Social suppression of reproduction in the naked mole-rat, *Heterocephalus glaber*:- Plasma LH concentrations and differential pituitary responsiveness to exogenous GnRH.

Laura-Anne van der Westhuizen

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by

Laura-Anne van der Westhuizen

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Department of Zoology
Faculty of Science
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Dedication

This thesis is dedicated to my parents Tessa and Jimmy, for all their love....

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ABSTRACT

The naked mole-rat provides possibly the most extreme example of socially-induced reproductive suppression observed in any mammal. The majority of colony members remain reproductively suppressed for their entire lives, nevertheless they are not sterile, and can become reproductively active soon after removal from the colony. The breeding female controls reproductive suppression in the non-breeding animals, and both males and females are sensitive to her presence and reproductive state. The breeding female is thought to maintain reproductive suppression in the non-breeding animals by preferentially directing agonistic behaviour towards those animals that pose the greatest threat to her reproductive supremacy.

Previous studies have investigated the phenomenon of reproductive suppression in the naked mole-rat, and have compared breeding and non-breeding animals selected at random from several colonies (Faulkes, 1990; Faulkes *et al.*, 1990a; 1990b; Faulkes & Abbott, 1991), but none has looked at the individuals of entire colonies. Moreover, the time in the breeding female's reproductive cycle when data have been collected has never been standardised. The aim of this study was to conduct the first large-scale investigation of reproductive suppression in the individuals of entire colonies of naked-mole-rats, over the reproductive cycle of the breeding female.

The study was conducted on two colonies of naked mole-rats. In order to standardise the time in the breeding female's reproductive cycle during which data would be collected, the gestation period was divided into three time intervals, early (days 1-15), mid (days 30-40) and late (days 55-65) pregnancy, with the assumption that mating occurred on day 10 after parturition. During each time interval, behavioural data and blood samples were collected, and GnRH challenges were performed on all the colony members over 26 g. An in-vitro bioassay based on the production of testosterone by dispersed mouse Leydig cells was used to measure LH concentration in plasma samples collected before and 20 min after an exogenous dose of 0.1 μg GnRH.

The results of this study support the findings of these previous studies, by providing conclusive evidence for reproductive suppression in non-breeding naked mole-rats, and by confirming that non-breeding females are more suppressed than non-breeding males. However, they also showed that there is variation between individuals in terms of both basal plasma and post-GnRH plasma

LH concentrations, implying that certain individuals are more suppressed than others. This variability is more pronounced amongst the non-breeding males than the non-breeding females of naked mole-rat colonies. Relatively few interactions occur between the breeding female and the majority of the colony members, and there is no clear relationship between plasma LH and the amount of aggression received from the breeding female. However, it appears that age is an important factor, and older colony members have higher plasma LH concentrations than younger animals, and, moreover, receive most of the breeding female's aggression.

A take-over event in one of the study colonies provided a unique opportunity to investigate "natural" succession (*i.e.* without the planned removal of the breeding female) in a naked mole-rat colony. The breeding female was physically challenged by a non-breeding female which had been the primary recipient of her aggression prior to the take-over. During the fighting that followed, seven females, including the breeding female, and five males were killed. The female that finally became the new breeding female was the heaviest female at the start of the study, but she only became involved in the fighting once the two next heaviest females had been killed. Nevertheless, age appears to be more important than body mass in determining which animals become involved in the fighting accompanying succession. During this particular take-over event, all the offspring of the original breeding pair, except the new breeding female and the three putative breeding males were killed, yet not all the heaviest animals in the colony were killed. Therefore, although it is not possible to predict which female will become the new breeding female, it is likely that she will be among the oldest and heaviest females in the colony. It is possible, to a certain extent, to predict which group of females, and to a lesser degree which males, are the most likely to become involved in the intra-colonial fighting accompanying colony succession. Finally, it is interesting that the take-over event was preceded by the death of the breeding male 48 days earlier, and it appears that the death of the breeding male in a colony may have more important consequences, in terms of colony succession, than previously considered.

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Chapter 1

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GENERAL INTRODUCTION

1.1 General

The naked mole-rat is a hystricomorph rodent belonging to the family Bathyergidae. The first sample specimen was collected in Ethiopia and described in 1842 by Eduard Rüppell, who gave it the name *Heterocephalus glaber* (Rüppell,1842). *Heterocephalus glaber,* meaning "different-headed, smooth", provides an apt description of the appearance of this animal which, unlike the rest of the Bathyergidae, lacks a pelage. Its head is dorso-ventrally flattened, with large extra-buccal incisors, tiny, vestigial eyes, and only the slightest trace of pinnae.

Bathyergid mole-rats are endemic to sub-Saharan Africa, and the geographic distribution of the naked mole-rat is restricted to the arid regions of the Horn of Africa including parts of Ethiopia, Kenya and Somalia. These regions are characterised by irregular rainfall with a low average of approximately 200-400 mm of rain per year (Honeycutt, Allard, Edwards & Schlitter, 1991a). In general, the localities in which the naked mole-rat occurs are characterised by fine, sandy soils which, although extremely hard during dry seasons, soften substantially after rain (Jarvis & Bennett, 1991). This is important as the naked mole-rat is entirely subterranean and constructs extensive burrow systems in which to live.

Early studies on the naked mole-rat tended to focus on taxonomy and anatomy, and it was only in 1957 that the first comprehensive study was conducted. Following research in the wild and captivity, Osman Hill and his co-workers described the habitat and burrow system structure, anatomy and parasitology, as well as the first details of the behaviour of the naked mole-rat (Hill, Porter, Bloom, Seago, & Southwick, 1957). Jarvis (1981) was the first to breed naked mole-rats in captivity and to report on the reproductive and behavioural divisions of labour exhibited by this animal. Brett (1986) has made a significant contribution to the current knowledge of the ecology and behaviour of wild naked mole-rat colonies, and more recently O'Riain (1996) has provided a detailed qualitative and quantitative description of the behavioural and morphological development of naked mole-rats from birth through to adulthood. Most of what is understood

PLATE 1: *Heterocephalus glaber*, meaning "different-headed smooth", provides an apt description of the appearance of the naked mole-rat. It is almost completely hairless and its head is dorso-ventrally flattened, with large extra-buccal incisors. Being entirely subterranean it has loose skin, short limbs, tiny, vestigial eyes, and only the slightest trace of pinnae. *Above*, A group of naked mole-rats huddling in the communal nest chamber.

about the reproductive physiology of the naked mole-rat is due to the work of Faulkes (1990) who was the first to investigate the physiological mechanisms involved in reproductive suppression in non-breeding naked mole-rats, and the social and environmental factors bringing about the extreme reproductive suppression exhibited by this animal.

1.2 Colony composition and social organisation

Subterranean mammals are generally solitary, but coloniality does occasionally occur (Nevo, 1979). Of the seven families of subterranean rodents, the Bathyergidae display the widest range in both social structure and body mass. The five bathyergid genera have social structures ranging from a solitary to a eusocial lifestyle; mole-rats of the genera *Bathyergus*, *Georychus* and *Heliophobius* are solitary while those of the genera *Cryptomys* and *Heterocephalus* are social (Jarvis and Bennett, 1991). The naked mole-rat, *H. glaber*, is the most highly social of all the Bathyergidae.

The naked mole-rat lives in colonies averaging 70-80 individuals, with a wide variation in colony size. Colonies have been found to contain as few as two and as many as 295 individuals, and the variability appears to reflect food availability and founding events (Brett, 1991a; Sherman, Jarvis & Braude, 1992; Braude, pers. com.).

One of the most widely studied aspects of naked mole-rat biology is its highly complex social system. Jarvis (1981) described the social organisation of naked mole-rat colonies and showed that it is very similar to that seen in eusocial (Michener, 1969) insects. Wilson (1971) proposed three criteria for defining eusocial species: a reproductive division of labour, an overlap of at least two generations and co-operative care of the young. The naked mole-rat meets all three criteria, and, although eusociality was previously believed to be restricted to insects, Jarvis (1981) has proposed that the naked mole-rat is in fact a eusocial mammal.

The naked mole-rat exhibits a distinct reproductive division of labour, and despite relatively large colony sizes (Brett, 1986), reproduction is usually monopolised by a single female and 1-3 males per colony (Jarvis, 1981; Brett, 1986, Jarvis, 1991a; Lacey & Sherman, 1991). The remaining colony members are reproductively suppressed and are classified as non-breeders (Jarvis, 1981). Many generations of naked mole-rats appear to remain in the natal burrow system, contributing either directly or indirectly to the care of the offspring born of a single reproductive female. Although every animal in the colony is potentially a reproductive, laboratory data indicate that the majority of individuals will remain reproductively suppressed for their entire lifespans (Jarvis, 1991a).

Naked mole-rat colonies therefore consist of the breeding female, breeding male(s) and a number of non-breeding animals which are either offspring or close relatives of the breeding female (Jarvis, 1991a). As a result of consanguineous mating, the naked mole-rat has one of the highest inbreeding coefficients ($F = 0.45 \pm 0.18$) recorded among wild animals, and individuals within colonies are extremely similar genetically (Faulkes, Abbott & Mellor, 1990c; Reeve, Westneat, Noon, Sherman & Aquadro, 1990; Honeycutt, Nelson, Schlitter & Sherman, 1991b).

The breeding female is usually one of the largest animals in the colony, while the breeding males, although they may start out as large animals, are often emaciated as a consequence of a marked mass loss over time (Jarvis, O'Riain & McDaid, 1991). The breeding animals, together with the younger colony members, perform most of the pup-care activities, but rarely participate in colony-maintenance or defence activities, which are performed by the non-breeders (Lacey & Sherman, 1991). A recent long-term study (O'Riain, 1996) has suggested that the breeding female is the only colony member exhibiting morphological and behavioural specialisation. There appears to be a generalised social system, with a lack of temporal worker castes and morphological specialisation among non-workers, although the oldest colony members, from the first litter, are larger than the individuals of subsequent litters (O'Riain, 1996). Two exceptions to the apparent lack of morphological specialisation are the colony defenders, which tend to be the largest, oldest non-breeding animals in a colony (Lacey & Sherman, 1991; O'Riain, 1996) and dispersers, which are significantly heavier than non-dispersers due to a higher percentage of body fat (O'Riain, Jarvis & Faulkes, 1996). The morphology, behaviour and development of the naked mole-rat appears to be flexible, and the social organization within colonies allows for rapid short-term behavioural, as well as long-term morphological, adjustments to changing social and environmental conditions (O'Riain, 1996).

Dominance interactions, which strongly influence the social system, occur primarily between breeders and non-breeders, but also among non-breeders (O'Riain, 1996). There are several types of agonistic behaviours exhibited by the naked mole-rat including, amongst others, shoving (Lacey, Alexander, Braude, Sherman & Jarvis, 1991). Shoving occurs more frequently than the other forms of mild aggression observed in naked mole-rat colonies. It occurs when one animal pushes another backwards while in nose-to-nose contact with that animal, and data indicate that the breeding female initiates more shoves per unit time than any other colony member (Reeve & Sherman, 1991). The function of shoving is equivocal. Reeve and Sherman (1991) have suggested that it may be functionally related to the maintenance of reproductive dominance and/or the stimulation of colony activity. The work-conflict hypothesis (Reeve, 1992) postulates potential conflict within naked mole-rat colonies, between the non-breeding animals and the breeding female, in terms of how much aid they should provide in support of her reproductive fitness. The hypothesis predicts that the breeding female should preferentially direct aggression, in the form of shoving, towards large non-breeders, which represent potential breeders, and also towards less related non-breeders, which have less to gain in terms of inclusive fitness, to incite them to provide aid (Reeve, 1992). However, a recent study has found no evidence for the work-conflict hypothesis and has suggested that shoving by the breeding female may have functions other than the incitement of activity, such as the maintenance of reproductive suppression in the non-breeding animals (Jacobs & Jarvis, 1996).

Naked mole-rat colonies are not perfectly harmonious, and, in addition to shoving, a number of other agonistic behaviours, including tooth-fencing, tugging and biting, can be observed (Lacey *et al.*, 1991). The breeding female is the most aggressive colony member (Reeve & Sherman, 1991), but one of the features of captive colonies is the great deal of variability in the amount of aggression shown by the breeding female (Jarvis, 1991a). The reproductive animals, particularly the breeding female, are central to the organization of naked mole-rat colonies (Jarvis, 1991a), and the breeding female appears to maintain control of the colony by the use of aggression. Aggression by the breeding female in naked mole-rat colonies may be analogous to queen aggression in small-colony social insects such as the paper wasp, *Polistes fuscatus*, in which the queen's control of worker activity and reproduction is mediated behaviourally (Reeve & Gamboa, 1983; 1987).

1.3 Reproduction in the naked mole-rat

Although there have been reported instances of more than one breeding female in captive (Jarvis, 1991a) and wild (Sherman *et al.*, 1992; Braude, pers. com.) naked mole-rat colonies, a single breeding female per colony appears to be the norm. The majority of the animals in a colony are reproductively quiescent, and only the breeding female and breeding male(s) are involved in reproduction (Jarvis, 1981; Brett, 1986, Jarvis, 1991a; Lacey & Sherman, 1991).

Under experimental conditions, cycling non-pregnant breeding females exhibit patterns of urinary progesterone which indicate a mean ovarian cycle length of 34.4 ± 1.6 days, with a follicular phase of 6.0 ± 0.6 days and a luteal phase of 27.5 ± 1.3 days (Faulkes, Abbott, & Jarvis, 1990a). Normally, however, the breeding female is pregnant and/or lactating throughout her tenure as the breeding female

(Jarvis, pers. com.). Oestrus occurs postpartum, and although sexual interactions such as ano-genital nuzzling and sniffing are carried out throughout the breeding cycle (pers. obs.), the breeding female exhibits a behavioural oestrus of 24 h or less (Lacey *et al.*, 1991). Mating takes place 7-11 days after the birth of a litter, while the breeding female is still lactating (Jarvis, 1991a; Lacey & Sherman, 1991). Lactation length is variable, but by about 3-4 weeks post-partum, the pups obtain most of their nutrients from solid foods and from coprophagy, and nursing becomes infrequent (Jarvis, 1991a).

The naked mole-rat is an aseasonal breeder, and both captive and wild colonies are capable of producing 4-5 litters per year (Brett, 1991a; Jarvis, 1991a). The interval between successive litters is 77-84 days, with a gestation length of 66-77 days (Jarvis, 1991a; Lacey & Sherman, 1991). There is a great deal of variation in terms of litter size, and litters can contain as few as one or as many as 27 pups. In general, litters born in captivity often consist of more than 12 pups (Jarvis, 1991a), while in wild colonies it appears that 5-15 pups are born per litter (Brett, 1986; 1991a; Jarvis, 1991a).

There appears to be strong behavioural bonding between the breeding female and breeding male(s), who spend a large amount of time together in the nest and frequently engage in mutual ano-genital nuzzling and sniffing interactions (Jarvis, 1991a; Lacey *et al*, 1991). The breeding animals are usually the only individuals in a colony to engage in ano-genital nuzzling and other sexual behaviours on a regular basis (Lacey & Sherman, 1991) and, unless there is colony strife, these behaviours occur only occasionally between the breeders and non-breeders and among the non-breeders of both sexes (Jarvis, 1991a).

1.4 Reproductive suppression

Reproductive suppression occurs in many mammals, and the factors responsible for bringing it about can be divided into three main categories: (1) Environmental factors, (2) Lactation and (3) Social factors (Abbott, 1988).

The environmental factors bringing about reproductive suppression are primarily associated with seasonal changes in photoperiod, temperature, rainfall and food availability. Many mammalian species, particularly those living in temperate regions, have adopted seasonal breeding as a means for maximising reproductive success, with mating taking place at a specific time each year to ensure that the birthing period is synchronised with a time of year when food is abundant (Follett, 1985). Two examples of seasonal breeders are the red deer (Lincoln and Short, 1980) and the sheep (Karsch, Bittman, Foster, Goodman, Legan & Robinson, 1984).

In seasonal breeders, the most common environmental cue determining when reproduction occurs is photoperiod (Bronson & Heideman, 1994), and there is substantial evidence to suggest that the photoperiodic control of the release of the hormone melatonin by the pineal gland, plays an important role in regulating hypothalamic-pituitary-gonadal activity (Turek & Van Cauter, 1994). It is believed that it is not the melatonin *per se*, but the diurnal changes in melatonin secretion, that functions as a timing signal to co-ordinate internal events with the environmental light/dark cycle (Ganong, 1991). Changes in the secretion of melatonin result in a reduction in the release of gonadotrophin releasing hormone (GnRH) from the hypothalamus, leading to insufficient stimulation of synthesis and release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) by the anterior pituitary. Consequently, the gonads are not stimulated and the suppression of ovulation or reduced sperm production results in impaired fertility (Abbott, 1988).

The naked mole-rat is entirely subterranean, living in extremely thermostable burrow systems (McNab, 1966). It rarely encounters light, hence its pineal gland is atrophic (Quay, 1981) and its activity patterns appear to be independent of ambient light cycles (Davis-Walton & Sherman, 1994). It an aseasonal breeder (Jarvis, 1991a), and although rainfall is required to soften the soil before the plant roots and tubers on which it feeds can be located, (Jarvis and Sale 1971; Jarvis & Bennett, 1991), the environmental factors which control reproduction in seasonal breeders do not bring about reproductive suppression in this animal. It is interesting, however, that some species in the family Bathyergidae, which live under similar environmental conditions to the naked mole-rat, are seasonal breeders, and others are aseasonal (Jarvis & Bennett, 1991).

In addition to environmental factors, another major naturally-occurring cause of reproductive suppression is lactation. Although the impact of lactation on the fertility of small mammals with high reproductive rates is relatively low, in larger, seasonally breeding mammals, the inhibitory effects of lactation often complement seasonal reproductive suppression, bringing about the annual spacing of births (Short, 1984).

From studies on rats and pigs it appears as though endogenous opioids might be involved in causing the high prolactin and low LH concentrations characteristic of lactation (Sirinathsinghji & Martini, 1984; Mattiolo, Conte, Galeati & Seren, 1986) and that both are the result of the same factor (Mattiolo *et al.*, 1986). It was previously believed that prolactin itself was responsible for the inhibition of GnRH release, but, although suckling does result in the release of prolactin, the principle inhibition seems to arise from the suckling stimulus itself (Kann & Martinet, 1975). Suckling delays the resumption of ovarian activity by suppressing the normal pulsatile secretion of LH, apparently via the suppression of GnRH release from the hypothalamus (Glasier, McNeilly & Baird, 1986), but the hypothalamic mechanism linking the incoming neural signal with the GnRH pulse generator, is unknown (McNeilly, 1994).

Lactation does not play a role in naked mole-rat reproductive suppression as breeding females exhibit a post-partum oestrus, with mating occurring 7-11 days after the birth of a litter, while she is still lactating (Lacey & Sherman, 1991; Jarvis, 1991a).

Social suppression of reproduction is the third naturally-occurring cause of reproductive suppression, and occurs whenever reproduction is somehow inhibited by an individual's interactions with, and the reproductions of, other individuals (Wasser & Barash, 1983). It is recognised in a wide range of mammalian species including the dwarf mongoose, *Helogale parvula* (Rasa, 1973; Rood, 1980), the African wild dog, *Lycaon pictus* (Frame, Malcom, Frame & van Lawick, 1979), the prairie vole, *Microtus ochrogaster* (Getz & Carter, 1980), the gelada baboon, *Theropithecus gelada* (Dunbar, 1980), the yellow baboon, *Papio cynocephalus* (Wasser & Barash, 1983), the marmoset monkey, *Callithrix jacchus, jacchus* (Abbott, McNeilly, Lunn, Hulme & Burden, 1981; Abbott, 1984), the cotton top tamarin, *Sanguinus oedipus*, (French, Abbott & Snowdon, 1984), as well as in the Damaraland mole-rat, *Cryptomys damarensis* (Bennett & Jarvis, 1988; Bennett, Jarvis, Millar, Sasano & Ntshinga, 1994; Bennett, 1994.), the common mole-rat *Cryptomys h. hottentotus* (Bennett, 1989), the Mashona mole-rat, *Cryptomys darlingi* (Bennett, Jarvis & Cotterill, 1994) and the naked mole-rat, *Heterocephalus glaber* (Jarvis, 1981).

In group-living species with hierarchical social organisations, the dominant individuals of the group may suppress reproduction in the subordinate individuals, with the degree of suppression varying greatly from one species to the next. In the dwarf mongoose, some subordinates may become pregnant, but their litters are not raised, while others may mate but not become pregnant, implying a post-copulatory block to reproduction (Rood, 1980). Similarly, in the gelada baboon, subordinate females maintain a level of sexual activity, but conception and birth rates are lower than in dominant females (Dunbar, 1980). On the other hand, in species such as the marmoset monkey (Abbott, 1988) and the cotton top tamarin (French *et al.*, 1984), suppression is more extreme, and reproduction is restricted to a single female in each social group.

There are two major ways in which socially-induced reproductive suppression can be imposed on an individual: one involves the release of primer pheromones by conspecifics, and the other occurs via behavioural intimidation.

In several mammalian species, particularly rodents, primer pheromones have been shown to play an important role in regulating reproductive physiology. Primer pheromones are chemical signals released by conspecifics, which elicit a physiological response in the recipient animal (Vandenberg, 1994). The effects of primer pheromones have been well studied in laboratory mice, and primer pheromones appear to fall into two main categories: those which accelerate sexual development and stimulate reproduction, and those which delay sexual development and inhibit reproduction. In female mice that have been exposed to male urine, the effects include the acceleration of puberty (Vandenberg, 1969), the induction of regular oestrous cycles (Whitten, 1956) and the blocking of pregnancy in newly-mated females which have been exposed to the urine of a foreign male (Bruce, 1965). In female mice that have been exposed to female urine however, puberty is delayed (Cowley & Wise, 1972) and oestrous suppressed (Whitten, 1959). It is likely that primer pheromones have a common endocrinological effect, bringing about changes in prolactin (Keverne, 1983) and LH (Bronson & Desjardens, 1974) secretion from the anterior pituitary.

In group-living species, communicative interactions among individuals generate various forms of stress within the group, that can have profound influences on endocrine physiology and thus reproduction. Stress produced by behavioural intimidation plays an important role in reproductive suppression, particularly in primates. In female gelada baboons, low-ranking females suffer from a loss of fecundity which can only be explained in terms of the stress brought about from harassment by higher ranking females (Dunbar, 1989). In humans too, stress is becoming increasingly identified with GnRH infertility in women (Dunbar, 1985).

The most extreme form of socially-induced reproductive suppression occurs where females require help to raise their young in order to maximise survival rate in the young (Wasser & Barash, 1983). In the common marmoset there is only one breeding female and one breeding male per group, and reproductive suppression is brought about by both behavioural mechanisms, resulting in sexual inadequacy, and physiological mechanisms resulting in the suppression of ovulation (Abbott, 1989).

In well-established families of marmoset monkeys, dominant-subordinate interactions are rare, and although sons can ejaculate motile spermatozoa and daughters can ovulate, mating occurs almost exclusively between the parents. Thus, in families, otherwise fertile offspring are restrained from exhibiting sexual behaviour by some form of inbreeding taboo. In newly established peer groups on the other hand, dominant-subordinate interactions are very common and reproduction is curtailed by the obstruction of subordinate sexual behaviour by the dominant male and female, and the suppression of ovarian cyclicity in all subordinate females (Abbott, 1984).

