The biology of reproduction of the Tete veld rat, *Aethomys ineptus* and the Namaqua rock mouse, *Aethomys namaquensis* (Rodentia: Muridae)

By

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Dedicated to my wife and to our precious children for their unconditional love, support, and encouragement.

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CHAPTER 1

General Introduction

Background to the genus *Aethomys*

The Genus *Aethomys* has a wide distribution in East, Central, and southern Africa, and extends marginally into West Africa (Musser & Carleton 1993, Linzey *et al.* 2003). These species are generalists in their habitat preferences and are found in savanna and grassland habitats (De Graaff 1981; Skinner and Smithers 1990; Linzey & Kesner 1997). While species such the Red veld rat, *Aethomys chrysophilus* prefer thick grass, hollow tree trunks and logs, crevices, and termitaria as forms of cover, other species such as the Namaqua rock mouse, *A. namaquensis* prefer rocky habitats (De Graaff 1981; Skinner and Smithers 1990). Some species are commensal with man, especially in agriculturally well developed areas where they destroy crops and stored grain (Wilson 1970, 1975; Smithers 1971; De Graaff 1981).

Species within the genus *Aethomys* may be nocturnal, terrestrial, arboreal, gregarious, or may occur in small family units (Skinner & Smithers 1990). Preferred food items include seeds, fruits, grain and in some instances, insects (Watson 1987; Woodall and Mackie 1987). Information pertaining to the reproductive patterns within the genus intimate that some species procreate throughout the year whereas other species are reproductively quiescent during the cooler winter months (Smither 1971; Skinner & Smithers 1990).

Although the genus has historically undergone a number of nomenclatural changes, it is currently subdivided into two subgenera, namely: *Micaelamys* which

comprised of *A. namaqensis* and *A. granti* and the nominate subgenus *Aethomys* which includes *A. bocagei, A. chrysophilus, A. hindei, A. ineptus, A. kaiseri, A. nyikae, A. silindensis, A. stannarius and A. thomasi* (Davis 1975; Chimimba 1997; Russo 2003). However, the systematic status of the genus from the subgenus to the infraspecific levels, is largely uncertain because of the presence of morphologically similar and yet cytogenetically diverse taxa.

In southern Africa for example, studies have shown that "*A. chrysophilus* (*sensu lato*)" includes two distinct species that have been designated the Tete veld rat, *A. ineptus* and the nominate Red veld rat, *A. chrysophilus*. Although these two species are morphologically very similar, they are cytogenetically distinct (Gordon & Rautenbach 1980; Visser & Robinson 1986), and also differ in their protein electrophoretic patterns, gross sperm and bacular morphology as well as in subtle cranio-metric characteristics (Gordon & Watson 1986; Breed *et al.* 1988; Chimimba *et al.* 1999).

The aim of study

While the current focus on small mammal systematics is mainly directed at differentiating species, particularly sibling species, there is a critical need to incorporate other aspects of their biology, such as ecology, behavior, and reproductive biology. To this end, the main aim of this study was therefore to evaluate the reproductive biology of *A. ineptus* and *A. namaquensis* which represent members of two currently recognised subgenera, from two localities in Gauteng and Mpumalanga Provinces, South Africa, in an attempt to contribute towards the increasing body of knowledge on African small mammal biology. While little information on reproduction is available for *A.*

namaquensis, the present study represents the first reproductive study of *A. ineptus* ever since the species was formally recognised.

The objectives of study

The main objectives of this study are:

1) To determine the seasonality of reproduction in the Tete veld rat for wich there are no data from populations positively cofirmed to be *A. ineptus*.
2) To determine the seasonality of reproduction in the Namaqua rock mouse. Only little information on the reproductive biology is known from a population in Botswana (Smithers 1971)
3) To determine whether the Tete veld rat and the Namaqua rock mouse are photoperiodically responsive. This will shed light on whether these two species may potentially utilize photoperiod to activate reproduction.

Relevance of study

Some species within the genus *Aethomys* have been reported to undergo periodic population explosions, and this has led to some species being implicated in the epidemiology of plague, Rift Valley Fever, and Schistosomiasis, and in causing damage to agricultural products (Hallet *et al.* 1970; Smithers 1975a; Swanepoel *et al.* 1978; Gordon & Rautenbach 1980; De Graaf 1981). In addition, *A. namaquensis* has been reported to be a vector of several tick species such as *Ixodes rubicundus* and *Rhipicephalus puntatus* that cause paralysis in domestic animals (Fourie *et al.* 1992; Fourie *et al.* 1988a, 1988b). The species has also been reported to host microorganisms that cause tick bite fever in humans and Q-fever in both humans and animals (Howell *et*

al., 1978). Therefore, the present study may have important medical, veterinary, economic, and agricultural implications. Given that reproduction is central to these population eruptions, the fundamental understanding of reproduction in *A. ineptus* and *A. namaquensis*, may either directly or indirectly, assist agricultural, economic, human and animal health authorities in controlling these potentially problematic rodents.

Approach

The study of reproduction requires significant amounts of knowledge about factors, such as the onset of rainfall, availability of food, photoperiod, ambient temperature, and social cues, that may influence the breeding period of both small and large animals (Bronson 1989).

Reproduction is important for all living organisms, as it is the only way through which genes are passed on to future generations through which the survival of species may be sustained. In nature, animals display different reproductive strategies. In some, reproduction may be confined to one specific time of the year and in others, it may occur throughout the year (Prendergast *et al.* 2001). For example, Australian musky rat-kangaroos, *Hypsiprymnodon moschatus*, are seasonally breeding rodents that initiate breeding from March and cease reproducing in October (Dennis & Marsh 1997). The Deer mouse, *Peromyscus maniculatus,* is an aseasonal breeder and has been found to breed throughout the year in Kansas, Coastal South Carolina, Florida, eastern Washington and southern Mexico (Bronson 1985). These reproductive patterns are often a result of variations in environmental conditions such as suitable diet, physical, and social conditions (Bronson 1985). Thus, the majority of organisms may use environmental cues such as the onset of rainfall, photoperiod as well endogenous annual

rhythms, and social cues to initiate reproductive events such as gonadal growth, steroidogenesis, gametogenesis and mating to occur when environmental conditions are favourable for the survival and growth of offspring (Jameson 1988). Offspring are thus born during the time of the year when food is most plentiful and favorable socio-biological factors are in place (Ims 1990).

Environmental cues include variables such as rainfall, food availability, temperature, and photoperiod. In climates that experience a significantly dry season, such as many semi-arid areas in sub-Saharan Africa, the onset of rainfall stimulates growth of numerous annuals, forbes, herbs, trees, grass and fruits that form basic diets for many animals (Beatley 1969, Nilsson 2001). In general, animals may only reproduce when extra energy is available after essential energy requirements for processes such as cell maintenance, locomotion, metabolism, respiration, and thermoregulation have been met (McNab 1963; Millar 1977; Bronson 1984; Bronson & Marsteller 1985; Peters 1983). For example, deprivation of food in male deer mice, *Peromyscus maniculatus*, and in CF-1 female mice has been shown to depress the process of spermatogenesis and ovulation respectively (Blank & Desjardins 1984; Bronson & Marsteller 1985).

Homeothermic rodents need to maintain body temperature in order to survive. A decrease in body temperature increases the thermoregulatory requirements of an animal and may subsequently decrease the amount of energy that may be free for reproduction (Bronson 1985; Bronson & Pryor 1983; Sicard *et al.* 1993). Testicular development and spermatogenesis are enhanced in the desert pocket mouse, *Perognatus formosus*, at ambient temperature of 13-23°C but significantly depressed at 33°C (Kenagy & Bartholomew 1981). Elevated ambient temperature has also been shown to decrease

conception rates and embryonic survival in pigs (Hoagland & Wettemann 1984). Since temperature fluctuations are characteristic of many habitats, and as a result of adaptations to certain climates, animals have developed different temperature tolerances within which reproductive success may be achieved.

Many mammals make use of changes in day length to time reproductive events. Many rodents cease to breed when day length falls below some critical minimum during late summer and early autumn (Prendergast *et al.* 2001). For example, testicular function in the desert pocket mouse, *Perognathus formosus,* was stimulated when subjected to a 16L:8D photoperiod and was inhibited when exposed to 8L:16D day length (Kenagy & Bartholomew 1981). Photoperiodism has also been shown to affect somatic and reproductive development. In photoperiodic rodents, offspring born in late summer have been found to have somatic and reproductive development delayed by 4-5 months before reaching maturity, whereas those born into increasing day length reach maturity within 40-50 days (Bronson 1985; Forger & Zucker 1985).

Social interactions between organisms also affect reproduction at different levels. It has been shown that male *Peromyscus aztecus*, housed together with female conspecifics showed significantly higher testicular, and epididymal masses as well as higher testicular sperm counts than those that were housed individually (Demas & Nelson 1998). Social cues in colonial animals are used to regulate reproduction whereby only certain individuals carry out the reproductive process (Bronson 1985). Certain male and female rodents live in isolated burrows. They monitor the reproductive status of others semiochemically by detecting pheromones within the urine that are used to mark the ground during foraging. These chemical cues denote the sex, and sexual status of the

species (Bronson 1985). Upon locating each other, behavioural responses in rodent conspecifics activate the secretion of luteinizing hormone (LH), gonadotrophin-releasing hormone (GnRH) and testosterone resulting in mating (Bronson 1989).

Among all these potentially influencial factors, the approach in the present study is to investigate factors that may influence reproduction in *A. ineptus* and *A. namaquensis* focusing on: 1) seasonality of reproduction based on ovarian and testicular histology, plasma progesterone, and oestradiol-17ß, and testosterone hormone concentrations, and the presence of embryos; 2) photoperiodic responsiveness; and 3) reproductive age with reference to body mass.

Thesis outline

The first (Chapter 2) and the second (Chapter 3) parts are aimed at determining the seasonality of reproduction in the Tete veld rat and the Namaqua rock mouse respectively, through a detailed monthly analysis of ovarian histology, plasma progesterone, oestradiol-17ß hormones and the presence of embryos in females. In males, testicular histology and plasma testosterone concentration were also assessed on monthly basis.

Chapter 4 examines the nature and extent of photoperiodic responsiveness to long and short days in the Tete veld rat and the Namaqua rock mouse, by subjecting the two species to two different lighting regimes. The final section of the thesis provides a synthesis that summarizes the findings of the entire study.

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CHAPTER 2

Seasonality of reproduction in the Tete veld rat, *Aethomys ineptus* (Rodentia: Muridae) from southern Africa

ABSTRACT

Body mass, reproductive-tract morphometrics, gonadal histology, and plasma testosterone in males and plasma oestradiol-17β and progesterone concentrations in females were studied throughout the year in wild Tete veld rats, *Aethomys ineptus* from South Africa. The main objective of the study being to discern if there was an inclination of this species towards seasonal breeding.

A total of 59 males collected over one calendar year revealed that seminiferous tubule diameters were significantly greater from September through to February but gradually decreased from March to August. Spermatogenesis was observed during winter months. However, the number of spermatozoa in the epididymides was lower when compared to the spring, summer, and autumn. Testicular mass and volume also regressed from June to August but exhibited recrudescence from September through to May. Similarly, mean plasma testosterone was significantly higher from October through to February, but was lower from March to September.

A total of 67 females were caught during a single calendar year. Ovarian histology revealed that corpora lutea were present throughout the year, but were reduced in number during winter months. Mean plasma progesterone concentration reflected this pattern, and were significantly higher from September through to April, but relatively lower between May and August. Gravid females were found from October through to

April. A conspicuous absence of gravid or lactating females was found from May through to September.

The Tete veld rat appears to be a seasonal breeder with reproduction confined predominantly to summer and autumn months of the southern hemisphere when food resources are optimal. The presence of follicular development throughout the year in females and spermatozoa in the epididymis of males suggest that *A. ineptus* may be capable of opportunistic breeding throughout the year when food resources are available and climatic variables are mild.

INTRODUCTION

Small mammals synchronize reproduction to coincide with favourable environmental conditions (ultimate factors) that promote rapid growth and maximal survival of offspring (Ims 1990). Seasonally breeding mammals may use the onset of rainfall, photoperiod, ambient temperature, and social cues to initiate reproductive events such as steroidogenesis, gametogenesis, spermatogenesis, mating, and conceiving to occur during the favourable time of the year (Jameson 1988).

Rainfall triggers growth of numerous plant species such as herbs, flowers, plant parts and insects that form basic diet for many small mammals (Beatley 1969; Nilsson 2001). Thus, seasonally breeding mammals confine reproduction to periods of rainfall. However, there is also a tendency for some animals to reproduce at anytime of the year whenever an opportunity arises (Prendergast *et al.* 2001). For example, the pouched mouse, *Saccostomus campestris*, is an opportunistic breeder, capable of breeding at any time of the year whenever environmental conditions permit (Tinney *et al.* 2001). Quality and quantity of basic nutrients play a significant role in the reproductive process.

An animal in a nutritionally deficient environment will not be able to successfully reproduce since substantial amounts of energy are required (Thompson & Nicoll 1986), and the energy is primarily geared towards other physiological process such as metabolism and thermoregulation prior to reproduction.

Mammalian reproduction is largely influenced in many aspects by ambient temperature. Homeothermic animals, which include many rodents, need to maintain body temperature in order to survive. A decrease in body temperature increases thermoregulatory requirements of an animal and may subsequently decrease the amount of energy made available for reproduction (Bronson 1985; Bronson & Pryor 1983; Sicard *et al.* 1993). Ambient temperature has adverse effects on testicular development and spermatogenesis in the desert pocket mouse, *Perognatus formosus*, at 33^0C but these reproductive events are enhanced at temperatures between 13-23^0C. Since ambient temperature fluctuates occasionally in many environments, mammals have learnt to avoid breeding when such changes lower survival rates of offspring and reproduction is postponed until the right conditions return (Prendergast *et al.* 2001).

Seasonally breeding mammals may use photoperiod to initiate reproductive events at the onset of the breeding period. Many rodents cease to breed when daylength becomes shorter during late summer and early autumn (Prendergast *et al.* 2001). For example, studies on the grasshopper mouse, *Onychomys leucogaster*, have shown that reproductive features such as gonadal recrudesence is inhibited on a short photoperiod of 10L:14D while stimulated on a long photoperiod of 14L:10D (Frost & Zucker 1983). Similar studies on *P. formosus*, have shown that testicular development and recrudesence is stimulated on a long photoperiod of 16L:8D but inhibited on a short photoperiod of

8L:16D (Kenagy & Bartholomew 1981). Bronson (1985) and Forger & Zucker (1985) reported on effects of day length on body growth. Offspring born in late summer were found to have reproductive development delayed by 4-5 months to reach maturity whereas those born into an increasing daylength reach maturity within 40-50 days.

Onset of reproduction may be signalled through social cues. Extensive studies on insects, rodents, swine, sheep, goats, and cattle have shown the importance of pheromones in reproduction (Rekwot *et al.* 2001). Some rodent species use urinary and other semiochemical compounds to indicate their reproductive status to conspecifics (Bronson 1985). Females housed together with males reached reproductive maturity earlier than those housed alone, and it has also been shown that male *Peromyscus aztecus*, housed together with female conspecifics showed significantly higher testicular, epididymal masses, as well as higher testicular sperm counts than those that were individually housed (Vandenbergh 1967, 1969; Vandenbergh *et al.* 1972; Demas & Nelson 1998).

The aim of this study is to determine if the Tete veld rat is a seasonal or aseasonal breeder and to investigate the male and female reproductive parameters that may allow to discern this.

MATERIALS AND METHODS

Trapping and handling of animals

A total of 126 specimens (67 females and 59 males) were caught monthly between February 2002 and May 2003 at Roodeplaat Nature Reserve (25° 34'S 28° 22'E) in Gauteng Province, South Africa, using Sherman live traps. A mixture of peanut butter, syrup, oat meal, and fish oil was used as bait. The animals were kept in polyurethane

cages with wood shavings as bedding. Mice pellets and water were provided *ad libitum* during the time prior to processing.

Determining reproductive status and processing of specimens

Animals were kept alive in the laboratory for a maximum of three days. Females were examined for reproductive status. Prominent teats, perforated vagina, and the number of embryos in gravid females were used to provisionally determine the reproductive status of females. Each animal was sacrificed using halothane and body mass obtained using a Mettler digital balance. Using a 1.0 ml heparin coated syringe, blood samples were collected by exsanguinations from the heart, centrifuged at 3000 rpm for 15 minutes and the plasma fraction stored at –20°C until analysed. Ovaries and testes were removed and testicular masses obtained. A pair of digital callipers was used to record the length and width of testes. Testicular volume was calculated using the formula for the volume of an ellipsoid described by Woodall and Skinner (1989) as:

$$V=4/3\ \pi ab^2$$

Where: a = 1/2 maximum length and, b = 1/2 maximum breadth.

Ovaries and testes were then fixed in Bouin's fluid for a minimum of 24 hours prior to being rinsed and stored in 70% ethanol. The heart, liver, kidneys and muscle tissues were obtained and placed into eppendorf vials, and frozen for subsequent mtDNA analysis. Skulls were prepared using standard museum procedures for preparing museum skull specimens as described below.

Verification of species

Skulls were boiled for approximately 2 hours, cleaned using forceps, and placed into bleach for 20-30 minutes. The skull were allowed to dry before being examined using a dissecting microscope. DNA sequences of Roodeplaat Dam Nature Reserve samples were obtained using the service of a molecular laboratory (Department of Genetics, University of Pretoria) to positively identify *A. ineptus* which otherwise is indistinguishable from its sibling species, *A. chrysophilus* based on external, cranial, and dental morphology. The following was observed from the study:

1) The Tete veld rat has two cusps on the first molar of the lower jaw.
2) The DNA sequences confirmed that specimens from Roodeplaat Dam Nature Reserve are *A. ineptus*.

Histology of ovaries

The histology of ovaries of 67 females were prepared following the guidelines of Ross *et al.* (1995) and Leeson *et al* (1985). A standard sequential dehydration procedure was used to prepare the tissues for embedding into paraffin wax. After embedding, ovaries were serially sectioned at 7.0 μm using a rotary microtome. Each ovary was sectioned in its totality and mounted onto microscopic slides using albumin as an adhesive. Slides were placed into an oven for a minimum of 24 hours and subsequently, stained in Ehrlich's haematoxylin, and counter-stained in eosin. A Nikon digital camera (DMX 1200) was used to view, photograph and count follicles.

Both ovaries of all females were sectioned in totality and mounted. Sections were examined in consecutive order using a light microscope at 100x, 200x and 400x magnifications. Follicles were counted by drawing and mapping the distribution of the

respective follicles in each ovary. Ovarian follicles were counted and categorised according to Ross *et al.* (1995) as follows:

1) Primordial follicles (Prm) were the smallest of all follicles found in the ovary. They were located in the stroma of the cortex immediately below the tunica albuginae. The primordial follicles were surrounded by a single layer of squamous follicular cells with the outer surface of the follicular cells kept together by a basal lamina (Plate 2.1). The Primary follicles (Pr) were characterized by an enlarged oocyte and were surrounded by cuboidal cells. The zona pellucida appearing between the oocyte and the adjacent follicular cells becomes more pronounced (Plate 2.1).
2) Follicles produced in the ovary, which do not reach maturity, degenerate and disappear from the ovary through follicular "atresia"(Atr) (Plate 2.1).
3) Secondary follicles (Sc) were characterized by a fluid-containing antrum. The follicular layers become 6-12, antral spaces starts to appear within the follicular cells. This antrum contains a hyaluronic acid-rich fluid or liquor folliculi. As this fluid continues to accumulate among the glanulosa cells, the antrum becomes larger and forcing the oocyte to be pressed towards the follicular edge (Plate 2.2).
4) At the Graafian follicle (Grf) stage, the antrum does not increase any further. The oocyte is pressed to one edge of the antrum and held in place by a cumulus oophorus, whereas the antral lumen is surrounded by membrana granulosa (Plate 2.3).
5) When the oocyte is expelled during ovulation, a corpus hemorrhagicum (Ch) is formed (plate 2.4).

6) During fertilization, the corpus luteum (CL) of pregnancy is formed. The CL is the source of progesterone during the early stages of pregnancy (Plate 2.5).
7) The corpus luteum lasts for several months when it starts to degrade to form a corpus albican (Cal) (Plate 2.6).

In addition to the follicular count, the presence of placental scars and foetuses were recorded.

Histology of testes

All testes were sectioned, mounted, and stained following Ross *et al.* (1995). In order to determine the diameters of seminiferous tubules, several sections of the testes with circular tubules, were selected and photographed at 200x and 400x magnifications using a Nikon digital camera (DMX 1200). The diameters were determined using Image Tools Software Version 3.00. All male specimens were examined for signs of spermatogenesis, spermatozoa, and stages of testicular development during the twelve months of the year and the following observed:

1) The seminiferous tubules (St) were lined with seminiferous epithelium, which was made up of the supporting cells of Sertoli and spermatogenic cells. Different stages of spermatogenic activity were observed at different parts of the year depending on the stages of testicular recrudesces (Plate 2.7).
2) The epididymis (Ep) consisted of the ductuli efferens that forms the head, the convoluted body, the tail, and the excretory Ductus deferens. All specimens in

this study were examined for the presence of spermatozoa in the epididymis during the twelve-month study period (Plate 2.8).

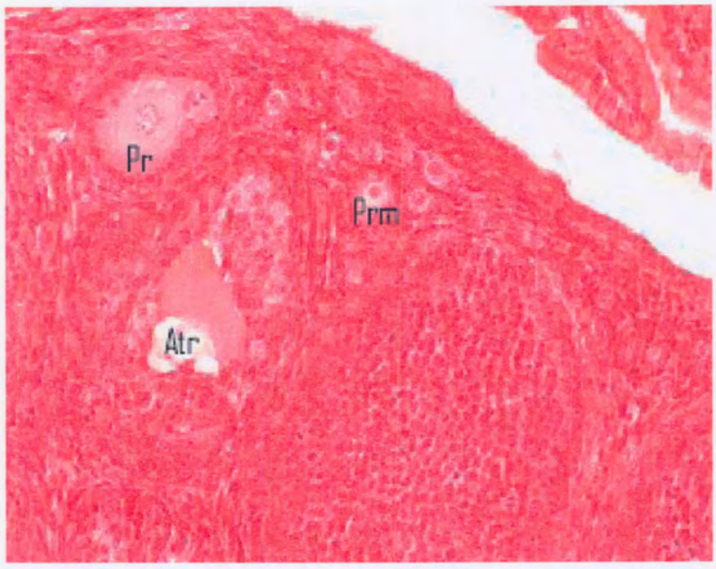

Plate 2.1. Primordial (Prm) follicles in the ovarian cortex, Primary (Pr) and Atretic (Atr) follicles, observed in a female Tete veld rat ovary.

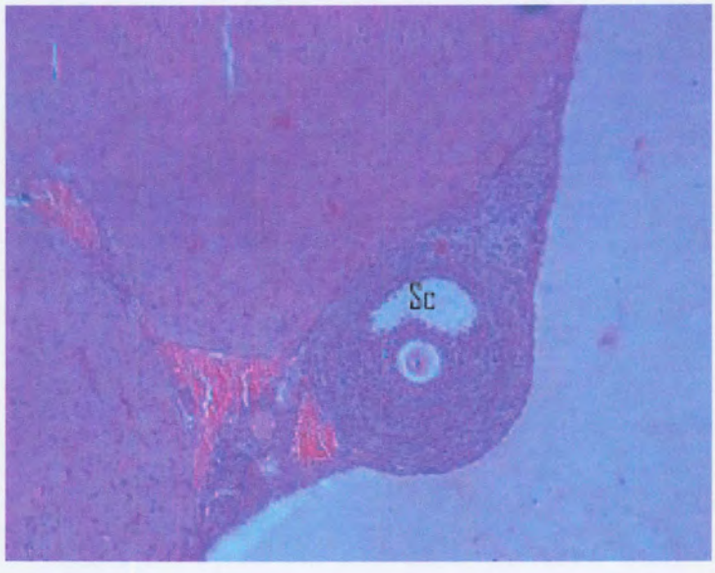

Plate 2.2. Secondary (Sc) follicle observed in a female Tete veld rat ovary. Fluid-containing antrum is seen within the follicular cells surrounding the oocyte.

