Chapter 1

General introduction

Seasonal reproduction

Interactions between organisms and their physical environment as well as with other species result in many species exhibiting a restricted breeding period. Reproduction confined to a specific season, requires an animal to be able to time reproduction to ensure that the young are born at the most favourable time of the year, to promote rapid growth and maximal survival (Ims 1990). Seasonal reproduction involves the association of a series of physiological events from gonadal growth, steroidogenesis, gametogenesis, mating and finally the appearance of the young, all occurring within a particular season of the year (Jameson 1988).

Great advantages accompany seasonal reproduction. For example the musky rat-kangaroos, *Hypsiprymnodon moschatus,* produce their young when the two main food resources in their diet, litter fauna and fruits, are in great abundance (Dennis & Marsh 1997). Furthermore, regression of the reproductive organs during the non-breeding period can also be of benefit to an animal. Gonadal regression allows the animal to utilise energy for other activities, such as food gathering, rather than maintaining reproductive organs when not in use (Woodall & Skinner 1989).

Two main groups of cues are used for the onset of seasonal reproduction, namely internal and environmental cues. Endogenous rhythms constitute the internal cues, while environmental cues include factors such as food availability, humidity, temperature and photoperiod.

Mole-rats may use the onset of rainfall and photoperiod to trigger reproduction (Jarvis 1969; Moolman *et al.* 1998; Pevet *et al.* 1984). Temperature is yet another environmental cue that may be important for the onset of reproduction within the

subterranean mole-rats. Although temperature fluctuations in the burrow systems of mole-rats are muted compared to the seasonal ambient fluctuations, there is still some seasonality to the fluctuations (Bennett *et al.* 1988). The foraging burrows of mole-rats do not exceed depths of 30 cm and fluctuations in surface air temperature, influence soil temperatures (Gates 1962; Jarvis 1969; Bennett *et al.* 1988). Furthermore, Bennett *et al.* (1988) found marked seasonal differences in the mean burrow temperatures of mole-rats occurring in mesic and arid habitats, while in the tropics little seasonality in burrow temperature occurred. The presence or absence of seasonal temperature changes in the burrows may be an important determinant in the onset of reproduction in seasonally reproducing mole-rats (Bennett *et al.* 1988).

Another important indirect environmental cue is rainfall (Jarvis 1969). Recrudescence of digging results from rainfall, the result of such excavation being surface mounds of freshly displaced soil. The mole-rats extend their burrow systems and this increases the opportunities for non-reproductive animals to disperse from their natal colony to form their own colonies (Moolman *et al.* 1998). In addition, rainfall influences vegetation growth, thereby affecting availability of food resources (Dennis & Marsh 1997).

Of all the environmental cues, photoperiod is perhaps the most commonly used synchronising agent determining the breeding period of animals, as day length stays constant from year to year (Karsch *et al.* 1984; Gardiner *et al.* 1999). Since mole-rat species are subterranean most individuals are rarely, if ever, exposed to light. Individuals of the blind mole-rat species (*Spalax ehrenbergi*), for example, only receive light stimulus during winter, when they extend their burrow systems, pushing excavated soil to the surface (Shanas & Terkel 1996). However, the light stimulus in winter would not be enough for these animals if the development of their reproductive system were dependent on it. It is necessary for females to be exposed to light stimulus in summer to determine the onset of the reproductive process, to ensure that these animals would be able to breed during the winter months. Light impulses are received during the summer period, thus emphasising the importance of the mole-rat's reproductive circannual rhythm (Shanas & Terkel 1996; Shanas *et al.* 1995).

Trends in reproduction within the subterranean rodents

In solitary subterranean rodents there is sole occupancy of the burrow system. Plural or multiple occupancy arising only during the mating period or when the mother has offspring (Bennett & Jarvis 1988). The pups remain in the maternal burrow system for a short period that may not exceed 60 days in most cases. After this time, the mother will actively and aggressively expel the pups from the system (Bennett & Jarvis 1988; Bennett *et al.* 1991). The social subterranean rodents differ from the solitary species in that the breeding animals share a burrow throughout the year, the offspring do not disperse but rather share it with the parents throughout the year or extended periods of time. Indeed, they become extended families and show varying degrees of philopatry.

World wide, subterranean rodents are typically highly xenophobic and aggressively defend their natal burrow system. The presence of a single animal in a burrow system means that for breeding to take place, the extremely strong barriers of territoriality and aggression must be broken down. Advertisement of sex, status and the intention to breed must also be conveyed to conspecifics. This is achieved usually by seismic communication, which can take on a variety of forms. The blind mole-rat, *Spalax ehrenbergi*, uses head drumming (Rado *et al.* 1987; Heth *et al.* 1987) whereas incisor tapping is used in the rhizomyid *Tachyoryctes splendens* (Jarvis 1969). Hind foot drumming has been reported in the Geomyidae (the gophers; O.J. Reichman pers. comm.) and in three bathyergids, *Georychus capensis* (Bennett & Jarvis 1988), the Namaqua and Cape dune mole-rat (Bennett *et al.* 1991; Jarvis & Bennett 1991). This long distance communication allows members of the opposite sex to come together to procreate.

In contrast, the social species of subterranean rodents can reproduce when the occasion arises. There is no need for a seismic component to reproduction since the breeding animals occur together in the same burrow system. Courtship in the social bathyergids is less abrupt than that which occurs in the solitary species. Because the animals co-habit the same tunnels, pair bonds are formed by the breeding animals. There is considerable foreplay prior to mounting and mating. In seasonally breeding social species mounting and attempted mating is observed outside of the breeding season and this may strengthen the pair bond (Bennett *et al.* 1999).

Social suppression of reproduction in colonial bathyergids

The African mole-rats are fascinating since they exhibit both a range of social organisation and mechanisms to restrict reproduction to a single breeding female (Bennett *et al.* 1997).

Amongst outbred social mole-rats that inhabit mesic habitats, where the opportunities for dispersal and of becoming reproductive in a new colony are potentially high, one would predict that the staying incentives offered by the breeders to subordinates would be higher than in areas with strong environmental constraints. In these species incest avoidance would be the underlying form of monopolisation of reproduction and physiological suppression would be absent (Bennett *et al.* 1999).

In marked contrast to the mesic species, the naked mole-rat inhabits areas in which the ecological constraints are high and therefore theoretically the reproductive female need offer few, if any staying incentives. Naked mole-rats exhibit the most extreme form of social suppression found in the Bathyergidae, with there being physiological suppression in both sexes (Faulkes *et al.* 1990; 1991). The absence of incest avoidance necessitates a stringent reproductive control in the form of physiological suppression.

Bennett *et al.* (1997) suggested that within the family Bathyergidae, there is a continuum of socially-induced infertility occurring amongst different species inhabiting regions of varying degrees of aridity. Hence, in mesic habitats where opportunities for dispersal and of becoming reproductively active in new colonies are great, there is predominantly an incest avoidance component to social suppression and colony members are obligate outbreeders. In marked contrast, the naked mole-rat is an obligate inbreeder, exhibits physiological suppression in both sexes of non-reproductives and shows dominant control. The Damaraland mole-rat (*Cryptomys damarensis*), an obligate outbreeder, lies between these two extremes in that non-reproductive females have both a behavioural and physiological suppression operating upon them (Bennett *et al.* 1996).

The study animal: *Cryptomys hottentotus pretoriae* (Faulkes 1997)

Within the family Bathyergidae a sociality continuum exists, with species ranging from strictly solitary through to the true eusocial species (Jarvis & Bennett 1991; 1993). The genus *Cryptomys* has a wide geographical distribution throughout Africa and contains social representatives that range from loosely social species, such as *Cryptomys hottentotus hottentotus* to the true eusocial mole-rat, *C. damarensis* (Jarvis & Bennett 1991; De Graaff 1964). According to Jarvis *et al.* (1994) the frequency of rainfall and the degree of aridity in the habitat, plays a role in the social organisation of each mole-rat species. Indeed two very important ecological factors, the amount of precipitation per year and the distribution and abundance of food, are directly linked to the social status of each species (Jarvis *et al.* 1994). Thus, eusocial species, such as *C. damarensis* and *Heterocephalus glaber* occur in very arid habitats. These animals reproduce throughout the entire year, with no seasonal component linked to their reproduction. Solitary species such as *G. capensis* are known to occur in the more mesic regions of the Cape and reproduce seasonally. *Cryptomys hottentotus pretoriae,* the highveld mole-rat, is of special interest in that the individuals lead a colonial lifestyle and yet occur in a habitat with fairly predictable rainfall that should select for a solitary lifestyle (Moolman *et al.* 1998).

The highveld mole-rat is a social, subterranean, rodent mole-rat that occurs in colonies of up to twelve individuals (L. Janse van Rensburg, unpubl. data). The highveld mole-rat is phylogenetically closer to *Cryptomys hottentotus natalensis* than to *C. h. hottentotus* (Faulkes *et al.* 1997). They occur on the verdant highlands of South Africa, characterised by cold dry winters and hot moist summers (South African Weather Bureau).

The highveld mole-rat occur in colonies comprising of one reproductive female and one or two reproductive males that are responsible for the procreation of the new colony members (Moolman *et al.* 1998). Non-reproductive females have functional ovaries, suggesting these animals are only reproductively quiescent and not sterile. The non-reproductive males are not physiologically different from the reproductive males in

that their testes are of similar size and both groups undergo spermatogenesis. Non-reproductive males are behaviourally suppressed preventing them from reproducing with the reproductive female (Bennett *et al.* 1994).

It is still unclear what triggers the onset of reproduction in the highveld mole-rat. As suggested by Moolman *et al.* (1998) it is most likely that the highveld mole-rat uses rainfall as a cue for dispersal, since rainfall is frequent and predictable in these habitats (South African Weather Bureau). Opportunities therefore frequently arise for non-reproductive animals to disperse and form their own colonies. The small colony sizes of the highveld mole-rat tend to suggest that emigration from the colony is a common affair. Photoperiod is a potential *zeitgeber* that may have an important influence on the reproduction of the highveld mole-rat and this cue was also investigated during the course of the study.

Aims of the thesis

Knowledge regarding the reproductive biology of *C. h. pretoriae* is extremely fragmentary. A study on the molecular phylogeny by Faulkes *et al.* (1997) and a study undertaken on the social structure and dominance hierarchy by Moolman *et al.* (1998) provide us with the only information about this sub-species. The primary aim of this thesis is to investigate the reproductive cycle of the highveld mole-rat. The common mole-rat, a close relative of the highveld mole-rat, occurs in the winter rainfall region of the Cape province. It is a seasonal breeder that produces two litters per annum (Spinks *et al.* 1999). The highveld mole-rat in comparison occurs in the summer rainfall regions on the escarpment of South Africa, but to date we do not know if it is a seasonally or aseasonally breeding animal.

In chapter 2 the reproductive activity of both males and females are discussed. Intensive histological procedures were used to establish the reproductive cycle of the highveld mole-rat to elucidate whether it is a seasonal of aseasonal breeder. In addition hormone assays were executed to establish if the data obtained would co-incide with the data found during the histological part of the study. Sperm data for the males were

analysed to determine whether any patterns might occur and whether these patterns concur with the reproductive pattern found in the females. In chapter 3 relative age classes, mass and reproductive status was taken into account to determine if the oldest animals in a colony are the breeders. Dispersal and the establishment of new colonies by these vertebrate alates is believed to involve the older and stronger individuals within the colony. Thus, it is suggested that these animals would be the oldest or amongst the oldest members within the colony. Morphometric data was included to determine if sexual dimorphism is present. In addition the morphometric data was analysed for four separate localities, to determine any differences that might occur between different populations from the four localities. The possible role of photoperiod as a cue for the onset of reproduction was investigated in chapter 4, where mole-rats were exposed to two different light regimes. Due to the subterranean nature of this mole-rat it is interesting to explore the possible affect photoperiod might have on reproduction and whether or not it might be some other environmental factor such as rainfall that might play a more important role in the onset of reproduction.

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Chapter 2

Seasonal breeding in the highveld mole-rat, *Cryptomys hottentotus pretoriae*

ABSTRACT

Cryptomys hottentotus pretoriae is a co-operatively breeding rodent that exhibits seasonal breeding and a reproductive division of labour. Body mass, reproductive tract morphometrics, ovarian histology and plasma oestrogen and progesterone concentrations were studied in 189 females from 49 colonies. Although the birth of the offspring is confined to the months of May through to November, qualitative analysis of ovarian histology revealed that females retained reproductive function during the summer non-breeding period (December – March). Seasonal differences were found in ovarian morphometrics and progesterone and oestrogen concentrations which are associated with enhanced follicular activation in April and May and subsequent conceptions from May through to November during the breeding period.

The continuance of ovarian function during the non-breeding period as evidenced by the production of corpora lutea in reproductive females parallels the situation found in the male members of the colony. Interestingly, the non-breeding period coincides with the period of maximal dispersal opportunities in the summer rainfall areas inhabited by the highveld mole-rat. Non-reproductive females, while exhibiting some follicular development failed to ovulate in the confines of the colony, as evidenced by a lack of corpora lutea. The endocrinological data supports the lack of ovulation in these socially suppressed non-reproductive females.

Body mass, reproductive tract morphometrics, testicular histology and plasma testosterone concentrations were studied in 92 males from 37 colonies. The available evidence suggests that there is a gradual increase in testicular mass and volume with

increasing proximity to the breeding season, but after September, the testicular parameters begin to fall. Seminiferous tubule diameter was significantly greater in reproductive males but there was no obvious change with season. In general, testosterone concentrations are higher in the reproductive males, with the highest titres occurring around July and August. All available evidence supports a continuance of reproductive activity during the non-breeding season. It is speculated that reproductive activation in the non-reproductive males may facilitate inter-sexual recognition and hence facilitate bond formation for independent reproduction.

Bimonthly sampling of males to investigate sperm motility, revealed no significant difference between the sperm kinematics of reproductive (n = 14) and non-reproductive males (n = 17).

The available data suggests that there are two main periods of both follicular and testicular activity, these being June and September. It is possible that for an animal with an estimated gestation of two months, that the reproductive potential of producing two litters during the breeding season arises with pups being born at the end of July through to November.

Evidence from this study suggests that during the non-breeding season, moist workable soils facilitate the dispersal of previously non-reproductive animals from their natal colonies and subsequent colony genesis arises from previously suppressed females and males.

INTRODUCTION

Reproduction is important to the biology of all organisms and is the means by which an individual perpetuates copies of its genes. Considering the fundamental role of reproduction in organismal biology, it is surprising that little research has been afforded to subterranean rodent moles (Bennett *et al.* 2000). Reproduction in subterranean rodents is ecologically constrained by the burrow environment. Indeed, subterranean rodents rarely, if ever, venture onto the surface and therefore many of the common proximate cues normally utilised by seasonal breeders, such as photoperiod are precluded from use.

In the labyrinths of the burrow system, other environmental cues may trigger the onset of breeding such as thermoperiod, changes in soil moisture content or the associated sudden flush of vegetation associated with good precipitation (Bennett *et al.* 1988). Seasonal rainfall, which results in a softening of the soil may facilitate the extension of existing burrows as well as enabling opposite sexed conspecifics to come together for the process of procreation.

