CHAPTER 1:

GENERAL INTRODUCTION

Reproductive regulation in mammals

In mammals, the hypothalamo-pituitary-gonadal (HPG) axis regulates reproduction. This axis is controlled by the central nervous system (CNS) which responds to both internal and external stimuli. Gonadotropin releasing hormone (GnRH) plays a crucial role in the regulation of reproduction. The synthesis of GnRH is stimulated by the CNS, while GnRH regulates the synthesis and secretion of pituitary gonadotropes (Batailler *et al*. 2004), thereby providing a link between the neural and endocrine systems.

GnRH involved with reproduction is produced in and secreted from a specific sub-population of GnRH neurons in the preoptic area (POA) and the mediobasal hypothalamus (MBH) of the brain (Clarke 1987). The afferents of these neurons project to the median eminence (ME) where GnRH is stored. From here, GnRH is released into the pituitary portal system to the anterior pituitary where it stimulates the release of follicle stimulating hormone (FSH) and luteinising hormone (LH) into the circulatory system (Page & Dovey-Hartman 1984). In female mammals the release of GnRH from the median eminence is related to the mode of ovulation (Milligan 1980). In spontaneously ovulating species, GnRH is released from the median eminence in a pulsatile fashion that leads to cyclical production and release of gonadal hormones to generate continuous reproductive cycles (Bakker & Baum 2000). In contrast, in mammals exhibiting induced ovulation external stimulation is required to release GnRH that produces the preovulatory LH surge. Such stimulation is typically received from coitus. The primary endocrine response to coitus is the release of LH from the anterior pituitary which results in ovulation (Bakker & Baum, 2000).

The gonadotropes primarily control gonadal function. In females, FSH released from the pituitary is responsible for follicular development in the ovaries. The maturing follicle secretes mainly oestrogen and a small amount of progesterone. Higher levels of oestrogen in the relative absence of progesterone cause the GnRH pulse frequency to increase from the GnRH neurons while the amplitude decreases (Clarke & Pompolo 2005). The rise in GnRH release triggers a surge in LH that induces ovulation. The follicle ruptures and the oocyte is released into the fallopian tube. The remnants of the follicle remain in the ovary, and develop into a corpus luteum that secretes mostly progesterone and a small amount of oestrogen. These hormones are required to develop and maintain the endometrium for implantation and growth of the embryo. The elevated levels of oestrogen and progesterone yield GnRH secretion with a high amplitude and low frequency (Clarke 1987) that inhibits the production of FSH and LH in the pituitary. If implantation does not take place, the corpus luteum degenerates into a corpus albicans in response to the diminishing level of plasma LH, the oestrogen and progesterone levels decline and the negative feedback to the pituitary terminates (Clarke 1995) (Fig. 1.1a, Fig. 1.2).