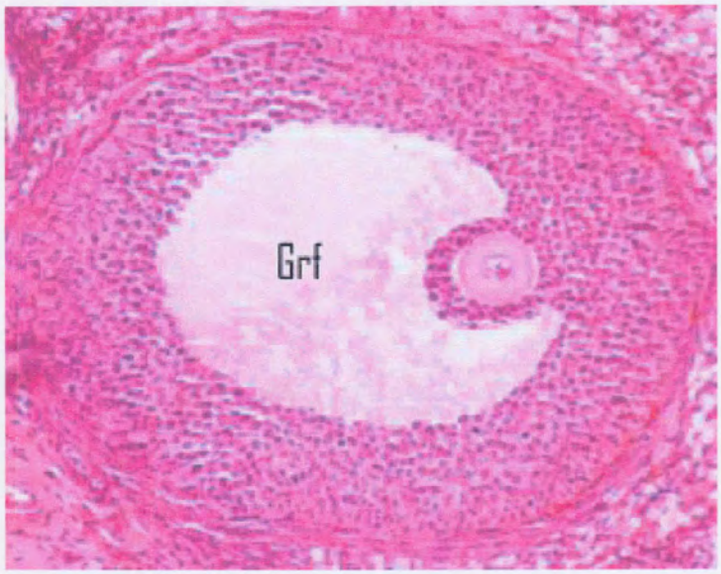

Plate 2.3. Graafian (Grf) follicle observed in a female Tete veld rat ovary. The oocyte is pushed to the edge as a result of accumulation of fluid into the antrum.

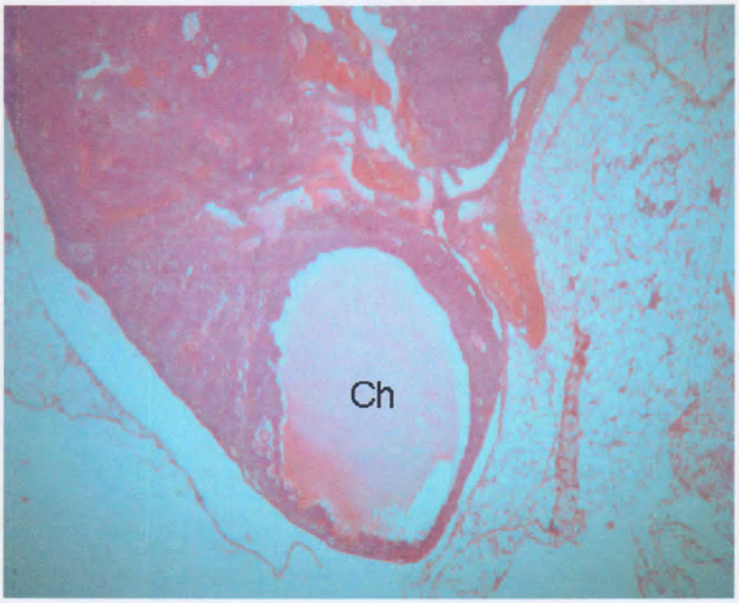

Plate 2.4. Corpus hemorrhagicum (Ch) observed in a female Tete veld rat ovary, formed after the oocyte has been expelled from the follicle during ovulation.

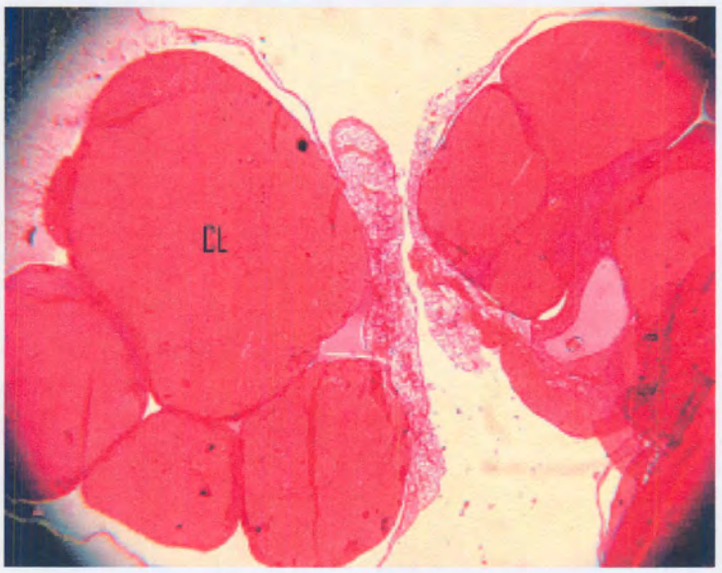

Plate 2.5. Corpora lutea (CL) observed in a female Tete veld rat ovary. CL produces progesterone during early stages of pregnancy.

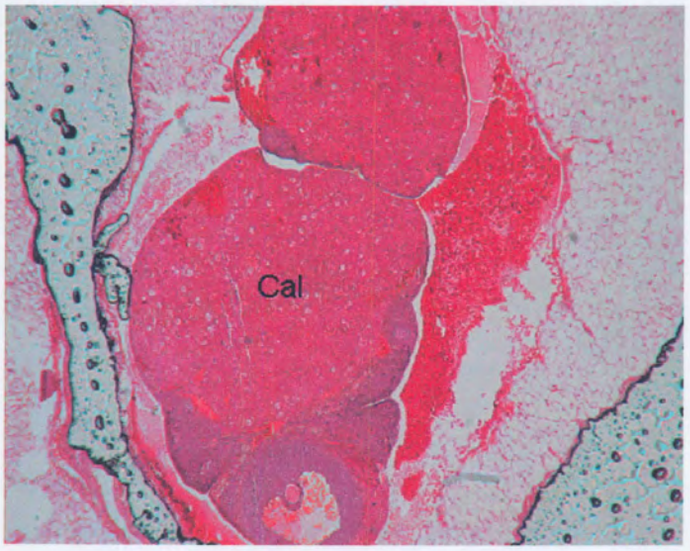

Plate 2.6. Corpus albican (Cal) from a female Tete veld rat ovary, formed after the corpus luteum has degerated.

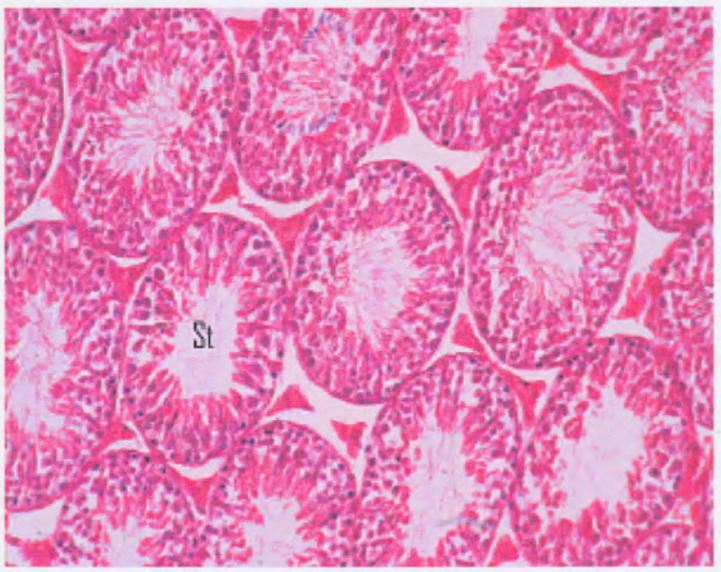

Plate 2.7. Seminiferous tubules (St) from a male Tete veld rat testis. Spermatogenic activity is observed as spermatozoa are seen lined up with their heads toward the lining of tubule epithelia (spermatogenic cells).

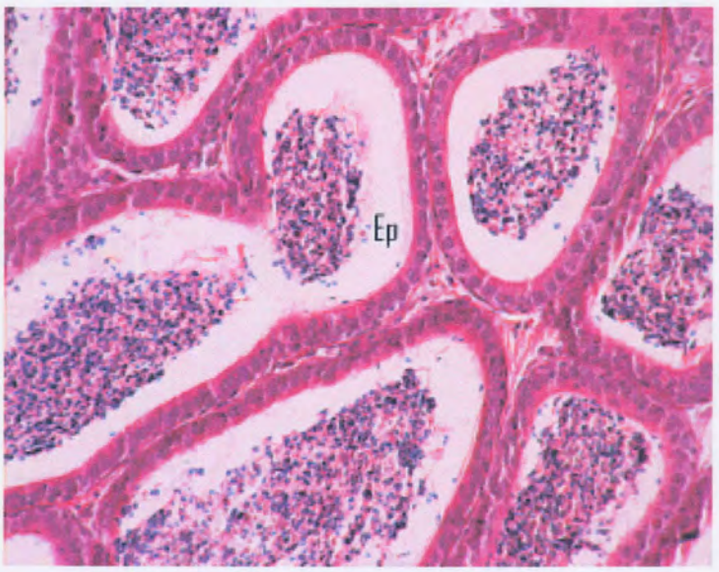

Plate 2.8. Epididymides (Ep) from a male Tete veld rat testis. Epididymides are filled with spermatozoa.

Analysis of female hormones

Progesterone concentration was determined in 67 females that were collected throughout the year. A non-extraction assay using Coat-A-Count progesterone kits (Diagnostic Products Corporation, USA) was used to determine progesterone concentrations. The antiserum is highly specific to the naturally occurring steroids with a cross reactivity of < 0.5%, and has a cross reactivity of 2.0% and 2.4% with 20-α-dihydroprogesterone and 11-deoxycortisol respectively. No purification or separation by chromatography was required.

Validation for progesterone

The hormonal assays were validated for use in the Tete veld rat by testing the homogeneity/parallelism of the standard curve slope and that of the serial double dilutions curve (ranging from a ratio of 1:1 to 1:8) of the plasma progesterone. There was no significant difference between the two slopes (ANCOVA: $F = 0.51$, $P > 0.05$) (Fig 2.1). The intra assay coefficient of variation for plasma pool was 6.98% (n = 20) . The sensitivity of the assay was 0.032 ng/ml.

Determination of Oestradiol-17β

Oestradiol-17β was determined in 47 females using Coat-A-Count oestradiol-17β kit (Diagnostic Products Corporation, USA). The method is a solid-phase radioimmunoassay that does not require purification of steroids or separation by chromatography. The antisera is highly specific for oestradiol-17β, with a very low cross-reactivity with any other steroids present in the plasma.

Validation for oestradiol-17β

Serial dilution of plasma oestradiol-17β from a reproductive female paralleled the reference preparation, thus the slopes did not differ significantly (ANCOVA: $F = 7.37$; $P > 0.11$) (Fig. 2.2), following log-logit transformation of the data (Chard 1987). The intra-assay coefficient of variation was 22.0% ($n = 22$) and the sensitivity of the assay was 2pmol/L.

Analysis of male hormones

Testestorone was analysed in 59 males using a Coat-A-Count total testestorone Kit (Diagnostic Products Corporation, USA). This is a solid-phase radioimmunoassay . The assay does not require any extraction or chromatography. Antisera is highly specific for testosterone and has a very low cross reactivity with other compounds. Cross reactivity with dihydrotestosterone is less than 5%.

Validation of Testosterone

Serial dilution of plasma testosterone was parallel to the curve of the standard samples. The slopes of the two curves were not significantly different from each other (ANCOVA: $F = 0.045$; $P = 0.84$) (Fig. 2.3) following a log-logit data transformation (Chard 1987). The intra essay coefficient of variation was 12.8 % ($n = 20$) and the sensitivity of the assay was 1.39 nmol/L.

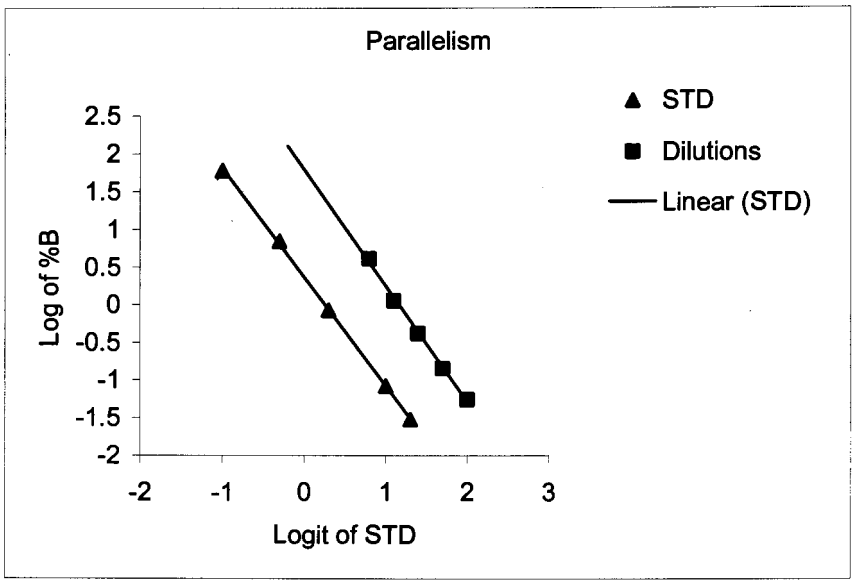

Fig. 2.1. Serial dilutions of plasma progesterone (■) of a female Tete veld rat, showing parallelism with a reference preparation curve (▲) thus validating the hormonal assay.

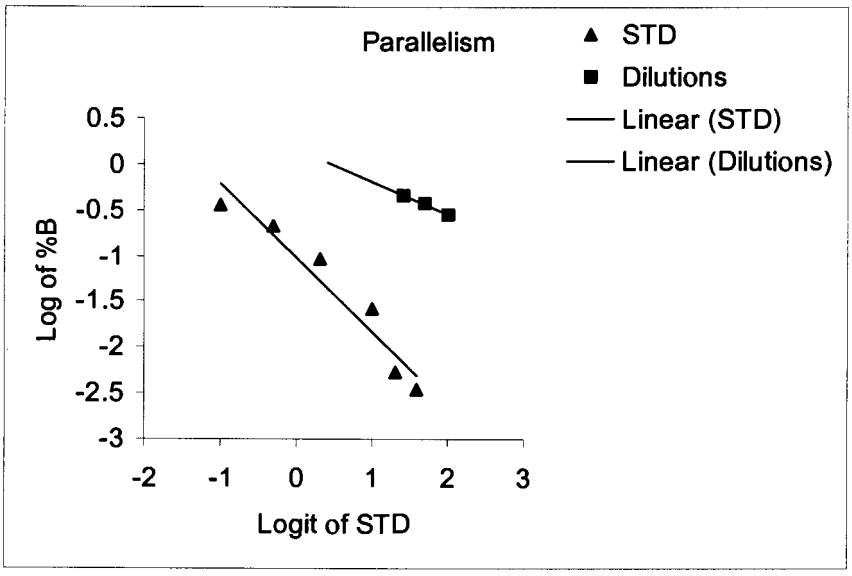

Fig. 2.2. Serial dilutions of plasma oestradiol-17β (■) of a female Tete veld rat, showing parallelism with a standard samples curve (▲) thus validating the hormonal assay.

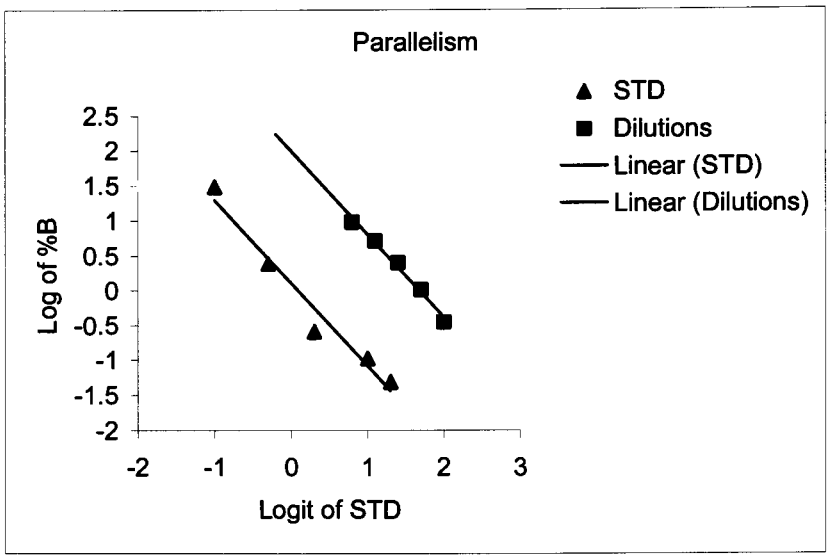

Fig. 2.3. Serial dilutions of plasma testosterone (■) of a male Tete veld rat, showing parallelism with a standard testosterone curve (▲) thus validating the hormonal assay.

Analysis of data

The data was analysed using Microsoft Excel™ spreadsheets and Statistical version 6.0™. Analysis of Covariance (ANCOVA) was used to test for parallelism between the curve of plasma dilutions and standard samples. Tukey HSD was used to test for differences in seminiferous diameters, testicular mass, testicular volumes, and testosterone between the months. A General Linear Model (GLM) was used to test for significant differences in follicular development between months.

RESULTS

The months are divided into four seasons of the year as follows: December to February (summer), March to May (autumn), June to August (winter) and September to November (spring). The statistical results are expressed as the mean ± S.E (standard error).

In both males and females, there were no significant differences amongst the body masses of animals collected during each month of the year (Males = 80.81 ± 2.29, n = 58, females = 71.7 ± 2.54, n = 65) (Tukey HSD: $P > 0.05$) (Figs. 2.4 & 2.5). Gravid females were found between October and April (Table 2.1). No gravid females were present in the samples obtained between May and September.

Histology of ovaries

Primordial follicles were found throughout the year. No significant differences were found in the number of primordial follicles (Prm) between months (GLM: $R^2 = 0.26$; $F = 0.68$; $P > 0.05$) (Fig. 2.6). The primary follicles (Pr) were found throughout the entire sampling period. No significant differences were found between months (GLM, R^2 =

0.13; $F = 1.94$; $P > 0.05$) and no patterns were found in the distribution of primary follicles during the entire sampling period (Fig. 2.7).

Secondary follicles (Sc) were found throughout the year and no significant differences were found between months (GLM: $R^2 = 0.053$; $F = 1.09$; $P > 0.05$). No patterns were observed in the distribution of secondary follicles during the year (Fig. 2.8). Graafian follicles (Grf) were found throughout the sampling period. Between June and February, Graafian follicles were significantly higher (GLM: $R^2 = 0.30$; $F = 5.78$; $P < 0.0015$) and lower between March and May (Fig. 2.9).

The data for corpora lutea had to be log transformed, as they were not normally distributed. The number of corpora lutea was significantly higher between December and August but lower between September and November (GLM: $R^2 = 0.34$; $F = 2.28$; $P < 0.05$) (Fig. 2.10). Corpora hemorrhagicum were found between October and January through to April. No corpora hemorrhagicum were found between May and September (Fig. 2.11). Corpora albicans were observed during July (Fig. 2.12). Log transformation was performed to normalize the data on atretic follicles. The number of atretic follicles was significantly higher between September and May (GLM, $R^2 = 0.15$; $F = 2.45$; $P < 0.05$) (Fig. 2.13).

Histology of testes

Seminiferous tubule diameters were significantly higher between October and February (Tukey HSD test: $P < 0.05$) (Fig. 2.14). During September through to May, all males had spermatozoa in the seminiferous tubules as well as in the epididymis. Some of the specimens sampled between June and August possessed few spermatozoa in the epididymis, with very little spermatogenic activity in seminiferous tubules.

Testicular mass and volume

Testicular mass was significantly higher between September and March and low between April and August (Tukey HSD test: $P < 0.02$) (Fig. 2.15). Testicular volume was significantly higher between September and May (Tukey HSD test: $P < 0.05$) and low between June and August (Fig. 2.16).

Table 2.1. Number of embryos and percentage of lactating *Aethomys ineptus* females collected during one calendar year.

	J	F	M	A	M	J	J	A	S	O	N	D
Total number of embryos	5	8	10	17	0	0	0	0	0	20	13	7
% Lactating ♀s	33	80	83	76	0	0	0	0	0	86	80	66

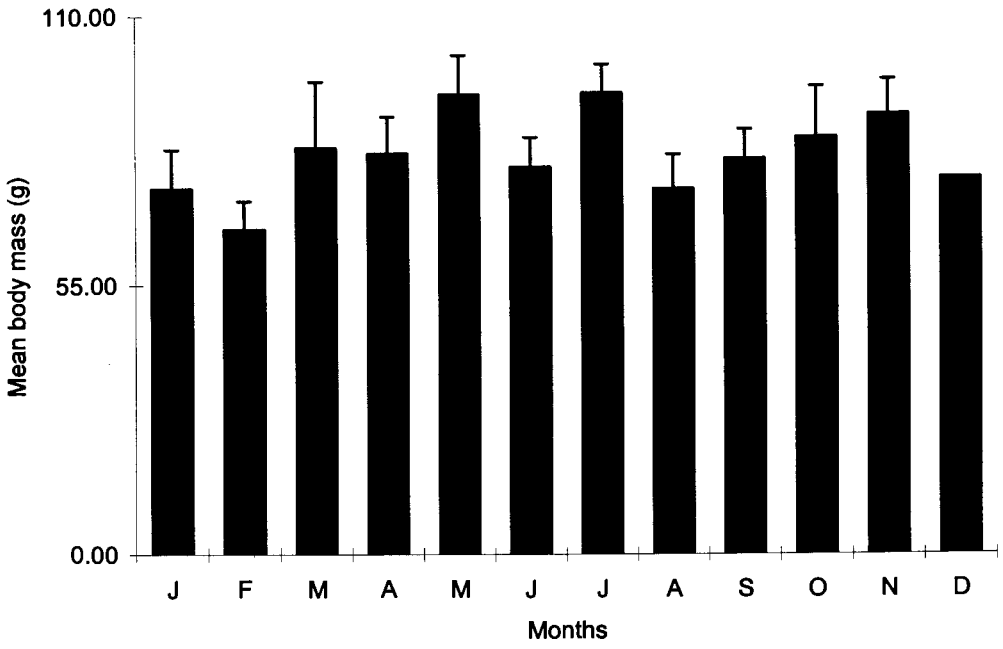

Fig. 2.4. Mean body mass ± S.E. of male Tete veld rats measured over a twelve-month period. No significant differences were found in mean body masses between months.

