CHAPTER 3:

GONADAL STEROID HORMONE CONCENTRATIONS IN THE SOLITARY CAPE MOLE-RAT AND THE SOCIAL NATAL MOLE-RAT

Abstract

Urinary gonadal steroid concentrations were measured and compared in the summer and winter for both the Cape mole-rat *(Georychus capensis*) and the Natal mole-rat (*Cryptomys hottentotus natalensis*). The Cape mole-rat breeds seasonally, with sexual activity and pregnancy recorded during the winter months in the southern hemisphere. Despite the fact that it has a distinct breeding season, the seasonal differences in the urinary hormone concentrations in both male and female Cape mole-rats were not statistically significant. This suggests that the Cape mole-rat is an opportunistic breeder and is able to make use of favourable environmental conditions when they occur during the year.

No seasonal differences in urinary sex steroid concentrations were found in either male or female Natal mole-rats. The endocrinology complements histological and post mortem data for presence of embryos in reproductive females recorded during the entire year and support the notion that the Natal mole-rat is not a seasonal breeder.

Dominant, reproductive and subordinate, non-reproductive males displayed comparable testosterone levels and the reproductive and non-reproductive females exhibit comparable oestrogen concentrations. The comparable concentrations of sex steroids in reproductive and non-reproductive animals implies that lack of reproduction in subordinate animals is likely the result of incest avoidance rather than a physiological component of suppression. Urinary progesterone concentrations of the dominant, reproductive females were found to be significantly higher than that of the subordinate, non-reproductive females, and histological data indicate that follicular development does not progress to the stage of the corpus luteum. Furthermore a resultant

LH surge to induce ovulation does not take place in subordinate animals. The reproductive physiology of the Natal mole-rat compares with loosely social mole-rat species inhabiting mesic areas.

Introduction

Gonadal hormones

Cholesterol is the common precursor for all steroid hormones. It is first converted to pregnenolone, a rate-limiting step prior to the manufacture of steroid hormones (Hsu *et al*. 2006) (Figure 3.1).

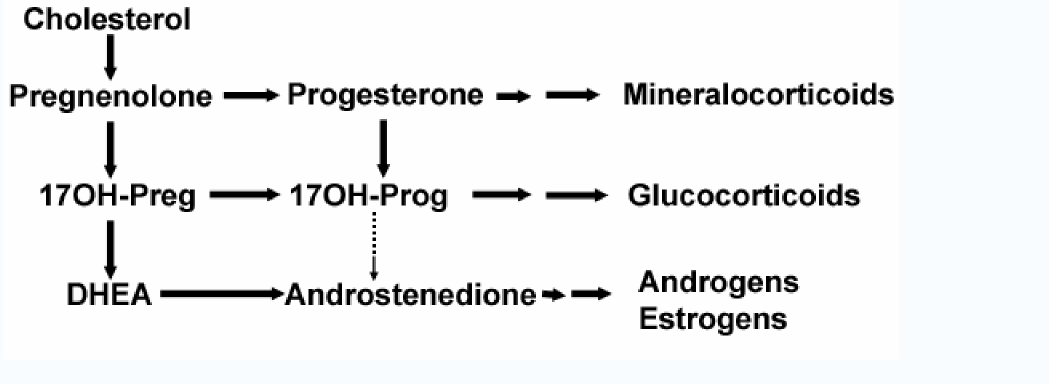

Figure 3.1: The pathway of steroid biosynthesis.

Testosterone

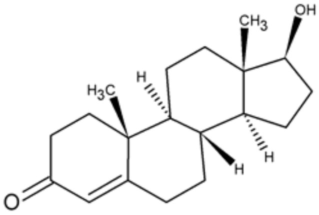

Figure 3.2: Molecular structure of testosterone.

Testosterone is secreted primarily by the testes in males, although small amounts are also secreted by the ovaries in females and the adrenal glands of both sexes. It is the principal male sex hormone, and is responsible for the development of male secondary sex characteristics (Frandson & Spurgeon 1992).

In the testes, testosterone is synthesized mainly in the interstitial Leydig cells and is regulated by luteinising hormone (LH) from the anterior pituitary. In the Sertoli cells, testosterone activates the androgen receptor to initiate and maintain spermatogenesis, and inhibit germ cell apoptosis (Dohle *et al*. 2003).

Testosterone is transported to its target organs in the blood and while being transported, it is bound to a plasma protein called sex hormone binding globulin (SHBG) (Anderson 1974). The main function of testosterone is activation of androgen receptors and it is converted to oestradiol.