Reproductive suppression appears to be less stringent among subordinate male marmosets than subordinate female marmosets. Subordinate males are prevented from reproducing by behavioural means only, while subordinate females can suffer from complete infertility. In addition, subordinate males may occasionally mate with the dominant female, despite the behavioural constraints imposed upon them (Abbott, 1984). Subordinate female marmosets are characterised by low plasma LH concentrations and reduced pituitary LH response to GnRH (Abbott *et al*, 1981), and the social suppression of fertility appears to be mediated by impaired hypothalamic GnRH secretion (Abbott, Hodges & George, 1988). There is evidence to suggest that there are two physiological causes of reproductive suppression in subordinate female marmoset monkeys. One is brought about by increased sensitivity of the anterior pituitary to the negative feedback effect of oestradiol, resulting in a reduced secretion of LH . There is also possibly a brain opioid-mediated mechanism causing suppression of the secretion of GnRH and LH, which is completely separate from the oestradiol negative feedback mechanism (Abbott, 1989).

In addition to *Heterocephalus*, colonies of the social bathyergid genus *Cryptomys* also exhibit a reproductive division of labour. Reproduction is restricted to one female and 1-3 males per colony, with the remaining animals being reproductively suppressed. (Jarvis & Bennett, 1991). In the Damaraland mole-rat, *C. damarensis*, reproductive suppression is manifest physiologically and behaviourally in the non-breeding females, and behaviourally in the non-breeding males, as in the marmoset monkey (Bennett, 1994; Bennett *et al.*, 1994). In addition to the physiological suppression of non-breeding females, which occurs in the presence of the breeding female, there appears to be an element of incest avoidance arising from familiarity. In colonies where the breeding female has died or has been removed, the colony remains reproductively quiescent. However, sexual activity resumes following the introduction of a foreign male into the colony (Bennett, Faulkes & Molteno, 1996; Rickard & Bennett, 1997).

In the Mashona mole-rat, *C. darlingi*, reproduction is restricted to a single breeding pair, but reproductive suppression of the non-breeding animals is not physiological. There is no significant difference between the breeding and non-breeding animals of either sex in terms of circulating plasma LH concentration or anterior pituitary sensitivity to exogenous GnRH. The Mashona mole-rat is an obligate outbreeder, and reproductive suppression appears to arise from incest avoidance (Bennett, Faulkes & Spinks, 1997).

Reproductive suppression thus occurs in many mammalian species and can be mediated by a number of factors. The degree of reproductive suppression varies greatly from one species to the next, but the most extreme forms occur in those group-living species which exhibit social suppression of reproduction.

1.5 Reproductive suppression in the naked mole-rat

As outlined above, socially induced reproductive suppression occurs in a wide variety of mammals, but the naked mole-rat provides possibly the most extreme example (Abbott, Barrett, Faulkes & George, 1989). Despite relatively large colony sizes, usually only one female and 1-3 males per colony are involved in reproduction (Jarvis,. 1981; Brett, 1986, Jarvis, 1991a; Lacey & Sherman, 1991). In captivity, the breeding female may monopolise reproduction for up to 15 years, and although every animal born to a colony is potentially a breeder (Jarvis, 1991a) the majority never obtain the opportunity to reproduce.

There are a number of features distinguishing breeding and non-breeding animals, and although both female and male non-breeding naked mole-rats exhibit reproductive suppression, it appears to be less clear-cut in males.

Breeding and non-breeding females are anatomically, histologically and endocrinologically distinct. The external genitalia of the non-breeding females are very similar to those of the males, but the breeding females (and non-breeding females that are close to breeding) can be distinguished from the non-breeding females in having either a perforate vagina or a very thin vaginal closure membrane (Jarvis, 1991a). The ovaries and reproductive tracts of non-breeding females are greatly reduced in size relative to body mass (Hill *et al.*, 1957; Faulkes, 1990) and the ovaries of breeding females are approximately 2-3 times larger and thicker than those of non-breeding females (Kayanja & Jarvis, 1971). The ovaries of non-breeding females are quiescent, with reduced follicular development and an absence of post-ovulatory corpora lutea, indicating that ovulation does not occur (Faulkes, 1990).

Both pregnant and non-pregnant breeding females have elevated levels of urinary and plasma progesterone, while non-breeding females have extremely low, often undetectable levels, indicative of an absence of ovarian activity. Breeding females also have higher plasma LH concentrations than non-breeding females (Faulkes *et al.*, 1990a). The results of single and multiple GnRH challenges have suggested that the anterior pituitary in non-breeding females is less sensitive to low doses of exogenous GnRH, possibly due to a lack of priming by endogenous GnRH. The socially-induced block to reproduction in female naked mole-rats may therefore be due to the inhibition of hypothalamic GnRH secretion (Faulkes, Abbott, Jarvis & Sherriff, 1990b).

Non-breeding males have lower urinary testosterone and plasma LH levels than breeding males, and, like non-breeding females, exhibit reduced pituitary sensitivity to single low doses of GnRH (Faulkes *et al.*, 1990b). However, despite the evident endocrinological deficiencies, reproductive suppression in non-breeding males is not absolute as in non-breeding females. The gross anatomy of the reproductive tract is not obviously different in breeding and non-breeding male naked mole-rats, and histological examination has revealed that spermatogenesis occurs in the testes of both non-breeding and breeding males, implying that criteria other than the presence of spermatozoa must determine which males mate (Jarvis, 1991a). Although these similarities exist, breeding males have larger testis weights relative to body weight, higher sperm counts and lower incidences of non-motile sperm, when compared with non-breeding males (Faulkes, Trowell, Jarvis & Bennett, 1994).

It is believed that the breeding female controls reproductive suppression in the non-breeding animals, but how she does this is still unclear. Initially it was thought that reproductive suppression might be brought about by primer pheromones, secreted into the communal toilet chamber by the breeding female (Jarvis, 1981). However, primer pheromones do not appear to play a major role in the suppression of reproduction in non-breeding naked mole-rats, and the primary factor bringing about reproductive suppression appears to be behavioural cues from the breeding female (Faulkes & Abbott, 1993). The breeding female is the most aggressive colony member (Jarvis, 1991a; Reeve & Sherman, 1991) and it is likely that she employs agonistic behaviours to suppress reproductive function in the non-breeding animals, in order to maintain reproductive dominance.

Both males and females are sensitive to the presence and reproductive state of the breeding female. A curious phenomenon which has been observed in a number of captive colonies is the cyclical development of nipples in non-breeding animals of both sexes. Nipples begin to enlarge in the second half of pregnancy, reaching maximum size just prior to parturition of the breeding female (Jarvis, 1991a; Jarvis, pers. com.). Furthermore, in a study by Westlin, Bennett and Jarvis (1994) it was found that non-breeding females with detectable urinary concentrations of oestradiol-17*B*, many of which had perforate vaginas throughout the study period, showed a significant increase in urinary concentration of this hormone 6 days prior to parturition. In those females with undetectable concentrations of oestradiol-17*B*, the proportion with perforate vaginas increased from 6 days prepartum to a peak on the day of parturition.

There thus appears to be a relaxation of reproductive suppression at certain times of the breeding cycle, and it is thought that the control of the breeding female is weakest during late pregnancy (Jarvis, 1991a). When the breeding female is highly gravid she is less agile, and consequently unable to patrol the burrow system as easily as at other times, and Reeve & Sherman (1991) have found that the breeding female's rate of shoving of the other colony members decreases as pregnancy progresses.

Despite the fact that the majority of non-breeding male and female naked mole-rats remain reproductively suppressed throughout their entire lives (Jarvis, 1991a), they are not sterile. Reproductive activation can occur relatively quickly in both males and females which have been removed from their colonies. Urinary testosterone levels of non-breeding males can rise to levels comparable with those of breeding males within 6 days of separation (Faulkes, 1990) while non-breeding females may develop perforate vaginas and elevated progesterone levels, indicative of ovarian cyclicity, within 8 days after separation from the colony (Faulkes *et al.*, 1990a). In addition, during colony succession events following the death of removal of the breeding female, several non-breeding females may simultaneously become reproductively active and fight for the position of breeding female. Furthermore, there have been several reported cases in which a second breeding female has emerged in a colony and been tolerated by the original breeding female long enough to produce one or more litters. On one occasion, an invasion of a neighbouring captive colony into a colony where the breeding female had just had pups, led to a second female's becoming pregnant, implying that this female was reproductively active at the time of the invasion (Jarvis, 1991a). Therefore, although the form of reproductive suppression exhibited by the non-breeding animals of naked mole-rat colonies is extreme, it is readily reversible, and reproductively quiescent animals have the potential to become reproductively active should appropriate social conditions arise.

1.6 Conclusions and Aims of Research

The purpose of this introductory chapter was to provide a brief introduction to the naked mole-rat, to describe those features of its social structure and reproductive physiology that are pertinent to the present study, and to provide an overview of the phenomenon of reproductive suppression, of which the naked mole-rat provides possibly the most extreme example.

Dr C.G. Faulkes (1990) was the first to investigate the physiological mechanisms involved in reproductive suppression in non-breeding naked mole-rats, and the social and environmental factors bringing it about. His work greatly contributed to our current knowledge on reproductive suppression in the naked naked-mole rat, and provided a concrete foundation for further work on this subject. Several studies have investigated the physiological differences between breeding and non breeding animals (Faulkes *et al.* 1990a; Faulkes *et al.*, 1990b; Faulkes *et al.*, 1991a; Faulkes *et al.*, 1991b), but subjects have been randomly selected from several colonies. No study has yet looked at the reproductive physiology of all the individuals within entire colonies, or related individual hormone characteristics to the reproductive cycle of the breeding female.

Thus, the aim of the present study was to perform the first large-scale investigation into the phenomenon of reproductive suppression in the individuals of entire colonies of naked mole-rats over the reproductive cycle of the breeding female. In addition, a natural take-over event in one of the study colonies provided a unique opportunity to investigate the changes which occur in non-breeding animals during colony succession, and to provide the first detailed qualitative description of a natural succession occurrence.

Chapter 2

Chapter 2

MATERIALS AND METHODS

2.1 Study Colonies

Two colonies of naked mole-rats were used for the study; colony 100 and colony 7400. These colonies were chosen because they had been the subjects of a long-term behavioural study by O'Riain (1996). Both colonies were started from breeding pairs (multiparous females and their mates), in 1987. The individual mole-rats of the two colonies had all been numbered at weaning by clipping unique combinations of toes, and could therefore easily be identified.

2.1.1 History of colony 100

The original breeding male of colony 100 died in 1989, as a result of an infection in the colony, and was replaced by #9, one of the three oldest non-breeding males in the colony. The infection that killed the original breeding male also resulted in the severe swelling of the feet of the breeding female, and for approximately 70 days she was unable to patrol the burrow system or physically dominate her offspring. During this time one of the non-breeding females #15, became sexually active, producing a single litter which did not survive. Once the breeding female had regained her strength, #15 returned to a state of reproductive suppression.

2.1.2 Colony 100 during the study

At the start of the present study, in August 1995, colony 100 consisted of 19 males and 25 females. However, during the course of the study two non-breeding males (#5 and #6) escaped from the colony and could not be reintroduced, and were therefore left out of the analyses. During the study period the breeding female of colony 100 gave birth to two litters, one in September and one in December 1995. None of the pups of either litter survived. Details of the individuals of colony 100 are given in Table 2.1. Mass is expressed as the mean mass of each individual during the study period, and relative age is indicated by litter number.

TABLE 2.1: Details of the individuals of colony 100 (July - December 1995).

Animal	Sex	Litter	Mass (g)
Breeding female	BF	NA	NA
12	F	2	53.8
13	F	2	58.4
15	F	2	47.9
23	F	3	41.8
1	F	4	35.2
25	F	5	41.2
43	F	7	40.3
45	F	7	54.4
48	F	8	41.7
5F	F	8	37.1
57	F	10	25.5
70	F	11	42.4
71	F	11	35.3
72	F	11	39.0
76	F	11	33.3
90	F	11	34.6
92	F	12	33.8
94	F	12	35.2
98	F	12	43.2
CC	F	13	37.3
91	F	13	26.5
95	F	13	35.8
96	F	13	31.9
7	F	14	25.4
9	BM	1	49.0
5	M	2	54.9
6	M	2	76.4
10	M	2	58.8
14	M	2	49.7
16	M	2	73.8
17	M	2	64.5
18	M	2	41.7
20	M	4	51.1
28	M	6	55.1
29	M	6	38.0
30	M	6	67.9
47	M	7	34.4
XX	M	7	69.4
51	M	8	42.1
52	M	8	50.1
54	M	9	34.9
5M	M	8	38.2
200	M	14	24.4

Animals < 26 g excluded from final analyses
Escapees excluded from final analyses

2.1.3 History of colony 7400

The original breeding male of colony 7400 died in January 1992, after which the breeding female bred only once more prior to her own death in August 1992. It was not possible to determine the identity of the temporary breeding male as copulation was not observed. Following the death of the original breeding female, two of the oldest non-breeding females fought for the position, and after 5 months #4 killed the other contender. Despite repeated participation in pre-copulatory behaviours, #4 apparently did not mate with her siblings as she did not become pregnant. However, following the accidental introduction of a foreign male (#FO) into the colony in 1993, #4 began reproducing almost immediately, giving birth within 100 days following the introduction of #FO.

On 29 June 1995, 33 days prior to the commencement of this study, the existing breeding male of colony 7400 (#FO) died as a result of wounds inflicted during inter-colonial fighting on 16 June 1995, when a neighbouring managed to escape and break into the system. Thereafter, although no copulations were observed, the breeding female was seen to engage in mutual ano-genital nuzzling, a behaviour which typically occurs between the breeding female and breeding males, with three of the males (#9, #11 and # 20). Prior to the introduction of #FO the breeding female had engaged in pre-copulatory behaviour with several males including these three. Following the introduction of #FO, the breeding female had killed all the males with whom she had previously engaged in pre-copulatory behaviour, with the exception of these three males (O'Riain, 1996).

2.1.4 Colony 7400 during the study

A few days after the birth of her next litter (12 or 13 August 1995), which had presumably been sired by #FO before his death, the breeding female was wounded by a non-breeding female (#27) towards whom she had displayed heightened aggression during late pregnancy and a few days post-partum. A take-over event followed during which seven females, including the breeding female, and five males were killed before the existing breeding female (#28) took over. Following the take-over event, males #9, #11 and #20 were the only males to engage in ano-genital nuzzling with the new breeding female, and for the purposes of this study they were therefore considered as the putative breeding males.

After the take-over event colony 7400 consisted of 11 males and 11 females. Details of the individuals of colony 100 prior to and after the take-over event are given in Table 2.2. Mass is expressed as the mean mass of each individual before and after the take-over event, and the mean mass of each individual over one breeding cycle of the new breeding female (#28).

TABLE 2.2: Details of the individuals of colony 7400 (July 1995 - March 1996).

Animal	Sex	Litter	Mass (g) Before take-over	Mass (g) After take-over	Mass (g) Over cycle
1	BF	1	[illegible]		
14	F	3	[illegible]		
23	F	5	[illegible]		
27	F	5	[illegible]		
28	F	6	61.1	63.3	NA - BF
31	F	6	[illegible]		
[illegible]	F	6	[illegible]		
[illegible]	F	7	[illegible]		
47	F	9	37.2	38.5	39.4
52	F	9	25.6	[illegible]	[illegible]
4	F	10	44.5	45.7	49.1
6	F	12	31.6	32.9	36.1
7	F	12	41.8	45.6	47.7
75	F	13	33.1	34.6	39.7
80	F	14	23.6	28.6	33.5
82	F	14	23.0	28.2	34.8
83	F	14	25.7	29.9	35.2
84	F	14	25.2	31.0	34.1
9	BM	2	53.1	55.0	53.0
11	BM	3	74.7	72.9	73.0
20	BM	3	61.9	60.4	57.7
[illegible]	M	5	47.5		
[illegible]	M	5	[illegible]		
29	M	6	[illegible]		
34	M	7	[illegible]		
[illegible]	M	7	47.9		
49	M	9	46.4	50.7	47.1
51	M	9	43.2	47.2	44.0
57	M	11	35.1	37.8	40.8
58	M	11	34.3	34.9	35.8
71	M	13	30.2	33.6	37.9
72	M	13	27.1	28.7	36.8
77	M	13	33.8	35.5	39.6
81	M	14	[illegible]	25.8	[illegible]

Animals < 26 g not sampled prior to the take-over event
Animals killed during the take-over event

2.2 Maintenance in Captivity

2.2.1. Artificial Burrow System/Housing

The artificial burrow systems in which the two colonies were housed during the study period were identical and comprised approximately 3.5 m of transparent Perspex tunnels (4.5cm wide by 5.3cm high) with removable lids, connected by several T- and L-shaped Perspex joints. The length of each system was increased by ± 3 m with the addition of a metal run. The Perspex tunnels connected four containers which served as a nest, food chamber and two toilet chambers. The containers were covered with sheets of glass, held in place with bricks, which could easily be removed in order to allow easy access for cleaning of the containers and capture of the animals.

The constant-environment room, in which the systems were housed, was kept at a temperature of 30°C and a relative humidity of 50%. Each system was provided with a lamp containing a 40 W bulb as an additional heat source.

Shredded paper towelling and wood shavings were provided as nesting material, and the toilet and food chamber floors were covered with wood shavings. The toilet and food areas were cleaned daily and the tunnels approximately every 1-2 months. The nest chamber was left as undisturbed as possible and cleaned only when it became very soiled, and each time some of the old nesting material was mixed with the new, in order to maintain colony odour.

2.2.2 Feeding Regime

The mole-rats were fed on a variety of fresh fruits and vegetables, all of which had been washed in a dilute solution of Biocide D, and thoroughly rinsed, prior to being stored. This was done to prevent the introduction of pathogenic bacteria such as *Escherichia coli*, into the colonies. The fruits and vegetables that were most commonly fed to the mole-rats included sweet potato, carrots, butternut, pumpkin, gemsquash, spinach, hubbard squash, peas, sweetcorn, apples, pears, grapes and occasionally clover. The mole-rats were fed once daily and provided with an *ad libitum* food supply consisting of a mixture of diced fruit and vegetables as well as a larger piece of either apple, gemsquash, butternut or sweet potato. The daily diet was supplemented with a portion of Pronutro™, a high protein breakfast cereal, which was moistened with water. It was not necessary to provide drinking water as the naked mole-rat obtains all its water requirements from its food (Jarvis, 1991b).

2.3 Data Collection

Blood samples and behavioural data were collected over a period of approximately 7 months between 1 August 1995 and 7 March 1996, during which time all the animals, except the breeding females, were weighed every 1-4 weeks. In the naked mole-rat, mating occurs approximately 8-11 days postpartum, and is followed by a gestation period of approximately 72 days (range 66-77 days) (Jarvis, 1991a; Lacey & Sherman, 1991). As the study relied on successful pregnancies, no data were collected for a period of 10 days postpartum to ensure minimal disturbance of the colonies. To facilitate identification, the unique toe-clip number of each mole-rat, except the breeding female in each colony, was marked on its back with a black felt-tipped permanent marker. The markings usually lasted for about 5 days, and animals were only marked for the period during which behavioural data and blood samples were to be collected.

Data were collected over the equivalent of two complete cycles of the breeding female of colony 100 and over one cycle of the new breeding female of colony 7400. Data were also collected prior to and after the take-over event in colony 7400. The dates of data collection are given in the relevant chapters.

2.3.1 Behavioural Observations

All behavioural observations were recorded using ***THE OBSERVER***, a software package for behavioural research developed by Noldus Information Technology (Wageningen, The Netherlands). Details of the recording techniques employed and the number of hours of behavioural observation undertaken, are given in the relevant chapters.

Naked mole-rats are particularly sensitive to sound and vibration so the collection of behavioural data was not started until at least 10 min after the observer had entered the room. Similarly, if the study colony was disturbed by outside noises during the data collection session, the recording was immediately suspended for at least 15 min, or until it had been established that activity in the study colony had returned to normal.

2.3.2 Blood Sampling

A total of 680 blood samples from two colonies and 70 animals was collected and assayed for this study. Animals were hand-held and a clearly-visible superficial vein on either the hind or forefoot was pricked with a 0.9 x 40 mm hypodermic needle. Blood was collected by capillary action using heparinised micro-haematocrit tubes (80 µl), and 2-6 tubes were filled per bleed. This

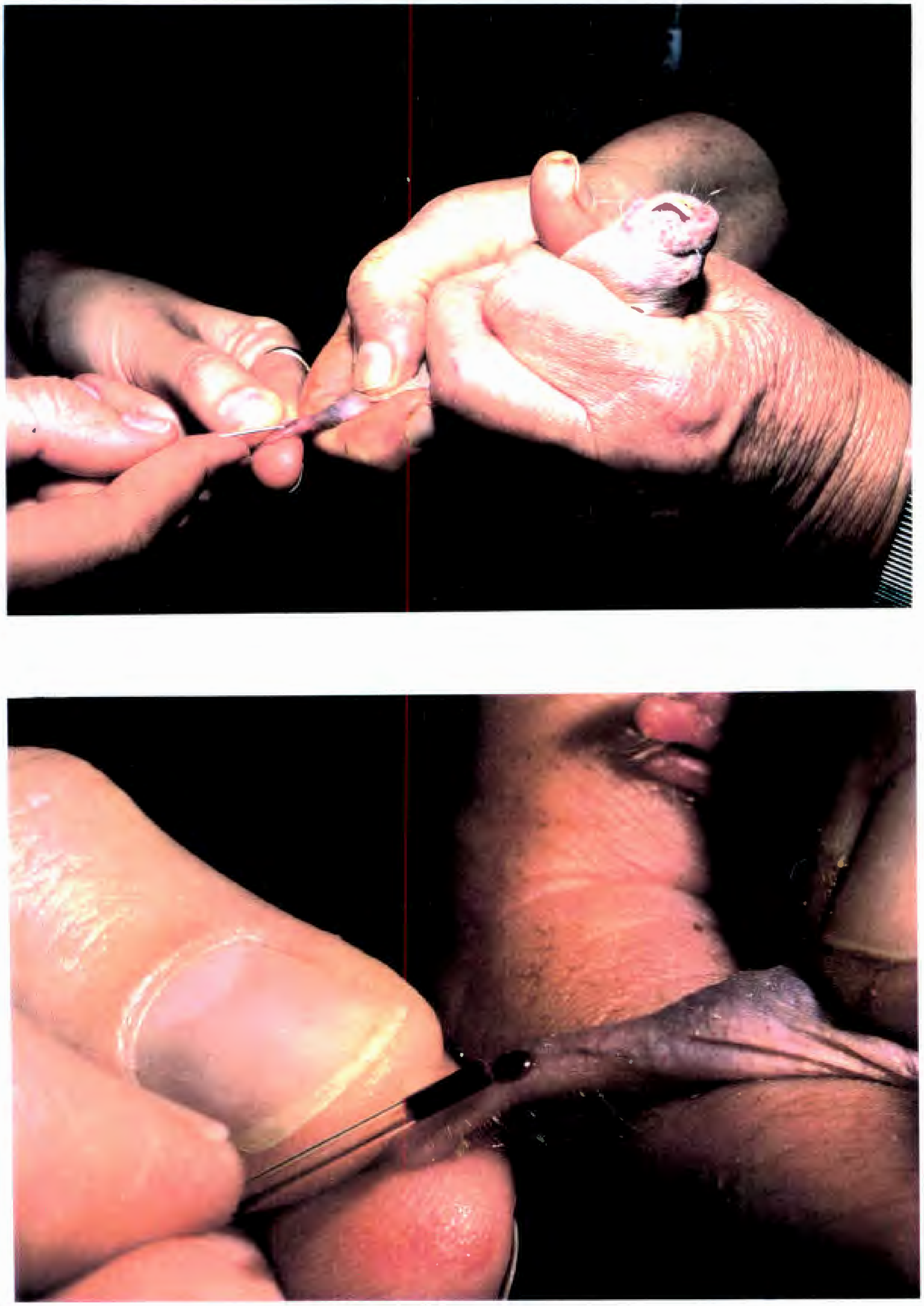


PLATE 2: The procedure used to collect blood samples. *Top*, An animal is hand-held and a superficial vein on the hind foot (or forefoot) is pricked with a 0.9 x 40 mm hypodermic needle. *Bottom*, Blood is then collected by capillary action using heparinised micro-haematocrit tubes.

method of blood collection proved to be superior and less disturbing to the animals than the previously employed technique which involved cutting the tip of the tail with a scalpel (Faulkes, 1990; Faulkes *et al.* 1990a; Faulkes *et al.*, 1990b; Faulkes *et al.*, 1991a; Faulkes *et al.*, 1991b). There was no evidence of tissue damage 1-2 days following bleeding of an animal using the present technique. After blood collection, the micro-haematocrit tubes were sealed and centrifuged at 500 g. The plasma thus obtained was stored in sealed 1 ml nuncs (Labotec, Cape Town) at -70°C (at the University of Cape Town) and then at -40°C (at the University of Pretoria) until LH determination.