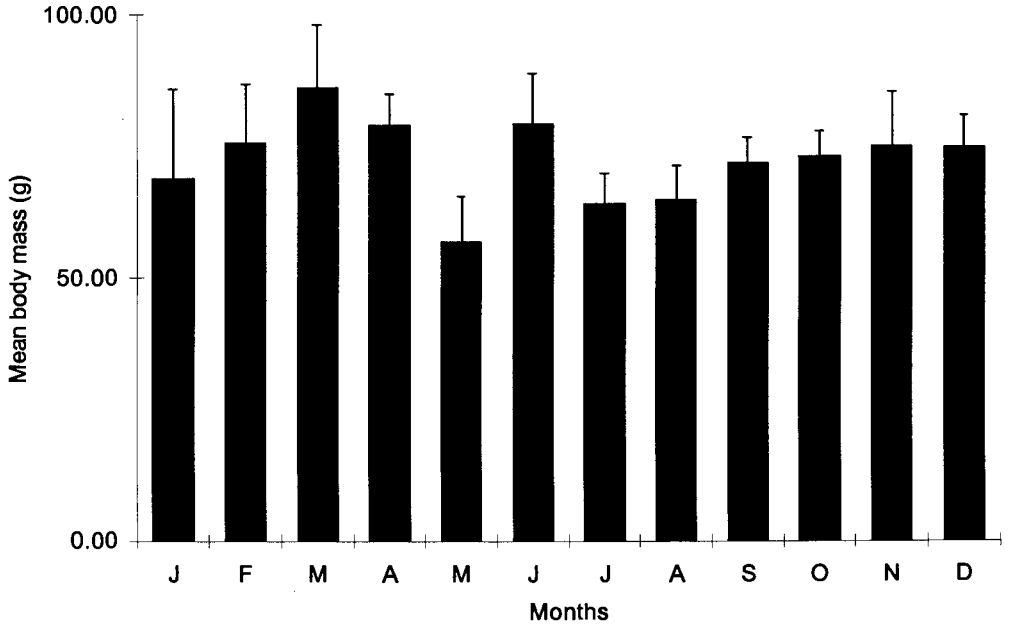

Fig. 2.5. Mean body mass ± S.E. of female Tete veld rats measured over a twelve-month period. No significant differences were found in body masses between months.

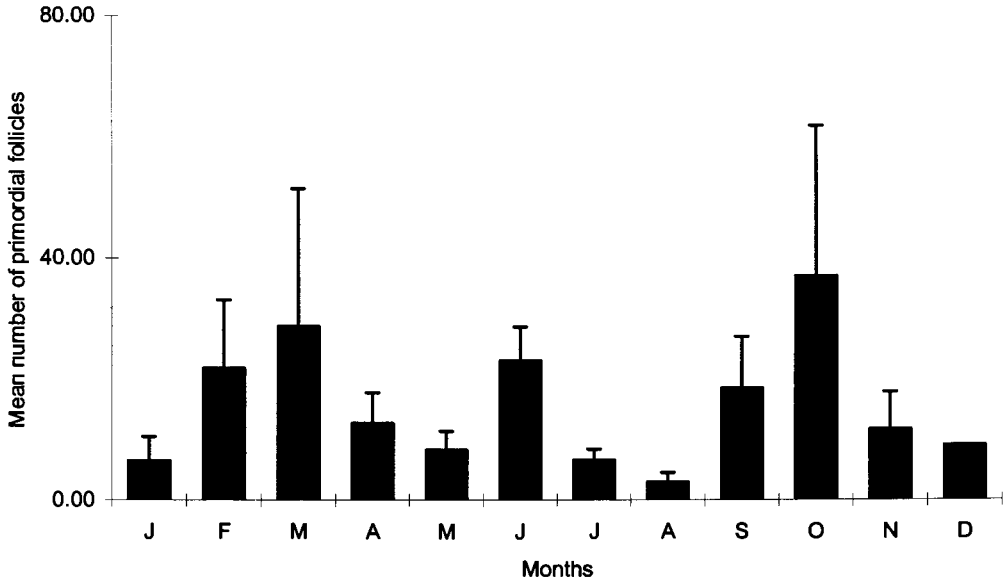

Fig. 2.6. Mean number ± S.E. of primordial follicles of female Tete veld rats assessed over a twelve-month period. Mean numbers of primordial follicles did not differ significantly between months.

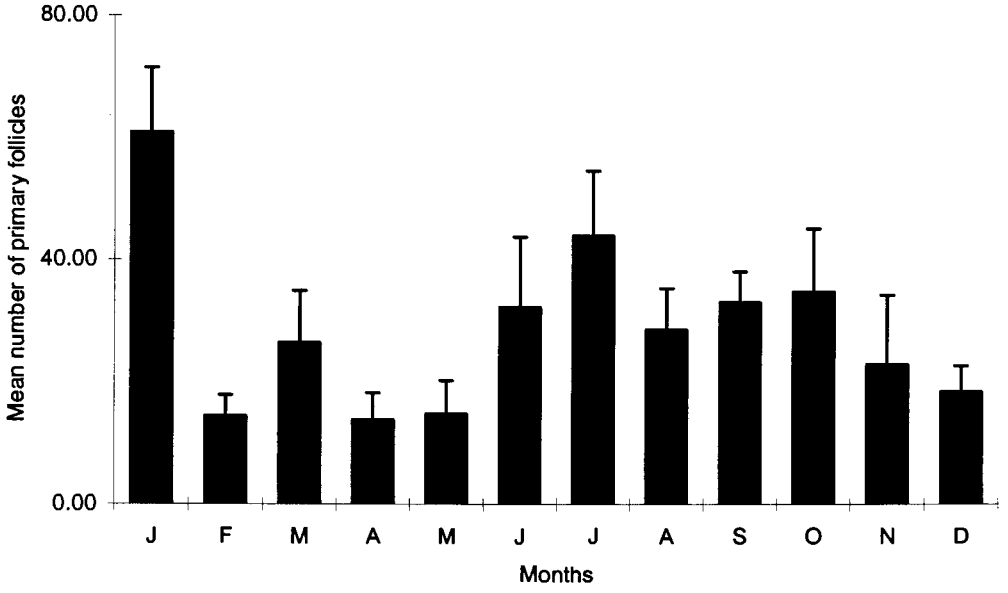

Fig. 2.7. Mean number ± S.E. of primary follicles of female Tete veld rats assessed over a twelve-month period. Mean number of primary follicles did not differ significantly between months.

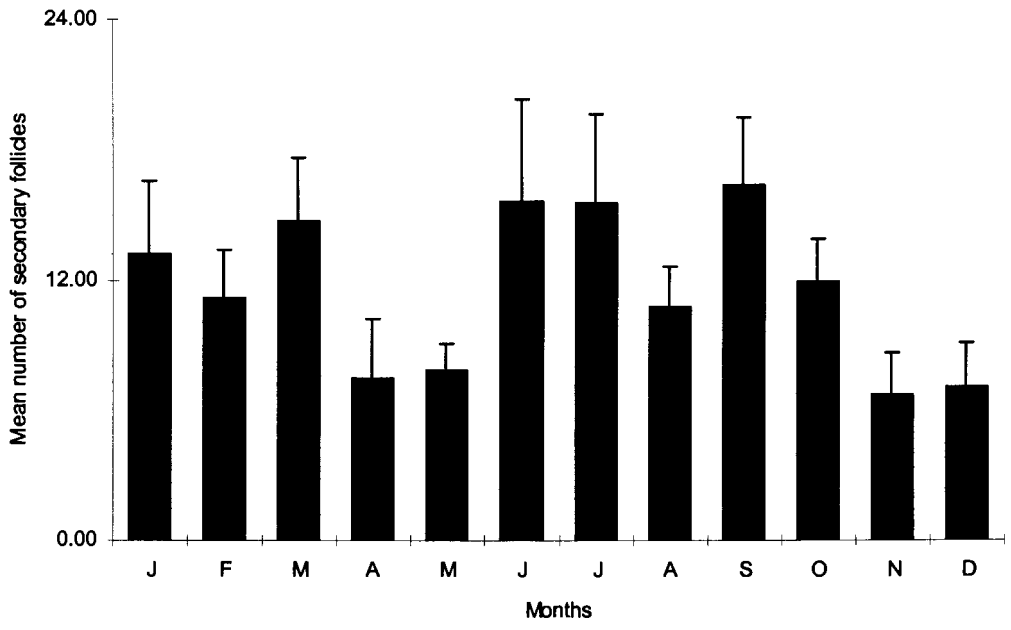

Fig. 2.8. Mean number ± S.E. of secondary follicles of female Tete veld rats assessed over a twelve-month period. Mean number of secondary follicles did not differ significantly between months.

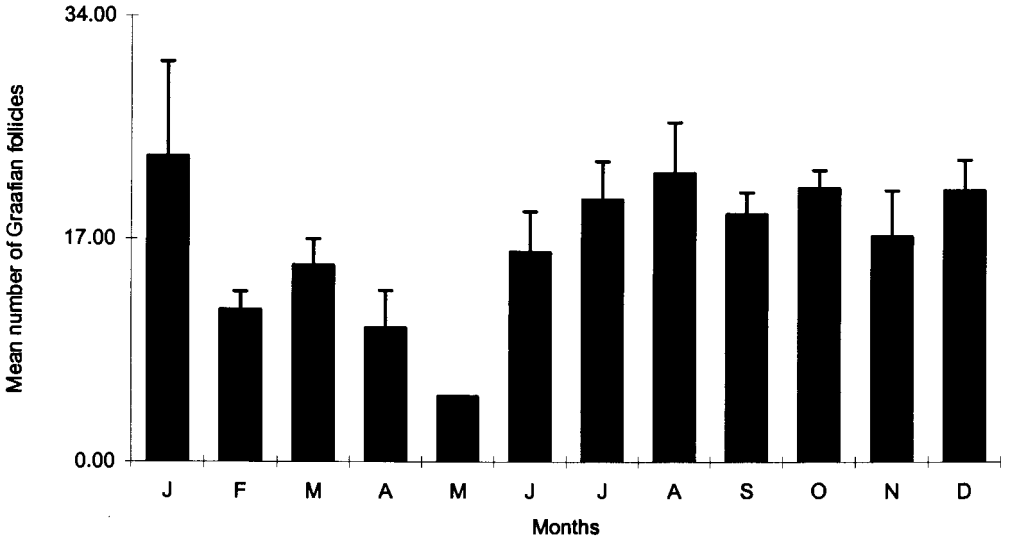

Fig. 2.9. Mean number ± S.E. of Graafian follicles assessed in female Tete veld rats over a twelve-month period. Mean numbers of Graafian follicles were significantly higher between June and February and lower between March and May.

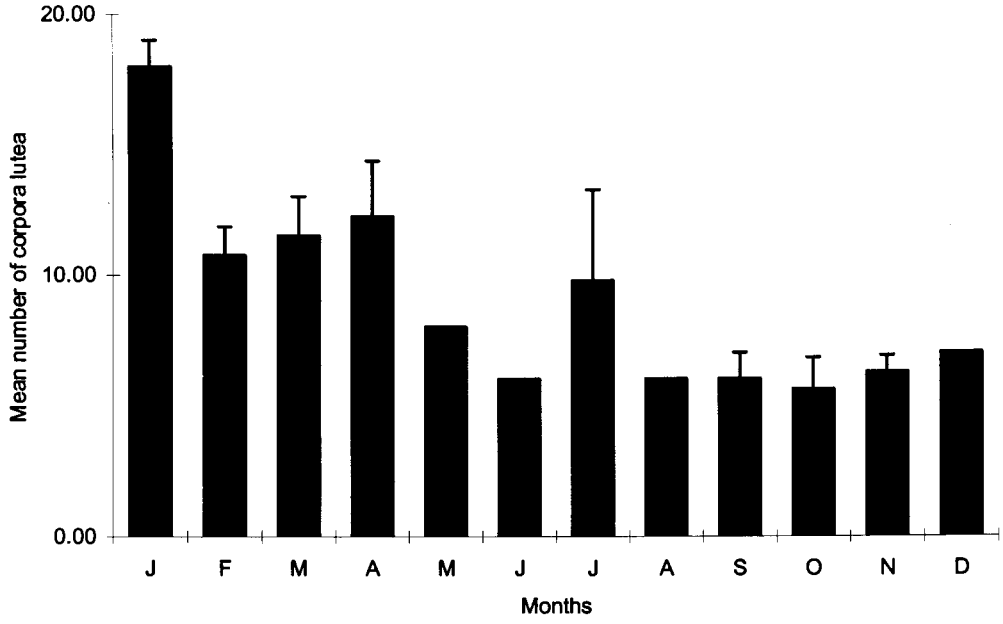

Fig. 2.10. Mean number ± S.E. of corpora lutea assessed in female Tete veld rats over a twelve-month period. Corpora lutea were found throughout the year and were significantly higher between December and August and lower between September and November.

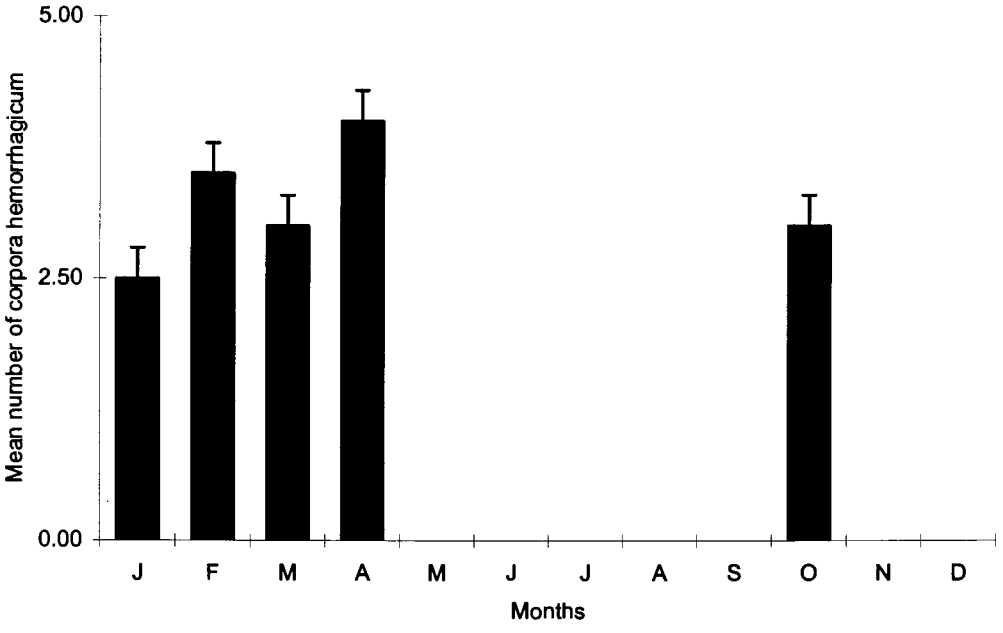

Fig. 2.11. Mean number ± S.E. of corpora hemorrhagicum assessed in female Tete veld rats over a twelve-month period. Corpora hemorrhagicum were found in spring, summer and autumn. No corpora hemorrhagicum were observed in winter.

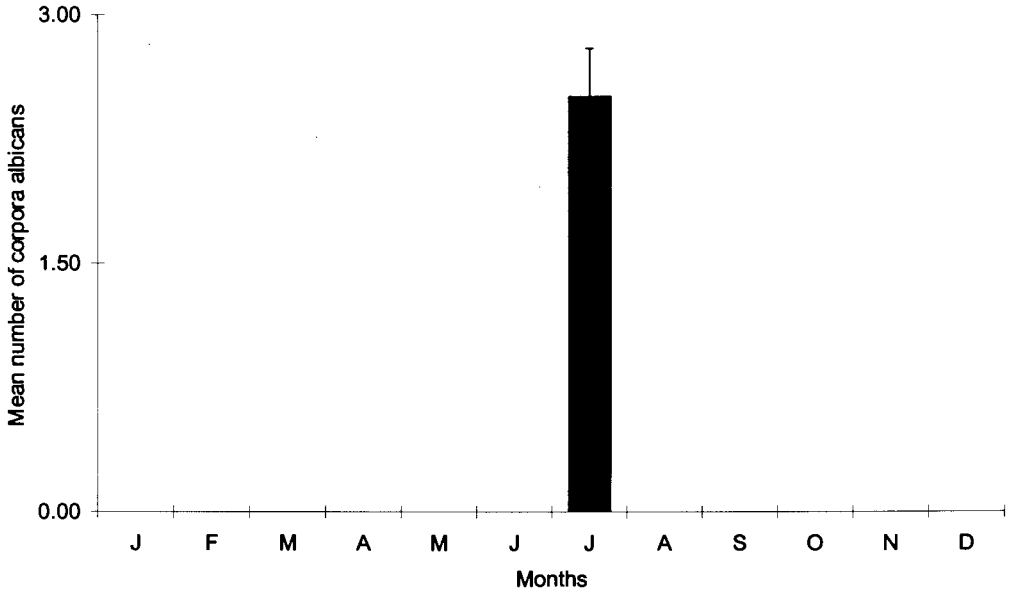

Fig. 2.12. Mean number ± S.E. of corpora albicans assessed in female Tete veld rats over a twelve-month period. Corpora albicans were only observed in July.

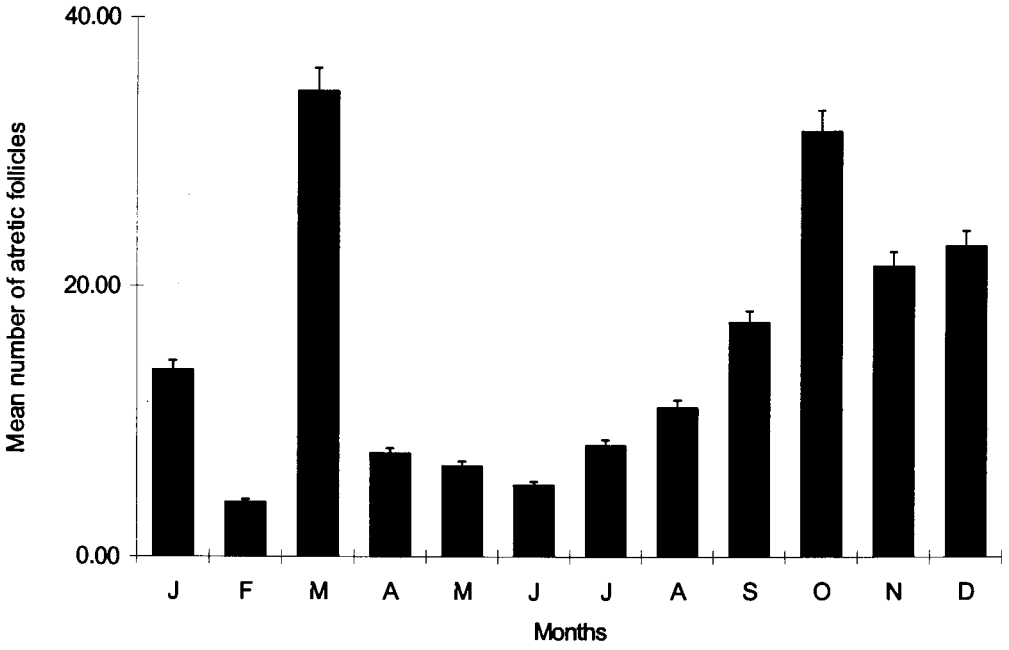

Fig. 2.13. Mean number ± S.E. of atretic follicles assessed in female Tete veld rats over a twelve-month period. Atretic follicles were significantly higher between September and May, and lower between June and August.

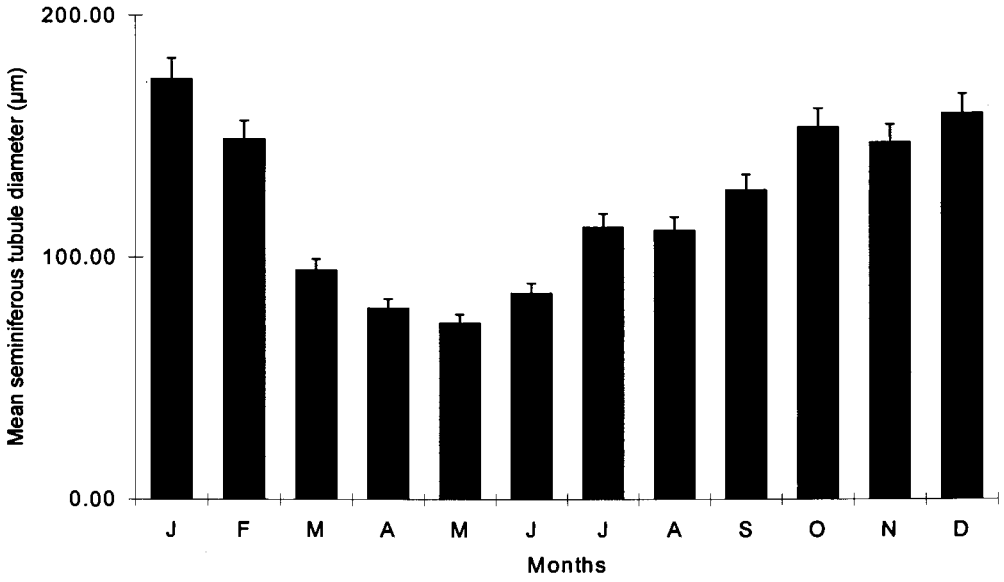

Fig. 2.14. Mean seminiferous tubule diameter ± S.E. in male Tete veld rats during a twelve-month period. Seminiferous tubule diameters were significantly higher between September and May, and lower between June and August.

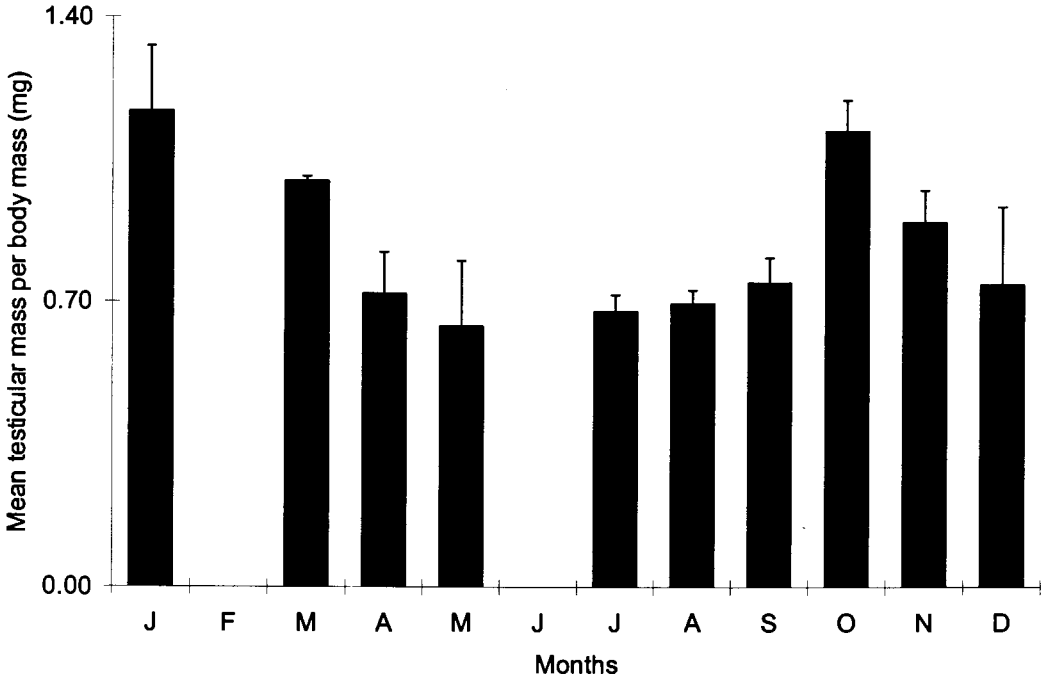

Fig. 2.15. Mean testicular mass per body mass ± S.E. of male Tete veld rats over a twelve-month period. Testicular mass per body mass was significantly higher between September and March, and lower between April and August.