Oestrogen

Oestrogens are a group of steroid compounds important in the oestrous cycle. Major oestrogens are oestradiol, oestriol and oestrone, from which oestradiol is the most prominent. Oestradiol is converted from testosterone via the enzyme aromatase (Balthazart *et al*. 1996). It functions as the primary female sex hormone and promotes the development of secondary sexual characteristics (Havelock *et al*. 2004). Although oestradiol is present in both males and females, the concentration in females is much higher than in males (Frandson & Spurgeon 1992).

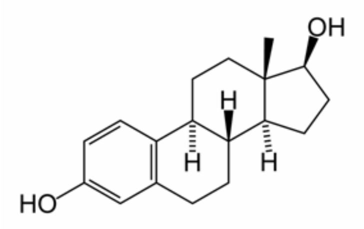

Figure 3.3: Molecular structure of oestradiol.

During the normal course of the reproductive cycle the ovarian follicles are the primary sites of oestrogen synthesis (Poutanen *et al*. 1995), however during pregnancy, oestrogen is mainly produced in the placenta. Small quantities of oestrogen are also produced in the adrenal glands and in the testes of males. Oestrogenic hormones are secreted at varying rates during the menstrual cycle throughout the period of ovarian activity (Figure 3.5).

Oestradiol acts as a growth hormone for the tissue of reproductive organs. It appears to be necessary for maintenance of oocytes in the ovary. Oestradiol produced by the growing follicles trigger hypothalamic-pituitary events that lead to the luteinising hormone surge that induces ovulation.

Progesterone

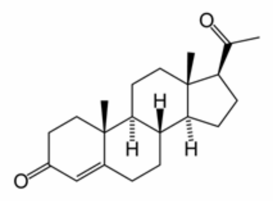

Figure 3.4: Molecular structure of progesterone.

Progesterone is primarily synthesized by the ovarian corpus luteum and the placenta. It is considered essential for the preparation and maintenance of mammalian pregnancy. Initially, the corpus luteum is responsible for the majority of progesterone production, but during the final three quarters of the pregnancy the placenta is recognised as the primary source of progesterone (Henson 1998). Small quantities of progesterone are also manufactured in the adrenal glands.

Circulating progesterone levels are characteristically low during the follicular phases of the reproductive cycle, it shows a sharp increase during the luteal phase, reaching a peak after the LH surge, after which it declines rapidly, unless pregnancy occurs (See fig. 3.4) (Clarke & Pompolo 2005).

Gonadotropins of the anterior pituitary regulate secretion of ovarian hormones, oestradiol and progesterone, hypothalamic control of pituitary gonadotropin production is in turn regulated by plasma concentrations of oestrogen and progesterone. A complex feedback system results in the cyclical phenomenon of ovulation.

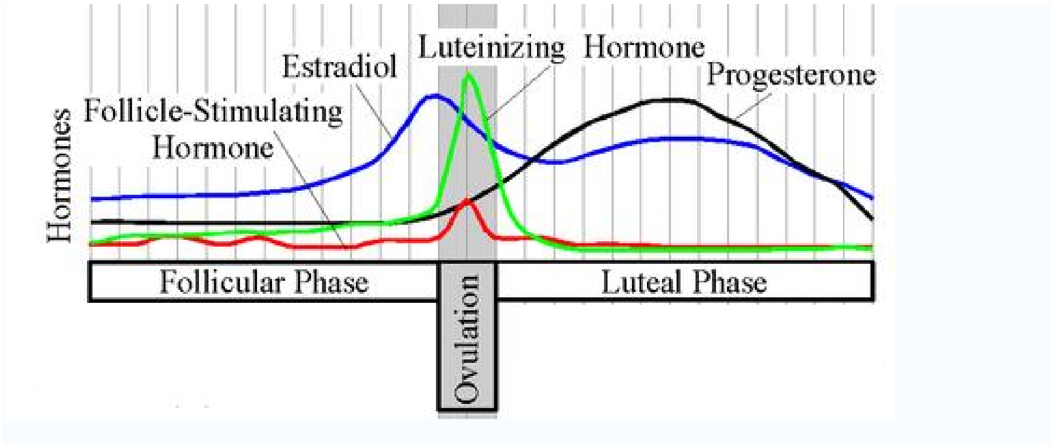

Figure 3.5: Hormonal levels during the course of the female reproductive cycle.

Chapter 3a

Cape mole-rat (*Georychus capensis*)

The Cape mole-rat (*Georychus capensis*) is a solitary rodent mole that is xenophobic and highly aggressive towards conspecifics (Nevo 1979, Bennett & Jarvis 1988a). Foot drumming is used to convey information about territorial boundaries, such that neighbouring tunnel systems may come to a metre from each other, but do not interlink (Bennett & Jarvis 1988a).