Blood was only collected from animals with body masses greater than 26 g as it was not possible to obtain sufficient quantities of blood for hormonal analysis from smaller animals. Blood was not collected from the breeding female of either colony in order to avoid unnecessary stress and to prevent the possible termination of pregnancy.

2.3.3 GnRH Administration

The GnRH was synthesised in the laboratory of R.P. Millar at the MRC Regulatory Peptides Research Unit in the Department of Chemical Pathology of the University of Cape Town Medical School. The purity of the GnRH was greater than 98% homogeneity (Millar, Flanagan, de L. Milton & King, 1987). Ampoules of 2 µg GnRH per ml physiological saline, were stored at -70°C until required. Prior to administration, dilutions were made to give a GnRH concentration of 0.1 µg GnRH per 200 µl physiological saline. Following an initial blood collection, a 0.1 µl dose of GnRH was administered subcutaneously as a single 200 µl bolus injection. A further blood sample was collected from each animal 20 min following the administration of GnRH.

Previous studies have shown that the difference in LH response between breeding and non-breeding male and female naked mole-rats is greatest following a dose of 0.1 µg GnRH when compared with higher doses of 0.5 µg and 1.0 µg (Faulkes, 1990; Faulkes *et al.*, 1990b; Faulkes *et al.*, 1991a). One of the aims of the present study was to determine whether any individuals could be distinguished as being less reproductively suppressed than others, in terms of the relative sensitivity of their pituitaries to exogenous GnRH. Since the use of a higher dose of GnRH might have masked subtle differences between individuals, 0.1µg GnRH per 200 µl physiological saline was selected as the optimal dose for the purposes of this study.

Similarly, previous studies have shown plasma LH concentrations to be maximal 20 min following 0.1 µg injection of GnRH in both breeding and non-breeding female naked mole-rats

(Faulkes, 1990; Faulkes *et al.* 1990b). A 20 min interval is now commonly employed in studies involving GnRH challenges on the Bathyergidae (Bennett, Jarvis, Faulkes & Millar, 1993; Bennett *et al.*, 1996; Bennett *et al.*, 1997) and was used in the present study. Blood was therefore collected immediately prior to injection of GnRH and again 20 min later.

It has already been shown that male and female, breeding and non-breeding naked mole-rats show no response to control subcutaneous injections of 200 µl saline (Faulkes *et al.* 1990b; Faulkes *et al.*, 1991a) and that the injection itself therefore has no effect. For this reason, and because of the limited time available in which to assay a very large number of samples, no saline controls were performed in this study.

2.4 Hormone Determinations

2.4.1 Luteinizing Hormone Bioassay

An *in vitro* bioassay, based on the production of testosterone by dispersed mouse Leydig cells (Van Damme, Robertson & Diczfalusy, 1974), was used to measure levels of luteinizing hormone (LH) in the plasma samples. This assay has been described previously (Harlow, Gems, Hodges & Hearn, 1984; Hodges, Cottingham, Summers & Yingnan, 1987; Abbott *et al.*, 1988) and the method has been validated for use in the naked mole-rat (Faulkes, 1990; Faulkes *et al.*, 1990b; Faulkes *et al.*, 1991a).

Materials and reagents

1. Bioassay buffer

A stock buffer was made up by dissolving:

0.296 g $NaH_2PO_4.2H_2O$
2.900 g $Na_2HPO_4.12H_2O$
4.385 g NaCl

in 1 l of distilled water, and stored at 4°C until required.

The bioassay buffer was prepared by adding 0.1 g bovine serum albumin (BSA) to 100 ml of the stock buffer to give a concentration of 0.1% w/v.

2. Leydig cell incubation medium:

12.0 ml BME 10 X (Earle's salts with L-Glutamine)
2.1 ml $NaHCO_3$ (7.5%)

2.0 ml calf serum
100.0 ml distilled water

were mixed in a conical flask, placed on ice, and gassed slowly with 95% O_2 : 5% CO_2 for 10 min, immediately prior to use.

3. Standard

The standard used was the human LH pituitary preparation (2nd International Standard, 1988, code no. 80/522; NIBSC, Hertfordshire, England) over the range 400 - 3.125 µi.u./100 µl. The 35 i.u. LH sample in the ampoule was dissolved in 70 ml of distilled water to give a concentration of 500 mi.u./ml. This solution was then divided into 7 x 10 ml aliquots, one of which was further diluted in 90 ml of distilled water to give a concentration of 50 mi.u./ml. This was then split into aliquots which were stored frozen at -40°C until required. For each bioassay, 160µl of the 50 mi.u./ml stock was diluted in 1840 µl of bioassay buffer to give a top standard of 400 µi.u./100 µl. Of this top solution, 500 µl was then serially diluted in assay buffer to give solutions of 200, 100, 50, 25, 12.5, 6.25 and 3.125 µi.u./100 µl.

4. Quality controls

Quality controls were prepared by serially diluting a sample of the 400 µi.u./100 µl standard to give concentrations of 12.5 and 3.125 µi.u./100 µl.

Assay Protocol

1. Preparation of Leydig cells

The testes of a male mouse (NMRI strain, South African Vaccine Producers, Edenvale) aged ± 6 weeks, which had been freshly killed by cervical dislocation, were removed and placed in a petri dish with a few millilitres of incubation medium. The testes were decapsulated and minced coarsely with two pairs of forceps. A further 5 ml of incubation medium was added to the petri dish, and the tissue was homogenised by sucking up and blowing out several times with a plastic pipette. The homogenate was then transferred to a polythene beaker and mixed slowly on a magnetic stirrer at 4°C for 10 min, after which the cells were filtered through nylon mesh into a polythene conical flask. The cell preparation was incubated for 1 h in a shaking water bath at 34°C, with an atmosphere of 95% O_2 :5% CO_2 (Carbogen 5, Afrox, Pretoria).

After the 1 h incubation period, the cell suspension was dispensed, in equal amounts, into six plastic tubes, and centrifuged for 5 min at 2500 rpm and 4°C. The supernatant was decanted off and the pellets in each tube were resuspended in a few millilitres of incubation medium. The centrifugation process was repeated, the supernatant decanted off and the resultant pellets, containing the Leydig cells, were resuspended in 10 ml of incubation medium, in a polythene beaker. The cell suspension was thoroughly mixed at 4°C on a magnetic stirrer. The Leydig cells were counted on a haemocytometer using light microscopy at a magnification of x100. The cells were counted in the squares indicated in Figure 2.1. Very small and very expanded cells were not included in the count. The cell suspension was diluted accordingly, to give approximately 500 000 cells/ml, and mixed thoroughly on a magnetic stirrer.

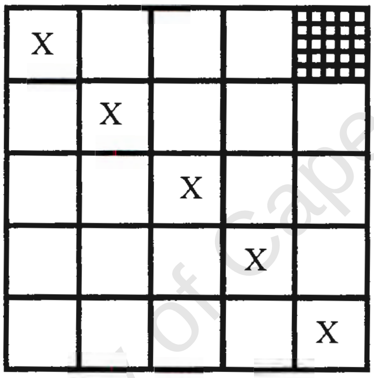


FIGURE 2.1: Graphic representation of the counting slide. The number of cells in the diagonal squares indicated with X's were counted.

2. Preparation of samples for Leydig cell incubation

During the 1 h incubation period of the Leydig cell preparation, the samples were prepared for the assay procedure. Glass assay tubes were labelled appropriately, and placed in metal assay racks in the following order:

Zero	**triplicate**
Standards (1-8)	**triplicate**
Quality controls	**duplicate or quadruplicate**
Samples (1:20 and 1:40)	**duplicate**
Zero	**triplicate**

The plasma samples were prepared to be assayed in duplicate at two dilutions of 1:20 and 1:40 (from 25 µl of plasma) and the necessary dilutions were made in assay buffer. Faulkes (1990) found it necessary to assay some samples, which fell below the sensitivity of the assay, at dilutions of 1:10 and 1:20, but most were done at 1:20 and 1:40. Dilutions of 1:10 were not possible in this study due to the limited volume of plasma available. The dilutions of the standards and quality controls have already been described above. Aliquots of 100 µl of assay buffer (zero), diluted standard, quality control and sample were pipetted into the appropriate assay tubes.

3. Assay procedure

An aliquot of 200 µl of the incubated Leydig cell suspension was pipetted into each of the assay tubes, to which 100 µl of the appropriate samples had already been added. The assay tubes were then incubated for 3 h in a shaking water bath at 34°C, with an atmosphere of 95% O_2 : 5% CO_2. After the incubation period, the assay racks were removed and placed in a waterbath at 100°C for 15 min in order to destroy the Leydig cells, thereby inhibiting further testosterone production. The assay tubes were then cooled on ice and 150 µl of testosterone assay buffer (PBS) added to each. The tubes were stored at -20°C until the samples could be radioimmunoassayed, which was generally within 24 h.

2.4.2 Testosterone Radioimmunoassay

Testosterone produced by the mouse Leydig cells as a result of the action of LH in the standards, quality controls and samples, was determined by radioimmunoassay as follows:

Materials and reagents

1. Assay buffer (PBS):

Distilled, ionised H2O	1000 ml
$Na_2HPO_4.12H_2O$	22.5 g
$NaH_2PO_4.2H_2O$	6.1 g
NaCl	9.0 g
NaN_3	1.0 g
Gelatin	1.0 g

The gelatin was dissolved in 100 ml of warm distilled water and the other chemicals were dissolved in 750 ml of distilled water. PBS buffer was prepared by adding the dissolved gelatin to the chemical mixture and made up to 1000 ml with the remainder of the water. The final PBS buffer solution had a pH of 6.8-7.0.

2. Dextran-coated charcoal (0.156%):

Charcoal	0.78 g
Dextran (T70)	0.078 g
PBS	500 ml

The charcoal was added to 450 ml PBS and the dextran to 50 ml PBS. The solutions were stirred separately for 1 h on a magnetic stirrer, after which the dextran solution was added to the charcoal solution and mixed for a further 3 h. The final charcoal solution was stored at 4°C for 24 h before use.

3. Antiserum:

R67, an antiserum raised in rabbits against testosterone-3-carboxymethyl-oxime, conjugated to bovine serum albumin, was supplied by the MRC Regulatory Peptides Research Unit in the Department of Chemical Pathology of the University of Cape Town Medical School. Aliquots of 25 µl were pipetted out from a 1 ml raw stock and stored at -20°C. Immediately prior to use, 25 µl of the antibody was diluted in 15 ml PBS.

4. Label:

[1,2,6,7,-3H] testosterone TRK 402 (sp. act. 80 - 105 Ci/mmol; radiochemical Centre Amersham, Bucks, UK) was used in the assay. The tritiated testosterone (250 µCi) aliquot in the supplier's vial was dissolved in ethanol to make up a stock solution with an end volume of 25 ml. To prepare the tritiated testosterone for use in the assay, 0.1 ml of the stock solution (1 µCi) was pipetted into a counting vial and dried under nitrogen gas (Afrox, Pretoria). A volume of 10 ml PBS was added to the dried label, the solution was mixed well and left to stand at room temperature for 1 h. The diluted label was then stored at 4°C.

Assay protocol

1. Assay procedure

Glass assay tubes were labelled and 100 µl of the bioassayed samples, standards and QC's, to which 150 µl of PBS had been added previously, were pipetted into the appropriate tubes. PBS was added to each of the radioimmunoassay zero (B_0) and NSB tubes. Each tube, except the NSB's, received 100 µl of the diluted R67 testosterone antibody, and all the tubes were covered and vortexed for 1 min. A 100 µl aliquot of testosterone label (± 10 000 cpm) was added to each tube, and the tubes were covered and vortexed for 1 min.

The contents of the radioimmunoassay tubes after all the reagents had been added were as follows:

Tubes	Sample	Buffer	Antibody	Label
4 x B_0	/	100	100	100
2 x NSB	/	200	/	100
3 x Zero	100	/	100	100
3 x S1-S8	100	/	100	100
2 x QC Low	100	/	100	100
2 x QC High	100	/	100	100
1 x each bioassay tube	100	/	100	100
3 x Zero	100	/	100	100

All volumes in µl.

After vortexing, the tubes were incubated in a water bath for 1 h at 37°C. The tubes were then removed from the water bath and left to stand at room temperature for 15 min, followed by a further 1 h incubation period at 4°C.

2. Separation of unbound label

Half an hour before the end of the 4°C incubation period, the charcoal was removed from the refrigerator and placed on a magnetic stirrer in a bucket of ice. At the end of the incubation hour, 0.75 ml of dextran-coated charcoal was pipetted into each assay tube and a stopwatch set to 12 min. The tubes were covered and vortexed, and then placed in a centrifuge, pre-set to 4°C, until the end of the 12 min. The tubes were centrifuged at 2500 rpm for 15 min, after which they were kept on ice while the supernatants of each were toppled into scintillation vials containing 4 ml scintillation fluid (Ultima Gold™, Analytical and Diagnostic Products, Weltevreden Park, Johannesburg)

3. Scintillation counting

The vials were vortexed for 1 min and left for at least 1 h before being placed in the scintillation counter. Each vial was counted for 2 min on Protocol 10 in a Hewlett Packard Tricarb 1500 liquid scintillation counter. Results were expressed as counts per minute (cpm).

4. Assay sensitivity and coefficients of variation

The sensitivity of the assay used by Faulkes (1990), determined as 90% binding, was 10 μi.u./tube, which was equivalent to 1.0 mi.u./ml plasma at the minimum sample dilution of 1:10. The sensitivity of the present assay was, however, lower than that used by Faulkes. The sensitivity, determined as 95% binding, was 14.45 μi.u./tube, which was equivalent to 2.89 mi.u./ml plasma at the minimum sample dilution of 1:20. There was insufficient plasma to re-assay, at a lower dilution of 1:10, those samples which gave values greater than 95% binding. Therefore, in order to facilitate statistical analysis, samples which were below the sensitivity of the assay were assigned a value of 1.0 mi.u./ml plasma, the equivalent sensitivity, at a 1:10 dilution, of the assay used by Faulkes. The intra- and inter-assay precisions, expressed as the coefficient of variation for repeated measures of a quality control (12.5 μi.u./μl) were 10.72% (n = 5) and 17.31% (n = 26) respectively. Three assays were run with quality controls of 3.125 μi.u./μl and gave an inter-assay coefficient of variation of 12.79 % (n = 3). Figure 2.3 shows a typical standard curve. The mean ± s.e.m. non-specific binding values (NSB counts ÷ total counts) were 2.625 ± 0.086 % (n = 29). The mean ± s.e.m. percentage binding for the zero's ((zero counts - NSB) ÷ total counts) were 26.074 ± 0.831 % (n = 29) and 13.22 ± 0.759% (n = 29) for the testosterone assay and bioassay respectively.

2.4.3 Assay Validation

A test of parallelism was used to validate the use of the LH bioassay in the naked mole-rat (See Figure 2.2). A volume of 75 μl of plasma, taken from a pool of plasma samples which had been collected 20 min after GnRH administration, and which had been shown to contain relatively high LH concentrations, was diluted in 425 μl of bioassay buffer. Serial dilutions were made over the range 1:6.67 to 1: 426.67. A Mann-Whitney *U*-Test revealed an absence of significant interaction between the LH standard and plasma dilutions in terms of either %B/Bo ($U = 35$, $P > 0.2$, n = 8 and 7) or LH concentration ($U = 21$, $P > 0.2$, n = 8 and 5). Faulkes (1990) has previously validated the LH bioassay for use in the naked mole-rat by tests of parallelism in which a two-way

ANOVA repeated measures design on log-transformed data demonstrated the absence of a significant interaction between the LH standard preparation and dilutions of naked mole-rat pituitary homogenate and dilutions of female naked mole-rat plasma containing relatively high concentrations of LH.

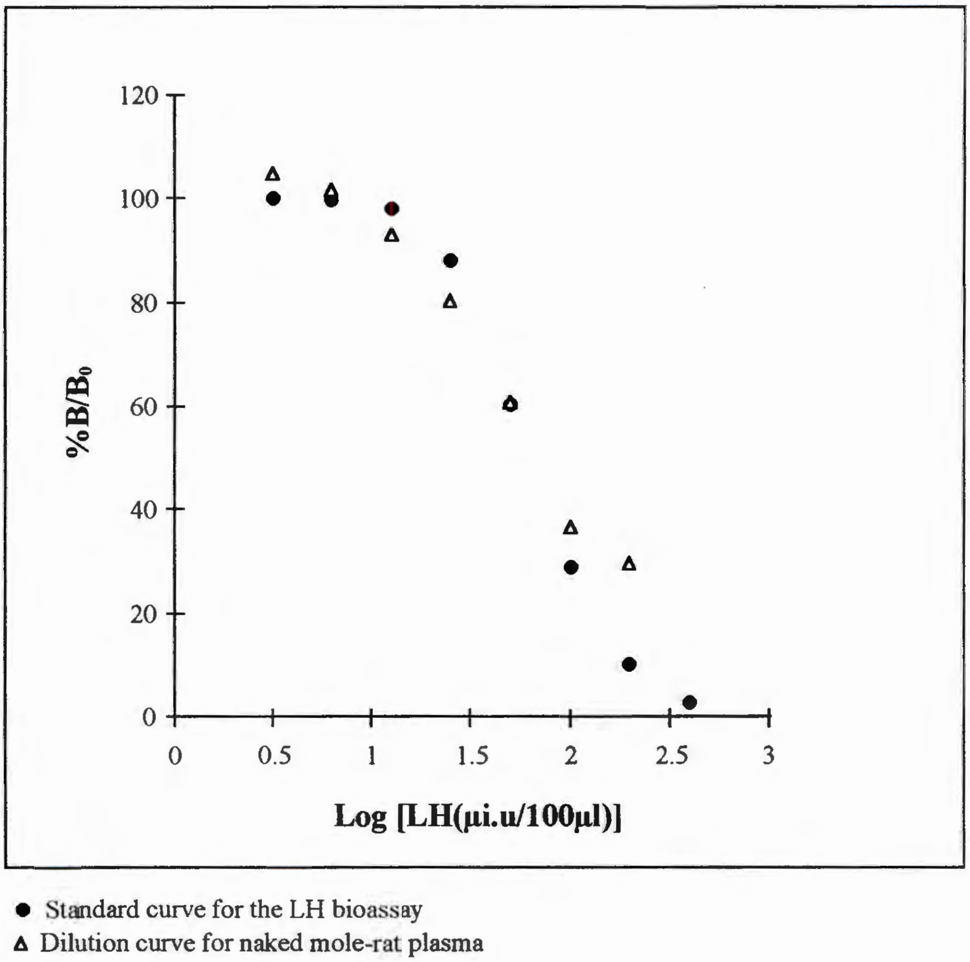


FIGURE 2.2: A specimen standard curve for the LH bioassay and dilution curve for naked mole-rat plasma. Concentration of the LH standard is expressed on a logarithmic scale and plotted against %B/B_0 .

2.4.4 Calculation of LH concentrations

The %B/B$_0$ for the standards and samples run in each assay were calculated according to the following equation:

$$\boxed{\frac{\textbf{Mean of sample (cpm) - Mean of NSB (cpm)}}{\textbf{Mean of bioassay zeros (cpm) - Mean of NSB (cpm)}} \times 100}$$

... Equation 2.1

For each assay, %B/B$_0$ of standards 1 - 8 were plotted against the corresponding LH concentrations (expressed as a logarithmic values) in the program **Curvefit 1.2** (Macintosh©). The program was then able to fit standard curves, similar to that seen in Figure 2.2, to each set of points, according to the following equation:

$$\boxed{y = d + \frac{(a - d)}{1 + (x/c)^b}}$$

... Equation 2.2

where y = %B/B$_0$ and x = the log of the corresponding LH concentration (Dudley, Edwards, Ekins, Finney, McKenzie, Raab, Rodbard & Rodgers, 1985). In addition the program calculated the values of the constants a, b, c and d from the best-fit curve, using Equation 2.2.

Once the values of a, b, c and d had been calculated from the standard curve of each assay, the logarithmic values of the LH concentrations of the samples were calculated using the following equation:

$$\boxed{x = \left(\sqrt[b]{\frac{a - d}{y - d} - 1}\right) \times c}$$

... Equation 2.3

where y = %B/B$_0$ of each sample, calculated using Equation 2.1. The logarithmic values were then converted to give the corresponding LH concentrations (μi.u./100μl) of the samples.

In order to correct for dilutions, the following equation was used:

$$\boxed{\frac{x \text{ x Dilution}}{100} = \text{LH (mi.u/ml)}}$$

... Equation 2.4

where x is the concentration in μi.u./100 μl, and "Dilution" is 20 for a 1/20 dilution and 40 for a 1/40 dilution.

The LH concentrations of a number of samples from a single assay were calculated using this method of calculating the LH concentrations of samples from a standard curve using **Curvefit 1.2** and the above equations, and, independently, by the method employed at the Institute of Zoology, London. The same values were obtained by both methods.

2.5 Statistical Analyses

The data did not meet with the basic requirements for the valid application of parametric techniques, (namely normal distribution and homoscedastic variances), even following logarithmic transformation. Therefore, only non-parametric tests were employed throughout the study. The statistical tests used to analyse the behavioural and hormonal data are described in the relevant chapters. Data are presented as mean ± s.e.m.. Unless otherwise stated, all statistical tests were made with reference to Zar (1984).

Chapter 3

Chapter 3

REPRODUCTIVE SUPPRESSION IN THE NAKED MOLE-RAT:-
INVESTIGATION OF THE INDIVIDUALS OF ENTIRE COLONIES OVER THE REPRODUCTIVE CYCLE OF THE BREEDING FEMALE

3.1 Introduction

One of the most fascinating aspects of naked mole-rat biology is the monopoly of reproduction by the breeding animals, with the concomitant suppression of reproduction in the non-breeding animals of both sexes. The anatomical, histological and endocrinological differences between breeding and non-breeding animals have been discussed in Chapter 1, section 1.5, and it is clear that reproductive suppression is more definitive in non-breeding females than in non-breeding males.

In non-breeding females of both wild and captive colonies, the socially induced block to reproduction is the result of a failure to ovulate (Faulkes *et al.*, 1990a). Non-breeding females have very low or undetectable plasma LH concentrations in comparison with breeding females (Faulkes, 1990; Faulkes *et al.*, 1990a). In addition, breeding and non-breeding female naked mole-rats exhibit differential pituitary secretion of LH following the administration of exogenous GnRH, and in non-breeding females the anterior pituitary appears to be less sensitive to single low doses of exogenous GnRH than in breeding females. At a higher dose of 1.0 μg GnRH, the magnitude of the LH response has been found to be no different in breeding and non-breeding females, suggesting that the non-breeding female pituitary contains a pool of bioactive LH which can be released under the influence of a sufficiently large GnRH stimulus. However, at lower doses of 0.5 μg and 0.1 μg GnRH, while the magnitude of the LH response in breeding females is not significantly reduced, that in non-breeding females is significantly lower, such that at a dose of 0.1 μg GnRH, non-breeding females produce only a small increase in plasma LH (Faulkes, 1990; Faulkes *et al.*, 1990b). Therefore, the socially-induced block to ovulation in non-breeding female naked mole-rats may be the result of insufficient secretion of LH from the anterior pituitary (Faulkes *et al.*, 1990a), possibly due to the inhibition of hypothalamic GnRH secretion (Faulkes *et al.*, 1990b).