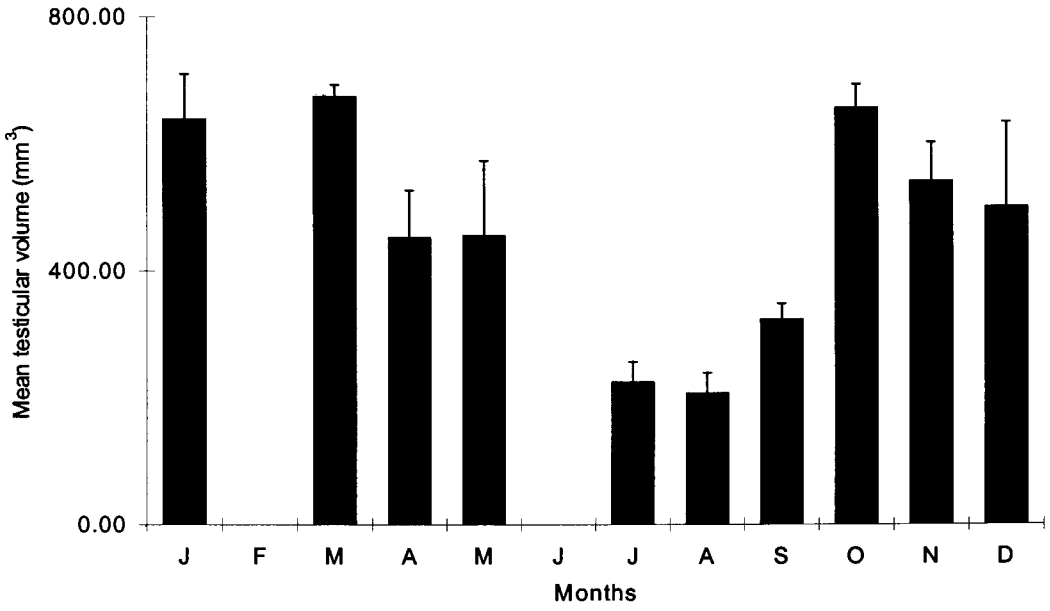

Fig. 2.16. Mean testicular volume ± S.E. of male Tete veld rats over a twelve-month period. Testicular volume was significantly higher between September and May, and lower between June and August.

Reproductive hormones

Plasma progesterone

Plasma concentration of progesterone was significantly higher between October and April (GLM: $R^2 = 0.28$; $F = 4.97$; $P < 0.0038$), and lower between May and September. There is a clear pattern showing that plasma progesterone concentration is relatively low during winter months (Fig. 2.17). Plasma oestradiol-17β concentrations showed no discernable pattern. The plasma oestradiol-17β concentration corresponded with the follicular development at all stages. There were no significant differences found in plasma concentration of oestradiol-17β between months throughout the sampling period (Tukey HSD test: $P > 0.05$) (Fig. 2.18).

Testosterone

Testosterone concentration was significantly higher between December and February (Tukey HSD test: $P < 0.05$), decreased from February, and remained relatively low until November (Fig. 2.19). The testosterone profile coincides with seminiferous tubule diameter and the progesterone concentration profiles of females.

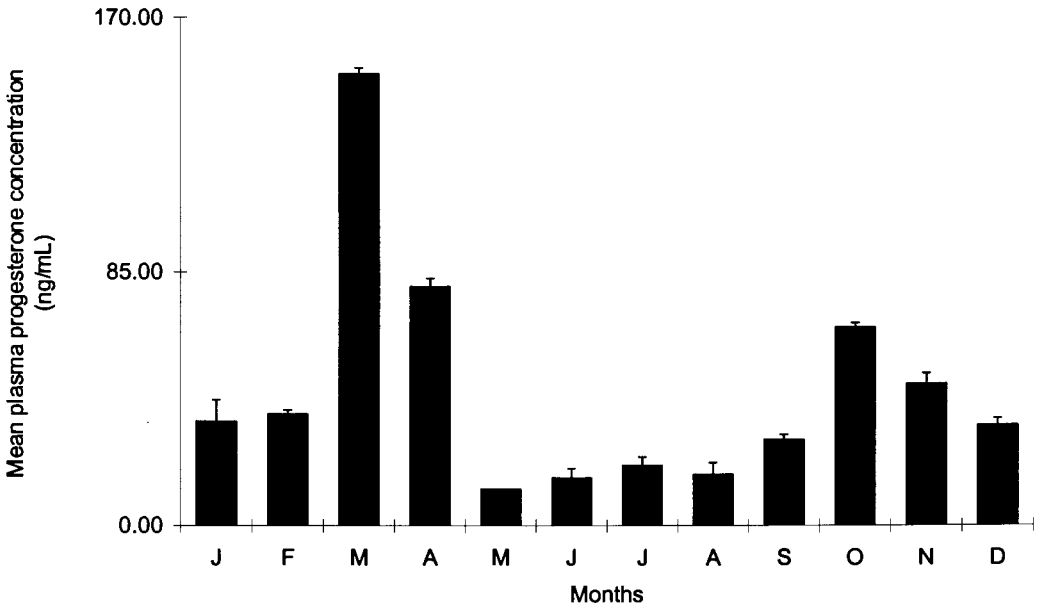

Fig. 2.17. Mean plasma progesterone concentration ± S.E. of female Tete veld rats over a twelve-month period. Plasma progesterone concentration was significantly higher between October and April, and lower between May and September.

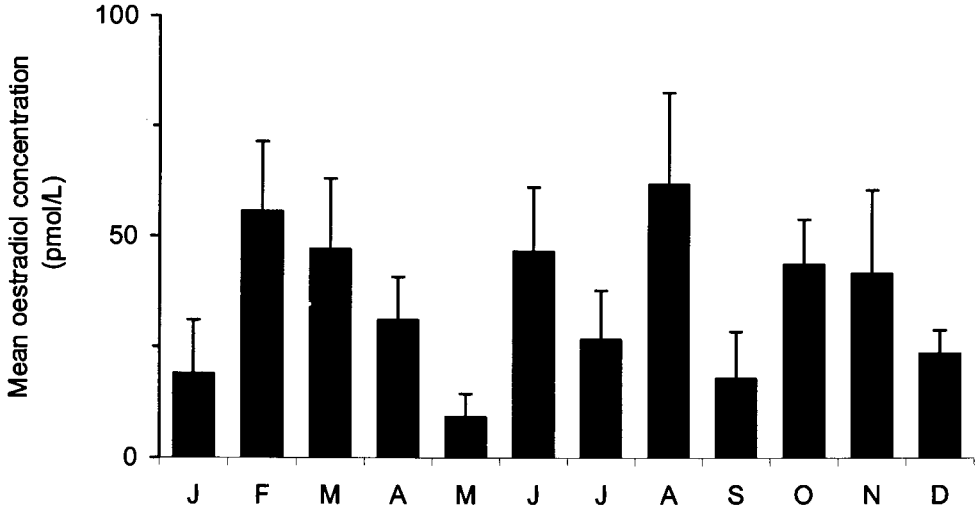

Fig. 2.18. Mean plasma oestradiol-17β concentration ± S.E. of female Tete veld rats over a twelve-month period. There were no significant differences in plasma oestradiol-17β concentrations between months.

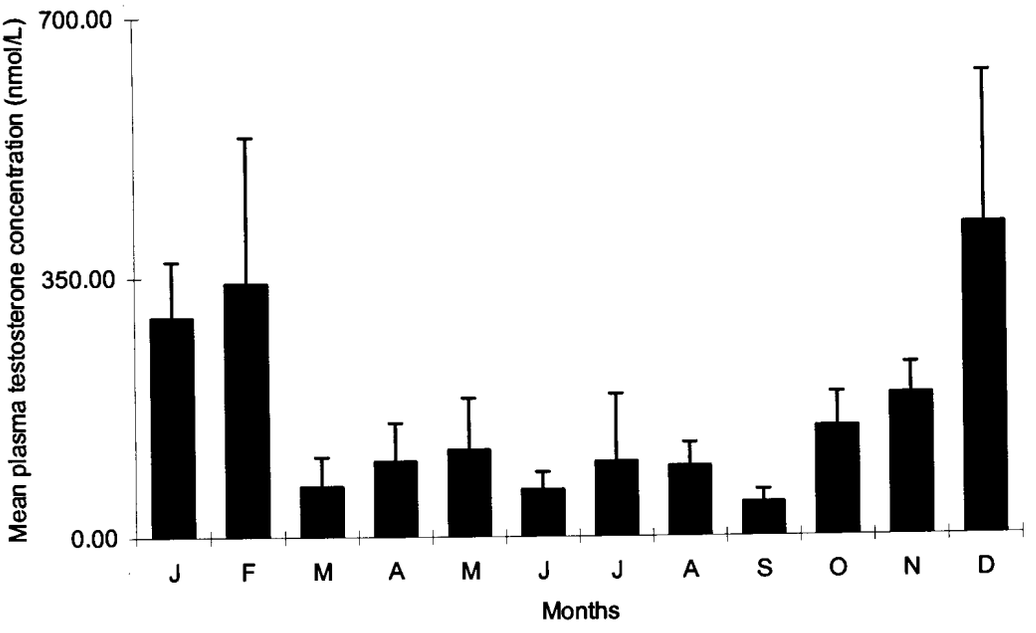

Fig. 2.19. Mean plasma testosterone concentration ± S.E. of male Tete veld rats over a twelve-month period. Plasma testosterone concentration was significantly higher between December and February, and lower between March and November.

DISCUSSION

Reproduction is an energertically demanding process that requires an animal to carry out procreation during such times when energy supplies are adequate for recrudescence of gonads, to nourish the developing foetus, and maintain the lactating mother's physiological demands (Thompson & Nicoll 1986; Flowerdew 1987).

Seasonal recrudescence of gonadal activity is carried out some time prior to mating and is accompanied by an increase in testicular mass or size in males and in follicular development in females. In the Tete veld rat, the findings of this study suggest that the major component of its breeding season occurs between October and April.

Follicles at various developmental stages were found throughout the year in the Tete veld rat. According to Clark (1981), regression of follicular development should be readily apparent in small mammals exhibiting a clear-cut seasonal reproductive strategy, but this does not appear to be the case in the Tete veld rat. Primordial, primary, secondary, and Graafian follicles were present in ovaries during all months of the year. The presence of Graafian follicles during every month suggests that this species is capable of breeding throughout the year. Atretic follicle production increased steadily from July to March.

Corpora lutea where found throughout the year. However, corpora hemorrhagicum were only found in October and between January and April. This, coupled with gravid females being present only between October and April, suggests that the Tete veld rat is possibly an opportunistic breeder. Tinney *et al.* (2001) reported a similar reproductive strategy in the pouched mouse, *S. campestris*, where reproduction during winter is inhibited as a result of a reduction in either food quantity or quality.

Thus, the Tete veld rat may be an opportunistic breeder that may use food availability to initiate reproductive events (Prendergast *et al.* 2001; Bronson 1989).

Plasma progesterone concentrations were significantly lower between May and August and gradually increased from September to April. This may imply that ovulation and pregnancy mainly occurs between September and April. This species perhaps adopts a seasonally reproductive quiescence in which breeding at energetically challenging times of the year risks fitness thus defers reproduction until when favourable environmental conditions return ((Prendergast *et al.* 2001, Nelson *et al.* 1983; Sicard *et al.* 1993).

Oestradiol-17β was measurable throughout the sampling period. Three peaks were discerned in November, another between March and April, and between July and August. However, this pattern was not consistent with the observed follicular development. This is contrary to Bedford *et al.* (1972) who reported that plasma oestrogen concentration increased during pregnancy. However, in this study, pregnancy was only observed between October and April and oestradiol would be expected to be high during this period.

The increased diameters of seminiferous tubules coincided with the rise in plasma progesterone concentration in females. The seminiferous tubule diameters were significantly larger between September and April, and decreased gradually during winter months (May-August). This may imply that males are reproductively active during spring, summer, and autumn but are less active during winter. The presence of spermatozoa in some of the epididymides and little spermatogenic activity during winter further supports an opportunistic breeding capability, which may be triggered by the

availability of quantity and/or quality of nutrients (Meredith *et al.* 1986; Sisk & Bronson 1986; Bronson 1989). Thus, the Tete veld rat may use multiple cues (e.g., food, ambient temperature, and social cues) other than photoperiod alone (see chapter 4) to initiate reproductive events during periods when environmental conditions become favourable (Bernard & Hall 1995).

Testicular mass as expressed against body mass was significantly higher between September and May. It was significantly lower during winter (June-August). Testicular volume followed the same trend. These changes in dimensions are reflected in both the change in diameter of the seminiferous tubules and the rise in plasma testosterone concentrations in males and the rise in plasma progesterone concentrations in females. The change in testicular mass and volume suggests that reproduction in the Tete veld rat may be retarded during winter months (June-August) perhaps due to an insufficient food resource base (Blank & Desjardins 1984). Food deprivation was found to retard reproduction in the water vole, *Arvicola terrestris* (Hamilton & Bronson 1985) and supplementation enhanced reproductive activity in the rock mouse, *Peromyscus difficilis* (Galindo-Leal & Krebs 1998). Thus, reproduction during winter may be minimized due to low availability of food and harsh winter conditions.

Plasma testosterone concentrations were significantly higher during summer months but dropped between March and November. The rise in testosterone concentrations in males mirrors the rise in progesterone concentrations in females. This suggests that males are more reproductively active during summer than winter months.

In conclusion, the Tete veld rat has the potential for opportunistic breeding and is capable of breeding throughout the year with reproduction being controlled by multiple

cues (Bronson 1989; Prendergast *et al.* 2001) in which food availability, ambient temperature, and social cues may be some of the proximate cues. The peak of reproduction occurs between September and April when all females sampled during this period were either pregnant or lactating. No pregnant or lactating females were found between May and September. Males had spermatozoa in the epididymides during winter months and this supports the notion that the Tete veld rat may be an opportunistic breeder. The winter breeding capacity may be inhibited by a reduction in food quantity and/or quality, and possibly by the lower ambient temperatures of winter as in the case of the pouched mouse, *S. campestris* (Tinney *et al.* 2001).

Future research should be conducted to assess the reproductive pattern of *A. chrysophilus*, a sibling species to *A. ineptus*. Such information would contribute significantly to resolving systematic problems involing sibling species. The reproductive biology of medically and agriculturally important rodents is of vital importance in cases of epidemiological problems as they have been implicated in the spread of plague, diseases, and in destroying crops and stored products in agriculturally developed areas.

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CHAPTER 3

Seasonality of reproduction in the Namaqua rock mouse, *Aethomys namaquensis* (Rodentia: Muridae) from southern Africa

ABSTRACT

Body mass, reproductive-tract morphometrics, gonadal histology, plasma progesterone, and oestradiol concentrations in females, and plasma testosterone concentration in males were studied throughout the year in wild caught Namaqua rock mouse, *Aethomys namaquensis* from southern Africa to evaluate the breeding period in the species. The number of Graafian follicles, corpora lutea and concentrations of plasma progesterone, and oestradiol-17β studied in 102 females collected over twelve months, were significantly higher between September and early March. Gravid and lactating females were captured between October and early March. No lactating females were found between April and September. A total of 88 males were collected during one calendar year. Testicular mass expressed against body mass, testicular volume, seminiferous tubule diameter, and circulating plasma testosterone concentration were significantly higher between September and February. No testicular activity was observed between March and September. These data suggest that the Namaqua rock mouse is a seasonal breeder with reproduction confined to the rainy summer months of the southern hemisphere during which nutritional supplies are abundant.

INTRODUCTION

In most mammalian species, reproduction is timed to ensure that birth of the offspring occurs at the time of the year that maximizes growth and survival (Fitzgerald & McManus 2000). In some short-lived species however, reproduction may occur during any time of the year when favourable opportunities arise (Prendergast *et al.* 2001; Fitzgerald & McManus 2000). Reproduction in opportunistic breeding rodents such as the pouched mouse, *Saccostomus campestris*, may be controlled by more than one environmental cue such as food availability and not by photoperiod alone (Bernard & Hall 1995; Tinney *et al.* 2001).

Seasonally breeding rodents such as the four-stripped field mouse, *Rhabdomys pumilio* (Jackson & Bernard 1999), white-footed mouse, *Peromyscus leucopus* (Young *et al.* 2000), the desert pocket mouse, *Perognathus formosus* (Kenagy & Bartholomew 1981), the house mouse, *Mus musculus* (Hamilton & Bronson 1985), and the deer mouse, *Peromyscus maniculatus* (Nelson *et al.* 1992) rely mainly on photoperiod to regulate reproduction. In some seasonal breeders, such as the four-stripped field mice, breeding has been reported to occasionally occur during winter months (Young *et al.* 2000). Such cases confirm that reproduction in seasonal breeders may also be controlled by other environmental cues such as rainfall, temperature, and secondary plant compounds (Bronson 1989; Nelson *et al.* 1992; Pinter & Negus 1965; Beatley 1969; Perrin 1980; 1986).

Inhibition of reproduction during winter months may be due to harsh ambient temperatures, which determine individual thermoregulatory demands. Homeothermic animals, such as rodents need to maintain a near constant body temperature in order to survive. A decrease in body temperature increases the thermoregulatory requirements of

an individual and may subsequently decrease the amount of energy that may be available for reproduction (Bronson 1985; Bronson & Pryor 1983; Sicard *et al.* 1993). Other studies have shown that testicular development and spermatogenesis in some rodents, such as the desert pocket mouse, *P. formosus*, become depressed at high temperatures (33°C) and enhanced at temperatures between 13-23°C. Some mammals may cue in to ambient temperature for reproduction. As ambient temperature fluctuates in many environments, some small mammals avoid breeding when such changes reduce survival rates of offspring, and as a consequence, reproduction is postponed until favourable conditions return (Prendergast *et al.* 2001).

Although the Namaqua rock mouse is widely distributed in southern Africa, little is known about its reproductive biology in the subregion. The primary objective of this study is therefore to assess the pattern of reproduction in the Namaqua rock mouse in southern Africa.

MATERIALS AND METHODS

Trapping and handling of animals

Specimens were collected from Ezemvelo Nature Reserve (25° 41'S 28° 56'E in Mpumalanga Province, South Africa. Animals were trapped using Sherman traps with a mixture of peanut butter, syrup, oats, and fish oil used as bait. Animals were kept in polyurethane cages during transportation as well as in the laboratory. Mice pellets and water were provided *ad libitum*. Animals were given three days to acclimatize before being sacrificed and various reproductive data obtained.

Determination of reproductive status and processing of specimens

Females were examined for prominent teats and perforated vagina to established their reproductive status. Placental scars were also used to determine whether females had given birth prior to the time of capture. The number of embryos and offspring born during the period following capture and prior to processing time were recorded.

Animals were sacrificed in the laboratory using halothane anaesthetic. Body masses were obtained using a Mettler digital balance. Blood was obtained through exsanguinations from the heart, and was centrifuged at 3000 rpm for 15 minutes. The plasma fraction was stored at –20°C until analysis. The heart, kidneys, muscle, and liver were collected and stored in epindorf vials and frozen for subsequent mtDNA analysis. Ovaries and testes were removed and placed in Bouin's fluid for at least 24 hours before being rinsed and stored in 70% alcohol. Skulls were prepared using standard museum procedures.

Identification of species

Skulls were boiled for approximately 2 hours, cleaned using forceps, and the brain tissue washed out using disposable pipettes. Skulls were then placed in bleach diluted with water (1:1 ratio) for 30 minutes. Skulls were subsequently examined using a dissecting microscope.

Histology of ovaries

The histology of ovaries of 102 females was conducted following the guidelines of Ross *et al.* (1995) & Leeson *et al.* (1985). A standard sequential dehydration procedure was used to prepare tissues for embedding into paraffin wax. After embedding, ovaries were

serially sectioned using a rotary microtome. Each ovary was sectioned in its totality and mounted onto microscopic slides with albumin as an adhesive. Slides were placed into an oven for at least 24 hours or longer, stained in Ehrlich's haematoxylin, and counter stained in eosin. A Nikon digital camera (DMX 1200) was used to view, photograph, and to count follicles.

Follicles were differentiated as follows:

1. Primordial follicles were the smallest of all follicles found in the ovary. A single layer of flat follicular epithelial cells surrounded the oocyte. The follicles were located in the stroma of the cortex. The primordial follicles were surrounded by a single layer of squamous follicular cells with the outer surface of the follicular cells kept together by a basal lamina (Plate 3.1).
2. In primary follicles, the oocyte is enlarged and the cells that surround the follicle appeared cuboidal. The zona pellucida appearing between the oocyte and the adjacent follicular cells becomes more pronounced. Primary follicles were surrounded by one layer or several layers of cuboidal or columnar cells (Plate 3.1).
3. Secondary follicles were characterized by a fluid-containing antrum. As this fluid continues to accumulate among granulosa cells, the antrum becomes larger and forces the oocyte to be pressed towards the follicular edge. Multiple follicular layers surround the oocyte at this stage (Plate 3.2).
4. At the Graafian follicle stage, the antrum does not increase any further. The oocyte is pressed to one edge of the antrum and held in place by cumulus

oophorus cells, whereas the antral lumen is surrounded by membrana granulosa (Plate 3.3).

5. Follicles that do not reach maturity are removed from the ovary by follicular "atresia" (Plate 3.4).
6. When the oocyte is expelled during ovulation, a corpus hemarrhagicum is formed (Plate 3.5).
7. During fertilization or when ovulation has taken place, the product is the corpus luteum. This is the source of progesterone during the early stages of pregnancy (Plate 3.6).
8. The corpus luteum lasts for the duration of pregnancy but begin to degrade to form a corpus albican (Plate 3.7).

Histology of testes

All the testes were sectioned, mounted, and stained following Ross *et al.* (1995). In order to determine the diameters of seminiferous tubules, several sections of the testes with circular tubules were selected and photographs taken at 200x and 400x magnification using a Nikon digital camera (DMX 1200). Diameters were determined using Image Tools software version 3.00. Following Ross *et al.* (1995), all male specimens were examined for signs of spermatogenesis, spermatozoa and stages of testicular development during the twelve months study period, leading to the following observations:

1. Seminiferous tubules were lined with seminiferous epithelium, which consisted of supporting cells of Sertoli and spermatogenic cells. Different stages of spermatogenic activity were observed at different times of the

twelve-month study period depending on stages of testicular development (Plate 3.8).

2. The epididymides consisted of the ductuli efferens that forms the head, the convoluted body, the tail, and the excretory ductus deferens. All male specimens were examined for the presence of spermatozoa in the epididymis during the twelve-month study period (Plate 3.9).

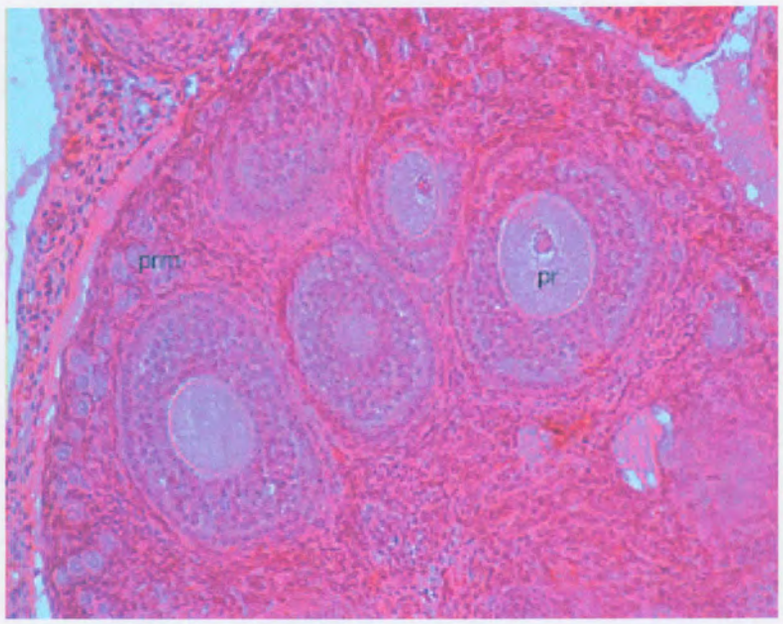

Plate 3.1. Primordial (prm) and primary (pr) follicles from a female Namaqua rock mouse ovary. Primordial follicles were located in the cortex and are the smallest follicles found in the ovary.