Members of the Bathyergidae are unique in that they display a broad spectrum of social organisation ranging from strictly solitary through to eusocial representatives (Jarvis *et al.* 1994; Bennett & Faulkes 2000).

Many subterranean rodents are strictly solitary, highly xenophobic and aggressively defend their natal burrow system from conspecifics (Nevo 1979). In these solitary species, reproduction is usually a brief affair, during which strong barriers to aggression are broken down and mating is thus short-lived (Bennett & Jarvis 1988). In solitary species, plural occupancy of the burrow occurs briefly during the breeding season or when the female has young (Bennett & Jarvis 1988).

Along the gradient of social organisation lies a number of social species of the genus *Cryptomys*. All solitary species of Bathyergidae reproduce seasonally, whereas the majority of social mole-rats exhibit no seasonal component to their reproduction, thus reproducing throughout the year (Bennett *et al.* 1991). However, to date one social species from the winter rainfall region of the western and northern Cape Province has been found to be a seasonal breeder (Spinks *et al.* 1997; 1999). Spinks *et al.* (1997; 1999) have shown that the social common mole-rat, *C. h. hottentotus*, exhibits a marked seasonality to reproduction, rearing young during the southern hemisphere summer (late November through to January).

The highveld mole-rat, *C. h. pretoriae* occurs in the summer rainfall regions of the highveld in South-Africa. The sub-species *C. h. pretoriae* is phylogenetically closely related *to C. h. hottentotus*, the common mole-rat, also a social, seasonal breeder (Faulkes *et al.* 1997). The highveld mole-rat occurs in colonies of similar size as the common mole-rat, 2-12 individuals per colony (Moolman *et al.* 1998; L. Janse van Rensburg, unpubl. data). The warm wet summers and dry cool winters of the highveld show a distinct seasonality. This seasonal component and the close phylogenetic relationship

between the common mole-rat and the highveld mole-rat suggests that this social subterranean rodent may also exhibit the potential for seasonal reproduction.

Moolman *et al.* (1998) found the highveld mole-rat to be a loosely social species with no distinct dominance hierarchy. While colonies exhibit a marked reproductive division of labour, with up to two males and one female being responsible for procreation, no secondary work division of labour is apparent (Moolman *et al.* 1998). This is in contrast to the situation found in the Damaraland mole-rat (*Cryptomys damarensis*) (Bennett & Jarvis 1988). Given that the environment in which the highveld mole-rat occurs, exhibits an annual seasonal component to it in the form of a change in both temperature and precipitation, my *a priori* prediction was that this social subterranean rodent mole should exhibit a seasonality to reproduction both in the production of young and also in the recrudescence and regression of the gonads.

In order to address this question, I adopted an approach of sampling a population of highveld mole-rats in the Gauteng Province of South Africa, on a monthly basis for an entire calendar year. Post-mortem examinations have proven invaluable in determining whether subterranean rodents have a seasonal component to reproduction (see Bennett *et al.* 2000 for review). Basic reproductive parameters, such as duration of pregnancies, physical dimensions of reproductive organs and patterns of follicular development and sperm production have been quantified for the tuco tuco, Ctenomyidae (Malizia & Busch 1991), the pocket gophers, Geomyidae (Miller 1946; Smolen *et al.* 1980), the African mole-rats, Bathyergidae (Jarvis 1969; van der Horst 1972; Bennett & Jarvis 1988) and the mediterranean mole-rats, Spalacinae (Redi *et al.* 1986).

Social subterranean rodents have reproduction monopolised by a single female, with all other females being reproductively quiescent or exhibiting socially-induced infertility (Bennett *et al.* 1993; 1994b; 1996; 1997). The reproductive status of an animal is readily identifiable and consequently an examination of complete colonies allows the determination of the reproductive state of this individual at any point in the year. However, the repression of reproduction in non-reproductive females also provides an opportunity to investigate whether relaxation of suppression occurs at any particular part of the season in these otherwise behaviourally infertile females.

In the social bathyergid mole-rats suppression of reproduction in non-reproductive males does not appear to be physiological in nature. Faulkes *et al.* (1994) found no apparent suppression of sperm production or sperm motility in non-reproductive Damaraland mole-rats. However, they found that suppression of reproductive hormones in *Heterocephalus glaber* might correlate with reduced fertility in the non-reproductive males, because the majority of these males produced fewer, non-motile spermatozoa than the reproductive males. However, Faulkes *et al.* (1994) also found that a number of non-reproductive males were reproductively active and thus potentially fertile. Whenever non-reproductive males were removed from their natal colonies, their body and testes size increased and they produced a greater number of motile spermatozoa. In this study, the presence of sperm production and sperm motility, in both reproductive and non-reproductive males throughout the study period were examined to determine the potential non-reproductive males may possess to successfully reproduce when they disperse from their natal colony. I also aimed to determine if any relationship might occur between increased sperm motility and increased testes volume and mass.

The aims of this chapter were threefold. 1) to determine if the highveld mole-rat is a seasonal breeder, 2) to assess if there is relaxation of reproduction in non-reproductive members of the colony at any point in the year and 3) to determine if seasonality of breeding (if present) can be linked to rainfall.

MATERIALS AND METHODS

Capture

A minimum of three colonies of the highveld mole-rat were caught on a monthly basis using modified Hickman live traps (Hickman 1979). The capture period lasted from January 1998 to April 1999 and a total of 126 males and 260 females from 55 colonies constituted the source of my study. Ninety-six of the animals caught were used for a melatonin study (Chapter 3). The animals were captured on golf courses and in gardens in the environs of Gauteng: Pretoria (25°45'S 28°10'E) (Tygerpoort, Monumentpark golf course, Dienste golf course), Johannesburg (26°12'S 28°05'E) (Johannesburg

Countryclub, Modderfontein golf course, Bryanston golf course, Esselinpark golf course), Krugersdorp (26°06'S 27°46'E) (Palm nursery), Vanderbijlpark (26°42'S 27° 49'E) (Industrial areas and small holdings).

Colonies were caught out in their entirety, a colony being deemed fully trapped out if open sections of the burrow where animals had been trapped were not blocked with soil after 3 days.

Housing

The animals were housed in plastic crates (49.5cm x 28cm). Wood shavings and paper towelling were provided as nesting material. The mole-rats were fed sweet potato, gem squash, carrots and apples on a daily basis. The animals were kept in a climate room at a constant temperature of 25 ± 1°C. The animals were maintained in the laboratory for as short a time as possible. However, to ensure that post-mortem examination was as accurate as possible, functionally complete colonies were maintained together for a minimum of a week after all individuals in a system had been trapped out.

Determination of reproductive status

In the laboratory, the animals were sexed and their reproductive status determined. Prominent axillary teats were one of the characteristics used to identify the reproductive female from non-reproductive females. Many of the females caught, exhibited a perforate vagina, irrespective of whether they were reproductive or non-reproductive. During histological procedures confirmation of the reproductive status of the females was supported by the presence of foetuses and placental scars present on the uterine horns, as well as by the presence of corpora lutea or corpora albicans in the ovary. The reproductive male was discerned based on its physical size and its observed copulation with the reproductive female in the laboratory whenever possible.

Histological procedures

After capture and prior to being killed each individual was weighed using a Sartorius 1213MP scale (max = 3000.0g, Zeiss, Germany). The animals were then deeply anaesthetised with halothane, causing death. Blood samples were obtained by

exsanguination from the heart. The blood was centrifuged at 3000rpm and the plasma fraction obtained was immediately stored at -20°C.

The animals were then dissected in order to remove the reproductive tracts. The material was fixed in Bouins fluid for approximately 16 hours prior to being rinsed and stored in 70% ethanol. Prior to being processed, the fixed gonads were weighed using a Sartorius scale (max = 100g, d = 0.1mg, Zeiss, Germany). Both ovaries of the females and both testes of the males were weighed. For statistical analyses the mean of the gonads for each individual was determined.

The material was sequentially dehydrated and embedded in paraffin wax. The ovaries and testes were serially sectioned at 7µm, mounted on glass slides, stained in Ehrlich's haemotoxylin and counter stained in eosin (Drury & Wallington 1967). Using a vernier calliper, the maximum length and width of each testes and ovary was measured. Ovarian volume as well as testicular volume was calculated using the formula for the volume of an ellipsoid, as described by Woodall & Skinner (1989); $V = 4/3\pi ab^2$, where a = ½ maximum length and b = ½ maximum breadth.

Female histology

Each ovary (n = 180) was sectioned in its totality and mounted. Sections were then examined in consecutive order using a light microscope at the following magnifications: x100, x200 and x400. Ovarian follicles were categorised and counted based on follicular development and atretic changes. Bloom & Fawcett (1962) and Bennett *et al.* (1994a) were used as a guide to identify and categorise the various follicular stages. The following follicle counts were made during the study:

1. Primordial follicles (P) were numerous and located at the periphery of the ovary, just interior to the tunic albuginea (Plate 1).
2. The development of primordial follicles into primary follicles (Pr) is characterised by the transition of the flattened or squamous follicular cells into cuboidal cells (Plate 2). The primary follicle is much larger than the primordial follicle and contains an enlarged oocyte surrounded by one or more layers of cuboidal cells.

3. The transition from primary to secondary follicles is marked by the appearance of several irregular spaces in the stratum granulosum, filled with clear liquor folliculi (Plate 3). An increase in the amount of this liquid causes the cell to increase in size and develop into the next follicular stage.
4. The Graafian follicle was defined as having a large continuous fluid filled antrum with the oocyte pressed to one side of the follicle (Plate 4).
5. A ruptured Graafian follicle, named the corpora haemorrhagicum is another distinct feature of a female in a state of ovulation. The ovum loosens itself from the cumulus oophorus and as soon as the follicular wall ruptures the ovum is released (Plate 5).
6. During an organisms lifetime only a fixed amount of ova are discharged during ovulation. The remainder of the follicles degenerate and disappear. This involution of a follicle is termed "atresia" (A) (Plate 6).
7. After the Graafian follicle ruptured it is transformed into a corpus luteum (Cl) (Plate 7). The corpus luteum serves as a source of progesterone during pregnancy.
8. After pregnancy the corpus luteum will regress until it is reduced to a scar, the corpus albicans (Plate 8).

In addition to the follicular count the presence of placental scars and foetuses were recorded, including the presence of very young animals within colonies.

Male histology

Only a few selected sections, from the mid region of the testes were mounted. Sections were then examined using a light microscope at the following magnifications: x100, x200 and x400. Thirty randomly selected, cross-sectioned seminiferous tubules from each male specimen, were chosen and their diameter measured with the aid of an eyepiece micrometer (Vickers instruments).

The following was studied during the histological part of the study using Bloom & Fawcett (1962) as a reference:

1. The seminiferous tubules are lined by the seminiferous epithelium (Se), which consists of two types of cells the supporting cells of Sertoli and spermatogenic cells (Plate 9).
2. The epididymis is an elongated organ attached to the posterior surface of the testis (Plate 10). It is made up of the convoluted proximal part of the excretory duct system.

The results are based on the assumption that any changes in size that might have occurred as a result of fixation were constant across all the samples measured. Thus, all measurements are relative and not absolute (Spinks *et al.* 1997).

Sperm collection

Male highveld mole-rats were put down by halothane inhalation on a bimonthly basis. The testes and epididymis were dissected free from the surrounding connective tissue and fat. The material was placed in a 35mm plastic petri dish filled with preheated Ham's F10 (Sigma Cat No. N6635) culture medium with L-glutamate and supplemented with 1.2 g/l sodium bicarbonate. This medium is used to activate and preserve sperm mobility. By puncturing the vasa deferens the sperm appeared as a thick white fluid and were collected using a pipette. A volume of 10µl of sperm suspension was transferred to an object slide and covered with a cover slip. The slide was placed on a microscope stage pre-heated to 36°C and 5-10 minutes of free-swimming sperm were videotaped using a VHS recorder at 320x magnification.

After the extraction of the sperm, the testes and epididymis were fixed in Bouins fluid for 16 hours, rinsed and stored in 70% ethanol for further histological studies (See male histology, materials and methods).

Tracking sperm movement

Images were recorded at 30 frames per second and played back at 1/10 of the normal speed. Frame by frame analysis was facilitated by coupling the videotape – output to a computer based image analysis system. (Sperm Motility Quantifier, Wirson Scientific and Precision Equipment, Auckland Park, Johannesburg). As the sperm moved across

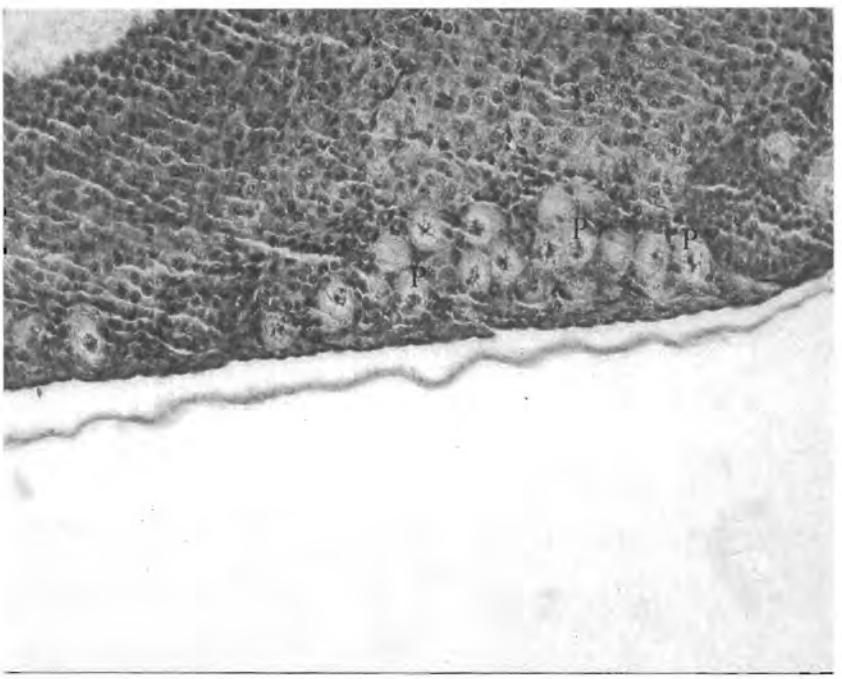

Plate 1. Primordial follicles.

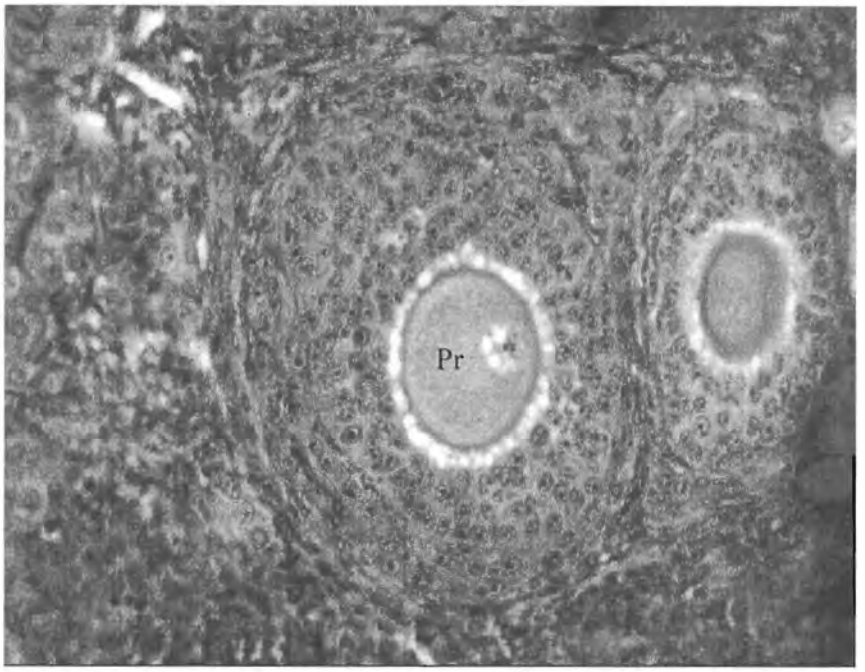

Plate 2. Primary follicle.