Aggressive and territorial behaviour is halted briefly for courtship and mating. This behaviour is initiated via foot drumming that, depending on the frequency, relates information about sex and reproductive state to surrounding mole-rats (Bennett & Jarvis 1988a). In the Cape mole-rat, breeding is restricted to the wet winter months. Mole-rats have a relatively long gestation time for their body size (Bennett & Faulkes 2000), such that young are produced towards the end of the winter and into early summer. The Cape mole-rat appears to have the potential to produce two litters per season. The Cape mole-rat is an induced ovulator, and thus does not show a constant cyclical pattern of ovulation (Van Sandwyk & Bennett 2005). The act of coitus stimulates the hypothalamus to produce gonadotropin releasing hormone (GnRH) which in turn triggers the release of follicle stimulating hormone (FSH) from the anterior pituitary. FSH promotes follicular development, from where oestrogen is secreted. Increased levels of oestrogen instigate a surge of LH that results in ovulation (Knobil 1988).

In most seasonally breeding mammals, the reproductive system undergoes some level of regression during the reproductively quiescent season. In seasonally breeding females, ovulation occurs only during the part of the year that is optimal for reproduction whereas in males testicular size is reduced and sperm production is down-regulated or terminated. Furthermore, gonadal steroid levels are secreted at much lower levels (Gerlach & Aurich 2000). The Cape mole-rat breeds seasonally, however there is no data on gonadal steroid hormone profiles for wild captured mole-rats both during the breeding season (August) and out of the breeding season (February). The aim of this study was to determine whether there is a seasonal difference in urinary steroid concentrations.

Material and methods

Gonadal steroid concentrations were measured from urine samples using Coat-a-count kits (Diagnostic Products Corporation, Los Angeles, California, USA). All hormone assays have been validated for use in mole-rats (Bennett & Jarvis 1988a, Bennett *et al*. 1994.)

Urine concentrations of steroid hormones were standardized by measuring the creatinine content in each sample (Bonney *et al*. 1982).

Refer to Chapter 2 for detailed experimental procedures.

Statistical analysis

Due to the small sample sizes of some of the groups, non-parametric Mann-Whitney U tests were performed to determine whether there were any statistical differences between the experimental groups.

Results

- Testosterone

Urinary testosterone concentrations in male Cape mole-rats were slightly higher during the breeding season (winter) compared to out of the breeding season (summer), but the difference was not significant (Mann Whitney U-test: $n_1=4$, $n_2=5$, U=7, Z=0.735, p=0.462) (Figure 3.6).

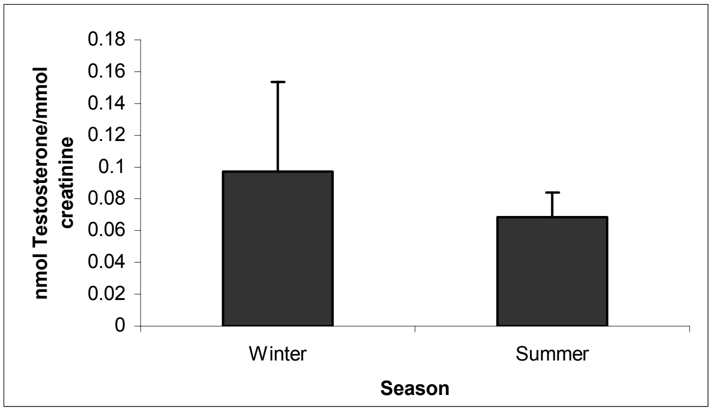

Figure 3.6: Urinary testosterone concentrations (nmol/mmol creatinine) measured in and out of the breeding season in the male Cape mole-rat.

- Oestrogen

The urinary oestrogen concentrations in Cape mole-rat females were higher during the breeding season (winter) than out of the breeding season (summer). This difference however, was not statistically significant (Mann Whitney U test: n_1=14, n_2=12, U=50, Z=1.75, p=0.08) (Figure 3.7).