Whereas the ovaries of most non-breeding female naked mole-rats are quiescent, histological examination has revealed that spermatogenesis occurs in the testis of the majority of non-breeding male naked mole-rats (Jarvis, 1991a). Nevertheless, non-breeding males have lower sperm counts and higher incidences of non-motile sperm than breeding males (Faulkes *et al.*, 1994), and, like non-breeding females, have significantly lower plasma LH concentrations than breeding males, and an apparent lack of pituitary sensitivity to single low doses of exogenous GnRH (Faulkes *et al.*, 1991a). The magnitude of the LH response in non-breeding males is dose-dependent. After a dose of 1.0 µg GnRH, the magnitude of the LH response in non-breeding males has been found to be no different to that in breeding males, but lower than that in breeding males after a dose of 0.5 µg or 0.1 µg GnRH. As with breeding females, the magnitude of the LH response in breeding males is not significantly different across the doses (Faulkes *et al.*, 1991a).

Various aspects of reproductive suppression in the naked mole-rat have been investigated, and the results of several studies have shown that both non-breeding females and non-breeding males are reproductively suppressed (Faulkes *et al.*, 1989; Faulkes *et al.*, 1990a; Faulkes *et al.*, 1990b). However, previous studies have used only small sub-samples of colonies in order to make comparisons between breeding and non-breeding males and females, but none has looked at the individuals of entire colonies. Moreover, the time in the breeding female's reproductive cycle when data have been collected has never been standardised. The differential agonistic treatment of particular individuals by the breeding female (Reeve & Sherman, 1991; Margulis, Saltzman & Abbott, 1995; O'Riain, 1996), the occurrence of perforate vaginas in certain non-breeding females (Westlin *et al.*, 1994) and the range of individual reproductive hormone concentrations (Faulkes, 1990; Westlin *et al.*, 1994; Margulis *et al.*, 1995; O'Riain, 1996;) and pituitary responsiveness to exogenous GnRH challenges (Faulkes, 1990), suggest that not all non-breeding animals are equally suppressed. Furthermore, it appears that suppression is dynamic and may be influenced by the reproductive state of the breeding female (Faulkes, 1990; Jarvis, 1991a; Westlin *et al.*, 1994). It was therefore considered necessary to conduct this first study to investigate the phenomenon of reproductive suppression in the individuals of entire colonies of naked mole-rats within pre-determined time periods during the reproductive cycle of the breeding female.

The aims of the study were:

1. To obtain "baseline levels" of LH for the individuals of entire colonies of naked mole rats
2. To perform GnRH challenges on all the males and non-breeding females in order to determine whether non-breeders show variability in the degree of suppression.

3. To determine whether there is a relaxation of reproductive suppression at a particular time of the cycle.

4. To carry out behavioural observations in order to correlate the behaviour of the breeding female towards the non-breeders, with their individual endocrinological profiles.

5. To see if there is any relationship between plasma LH and body mass, age or the degree of agonism shown by the breeding female towards the non-breeding animals.

3.2 Methods

3.2.1 . Subjects

The study was carried out on two colonies of naked mole-rats, colony 100 and colony 7400, the particulars of which are discussed in Chapter 2, section 2.1 and the details of which are given again in Tables 3.1 and 3.2. Initially the study was to be performed on the two colonies over two breeding cycles. However, as a result of the take-over event in colony 7400 (Chapter 2, section 2.1.4), only one complete breeding cycle (early, mid and late pregnancy) could be investigated in this particular colony, once the new breeding female had begun reproducing. The data collection in colony 100 was started during mid pregnancy, and data were collected over the equivalent of two breeding cycles, of which one was complete (Cycle 1), while the other (Cycle 2) comprised data collected during mid and late pregnancy at the beginning of the study, and early pregnancy at the end of the study.

3.2.2 Data Collection

In order to standardise the time in the breeding cycle during which the data would be collected, the gestation period was divided into three time intervals: early (days 1-15), mid (days 30-40) and late (days 55-65) pregnancy, with the assumption that mating occurred on day 10 after parturition. During each time interval, behavioural observations were first conducted over a period of 2-6 days, followed by the collection of blood samples and the performance of GnRH challenges. Only three time intervals were selected as it was felt that more frequent bleeding of the animals would have resulted in too much disturbance to the colony. Depending on the number of animals involved, the bleeding and GnRH challenges were done either in 1 day or over 2 consecutive days. Details of when in the breeding cycle the data were collected from colony 100 and colony 7400 are given in Table 3.3 and Table 3.4 respectively.

TABLE 3.1: Details of the individuals of colony 100 (July - December 1995).

Animal	Sex	Litter	Mass (g)
Breeding female	BF	NA	NA
12	F	2	53.8
13	F	2	58.4
15	F	2	47.9
23	F	3	41.8
1	F	4	35.2
25	F	5	41.2
43	F	7	40.3
45	F	7	54.4
48	F	8	41.7
5F	F	8	37.1
57	F	10	25.5
70	F	11	42.4
71	F	11	35.3
72	F	11	39.0
76	F	11	33.3
90	F	11	34.6
92	F	12	33.8
94	F	12	35.2
98	F	12	43.2
CC	F	13	37.3
91	F	13	26.5
95	F	13	35.8
96	F	13	31.9
7	F	14	25.4
9	BM	1	49.0
5	M	2	54.9
6	M	2	76.4
10	M	2	58.8
14	M	2	49.7
16	M	2	73.8
17	M	2	64.5
18	M	2	41.7
20	M	4	51.1
28	M	6	55.1
29	M	6	38.0
30	M	6	67.9
47	M	7	34.4
XX	M	7	69.4
51	M	8	42.1
52	M	8	50.1
54	M	9	34.9
5M	M	8	38.2
200	M	14	24.4

Animals < 26 g excluded from final analysis

Escapees excluded from final analysis

TABLE 3.2: Details of the individuals of colony 7400 (January - March 1996).

Animal	Sex	Litter	Mass (g)
28	F	6	NA - BF
47	F	9	39.4
52	F	9	32.1
4	F	10	49.1
6	F	12	36.1
7	F	12	47.7
75	F	13	39.7
80	F	14	33.5
82	F	14	34.9
83	F	14	35.2
84	F	14	34.1
9	BM	2	53.0
11	BM	3	73.0
20	BM	3	57.7
49	M	9	47.1
51	M	9	44.0
57	M	11	40.8
58	M	11	35.8
71	M	13	37.9
72	M	13	36.8
77	M	13	39.6
81	M	14	29.1

3.2.2.1. Behavioural Data

Behavioural data were collected using ***THE OBSERVER*** as described in Chapter 2, section 2.3.1. Ten hours of behavioural data were collected from each colony during each of the early, mid and late pregnancy time periods, with a total of 60 h from colony 100 and 30 h from colony 7400. Initially the behavioural observations were to constitute a total of 10 h of focal study on the breeding female during each period of the reproductive cycle, in order to record all her behaviours, including the agonistic interactions directed towards the other colony members. However, during the take-over event in colony 7400, it became clear that this excluded valuable data on the behaviours of other colony members. Consequently, for the present part of the study, behavioural observations on the two colonies during each time interval of the cycle, comprised 5 h of focal study on the breeding female, as well as 5 h of focal study on the nest in order to record the agonistic and sexual behaviours performed by the rest of the colony members.

The number of hours of behavioural data collected, was selected on the basis of comparative studies. Reeve and Sherman (1991) investigated intra-colonial aggression in six captive colonies of naked mole-rats, and recorded between 9.18 h and 63.7 h of behavioural observation from each colony. Margulis *et al.* (1995), looking at reproductive succession in naked mole-rats, divided the study period into three time intervals, during which 3.3 h of focal observations were performed, as 20 10 min sessions, on each of the 12 females of interest. A total of 60 h of behavioural observation on colony 100, of which 30 h were focal on the breeding female, and 30 h on colony 7400, of which 15 h were focal on the breeding female, were therefore considered sufficient for the purposes of this study.

TABLE 3.3: Dates of the behavioural studies and blood collection for colony 100 over two breeding cycles.

Time in cycle	**Behaviour** (Day in cycle)	**Date**	**Blood Collection** (Day in cycle)	**Date**
CYCLE 1				
Early	5, 6 + 9	5, 6 + 9/10/95	10 + 11	10 + 11/10/95
Mid	33, 34, 37 + 38	2, 3, 6 + 7/11/95	39 + 40	8 + 9/11/95
Late	55, 57 + 58	25, 27 + 28/11/95	59 + 60	29 + 30/11/95
CYCLE 2				
Early	1, 2 + 3	18, 19 + 20/12/95	4 + 5	21 + 22/12/95
Mid	33 + 34	15 + 16/8/95	39 + 40	21 + 22/8/95
Late	50, 51, 54 + 55	31/8, 1, 4 + 5/9/95	56 + 57	6 + 7/9/95

TABLE 3.4: Dates of the behavioural studies and blood collection for colony 7400 over one breeding cycle.

Time in cycle	**Behaviour** (Day in cycle)	**Date**	**Blood Collection** (Day in cycle)	**Date**
Early	9, 10 + 11	15, 16 + 17/1/96	12	18/1/96
Mid	30, 31, 32 + 33	5, 6, 7 + 8/2/96	34	9/2/96
Late	55, 58, 59 + 60	1, 4, 5 + 6/3/96	61	7/3/96

3.2.2.2. Hormonal Data

Blood samples were collected prior to and 20 min after a subcutaneous injection of 0.1 µg GnRH in 200 µl physiological saline, during early, mid and late pregnancy, as described in Chapter 2, section 2.3.2. Concentrations of LH were measured using an in-vitro bioassay based on the production of testosterone by dispersed mouse Leydig cells, the details of which are given in Chapter 2, section 2.4.

3.2.3 Data Analyses

3.2.3.1 Behavioural Data

The behaviours included in the analyses are described in Appendix I. The individual agonistic behaviours were grouped together, as were the sexual behaviours. Behavioural data were expressed as a percentage of the total agonistic and sexual interactions initiated by the breeding female towards the non-breeding females and males respectively, and as a percentage of the total agonistic and sexual interactions initiated or received by the non-breeding females and males of each colony.

3.2.3.2 Hormonal Data

Kruskal Wallis tests were used to determine whether there were any differences between early, mid and late pregnancy in terms of the basal and post-GnRH plasma LH concentrations. Mann-Whitney *U*-tests were used to investigate differences between the non-breeding females and males in terms of plasma LH concentrations before and 20 min after a single s.c. injection of 0.1 µg GnRH.

3.2.3.3 Combined Data

Spearman's rank correlation coefficient was used to determine whether there was any relationship between either basal or post-GnRH plasma LH concentrations and body mass, relative age or the percentage of the total agonistic interactions initiated by the breeding female towards the non-breeding females and non-breeding males. Spearman's rank correlation coefficient was also used determine whether or not there was a relationship between the percentage of the total agonistic interactions initiated by the breeding female towards the non-breeding females and non-breeding males, and their body mass or relative age.

In order to determine whether, at an individual level, agonism from the breeding female was influenced by an individual's plasma LH concentrations (or *vice versa*), Spearman's rank correlation coefficient was used to determine whether or not there was a relationship between the percentage of the agonistic interactions received from the breeding female and plasma LH, in those non-breeding females and non-breeding males of colony 100 that each received greater than 5% of the total agonistic interactions initiated by the breeding female towards the respective genders.

3.3 Results

Individual plasma LH concentrations

The plasma LH concentrations of the individual mole-rats of colony 100 (Cycle 1 and Cycle 2) and colony 7400, before and 20 min after a single s.c. injection of 0.1 μg GnRH, are presented in Figures 3.1 to 3.3, and the mean basal and post-GnRH plasma LH concentrations of each individual, over two cycles in colony 100 and over one cycle in colony 7400, are presented in Figures 3.4 and 3.5.

There was a great deal of variation between the individual mole-rats in terms of both basal and post-GnRH plasma LH concentrations. In addition, the plasma LH concentrations of the individual mole-rats varied from one part of the cycle to the next, and there was no individual in either colony that could be identified as having consistently higher concentrations than all the other mole-rats of the same colony throughout the breeding cycle. However, some individuals had consistently low LH concentrations throughout the cycle. Basal plasma LH concentrations and LH response to 0.1 μg exogenous GnRH were used in an attempt to broadly categorise the individuals.

Colony -100

In colony 100 there were nine non-breeding females that had basal plasma LH concentrations that were consistently below the sensitivity of the assay (Group 1). Four of these females also showed no pituitary response to exogenous GnRH (#71, #CC, #90, and #92) while five others showed some pituitary response, but in fewer than 50% of the challenges (#48, #70, #94, #98 and #91). Another group (Group 2), consisting of 13 non-breeding females, had detectable basal plasma LH concentrations at some time in the study, with four showing pituitary responsiveness to exogenous GnRH in less than 50% of the challenges (#25, #72, #76 and #95) and nine in 50% or more of the challenges (#12, #13, #15, #23, #1, #43, #45, #5F, and #96). The females of Group 1 had significantly lower basal plasma LH concentrations (1.00 ± 0.00 mi.u./ml, n = 9) than those of Group 2 (1.93 ± 0.19 mi.u./ml, n = 13) ($U = 117$, $P < 0.0005$) and also significantly lower post-GnRH plasma LH concentrations (2.01 ± 0.36 mi.u./ml, n = 9) than Group 2 (3.80 ± 0.39 mi.u./ml, n = 13) ($U = 100$, $P = 0.0025$). Further differences between these two groups, in terms of body mass, age and percentage of the total agonistic interactions received from the breeding female, will be discussed later.

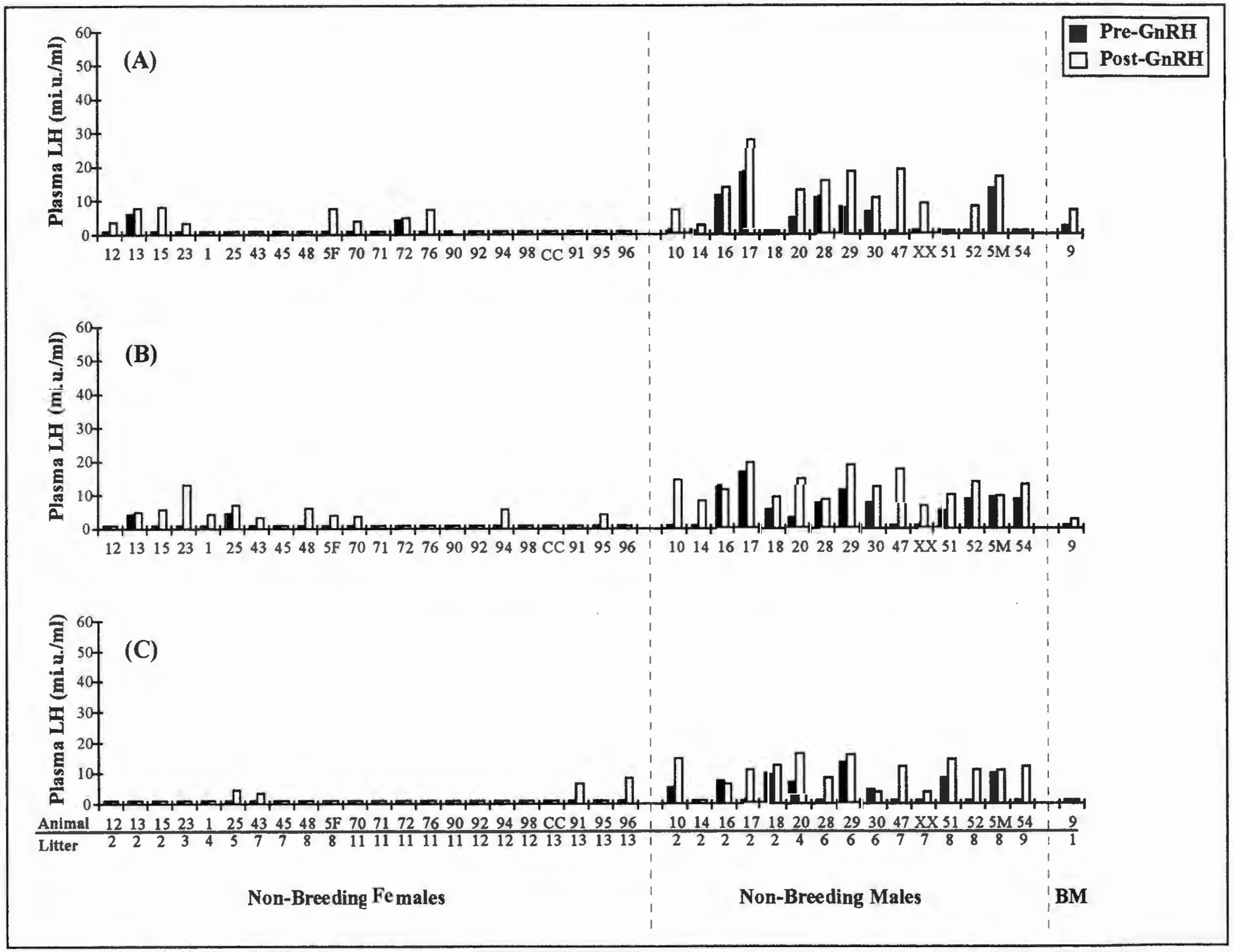


FIGURE 3.1: Plasma LH concentrations of each non-breeding female and male of colony 100 (Cycle 1) during early (A), mid (B) and late (C) pregnancy, before (Pre-GnRH) and 20 min after (Post-GnRH) a single s.c. injection of 0.1 µg GnRH.

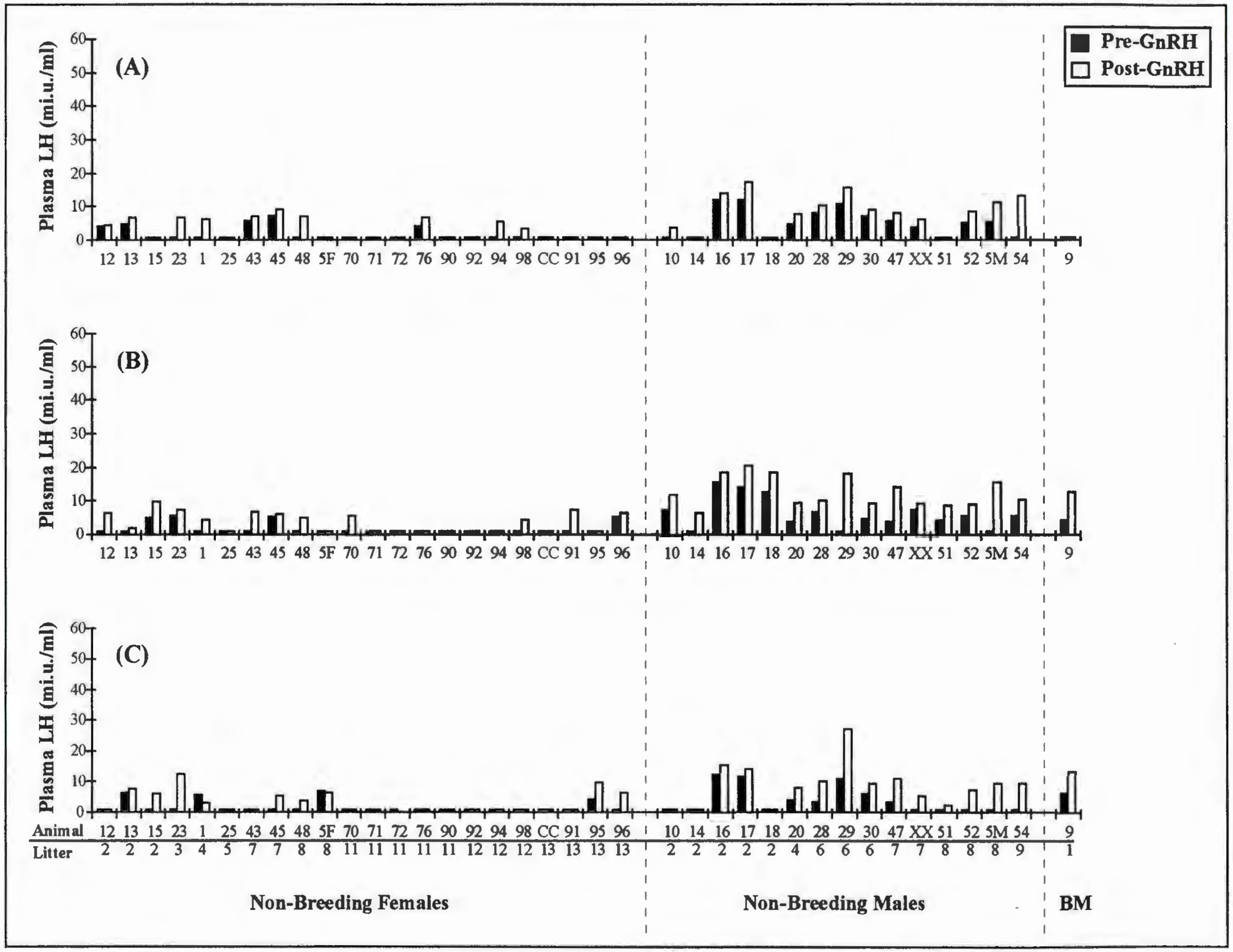


FIGURE 3.2: Plasma LH concentrations of each non-breeding female and male of colony 100 (Cycle 2) during early (A), mid (B) and late (C) pregnancy, before (Pre-GnRH) and 20 min after (Post-GnRH) a single s.c. injection of 0.1 μg GnRH.

Only three of the non-breeding males (16, #20 and #30) had consistently detectable basal plasma LH concentrations throughout the study, males (#17, #28, #29, #52 and #5M) had undetectable concentrations in one or two of the six samples, while the rest (#10, #14 and #18, #47, #XX and #54) had undetectable basal plasma LH concentrations in three or more of the samples. The only non-breeding male to have basal plasma LH concentrations that were consistently below the sensitivity of the assay, was male #14. All the non-breeding males of colony 100, with the exception of #14 and #18, consistently showed pituitary responsiveness to exogenous GnRH. Both #14 and #18 showed no response to three of the six GnRH challenges, over the two breeding cycles. However, among the males there were no distinctive groups as in the females.

Males which have previously been identified as dispersers by O'Riain (1996) in 1994 were #16, #28, #29 and #30. From Figures 3.1, 3.2 and 3.4(B) it can be seen that these males did not appear distinct from the other non-breeding males in terms of their hormone profiles. However, statistical analysis showed that, as a group, their basal plasma LH concentrations (8.40 ± 1.38 mi.u./ml, n = 4) were significantly higher than those of the other non-breeding males (4.37 ± 0.92 mi.u./ml, n = 11) ($U = 38$, $P = 0.025$), although there was no significant difference between their post-GnRH plasma LH concentrations (12.93 ± 2.18 mi.u./ml, n = 4) and those of the other non-breeding males (9.79 ±.1.23 mi.u./ml, n = 11) ($U = 32$, $P > 0.1$) (Mann-Whitney *U*-Test).

Colony -7400

In colony 7400, four non-breeding females had basal plasma LH concentrations that were consistently below the sensitivity of the assay (#4, #7, #82, 83) (Group 3), of which #7 was the only one to show no pituitary response to exogenous GnRH over the three time periods. The remaining six non-breeding females in the colony (#47, #42, #6, #75, #80, #84) (Group 4), all had detectable basal plasma LH concentrations at some stage during the cycle. The females of Group 3 had significantly lower basal plasma LH concentrations (1.00 ± 0.00 mi.u./ml, n = 4) than those of Group 4 (2.08 ± 0.16 mi.u./ml, n = 6) ($U = 24$, $P = 0.005$), and also significantly lower post-GnRH plasma LH concentrations (1.93 ± 0.34 mi.u./ml, n = 4) than Group 4 (5.70 ± 1.36 mi.u./ml, n = 6) ($U = 23$, $P = 0.01$). The two groups did not differ significantly in terms of body mass, age or percentage of the total agonistic interactions received from the breeding female, possibly because data were collected two months after the take-over event in which the oldest, and most of the largest, non-breeding females had been killed. The two groups are therefore not discussed any further, and for convenience, the results of the statistical analyses are given in Appendix II (Table 1).