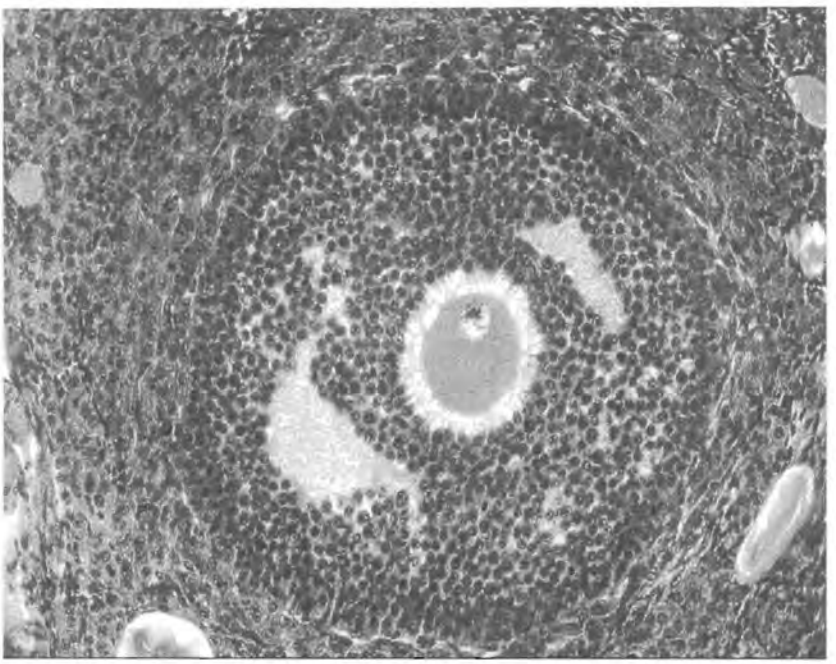

Plate 3. Secondary follicle.

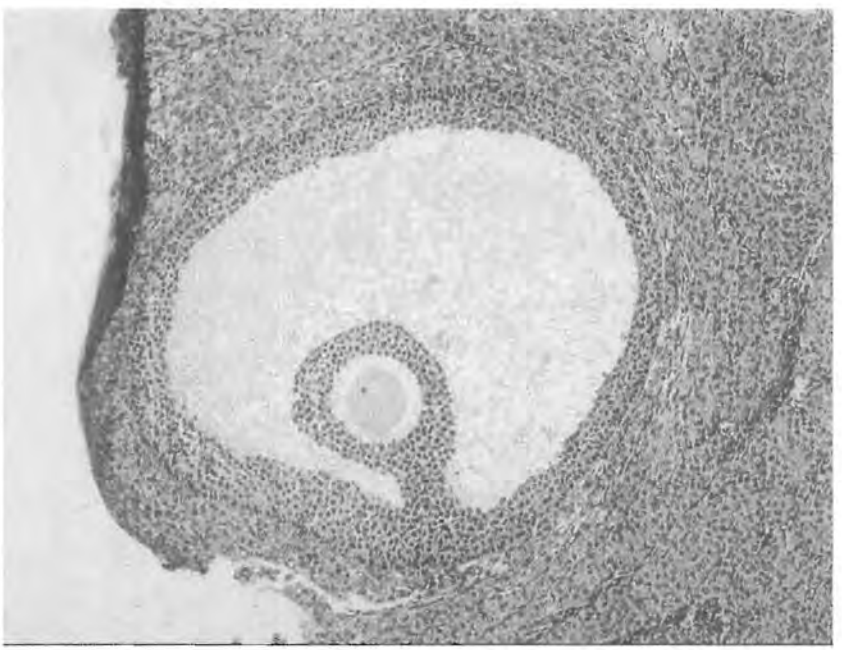

Plate 4. Graafian follicle.

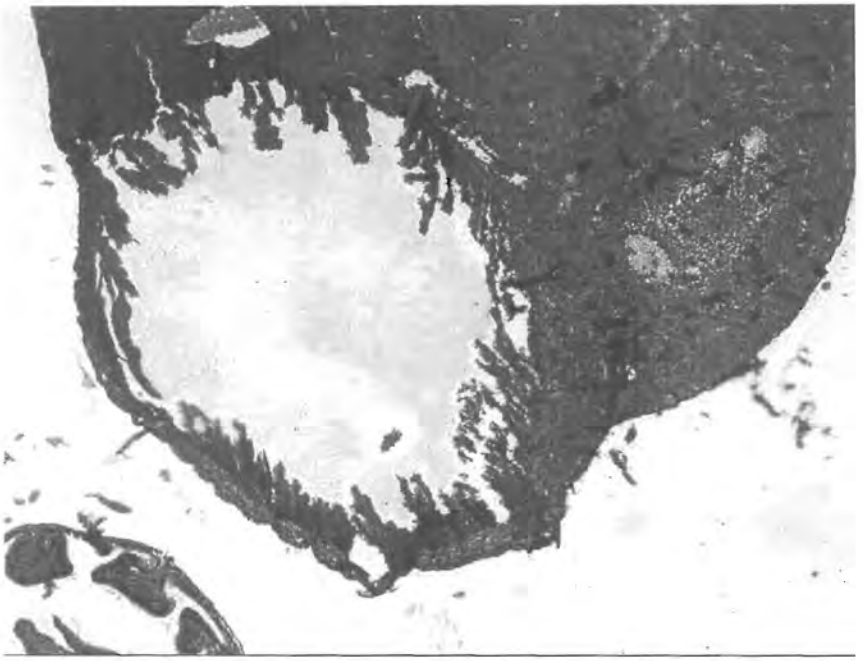

Plate5. Corpus haemorachicum.

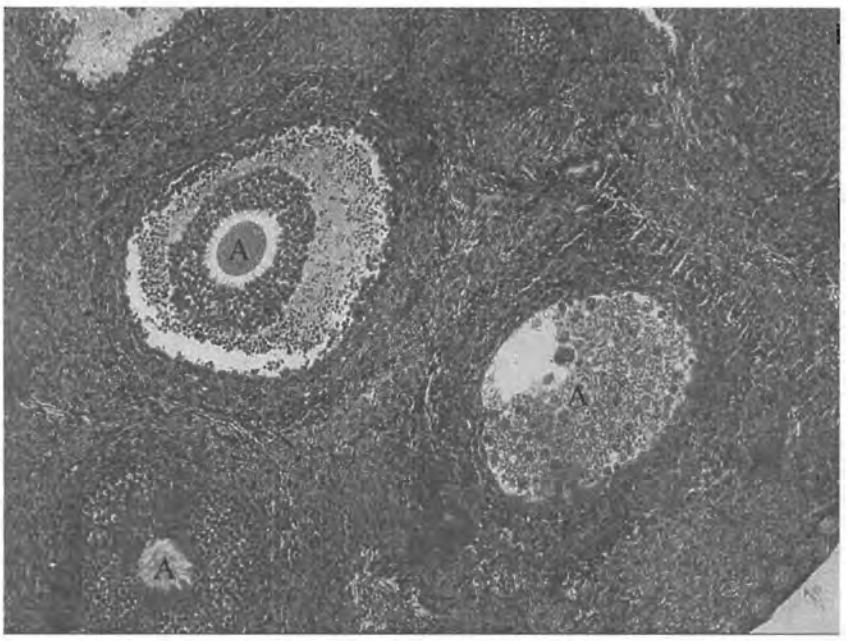

Plate 6. Atretic follicles.

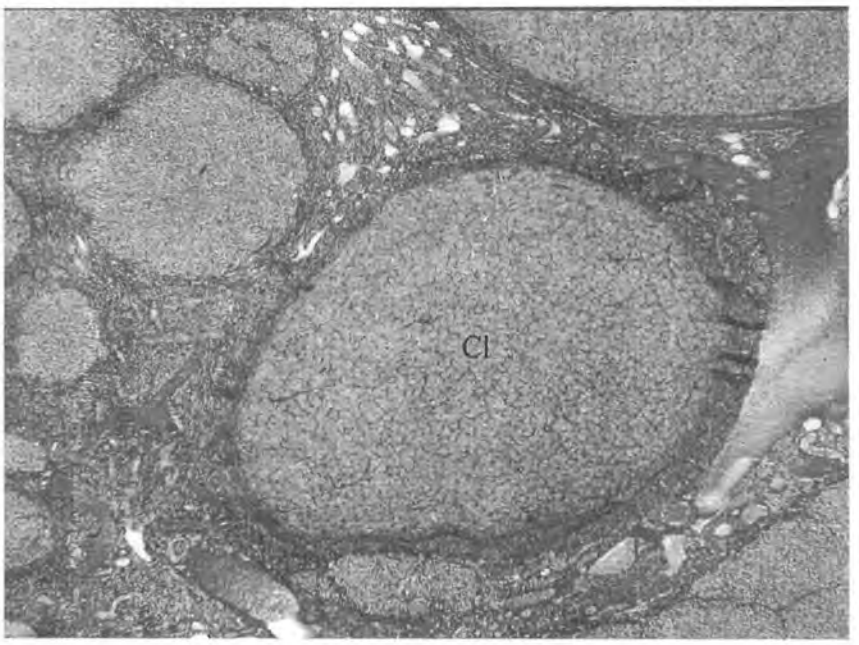

Plate 7. Corpora luteum.

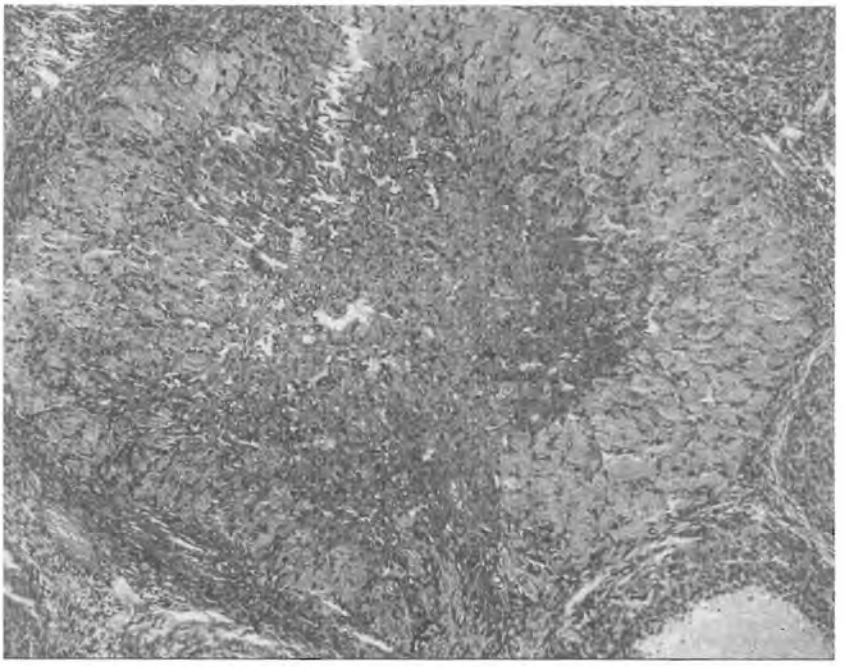

Plate 8. Corpus albicans.

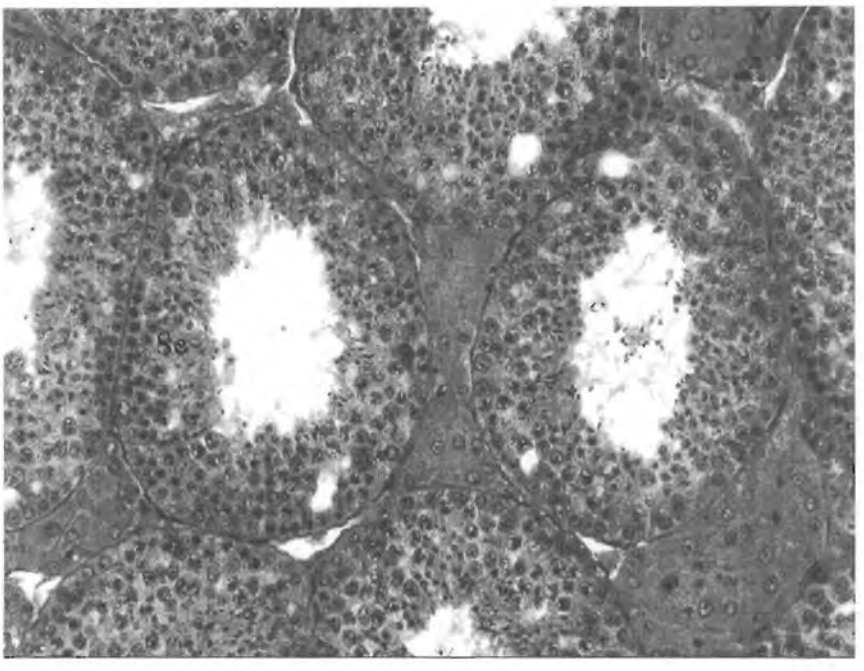

Plate 9. Seminiferous tubules.

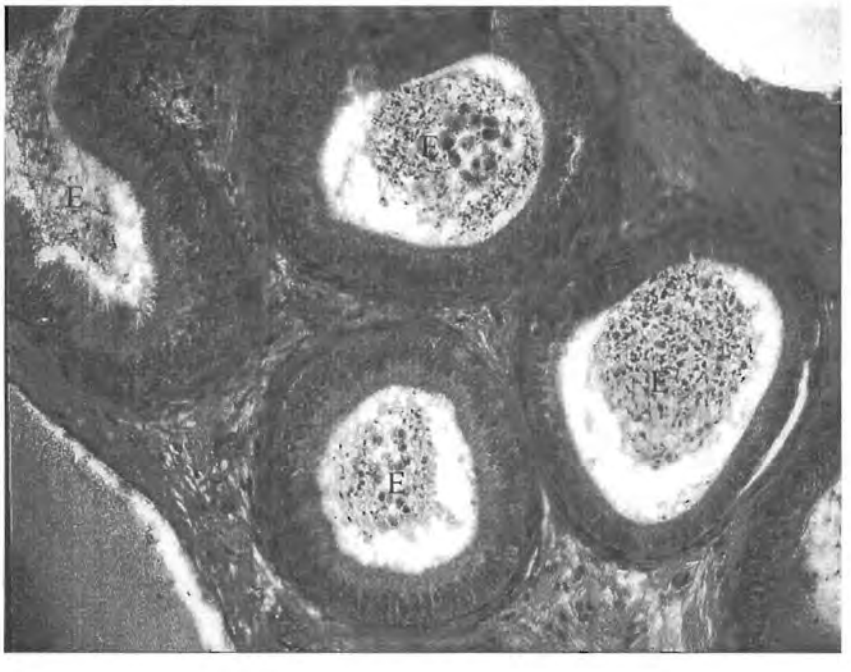

Plate 10. Epididymis

the screen the computer automatically tracked the position for the sperm head/midpiece junction for each frame.