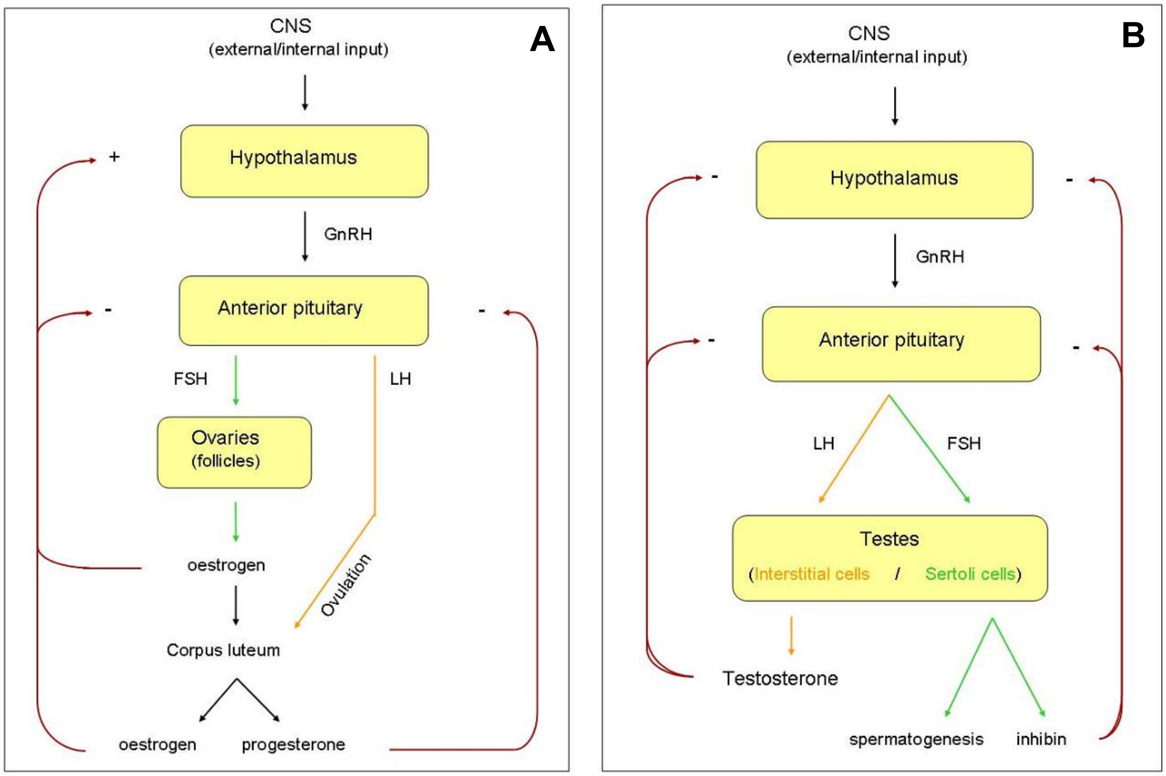

Figure 1.1: Schematic representation of the regulation of the hypothalamo-pituitary-gonadal axis in (a) females and (b) males.

In males, FSH and LH regulate spermatogenesis. FSH initiates sperm production while LH stimulates the production of testosterone in the interstitial cells (Frandson & Spurgeon 1992). Testosterone is the male hormone that maintains the male sexual characteristics. Under the influence of testosterone and other hormones, immature sperm cells develop into mature sperm called spermatozoa and are stored in the epididymus (Martan 1969). Males do not produce a LH surge due to insufficient levels of oestrogen (Parvizi 2000), or in the case of male non-human primates, rats and sheep, as a result of an inability of the hypothalamus to respond to a rapid and large LH increase in estradiol with a GnRH surge (Steiner *et al.* 1976) (Fig.1.1b).