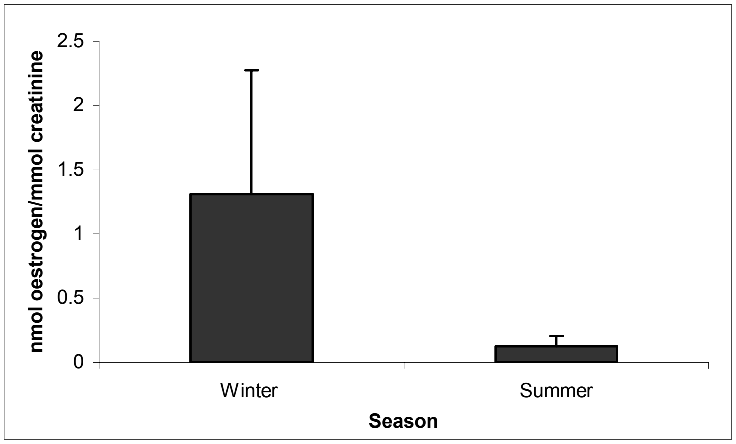

Figure 3.7: Urinary oestrogen concentrations (nmol/mmol creatinine) measured in and out of the breeding season in the female Cape mole-rat.

- Progesterone

The female Cape mole-rats have a higher concentration of progesterone during the breeding season (winter) compared to out of the breeding season (summer), however, this difference was not significant (Mann Whitney U-test: n_1=14, n_2=12, U=51, Z=1.69, p=0.089) (Figure 3.8).

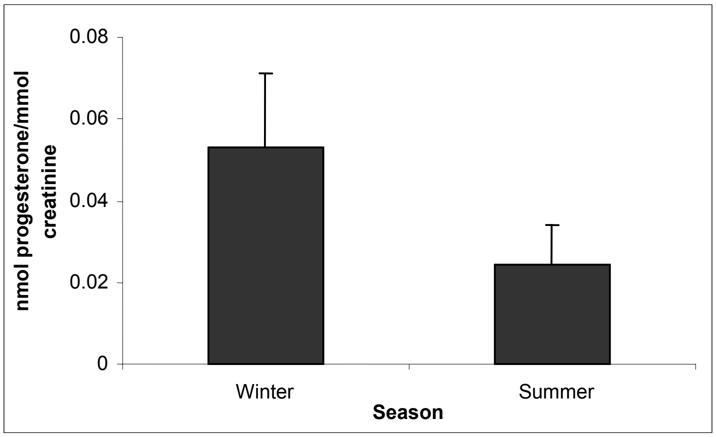

Figure 3.8: Urinary progesterone concentrations (nmol/mmol creatinine) measured in and out of the breeding season in the female Cape mole-rat.

Discussion

Seasonal breeding is common among solitary subterranean rodents (Andersen & MacMahon 1981, Bennett & Jarvis 1988a, Hansen 1960, Herbst *et al*. 2004, Jarvis 1969, Rado *et al*. 1992, Vaughan 1962). As a result, young are produced at a time of the year most favourable for their survival. Typically, the reproductive state of seasonally breeding animals is reflected in the physiology of their reproductive systems (Clarke 1981). Reproductive function is disrupted during periods when environmental conditions are unfavourable for breeding. In general, ovulation is suspended in females, whereas sperm production and quality is affected in males (Gerlach & Aurich 2000). GnRH plays a key role in reproductive function, and gonadal steroids both stimulate and inhibit its release. Negative feedback from gonadal hormones mainly reduces pulse frequency of GnRH release which imposes an inhibitory effect downstream on the rest of the reproductive system. Gonadal hormone concentrations that, outside the breeding season, inhibit GnRH secretion have no effect or are stimulatory during the breeding season (Karsch *et al*. 1984, 1993, Lincoln 1984). Changes in gonadal steroid feedback are at least partly responsible for seasonal changes observed in GnRH release (Ebling *et al*. 1994).

In wild captured male Cape mole-rats, urinary testosterone concentrations were not significantly higher during the breeding season (winter) than in the non-breeding season (summer). This contradicts the findings of Bennett and Jarvis (1988a), who found an increase in urinary testosterone concentrations at the onset of the breeding season. The non-significant result may be attributed to the small number of male mole-rats captured, and the samples in the two studies are likely to be collected at different times during the breeding season. Male Namaqua dune mole-rats (*Bathyergus janetta*) and Cape dune mole-rats (*Bathyergus suillus*), exhibit heightened testosterone concentrations at the onset of the breeding season (Herbst *et al*. 2004, Hart & Bennett 2006). Both of these species show two distinct peaks in testosterone levels during the breeding season, one at the beginning of the breeding period and one towards the end. The difference in testosterone concentrations between breeding and non-breeding seasons was significant in the Cape dune mole-rat but not so in the Namaqua dune mole-rat (Herbst *et al*. 2004, Hart & Bennett 2006).