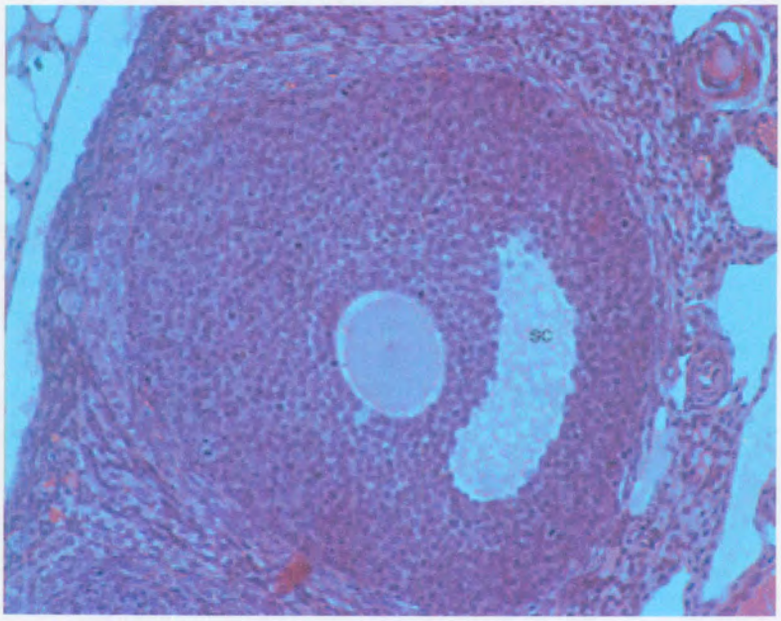

Plate 3.2. Secondary (sc) follicle from a female Namaqua rock mouse ovary. A fluid containing antrum is seen within the granulosa or follicular cells

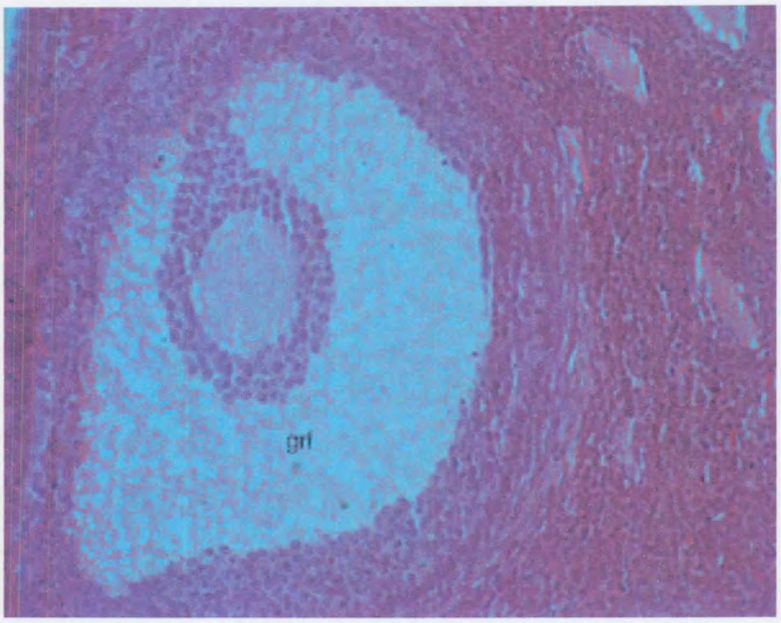

Plate 3.3. Graafian (grf) follicle from a female Namaqua rock mouse ovary. Antrum has reached almost maximum size and an increase in size is minimal at this stage.

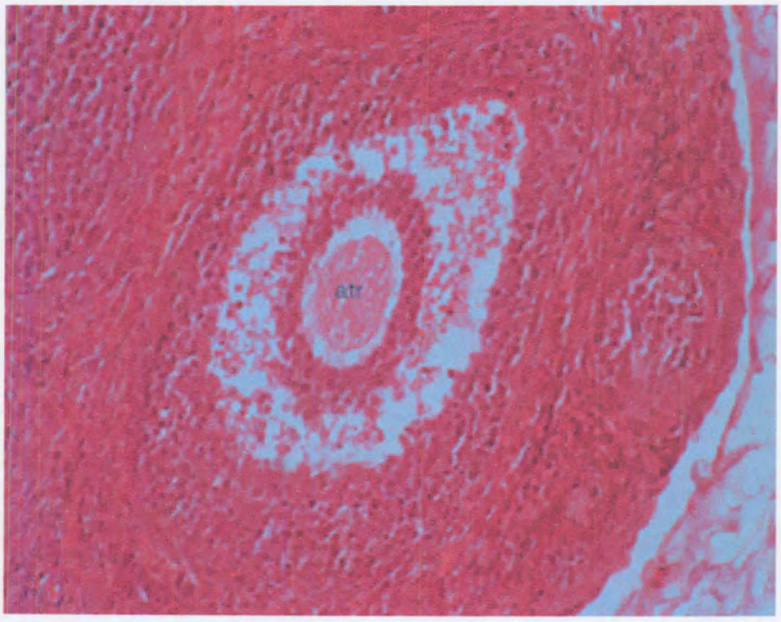

Plate 3.4. Atretic (atr) follicle from a female Namaqua rock mouse ovary. Excessive follicles are removed from the ovary through atresia.

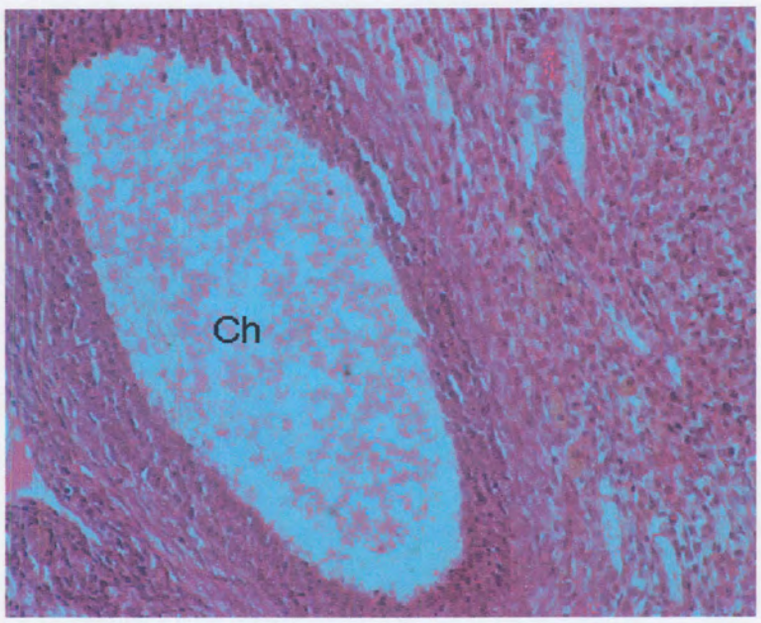

Plate 3.5. Corpus hemorrhagicum (Ch) from a female Namaqua rock mouse ovary. Corpus hemorrhagicum is produced after ovulation.

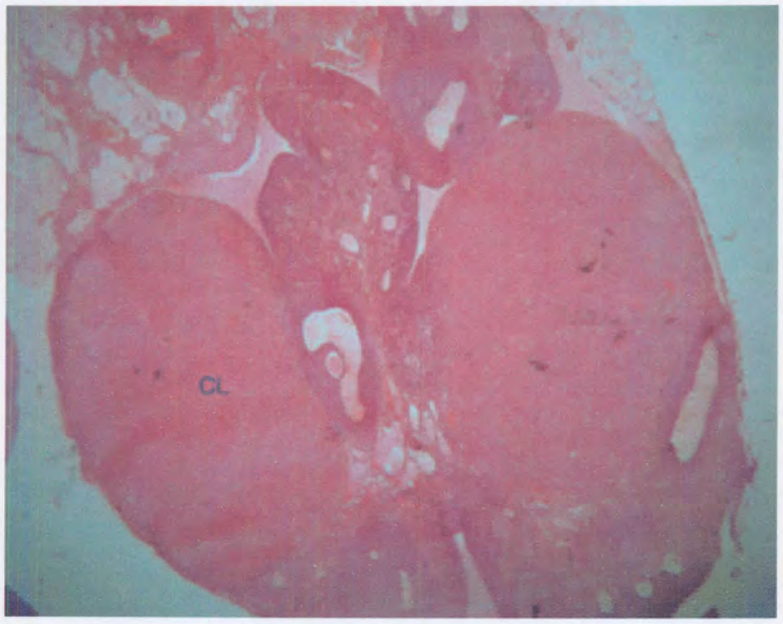

Plate 3.6. Corpus luteum (CL) from a female Namaqua rock mouse ovary. CL becomes the source of progesterone during early stages of pregnancy.

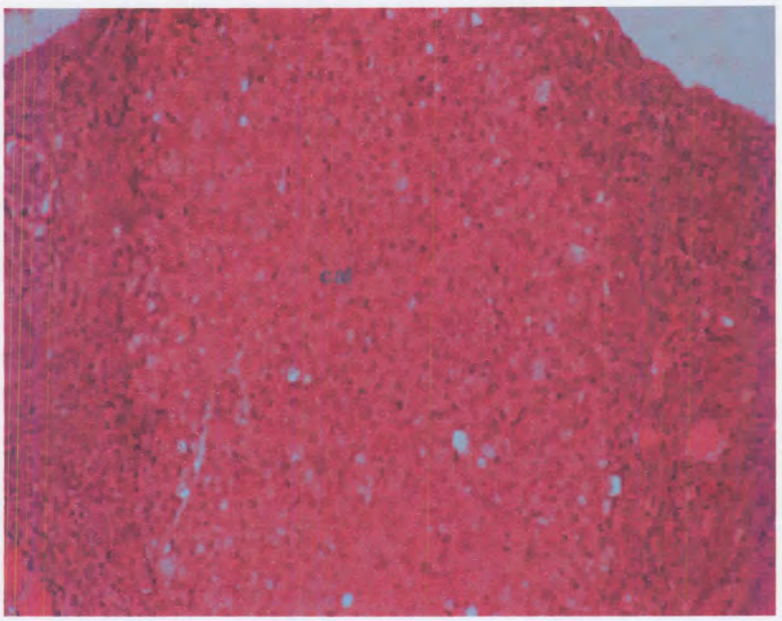

Plate 3.7. Corpus albican (cal) from a female Namaqua rock mouse ovary. A corpus albican is formed when a corpus luteum degenerates.

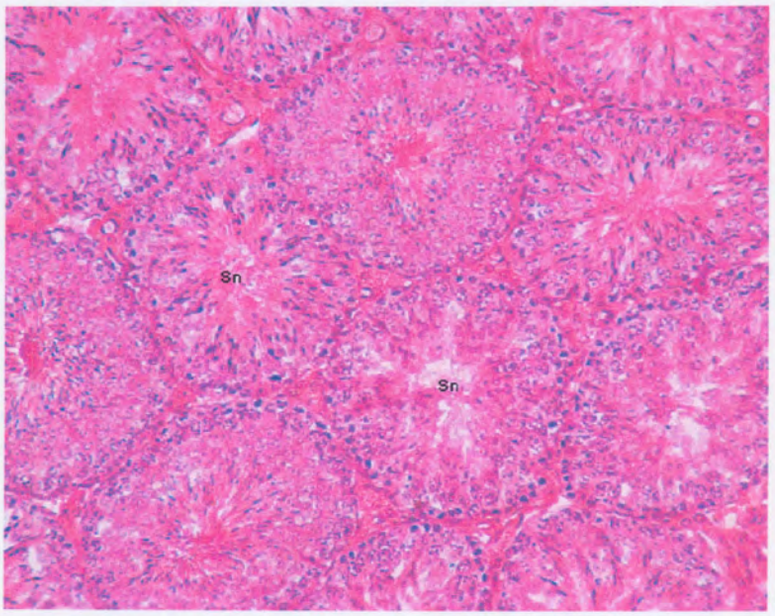

Plate 3.8. Seminiferous tubules (Sn) from a male Namaqua rock mouse testis. Spermatozoa can be seen as their heads point towards the seminiferous tubule epithelial cells (spermatogenic cells).

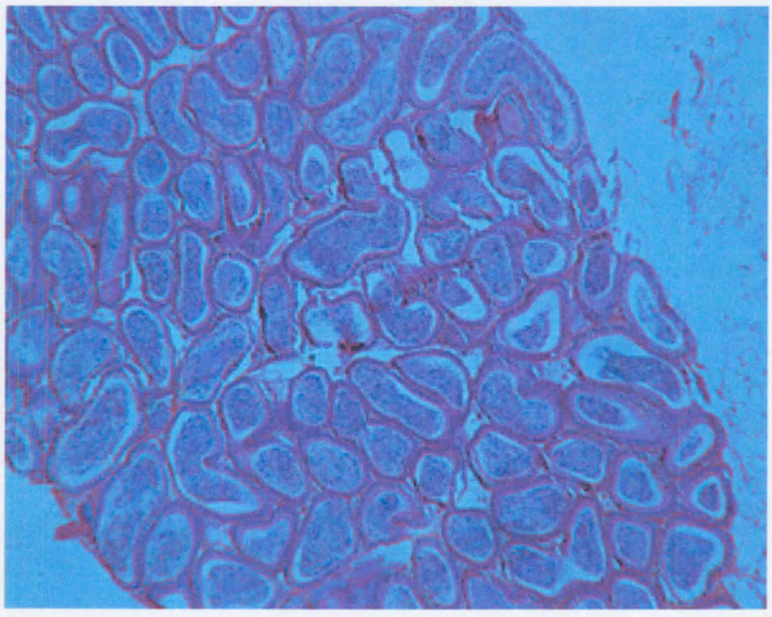

Plate 3.9. Epididymides from a male Namaqua rock mouse testis filled with spermatozoa.

Reproductive hormones

Progesterone

Progesterone concentration was determined in 102 females that were collected over a 12-month period, using a Coat-A-Count progesterone kit (Diagnostic Products Corporation, USA), a solid-phase radioimmunoassay method. The antiserum is highly specific to the naturally occurring steroids with a cross reactivity of less than 0.5%. It has a cross reactivity of 2.0% and 2.4% with 20-α-dihydroprogesterone and 11-deoxycortisol, respectively. Steroids were neither purified nor separated by chromatography.

Validation for progesterone

Following serial dilutions of plasma progesterone, parallelism was obtained between the serial dilutions curve and the standard samples curve. There was no significant difference between the two slopes (ANCOVA: $F = 4.44$; $P > 0.08$) (Fig. 3.1).

Oestradiol-17β

Oestradiol-17β was also determined in 57 females using Coat-A-Count oestradiol kit (Diagnostic Products Corporation, USA). The method is also a solid-phase radioimmunoassay that does not require extraction or separation of steroids. The antisera is highly specific for oestradiol-17β with very low cross reactivity with any other steroids present in the plasma.

Validation for oestradiol-17β

Serial dilution of plasma oestradiol-17β from a reproductively active female paralleled the reference preparation, thus the slopes did not differ significantly (ANCOVA: $F = 4.31$; $P > 0.17$) following log-logit transformation of the data (Chard 1987) (Fig. 3.2).

The intra and inter-assay coefficient of variation was 26.74% and the sensitivity of the assay was 2 pmol/L.

Testosterone

Testestorone was analysed in 88 males using a Coat-A-Count testestorone kit (Diagnostic Products Corporation, USA). This solid-phase radioimmunioassay does not require extraction or purification. Antiserum is highly specific for testosterone and has a very low cross reactivity with other compounds. Cross reactivity with dihydrotestosterone is less than 5%.

Validation of Testosterone

Serial double dilution of plasma testosterone was parallel to the standard curve of the reference preparation. The slopes of the two curves were not significantly different from each other (ANCOVA: $F = 5.56$; $P > 0.14$) following log-logit transformation (Chard 1987) (Fig. 3.3). The intra-assay coefficient of variation was 12.7% (n = 20) and the sensitivity of the assay was 1.39 nmols/l.

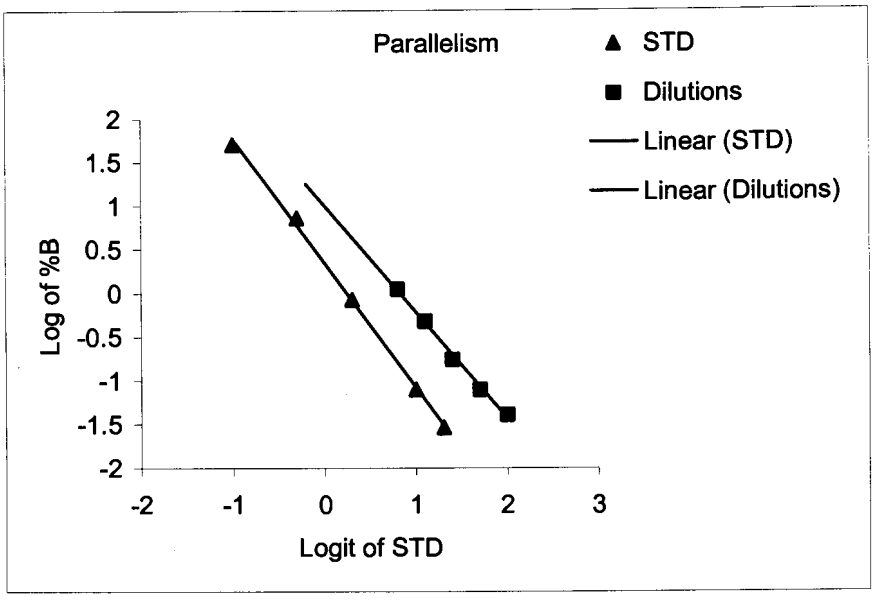

Fig. 3.1. Serial dilution of plasma progesterone (■) from a female Namaqua rock mouse, showing parallelism with a reference preparation curve (▲) thus validating the hormonal assay.

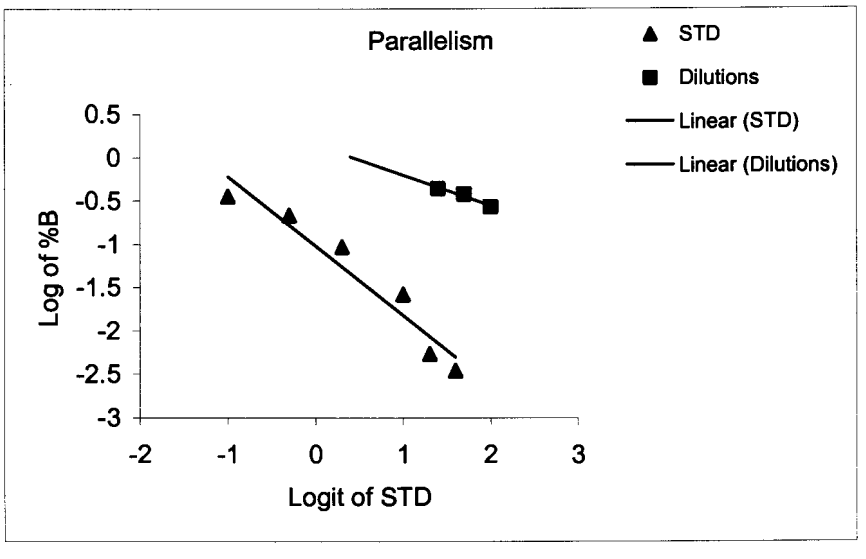

Fig. 3.2. Serial dilution of plasma oestradiol-17β (■) from a Namaqua rock mouse showing parallelism with a reference oestradiol-17β curve (▲) thus validating the hormonal assay.

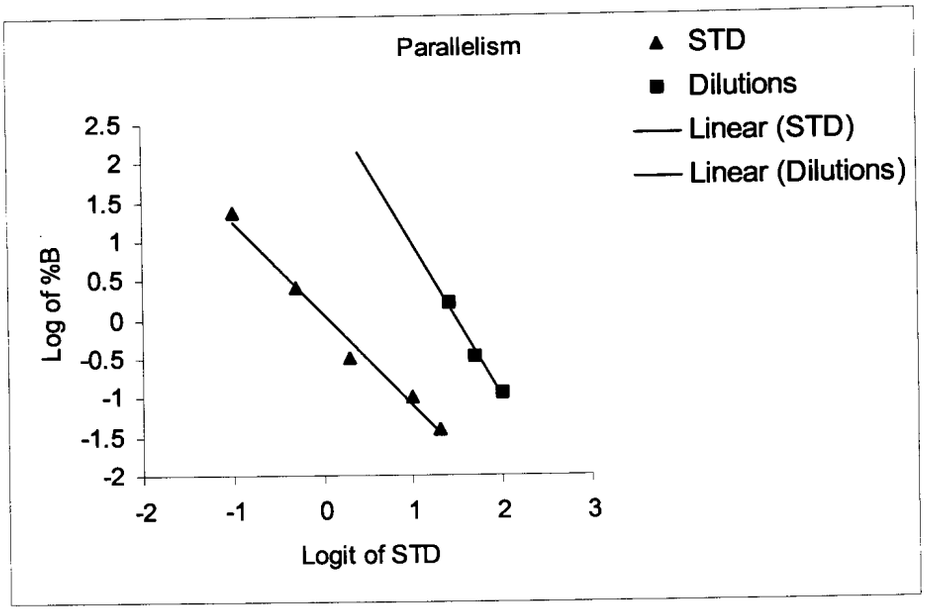

Fig. 3.3. Serial dilutions of plasma testosterone (■) from a Namaqua rock mouse, showing parallelism with a reference testosterone curve (▲) thus validating the hormonal assay.

Data Analysis

The data was analysed using Microsoft Excel™ spreadsheets and Statistica version 6.0™ Analysis of Covariance (ANCOVA) was used to validate hormonal assays and Tukey HSD test was used to test for differences in seminiferous tubule diameter, testicular mass, testicular volumes, and hormonal concentration between 12 months of the study period. A General Linear Model (GLM) was used to test for differences in follicular development between months.

RESULTS

The results are expressed as the mean ± S.E (standard error). Gravid and lactating females were observed between October and March. No gravid or lactating females were recorded between April and September. Placental scars were only found in females collected between December and February. No placental scars or animals with perforated vaginas were recorded between April and September (Table 3.1).

In both males and females, body mass was significantly higher during the breeding period (October-early March) and lower during the non-breeding period (mid-March-September) (Tukey HSD: $P < 0.05$) (Figs. 3.4 & 3.5).

Ovarian histology

Primordial follicles were recorded throughout the twelve-month study period. A peak in primordial follicles was observed in July and the numbers remained relatively low between August and June. Significant differences in the number of primordial follicles were found during July (GLM: $R^2 = 0.29$; $F = 13.27$; $P < 0.05$) but no significant differences were found between other months of the sampling period (Fig. 3.6).

Primary follicles were found throughout the twelve-month study period. The number of primary follicles was significantly lower between February and May (GLM: $R^2 = 0.28$; $F = 9.03$; $P < 0.000003$). There were no significant differences in the number of primary follicles between June and January (Fig. 3.7).

Secondary follicles were observed throughout the twelve-month study period. There was no distinct pattern in the distribution of secondary follicles and no significant differences were found in the number of secondary follicles during the twelve-month study period (GLM: $R^2 = 0.19$; $F = 1.87$; $P > 0.05$) (Fig. 3.8).

The number of Graafian follicles was significantly higher between September and February (GLM: $R^2 = 0.74$; $F= 39.76$; $P < 0.05$) and lower between March and August. No Graafian follicles were found during May (Fig. 3.9).

Corpora lutea were significantly more abundant between September and March (GLM: $R^2 = 0.41$; $F = 22.25$; $P < 0.05$). No corpora lutea were found between April and August (Fig. 3.10). Corpora hemorrhagicum were observed during spring, summer and autumn with a peak in October. No corpora hemorrhagicum were found between May and August (Fig. 3.11). Corpora albicans were found in March and in May but not in other months of the study period (Fig. 3.12).