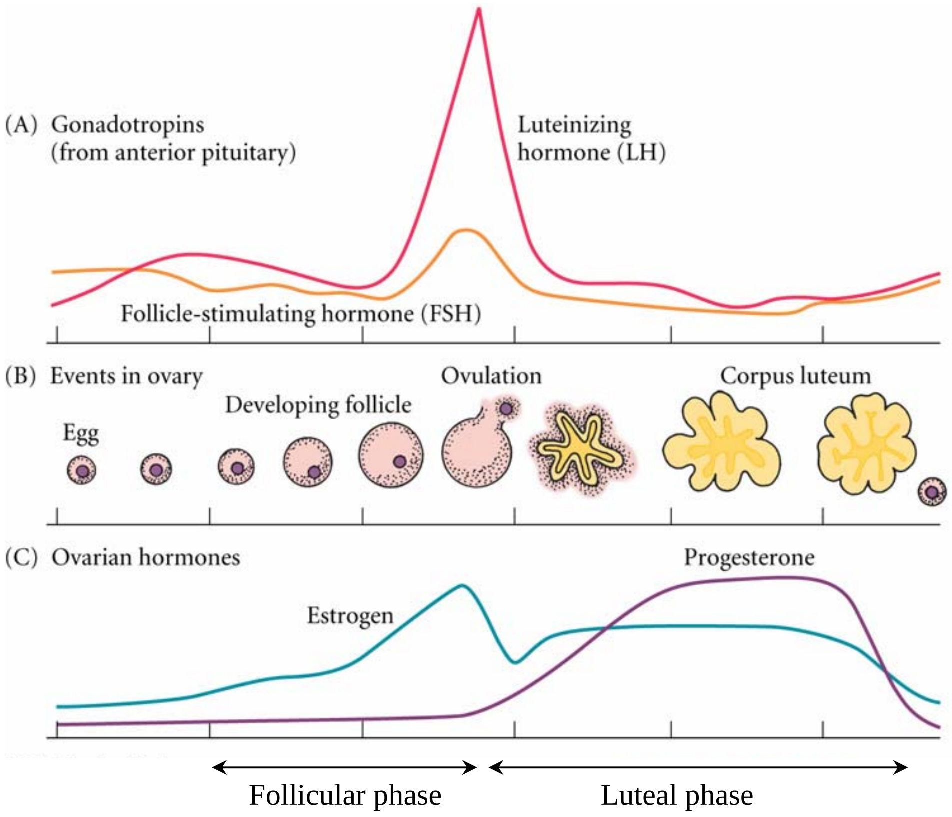

Figure 1.2: (A) Gonadotropic hormone profiles during the oestrous cycle, (B) Development of the egg into a corpus luteum, (C) Ovarian hormone profiles during the oestrous cycle.

- ## Seasonal breeding

Many species inhabiting environments with moderate to extreme seasonal fluctuations in temperature and rainfall display a reversible, annual cycle of fertility that directs their reproductive activity (Bronson & Heideman 1994, Lehman *et al*. 1997). Hence, young are usually born when environmental conditions are most favourable for optimum survival of the offspring.

Physiologically, the change in seasonal reproductive status is controlled by modifications in the activity of the HPG axis through variations in the pulsatile LH secretion. During the non-breeding season, there is a marked change in the responsiveness of the GnRH system to negative feedback of the gonadal hormones (Karsch & Moenter 1990, Lehman *et al*. 1997). In the breeding season, oestrogen inhibits the GnRH pulse amplitude while progesterone suppresses the pulse frequency. However, during the non-breeding period, the GnRH pulse frequency is inhibited by both these hormones (Goodman 1996). Outside the breeding period, there is a complete down-regulation of reproductive function in females of species such as sheep and horses (Lehman *et al.* 1997). The secretion of GnRH decreases dramatically, consequently the pituitary cannot release significant amounts of LH and FSH. Hence, gonadal steroid production diminishes (Eagle & Tortonese 2000).

In males, reproductive activity outside the breeding season is suppressed to a lesser degree than in females (Dacheux *et al.* 1981). Testicular size, testicular and plasma testosterone, sperm production and reproductive behaviour are reduced (Berndston & Desjardins 1974, Hoffmann 1979) but species differ in the degree of reproductive down-regulation. In some species like the horse, fertility is maintained at a reduced level (Guillaume 1996), whereas in some other species like hamsters (Turek *et al*. 1975), the grey squirrel (Tait & Johnson 1982) and brown bears (Tsubota *et al*. 1997), a near complete cessation of spermatogenesis occurs. In addition, intra specific variation also occurs. Some male prairie voles (*Microtus ochrogaster*) undergo complete gonadal regression during short days while others fail to respond to photoperiod and do not show any inhibition of reproduction (Kriegsfeld & Nelson 1999).

However, ‘out of season’ breeding occurs in virtually all rodent populations (Moffat *et al*. 1993). The house mouse (*Mus musculus*) reproduces seasonally when in seasonal habitats, but in a constant environment, offspring is produced throughout the year (Bomford 1987, Efford *et al*. 1988). In the badger, testicular activity shows seasonal fluctuations, spermatogenesis occurs throughout the year (Audy *et al*. 1985). Thus although reproductive activity is interrupted during the non-breeding season, the hypothalamo-pituitary axis appears to remain active. This provides the possibility for opportunistic breeding throughout the year whenever conditions are favourable.

A number of environmental factors have been shown to play a role in the regulation of seasonal breeding, such as photoperiod, temperature, and humidity (Bronson & Heideman 1994). In mammals, photoperiod is the most prominent environmental cue used for timing their annual reproductive cycles. It is the single most reliable environmental cue to indicate the time of the year (Saunders 1977). The reproductive system is activated or shut down when the photoperiod reaches a certain critical day length.

Subterranean mammals spend the majority of their lives underground in burrows and therefore do not come into regular contact with environmental light cycles (Ben-Shlomo *et al*. 1995, Oelschläger *et al*. 2000, Ricco & Goldman 2000). Thus, light probably does not play a significant role in seasonal reproductive cycles in these species.

