs (*Acinonyx jubatus*) by vaginal cytology. Zoo Biology, 11**,** 139-151.

Bertschinger,H.J., Meltzer,D.G.A., van Diyk,A., Coubrough,R.I., Soley,J.T. & Collett,F.A. 1984. Cheetah lifeline. Nuclear Active, 30**,** 2-7.

Brown,J.L., Wildt,D.E., Wielebnowski,N., Goodrowe,K.L., Graham,L.H., Wells,S. & Howard,J.G. 1996. Reproductive activity in captive female cheetahs (*Acinonyx jubatus*) assessed by faecal steroids. Journal of Reproduction & Fertility, 106**,** 337-346.

Caro,T.M. 1994. Cheetahs of the Serengeti plains: group living in an asocial species. The University of Chicago Press.

Caro,T.M. & Laurenson,M.K. 1994. Ecological and genetic factors in conservation: A cautionary tale. Science, 263**,** 485-486.

Caughley,G. 1994. Directions in conservation biology. Journal of Animal Ecology, 63**,** 215-244.

Clutton-Brock,T.H.&.P.G.A. 1995. Sexual coercion in animal societies. Animal Behaviour, 49**,** 1345-1365.

Engel,J. 1997. Signifikante Schule der schlichten Statistik. Fürth: Filander Verlag GmbH.

Fritsch,R. & Gerwing,M. 1993. Sonographie bei Hund und Katze. 1st edn. Stuttgart: Ferdinand Enke Verlag.

Goodrowe,K.L., Howard,J.G., Schmidt,P.M. & Wildt,D.E. 1989. Reproductive biology of the domestic cat with special reference to endocrinology, sperm function and in vitro fertilization. Journal of Reproduction & Fertility, Supplement 39**,** 73-90.

Grisham,J. & Marker-Kraus,L. Cheetah (*Acinonyx jubatus*). Bowdain, J., Wiese, R. J., Willis, K., and Hutchins, M. 1994. Bethesda, MD, American Zoo and Aquarium Association. Annual report on conservation and science 1993-1994.

Hermes,R., Göritz,F., Maltzan,J., Blottner,S., Proudfoot,J., Fritsch,G., Fassbender,M., Quest,M. & Hildebrandt,T.B. 2001. Establishment of assisted reproduction technologies in female and male African wild dogs (*Lycaon pictus*). Journal of Reproduction & Fertility, Supplement 57**,** 315-321.

Hermes,R., Hildebrandt,T.B., Göritz,F., Lengwinat,T., Jewgenow,K. & Holt,M.E. 1997. Sonographie der Trächtigkeit beim europäischen Reh (*Capreolus capreolus*). Verh. ber. Erkrg. Zootiere, 38**,** 169-173.

Hildebrandt,T.B. & Göritz,F. 1998. Use of ultrasonography in zoo animals. (Ed. by M.E.&.R.E.Fowler), pp. 44-54. Philadelphia: WB Saunders.

Hildebrandt,T.B., Maltzan,J., Hermes,R., Wiesner,H., Blottner,S. & Göritz,F. 2000. Ultrasonographic evaluation of reproductive soundness and ultrasound-guided artificial insemination (AI) in cheetah. In: Proceedings of the Fourth International Symposium on Canine and Feline Reproduction – Advances in reproduction in dogs, cats and exotic carnivores (Ed. by P.W. Concannon), p 60.

Immegart,H.M. 1997. Abnormalities of pregnancy. In: Current therapy in large animal theriogenology (Ed. by R.S. Youngquist), pp. 113-128. Philadelphia London Toronto Montreal Sydney Tokyo: W.B.Saunders Company.

Kelly,M.J., Laurenson,M.K., Fitzgibbon,C.D., Collins,D.A., Durant,S.M., Frame,G.W., Bertram B.C.R. & Caro,T.M. 1998. Demography of the Serengeti cheetah (*Acinonyx jubatus*) population: The first 25 years. Journal of Zoology, 244**,** 473-488.

Kriek,N., Rogers,P.S., Schultheiss,W.A., van Vuuren,M. & Berry,W.L. 1998. Neonatal mortalities in captive-bred cheetahs. Proceedings of a Symposium on Cheetahs as Game Ranch Animals, 114-115. Onderstepoort, South Africa. Penzhorn, B. L.

Laurenson,M.K. 1994. High juvenile mortality in cheetahs *(Acinonyx jubatus)* and its consequences for maternal care. Journal of Zoology, 234**,** 387-408.