Only two of the non-breeding males (#58 and #72) of colony 7400 had consistently undetectable basal plasma LH concentrations. Every non-breeding male of colony 7400 showed pituitary responsiveness to all three of the GnRH challenges that were performed on the colony, as did the breeding males, although #9 and #11 showed only a slight responses during early pregnancy.

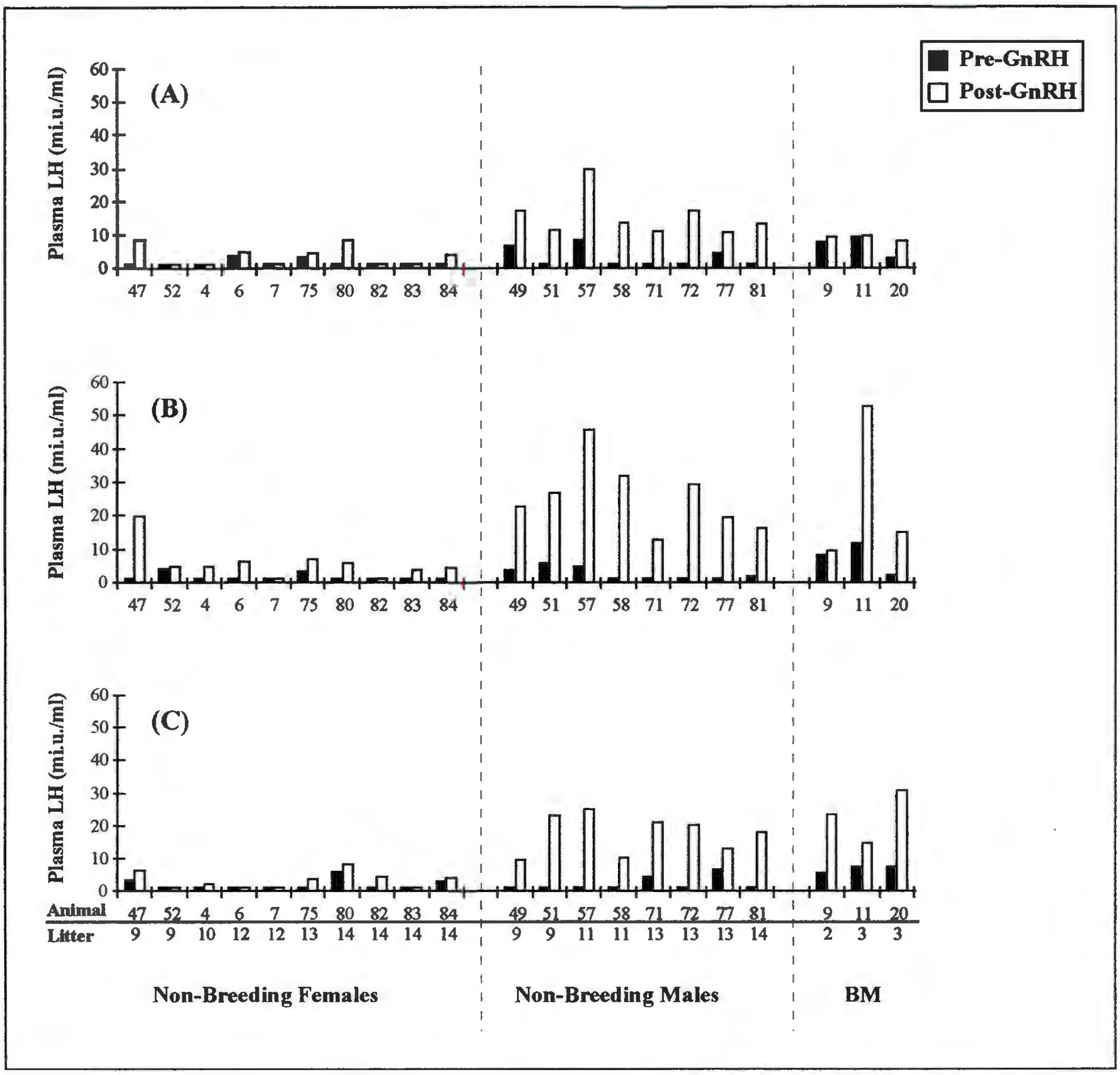


FIGURE 3.3: Plasma LH concentrations of each non-breeding female and male of colony 7400 during early (A), mid (B) and late (C) pregnancy, before (Pre-GnRH) and 20 min after (Post-GnRH) a single s.c. injection of 0.1 µg GnRH.

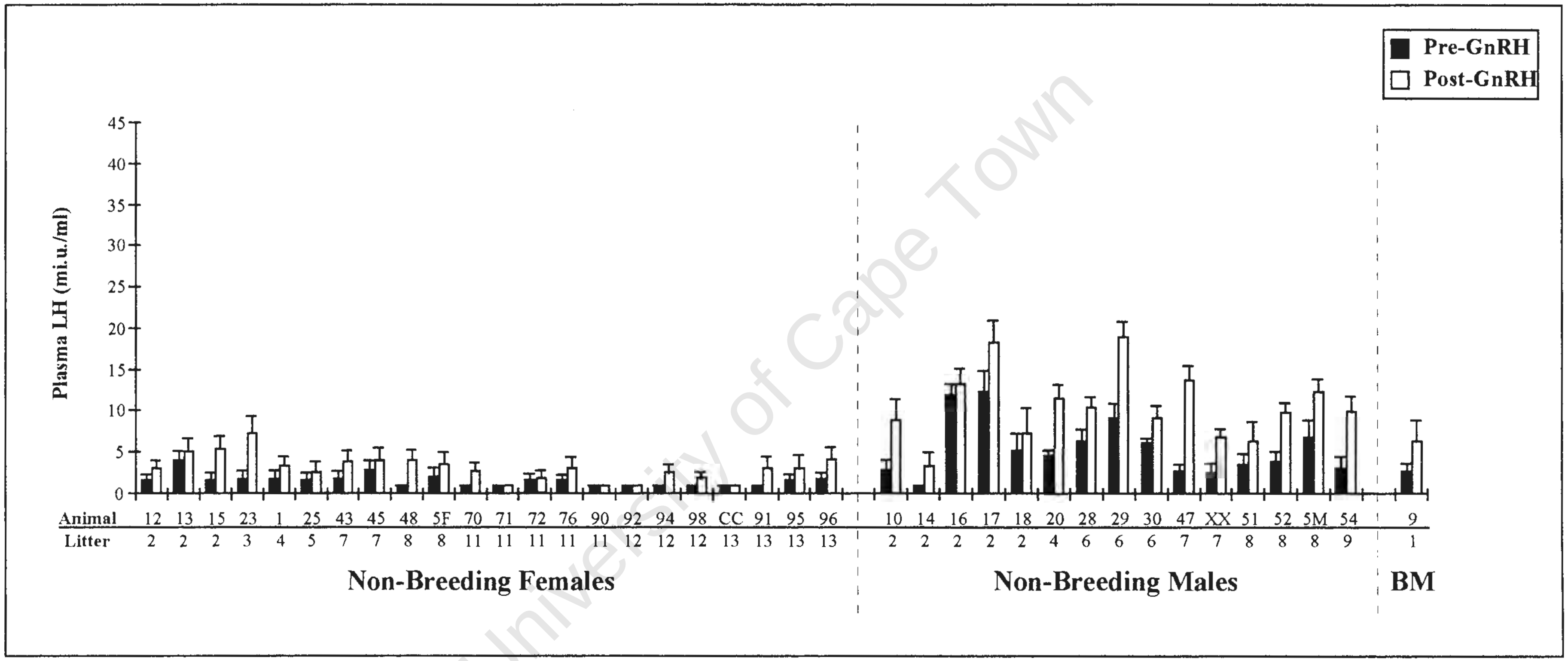


FIGURE 3.4: Mean plasma LH concentration (± s.e.m.) of each non-breeding female and male of colony 100, over two breeding cycles, before (Pre-GnRH) and 20 min after (Post-GnRH) a single s.c. injection of 0.1 μg GnRH.

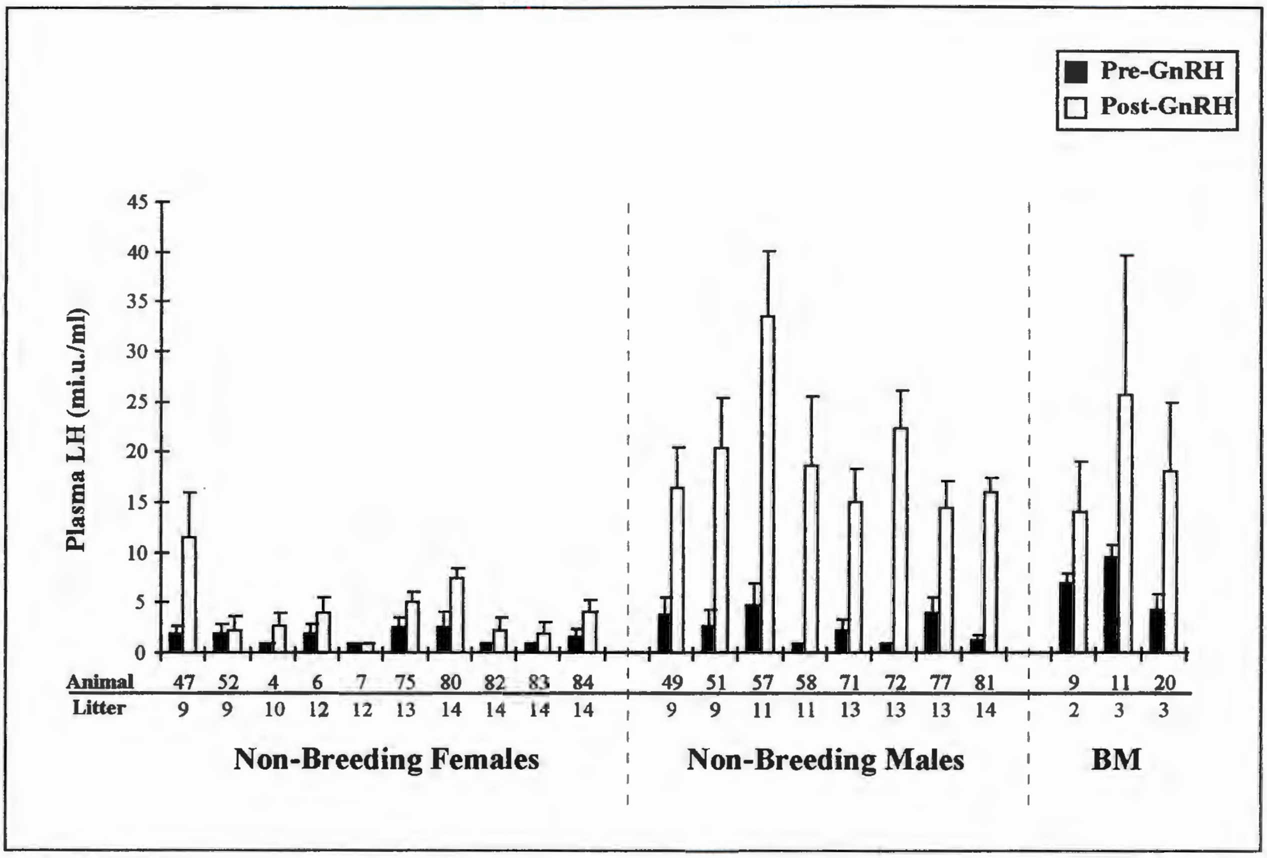


FIGURE 3.5: Mean plasma LH concentration (± s.e.m.) of each non-breeding female and male of colony 7400, over one breeding cycle, before (Pre-GnRH) and 20 min after (Post-GnRH) a single s.c. injection of 0.1 μg GnRH.

Figures 3.6 and 3.7 show the differences in the pooled mean basal and post-GnRH plasma LH concentrations of the non-breeding females and non-breeding males of colony 100 (over two cycles) and colony 7400 (over one cycle).

Colony -100

In colony 100, the mean basal plasma LH concentrations of the males (5.44 ± 0.88 mi.u./ml, n = 15) were significantly higher than those of the females (1.55 ± 0.15 mi.u./ml, n = 22) (*U* = 304.5, *P* < 0.0005), as were the post-GnRH concentrations (10.63 ± 1.10 mi.u./ml, n = 15, and 3.06 ± 0.33 mi.u./ml, n = 22, respectively) (*U* = 319, *P* < 0.0005) (Mann-Whitney *U*-test).

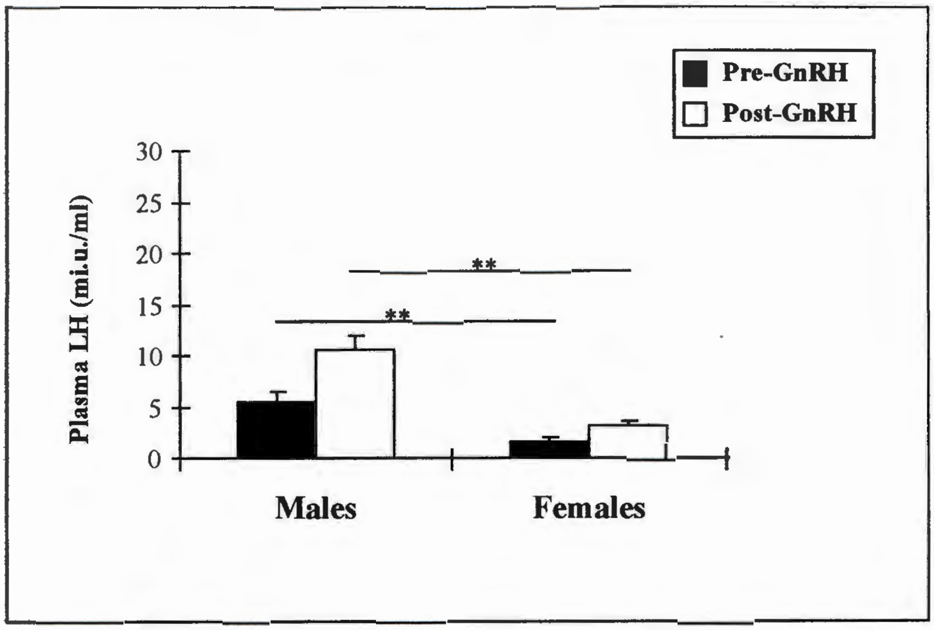
Colony -7400

In colony 7400 the mean basal plasma LH concentrations of the males (2.57 ± 0.51 mi.u./ml, n = 8) were higher than those of the females (1.65 ± 0.20 mi.u./ml, n = 10), although not significantly higher ($U = 56$, $P = 0.1$), but the post-GnRH concentrations of the males (19.56 ± 2.22 mi.u./ml, n = 8) were significantly higher than those of the females (4.19 ± 1.00 mi.u./ml, n = 10) ($U = 80$, $P < 0.0005$) (Mann-Whitney *U*-test).

When the results from the two colonies were compared, it was found that there was no significant difference between the non-breeding females of colony 100 and colony 7400 in terms of either basal plasma LH concentrations (1.55 ± 0.15 mi.u./ml, n = 22, and 1.65 ± 0.20 mi.u./ml, n = 10, respectively) ($U = 128$, $P > 0.1$) or post-GnRH plasma LH concentrations (3.06 ± 0.33 mi.u./ml, n = 22, and 4.19 ± 1.00 mi.u./ml, n = 10, respectively) ($U = 125$, $P > 0.1$). However, the males of colony 100 had significantly higher basal plasma LH concentrations (5.44 ± 0.88 mi.u./ml, n = 15) than the non-breeding males of colony 7400 (2.57 ± 0.51 mi.u./ml, n = 8) ($U = 92$, $0.01 < P < 0.025$), while the post-GnRH plasma LH concentrations were significantly higher in the non-breeding males of colony 7400 (19.56 ± 2.22 mi.u./ml, n = 8) than in the non-breeding males of colony 100 (10.63 ± 1.10 mi.u./ml, n = 15) ($U = 101$, $0.0025 < P < 0.005$) (Mann-Whitney *U*-Test).

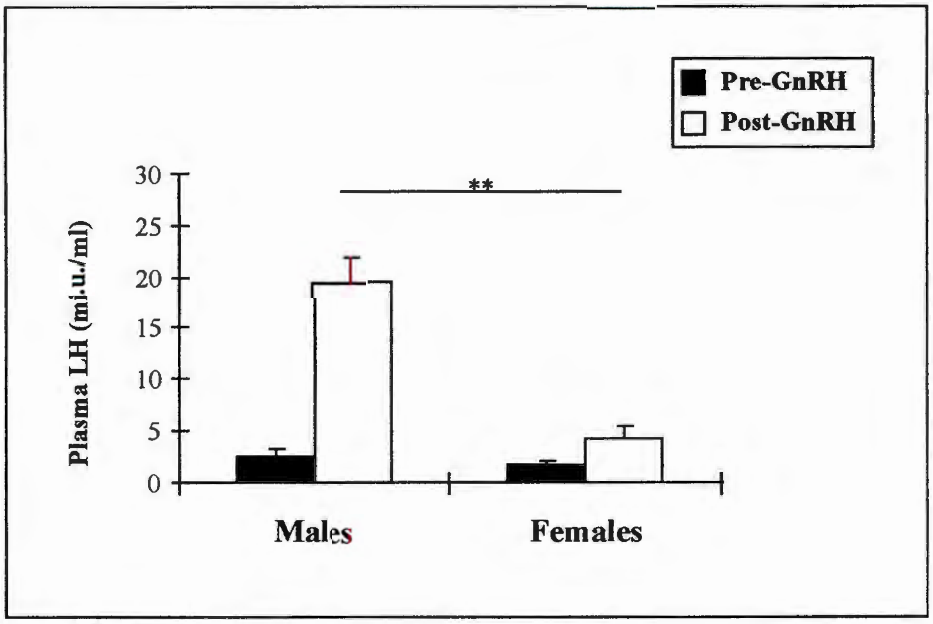
Plasma LH concentrations throughout the breeding cycle

The pooled mean basal and post-GnRH plasma LH concentrations of the non-breeding females and males of colony 100 and colony 7400 during early, mid and late pregnancy, are presented in Figures 3.8 to 3.10. Although there appears to be a slight elevation in the post-GnRH concentrations during mid pregnancy in the males and females of both colonies, there were no significant differences in terms of either the basal or post-GnRH plasma LH concentrations between early, mid and late pregnancy (Kruskal Wallis tests). For convenience, the results of the statistical analyses are presented in Appendix II (Table 2). From Figures 3.1 to 3.3 it can also be seen that during mid cycle a greater proportion of individuals showed an LH response following an exogenous dose of 0.1 µg GnRH, than during early or late cycle of the breeding female.



** P < 0.0005

FIGURE 3.6: Mean plasma LH concentrations (± s.e.m.) of the non-breeding females (n = 22) and non-breeding males (n =15) of colony 100, over two breeding cycles, before (Pre-GnRH) and 20 min after (Post-GnRH) a single s.c. injection of 0.1 μg GnRH.



** P < 0.0005

FIGURE 3.7: Mean plasma LH concentrations (± s.e.m.) of the non-breeding females (A) (n = 10) and non-breeding males (B) (n =8) of colony 7400, over one breeding cycle, before (Pre-GnRH) and 20 minutes after (Post-GnRH) a single s.c. injection of 0.1 μg GnRH.

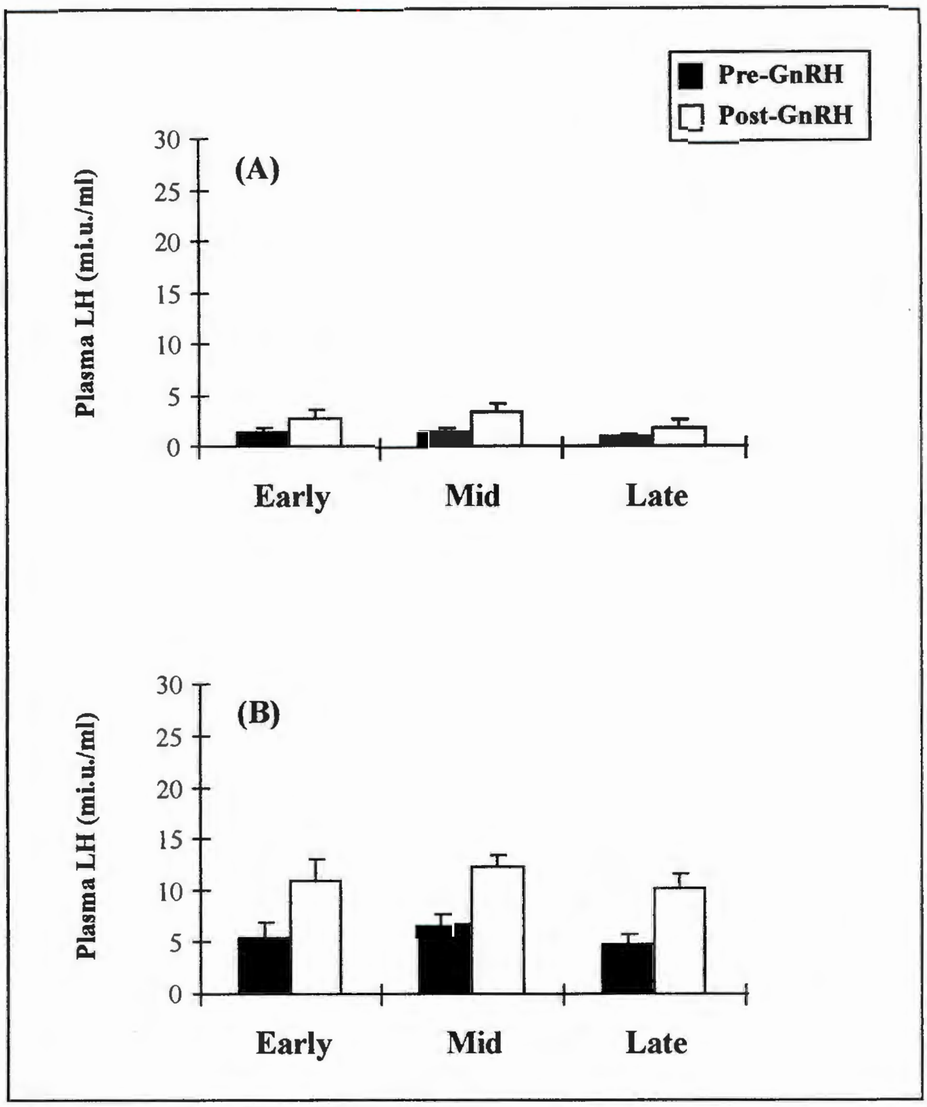


FIGURE 3.8: Plasma LH concentrations (mean ± s.e.m.) of the non-breeding females (A) and non-breeding males (B) of colony 100, during early, mid and late pregnancy (Cycle1), before (Pre-GnRH) and 20 min after (Post-GnRH) a single s.c. injection of 0.1 µg GnRH.