Temperature is another environmental factor that shows seasonal variation. Although not as reliable as photoperiod, some species have been shown to entrain activity rhythms to temperature cycles. Although temperature is much buffered underground, seasonal differences are detectable closer to the soil surface. A number of animals have been shown to entrain activity rhythms to temperature cycles (Aschoff & Tokura 1986, Gavaud 1991, Lopez-Olmeda *et al.* 2006, Rajaratnam *et al*. 1998, Tokura & Aschoff 1983, Yoshii *et al*. 2005), and in certain animals annual cycles are dependent on ambient temperature.

Around the equator, there is no annual change in photoperiod. However, rainfall tends to be strongly seasonal. Seasonal rainfall influences the availability of food and promotes seasonal breeding according to food resources (Brosset 1986).

In addition to abiotic factors, social cues can also strongly influence reproductive status of animals. Behavioural interactions between different sexes can initiate neuroendocrine events required for successful copulation, ovulation and pregnancy (Tai *et al*. 1997) In some cases sensory signals can stimulate the activation of gonadotropin releasing hormone neurons (Ferron & Gheusi 2003, Moffatt *et al*. 1995, Perret 1992, Richardson *et al*. 2004, Westberry & Meredith 2003), while in other cases coitus acts as the stimulating factor (Bakker *et al*. 2001, Dellovade *et al.* 1995, Rissman *et al.* 1997).

• Cooperative breeding

Cooperative breeding occurs in a number of small mammals (e.g. marmosets, suricates, mongooses, prairie voles and mole-rats) (Faulkes & Abbott 1997). The extent of cooperation varies from social groups where several males and females regularly breed to groups where reproduction is restricted to a single breeding female and one or two dominant males (Emlen 1991, Creel & Waser 1991). The non-breeding members of the colony are typically helping with various tasks such as foraging and tending to young (Clutton-Brock 2002, Clutton-Brock *et al*. 2001, Faulkes & Bennett 2001, Hodges 2005, Schaffner & French 1997, Solomon & Vandenbergh 1994).

Eusociality is typically associated with insects such as bees, wasps, ants and termites. An eusocial breeding strategy is considered as an evolutionarily advanced level of colonial living where groups of cooperatively breeding conspecifics have a reproductive division of labour, cooperative care of young and more than two overlapping adult generations (Wilson 1971, Wilson & Hölldobler 1995). On the eusociality continuum, social systems are rated on a scale from zero to one, such that in societies with a low skew (close to zero), all individuals have almost equal opportunities for breeding and those with a high skew (close to one) have a more despotic type of reproductive system (Sherman *et al*. 1995).

- African mole-rats

African mole-rats are subterreanean hystricomorph rodents endemic to sub-Saharan Africa (Skinner & Smithers 1990). The family Bathyergidae (African mole-rats) is composed of at least 16 species and five genera (Faulkes *et al*. 2004, Ingram *et al.* 2004). Of the five genera, three genera (*Georychus, Heliophobius* and *Bathyergus*) are solitary and two genera (*Cryptomys* and *Heterocephalus)* are social (Bennett & Faulkes 2000), displaying varying degrees of sociality and cooperative breeding. Social mole-rat species exhibit a reproductive division of labour; consequently reproduction in colonies is highly skewed and affects the lifetime reproductive success of subordinate animals (Faulkes & Bennett 2001).

Two species, the Damaraland mole-rat (*Cryptomys damarensis*) and the naked mole-rat (*Heterocephalus glaber*), are considered to be truly social or eusocial (Jarvis 1981, Jarvis & Bennett 1993). Eusociality is thought to have evolved independently in these two species, it is not observed in any other subterranean mammal but further studies may include other species of mole-rat within this definition (Faulkes *et al.* 1997, Jarvis & Bennett 1993).

Comparisons between group size and the environment where the different species occur has led to the idea that the degree of sociality observed across the family Bathyergidae is correlated to the aridity of the habitat and subsequent food availability (Faulkes & Abbott 1997, Jarvis & Bennett 1991, 1993, Jarvis *et al*. 1994). The aridity food distribution hypothesis (AFDH) has been proposed to explain the subsequent costs and risks associated with foraging and dispersal in arid areas (Jarvis *et al.* 1994). Mole-rats live in extensive underground burrows that are excavated with their teeth. The primary food source of mole-rats is geophytes, roots and tubers that are encountered as they burrow (Jarvis & Bennett 1991). In areas with a predictable and frequent rainfall pattern, food resources are evenly distributed and soil is readily workable for most of the year. Solitary species are confined to these mesic areas where a single animal can easily find sufficient food to sustain itself. In arid areas mole-rats are energetically restricted by dry, hard soil, and food resources are more clumped and further apart. Since mole-rats forage blindly, a larger colony size will increase the probability of encountering a localised food patch and reduce energetic costs and tooth wear. While solitary species are confined to mesic areas, social species are not excluded from those areas. However, social species that are adapted to more arid environments tend to have larger colony sizes as the total energetic costs of foraging are reduced (Jarvis *et al.* 1994)