Atretic follicles were found throughout the sampling period and were significantly higher between May and August (GLM: $R^2 = 0.16$; $F = 6.07$; $P < 0.05$) and were low between September and April (Fig. 3.13).

Testicular histology

Testicular histology showed that the size of seminiferous tubules was significantly larger between August and February (Tukey HSD: $P < 0.05$) and lower between March and July

(Fig. 3.14). Spermatozoa in epididymides and spermatogenic activities in tubules were observed between September and February. No spermatozoa or spermatogenic activity was observed between March and August in the epididymides and seminiferous tubules, respectively.

Testicular mass and volume

Testicular mass expressed against body mass was significantly higher between September and February (Tukey HSD: $P < 0.05$) and was lower between March and August (Fig. 3.15). Testicular volume was also significantly higher between September and February and lower between March and August (Tukey HSD: $P < 0.05$) (Fig. 3.16). The trend in testicular mass and volume coincided with plasma testosterone concentration changes.

Table 3.1. Number of embryos and percentage of lactating *Aethomys namaquensis* females collected during one calendar year.

	J	F	M	A	M	J	J	A	S	O	N	D
Total number of embryos	23	30	3	0	0	0	0	0	0	14	16	13
% Lactating ♀s	100	91	11	0	0	0	0	0	0	85	97	99

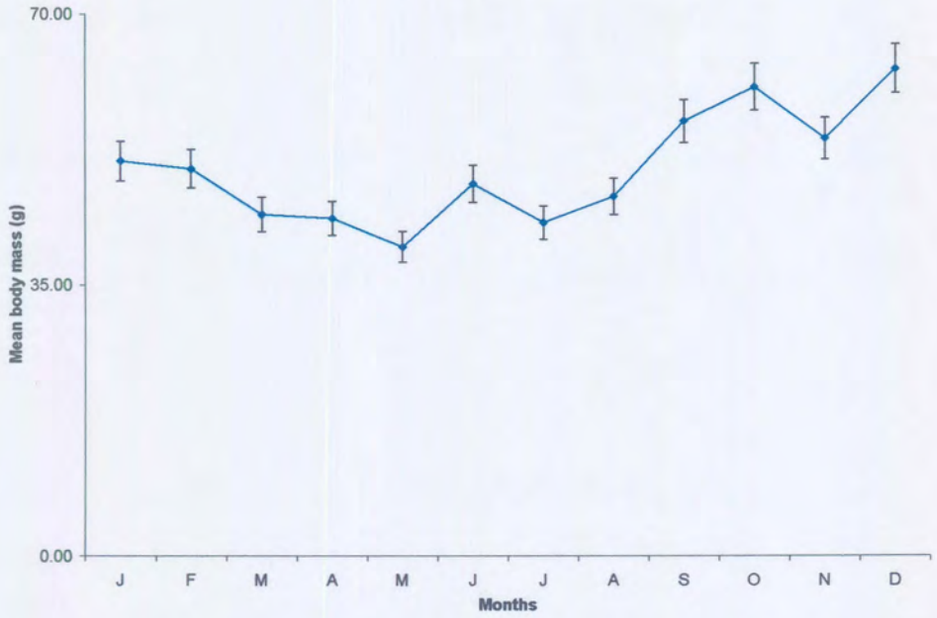

Fig. 3.4. Mean body mass ± S.E. of Namaqua rock mouse males over a twelve-month period. Body mass was significantly higher during the breeding season and lower during the non-breeding season.

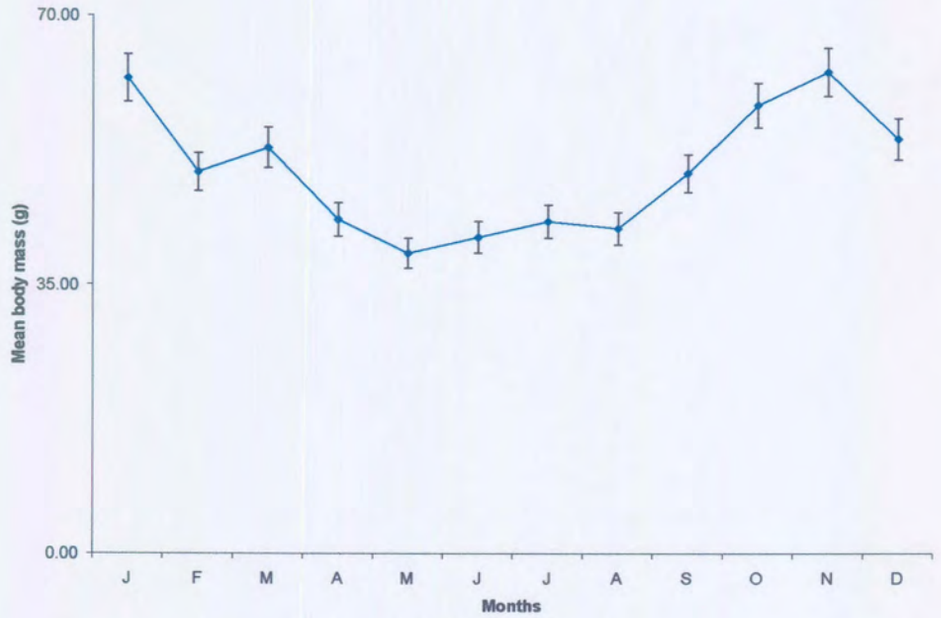

Fig. 3.5. Mean body mass ± S.E. of Namqua rock mouse females over a twelve-month period. Body mass was significantly higher during the breeding period, and lower during the non-breeding period.

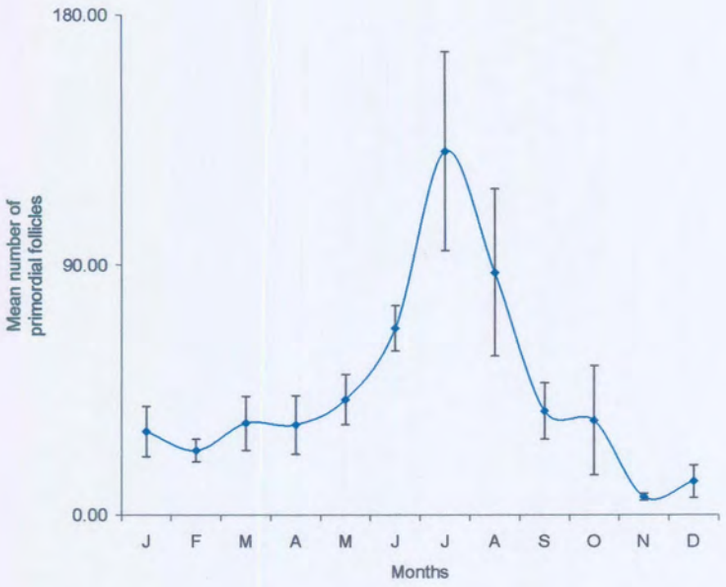

Fig. 3.6. Mean number ± S.E. of primordial follicles in the ovaries of the Namaqua rock mouse females during a twelve-month period. A peak is observed during July, two months before the start of the breeding season.

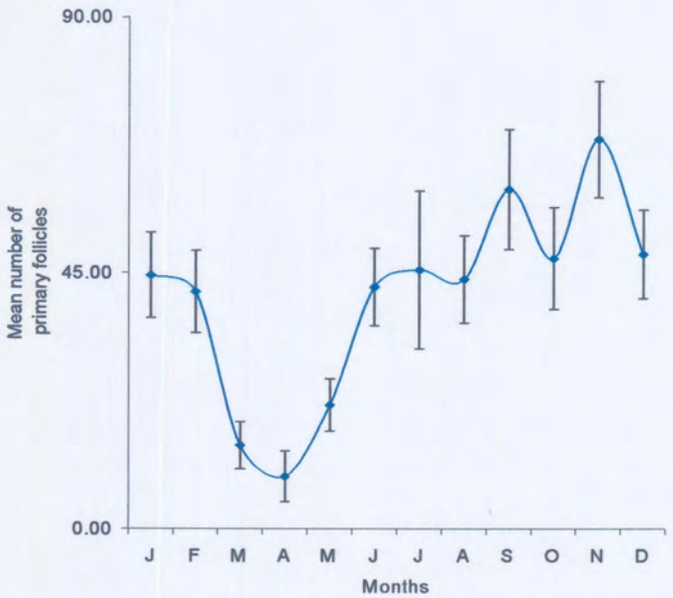

Fig. 3.7. Mean number ± S.E. of primary follicles in the ovaries of the Namaqua rock mouse females during a twelve-month period. Primary follicles remained numerous between July and February, but lower between March and May.

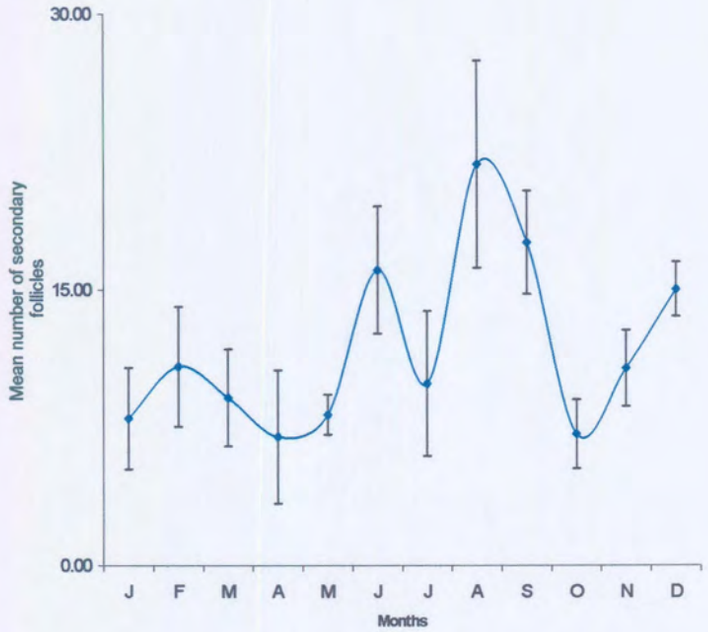

Fig. 3.8. Mean number ± S.E. of secondary follicles in the ovaries of the Namaqua rock mouse females during a twelve-month period. No significant differences in the mean number of secondary follicles were found between the months.

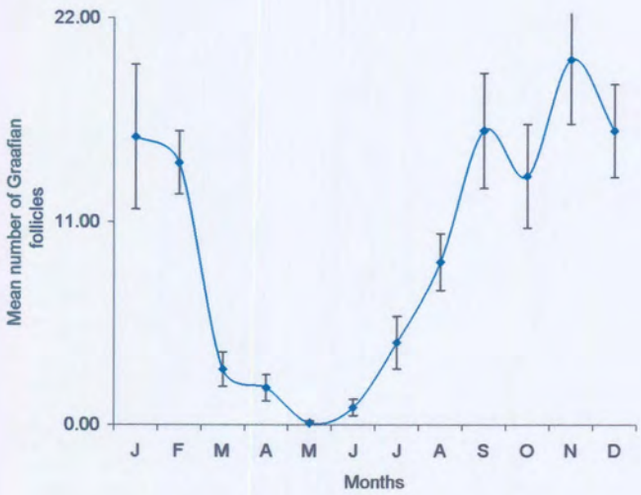

Fig. 3.9. Mean number ± S.E. of Graafian follicles in the ovaries of the Namaqua rock mouse females during a twelve-month period. Graafian follicles were higher during the breeding period, and lower during non-breeding period.

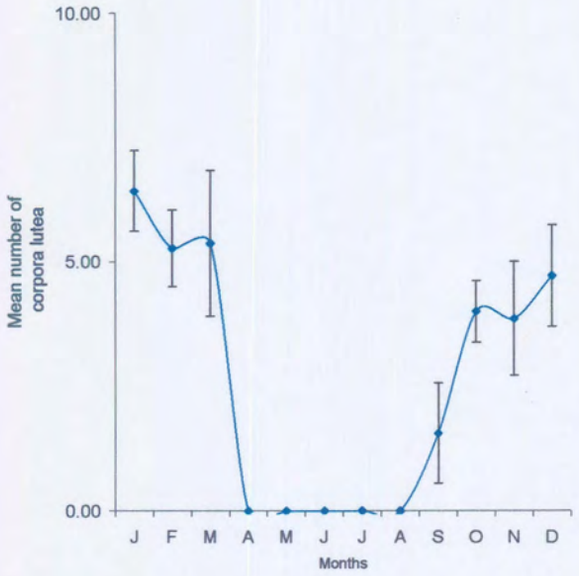

Fig. 3.10. Mean number ± S.E. of corpora lutea from the ovaries of the Namaqua rock mouse females during a twelve-month period. Corpora lutea are absent during the non-breeding period.

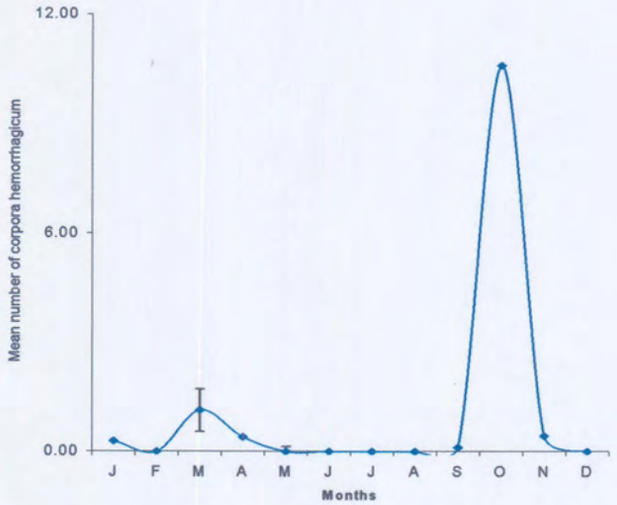

Fig. 3.11. Mean number ± S.E. of corpora hemorrhagicum in ovaries of the Namaqua rock mouse females during a twelve month period. A peak is observed during the start of the breeding period.

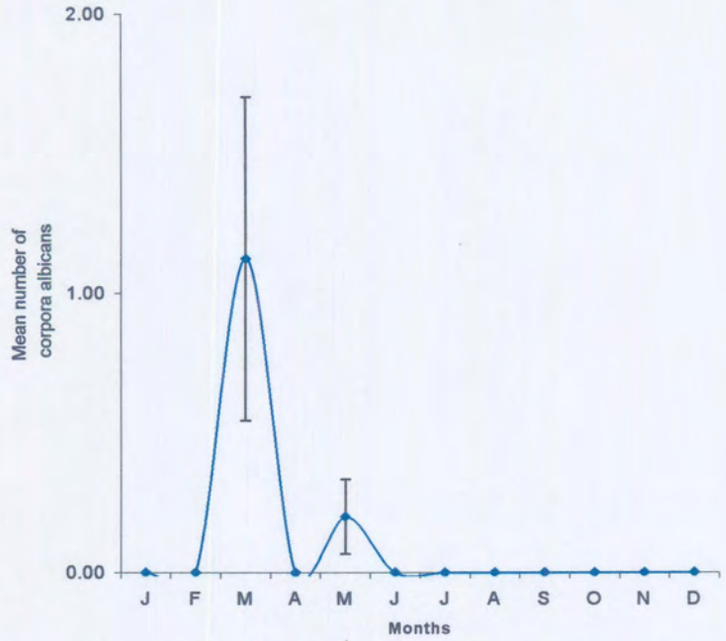

Fig. 3.12. Mean number ± S.E. of corpora albicans observed in the Namaqua rock mouse females during a twelve-month period. A peak is observed at the end of the breeding period.

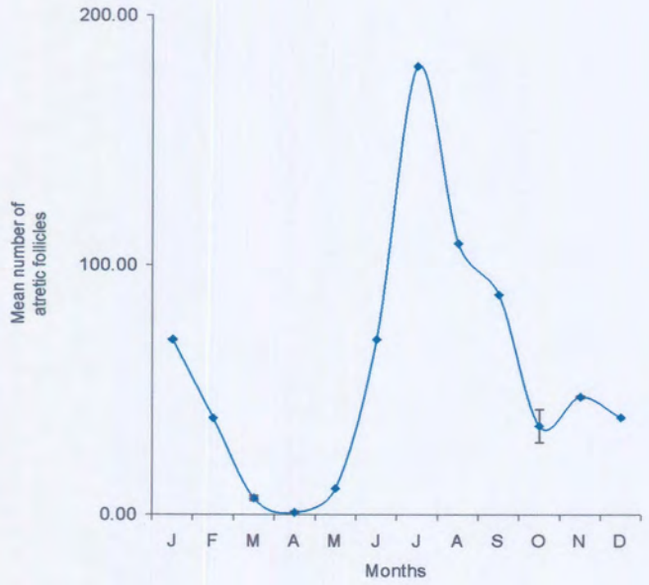

Fig. 3.13. Mean number ± S.E. of atretic follicles in the Namaqua rock mouse ovaries during a twelve-month period. A peak is observed during July which coincides with a peak observed in primordial follicles.

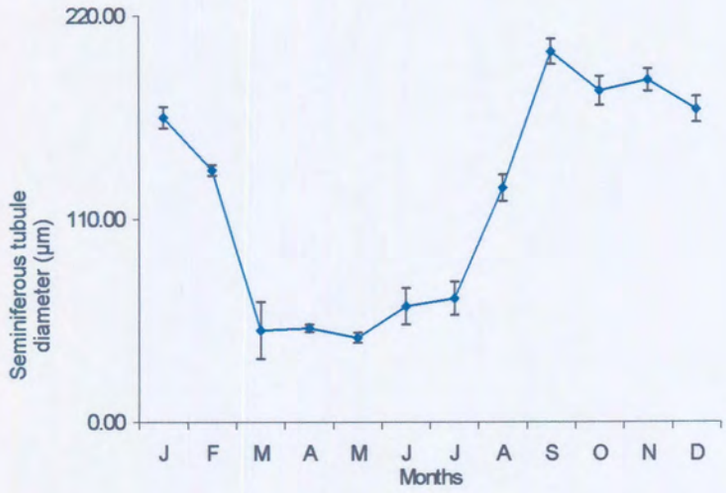

Fig. 3.14. Mean seminiferous tubules diameter ± S.E. in the Namaqua rock mouse males during a twelve-month period. Seminiferous tubule diameters are higher during the breeding season, and lower during the non-breeding period.

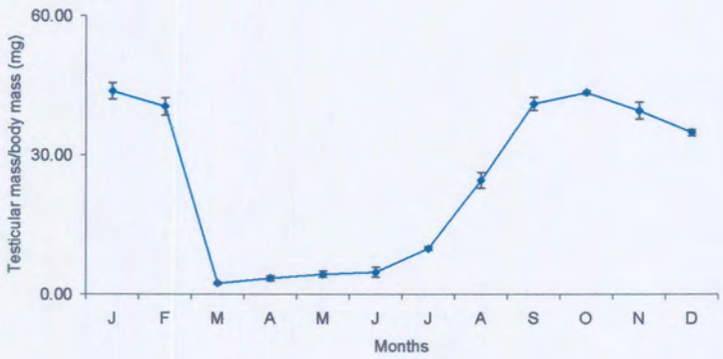

Fig. 3.15. Mean testicular mass per body mass ± S.E. in the Namaqua rock mouse males during a twelve-month study period. Testicular mass per body mass remained higher during the breeding season and lower during the non-breeding period.

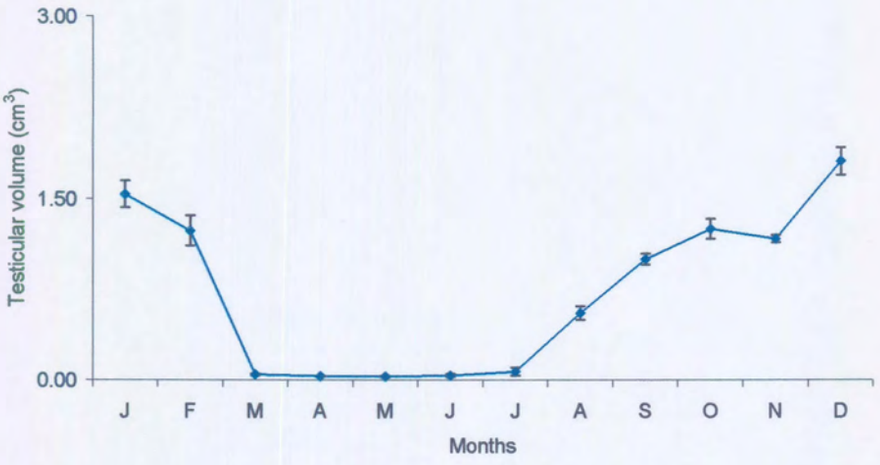

Fig. 3.16. Mean testicular volume ± S.E. in the Namaqua rock mouse males during a twelve-month period. Testicular volume remained higher during the breeding period, and lower during non-breeding period.

Female reproductive hormones

Plasma progesterone concentration showed a distinctive pattern. Progesterone concentration was significantly higher between October and March (GLM: $R^2 = 0.34$; $F = 16.72$; $P < 0.05$) and lower between April and September (Fig. 3.17). A similar pattern was also observed in plasma oestradiol-17β concentration in which it was significantly higher between October and March (GLM: $R^2 = 0.14$; $F = 5.29$; $P < 0.002$) and lower from April to September (Fig. 3.18).

Male reproductive hormone

Plasma testosterone was significantly higher between September and March (GLM: $R^2 = 0.15$; $F = 4.81$; $P < 0.004$), and lower between April and August (Fig. 3.19). Plasma testosterone concentration coincides with the changes in seminiferous tubule sizes, testicular mass per body mass, and testicular volume.

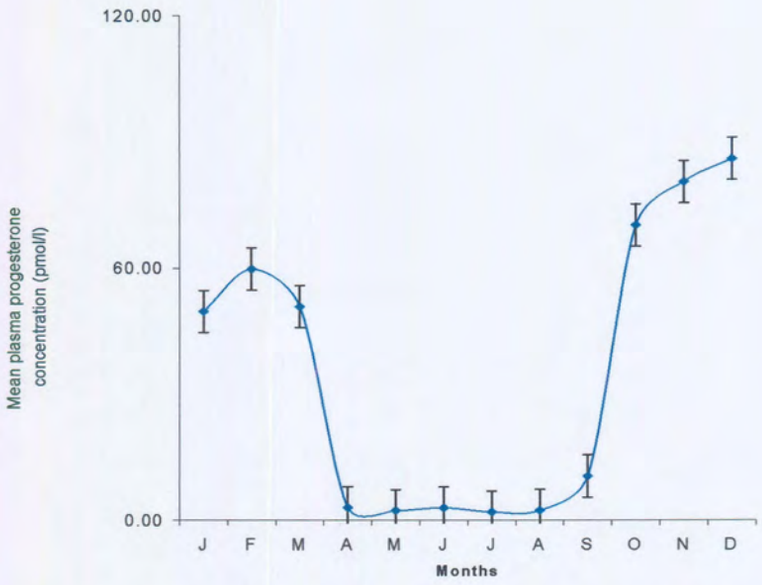

Fig. 3.17. Mean plasma progesterone concentration ± S.E. of the Namaqua rock mouse females during a twelve-month period. Circulating progesterone concentration was higher during the breeding season, and lower during the non-breeding period.