Female Cape mole-rats displayed higher circulating basal oestrogen and progesterone concentrations during the breeding season, but due to large intra-specific variation this difference was not significant for either of the steroids. High intra-specific variation could stem from the inclusion of samples taken from females that have and have not mated. Since these animals are induced ovulations, coitus will alter hormone profiles. Sharp elevations in oestrogen and progesterone levels were recorded for the Cape dune mole-rat and the Namaqua dune mole-rat (Herbst *et al*. 2004, Hart & Bennett 2006). In the Cape dune mole-rat, only the progesterone concentration was significantly higher during the breeding season than out of the breeding period (Hart & Bennett 2006), which may be as a result of the large numbers of pregnant females. The hormonal concentrations of the Namaqua dune mole-rat were not significantly different in and out of the breeding season (Herbst *et al*. 2004), which corresponds with the findings of the current study.

It is possible that the Cape mole-rat is a seasonal breeder, the lack of distinct differences in gonadal steroid concentrations in and out of the breeding season may be the result of sampling methods. Animals were captured in August, towards the end of the breeding season. Bennett and Jarvis (1988a) showed the highest testosterone levels to occur in June and July at the onset of the breeding season therefore animals sampled in the present study might well have been captured when hormonal levels were already in decline after the initial part of the breeding season. The onset of winter rainfall in the Cape is around late May hence if these animals show a similar trend to the Namaqua dune mole-rat, hormonal concentrations would have started to increase soon after the first rainfall. However, the Cape mole-rat occurs sympatric with the Cape dune mole-rat, which shows a peak in hormonal levels during August.

At present, it is uncertain which environmental factor(s) are responsible for the seasonal control of reproduction in subterranean mole-rats. Photoperiod is the primary environmental cue used by aboveground animals to entrain their breeding patterns (Karsch *et al*. 1984). Mole-rats rarely venture above ground and therefore are not in frequent contact with photoperiodic cues. However, despite a regressed visual system, most mole-rat species are still able to entrain their daily activity rhythms to a circadian light cycle (Hart *et al.* 2004, Oosthuizen *et al.* 2003, Schöttner *et al.* 2006, Vasicek *et al*. 2005).

Temperatures in the burrow system are reasonably buffered, thus seasonal temperature changes do not fluctuate as much as aboveground (Bennett & Faulkes 2000). Yet, foraging tunnels close to the surface may relay seasonal temperature changes to mole-rats.

Rainfall influences both growth of plants, which affects food availability, and the moisture content of the soil that enables animals to excavate new tunnels (Dennis & Marsh 1997, Herbst *et al.* 2004). In addition, gonadal steroid concentrations appear to be well correlated with the rainfall profile. Breeding in the Namaqua dune mole-rat appears to be restricted to the period after the first winter rainfall (Herbst *et al*. 2004). Considering that in this study, seasonal gonadal hormone concentrations were not found to be significantly different in the Cape mole-rat, it is likely that, physiologically, this rodent mole has the capability to reproduce at any time of the year, but is restricted by unfavourable environmental conditions.

Chapter 3b

Natal mole-rat (*Cryptomys hottentotus natalensis*)

Species in the family Bathyergidae display a broad spectrum of social organisation and mating strategies, ranging from strictly solitary to highly social (Bennett *et al*. 1999). While all solitary species currently studied appear to breed seasonally, this appears to be the exception rather than the rule among the social representatives of this family (Bennett *et al*. 1991). Reproduction in the social mole-rat species is monopolised by a single reproductive female and between one and three male consorts, with all other natal members in the colony being reproductively suppressed by the breeders (Bennett *et al*. 1993, 1994, 1996, 1997). The extent of the suppression varies along with different life history strategies displayed by the different species.

The reproductive suppression of subordinate animals in eusocial mole-rat species is much more stringent than that observed in less social species. Subordinate naked mole-rat males and females are physiologically suppressed from reproducing. Females exhibit low levels of reproductive hormones and fail to undergo follicular development and subsequent ovulation (Faulkes *et al*. 1990a). In the males, hormonal levels are also inhibited (Faulkes *et al*. 1991), and although testosterone levels are sufficient to support spermatogenesis (Faulkes *et al.* 1994), the number of spermatozoa produced is significantly less than that of the reproductive animals and most are non-motile (Faulkes *et al*. 1994, Faulkes & Abbott 1997). Female subordinates of the Damaraland and highveld mole-rat are also physiologically suppressed, whereas the subordinate males exhibit reproductive hormonal levels comparable to those of the breeding males (Bennett *et al*. 1993, Van der Walt *et al*. 2001). In transiently social species such as the common mole-rat and Mashona mole-rat, subordinate animals are not physiologically suppressed from reproducing. Dominant and subordinate animals of both sexes exhibit similar steroid hormone concentrations (Herbst & Bennett 2001, Bennett *et al.* 1997, Spinks *et al*. 1997).