Alternatively, Burda *et al*. (2000) suggests that eusociality in mole-rats results from cooperative monogamy and is reinforced by a subterranean lifestyle. In this scenario, dispersal is restricted, and it allows for continuous rather than seasonal breeding, which would lead to rapid overlap of generations. They propose that mole-rats have an ancestral tendency to be solitary. Differing rates of change between social to solitary along the different phylogenetic lines may validate any variation in the different species.

• Reproductive skew in mole-rats

In social mole-rat species, breeding opportunities are monopolised by dominant animals; usually a single female and one or two males that are responsible for procreation in a colony (Jarvis 1981, Jarvis & Bennett 1993). This unequal distribution of reproduction creates a reproductive skew, which in terms of lifetime reproductive success, differs significantly between species. It is not clear exactly what role the dominant animals play in maintaining this reproductive skew, and the proximate mechanisms underlying this reproductive division of labour may differ even between closely related species (Faulkes & Bennett 2001).

A number of models have been proposed to explain reproductive skew in cooperatively breeding societies (for overview see Faulkes & Bennett 2007). Two of these are particularly applicable to explain the maintenance of a reproductive skew in mole-rats (Snowdon 1996). The dominant control model suggests that the dominant animals exert some form of reproductive control over subordinate individuals (Faulkes & Bennett 2001). The breeding animal benefits from assistance provided by non-breeding colony members and avoids reproductive competition by suppressing subordinate animals

(Snowdon 1996). Reproductive control ranges from infanticide of the offspring of a subordinate, aggression and interference with mating attempts, to suppression of the reproductive physiology of other members in the colony (Faulkes & Abbott 1997). Naked mole-rats differ from other mole-rat species in that they will spontaneously inbreed in captivity (Faulkes & Bennett 2007) although when given a choice, they do prefer outbreeding (Clarke & Faulkes 1999). There is a very high reproductive skew among members of their colonies and lifetime reproductive success is almost zero (Jarvis *et al.* 1994).

Alternatively, the self restraint model implies that incest avoidance may suffice to maintain the reproductive skew between closely related animals. Most cooperatively breeding mammals live in extended family groups, and because breeding with close relatives is often deleterious, most species have evolved mechanisms to prevent inbreeding (Cooney & Bennett 2000, Pusey & Wolf 1996). Species from the genus *Cryptomys* are obligate outbreeders, consequently a lack of unrelated breeding partners might be adequate to prevent subordinate animals from breeding (Cooney & Bennett 2000).

No individual factor can be singled out to explain delayed dispersal and group living. Non-breeding individuals may benefit from group living with respect to enhanced foraging efficiency and reduced risk of predation (Koenig *et al*. 1992). In mole-rats, ecological constraints are major factors that contribute to cooperative breeding. High energetic costs of dispersal may lead to individuals remaining in their natal colonies (Lovegrove & Wissel 1988, Lovegove 1991). Hamilton (1964) proposed that by helping closely related individuals rear their offspring, helpers gain indirect fitness benefits. Also, helpers frequently increase their own chances of becoming breeders by gaining experience (Emlen 1997). Thus, non-reproductive animals may delay their dispersal until opportunities arise, when environmental conditions are favourable or unrelated animals are present or they have gained sufficient skills to ensure successful reproduction independently.

• Reproductive suppression in mole-rats

In mole-rats, the extent and type of reproductive suppression is correlated with the degree of sociality (Figure 1.3). Reproductive suppression amongst subordinate animals is either behavioural or physiological or a combination of the two. Behavioural suppression entails interference with breeding attempts of subordinate animals by dominant animals or the subordinate individuals that refrain from breeding to avoid inbreeding (Snowdon 1996). Alternatively, reproduction is physiologically interrupted and in extreme cases, reproduction can be completely suppressed by blocking ovulation (Abbott 1987, Bennett *et al*.1999).