The sperm recordings were captured with a frame skip of zero at an analysis rate of 50Hz: 25 frames were analysed to monitor the sperm trajectory. All tracings complete, the computer automatically scaled the distances between points to microns and calculated all motility parameters (Olds – Clarke 1986).

The following sperm motility parameters as defined by Katz (1991) were measured:

1. Curvilinear velocity (VCL): time-averaged velocity of sperm head along its actual path or curvilinear trajectory.
2. Straight-line velocity (VSL): time averaged velocity of sperm head as projected along the straight line between its first and final detection positions.
3. Average path velocity (VAP): time-averaged velocity of sperm head projected along its spatial average trajectory.
4. Beat cross frequency (BCF): the beat cross frequency of the sperm head.
5. Linearity (LIN): a ratio of projected length of the curvilinear trajectory. (LIN = VSL/VCL).
6. Amplitude of lateral head displacement (ALH): maximum amplitude of lateral distances of the sperm head trajectory about its spatial average path.
7. Wobble (WOB): ratio of VAP to VCL and is an expression of the degree of oscillation of the curvilinear path about its spatial average path (WOB = VAP/VCL).
8. Straightness (STR): ratio of VAP to VSL and is an expression of the straightness of the average path. (STR = VAP/VSL).
9. Dance (DNC): defined by the product of VCL an ALH, and describes sperm motion as the space occupied by the sperm head path during one second. DNC = VCL x ALH.
10. Radian (RAD): the radius of the circle of which the total curvilinear track is an arc. By using the radian, circling sperm can be detected (RAD = (radius/π) x 180^{o}).
11. Curvature (CURV): it reflects the progressiveness of movement (CURV = 1 – (VSLpath/VCLpath).

Progesterone determination

Progesterone was measured in 29 reproductive females (RF) and 154 non-reproductive females (NRF). The plasma progesterone determinations were undertaken on duplicate 100µl samples using the coat-a-count progesterone kit (Diagnostic Products Corporation, USA).

The antiserum is highly specific for all naturally occurring steroids with a cross reactivity of <0.5% except for 20 α dihydroprogesterone and 11-deoxycortisol for which it was 2% and 2.4% respectively. Steroids were not purified and separated by chromatography. The assay is a non-extraction assay.

Validation for progesterone

All the hormone assays were validated for use in the highveld mole-rat as described by Bennett *et al.* (1994b). Plasma progesterone samples from a reproductive female provided a displacement curve parallel to the standard curve when serially double diluted (over the range 1:1 to 1:64). The slope of both curves did not differ significantly (ANCOVA, $F = 2.27$, $p>0.05$; Fig.1), following a log-logit transformation of the data (Chard 1987). The intra assay coefficient of variation for a plasma pool was 7.9% ($n = 10$). The sensitivity of the assay was 0.159nmols/l.

Oestradiol determination

Oestradiol was measured in 23 reproductive (RF) and 145 non-reproductive females (NRF). Oestradiol determinations were performed using a Coat-A-Count Oestradiol kit (Diagnostic Products Corporation, USA). This method requires neither extraction nor chromatography. The antiserum is highly specific for oestradiol, with a very low cross reactivity to any other compounds present in the plasma samples.

Validation for oestradiol

A serial dilution of plasma oestradiol from a reproductive female paralleled the reference preparation, thus the slopes did not differ significantly (ANCOVA, $F = 0.66$, $p>0.05$, Fig. 2), following a log-logit transformation of the data (Chard 1987). The intra assay

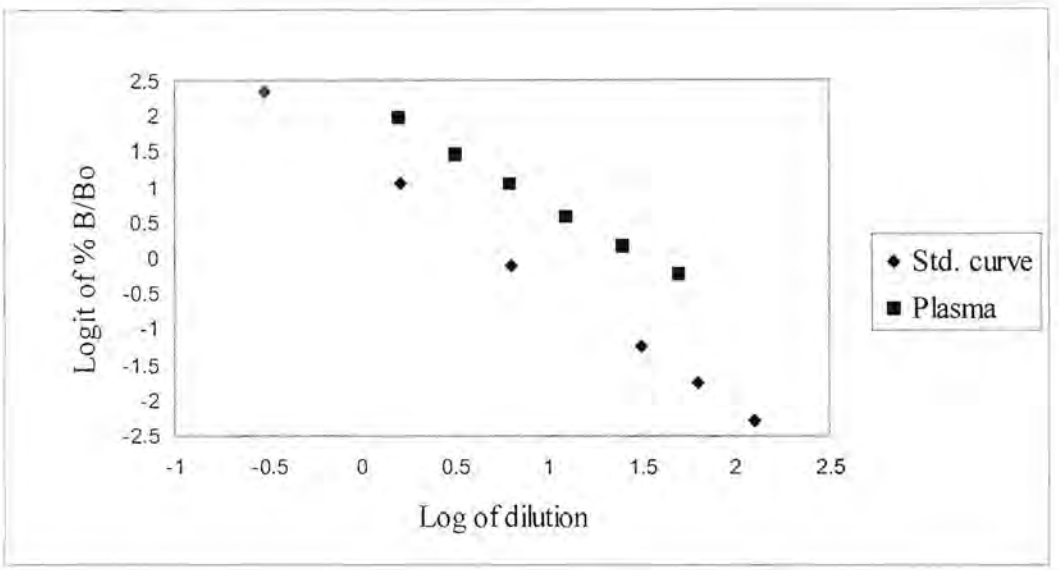

Fig. 1. Reference progesterone preparation (♦) and serial doubling dilution (■) of highveld mole-rat plasma, showing parallelism.

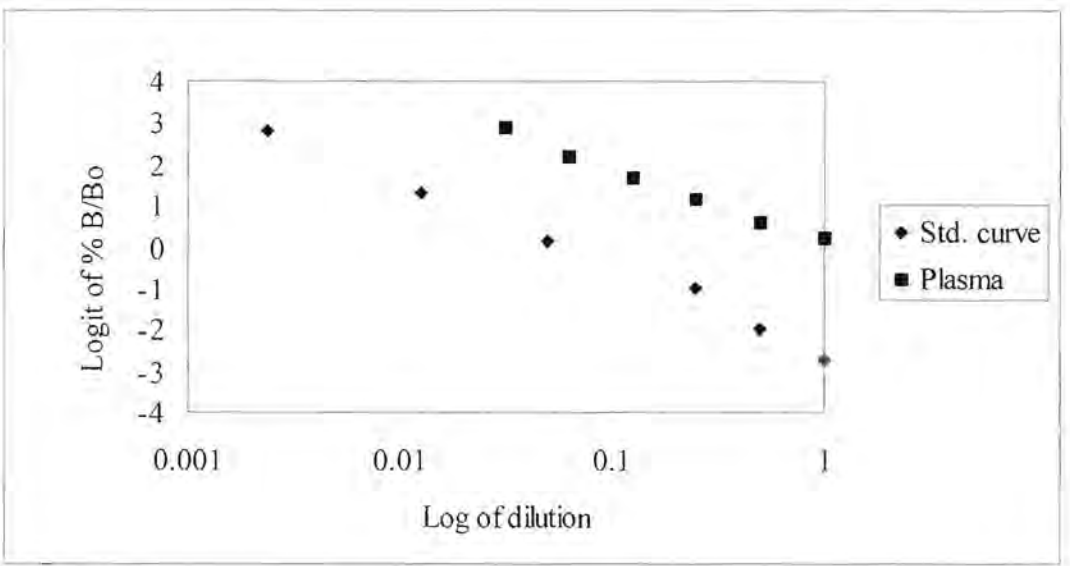

Fig. 2. Reference oestradiol preparation (♦) and serial doubling dilution (■) of highveld mole-rat plasma, showing parallelism.

coefficient of variation had a value of 17.38 (n = 6). The sensitivity of the assay was 0.002nmols/l.

Testosterone determination

Testosterone was determined for 43 reproductive males (RM) and 55 non-reproductive males (NRM). Total testosterone concentrations were measured using a Coat-A-Count total testosterone kit (Diagnostic Products Corporation, USA). Due to the simplicity of this method, neither extraction nor chromatography is required. The antiserum is highly specific for testosterone, with very low cross reactivity to other compounds. Crossreactivity with dihydrotestosterone is less than 5%.

Validation for testosterone

A serial double dilution of reproductive male plasma testosterone paralleled with the standard curve, thus validating the assay (ANCOVA, $F = 0.18$, $p>0.05$, Fig. 3). A log-logit transformation of the data was followed (Chard 1987). The intra assay coefficient of variation was equal to 4.9 (n = 6). The sensitivity of the assay was 0.011nmols/l.

Statistical analyses

Statistical analyses were performed using Statistica version 5.0™ and Prism, GraphPad Software™. The non-parametric, Mann Whitney U-test (Zar 1984), was used for comparisons between males and females with regard to their reproductive status.

After testing for normality within the hormone data sets, Kruskal Wallis and Dunn's multiple range tests (Zar 1984) were used to determine significant differences between reproductive and non-reproductive animals within months. Comparative testing between reproductive and non-reproductive male sperm was undertaken using a one way analysis of variance (ANOVA), after testing for homoscedacity. The sperm motility characters for reproductive and non-reproductive males were analysed using a Generalised Linear Model (GLM) (Zar 1984). 95% degree of confidence applies to all statistical tests.

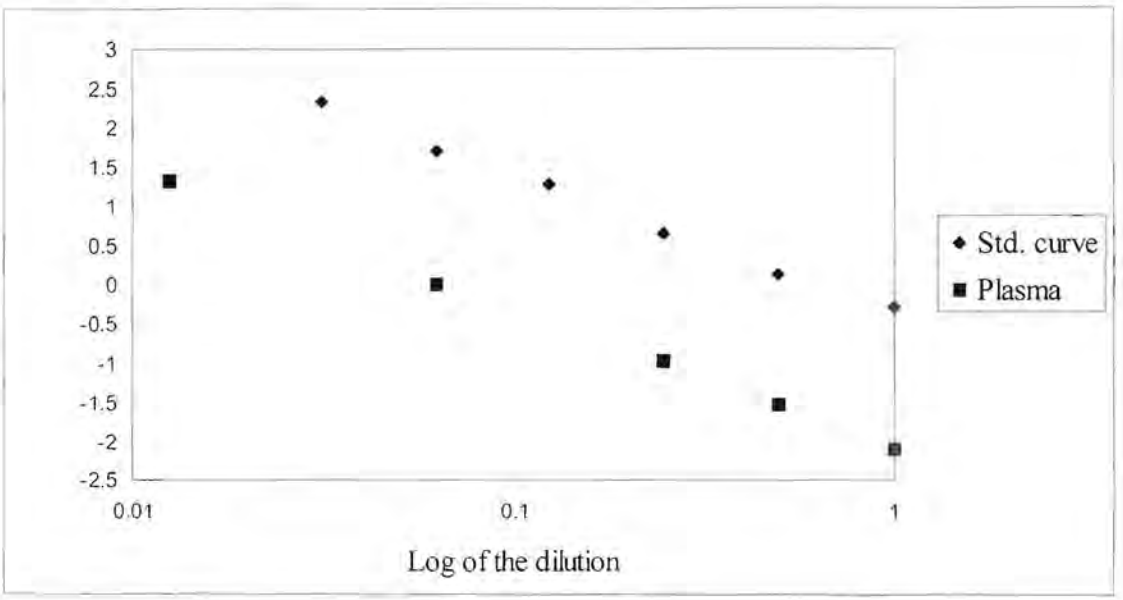

Fig. 3. Reference testosterone preparation (♦) and serial doubling dilution (■) of higveld mole-rat plasma, showing parallelism.

RESULTS

The ovarian histology

All follicle counts, measurements and hormone concentrations are expressed as mean ± S.E. (standard error) throughout for all animals studied. No S.E. bars are indicated on graphs, where sample size (n) = 1.

The number of primordial follicles was significantly higher for the non-reproductive females than for the reproductive females (NRF = 206.21 ± 9.02; RF = 115.29 ± 21.41) (Mann Whitney U-test, U = 961.000 $p<0.001$, n(NRF) = 150, n(RF) = 29), throughout the entire sample period (Fig. 4). There was no significant difference in the number of primary follicles between reproductive and non-reproductive females throughout the sampling period (NRF = 20.82 ± 1.26; RF = 25.47 ± 4.07) (Mann Whitney U-test, U = 2023.00, $p>0.05$, n(NRF) = 151, n(RF) = 29) (Fig. 5). No pattern was observed in the distribution of secondary follicles between reproductive and non-reproductive females throughout the year (NRF = 0.54 ± 0.01; RF = 0.59 ± 0.19) (Fig. 6).

The number of Graafian follicles were not significantly higher in the reproductive females when compared with non-reproductive females (NRF = 0.14 ± 0.03; RF = 0.10 ± 0.05) (Mann Whitney U-test, U = 2164.50, $p>0.05$, n(NRF) = 151, n(RF) = 29) (Fig. 7).

The reproductive females possessed Graafian follicles from February through to May with a peak observed during August (0.30 ± 0.35, n = 2). No Graafian follicles were found during June and July or for September through to January. In the non-reproductive females, Graafian follicles were present for nine months of the year, excluding the months of May, July and September (Fig. 7).

Reproductive females have a significantly higher mean number of atretic follicles than the non-reproductive females (NRF = 12.92 ± 0.62; RF = 19.53 ± 1.88) (Mann Whitney U-test, U = 14459.00 $p<0.001$, n(NRF) = 151, n(RF) = 29). Higher follicular activity in the reproductive females is evident from the statistical analyses ($p<0.05$) across the months of June to December (Fig. 8).

Corpora lutea were not present in any of the non-reproductive females sampled. However, reproductive females examined had corpora lutea present in their ovaries

throughout the sampling period, excluding the month of January (RF = 1.220 ± 0.21) (Fig. 9). Corpora albicans were only observed in August and October in the reproductive females (Fig. 10). During the study two reproductive females gave birth to three young each, in captivity within the months of May and July. The presence of very young individuals with masses not exceeding 40g (age class 1), were sampled from May through to December, excluding June, October and November.