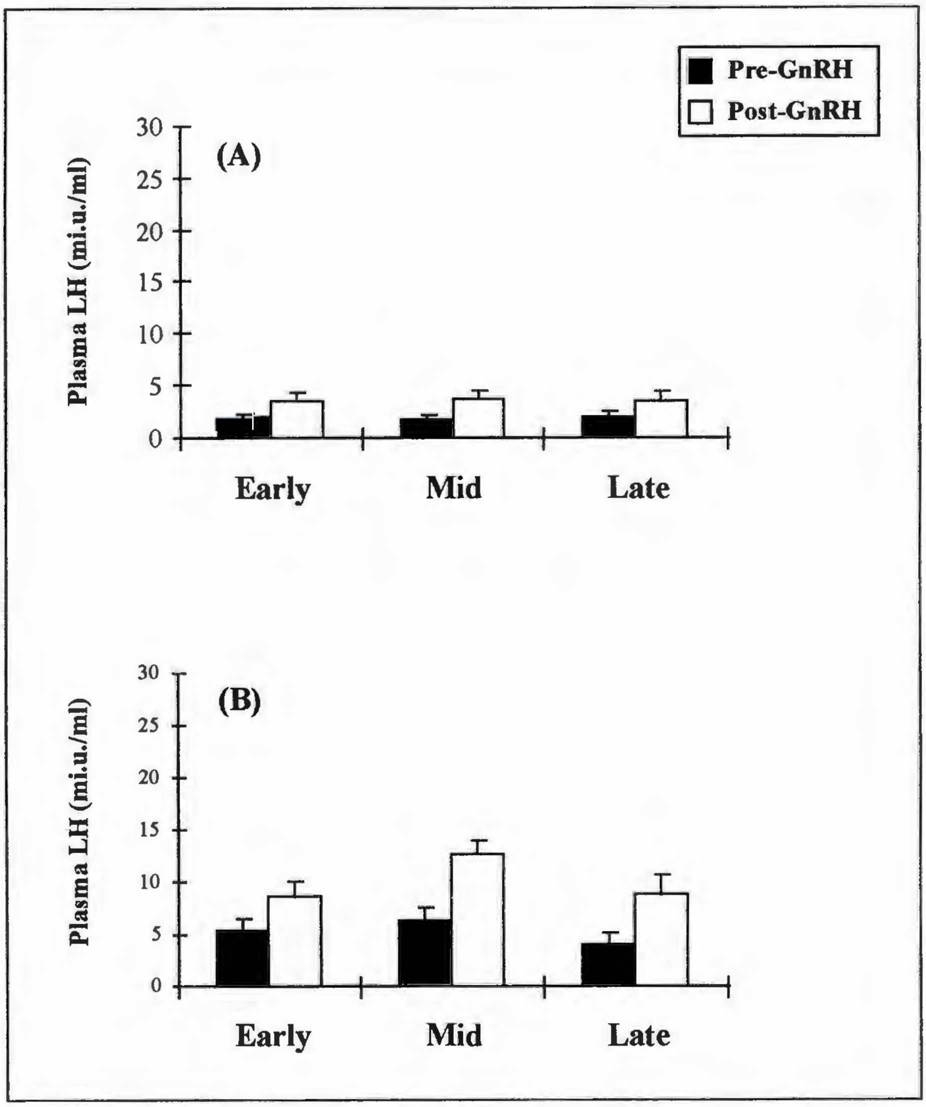


FIGURE 3.9: Plasma LH concentrations (mean ± s.e.m.) of the non-breeding females (A) and non-breeding males (B) of colony 100, during early, mid and late pregnancy (Cycle 2), before (Pre-GnRH) and 20 min after (Post-GnRH) a single s.c. injection of 0.1 µg GnRH.

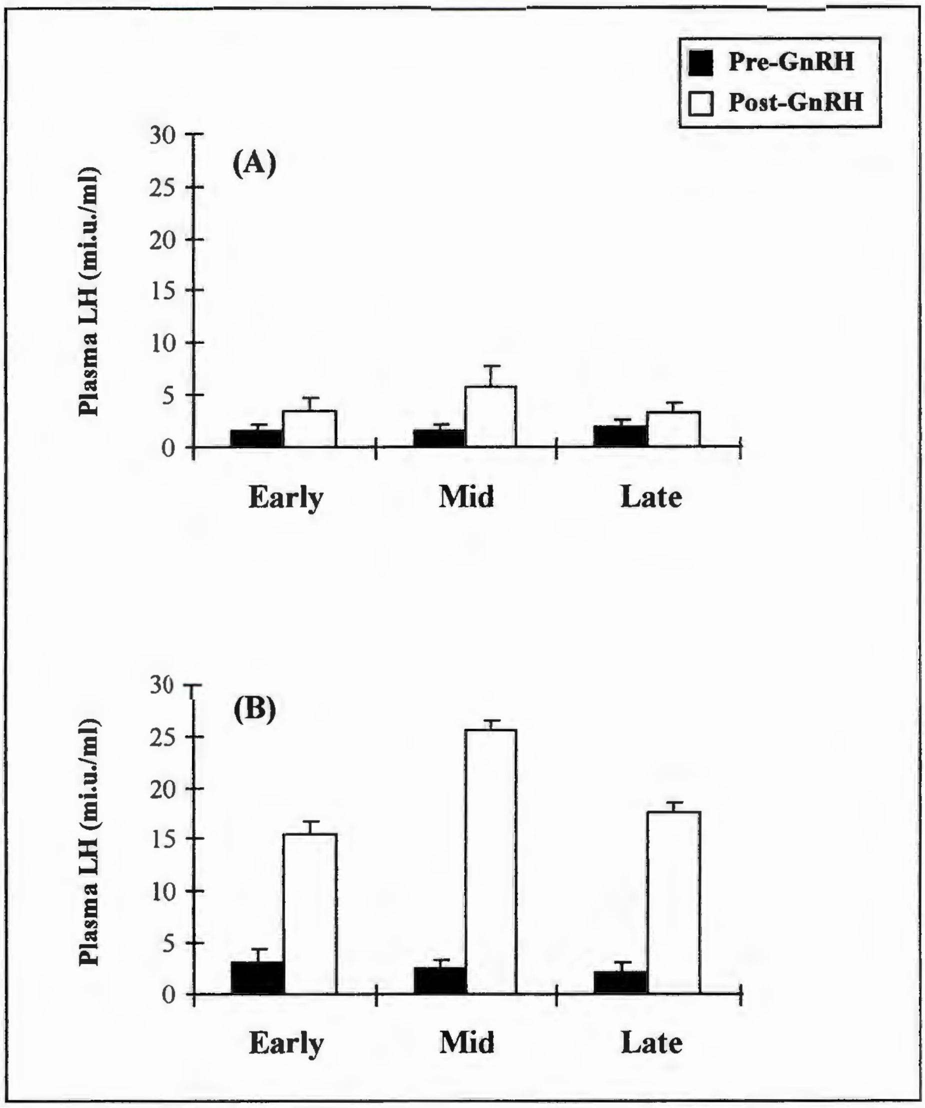


FIGURE 3.10: Plasma LH concentrations (mean ± s.e.m.) of the non-breeding females (A) and non-breeding males (B) of colony 7400, during early, mid and late pregnancy, before (Pre-GnRH) and 20 min after (Post-GnRH) a single s.c. injection of 0.1 μg GnRH.

Plasma LH concentrations and body mass

Colony 100

In the non-breeding females (n = 22) of colony 100 there was no significant correlation between body mass and mean basal (r_s = 0.327, $0.1 < P < 0.2$) or post-GnRH (r_s = 0.330, $0.1 < P < 0.2$) plasma LH concentrations. In addition, there was no significant difference in terms of body mass between the females of Group 1 (36.67 ± 1.75 g, n = 9), which had undetectable basal plasma LH concentrations, and the females of Group 2 (42.32 ± 2.40 g, n = 13), which had detectable basal plasma LH concentrations ($U = 76$, $P > 0.10$) (Mann-Whitney *U*-Test).

The non-breeding males (n = 15) too showed no significant correlation between body mass and mean basal (r_s = 0.207, $0.2 < P < 0.5$) or post-GnRH (r_s = -0.157, $P > 0.5$) plasma LH concentrations.

Colony 7400

In the non-breeding females (n = 10) of colony 7400 there was no significant correlation between body mass and mean basal (r_s = -0.344, $0.2 < P < 0.5$) or post-GnRH (r_s = -0.103, $P > 0.5$) plasma LH concentrations. The non-breeding males (n = 8) too showed no significant correlation between body mass and mean basal (r_s = 0.714, $0.05 < P < 0.1$) or post-GnRH (r_s = 0.167, $P > 0.5$) plasma LH concentrations.

Therefore, plasma LH concentration, and pituitary responsiveness to exogenous GnRH, do not appear to be related to body mass in the individuals of entire colonies of naked mole-rats.

Plasma LH concentrations and relative age

Colony 100

There was a significant positive correlation between basal plasma LH concentrations and relative age in the non breeding females of colony 100 (n = 22) (r_s = 0.45, $0.02 < P < 0.05$) but not between post-GnRH plasma LH concentrations and relative age (r_s = 0.394, $0.05 < P < 0.10$)

In addition, the females of Group 2 (n = 13) were significantly older than those of Group 1 (n = 9) ($U = 85.5$, $0.025 < P < 0.05$), as indicated by litter number. Mean litter for each group is omitted as litter number is an arbitrary value indicating relative, not absolute, age. The individual litter numbers can be found in Table 3.1.

In the non-breeding males of colony 100 (n = 16) there was no correlation between relative age and either basal plasma LH concentration (r_s = 0.068, $P > 0.50$) or post-GnRH plasma LH concentration (r_s = - 0.12, $P > 0.50$).

Colony 7400

In the non-breeding females of colony 7400 (n = 10) there was no significant correlation between relative age and either basal plasma LH concentration (r_s = 0.127, $P > 0.50$) or post-GnRH plasma LH concentration (r_s = 0.107, $P > 0.50$). In the non-breeding males (n = 8) too there was no significant correlation between relative age and either basal plasma LH concentration (r_s = 0.329, $0.20 < P < 0.50$) or post-GnRH plasma LH concentration (r_s = 0.419, $0.20 < P < 0.50$).

Plasma LH concentrations and agonistic interactions received from breeding female

Colony 100

In colony 100 there was a significant positive correlation between the percentage of the total agonistic interactions initiated by the breeding female towards the non-breeding females (n = 22) and basal plasma LH concentration (r_s = 0.461, $0.02 < P < 0.05$), but not with post-GnRH LH concentration (r_s = 0.219, $0.2 < P < 0.5$). In the non-breeding males (n = 15) there was no significant correlation in terms of either basal plasma LH concentration (r_s = 0.157, $P > 0.5$) or post-GnRH plasma LH concentration (r_s = -0.249, $0.2 < P < 0.5$).

The females of Group 2 cumulatively received 91.3% of the total agonistic interactions initiated by the breeding female towards the non-breeding females, while the females of Group 1 received 8.7%. The mean percentage received by the females of Group 2 (7.02 ± 3.46%, n = 13) was significantly greater than that received by the females of Group 1 (0.97 ± 0.73%, n = 9) (U = 87.5, $0.025 < P < 0.05$) (Mann-Whitney *U*-Test). Therefore, in colony 100, the breeding female directed the majority of her agonistic interactions towards those females with higher basal plasma LH concentrations.

Colony 7400

In colony 7400 there was no significant correlation between the percentage of the total agonistic interactions initiated by the breeding female towards the non-breeding females (n = 10) and either basal plasma LH concentration (r_s = -0.302, $0.2 < P < 0.5$), or post-GnRH LH concentration (r_s = -0.394, $0.2 < P < 0.5$). In the non-breeding males (n = 8) too there was no significant correlation in terms of either basal plasma LH concentration (r_s = 0.171, $P > 0.5$) or post-GnRH (r_s = 0.242, $P > 0.5$).

The results of the Spearman's rank correlation coefficient procedures that were used to determine whether there was a relationship between basal and post GnRH plasma LH concentrations and agonistic interactions from the breeding female, are given in Appendix II (Table 3). It was not possible to determine whether, at an individual level, agonism from the breeding female was influenced by an individual's plasma LH concentrations (or *vice versa*) because the majority of the tests revealed an absence of significant correlation

Agonistic interactions received from the breeding female and body mass

Colony 100

There was a positive correlation between the percentage of the total agonistic interactions initiated by the breeding female towards the non-breeding females (n = 22) and body mass, but it was not significant ($r_s = 0.413$, $0.05 < P < 0.10$). However there was a significant positive correlation between the percentage of the total agonistic interactions initiated by the breeding female towards the non-breeding males (n = 15) and body mass ($r_s = 0.812$, $P < 0.001$).

Colony 7400

There was no significant correlation between the percentage of the agonistic interactions initiated by the breeding female and body mass, in either the non-breeding females (n = 10) ($r_s = 0.127$, $P > 0.5$) or the non-breeding males (n = 8) ($r_s = 0.254$, $P > 0.5$).

Agonistic interactions received from the breeding female and relative age

Colony 100

In colony 100 there was a significant positive correlation between the percentage of the total agonistic interactions initiated by the breeding female towards the non-breeding females (n = 22) and relative age ($r_s = 0.533$, $0.01 < P < 0.02$). There was also a significant positive correlation between the percentage of the total agonistic interactions initiated by the breeding female towards the non-breeding males (n = 15) and relative age ($r_s = 0.625$, $0.01 < P < 0.02$).

Colony 7400

In colony 7400 there was no significant correlation between either the percentage of the total agonistic interactions initiated by the breeding female towards the non-breeding females (n = 10) and relative age ($r_s = 0.073$, $P > 0.50$), or between the percentage of the total agonistic interactions initiated by the breeding female towards the non-breeding males (n = 8) and relative age ($r_s = 0.541$, $0.10 < P < 0.20$).

Interactive behaviours

Tables 3.5 to 3.8 show the individual interactions of the non-breeding females and males of colony 100 and colony 7400. Values are expressed as a percentage of the total interactions initiated or received by the respective genders. The interactions under the headings "From BF" (breeding female) and "Towards BF" include those interactions recorded during each of the 5 h of focal study on the breeding female, as well as those that occurred between the breeding female and the other animals during each of the 5 h of focal study on the nest, recorded during early, mid and late pregnancy. The interactions under the headings "Initiated" and "Received", comprise those interactions recorded during each of the 5 h of focal study on the nest, recorded during early, mid and late pregnancy, including interactions initiated towards and received from the breeding female.

The average rate of agonistic interactions initiated by the breeding female of colony 100 was 2.02/h, while that of the breeding female of colony 7400 was 1.53/h. In terms of sexual interactions, the breeding female of colony 100 initiated an average of 1.47/h and engaged in an average of 2.53/h, while the breeding female of colony 7400 initiated sexual interactions at a rate of 4.33/h and engaged in an average of 6.8/h.

Colony -100

In colony 100, the breeding female initiated agonistic interactions towards 11 of the 22 non-breeding females (#12, #13, #15, #23, #43, #45, #5F, #72, #90, #92 and #95) over the two breeding cycles. Only three of these received >10% of the total agonistic interactions initiated by the breeding female towards the non-breeding females (n = 46). These were females #12, #13 and #15 who received 45.65%, 10.87% and 15.22% respectively of these interactions. Female #13 was the most aggressive non-breeding female in the colony. She was the only non-breeding female to initiate >10% of the agonistic interactions by the non-breeding females (n = 23) and was responsible for 60.87% of the total. None of the non-breeding females were seen to initiate agonistic interactions towards the breeding female.

The breeding female of colony 100 initiated sexual interactions towards five non-breeding females (#13, #15, #48, #CC and #95). Female #13, the heaviest non-breeding female in the colony, received 81.25% of all these sexual interactions (n = 48), while female #95 received 10.42%. Three other females (15, #48 and #CC) each received <10% of the total. The breeding female also received sexual interactions from 10 non-breeding females (#13, #15, #1, #48, #5F, #71, #90, #94, #98 and #91), however, only #13 initiated >10% of these interactions (n =35). Female #13, initiated 65.71% of the

TABLE 3.5: The individual behaviours of the non-breeding females of colony 100 (over 2 breeding cycles), expressed as a percentage of the total initiated or received by the non-breeding females.

Animal	Litter	Mass (g)	From BF	Towards BF	Initiated	Received
Agonistic Interactions:-			**(n = 46)**	**(n = 0)**	**(n =23)**	**(n = 27)**
12	2	53.8	45.65	0.00	8.70	11.11
13	2	58.4	10.87	0.00	60.87	7.41
15	2	47.9	15.22	0.00	0.00	18.52
23	3	41.8	2.17	0.00	4.35	3.70
1	4	35.2	0.00	0.00	0.00	3.70
25	5	41.2	0.00	0.00	0.00	3.70
43	7	40.3	2.17	0.00	8.70	0.00
45	7	54.4	2.17	0.00	0.00	3.70
48	8	41.7	0.00	0.00	0.00	3.70
5F	8	37.1	2.17	0.00	0.00	3.70
70	11	42.4	0.00	0.00	0.00	3.70
71	11	35.3	0.00	0.00	4.35	11.11
72	11	39.0	4.35	0.00	0.00	0.00
76	11	33.3	0.00	0.00	0.00	0.00
90	11	34.6	6.52	0.00	0.00	7.41
92	12	33.8	2.17	0.00	0.00	0.00
94	12	35.2	0.00	0.00	0.00	0.00
98	12	43.2	0.00	0.00	8.70	7.41
CC	12	37.3	0.00	0.00	4.35	0.00
91	13	26.5	0.00	0.00	0.00	7.41
95	13	35.8	6.52	0.00	0.00	3.70
96	13	31.9	0.00	0.00	0.00	0.00
Sexual Interactions:-			**(n = 48)**	**(n = 35)**	**(n = 41)**	**(n = 40)**
12	2	53.8	0.00	0.00	9.76	2.50
13	2	58.4	81.25	65.71	41.46	55.00
15	2	47.9	4.17	8.57	4.88	7.50
23	3	41.8	0.00	0.00	4.88	0.00
1	4	35.2	0.00	2.86	4.88	0.00
25	5	41.2	0.00	0.00	7.32	2.50
43	7	40.3	0.00	0.00	7.32	2.50
45	7	54.4	0.00	0.00	2.44	5.00
48	8	41.7	2.08	5.71	0.00	2.50
5F	8	37.1	0.00	2.86	2.44	0.00
70	11	42.4	0.00	0.00	0.00	2.50
71	11	35.3	0.00	2.86	0.00	5.00
72	11	39.0	0.00	0.00	0.00	0.00
76	11	33.3	0.00	0.00	4.88	0.00
90	11	34.6	0.00	2.86	0.00	0.00
92	12	33.8	0.00	0.00	0.00	2.50
94	12	35.2	0.00	2.86	0.00	2.50
98	12	43.2	0.00	2.86	4.88	0.00
CC	12	37.3	2.08	0.00	2.44	5.00
91	13	26.5	0.00	2.86	0.00	5.00
95	13	35.8	10.42	0.00	0.00	0.00
96	13	31.9	0.00	0.00	2.44	0.00

TABLE 3.6: The individual behaviours of the males of colony 100 (over 2 breeding cycles), expressed as a percentage of the total initiated or received by the males.

Animal	Litter	Mass (g)	From BF	Towards BF	Initiated	Received
Agonistic Interactions:-			**(n = 75)**	**(n = 1)**	**(n = 24)**	**(n = 51)**
9	1	49.0	8.00	0.00	29.17	15.22
10	2	58.8	2.67	0.00	20.83	8.70
14	2	49.7	5.33	0.00	4.17	13.04
16	2	73.8	6.67	0.00	8.33	8.70
17	2	64.5	44.00	100.00	25.00	23.91
18	2	41.7	4.00	0.00	0.00	4.35
20	4	51.1	1.33	0.00	0.00	0.00
28	6	55.1	4.00	0.00	4.17	6.52
29	6	38.0	0.00	0.00	0.00	0.00
30	6	67.9	12.00	0.00	0.00	10.87
47	7	34.4	0.00	0.00	0.00	0.00
XX	7	69.4	10.67	0.00	4.17	8.70
51	8	42.1	1.33	0.00	0.00	0.00
52	8	50.1	0.00	0.00	4.17	0.00
54	9	34.9	0.00	0.00	0.00	0.00
5M	8	38.2	0.00	0.00	0.00	0.00
Sexual Interactions:-			**(n = 40)**	**(n = 29)**	**(n = 58)**	**(n = 51)**
9	1	49.0	55.00	51.72	18.97	41.18
10	2	58.8	0.00	6.90	5.17	11.76
14	2	49.7	12.50	13.79	5.17	5.88
16	2	73.8	2.50	0.00	22.41	9.80
17	2	64.5	2.50	17.24	15.52	1.96
18	2	41.7	0.00	0.00	1.72	0.00
20	4	51.1	0.00	0.00	1.72	1.96
28	6	55.1	0.00	0.00	3.45	1.96
29	6	38.0	0.00	0.00	0.00	0.00
30	6	67.9	0.00	6.90	10.34	1.96
47	7	34.4	0.00	0.00	0.00	0.00
XX	7	69.4	22.50	0.00	5.17	23.53
51	8	42.1	0.00	0.00	3.45	0.00
52	8	50.1	5.00	0.00	5.17	0.00
54	9	34.9	0.00	0.00	0.00	0.00
5M	8	38.2	0.00	3.45	1.72	0.00

total sexual interactions initiated by the non-breeding females towards the breeding female. The sexual interactions that occurred between the breeding female and the non-breeding females were typically genital sniffing, but in the case of #13 many of the interactions with the breeding female were mutual ano-genital nuzzling.

The breeding female of colony 100 initiated agonistic interactions towards 10 of the 15 non-breeding males (#10, #14, #16, #17, #18, #20, #28, #30, #XX, and #51), as well as towards the breeding male

(#9). Only three received >10% of the total agonistic interactions initiated by the breeding female towards the males (n = 75). Males #30 and #XX received 12% and 10.67% respectively, and the primary recipient of the breeding females agonism, male #17, received 44% of these agonistic interactions. Male #17 was one of the three most aggressive males in the colony, being responsible for 25% of the total agonistic interactions initiated by males (n = 24). The breeding male (#9) was the most aggressive male in the colony initiating 29.17%, while #10 was responsible for 20.83%.

The breeding male (#9) received 55% of the total sexual interactions initiated by the breeding female towards the males (n = 40), with the majority of the interactions involving mutual ano-genital nuzzling. The breeding female also initiated sexual interactions, typically genital sniffing, towards five non-breeding males (#XX, #52, #17, #16, #14). Most received <10%, but #14 received 12.5% and #XX received 22.5%. In addition, #XX was the only non-breeding male to engage in mutual ano-genital nuzzling with the breeding female, although only on a couple of occasions. The breeding male (#9) initiated 51.72% of the total sexual interactions initiated by the males towards the breeding female (n = 29). The breeding female received sexual interactions such as genital sniffing and nuzzling from five non-breeding males (#5M, #30, #17, #14, #10). All except #14 and #17 initiated <10%, #14 initiated 13.79% and #17 initiated 17.24% of the total sexual interactions initiated by the males towards the breeding female. Although the majority of the non-breeding males were seen to initiate sexual interactions (not necessarily towards the breeding female) such as genital sniffing and nuzzling, only #30, #17 and #16 initiated >10% of the total sexual interactions initiated by males (n = 58) (10,34%, 15.51% and 22.41% respectively).

Colony -7400

The non-breeding female of colony 7400 initiated agonistic interactions towards six non-breeding females (#52, #4, #6, #7, #82 and #84), each of which received 10% or more of the total (n = 10). None of the non-breeding females initiated agonistic interactions towards the breeding female, and only four (#7, #75, #82 and #84) were seen to initiate any agonistic interactions, each being responsible for >10% of the total (n = 5). The only agonistic interaction initiated by these non-breeding females was tooth fencing.

The breeding female of colony 7400 did not engage in sexual interactions with any of the non-breeding females, and only four of the non-breeding females (#47, #4, #75 and #80) were seen to initiate any sexual interactions. Female #7 was the only non-breeding female to receive any sexual interactions.

TABLE 3.7: The individual behaviours of the non-breeding females of colony 7400 (over 1 breeding cycle), expressed as a percentage of the total initiated or received by the non-breeding females.