Studies of several loosely social species with relatively small colony sizes (the Mashona mole-rat, *C.darlingi*; the common mole-rat, *C.h.hottentotus;* the giant mole-rat, *C.mechowi*; Ansell's mole-rat, *C.anseli*) have revealed that there is no difference in the pituitary function of breeding and non-breeding animals. Subordinate males and females have comparable LH levels in response to a GnRH challenge to the dominant animals (Bennett *et al.* 1997, Bennett *et al*. 2000, Burda *et al*. 1995, Spinks *et al*. 2000). Hence, it appears that the reproductive skew is maintained by inbreeding avoidance alone.

In the Damaraland mole-rat (*C.damarensis*), physiological suppression of reproduction is apparent in subordinate females. Although their ovaries possess a degree of follicular development, no Graafian follicles are present (Bennett & Jarvis 1988b). Urine and plasma progesterone concentrations as well as LH concentrations are measurable but lower in non-breeding females (Bennett *et al*. 1999). However the pituitary show a reduced sensitivity towards exogenous GnRH compared to that of the reproductive females (Bennett *et al*. 1993).

No physiological block is apparent in subordinate male Damaraland mole-rats. Sperm number and motility is similar to that of the breeding animals (Faulkes *et al*. 1994). Also no differences were found in urinary and plasma testosterone (Bennett 1994) plasma LH or LH responses to exogenous GnRH (Bennett *et al*. 1993).

The naked mole-rat (*H.glaber*) is placed at the apex of this continuum, with the most stringent level of reproductive suppression exerted by dominant animals (Figure 1.3). A physiological block of reproduction is evident in non-breeding animals of both sexes (Faulkes *et al*. 1990a,b, Faulkes *et al*. 1991). The ovaries of subordinate naked mole-rat females have an immature appearance (Faulkes *et al.* 1990a). Urinary concentrations of oestrogen and progesterone are very low in these animals (Faulkes *et a*l. 1991, Westlin *et al*. 1994), and LH concentrations are significantly lower than that of breeding animals (Faulkes *et al*. 1990b). The LH response to exogenous GnRH suggests a reduced sensitivity of the pituitary to GnRH (Faulkes *et al*. 1990b, 1991), hence the LH production and secretion is insufficient to induce ovulation.

Subordinate male naked mole-rats have smaller testes and fewer testosterone secreting Leydig cells (Faulkes *et al.* 1994, Faulkes 1991). Although spermatogenesis takes place in subordinate males, the sperm number and motility are significantly reduced (Faulkes *et al*. 1991). All hormonal levels associated with reproduction in subordinate males are much reduced when compared to dominant males (Faulkes & Abbott 1991, Faulkes *et al*. 1991). It appears that with higher levels of sociality, dominant animals expend more energy on suppression of reproductive function of subordinate animals.