Laurenson,M.K., Caro,T.M. & Borner,M. 1992. Female cheetah reproduction. National Geographic Research & Exploration, 8**,** 64-75.

Laurenson,M.K., Wielebnowski,N. & Caro,T.M. 1995. Extrinsic factors and juvenile mortality in cheetahs. Conservation Biology, 9**,** 1329-1331.

Marker,L. 1998. Current status of the cheetah (*Acinony jubatus)*. Proceedings of a Symposium on cheetahs as Game Ranch Animals, 1-17. Onderstepoort, South Africa. Penzhorn, B. L.

Marker,L., Dickman,A.J., Jeo,R.M., Mills,M.G.L. & Macdonald,D.W. 2003. Demography of the Namibian cheetah. Biological Conservation, 114**,** 413-425.

Marker,L. & O'Brien,S.J. 1989. Captive breeding of the cheetahs (*Acinonyx jubatus*) in North American zoos (1871-1986). Zoo Biology, 8**,** 3-16.

Marker-Kraus,L. & Grisham,J. 1993. Captive breeding of cheetahs in North American Zoos: 1987-1991. Zoo Biology, 12**,** 5-18.

Marker-Kraus,L., Kraus,D., Barnett,D. & Hurlbut,S. Cheetah survival on Namibian farmlands. Cheetah Conservation Fund. 1996. Windhoek.

Mattoon,J.S.N.T.G. 1995. Ultrasonography of the genital system. In: Veterinary diagnostic ultrasound (Ed. by W.B.Saunders), pp. 141-164. Philadelphia.

Mendelsohn,J., Jarvis,A., Roberts,C. & Robertson,T. 2002. Atlas of Namibia - a portrait of the land and Its people. David Philip Publishers.

Menotti-Raymond,M. & O'Brien,S.J. 1993. Dating the genetic bottleneck of the African cheetah. Proceedings of the National Academy of Sciences of the United States of America, 90**,** 3172-3176.

Menotti-Raymond,M. & O'Brien,S.J. 1995. Hypervariable genomic variation to reconstruct the natural history of populations: Lessons from the big cats. Electrophoresis, 16**,** 1771-1774.

Morsbach,D. 1987. Cheetah in Namibia. Cat News, 6**,** 25-26.

Munson,L. 1993. Diseases of captive cheetahs (*Acinonyx jubatus*): Results of the Cheetah Research Council Pathology Survey, 1989-1992. Zoo Biology, 12**,** 105-124.

Myers,N. The Cheetah *Acinonyx jubatus* in Africa. IUCN Monograph. 1975. Switzerland, Morges.

O'Brien,S.J., Roelke,M.E., Marker,L., Newman,A., Winkler,C.A., Meltzer,D., Colly,L., Evermann,J.F., Bush,M. & Wildt,D.E. 1985. Genetic basis for species vulnerability in the cheetah. Science, 227**,** 1428-1434.

O'Brien,S.J., Wildt,D.E., Bush,M., Caro,T.M., FitzGibbon,C., Aggundey,I. & Leakey,R.E. 1987. East African cheetahs: evidence for two population bottlenecks? Proceedings of the National Academy of Sciences of the United States of America, 84**,** 508-511.

O'Brien,S.J., Wildt,D.E., Gildman,D., Merril,C.R. & Bush,M. 1983. The cheetah is depauperate in genetic variation. Science, 221**,** 459-462.

Stander,P., Hanssen,L. & de Goede,P. 2003. Field guide to the Official Policy on Large Carnivores in Captivity. Ministry of Environment and Tourism Directorate Scientific Services.

Terio,K.A., Marker,L., Overstrom,E.W. & Brown,J.L. 2003. Analysis of ovarian and adrenal activity in Namibian cheetahs. South African Journal of Wildlife Research, 33**,** 71-78.

Wielebnowski,N. 1996. Reassessing the relationship between juvenile mortality and genetic monomorphism in captive cheetahs. Zoo Biology, 15**,** 369.

Wildt,D.E., Brown,J.L., Bush,M., Barone,M.A., Cooper,K.A., Grisham,J. & Howard,J.G. 1993. Reproductive status of cheetahs (*Acinonyx jubatus*) in North American zoos: The benefits of physiological surveys for strategic planning. Zoo Biology, 12**,** 45-80.

Wildt,D.E., Platz,C.C., Seager,S.W.J. & Bush,M. 1981. Induction of ovarian activity in the cheetah (*Acinonyx jubatus*). Biology of Reproduction, 24**,** 217-222.

Wrogemann,N. 1975. Cheetah under the sun. Johannesburg: McGraw-Hill Book Company.