Little is known about the reproductive biology of the Natal mole-rat, and until now it has been unknown whether this species is breeding seasonally or continuous throughout the year. Since the Natal mole-rat is phylogenetically closely related to the common mole-rat and the highveld mole-rat, both of which breed seasonally (Faulkes et *al.* 1997), it was predicted to be a seasonal breeder. Urinary gonadal steroids concentrations were measured in the Natal mole-rats collected over an entire calendar year to establish whether there is any seasonal difference in the circulating urinary steroid concentrations.

Material and methods

Steroid concentrations were measured using Coat-a-count Radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, California, USA).

Urinary oestrogen and progesterone concentrations were monitored in the female, while testosterone concentrations were measured from blood plasma samples in males. Assays were validated for use in this species and creatinine levels were determined for urine samples.

Refer to Chapter 2 for complete experimental procedures.

Statistical analysis

As a result of small sample sizes of some of the groups, non-parametric Mann Whitney U-tests were performed on the different experimental groups to discover any significant differences between them.

Results

- Testosterone

The plasma testosterone concentrations showed no seasonal difference in either the reproductive (Mann Whitney U-test: n_1=17, n_2=16, U=128, Z=-0.29, p=0.77) or the non-reproductive (Mann Whitney U-test: n_1=27, n_2=35, U=405, Z=-0.93, p=0.352) Natal mole-rat males (Figure 3.9).

The plasma testosterone level was not different between reproductive and non-reproductive males during either the summer (Mann Whitney U-test: n_1=17, n_2=27, U=192, Z=0.90, p=0.37) or the winter (Mann Whitney U-test: n_1=16, n_2=35, U=235, Z=0.91, p=0.36) (Figure 3.9).

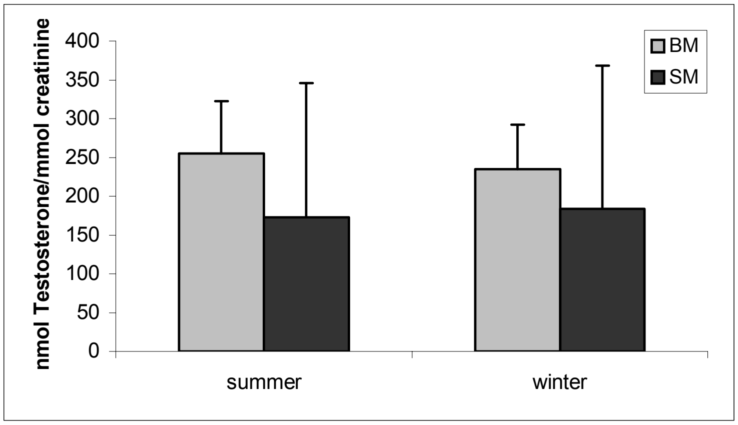

Figure 3.9: Plasma testosterone concentrations of dominant, reproductive and subordinate, non-reproductive Natal mole-rat males during the summer and winter. BM = Breeding male, SM = subordinate male

- Oestrogen

No significant difference was found in the urinary oestrogen concentrations in summer and winter for either the dominant, reproductive (n_1=7, n_2=9, U=25, Z=0.688, p=0.49) or subordinate, non-reproductive (n_1=29, n_2=25, U=270, Z=1.6, p=0.11) female Natal mole-rats (Figure 3.10).

In the summer, the non-reproductive females had a higher oestrogen concentration than the reproductive animals, although this was not significant (n_1=7, n_2=29, U=89, Z=-0.49, p=0.617). In contrast, the oestrogen concentration was higher in reproductive females during the winter, however not significantly so (n_1=9, n_2=25, U=105, Z=-0.29, p=0.769) (Figure 3.10).

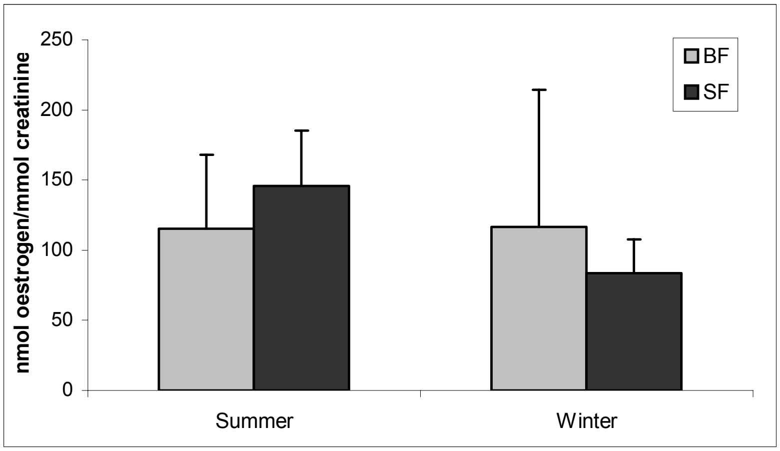

Figure 3.10: Urinary oestrogen concentrations (nmol/mmol creatinine) of dominant, reproductive and subordinate, non-reproductive female Natal mole-rats during summer and winter.