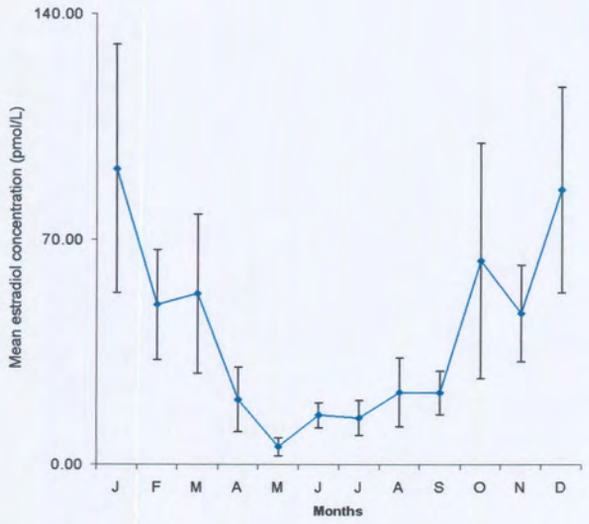

Fig. 3.18. Mean plasma oestradiol-17β concentration ± S.E. in the Namaqua rock mouse females during a twelve-month period. Oestradiol-17β was higher during the breeding period, and lower during the non-breeding period.

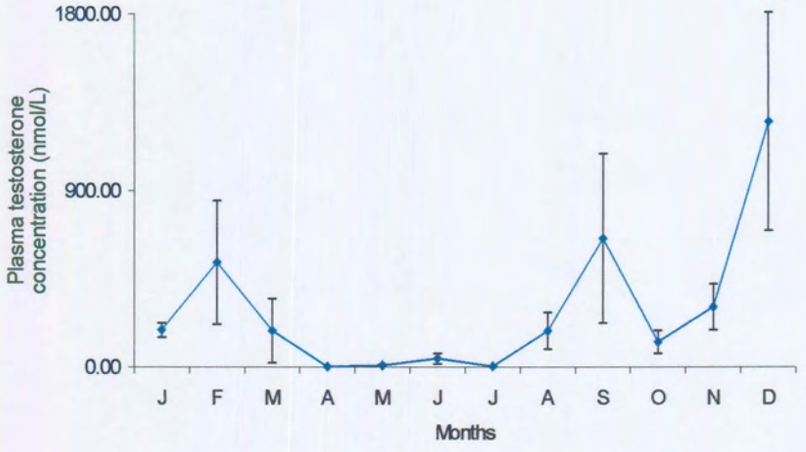

Fig. 3.19. Mean plasma testosterone concentration ± S.E. in the Namaqua rock mouse males during a twelve-month period. Circulating plasma testosterone was higher during the breeding season, and lower during the non-breeding period.

DISCUSSION

Contrary to the suggestion by Shortridge (1934), the Namaqua rock mouse is a seasonal breeder, with the breeding season starting from October and ending into March. According to Smithers (1971), gravid females in Botswana were observed between January and May. In this study, no gravid females were observed between April and September.

Gravid females, placental scars, and lactating females were observed between October and March. No gravid or lactating females were observed between April and September. This suggests that the Namaqua rock mouse breeds from mid-spring through to the beginning of autumn.

Follicular development of primordial, primary, and secondary follicles was observed throughout the twelve-month study period. The primordial follicles displayed a peak in July, suggesting the initiation of gonadal recrudescence in females two months prior to the breeding period. Primary follicles were higher during the breeding period and dropped steadily at the end of the breeding period, and this trend was also observed in secondary follicles. This trend of follicular development supports Clark (1981) who reported that regression of follicular development should be apparent in small mammals exhibiting a clear-cut seasonal reproduction.

Graafian follicles displayed a clear-cut pattern that is characteristic of seasonal breeding mammals. Graafian follicles increased significantly in number towards the beginning of the breeding season and dropped to very low levels at the end. Corpora lutea were only found during the breeding season and were not found during winter months. The presence of Graafian follicles and Corpora lutea only during specific

months of the year, strongly implies that the Namaqua rock mouse is a seasonal breeder which exhibits a breeding period from September through to March. Unlike the Tete veld rat (Chapter 2), the Namaqua rock mouse does not show any possibility of breeding during this period since gonadal recrudescence appears to be completely inhibited during winter months. A halt in breeding during winter months may allow the animal to direct its energy supplies towards other physiological needs, such as cellular maintenance, thermogenesis, and mobility (Bronson 1989; Jameson 1988; Klein & Nelson 1999). Support for a confined and distinct breeding season is confirmed by the prevalence of Corpus hemorrhagicum during the beginning of the breeding season, and corpus albicans during the end of the breeding season.

Circulating plasma progesterone concentrations were higher between September and March. This suggests that ovulation and fertilization in the Namaqua rock mouse take place during this time of the year. Very low levels of plasma progesterone were recorded during winter months and this supports the histological findings of reduced or inactive ovarian activity. The plasma oestradiol-17β concentrations mirrored the findings of progesterone analysis suggesting that seasonality of reproduction is confined to the rainy summer months of the year. The concommitant increase of oestradiol-17β with progesterone supports Bedford *et al.* (1972) who reported that the concentration of these hormones remains relatively high during pregnancy.

Testicular histology displayed a seasonal reproductive pattern as was the case with ovarian follicular development. Seminiferous tubule diameters were greater during the breeding season suggesting active spermatogenesis during this period. These observations are similar to those reported in other seasonal breeders such the Australian

bush rat, *Rattus fuscipes* (Irby *et al.* 1984), the Rock elephant shrew, *Elephantulus myurus* (Woodall & Skinner 1989), and the European rabbit, *Oryctolagus cuniculus* (Boyd & Myhill 1987). A lack of spermatogenesis between March and August is indicative of reproductive quiesence during this period of the year. Testicular mass and volume were significantly higher between September and February, reflecting a seasonally breeding period. Testicular recrudesence in most seasonal breeding animals is controlled by photoperiod (Prendergast *et al.* 2001; Flowerdew 1987; Bronson 1989; Young *et al.* 2000; Hastings *et al.* 1985) which appears to be one of the cues to male reproduction in the Namaqua rock mouse (see Chapter 4).

Testosterone concentration reflects the seasonal breeding pattern in the Namaqua rock mouse. This profile coincides with specific testicular mass and volume, seminiferous tubule sizes in the males, and ovarian, and hormonal profiles of females. The Namaqua rock mouse does not reproduce in winter months. This may be attributed to several factors such as nutrition, ambient temperature, and perhaps social conditions (Bronson 1984, 1989; Bronson & Perrigo 1987; Harvey & Purvis 1999; Nilsson 2001).

In conclusion, the Namaqua rock mouse is a seasonal breeder, exhibiting a breeding season that extends between October and March. Reproduction during mid-autumn and winter months is inhibited. The winter inhibition of reproduction may be due to nutrional deficiencies, harsh ambient temperatures, and the lack of rain during this time of the year. Since these rodents are granivores (Smither 1971) and feed on seeds (Shortridge 1934) nutrition could potentially be the main environmental factor that inhibits reproduction between mid-March and September in South Africa.

Observing the variation in the reproductive pattern in this study to what was observed in Botswana, requires further research to be conducted to evaluate the reproductive patterns of this species in other parts of the subregion. Geographic variation may become apparent and with possibilities of subspecies, the biology of reproduction within the Namaqua rock mouse could be valuable in understanding taxonomic complexities in species.

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CHAPTER 4

Photoperiodic responsiveness in the Tete veld rat, *Aethomys ineptus* and in the Namaqua rock mouse, *A. namaquensis* (Rodentia: Muridae) from southern Africa

ABSTRACT

Five male Tete veld rats, *A. ineptus* and eight male Namaqua rock mice, *A. namaquensis* were independently placed on a long photoperiod of 16L:8D and on a short photoperiod of 8L:16D for three months. This was done in order to determine the effect of photoperiod on testicular function based on testicular mass per body mass, testicular volume, seminiferous tubule diameters and changes in circulating plasma testosterone. On long day length, both species exhibited significantly higher testicular mass per body mass, higher testicular volume, and larger seminiferous tubule diameters. Plasma testosterone concentrations in the Tete veld rats were not significantly different between the two treatments, but the Namaqua rock mice showed significantly higher testosterone concentrations in males on long day than on short day length. This suggests that both the Tete veld rat and the Namaqua rock mouse are photoperiodically responsive to long days.

INTRODUCTION

Photoperiod is a major regulating factor controlling the timing of reproduction in many mammalian species. Many rodents cease breeding as the day length becomes shorter at the end of summer and the onset of autumn (Prendergast *et al.* 2001). Effects of photoperiod on reproduction have been investigated in many rodents such as in the Fischer 344 rat, *Rattus norvergicus F344* (Heideman & Sylvester 1997; Heideman *et al.* 1998), the California vole, *Microtus californicus* (Nelson *et al.* 1983), the vole, *Microtus agrestis* (Spears & Clark 1986), the four-stripped field mouse, *Rhabdomys pumilio* (Jackson & Bernard 1999), and the white-footed mouse, *Peromyscus leucopus* (Young *et al.* 2000).

Increasing day length coincides with the beginning of spring and reproductive recrudescence in seasonally breeding animals begins during this period in order to maximize the survival of the offspring (Jameson 1988, Flowerdew 1987). Survival and maximal growth rates of juveniles can be achieved during sufficient nutritional supplies (Vandenbergh *et al.* 1972; Irby *et al.* 1984; Nilsson 2001; Tinney *et al.* 2001), and favourable ambient temperatures (Benson & Morris 1971; Bronson & Pryor 1983; Bronson 1989), conditions of which are associated with spring and summer months of the year.

Although photoperiod is a major ultimate cue that controls reproduction in many animals, it has recently emerged that animals do not only rely on photoperiod but also on essential changes in environmental conditions (Prendergast *et al.* 2001). These may include proximate factors such as food availability, ambient temperature, and social factors (Anand *et al.* 2002). Some animals such as the California vole may

opportunistically stimulate gonadal development or prevent gonadal regression on short days when food is adequate (Nelson *et al.* 1983), whereas low humidity stimulates gonadal development in Kusu rats, *Arvicanthis niloticus* (Sicard *et al.* 1993).

Some rodent species such as the pouched mouse, *Saccostomus campestris,* mainly breed in summer during which reproduction is controlled by photoperiod. However, reproduction during winter appears to be inhibited by food (Tinney *et al.* 2001). Similar observations on the four-stripped mouse were reported by Jackson & Bernard (1999) when the inhibition of reproduction during winter was prevented by factors other than photoperiod. These studies confirm that although photoperiod is a major cue controlling reproduction, other factors may also be simultaneously influential together with day length. However, given that essential changes in environmental conditions vary widely, it may be possible that their predictability may be less reliable than photoperiod.

The aim of this study is to assess the effect of photoperiod on reproduction in the Tete veld rat, *Aethomys ineptus* and the Namaqua rock mouse, *Aethomys namaquensis* under controlled laboratory conditions.

MATERIALS AND METHODS

Trapping and handling of animals

A total of 10 male, *A. ineptus* and 16 male, *A. namaquensis* were caught using Sherman traps at Roodeplaat Dam Nature Reserve (25° 34'S 28° 22'E) in Gauteng Province and Ezemvelo Nature Reserve (25° 41'S 28° 56'E) in Mpumalanga Province, South Africa. A mixture of peanut butter, syrup, oats meal, and fish oil were used as bait. The animals were kept in polyurethane cages and wood shavings were provided as bedding. Five male Tete veld rats were placed on 16L:8D, and five on 8L:16D for 90 days, while two

groups of eight male Namaqua rock mice were subjected to the same experimental treatments. Mice pellets and water were provided *ad libitum* for the duration of the photoperiodic treatments.

Processing of specimens

After 90 days, animals were sacrificed using overdose of halothane anaesthetic and body mass obtained using a Mettler digital balance. Blood was obtained through exsanguinations from the heart and was centrifuged at 3000 rpm for 15 minutes. The plasma fraction was stored at –20°C until analysis, while the testes were removed and weighed, and the length and width of each measured using a pair of digital calipers. Testicular volume was calculated using the formula for the volume of an ellipsoid following Woodall and Skinner (1989) as follows:

$$V = 4/3\ \pi ab^2$$

Where: a = 1/2 maximum length and b = 1/2 maximum breadth.

Gonads were then placed into Bouin's fluid for at least 24 hours before being rinsed and stored in 70% alcohol. Skulls were prepared using standard museum techniques.

Identification of species

Skulls were boiled for approximately 2 hours and subsequently cleaned using forceps and blades, and brain tissue washed out using disposable pipettes. The skulls were placed in bleach (1:1 ratio of bleach to water) for 30 minutes and thereafter examined using a dissecting microscope. DNA sequences of Roodeplaat Dam Nature Reserve samples were obtained using the service of a molecular laboratory (Department of Genetics, University of Pretoria) to positively identify *Aethomys ineptus* which otherwise is

indistinguishable from its sibling species, *A. chrysophilus* based on external, cranial, and dental morphology.

Histology

All the testes were sectioned, mounted, and stained following the guidelines of Ross *et al.* (1995) and Leeson *et al.* (1985). In order to determine the diameters of seminiferous tubules, several sections of the testes with circular tubules were selected and photographed at 40x magnification using a Nikon digital camera (DMX 1200). Diameters were determined using Image Tools software version 3.00. Following Ross *et al.* (1995), all males were examined for signs of spermatogenesis, spermatozoa, and stages of testicular development.

Testosterone concentration

Plasma testosterone was analysed using a Coat-A-Count testesterone kit (Diagnostic Products Corporation, USA). The assay does not require any extraction or chromatography. Antiserum is highly specific for testosterone and has a very low cross reactivity with other compounds. Cross reactivity with dihydrotestosterone is less than 5%.

Validation of testosterone

Following serial double dilutions of plasma testosterone in both the Tete veld rat and the Namaqua rock mouse, parallellism were obtained between the standard curve within both species and that of the serial dilutions. For the Tete veld rat, there was no significant difference between the two curves (ANCOVA: $F = 0.05$; $P > 0.84$) (Fig. 4.1). A log-logit data transformation performed following (Chard 1987). The intra-assay and inter-

assay coefficient of variation was 5% and 9%, respectively. The sensitivity of the assay was 0.92 nmol.l^{-1}. Similarly, there was no significant difference between the serial dilution and the standard curve (ANCOVA: $F = 5.56$; $P > 0.14$) (Fig. 4.2) in the Namaqua rock mouse.

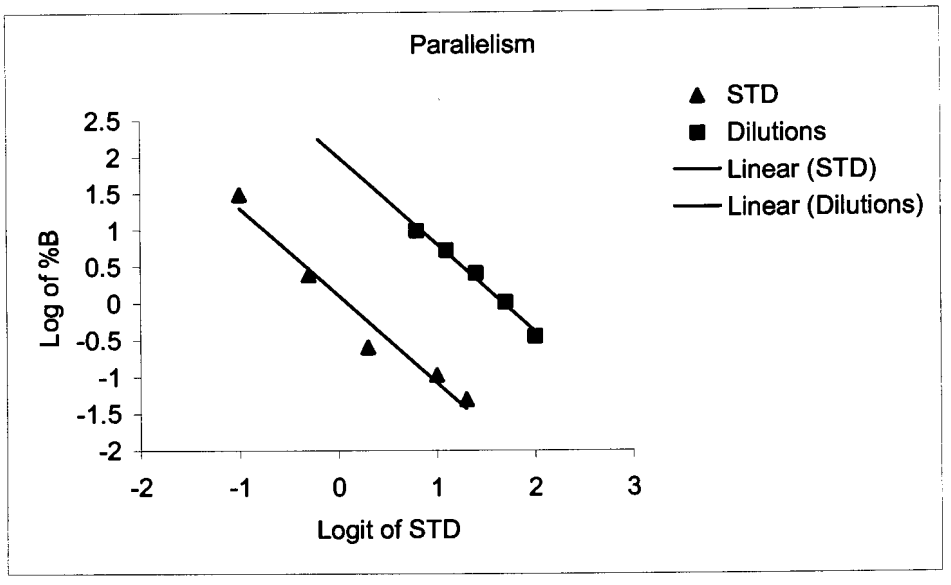

Fig. 4.1. Serial dilutions of testosterone from the Tete veld rat (■) showing parallelism with a reference preparation curve (▲) thus validating the hormonal assay.

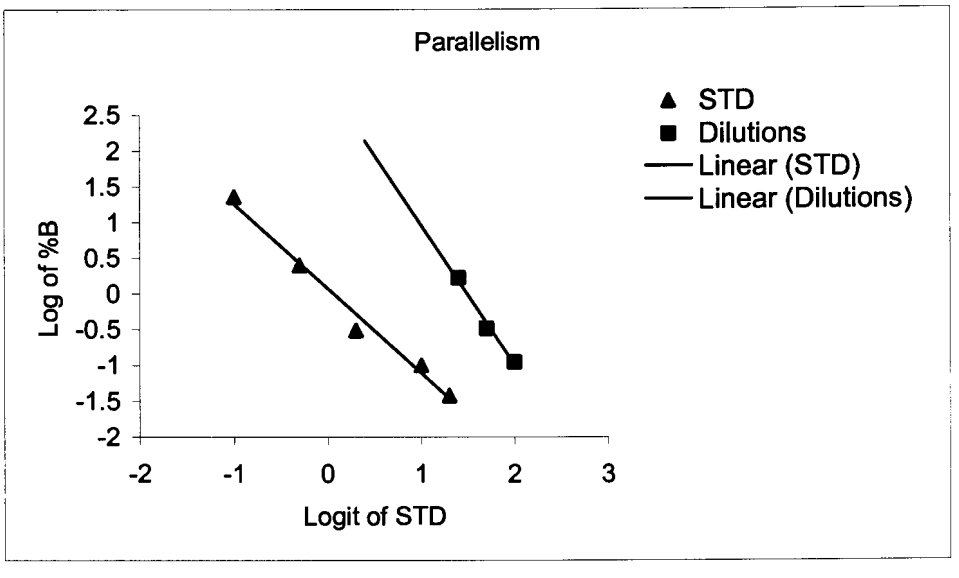

Fig. 4.2. Serial dilutions of testosterone from the Namaqua rock mouse (■) showing parallelism with a reference preparation curve (▲) thus validating the hormonal assay.

Data analysis

The data was analysed using Microsoft Excel™ spreadsheets and Statistical version 6.0™. Analysis of Covariance (ANCOVA) was used to validate hormonal assays and analysis of variance (ANOVA) was used to test for differences between males on long and short photoperiods. General Linear Model (GLM) was used to test for differences in seminiferous tubule diameter, testicular mass, testicular volume, and testosterone concentration.

RESULTS

The Tete veld rat

There was no significant difference in body mass between animals maintained on a long day length (LD) and those on a short day length (SD) (ANOVA: LD = 111.0 g ± 4.15; (n = 5); SD = 110.0 g ± 5.00 (n = 5); F = 0.0009; P > 0.98) (Fig. 4.3).

Testicular mass and volume, and seminiferous tubule diameter

Testicular mass expressed as a function of body mass was significantly higher for males on LD than males on SD (ANOVA: LD = 12.4 mg ± 0.63 (n = 5); SD = 7.65 mg ± 1.00 (n = 5); F = 16.26, P < 0.004) (Fig. 4.4). Similarly, testicular volume was significantly higher for animals on LD than those on SD (ANOVA: LD = 930 mm^3 ± 83.36 (n = 5); SD = 537.18 mm^3 ± 71.18 (n = 5); F = 12.86, P < 0.0071) (Fig. 4.5). Mean seminiferous tubule diameter was significantly higher in males on LD than on SD (ANOVA: LD = 105.42 µm ± 4.9 (n = 5); SD = 81.24 µm ± 4.9 (n = 5); F = 60.57, P < 0.00005 (Fig. 4.6).

Testosterone

The testosterone data was log transformed since it was not normally distributed. There was a large variation in plasma testosterone concentration among individuals under a long day photoperiodic treatment. No significant difference was found in plasma testosterone concentration between animals on long and short photoperiod (ANOVA: LD = 398.46 nmol.l^{-1} ± 197.05 (n = 5); SD = 89.24 nmol.l^{-1} ± 7.99 (n = 5), F = 2.43, P > 0.05) (Fig. 4.7).

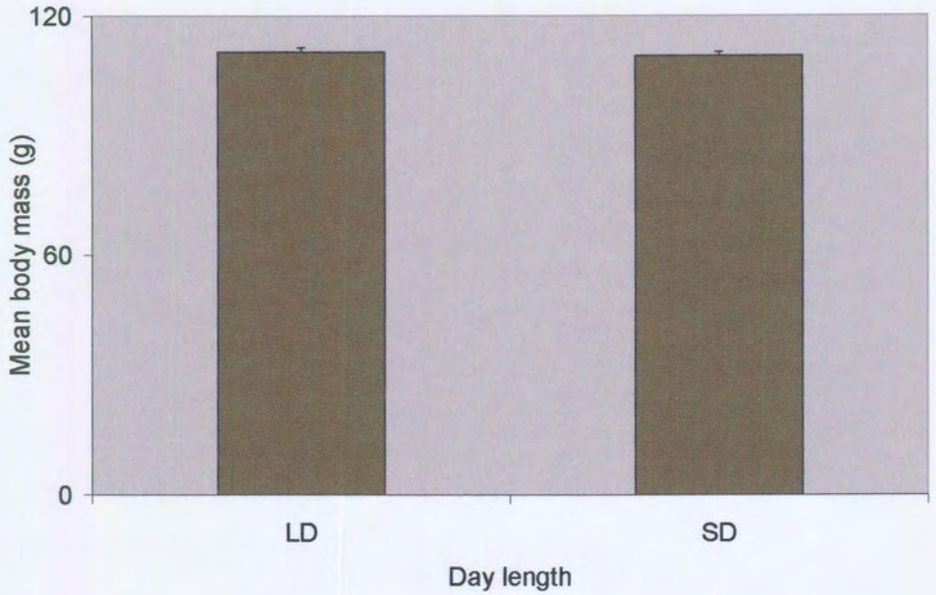

Fig. 4.3. Mean body mass ± S.E. of the Tete veld rat males subjected to long and short day lengths. Body mass of males on long day length was not significantly different from those on short day length.

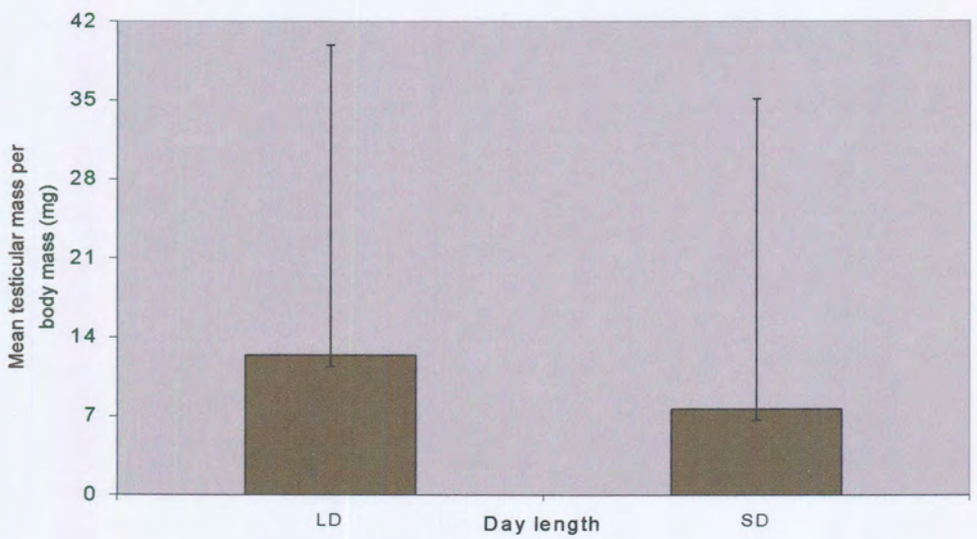

Fig. 4.4. Mean testicular mass per body mass ± S.E. in the Tete veld rat males subjected to long and short day lengths. Testicular mass per body mass of males on long day length was significantly higher than males on short day length.