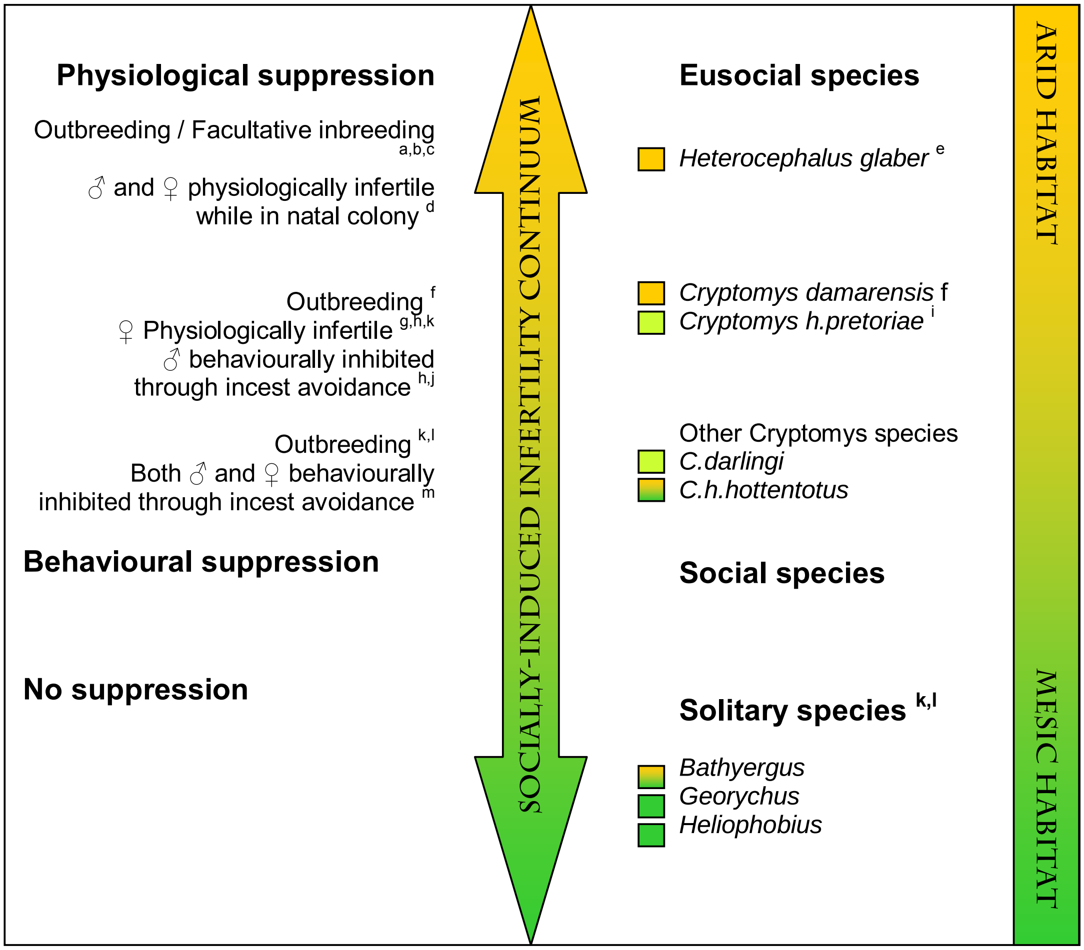

Figure 1.3: Species of the family Bathyergidae assembled according to the degree of sociality displayed and type of habitat in which they occur. (Modified from L. van der Walt 2003).

[a] Reeve *et al*. 1990; [b] Braude 2000; [c] Clarke & Faulkes 1999 [d] Faulkes *et al*. 1990a,b, 1991; [e] Jarvis 1981; [f] Jarvis & Bennett 1993; [g] Bennett *et al*. 1996; [h] Bennett 1994; [i] Moolman *et al*. 1998; [j] Van der Walt *et al*. 2001; [k] Jarvis & Bennett 1991; [l] Bennett & Faulkes 2000; [m] Spinks *et al*. 1997, 1999, 2000; ♀ - female; ♂ - male.

Study animals

- **The Cape mole-rat (*Georychus capensis*)**

The Cape mole-rat, *Georychus capensis* is a seasonally breeding, subterranean rodent mole (Bennett & Jarvis 1988a). It primarily inhabits coastal and mountain fynbos areas of the south-western Cape regions with a distinct seasonal rainfall pattern (Skinner & Smithers 1990).

The Cape mole-rat is strictly solitary, and like other solitary mole-rats, highly aggressive and territorial (Nevo 1961, Zuri *et al.* 1998). They display extreme levels of antagonistic behaviour towards conspecifics throughout most of the year (Guttman *et al*. 1975). This xenophobic behaviour is briefly broken down during the breeding season, when other mole-rats are tolerated in their tunnels for mating and subsequently the rearing of offspring (Bennett & Jarvis 1988a). The Cape mole-rat is an induced ovulator (Van Sandwyk & Bennett 2005), and courtship and copulation is confined to the rainy, winter months (Bennett & Jarvis 1988a). The gestation period is around 45 days and young are produced from August to December. Females appear to have the reproductive potential of producing two litters per season (Bennett & Jarvis 1988a).

Neighbouring individuals communicate through the soil by drumming their hind feet on the tunnel floor (Bennett & Jarvis 1988a, Narins *et al.* 1992). This seismic signalling is used to convey information about their territorial boundaries and, during the breeding season, sex and reproductive status (Bennett & Jarvis 1988a, Narins *et al*. 1992, Rado *et al*. 1998) (Plate 1.1).