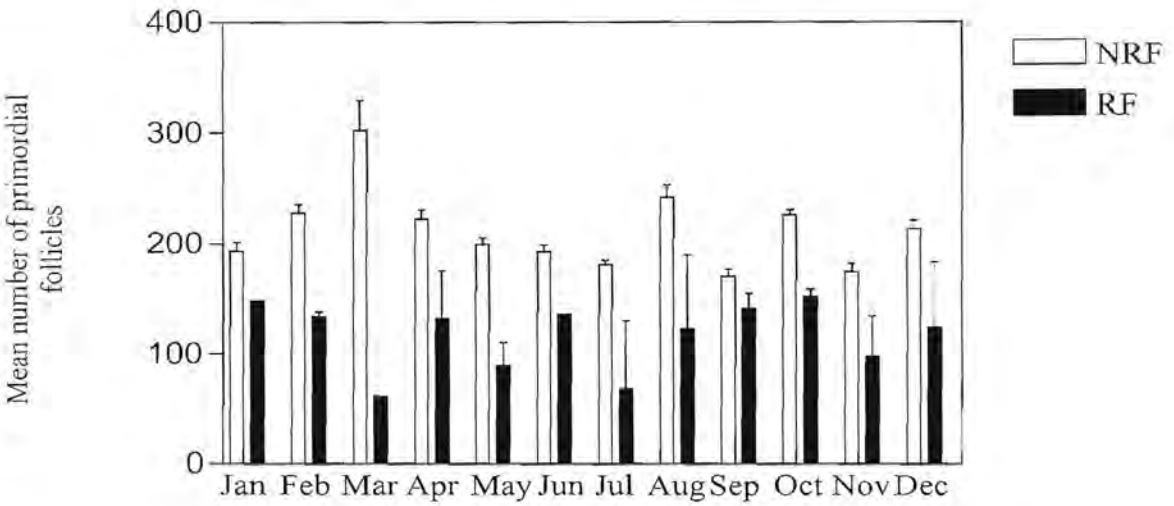

Fig. 4. The mean ± S.E. of primordial follicles in reproductive (RF) and non-reproductive female (NRF) ovaries.

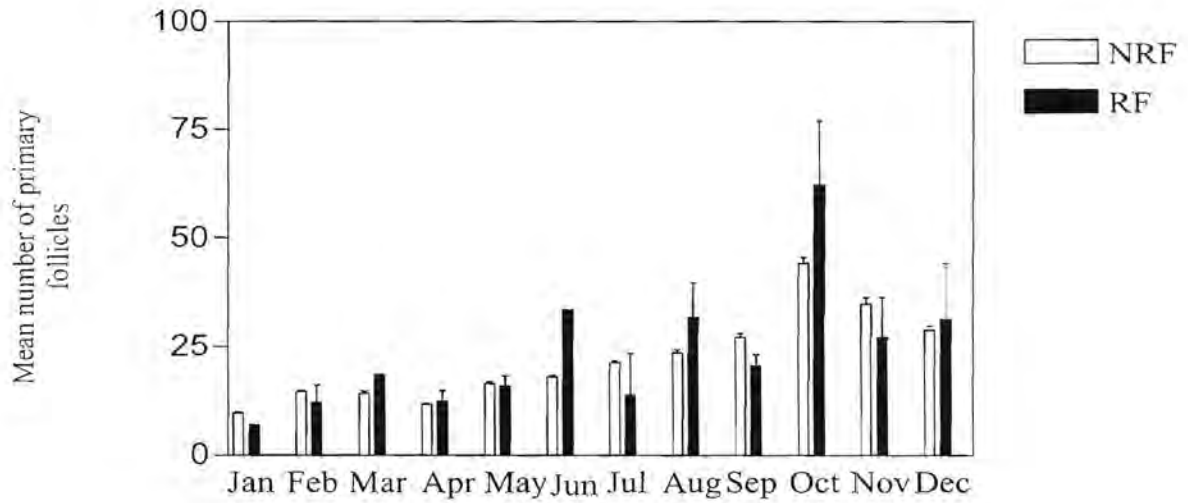

Fig. 5. The mean ± S.E of primary follicles in the reproductive (RF) and non-reproductive female (NRF) ovaries.

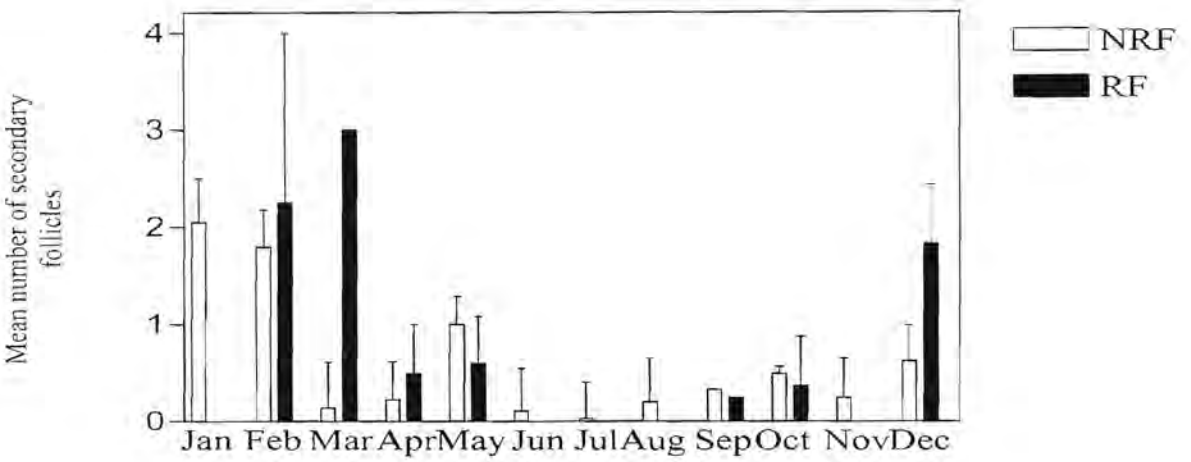

Fig. 6. The mean ± S.E. of secondary follicles in reproductive (RF) and non-reproductive female (NRF) ovaries.

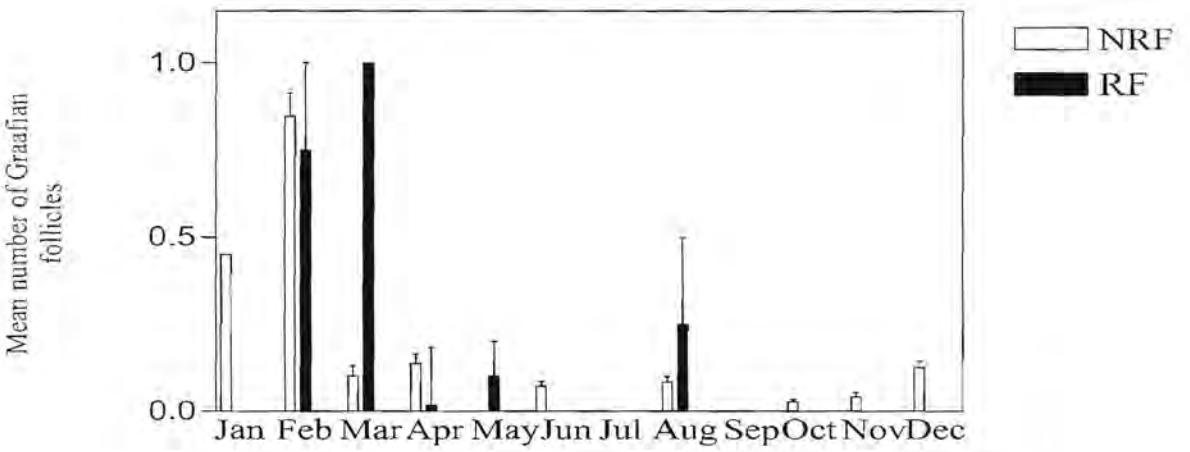

Fig. 7. The mean ± S.E. of Graafian follicles in the reproductive (RF) and non-reproductive female (NRF) ovaries.

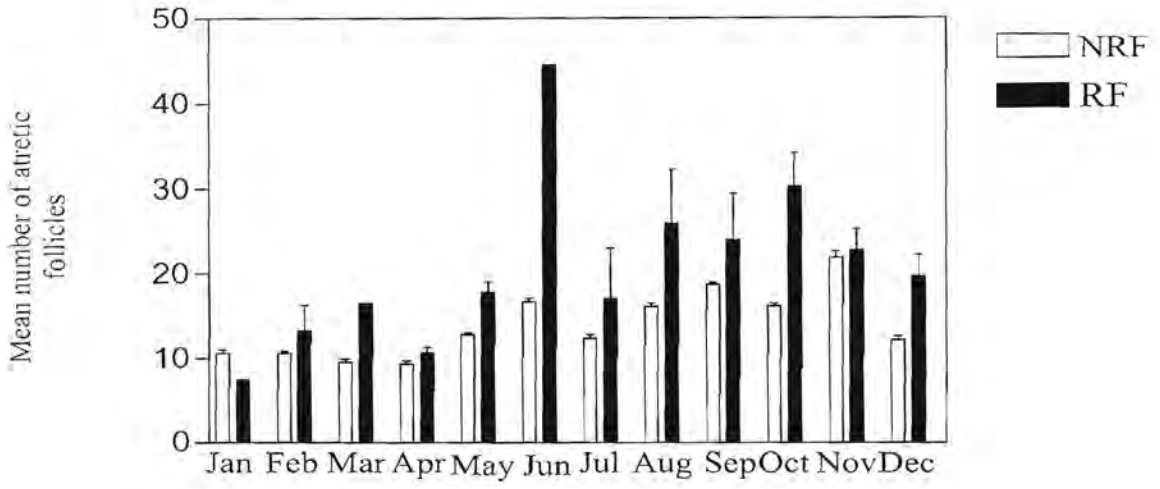

Fig. 8. The mean ± S.E. of atretic follicles in reproductive (RF) and non-rerproductive female (NRF) ovaries.

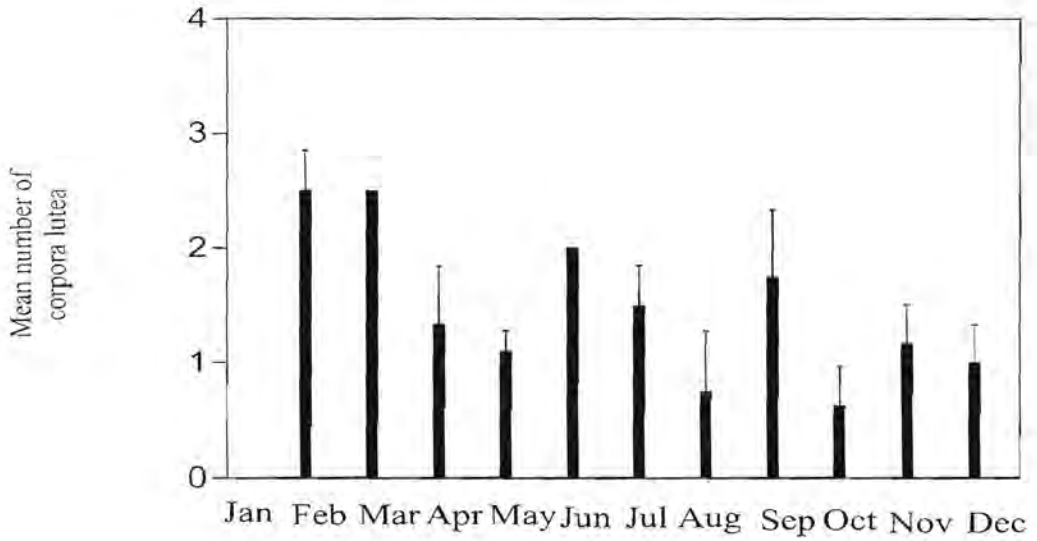

Fig. 9. The mean ± S.E. of corpora lutea counted in reproductive female (RF) ovaries.

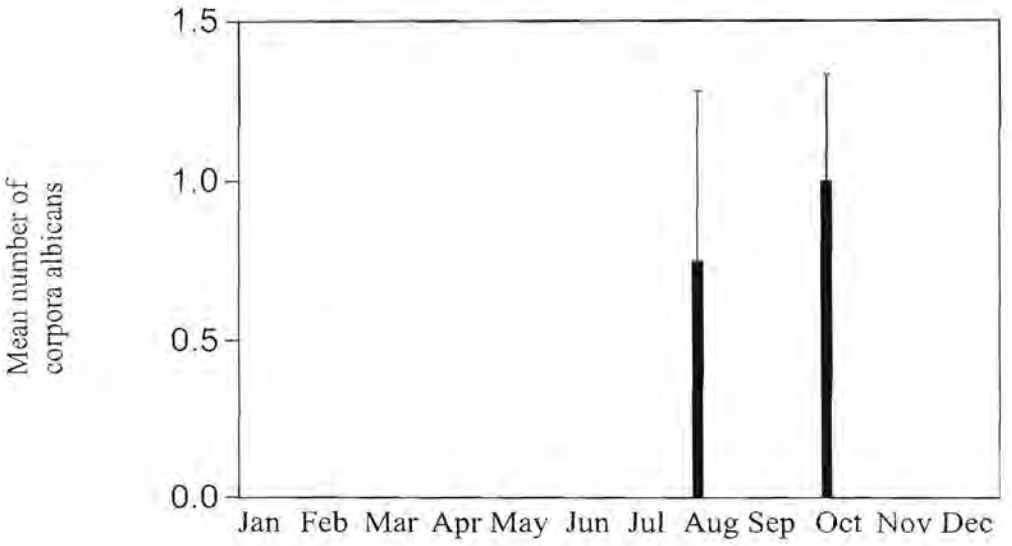

Fig. 10. The mean ± S.E. of corpora albicans in reproductive female ovaries.

Ovarian mass and volume

Mean ovarian mass in reproductive females exhibited a steady increase from April (0.06g ± 0.02, n = 3) to a peak value in July (0.14g ± 0.08, n = 2). A second, very subtle peak was observed in September (0.08g ± 0.01, n = 3) (Fig. 11). No ovarian mass and volume data were available for any females during February or any reproductive females during March (due to technical problems). The mean ovarian mass of non-reproductive females showed little fluctuation throughout the year. Reproductive females had a greater mean ovarian mass when compared to non-reproductive females (RF = 0.08g ± 0.01; NRF = 0.03g ± 0.00). (Mann Whitney U-test, U = 14.50, $p<0.05$, n(NRF) = 135, n(RF) = 26). The mean ovarian volume exhibited a trajectory of development similar to ovarian mass (NRF = 5.33mm³ ± 0.30, n = 154; RF = 18.61mm³ ± 3.38, n = 24) and reproductive females peaked in June (46.32mm³, n = 1) and July (44.73mm³ ± 28.85, n = 2) with the month of September (14.45mm³ ± 2.67, n = 3) showing a slightly greater volume than the month of August (13.95mm³ ± 8.97, n = 2) (Fig. 12).

Testicular histology

The mean seminiferous tubule diameter for reproductive males was significantly higher than that for the non-reproductive males (NRM = 149.28µm ± 4.71; RM = 183.32µm ± 4.02) (Mann Whitney U-test, U = 501.00, $p<0.01$, n(NRM) = 63, n(RM) = 39) (Fig. 13). Although highly significant differences occurred between reproductive and non-reproductive males, neither of the two male groups exhibited appreciable differences, between months within each of the groups (Fig. 13).

Testicular mass and volume

The mean testicular mass of the reproductive males increased albeit steadily from January (0.09g ± 0.05, n = 3) and reached a peak mass in July (0.34g ± 0.04, n = 2) (Fig. 14). A second peak in reproductive male testicular mass occurred in September (0.29g ± 0.02, n = 6), but then decreased towards December (0.16g ± 0.04 n = 4) (Fig. 14). The mean testicular mass of the reproductive males (0.21g ± 0.01) was significantly higher than the testicular mass of the non-reproductive males (0.10g ± 0.01) (Mann Whitney U-test, U= 10.00, $p<0.05$, n(NRM) = 51, n(RM) = 39).