- Progesterone

There are no seasonal differences in progesterone concentrations in either the reproductive (Mann Whitney U-test: n_1=7, n_2=9, u=21, z=-1.11, p=0.266) or non-reproductive (Mann Whitney U-test: n_1=28, n_2=25, U=309, Z=-0.73, p=0.465) Natal mole-rat females, although the range of the concentrations was much broader during the winter (figure 3.11).

Reproductive females have significantly higher progesterone concentrations than the non-reproductive females during both the summer (Mann Whitney U-test: n_1=7, n_2=28, U=37, Z=2.52, p=0.011) and winter (Mann Whitney U-test: n_1=9, n_2=25, U=23, Z=3.49, p=0.0004) (figure 3.11).

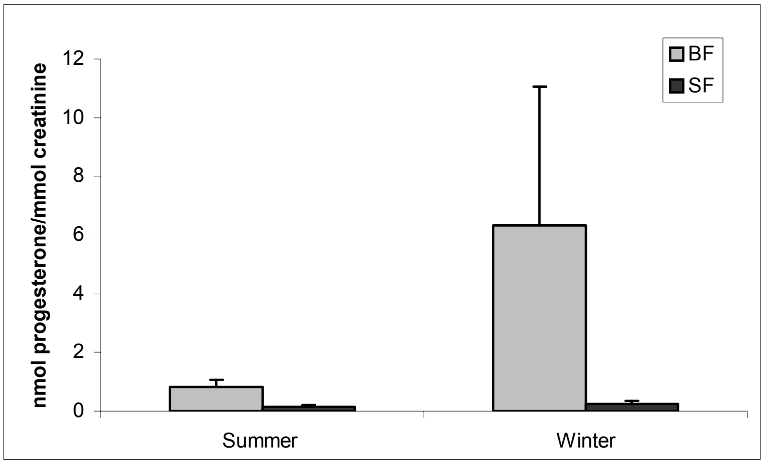

Figure 3.11: Urinary progesterone concentrations (nmol/mmol creatinine) during the summer and winter periods for reproductive and non-reproductive female Natal mole-rats.

Discussion

Breeding season

The majority of social mole-rat species do not breed seasonally (Bennett *et al*. 1999). Currently only two species of social mole-rat have been found to breed seasonally, the common mole-rat (*Cryptomys hottentotus hottentotus*) and the highveld mole-rat (*Cryptomys hottentotus pretoriae*) (Spinks *et al*. 1997, 1999, Janse v Rensburg *et al*. 2002). Despite being phylogenetically closely related to these two species, the Natal mole-rat (*Cryptomys hottentotus natalensis*) (Bennett & Faulkes 2000) does not appear to breed seasonally. No significant seasonal differences were found in any of the urinary oestrogen or plasma testosterone concentrations investigated in this study. The endocrine findings complement the post mortem findings of corpora lutea in the ovaries and embryos in the uterine horns of reproductive females during all months that mole-rats were captured (M.Oosthuizen, *pers.obs*.).

In the seasonally breeding social highveld mole-rat, oestrogen and progesterone plasma concentrations exhibited seasonal periodicity. In female reproductive mole-rats, both oestrogen and progesterone concentrations were elevated at the onset of winter. In contrast, testosterone concentrations in male highveld mole-rat males showed no seasonality (Janse van Rensburg *et al*. 2002).

Periodicity in environmental factors plays a major role in the seasonal regulation of reproduction (Bronson & Heideman 1994). Both the common and highveld mole-rats inhabit regions with very distinct seasonal variations in most environmental parameters. The regions occupied by the Natal mole-rat also display clear seasonal differences in temperature and rainfall, however, the yearly rainfall for the region in KwaZulu Natal where the animals for this study was captured, is approximately double than that of the Cape Town area where the common mole-rat occurs, and one and a half times that of the Pretoria area that the highveld mole-rat inhabits. For most of the year, food is readily available and the soil is workable such that dispersal is easily achievable. Therefore, it seems likely that rainfall is the determining factor for seasonal breeding in social mole-rats. In the Natal mole-rat, rainfall is not a limiting factor and reproduction is possible throughout the year. This species can therefore employ an opportunistic breeding strategy and make use of favourable conditions as they arise throughout the year.