- **The Natal mole-rat (*Cryptomys hottentotus natalensis*)**

Very little is known about the Natal mole-rat, *Cryptomys hottentotus natalensis*. Previously it has been thought to occur in groups of two or three animals (Hickman 1979a), however in the current study, colonies of up to 17 animals have been caught from a single burrow system (M. Oosthuizen, *pers.obs*). The known distributional range extends from KwaZulu-Natal to Mpumalanga in the eastern parts of South Africa (Skinner & Chimimba 2005).

The Natal mole-rat is closely related to the common mole-rat (*Cryptomys hottentotus hottentotus*) and the highveld mole-rat (*Cryptomys hottentotus pretoriae*) (Bennett & Faulkes 2000) and thus is predicted to exhibit similar reproductive characteristics. The Natal mole-rat is a cooperative breeder, with a single breeding female (M. Oosthuizen, *pers.obs*.) and 1 or possibly more breeding males, while the remaining individuals of the colony theoretically comprise the offspring of the breeding animals. To date, no information is available on the colony structure as no behavioural or genetic studies have been conducted. Information pertaining to the reproductive biology is sparse (Hickman 1982). The Natal mole-rat is an induced ovulator (Jackson & Bennett 2005), but it is unknown whether its breeding period is confined to a specific part of the year (Plate 1.2).

Plate 1.1: The Cape mole-rat (*Georychus capensis*).

Plate 1.2: The Natal mole-rat (*Cryptomys hottentotus natalensis*)

Aims

Two mole-rat species, the solitary Cape mole-rat (*Georychus capensis*) and the social Natal mole-rat (*Cryptomys hottentotus natalensis*) were the subjects of investigation in this thesis with the explicit purpose of increasing our knowledge on their reproductive systems.

To date there is limited information on the reproductive biology of the Cape mole-rat, whereas that pertaining to the Natal mole-rat is scant (Bennett & Jarvis 1988a, Hickman 1980). The objective of this study was to obtain a comprehensive understanding of all levels of the reproductive systems of these two species. It is known that the Cape mole-rat is a seasonal breeder, therefore the focus in this species will be on seasonal differences in the reproductive system. Before commencing the study, it was unknown whether the Natal mole-rat bred seasonally or not, thus seasonal differences were investigated along with the effect of reproductive status on the reproductive system. Data are analysed according to summer (non-breeding season for the Cape mole-rat) and winter (breeding season for the Cape mole-rat).

Chapter 2

The material and methods utilised in the various chapters are presented in this chapter.

Chapter 3

The objective of this chapter was to determine whether there are any seasonal differences in gonadal hormone levels in the Cape and Natal mole-rat and whether social status has an effect upon secretion of these hormones in the latter.

My *a priori* predictions are:

Cape mole-rat:

- There would be a seasonal difference in the levels of the hormones

Natal mole-rat:

- No seasonal difference in hormonal levels
- Distinct difference between breeding and non-breeding animals

Chapter 4

This chapter focuses on the effect of season and social status on the plasma LH concentrations before and after a GnRH challenge.

My *a priori* predictions are:

The solitary Cape mole-rat:

- Both males and females would exhibit differences in the basal concentrations of LH and differential responses to an exogenous GnRH challenge in and out of the breeding season.

The social Natal mole-rat:

- There would be no seasonal differences in the basal LH concentrations and response to a GnRH challenge in either sex during any part of the year.
- The basal LH concentrations and the response to a GnRH challenge would be similar between reproductive and non-reproductive animals of either sex.

Chapter 5

This chapter investigates the neuroanatomical and neuroendocrinological differences with regard to season in the Cape mole-rat and to social status in the Natal mole-rat. Having established that no seasonal differences are present in the Natal mole-rat, seasonal differences were not explored in this chapter. The neuroanatomy of the GnRH system in solitary and social mole-rats was compared.

My *a priori* predictions are:

Cape mole-rat:

- There would be a seasonal difference in the GnRH content of the median eminence of the female animals in and out of the breeding season.

Natal mole-rat:

- There would be a difference in the GnRH content of the median eminence in reproductive and non-reproductive animals of both sexes.

Chapter 6

This final chapter synthesizes the findings of this study and compares it in the light of the existing data of the family Bathyergidae. The findings of the study are placed into the broader context of the regulation of reproduction in the African mole-rats.