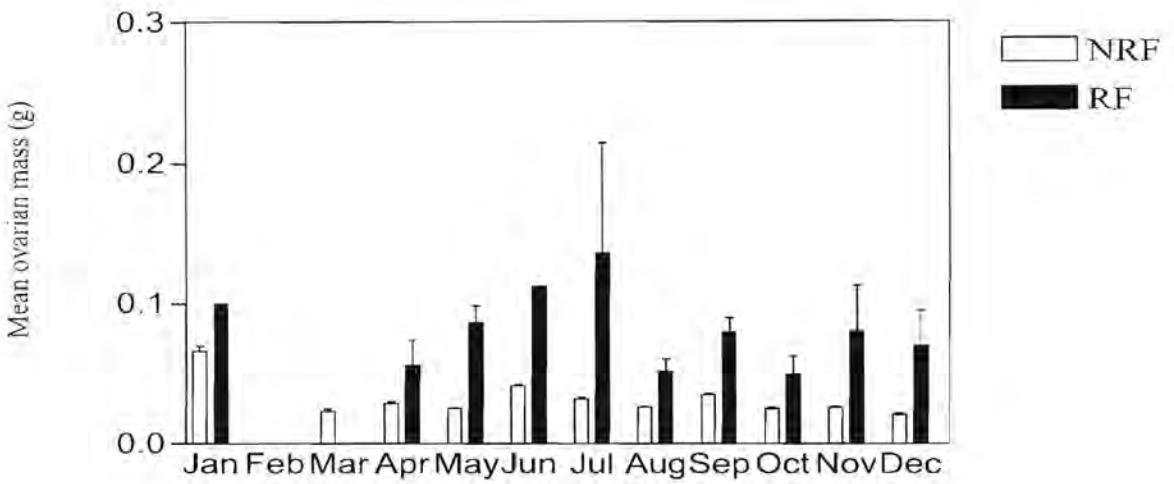

Fig. 11. The mean ± S.E ovarian mass for reproductive (RF) and non-reproductive females (NRF).

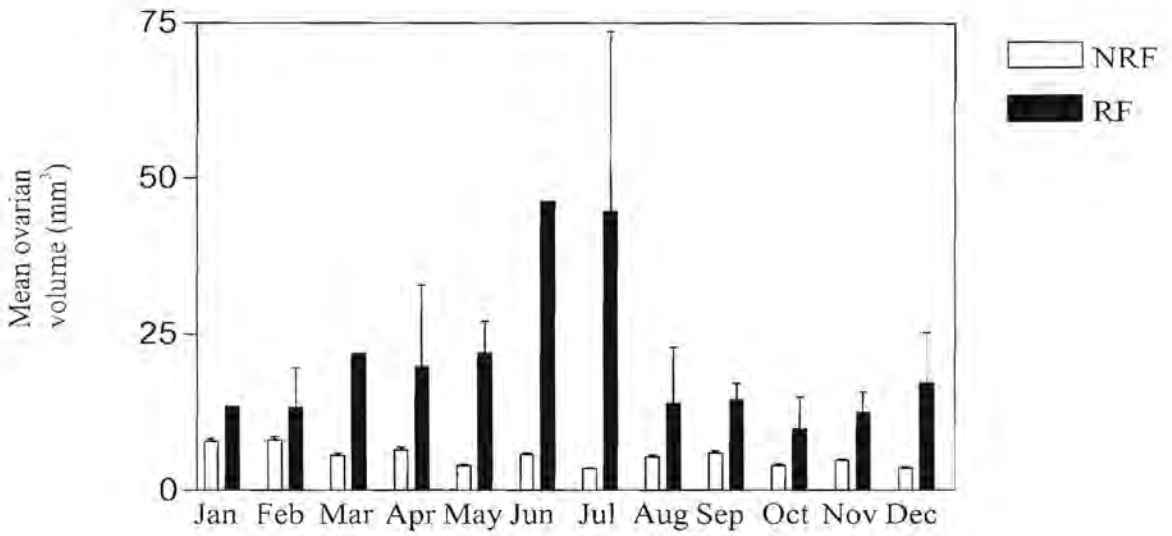

Fig. 12. The mean ± S.E ovarian volume for reproductive (RF) and non-reproductive females (NRF).

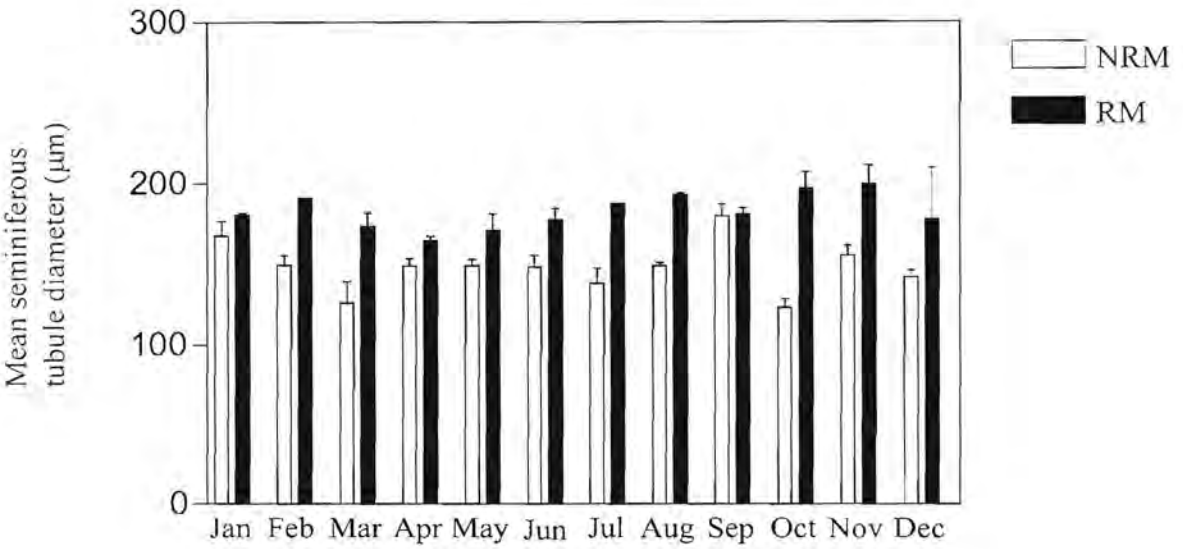

Fig. 13. The mean ± S.E. seminiferous tubule dimater for reproductive (RM) and non-reproductive males (NRM).

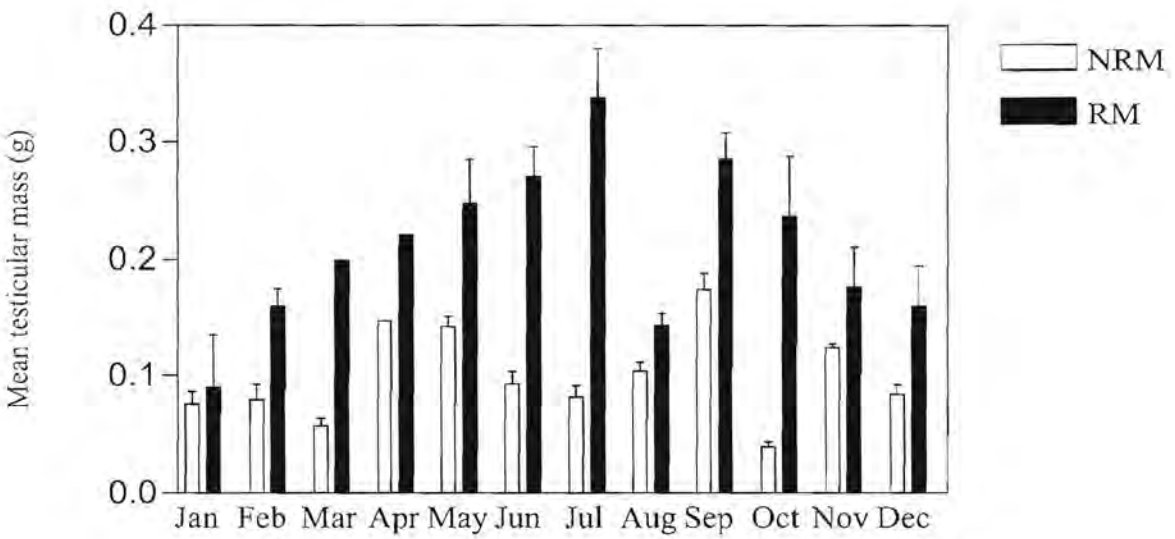

Fig. 14. The mean ± S.E. testicular mass for reproductive (RM) and non-reproductive males (NRM).

The testicular volume showed the same trend (Fig. 15). The first peak in testicular volume of the reproductive males (101.40mm^3 ± 16.17, n = 2) coincided with the first peak in ovarian mass for reproductive females in July (Fig. 11). In contrast to the testicular mass, the second peak in testicular volume occurred in October (99.48mm^3 ± 35.35, n =3) and not in September (Fig. 15).

Sperm motility

In table 1 the various sperm motility parameters were recorded for both reproductive and non-reproductive males. Without exception all the parameters showed no significant difference between reproductive and non-reproductive males. In addition, comparing the mass and age of the animals, as well as the number of sperm observed for both reproductive and non-reproductive males as co-variates in a Generalised linear model (GLM) only a significant difference could be observed for the beat cross frequency (BCF) variable between the masses of reproductive and non-reproductive males (GLM, $F = 4.65$, $p<0.05$) (Table 2). The remaining variables revealed no statistical significant differences. No significant differences between the kinematic parameters between reproductive and non-reproductive males were observed for either age or the number of sperm observed.

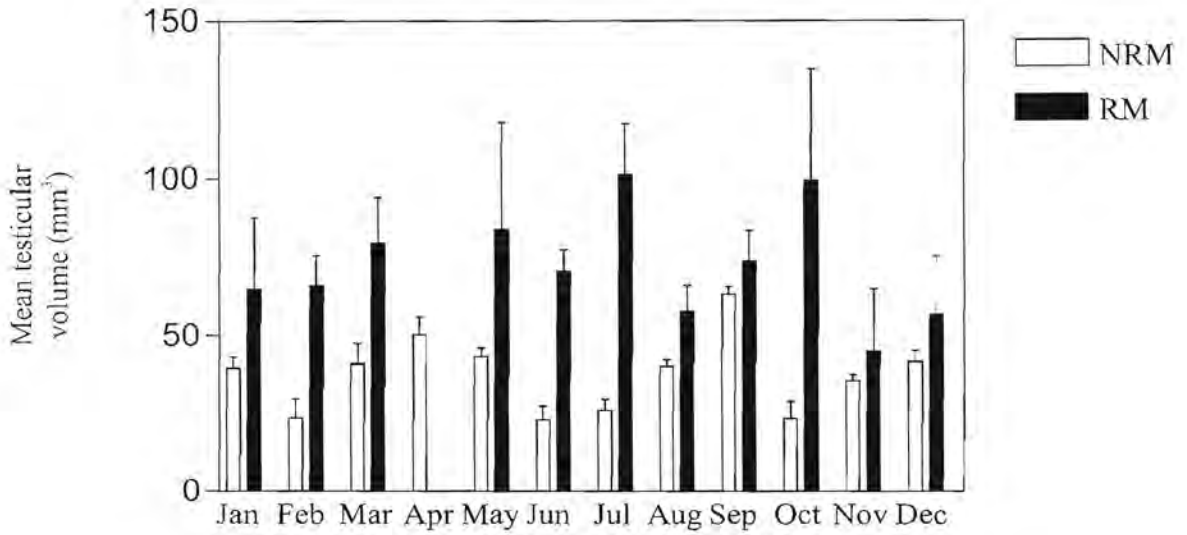

Fig. 15. The mean ± S.E. testicular volume for reproductive (RM) and non-reproductive males (NRM).

Table 1 Comparative sperm motility characteristics for highveld mole-rats of differing reproductive status. RM = Reproductive males (n=14), NRM = Non-reproductive males (n=17). The mean and standard error are indicated.

Variable	RM	NRM	F value	*p*
VCL	108.50 ± 5.05	107.76 ± 5.40	0.29	0.60
VSL	81.21 ± 3.91	82.19 ± 3.66	0.55	0.46
LIN	73.72 ± 2.17	75.72 ± 2.76	0.16	0.70
Mean ALH	1.44 ± 0.14	1.36 ± 0.21	4.22	7.16
Maximum ALH	3.92 ± 0.28	3.86 ± 0.45	1.01	0.32
BCF	27.32 ± 1.65	23.94 ± 1.94	1.43	0.24
DNC	368.08 ± 56.83	415.87 ± 142.72	4.22	4.91
DNC mean	11.96 ± 2.01	10.37 ± 2.52	0.32	0.58
VAP	91.68 ± 3.90	91.55 ± 3.55	0.96	0.34
WOB	0.85 ± 0.01	0.86 ± 0.02	0.38	0.54
STR	0.86 ± 0.02	0.87 ± 0.01	0.00	0.96
RAD	1.54 ± 0.09	1.50 ± 0.09	1.24	0.28
CURV	0.39 ± 0.02	0.34 ± 0.03	3.50	0.07

VCL = curvilinear velocity; VSL = straight line velocity; LIN = percentage linearity; ALH = amplitude of lateral head displacement; BCF = beat cross frequency; DNC = dance; VAP = average path velocity; WOB = Wobble; STR = straightness; RAD = Radian; CURV = curvature.

Table 2 Comparing reproductive (n=14) and non-reproductive males (n=17) with regard to mass, age and number of sperm observed (Count), to determine statistical differences. Generalised Linear Model ($p<0.05$).

	Mass		**Age**		**Count**	
Variable	F value	*p*	F value	*p*	F value	*p*
VCL	0.49	0.49	0.08	0.77	1.06	0.31
VSL	1.83	0.19	0.42	0.52	0.11	0.75
LIN	0.75	0.39	1.78	0.19	1.15	0.29
Mean ALH	0.07	0.79	0.05	0.82	0.08	0.79
Maximum ALH	0.16	0.69	0.04	0.84	0.20	0.66
BCF	4.65	0.04	1.63	0.21	1.48	0.23
DNC	0.10	0.75	0.40	0.53	0.28	0.60
DNC mean	0.02	0.88	0.18	0.68	0.45	0.51
VAP	2.65	0.12	0.18	0.68	0.46	0.50
WOB	1.42	0.24	1.52	0.23	0.82	0.37
STR	0.00	0.96	1.62	0.21	1.02	0.32
RAD	0.59	0.45	0.11	0.75	0.17	0.68
CURV	0.58	0.45	0.02	0.90	0.91	0.35

(Abbreviations - See table 1)

Hormones

The mean circulating progesterone concentrations measured in the reproductive females were significantly higher than in the non-reproductive females (NRF = 2.17nmol.l^{-1} ± 0.30; RF = 60.78 nmol.l^{-1} ± 16.30) (Mann Whitney U-test, U = 391.00, $p<0.01$, n(NRF) = 154, n(RF) = 29). Within the first three months of 1998 (January to March) the progesterone concentrations of the reproductive females were very low (5.51 nmol.l^{-1} ± 1.83, n = 4). These levels, however, increased and were much higher from April through to December (69.62nmol.l^{-1} ± 18.35, n = 25) (Fig. 16). Two peaks can be observed: the first peak in April (55.50nmol.l^{-1} ± 51.03, n = 3) and a second peak in September (163.41nmol.l^{-1} ± 39.03, n = 3). Within the months of May through to November a significant difference was found between reproductive females and non-reproductive females within months (Kruskal Wallis test, H = 78.58, $p<0.001$; Dunn's multiple range test, $p<0.05$).