Animal	Litter	Mass (g)	From BF	Towards BF	Initiated	Received
Agonistic Interactions:-			**(n = 10)**	**(n = 0)**	**(n = 5)**	**(n = 8)**
47	9	39.4	0.00	0.00	0.00	0.00
52	9	32.1	10.00	0.00	0.00	0.00
4	10	49.1	20.00	0.00	0.00	12.50
6	12	36.1	10.00	0.00	0.00	0.00
7	12	47.7	20.00	0.00	40.00	0.00
75	13	39.7	0.00	0.00	20.00	0.00
80	14	33.5	0.00	0.00	0.00	12.50
82	14	34.9	10.00	0.00	20.00	25.00
83	14	35.2	0.00	0.00	0.00	12.50
84	14	34.1	30.00	0.00	20.00	37.50
Sexual Interactions:-			**(n = 0)**	**(n = 0)**	**(n = 7)**	**(n = 3)**
47	9	39.4	0.00	0.00	28.57	0.00
52	9	32.1	0.00	0.00	0.00	0.00
4	10	49.1	0.00	0.00	42.86	0.00
6	12	36.1	0.00	0.00	0.00	0.00
7	12	47.7	0.00	0.00	0.00	100.00
75	13	39.7	0.00	0.00	14.29	0.00
80	14	33.5	0.00	0.00	14.29	0.00
82	14	34.9	0.00	0.00	0.00	0.00
83	14	35.2	0.00	0.00	0.00	0.00
84	14	34.1	0.00	0.00	0.00	0.00

The breeding female of colony 7400 initiated agonistic interactions towards all the males except the smallest, youngest non-breeding male (#81). Only breeding male #9 and non-breeding males #51 and #58 received >10% of these agonistic interactions (n = 36), with #9 being the primary recipient of the breeding female's aggression. The only males to initiate agonistic interactions towards the breeding female were the breeding males (#9, #11 and #20). The breeding males #9 and #20 were the most aggressive males in the colony, being responsible for 42.86% and 35.71% respectively of the total agonistic interactions initiated by the males (n = 14). Breeding male #11 and non-breeding males #49 and #57 were each responsible for <10% of the total.

In colony 7400, the only individuals to engage in sexual activities with the breeding female, were the putative breeding males (#9, #11, #20) who received 23.08%, 59.23% and 17.69% respectively of the total sexual interactions initiated by the breeding female. The breeding female neither initiated nor received sexual interactions from any of the non-breeding males. Among the males, the putative breeding males were responsible for the majority of the sexual interactions initiated by the males (n = 57) (#9 = 15.79%, #11 = 64.92%, #20 = 14.035%), and only two of the non-breeding males (#49 and #81) were seen to initiate sexual interactions, each being responsible for <5% of the total. Male #49 was the only non-breeding male to receive any sexual interactions, and received a single genital sniff.

TABLE 3.8: The individual behaviours of the males of colony 100 (over 2 breeding cycles), expressed as a percentage of the total initiated or received by the males.

Animal	Litter	Mass (g)	From BF	Towards BF	Initiated	Received
Agonistic Interactions:-			**(n = 36)**	**(n = 19)**	**(n = 14)**	**(n = 26)**
9	2	53.0	33.33	5.26	42.86	15.38
11	3	73.0	2.78	31.58	7.14	19.23
20	3	57.7	8.33	63.16	35.71	11.54
49	9	47.1	2.78	0.00	7.14	3.85
51	9	44.0	13.89	0.00	0.00	7.69
57	11	40.8	8.33	0.00	7.14	3.85
58	11	35.8	13.89	0.00	0.00	11.54
71	13	37.9	5.56	0.00	0.00	11.54
72	13	36.8	2.78	0.00	0.00	3.85
77	13	39.6	8.33	0.00	0.00	7.69
81	14	29.1	0.00	0.00	0.00	3.85
Sexual Interactions:-			**(n = 130)**	**(n = 75)**	**(n = 57)**	**(n = 79)**
9	2	53.0	23.08	16.00	15.79	25.32
11	3	73.0	59.23	70.67	64.91	56.96
20	3	57.7	17.69	13.33	14.04	16.46
49	9	47.1	0.00	0.00	3.51	1.27
51	9	44.0	0.00	0.00	0.00	0.00
57	11	40.8	0.00	0.00	0.00	0.00
58	11	35.8	0.00	0.00	0.00	0.00
71	13	37.9	0.00	0.00	0.00	0.00
72	13	36.8	0.00	0.00	0.00	0.00
77	13	39.6	0.00	0.00	0.00	0.00
81	14	29.1	0.00	0.00	1.75	0.00

3.4 Discussion

Low plasma LH concentrations, resulting from impaired endogenous hypothalamic GnRH secretion, have been implicated in the socially-induced suppression of reproduction observed in non-breeding naked mole-rats. Previous studies have shown that non-breeding females and non-breeding males have lower plasma LH concentrations than the breeding animals of their respective genders, and that this is reflected in the failure of non-breeding females to ovulate, and in the lower urinary testosterone concentrations of non-breeding males (Faulkes *et al.*, 1990a; Faulkes *et al.*, 1991a). The results of the present study, which was the first to examine the individuals of entire colonies, indicate that there is a great deal of individual variation in terms of basal plasma LH concentrations throughout the reproductive cycle of the breeding female. Although some individuals had consistently low basal plasma LH concentrations throughout the study, individual fluctuations meant that no animal could be singled out as having consistently higher concentrations than all the other mole-rats of the same colony. LH is secreted in a pulsatile manner (Turek & Van Cauter, 1994), and the individual variations observed in the present study probably reflect these temporal changes in circulating plasma LH concentrations. Nevertheless, although single plasma samples do not give information regarding the pulse frequency or amplitude of LH secretion, the mean basal plasma LH concentrations of the individuals of colony 100 over two cycles, and colony 7400 over one cycle, showed that certain individuals had mean basal LH concentrations that were clearly higher than those of the other non-breeding animals of their respective genders. These differences between individuals were, however, more apparent among the non-breeding males than among the non-breeding females.

It has previously been reported that non-breeding naked mole-rats of both sexes exhibit an apparent reduced pituitary sensitivity to low doses of exogenous GnRH, as evidenced by lower LH responses, relative to the breeding animals of their respective genders (Faulkes *et al*, 1990b; Faulkes *et al.*, 1991a). This may be the result of reduced concentrations of GnRH receptors on the anterior pituitary. Indeed, in rats, changes in pituitary responsiveness to exogenous GnRH during the oestrous cycle are paralleled by changes in pituitary GnRH receptor concentrations (Sandow, 1983). The apparent lack of anterior pituitary sensitivity in non-breeding naked mole-rats has been shown to be reversible following four consecutive 1 h injections of 0.5 μg GnRH in the males (Faulkes *et al.*, 1991a) and 0.1 μg GnRH in the females (Faulkes *et al.*, 1990b), implying that reduced sensitivity, possibly due to low concentrations of GnRH receptors on the anterior pituitary, may be the result of insufficient priming of the pituitary by endogenous GnRH.

As with the basal plasma LH concentrations, the present study revealed that there was also individual variation in terms of post-GnRH plasma LH concentrations in the non-breeding animals of both sexes, indicating temporal changes in individual pituitary sensitivity to exogenous GnRH. The mean post-GnRH plasma LH concentrations of the individuals of colony 100 over two cycles, and colony 7400 over one cycle, revealed that there were certain individuals that exhibited greater pituitary sensitivity to 0.1 μg exogenous GnRH than the other non-breeding animals of their respective genders. Moreover, as with the basal plasma LH concentrations, individual variation appeared to be greater among the non-breeding males than among the non-breeding females. Similarly, Faulkes (1990) has found a great deal of variation in male naked mole-rats, with some non-breeding males showing a degree of overlap with the breeding males, in terms of their urinary testosterone concentrations.

The mean basal and post-GnRH plasma LH concentrations of non-breeding female naked mole-rats have previously been found to be 1.3 ± 0.2 mi.u./ml and 2.9 ± 0.5 mi.u./ml respectively (Faulkes *et al.*, 1990b), and Faulkes *et al.* (1991a) have reported mean basal LH concentrations of 5.0 ± 0.8 mi.u./ml and post-GnRH plasma LH concentrations of 8.9 ± 0.5 mi.u./ml for non-breeding males. Therefore, despite individual variation, the mean basal and post-GnRH plasma LH concentrations of the non-breeding females of colony 100 and colony 7400, and non-breeding males of colony 100 were comparable with the findings of these studies, but the non-breeding males of colony 7400 had lower basal and higher post-GnRH plasma LH concentrations than these previously reported values. Nevertheless, as with urinary testosterone, there also appears to be a great deal of variation among non-breeding males in terms of plasma LH concentrations, and Faulkes *et al.* (1991a) have also reported mean basal plasma LH concentrations of 1.5 ± 0.8 mi.u./ml (n = 5) for non-breeding males. In both colonies the non-breeding males had higher basal LH concentrations than females, and, in addition, appeared to have higher anterior pituitary sensitivity, as indicated by the greater LH response to a low dose of 0.1μg GnRH.

The basal and post-GnRH plasma LH concentrations of breeding females have previously been measured to be 3.4 ± 0.8 mi.u./ml and 9.6 ± 2.0 mi.u./ml respectively (Faulkes *et al.*,1990b), while those of the breeding males have been measured at 10.7 ± 1.7 mi.u./ml and 33.7 ± 3.7 mi.u./ml respectively (Faulkes *et al.*, 1991a). Among the females of colony 100, only female #13 had a mean basal plasma LH concentration in excess of 3.4 ± 0.8 mi.u./ml, while in colony 7400 all of the non-breeding females had mean basal plasma LH concentrations below this value. In colony 100, all the non-breeding females had mean post-GnRH plasma LH concentrations below

9.6 ± 2.0 mi.u./ml, while in colony 7400, one female (#47) had a mean post-GnRH plasma LH concentration in excess of this value. Nevertheless, despite individual variation, the mean basal and post-GnRH plasma LH concentrations of the non-breeding females and non-breeding males of the two study colonies, were below the mean values previously measured for breeding animals of their respective genders.

An interesting point is that the basal and post-GnRH plasma LH concentrations of the breeding males of colony 100 and colony 7400 were surprisingly lower than the mean values previously measured for breeding males (Faulkes *et al.*, 1991a). Moreover, although the three breeding males of colony 7400 had the highest basal plasma LH concentrations of all the males in their colony, there were a number of non-breeding males that exhibited higher post-GnRH plasma LH concentrations. Even more surprisingly, in colony 100, the breeding male had relatively low basal and post-GnRH plasma LH concentrations compared with many of the non-breeding males in the colony. Faulkes (1990) investigated reproductive activation in non-breeding male naked mole-rats, and found that the plasma LH concentrations of non-breeding males that had been removed from their colonies and housed singly, were higher than those of non-breeding males that were subsequently housed with a female. This suggests that the breeding female, in addition to suppressing reproduction in the non-breeding animals, also has some control over the reproductive processes of the breeding male(s). Indeed, in the males that were subsequently housed with a female, urinary testosterone concentrations fluctuated over the ovarian cycle of the female partner, with peaks in urinary testosterone concentrations commonly coinciding with the follicular phase and oestrous period of the female's cycle (Faulkes, 1990). This implies an active suppression of pituitary secretion of LH, in breeding males, outside the follicular phase of the ovarian cycle, with a selective release from suppression, possibly accompanied by stimulation of the breeding male by the breeding female, leading to an increase in plasma LH concentrations, and presumably also plasma testosterone concentrations, at the time of oestrous (Faulkes, 1990).

In the present study, blood samples from the breeding males were collected throughout the reproductive cycle of the breeding female, but not at the time of oestrous. Nevertheless, it is improbable that the relatively low plasma LH concentrations of the breeding males of colony 100 and colony 7400, compared with values previously reported for breeding males (Faulkes *et al.*, 1991a), can be explained in terms of selective release from suppression, because, although Faulkes *et al.* (1991a) did not specify when in the breeding female's reproductive cycle the plasma samples were collected, it seems unlikely that collection of the majority of the 27 samples from 14 breeding males would have been restricted to the short 6 day follicular phase.

Faulkes (1990), having reported higher concentrations of plasma LH in breeding males than in non-breeding males, noted that it was not possible to comment on whether these higher plasma LH concentrations, and, consequently, higher testosterone production, actually had an effect on sperm numbers and viability, or simply reflected a greater degree of sexual behaviour. In colony 7400, the three breeding males were the only males to engage in sexual interaction with the breeding female, and the inter-colony differences between the plasma LH concentrations of the breeding males of colony 100 and the breeding male of colony 7400, when compared with the non-breeding males of their respective colonies, can possibly be explained in terms of the association between these males and their respective mates. In colony 100, the breeding male (#9) was well established, and, moreover, was the only breeding male in the colony. In colony 7400, on the other hand, there were three breeding males (#9, #11 and #20), all of which had only recently attained breeding status. The breeding female of colony 7400 (#28) had also only recently succeeded the previous breeding female (Chapter 4), and had probably not yet formed a strong behavioural bond with the three new breeding males. It is therefore possible that the three breeding males of colony 7400 needed higher plasma LH concentrations than the non-breeding males of the colony, to give them a competitive advantage. The well established breeding male of colony 100, on the other hand, who was already behaviourally bonded to the breeding female, and who also had no competitors, would not need such a advantage, and/or perhaps was under stricter control by the well established breeding female, as the low pituitary responsiveness to exogenous GnRH seems to indicate. Had plasma samples been collected at the time of oestrous, the breeding male of colony 100 may in fact have shown higher plasma LH concentrations.

The naked mole-rat is similar to the marmoset monkey in that in both species, reproductive suppression appears to be less stringent in the non-breeding males than in the non-breeding females. In newly established peer groups of marmoset monkeys, subordinate males are prevented from reproducing by behavioural means only, while subordinate females exhibit a complete absence of ovarian cyclicity (Abbott, 1984). In the naked mole-rat, both breeding and non-breeding males undergo spermatogenesis (Faulkes *et al*, 1991a), while ovulation is completely suppressed in the non-breeding females (Faulkes *et al*, 1990a). In the present study, the greater degree of individual variation in terms of basal plasma LH concentrations and pituitary sensitivity to exogenous GnRH among the non-breeding males, together with the fact that the non-breeding males of each colony had higher basal and post-GnRH plasma LH concentrations than the non-breeding females, confirm that the physiological suppression of reproductive function is less clear-cut among the non-breeding males of naked mole-rat colonies than among the non-breeding females.

In the present study, the elevation in post-GnRH plasma LH concentrations of the non-breeding females and non-breeding males of both colonies during mid pregnancy, albeit non-significant, implies an increase in anterior pituitary sensitivity to exogenous GnRH, in at least some individuals, during this time. From Figures 3.1 to 3.3 it is apparent that, although not all the individuals of colonies 100 and 7400 showed elevated LH responses during mid pregnancy, several of the colony members, particularly amongst the males, showed their highest responses during mid pregnancy.

From previous behavioural studies one would expect such an elevation to occur, if at all, during late pregnancy when the female is highly gravid and unable to patrol the burrow system as effectively as at other times of the cycle (Jarvis, 1991a; Reeve & Sherman, 1991). Further evidence that the breeding female's control is weak during late pregnancy has come from a study by Westlin *et al.* (1994) which found that the proportion of non-breeding females with perforate vaginas increased from 6 days prepartum to reach a maximum on the day of parturition. They also found that non-breeding females with detectable urinary concentrations of oestradiol-17ß, showed a significant increase in this hormone 6 days prior to parturition of the breeding female. However, the study concentrated on the period from 9 days prepartum to 13 days postpartum, and samples were not collected during the rest of the cycle. It is possible that, had they investigated the entire cycle, they might have found elevated hormone levels at other times, perhaps during mid-pregnancy.

The apparent increase in pituitary sensitivity during mid pregnancy is supported by an observation previously made by Faulkes (1990). Faulkes investigated the urinary testosterone profiles of several non-breeding male naked mole-rats before and after removal from their colonies. One point which he considered of significant interest to merit further discussion was the synchronous peak in urinary testosterone in two of the males, while they were still in the colony, and therefore under the influence of the breeding female. He commented on the fact that, if the observation was not merely a coincidence, then an unknown environmental factor was implicated. He stated that the phenomenon was unlikely to have been connected with a period of oestrous in the breeding female because at the time she had been mid-pregnant. From the results of the present study, in which pituitary sensitivity to exogenous GnRH was investigated in the individuals of entire colonies, it appears that Faulkes' observation was not merely a coincidence, and that an unknown environmental factor may indeed influence the degree of reproductive suppression in some of the non-breeding animals during mid pregnancy of the breeding female.

A possible factor acting during mid pregnancy could be the degree of behavioural thermoregulation exhibited by the breeding female during pregnancy. The naked mole-rat is unusual for a mammal in that it is a poor thermoregulator with a basal metabolic rate far lower than that expected for its size (McNab, 1966). It's poor ability to thermoregulate is probably the result of a lack of insulation, due to the absence of a pelage, a lack of sweat glands (Tucker, 1981) and a high surface area to volume ratio. It exhibits a high rate of heat transfer and it's body temperature has been shown to change with ambient temperature (Withers & Jarvis, 1980; Moon, Mustafa & Jørgensen, 1981; Buffenstein & Yahav, 1991) and it appears to be not a homoiotherm but rather a poikilotherm (Buffenstein & Yahav, 1991). Since reproduction in naked mole-rats is energetically expensive, it would be advantageous for the breeding female to employ energetically inexpensive thermoregulatory mechanisms, such as improved insulation or behavioural thermoregulation, in order to maintain an optimal temperature for gestation. This would allow more energy to be directed towards foetal development and growth. Buffenstein, Urison, Woodley, van der Westhuizen & Jarvis (1996) have suggested that a higher body temperature during pregnancy may enhance pup growth and development, by enhancing the rate of maternal-foetal energy transfer. Jarvis (1991a) has reported unquantified observations of extended basking during mid to late pregnancy. In the present study, in all three cycles during which behaviour was recorded, the incidence of basking by the breeding females of colony 100 and colony 7400 was greatest during mid pregnancy (see Appendix II, Table 4). Buffenstein *et al.* (1996) have found that the contribution of body fat to the total body composition increases significantly from early to mid pregnancy, and that during late pregnancy, when the reduction of conductive heat exchange and the maintenance of metabolic heat may be particularly important, the contribution of body fat to total mass exceeded 30%. They have suggested that this could explain the observed decline in the proportion of time spent basking during late pregnancy compared with mid pregnancy.

Although during mid pregnancy the breeding female is still able to patrol the burrow system with relative ease, the increased incidence of basking behaviour during this time may mean that she has less contact with the other colony members than at other times, which could explain the apparent slight relaxation of reproductive suppression at this time. Therefore, the apparent relaxation of reproductive suppression during mid pregnancy may not be connected with the reproductive state of the breeding female, but may simply be due to reduced contact between the breeding female and the non-breeding animals as a consequence of the increased frequency of basking behaviour exhibited by the breeding female at this time. However, in the present study, this was not reflected by the frequency of agonistic interactions by the breeding female, which did not appear to be lower during mid pregnancy than at other times in the cycle (see Appendix II, Table 5).

In naked mole-rat colonies, relatively few individuals are involved in interactive behaviours with the breeding female, and the majority are ignored (pers. obs.; O'Riain, 1996). The relationship between individual plasma LH concentrations and the degree of agonism directed by the breeding female towards the non-breeding animals of both sexes, was unclear. There was no evidence to suggest that, at an individual level, agonistic interactions from the breeding female were influenced by either basal or post-GnRH plasma LH concentrations (or *vice versa*), although one non-breeding female (#15) exhibited a strongly significant positive correlation between the relative amount of agonism received from the breeding female and post-GnRH, but not basal, plasma LH concentrations over two breeding cycles. However, the significant positive correlation between basal plasma LH and the percentage of the total agonistic interactions directed towards the non-breeding females by the breeding female of colony 100, indicates that, in this particular colony, the breeding female directed most of her agonism towards those females with higher basal plasma LH concentrations. These females may represent the greatest threat to her reproductive status. In colony 100, the non-breeding females were divided into two groups on the basis of whether or not they had detectable basal plasma LH concentrations throughout the study. The group which had significantly higher basal and post-GnRH plasma LH concentrations also received a significantly greater percentage of the breeding female's aggression than the non-breeding females of the other group. The relationship between plasma LH and breeding female aggression, observed among the non-breeding females of colony 100, was absent from colony 7400. This inter-colony difference may reflect the divergent histories of the two colonies. Colony 100 was a stable colony which still had the original breeding female, while colony 7400 had undergone two take-over events, the most recent of which had been completed only 2 months prior to the investigation of the breeding cycle. In fact, it was only the second pregnancy of the current breeding female. During the take-over event in colony 7400, most of the largest, oldest females had been killed, and this, together with the fact that it was a relatively unstable colony compared with colony 100, may explain the inter-colony differences in terms of the relationship between plasma LH and the aggression of the breeding females towards the non-breeding females. There was no relationship between plasma LH and the aggression of the breeding female towards the non-breeding males of either colony 100 or colony 7400. Unexpectedly, the breeding female of each colony directed more aggression towards the males than towards the non-breeding females. Prior to the take-over event in colony 7400, which is described in Chapter 4, the breeding female directed more aggression towards the non-breeding females than towards the non-breeding males.

In the present study there were inter-colony differences in terms of the agonistic and sexual interactions initiated by the breeding female of each colony. The breeding female of colony 100 initiated more agonistic interactions per hour than the breeding female of colony 7400, while the breeding female of colony 7400 initiated more sexual interactions per hour than the breeding female of colony 100. Moreover, the sexual interactions of the breeding female of colony 7400 occurred exclusively with the three putative breeding males, while in colony 100, the breeding female engaged in sexual interactions with a number of non-breeding animals as well as with the breeding male. As has already been discussed above, there were inter-colony differences in terms of colony history. In colony 7400 the breeding female was still relatively new, and the high frequency of sexual interactions in which she engaged with the breeding males may reflect the fact that strong behavioural bonding had not yet occurred between them. The high frequency of sexual interactions were probably to strengthen this bond during the early stages of the association.

Individual behaviours have already been discussed in some detail in the results section, but there are a number of individuals in colony 100 that merit further discussion. In colony 100, the primary recipient of the breeding female's agonistic interactions was male #17, who received 44% of the agonistic interactions initiated by the breeding female towards the males, and 27.27% of all the breeding female's agonistic interactions. In addition, this male had the highest mean basal plasma LH concentration, and second highest mean post-GnRH plasma LH concentration, of the males of colony 100. It is very interesting that this male had previously been seen attempting to mount the breeding female when she was in lordosis (O'Riain, pers. com.). This behaviour is extraordinary in a non-breeding male naked mole-rat, as non-breeding males usually show no reproductive interest in the breeding female when she is in oestrous (Jarvis, 1991a). In the present study too, male #17 showed sexual interest in the breeding female throughout the breeding cycle, and initiated more sexual interactions towards the breeding female than any other non-breeding male, although he received only a single genital sniff from the breeding female in 60 hr of behavioural observation.

Another non-breeding male of interest was #14. This male had the lowest mean basal and post GnRH plasma LH concentrations of all the males in colony 100, and received relatively few agonistic interactions from the breeding female. However, he was one of the few non-breeding males to receive sexual interactions from the breeding female and, in turn, to show sexual interest in the breeding female. In addition, during the behavioural observations of early-pregnancy in Cycle 2, the breeding female was observed to exhibit the lordosis reflex when she encountered #14 in the burrow system. Each time she encountered him she would sniff him, back into him and go into lordosis. However, #14 did not try to mount her and appeared to show no sexual interest in her at all during this time. This behaviour on the part of the breeding female was surprising as it has previously been reported that the breeding female does not solicit any individuals but the breeding males (Jarvis, 1991a). Although the breeding female had initiated sexual interactions towards #14 and received sexual interactions from him, the two had not been observed engaging in mutual ano-genital nuzzling, a behaviour that typically occurs between the breeding female and breeding males.