Social status

In social cooperative breeding communities, unequal distribution of reproductive success within such a group is not uncommon (Keller & Reeve 1994). In these communities, dominant animals may inhibit reproduction of subordinate animals with behavioural interactions (Abbott 1987, Abbott *et al*. 1988). In extreme cases, reproduction can be completely suppressed by a physiological block to ovulation (Bennett & Faulkes 2000).

In social mole-rat species, reproduction is highly skewed towards a single breeding pair, and subordinate reproduction is suppressed. The degree of suppression and the mechanism by which reproduction is controlled in subordinates, vary between species (Bennett & Faulkes 2000).

In the Natal mole-rat, there was no significant difference in the plasma testosterone concentrations of reproductive and non-reproductive males. This finding supports the notion that male non-reproductive mole-rats are not physiologically suppressed while in the confines of the natal colony. In the highveld mole-rat, a similar scenario was found in the males (Janse van Rensburg *et al*. 2002), and Damaraland mole-rat where reproductive and non-reproductive males also have comparable concentrations of circulating testosterone (Bennett 1994). Interestingly, in naked mole-rat males the non-breeding animals have significantly lower urinary testosterone levels than the breeding animals, implying that they are physiologically suppressed from reproduction (Faulkes *et al*. 1991). With the exception of the naked mole-rat (Faulkes *et al.* 1990b, Reeve *et al*. 1990, O'Riain *et al*. 1996), all evidence suggests incest avoidance and outbreeding in social bathyergid species (Bennett 1994, Burda 1995, Rickard & Bennett 1997, Spinks 1998). Since mole-rat colonies typically consist of the reproductive pair and several generations of their offspring (Jarvis *et al*. 1994), it appears that incest avoidance alone is sufficient to inhibit reproduction in subordinate male mole-rats of the genus *Cryptomys*.

Comparable concentrations of urinary oestrogen between reproductive and non-reproductive female Natal mole-rats imply that females are not physiologically suppressed and are capable of follicular development. On the contrary, oestrogen levels were extremely low in both subordinate highveld females (Janse van Rensburg *et al*. 2002), and subordinate naked mole-rat females (Faulkes *et al*. 1991), confirming a physiological block to reproduction at the level of the ovary. The naked mole-rat is not an obligatory outbreeder, necessitating the exercise of stringent reproductive control by the breeding queen (Faulkes *et al*. 1991), whereas the Natal, highveld and Damaraland mole-rats are all outbreeding species. The difference in reproductive suppression in the latter species may result from different ecological constraints placed upon them. Regular rainfall in the Natal midlands equates to frequent dispersal opportunities for the Natal mole-rat, whereas strictly seasonal rainfall patterns in the habitats of the highveld and Damaraland mole-rats inhibit dispersal and opportunities for independent breeding are less frequent (Jarvis *et al*. 1994).

Progesterone concentrations were substantially higher in reproductive female Natal mole-rats when compared to the non-reproductive females. If one takes into account that the Natal mole-rat is an induced ovulator (Jackson & Bennett 2005) the finding is not surprising, since for ovulation to occur and subsequent corpus luteum development to take place coitus must take place. The lack of opportunities for coitus in subordinate female Natal mole-rats due to incest avoidance substantiates this finding. Progesterone is primarily secreted by the corpus luteum (Frandson & Spurgeon 1992), therefore since follicular development does not reach the stage of the corpus luteum in subordinate females, progesterone concentrations should remain low. Similar results have been found in both the Damaraland mole-rat and the naked mole-rat (Clarke & Faulkes 1997, Clarke *et al.* 2000, Faulkes *et al*. 1991). The breeding female exhibits the highest concentration of progesterone in the colony and the subordinate females typically have very low progesterone levels (Clarke & Faulkes 1997, Clarke *et al.* 2000). In both of these species, progesterone levels increased significantly following the removal of the breeding female. This implies that the queen has a suppressive influence over the subordinate, non-reproductive females.

Thus, it appears that reproduction in both the male and female subordinate Natal mole-rats is not through physiological suppression but rather incest avoidance. High overall rainfall ensures constant availability of food sources and softer soils provide dispersal opportunities for much of the year and as a consequence reproductive control of the subordinate, non-reproductive animals does not need to be as stringent.