Oestradiol is a steroid hormone, secreted principally by the ovarian follicles and also by the corpora lutea, placenta and adrenals in the female. No clear trend was observed for the oestradiol concentrations (NRF = 0.33nmol.l^{-1} ± 0.08, n = 140; RF = 23.52nmol.l^{-1} ± 11.13, n = 28) (Fig. 17). The oestradiol concentrations for January through to March were very low for both the reproductive and non-reproductive females (NRF = 0.32nmol.l^{-1} ± 0.02, n = 23; RF = 1.06nmol.l^{-1} ± 0.63, n = 4). In April (64.76 nmol.l^{-1} ± 64.74, n = 3) and May (75.13nmol.l^{-1} ± 45.29, n = 5) very high oestradiol concentrations were observed for the reproductive females. Reproductive females obtained in the remaining months (June to December) had oestradiol concentrations which never exceeded 25 nmol.l^{-1} (NRF = 0.36nmol.l^{-1} ± 0.11, n = 93; RF = 5.28 nmol.l^{-1} ± 2.70, n = 16). In May, August, September and November significant differences were found between the reproductive and non-reproductive females (Kruskal Wallis test, H = 66.29, $p<0.001$; Dunn's multiple range test, $p<0.05$).

The testosterone concentrations of the reproductive males always exceeded that of the non-reproductive males in each respective month (NRM = 1.21nmol.l^{-1} ± 1.46; RM = 15.27 nmol.l^{-1} ± 1.87) (Mann-Whitney U-test, U = 635.00, $p<0.001$, NRM = 55 RM = 42). In both the reproductive and non-reproductive males the highest testosterone concentrations were recorded in June (NRM = 16.10nmol.l^{-1} ± 8.09, n = 4; RM = 28.90

nmol.l^{-1} ± 9.49, n = 5) and August (NRM = 18.18nmol.l^{-1} ± 1.86, n = 3; RM = 24.10nmol.l^{-1} ± 10.92, n = 7) (Fig. 18). Analysis of the all months sampled, revealed a highly significant difference between reproductive males and non-reproductive males in October (NRM = 1.49nmol.l^{-1} ± 1.23, n = 3; RM = 12.77nmol.l^{-1} ± 1.73, n = 3) (Kruskal Wallis test, H = 44.11, $p<0.01$; Dunn's multiple range test, $p<0.05$, NRM = 3, RM = 3). The testosterone concentrations for both reproductive and non-reproductive males show two distinct peaks, the first in June and a second in August. These peaks coincide with the progesterone peaks of the females (Fig. 16).

Rainfall

Rainfall data for the various regions in which trapping was undertaken were obtained from the South African Weather Bureau (Fig. 19). Single animals were captured only during months in which rainfall occurred (Fig. 20).

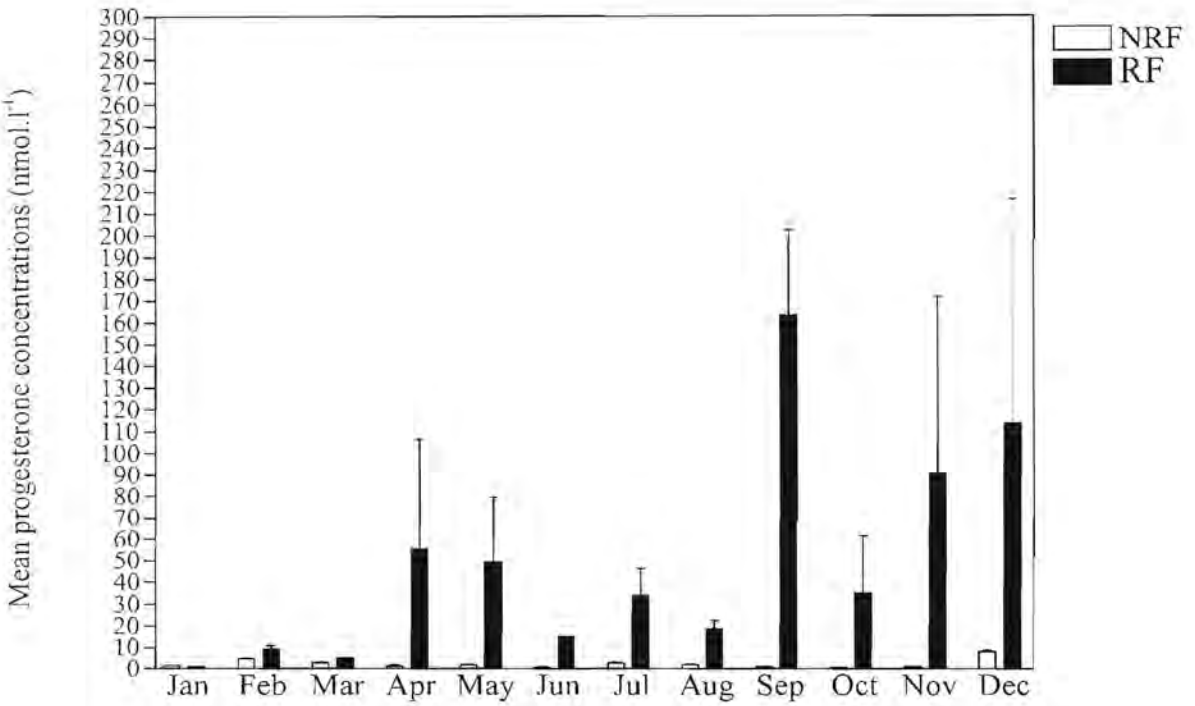

Fig. 16. The mean ± S.E. progesterone concentrations for reproductive (RF) and non-reproductive females (NRF).

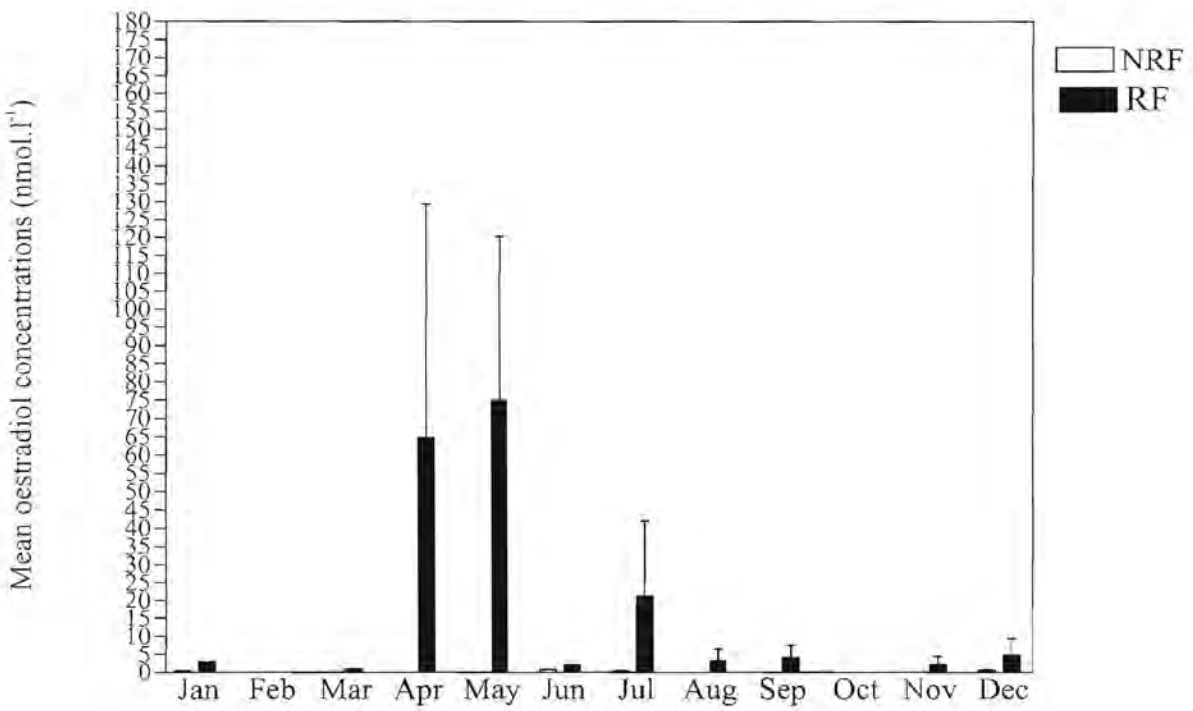

Fig. 17. The mean ± S.E. oestradiol concentrations for reproductive (RF) and non-reproductive females (NRF).

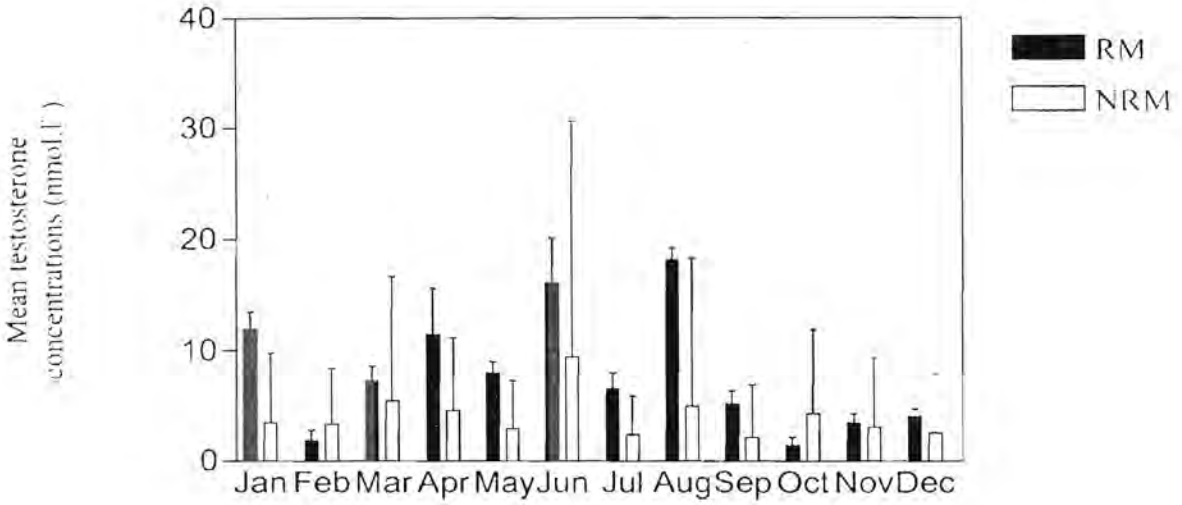

Fig. 18. The mean ± S.E testosterone concentrations for reproductive (RF) and non-reproductive males (NRM).

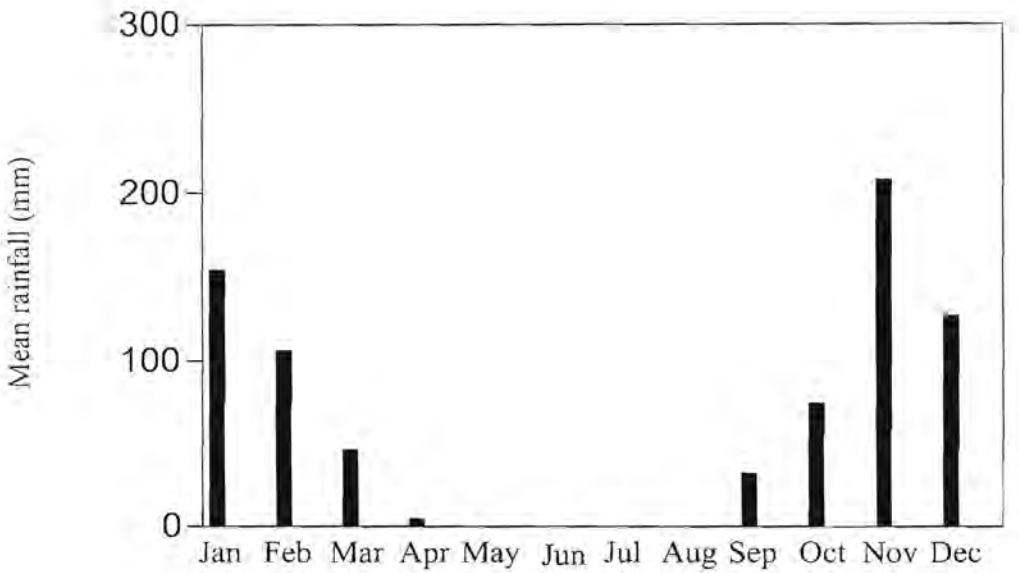

Fig. 19. The mean rainfall (mm) for each month during 1998 (South African Weather Bureau).

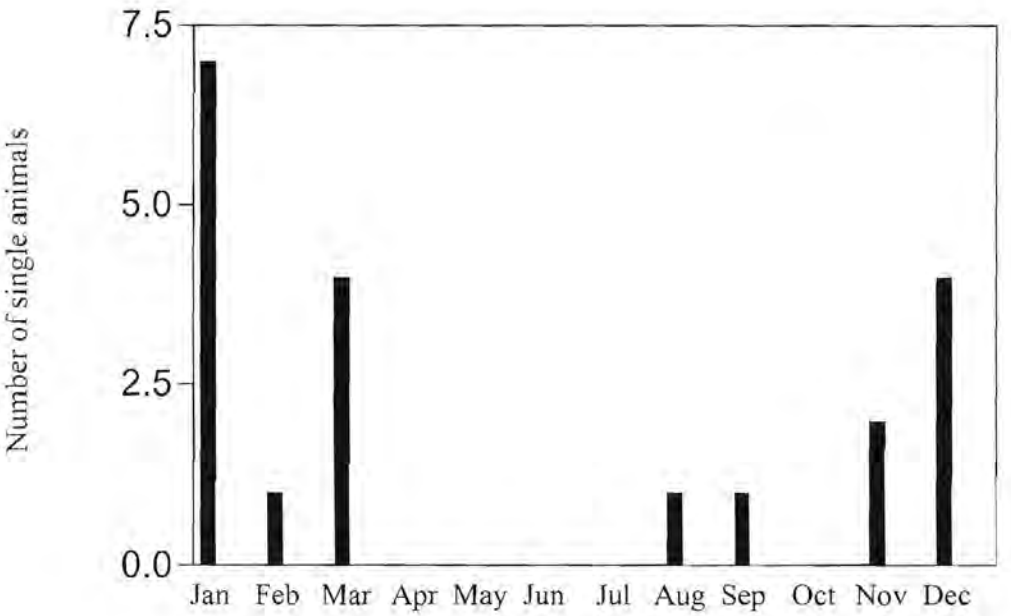

Fig. 20. The number of single animals sampled during 1998.