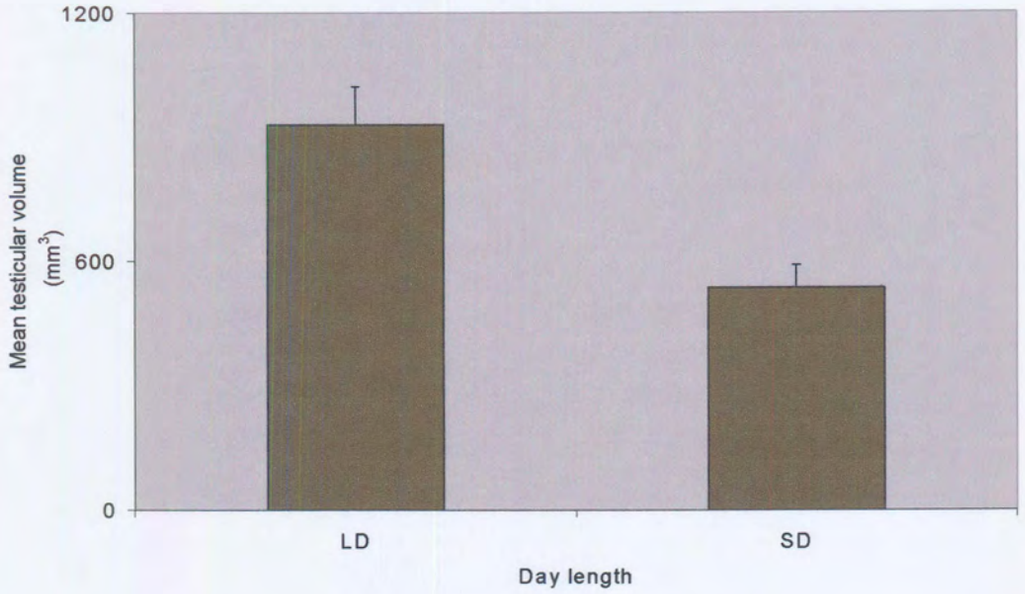

Fig. 4.5. Mean testicular volume ± S.E. in the Tete veld rat males subjected to long and short day lengths. Testicular volume of males on long day length was significantly higher than males on short day length.

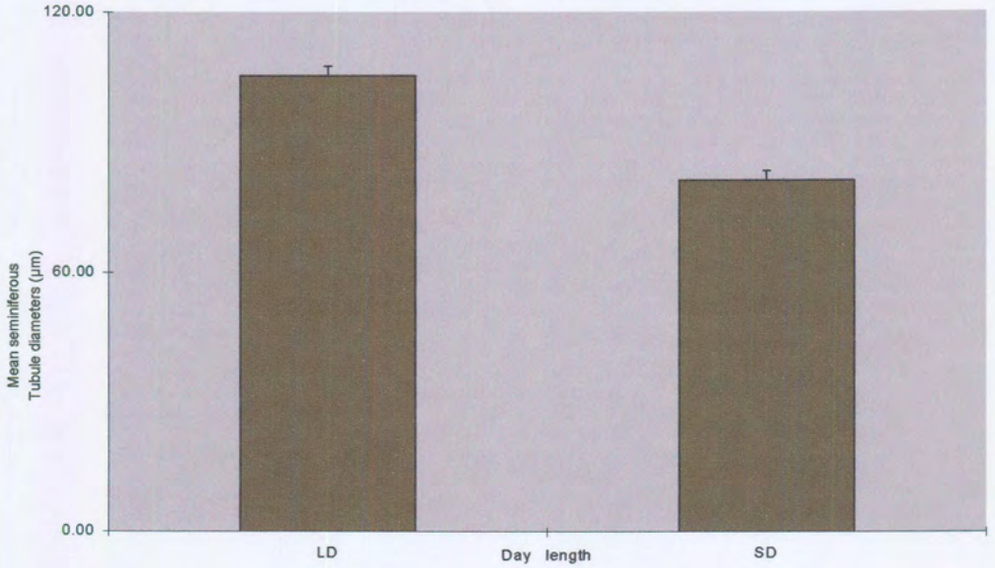

Fig. 4.6. Mean seminiferous tubule diameter ± S.E. in the Tete veld rat males subjected to long and short day lengths. Seminiferous tubule diameter of males on long day length was significantly larger than males on short day length.

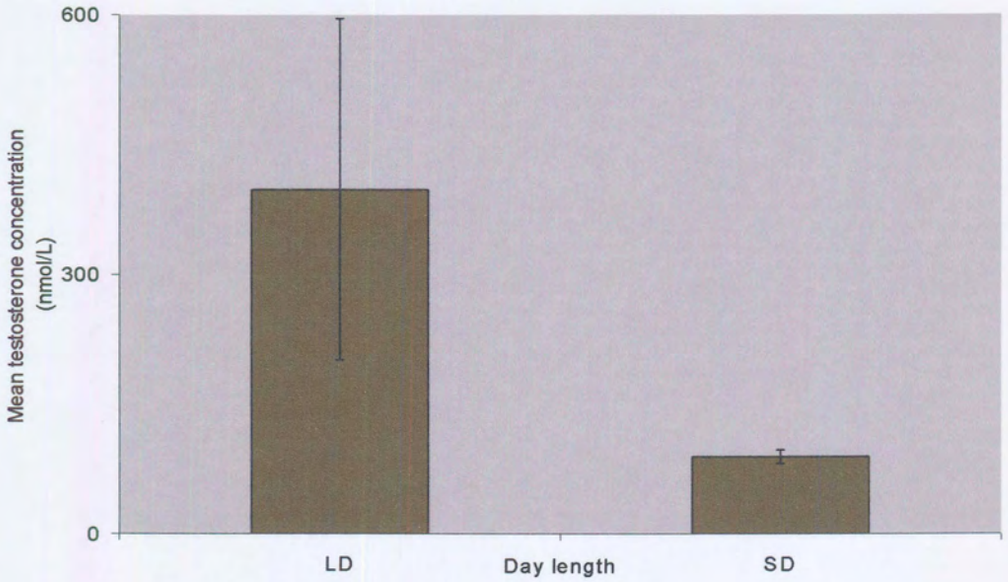

Fig. 4.7. Mean plasma testosterone concentration ± S.E. of the Tete veld rat males subjected to long and short day lengths. Plasma testosterone concentration in males on long day length was not significantly higher than males on short day length.

The Namaqua rock mouse

The body mass of animals on a long photoperiod were slightly higher than that of animals on short day length, but the difference was not statistically significant (ANOVA: LD = 76.0 g ± 2.36 (n = 8); SD = 73.0 g ± 3.56 (n = 8); F = 0.34, P > 0.57) (Fig. 4.8).

Testicular mass, volume, and seminiferous tubules

Testicular mass expressed against body mass was significantly higher for males on LD than those on a SD (ANOVA: LD = 32.5 mg ± 0.93 (n = 8); SD = 27.5 mg ± 0.76 (n = 8); F = 17.50, P < 0.009) (Fig. 4.9). Similarly, animals on LD showed a significantly higher testicular volume than animals on SD (ANOVA: LD = 1639.91 mm^3 ± 71.84 (n = 8); SD = 1293.08 mm^3 ± 60.16 (n = 8); F = 13.70, P < 0.0024) (Fig. 4.10). Mean seminiferous tubule diameters of males on LD were significantly larger than those on SD (ANOVA: LD = 144.29 µm ± 5.46 (n = 8); SD = 122.64 µm ± 3.21 (n = 8); F = 11.68, P < 0.004) (Fig. 4.11).

Testosterone

Mean plasma testosterone concentration in males on LD was significantly higher than those on SD (ANOVA: LD = 379.14 nmol/L ± 93.32 (n = 8); SD = 160.26 nmol/L ± 25.19 (n = 8); F = 5.13, P < 0.04 (Fig. 4.12).

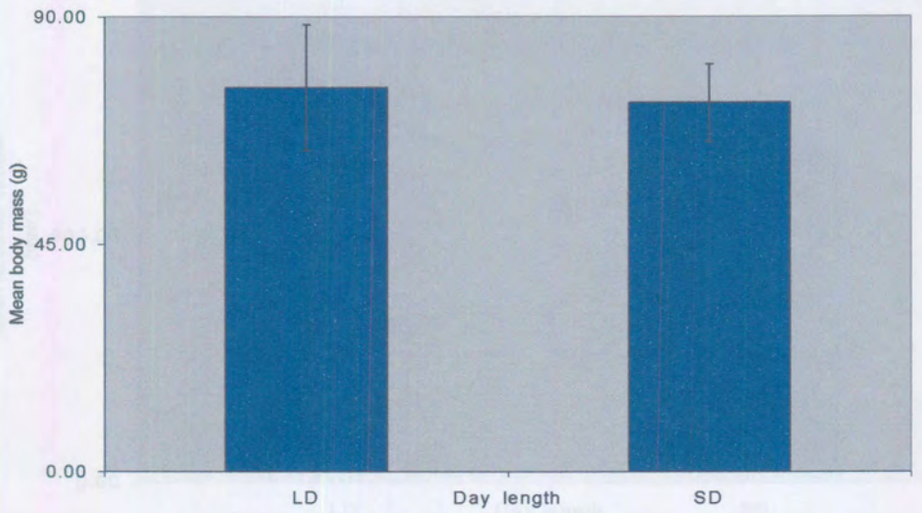

Fig. 4.8. Mean body mass ± S.E. of the Namaqua rock mouse males subjected to long and short day lengths. No significant difference was observed in body mass between the two treatments.

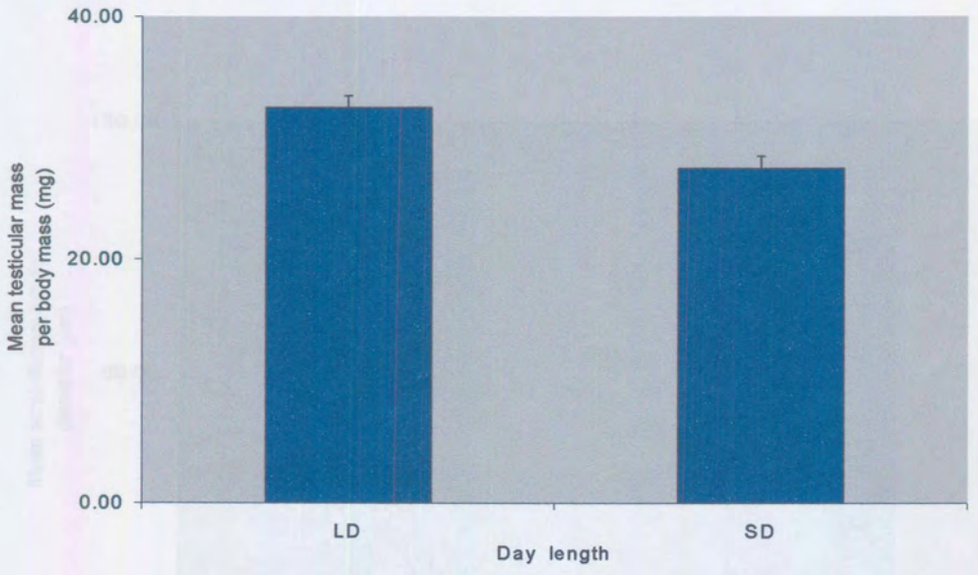

Fig. 4.9. Mean testicular mass per body mass ± S.E. in the Namaqua rock mouse males subjected to long and short day lengths. Males on long day length exhibited higher testicular mass per body mass than those on short day length.

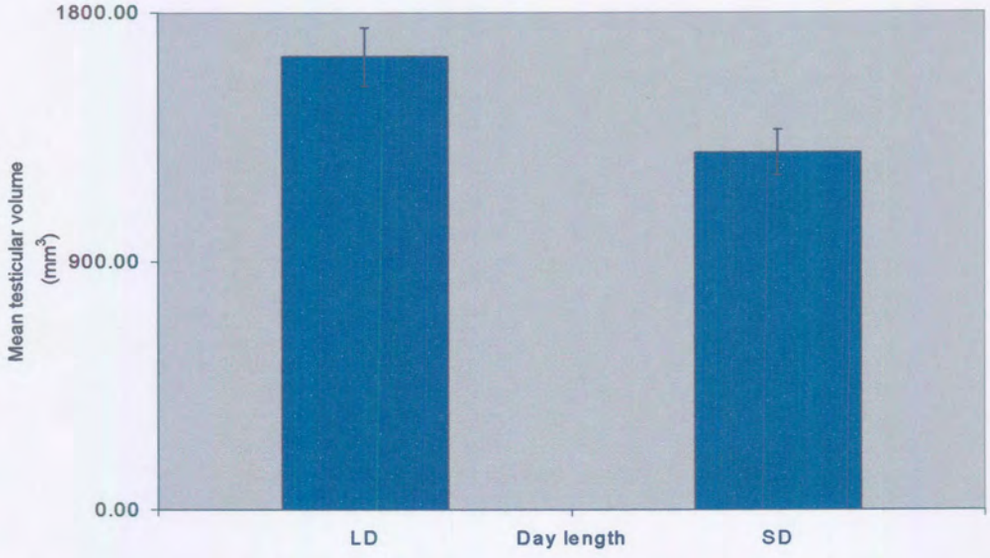

Fig. 4.10. Mean testicular volume ± S.E. in the Namaqua rock mouse males subjected to long and short day lengths. Males on long day length exhibited higher testicular volume than males on short day length.

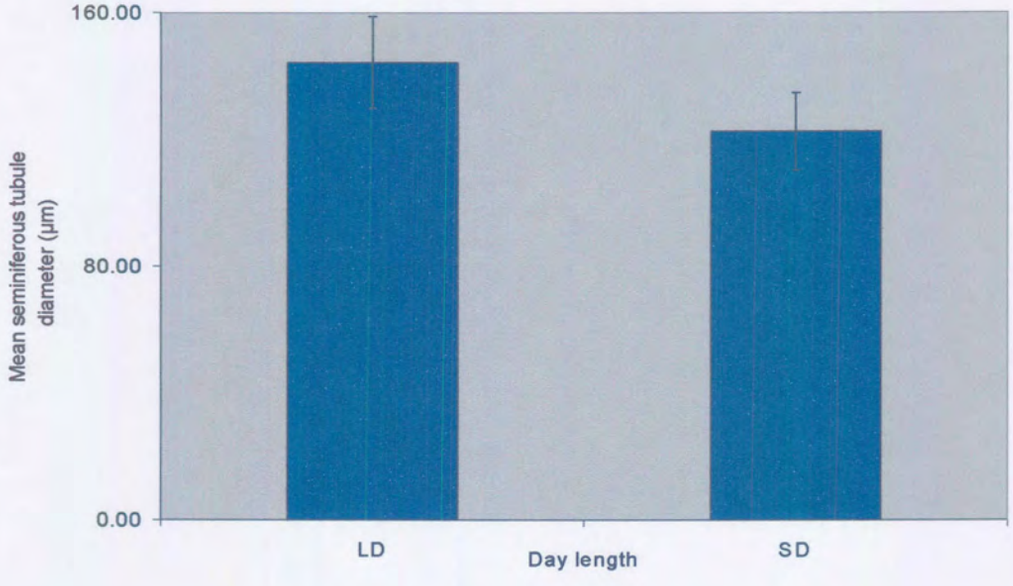

Fig. 4.11. Mean seminiferous tubule diameter ± S.E. in the Namaqua rock mouse males subjected to long and short day lengths. Males on longday length exhibited larger seminiferous tubule diameter than males on short

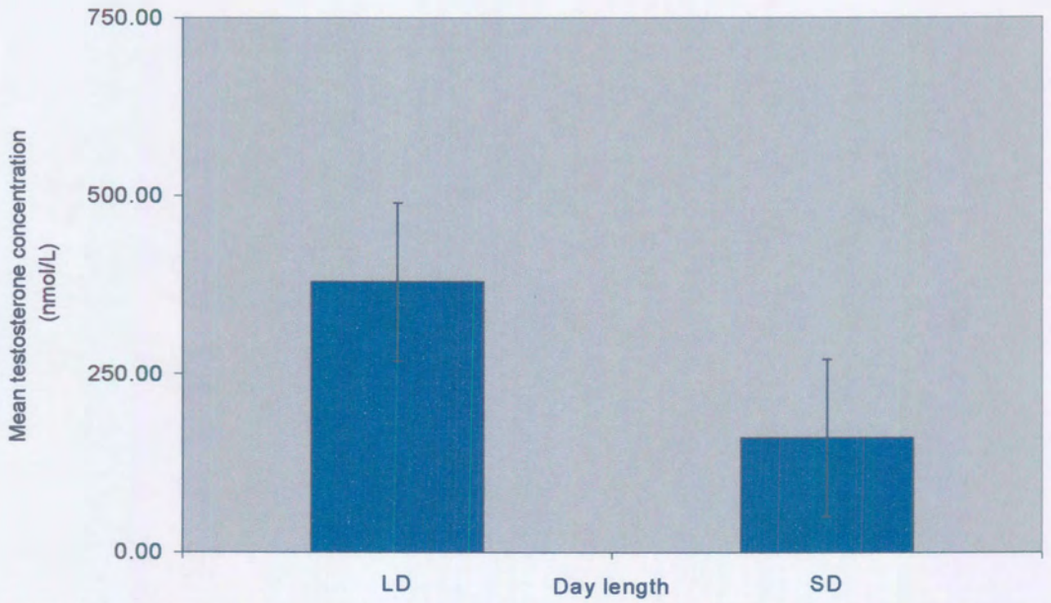

Fig. 4.12. Mean plasma testosterone concentration ± S.E. in the Namaqua rock mouse males subjected to long and short day lengths. Males on long day length exhibited higher testosterone concentration than males on short day length.

DISCUSSION

Male reproductive parameters in the Tete veld rat, *A. ineptus*, appear to be controlled by photoperiod. No significant differences were found in plasma testosterone concentration between animals maintained on a long day compared to those on short day photoperiodic treatment. One of the animals had very low plasma testosterone concentration while another had a very high concentration. This high variation in plasma testosterone concentration may have contributed to the statistically non-significant difference between the two Tete veld rat groups. Generally, however, testosterone concentration in animals on a long day was generally higher than those on a short day treatment. The significantly higher testicular mass, volume, and seminiferous tubule diameter in animals on long day compared to those on short day, suggest a photoperiodic control on reproduction in this species. Similar inhibitory effects of short day length on male reproductive characteristics have been reported in several other rodent species such as the grasshopper mouse, *Onychomys leucogaster*, in which reproductive features such as gonadal recrudescence is inhibited on a photoperiod of 10L:14D but stimulated on a photoperiod of 14L:10D (Frost & Zucker 1983). A similar study on the desert pocket mouse, *Perognathus formosus*, has shown that testicular development and recrudescnce was stimulated when subjected to a 16L:8D photoperiod but inhibited when subjected to 8L:16D daylength (Kenagy & Bartholomew 1981).

Bronson (1985) and Forger & Zucker (1985) reported that long day length could stimulate somatic growth. They found that photoperiodic rodents born late in the summer had somatic and reproductive development delayed by 4-5 months to reach maturity whereas those born under increasing daylength conditions reach maturity in 40-50 days.

This, however, was not the case in the present study since body mass of animals on both long and short photoperiods did not differ significantly. Nevertheless, this observation does not discount the fact that somatic effects may be obtained at a different day length than those on which these animals were exposed to as has been reported in Fischer 344 rats, *Rattus norvergicus F344* (Heideman *et al.* 1998).

The Namaqua rock mouse, *Aethomys namaquensis,* is a seasonal breeder with a breeding season confined to the rainy summer months (see Chapter 3). Male reproductive parameters based on testicular mass, testicular volume, seminiferous tubule diameter, and plasma testoterone appear to be strictly regulated by increasing daylength.

The experimental animals were placed on the two photoperiodic regimes in September, a time when the testes were fully developed (see Chapter 3 for comparison). The results of this study have shown that the testes of animals placed on a short day length regressed in size but not to the size observed during winter. Failure to reduce spermatogenic activity on a short day length has been reported in the Egyptian spiny mouse, *Acomys cahirinus,* Anderson's gerbil, *Gerbillus andersoni* (El-Bakry *et al.* 1998), the Meadow vole, *Microtus pennsylvanicus* (Christian 1980; Dark *et al.* 1983; Kerbeshian *et al.* 1994), the Deer mouse, *Peromyscus maniculatus* (Scheffer 1924; Whitsett & Miller 1982); the pouched mouse, *Saccostomus campestris* (Bernard & Hall 1995), and the Siberian hamster, *Phodopus sungorus* (Hoffmann 1978). In the Fischer 344 rat, *Rattus norvegicus F344,* Heideman *et al.* (1998) reported a total inhibition of testicular regression on day length of 16L:8D, partial regression on 14L:10D, and total regression on photoperiod of less or equal to 12.5L:11.5D. In the Namaqua rock mouse, it is possible that a critical photoperiod is required for complete testicular regression.

In conclusion, the results from this study suggest that male reproductive activities in both the Tete veld rat and the Namaqua rock mouse are influenced by exposure to different photoperiods. Other environmental factors such as rainfall, food availability, and ambient temperature may partially play a role in timing reproduction in this species, but photoperiod is probably the major cue for controlling the onset of reproductive parameters. Future research is needed to examine photoperiodic effects on reproduction from different geographic areas to assess the nature and extent of geographic variation.

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Summary

The distributional range of the Tete veld rat, *Aethomys ineptus*, extends from the Limpopo Province in the north through to the south of KwaZulu Natal. The Tete veld rat is a seasonal breeder, with the breeding period confined to the wet summer months of the year in the Gauteng Province of South Africa. The seasonality of reproduction in the Tete veld rat is confirmed by reproductive tract morphometrics, ovarian histology, plasma progesterone, and oestradiol-17β in females, and testicular histology and plasma testosterone concentrations in males. The presence of some spermatogenic activity and spermatozoa in the epididymides, as well as some follicular activity and raised circulating progesterone, and oestradiol-17β concentrations in some females during winter intimates that the Tete veld rat is possibly an opportunistic breeder. Reproduction during winter is presumably restricted by food availability and adverse winter conditions.

The Namaqua rock mouse, *Aethomys Namaquensis,* on the other hand is widely distributed in the southern African subregion. Reproductive tract morphometrics, ovarian histology, plasma progesterone and oestradiol-17β in females, and testicular histology, seminiferous tubule diameters and plasma testosterone concentrations in males confirm that the Namaqua rock mouse is a strictly seasonal breeder. The breeding period starts in October and extends to the end of February. The absence of Graafian follicles, corpora lutea, corpora albicans, corpus hemorrhagicum, lower plasma progesterone and oestradiol concentrations in females, and small seminiferous tubule diameters, and lower testosterone concentrations during winter months suggest that reproduction is completely inhibited during this period of the year.

Photoperiodic responsiveness was determined in both the Tete veld rat and the Namaqua rock mouse by exposing the animals to long day (LD) and short day (SD) lengths. Testicular mass expressed against body mass, testicular volume, and seminiferous tubule diameters were significantly larger and plasma testosterone concentrations were significantly higher in males subjected to a long day photoperiod than in males exposed to a short day. These findings suggest that both species are photoperiodically responsive and that photoperiod could potentially play a role in reproduction in both the Tete veld rat and the Namaqua rock mouse.

In conclusion, the results in this study suggest that the Tete veld rat is a seasonal breeder with the breeding period confined to the rainy summer months in South Africa. The breeding season starts in October and extends to April. Reproduction in the Tete veld rat appears to involve photoperiod.

The Namaqua rock mouse is a strictly seasonal breeder with a breeding period occurring between October and the end of February. Breeding during the winter months is completely inhibited. The Namaqua rock mouse may also utilize photoperiod to initiate reproductive events.