DISCUSSION

It is very likely that reproduction has been subjected to more evolutionary pressures than any other physiological system (Weir 1974). As a consequence a wide range of reproductive strategies are displayed in the animal kingdom. The family Bathyergidae is one such diverse group that incorporates solitary species reproducing seasonally to co-operatively breeding individuals that predominantly reproduce aseasonally.

Seasonal breeding represents an adaptation of animals to ensure maximum survival of their young (Louw 1993). Offspring are born at a time when environmental conditions are most favourable for growth and survival (Hickman *et al.* 1993).

Within the bathyergids, seasonal reproduction is usually confined to solitary species, such as *Georychus capensis* and *Bathyergus suillus* occurring in the mesic habitats of the Cape (Taylor *et al.* 1985; Bennett *et al.* 1991). The social species *C. damarensis* and *H. glaber*, exhibit a reproductive division of labour as well as distinct working groups within the confines of the colony. These mole-rats inhabit the arid regions of eastern and southern Africa (Faulkes *et al.* 1991; Jacobs *et al.* 1991) and thus, living in social groups enable these mole-rats to withstand very harsh environmental conditions and still maintain a high rate of reproductive success (Lovegrove 1988).

Within the hierarchical groups of co-operatively breeding mammals, reproductive suppression is a common phenomenon. Dominant individuals inhibit sexual activity in non-reproductive animals through behavioural or physiological suppression (Abbott 1987; Abbott *et al.* 1988). Such reproductive inhibition occurs in the naked mole-rats, *H. glaber* (Faulkes *et al.* 1990; 1991; Abbott 1984), wild dogs, *Lycaon pictus* (Malcolm & Marten 1982; Creel *et al.* 1997) and the dwarf mongooses, *Helogale parvula* (Rood 1980; Creel *et al.* 1992).

Within a mole-rat colony only one female and usually one or potentially two males are responsible for the procreation of new colony members (Bennett & Jarvis 1988; Faulkes *et al.* 1990; 1991; Jarvis & Bennett 1990; 1991; Bennett *et al.* 1997). Reproductive animals suppress the non-reproductive animals from reproducing (Faulkes *et al.* 1990; 1991; 1994; Bennett *et al.* 1993; 1994b; 1997; Spinks *et al.* 1997). Bennett *et al.* (1997) suggest that there is variation in the mechanisms of reproductive modulation

which can be correlated with environmental factors. The arid species (e.g. *H. glaber*) enforces physiological suppression, exhibiting extreme socially induced infertility where both non-reproductive males and females are physiologically suppressed from reproducing (Faulkes *et al.* 1990; 1991). Similarly *C. damarensis* exhibits physiological suppression as well as behavioural inhibition in the non-reproductive females with behavioural inhibition present in non-reproductive males (Bennett *et al.* 1994a; Bennett *et al.* 1994b; 1996). The mesic species (e.g. *Cryptomys darlingi*) practices behavioural suppression, in which incest avoidance is solely responsible for the maintenance of reproductive skew.

Mesic environments have frequent and predictable periods of rainfall and thus cater for frequent dispersal opportunities. Colonies that occur in these areas are very small, for example the Mashona mole-rat with a colony size of up to nine individuals (Bennett *et al.* 1997) and the common mole-rat colonies ranging from two to fourteen individuals (Spinks *et al.* 1997). Within these colonies it is of the utmost importance that the method used for reproductive suppression be quickly discarded when the opportunity for dispersal arises and the animals have the opportunity to procreate.

Until recently only the common mole-rat, *C. h. hottentotus* had been identified as being a social mole-rat, displaying seasonal reproduction (Bennett *et al.* 1991; Jarvis & Bennett 1991; Spinks *et al.* 1997). Spinks *et al.* (1997) suggested that *C. h. hottentotus* displays a cyclical reproduction because of invading a seasonal habitat. The common mole-rat occurs in the winter rainfall regions of the western and northern Cape Province. Long-term mark and recapture studies, revealed that the common mole-rat occurs in colonies of up to fourteen individuals and produces a maximum of two litters per annum (late November through to January) (Jarvis & Bennett 1991; Spinks 1998). This pattern of reproductive periodicity is typical of mammals, both surface dwelling and subterranean, that occur in seasonal habitats (Page *et al.* 1994; Mills *et al.* 1992; Kaplan & Mead 1994).

The invasion of *C. h hottentotus* into a seasonal, mesic habitat, which is predominantly inhabited by solitary mole-rats may be explained as a survival strategy that decreases or eliminates competition with other social mole-rat species and therefore increases its own survival and reproductive success.

The highveld mole-rat is phylogenetically closely related to the common mole-rat (Faulkes *et al.* 1997) and likewise, inhabits a seasonal habitat. According to Bennett *et al.* (1999) and Jarvis & Bennett (1991) the change in temperature and rainfall due to seasonality are important determinants of seasonal breeding in the solitary bathyergids. It would seem that these same environmental cues are responsible for seasonal reproduction in the common mole-rat that produce young during the southern hemisphere summer when the soil is workable and the food resource is readily harvested. I suggest that the highveld mole-rat uses the onset of the first rains during spring as a cue for dispersal and mating and then produce young during the southern hemisphere winter months (May to July).

Ovarian histology

The presence of primordial, primary and secondary follicles serve as an indicator of follicular development. According to Bloom & Fawcett (1962) primordial follicles are more abundant within the ovaries of non-reproductive females. This trend was consistent throughout the sampling period. The presence of primary and secondary follicles in both reproductive and non-reproductive females suggest that the ovaries undergo normal follicular development, supporting the assumption that non-reproductive females are not sterile but only reproductively quiescent in that ovulation does not occur. According to Weir (1974) and Bloom & Fawcett (1962) most follicles in both reproductive and non-reproductive ovaries do not survive maturation nor ovulation and degenerate to become atretic follicles and is a feature common to all mammalian ovaries (Mossman & Duke 1973). My study on the highveld mole-rat revealed that reproductive females possessed a higher follicular development, which leads to increased numbers of atretic follicles present in the ovaries. Similarly, non-reproductive females displayed high numbers of atretic follicles, which again supports the assumption made that the non-reproductive females have functional and active ovaries.

According to Clarke (1981) and Gorman & Stone (1990), seasonally breeding animals exhibit a regression of follicular development during the non-breeding time, thus no secondary or Graafian follicles are present in the ovaries. However, Spinks (1998) found that *C. h. hottentotus* shows follicular development during the non-breeding period

and thus suggested that reproductive function does not regress. The same trend is encountered in the highveld mole-rat. The predictability of the rainfall is an important determinant of reproduction, consequently a constant state of reproductive readiness would be advantageous, since the first rains might be advanced or delayed relative to the norm.

The high number of Graafian follicles in the ovaries of non-reproductive females out of breeding season may indicate a readiness in reproductive physiology for the anticipation of dispersal during the summer, with the onset of the first rains. Only non-reproductive females possessed Graafian follicles during June, October, November and December (breeding season). Most non-reproductive females were perforate, which would suggest that the non-reproductive females are not sterile but only inhibited from reproducing. Shanas *et al.* (1997) found a similar trend in the blind mole-rat, *Spalax ehrenbergi*. Although a seasonal breeder, the ovaries of the blind mole-rat females kept in a laboratory were found to be active out of the breeding season.

The reproductive females of the highveld mole-rat had a larger number of Graafian follicles at the beginning of the breeding season. Using the presence of corpora lutea as and indication of ovulation and pregnancy, the breeding season can be delineated as occurring from April until December. If one assumes that the gestation period is comparable to the sister taxon *C. h. hottentotus* of approximately 60 days, then it is feasible for the highveld mole-rat to produce two litters per annum.

Ovarian mass and volume

According to Weir & Rowlands (1974) the most striking feature of hystricomorph ovaries is the development of large amounts of luteal tissues and the presence of large corpora lutea. Weir & Rowlands (1974) suggested that the luteal tissues are developed to extend the gestation period or as in the chinchilla, it may serve as an extra source of progesterone.

Data obtained on the highveld mole-rat females show that the corpora lutea fill the ovary of a reproductive female during pregnancy. This gives the reproductive female ovary a granular-like surface appearance, unlike the smooth surface of the non-reproductive female ovaries. Besides the granular surface appearance of the ovaries of

reproductive females, they also exhibit very thick uteri as well as placental scars or foetuses. In contrast, the non-reproductive female ovaries were small and smooth. The uteri of the non-reproductive females are very thin and have a flaccid appearance.

In seasonally breeding mammals, temporal changes in the ovarian and uterine dimensions occur. An increase in ovarian size, mass and volume of reproductive females during the breeding season has been found in the European rabbits, *Oryctolagus cuniculus*, corn mice, *Calomys musculinus* and the red giant flying squirrels, *Petaurista petaurista* (Boyd & Myhill 1987; Mills *et al.* 1992; Lee *et al.* 1993). Spinks (1998) found a similar trend in the common mole-rat, *C. h. hottentotus*, where reproductive females showed an increase in ovarian mass and volume during the breeding season. The higher level of follicular development in the reproductive females correlated to a higher ovarian mass.

The present study shows that at the peak of the breeding season in the highveld mole-rat a large number of foetuses are present and the ovaries attain the greatest mass, due to the presence of large corpora lutea filling the stroma. Closer examination of the data reveals two subtle peaks in the mean number of corpora lutea present, occurring during June and September, which suggests that the highveld mole-rat may have the potential to produce two litters within a breeding season. The ovarian volume verifies the results of the ovarian mass, showing a higher ovarian volume in June, however, a second peak cannot be readily discerned.

Female hormones

Peaks in plasma oestradiol 17β concentrations occurred in April and May, indicative of enhanced follicular development at the beginning of the breeding season. Oestradiol concentrations for the reproductive females drastically decreased for the remainder of the breeding months. Whereas, progesterone concentrations, showed an increase within these months indicating ovulation or pregnancy. During pregnancy a lack of follicular development was observed in the highveld mole-rat, unlike that observed in the Cape porcupine (*Hystrix africaeaustralis*), where the number of large follicles increased with an extension of the gestation period (Van Aarde & Skinner 1986) and in the plains viscacha (*Lagostomus maximus*), where follicles develop throughout pregnancy to

ovulatory size (Weir 1971). Within the highveld mole-rat progesterone reached its highest concentrations in the breeding season (April to December), whereas very low concentrations were recorded during the non-breeding period (January to March). Two peaks are present in the progesterone data for reproductive females, the first in April and the second in September. The progesterone profiles in conjunction with the ovarian histological data suggest the highveld mole-rat being a seasonal breeder.

Testicular histology

Assuming the breeding season occurs from the beginning of April and ends in December and that the non-breeding season lasting for approximately 3 months (January – March), it would be advantageous to keep the testes functional and the seminiferous tubule diameter at a constant size. This would facilitate an increase in sperm production to enable the males to be reproductively functional for the breeding season (Spinks 1998). The present study reveals that although the seminiferous tubule diameter of the reproductive males are significantly larger than that of the non-reproductive males, they both maintained the seminiferous tubule diameter at constant levels throughout the year. During the breeding season the reproductive males exhibit two very subtle peaks in seminiferous tubule diameter which coincides with the months of the two litters of the reproductive females (June and September). Spinks (1998) found a similar trend in the common mole-rat males. There was no regression in spermatogenesis and a lack of any seasonal periodicity in male reproduction. The maintenance of reproductive activity is uncommon amongst seasonally breeding animals. However, male mole-rats need a ready supply of sperm throughout the year to seize any opportunity for reproduction that might arise during its lifetime.

Testicular mass and volume

Reproductive males show a gradual increase in testicular mass and testicular volume towards and during the breeding season, which reaches a peak within the month of July and also coincides with the peak reproductive month for the females. A second peak in testicular mass was observed in September, again this coincides with the second peak found in the progesterone concentrations for the reproductive females as well as the

number of corpora lutea. In turn, the testicular volume shows a very subtle peak during the month of October.

Sperm motility

Sperm motility is essential for the process of normal fertilisation (Katz *et al.* 1989). This demands the successful migration of the sperm to the ovum and then penetrating the cumulus oophorus and zona pellucida of the egg (Green 1988; Katz *et al.* 1989).

During the study no statistically significant differences were found between the sperm motility parameters of reproductive (n = 14) and non-reproductive males (n = 17). Neither mass, age nor the number of sperm had an influence on the sperm motility of reproductive and non-reproductive males. A similar trend was observed in the common mole-rat and the Damaraland mole-rat, where both reproductive and non-reproductive males exhibited no significant differences in their sperm motility (Spinks 1998; Faulkes *et al.* 1994). Thus, no apparent suppression of sperm motility exists in the non-reproductive males (Faulkes *et al.* 1994).

Due to the loosely social structure of the colonies, it may be possible for males to be in constant competition for the rights to breed with the reproductive female. Therefore by keeping the testes functional and sperm motile, males have the possibility of attaining reproductive rights. The frequent dispersal of the non-reproductive males would also favour males with highly motile sperm, ensuring the procreation of the species.

Male hormones

Reproductive males being the most dominant in the colony (Moolman *et al.* 1998) exhibited the highest testosterone concentrations throughout the entire sampling period of the study. However, no significant differences were found between the testosterone concentrations of reproductive and non-reproductive males, with the exception of October. In contrast to the male naked mole-rat, *H. glaber*, there are no physiological differences nor any distinct hormonal differences (Bennett *et al.* 1994b), since all males undergo spermatogenesis and exhibit similar testosterone levels. On closer examination, both reproductive and non-reproductive highveld mole-rat males displayed higher

testosterone concentrations a month prior to each of the two prominent reproductive months (July and September).

The approaching breeding season may result in increased aggression (due to high testosterone concentrations and reproductive competition) between males as they compete for the exclusive right to breed with the reproductive female or newly acquired females. The increase in testosterone occurs twice, just before each of the reproductive peaks found in the reproductive females. Presuming higher aggression between all the sexually mature males in a colony, with an increase in testosterone approaching the breeding season, I suggest that competition occurs twice between males for the right to be the breeding male. Further, I suggest that this might be because of no distinct dominance hierarchy found within the highveld mole-rat colonies (Moolman *et al.* 1998), which might lead to continuous competition for being the breeding male.

The importance of rainfall

The highveld mole-rat occurs in the summer rainfall regions in the highveld of South Africa. The rainfall data obtained from the South African Weather Bureau indicates that the months during which single animals were caught coincides with the months in which rainfall occurred. Thus, it is possible that these animals disperse towards the last months of the breeding season (September/October), right through to the beginning of the next breeding season (April), using rainfall as a cue for dispersal. Dispersing during the wet period, when the soil is workable, will optimise the distance over which these animals will be able to dig and minimise the energetic costs associated with burrowing (Jarvis & Bennett 1991). Food is readily available during the wet period and is not seen as a limiting factor.

Thus, in conclusion I suggest that the highveld mole-rat is a seasonal breeder, with a proposed breeding period lasting from April up to the end of December, with no reproductive activity during January, February or March. In addition a proposal is made that two litters are born during the breeding season in May/July and September.

Dispersal occurs with the onset of the first rains in September/October and continues up to the beginning of the breeding season in April/May promoting the establishment of new colonies.

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