Among the females, #13 was possibly the most intriguing. Not only was she the most aggressive non-breeding female in the colony, but she was also one of only three non-breeding females to receive >10% of the total agonistic interactions initiated by the breeding female towards the non-breeding females. In addition, she also received the majority of the sexual interactions initiated by the breeding female towards the non-breeding females, and moreover, was the only animal, apart from the breeding male (#9), to engage in frequent mutual ano-genital nuzzling with the breeding female. This is highly unusual as this particular behaviour usually occurs exclusively between the breeding female and male(s) and it is only during times of colony strife that non-breeding animals engage therein (Jarvis, 1991a; Chapter 4). Nevertheless, #13 was a frequent participant in this behaviour, and, furthermore, engaged in it exclusively with the breeding female. In addition to being behaviourally distinct from the other females, #13 also had the highest mean basal plasma LH concentration, over the two breeding cycles, of all the non-breeding females of colony 100, and the third highest mean post-GnRH plasma LH concentration.

Two other non-breeding females that are worth mentioning are #12 and #15. These two were the only other non-breeding females, apart from #13, to receive >10% of the total agonistic interactions initiated by the breeding female towards the non-breeding females. Although #15 had the second highest mean post-GnRH plasma LH concentration of the non-breeding females, neither had particularly high mean basal plasma LH concentrations. However, both belonged to the same litter as #13, which was the oldest litter in the colony, and, furthermore, they represented three of the four heaviest non-breeding females in the colony.

It has previously been shown that the latency of onset of reproductive activation in non-breeding female and male naked mole-rats which have been removed from their colonies, does not appear to be influenced by body mass and age prior to removal, illustrating that reproductive activation in non-breeding naked mole-rats may occur over a wide range of body sizes and ages (Faulkes, 1990). In the present study there was no correlation between either basal plasma LH or post-GnRH plasma LH concentration and body mass in either colony. In addition, among the females of colony 100, those of Group 1, which had consistently undetectable basal plasma LH concentrations, did not differ from those of Group 2 in terms of body mass. There was also no correlation between either basal or post-GnRH plasma LH concentration and relative age in the non-breeding males of colony 100 or in the non-breeding females or non-breeding males of colony 7400. There was, however, a significant positive correlation between basal plasma LH concentration and relative age in the non-breeding females of colony 100, reflecting the fact that the females of Group 2 were significantly older than those of Group 1.

The only significant positive correlation between the percentage of the total agonistic interactions initiated by the breeding female and body mass occurred in the non-breeding males of colony 100, indicating that the breeding female was directing her aggression at the larger males in this particular colony. There was, however, a significant positive correlation between the percentage of the total agonistic interactions initiated by the breeding female, and relative age, in both the non-breeding females and non-breeding males, of colony 100, although not in colony 7400. Previous studies have suggested that replacement reproductives, particularly females, often come from the ranks of the largest, oldest, most dominant non-breeding animals in the colony (Jarvis, 1991a; O'Riain, 1996; Clarke & Faulkes, 1997) and it is possible therefore, that these individuals may pose the greatest threat to the reproductive status of the breeding female. If this is the case then one would expect her to direct her agonism towards these individuals, in order to keep them reproductively suppressed. The results of the present study seem to indicate that age may be more important than body mass in determining which animals pose the greatest threat to the reproductive status of the breeding female, and are most likely to become reproductive first, should the opportunity arise. Indeed, although both mass and age appeared to be significant in determining which animals were killed in the take-over event described in Chapter 4, age seemed to be the more important of the two. It is interesting that, although the non-breeding animals that were killed during the take-over event were amongst the heaviest animals in the colony, there were younger animals, with greater body masses than some of those that were killed, that did not become involved in the fighting. Moreover, apart from the breeding males and the new breeding female, all the offspring of the original breeding pair, (*i.e.* the oldest colony members) were killed.

In conclusion, this was the first study to investigate reproductive suppression in entire colonies of naked mole-rats. Previous studies have compared breeding and non-breeding animals selected at random from several colonies (Faulkes, 1990; Faulkes *et al.*, 1990a; 1990b; Faulkes & Abbott, 1991), but none has looked at the individuals of entire colonies throughout the breeding cycle. The results of this study support the findings of these previous studies, by providing conclusive evidence for reproductive suppression in non-breeding naked mole-rats, and by confirming that non-breeding females are more suppressed than non-breeding males. However, the results of this study also indicate that there is variation between individuals in terms of both basal plasma and post-GnRH plasma LH concentrations implying that some individuals are more suppressed than others. This variability is more pronounced amongst the non-breeding males than the non-breeding females of naked mole-rat colonies. Relatively few interactions occur between the breeding female and the majority of the colony members, and there is no clear relationship between plasma LH and the amount of aggression received from the breeding female. However, it appears that age is an important factor, and older colony members have higher plasma LH concentrations than younger animals, and receive most of the breeding female's aggression.

Chapter 4

Chapter 4

BEHAVIOURAL AND HORMONAL CHARACTERISTICS OF THE MEMBERS OF A NAKED MOLE-RAT COLONY BEFORE AND AFTER A TAKE-OVER EVENT

4.1 Introduction

The naked mole-rat exhibits possibly the most extreme form of socially-induced reproductive suppression observed in any mammal (Abbott *et al.*, 1989). However, despite the fact that that the majority of non-breeding male and female naked mole-rats remain reproductively quiescent while in the presence of the breeding female, they can show signs of reproductive activation in as little as 8 days after being removed from the colony (Faulkes, 1990; Faulkes *et al.*, 1990a). This implies that non-breeding naked mole-rats are not sterile and that reproductive suppression is readily reversible when the social cues bringing it about are removed.

Upon the death or removal of the breeding female from a colony, several non-breeding animals may simultaneously become reproductively active until such time as a new breeding female has emerged. The events of a number of colony successions have been reported (Jarvis, 1991; Lacey & Sherman, 1991; Margulis *et al.*, 1995) and there appears to be a great deal of variability between different succession occurrences. Colony succession can occur within 10 days, with little aggression, or it can take more than a year, accompanied by violent fighting and the deaths of several animals. The succession of a breeding male appears to occur rapidly, possibly because colonies may have more than one breeding male and are rarely without at least one at any given time.

Although reproduction is usually monopolised by a single breeding female (Jarvis, 1981; Jarvis, 1985; Brett, 1986; Jarvis, 1991a; Lacey & Sherman, 1991; Faulkes *et al.*, 1991a), the occasional occurrence of two breeding females in a colony (Jarvis, 1991a; Sherman *et al*, 1992) provides evidence that non-breeding females are not all equally suppressed. In addition, reproductive suppression occurs at differential levels in certain individuals within a colony (Chapter 3) and some non-breeding females, although they may not actually breed, display evidence of reproductive activity while still in the natal colony. It has previously been suggested that this generally occurs just prior to parturition when the breeding female is highly gravid and her control over the other females is thought to be weakest (Jarvis, 1991a). At this time some non-breeding females may exhibit nipple development, increases in urinary oestradiol-17*B* concentrations or changes in the vaginal closure membrane (Jarvis, 1991a; Westlin *et al.*, 1994). Nearly all "natural deaths" in captive colonies occur when the breeding female is very gravid (Jarvis, pers. com.) and it has been suggested that the relaxation of reproductive suppression in some non-breeding females just prior to parturition may ensure that the breeding female is rapidly replaced should she die at this time (Jarvis, 1991a). Indeed, Jarvis (1991a) documents instances in which mating occurs within a couple of weeks after the death of the breeding female.

All animals born to a colony are potential reproductives (Jarvis, 1991a), although in reality most will never have the opportunity to breed. Replacement reproductives often come from the ranks of the largest, most dominant animals in the colony (O'Riain, 1996; Clarke & Faulkes, 1997). However, on some occasions small-sized females have achieved reproductive status following the death of the breeding female, and it is not always possible to predict correctly, from growth or behaviour, which non-breeders will assume reproductive roles upon the death of a reproductive animal (Jarvis, 1991a).

Most "natural" colony successions occur when the breeding female has died unexpectedly, and consequently data are unlikely to have been collected immediately prior to the event. Two recent studies have attempted to investigate, systematically, behavioural and endocrinological changes occurring in colonies during succession, by collecting data prior to and following the planned removal of breeding animals from colonies. Margulis *et al.* (1995) investigated the behavioural and endocrinological changes in the remaining non-breeding females following the removal of both the breeding female and breeding male from a single colony. The behaviours of primary interest were shoving and ano-genital nuzzling, and urinary progesterone was used as an indicator of ovulation or pregnancy. They found that intra-sexual aggression between female naked mole-rats appears to be facilitated by ovarian activation, which possibly also contributes to the attainment of breeding status. Clarke and Faulkes (1997) examined dominance and reproductive succession in captive colonies to investigate the relationship between urinary testosterone and cortisol levels, dominance rank, and reproductive status, and to determine whether behavioural and/or hormonal parameters can be used to predict breeding female succession. They found that dominance appears to be a good predictor of reproductive status, and that breeding females are succeeded by the next highest ranking females. Jarvis (1991a) has reported that when the breeding female dies, succession, or the onset of fighting for succession, usually occurs quickly, implying the existence of an established dominance hierarchy amongst the females.

Studies involving the artificial removal of breeding animals from colonies allow hormonal and behavioural data to be collected prior to and after succession, by simulating the deaths of breeding animals. Sometimes, however, fighting may break-out before the position of breeding female has been vacated by her death. In these cases the breeding female's reproductive status may be challenged by a non-breeding female, and colony succession is preceded by fighting between the breeding female the challenger (O'Riain, 1996). During the time of fighting, before the queen has been killed, there may be an opportunity for other non-breeding females to become reproductively activated. The take-over event in colony 7400, in which the reproductive position of the breeding female was challenged, provided a unique opportunity to examine differences between those animals that were subsequently killed during the course of a natural take-over event and those that were not, without the artificial removal of the breeding female. The colony was therefore, to some extent, primed for her death and the subsequent fighting for reproductive status.

The aims of this study were therefore to determine whether predictions could have been made, from endocrinological differences, body masses, ages and possibly differential behaviour of the breeding female towards certain individuals prior to the take-over event, as to which animals were most likely to become involved in the intra-colonial fighting during colony succession, and also to give a qualitative description of the behavioural changes which took place in the non-breeding animals involved in the fighting.

4.2 Methods

4.2.1 . Subjects

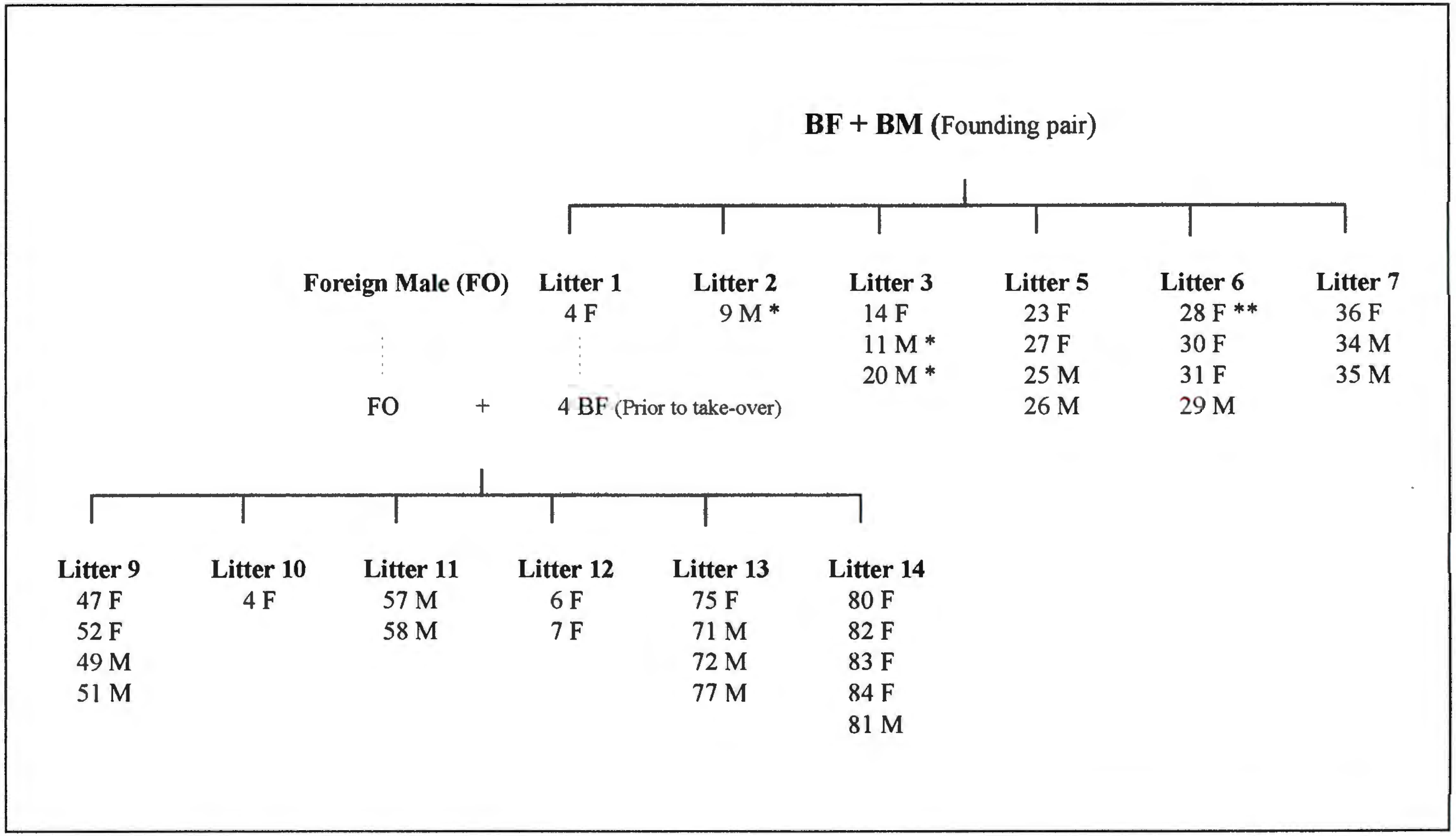
This part of the study was conducted on colony 7400. Details of the colony history and maintenance conditions have already been discussed in Chapter 2, sections 2.1 and 2.2. At the start of the study, prior to the take-over event, colony 7400 consisted of 34 animals, of which 18 were female and 16 were male. During the take-over event seven females, including the breeding female, and five non-breeding males were killed. For details on the individual colony members see Table 4.1. The relatedness of the individuals to one another is presented in Figure 4.1.

TABLE 4.1: Details of the individuals of colony 7400 (July - October 1995)

Animal	Sex	Litter	Mass (g) Before take-over	Mass (g) After take-over
1	BF	1	[illegible]	
[illegible]	[illegible]	[illegible]	[illegible]	
21	[illegible]	[illegible]	[illegible]	
27	F	[illegible]	[illegible]	
28	F	6	61.1	63.3
[illegible]	F	[illegible]	[illegible]	
[illegible]	F	6	[illegible]	
[illegible]	F	7	[illegible]	
47	F	9	37.2	38.5
52	F	9	25.6	[illegible]
4	F	10	44.5	45.7
6	F	12	31.6	32.9
7	F	12	41.8	45.6
75	F	13	33.1	34.6
80	F	14	[illegible]	[illegible]
82	F	14	[illegible]	28.2
83	F	14	[illegible]	[illegible]
[illegible]	F	14	[illegible]	31.0
9	BM	2	53.1	55.0
11	BM	3	74.7	72.9
20	BM	3	61.9	60.4
[illegible]	M	[illegible]	[illegible]	
[illegible]	M	[illegible]	[illegible]	
[illegible]	M	[illegible]	[illegible]	
[illegible]	M	[illegible]	[illegible]	
[illegible]	M	[illegible]	[illegible]	
49	M	9	46.4	50.7
51	M	9	43.2	47.2
57	M	11	35.1	37.8
58	M	11	34.3	34.9
71	M	13	30.2	33.6
72	M	13	27.1	28.7
77	M	13	33.8	35.5
81	M	14	22.8	[illegible]

Animals < 26 g not sampled prior to the take-over event

Animals killed during the take-over event



* Putative breeding males
** New breeding female following take-over

FIGURE 4.1: Diagram illustrating the relatedness of the individuals of the colony 7400, prior to the take-over event.

4.2.2 Data Collection

Prior to the take-over event, during late pregnancy (according to the divisions employed in Chapter 3) and again after parturition for 2 days prior to the initial fighting, a total of 13 h of behavioural data was recorded as a series of 30 min and 1 h focal studies on the breeding female. Blood samples were collected, and GnRH challenges performed on all colony members over 26 g, except the breeding female. During the take-over event, *ad libitum* sampling (Altmann, 1974) was used to monitor sexual and agonistic behaviours of the individual colony members. For 2 days after the fighting broke out, the colony was observed for a total of 7 h. One week after the initial fighting broke out, 10 h of behavioural observation was carried out over a period of 3 days. The colony was monitored over the following 3 months, and behaviour recorded *ad libitum*. Following the deaths of several animals, once a single female appeared to have become the new breeding female, a further 10 h of behavioural data were collected as a series of 1 h focal studies on the nest and surrounding area to record, *ad libitum*, the agonistic and sexual interactions occurring between the remaining colony members. Blood samples were collected and GnRH challenges performed on all the individuals remaining in the colony. It was not possible to determine the exact time in the breeding cycle during which these data were collected as it was the first pregnancy of the new breeding female. However, assuming that parturition occurred between days 66 and 77 of pregnancy, it can be concluded that the data were collected just before or during mid pregnancy (according to the divisions employed in Chapter 3).

The plasma samples were assayed for bioactive LH concentrations. All behavioural data were collected using ***THE OBSERVER*** as described in Chapter 2, section 2.3.1. Details of the blood sampling technique, GnRH administration and hormonal analyses are also given in Chapter 2, sections 2.3.2, 2.3.3 and 2.4 respectively. A summary of the dates of data collection is given in Table 4.2.

TABLE 4.2: Dates of the behavioural studies and blood collection for colony 100 before and after the take-over event.

Time in cycle	**Behaviour (Day in cycle)**	**Date**	**Blood Collection (Day in cycle)**	**Date**
Before take-over	56, 57, 58 + 59	1, 2, 3 + 4/8/95	63 + 64	8/8/95 + 9/8/95
After take-over	NA	24, 25, 30, 31/10	NA	15/11/95

4.2.3 Data Analyses

The results are divided into three broad sections: **i) Prior to take-over event**, where differences between those animals that were killed and those that survived, are investigated; **ii) During take-over event**, where a qualitative description of the take-over event is given and **iii) Before and after take-over event**, where changes that occurred in the surviving animals are investigated.

Mann-Whitney U-tests were used to determine whether there were statistical differences between those animals that were killed and those that were not, in terms of plasma LH concentrations and body masses. Wilcoxon matched-pair tests were used to determine whether there had been any hormonal changes in those animals that survived the take-over event. Spearman's rank correlation coefficient was used to determine whether there was a correlation between body mass and plasma LH concentration either prior to or following the take-over Results are expressed as mean ± s.e.m..

4.3 Results

i) Prior to the take-over event

Plasma LH concentrations and body masses of those killed and those not killed

The individual plasma LH concentrations of the mole-rats of colony 7400, before and 20 min after a single s.c. injection of 0.1 μg GnRH, prior to and following the take-over event are presented in Figures 4.2 and 4.3 respectively. There was a great deal of variability among the individual mole-rats, particularly the males, in terms of both basal and post-GnRH plasma LH concentrations. Prior to the take-over event, all those females that were not killed, including female #28, who eventually became the new breeding female, had basal plasma LH concentrations which were below the sensitivity of the assay. Of those females that were subsequently killed, however, all but one (#36) had detectable basal plasma LH concentrations. The post-GnRH plasma LH concentrations of those females that were subsequently killed were generally higher than those of the females that survived. No such clear distinctions in terms of basal plasma LH concentrations were evident among the males, although the post-GnRH plasma LH concentrations tended to be higher in those males that survived.

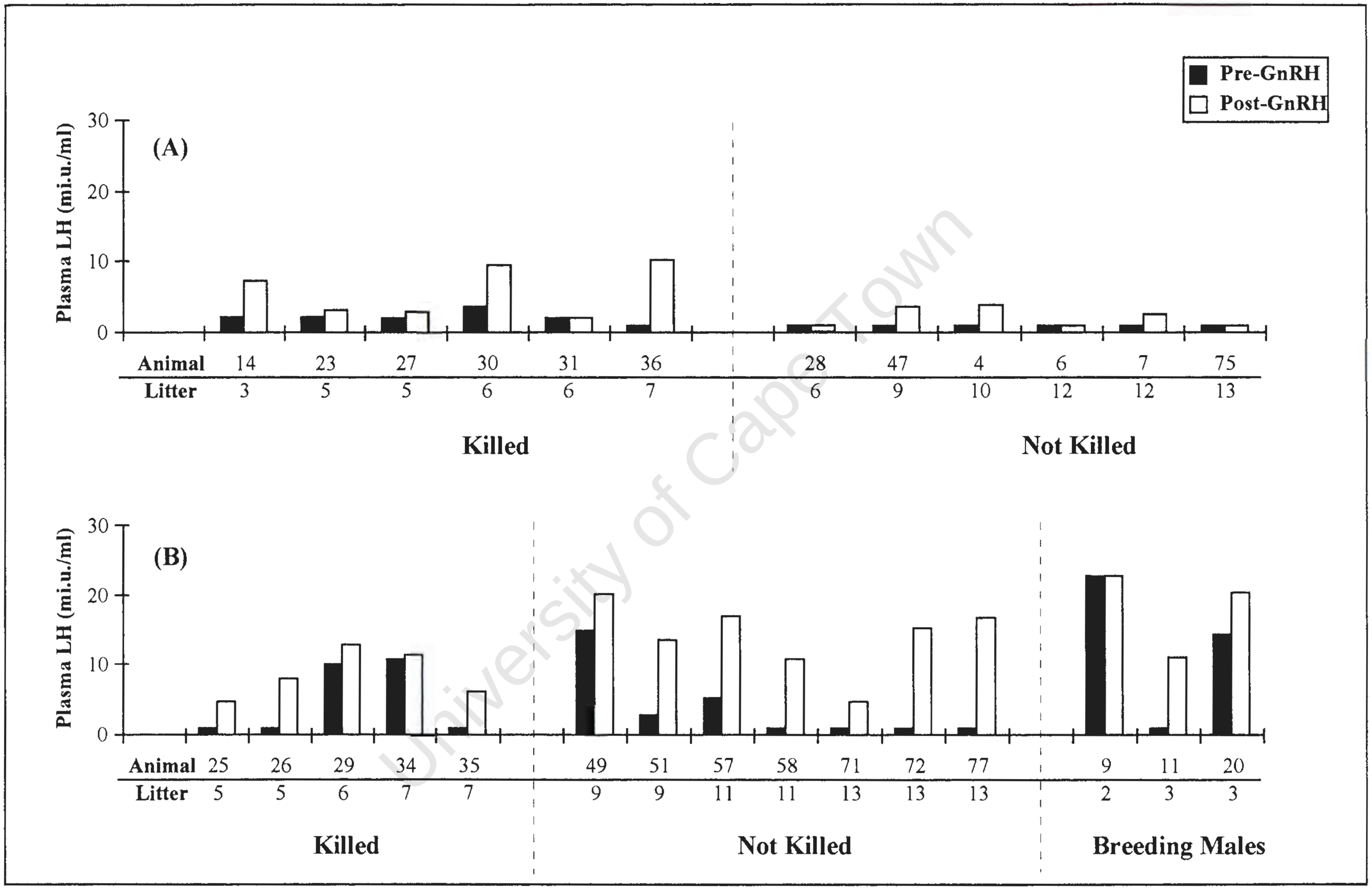


FIGURE 4.2: Plasma LH concentrations of the females (A) and males (B) of colony 7400, prior to the take-over event, before (Pre-GnRH) and 20 min after (Post-GnRH) a single s.c. injection of 0.1 μg GnRH.

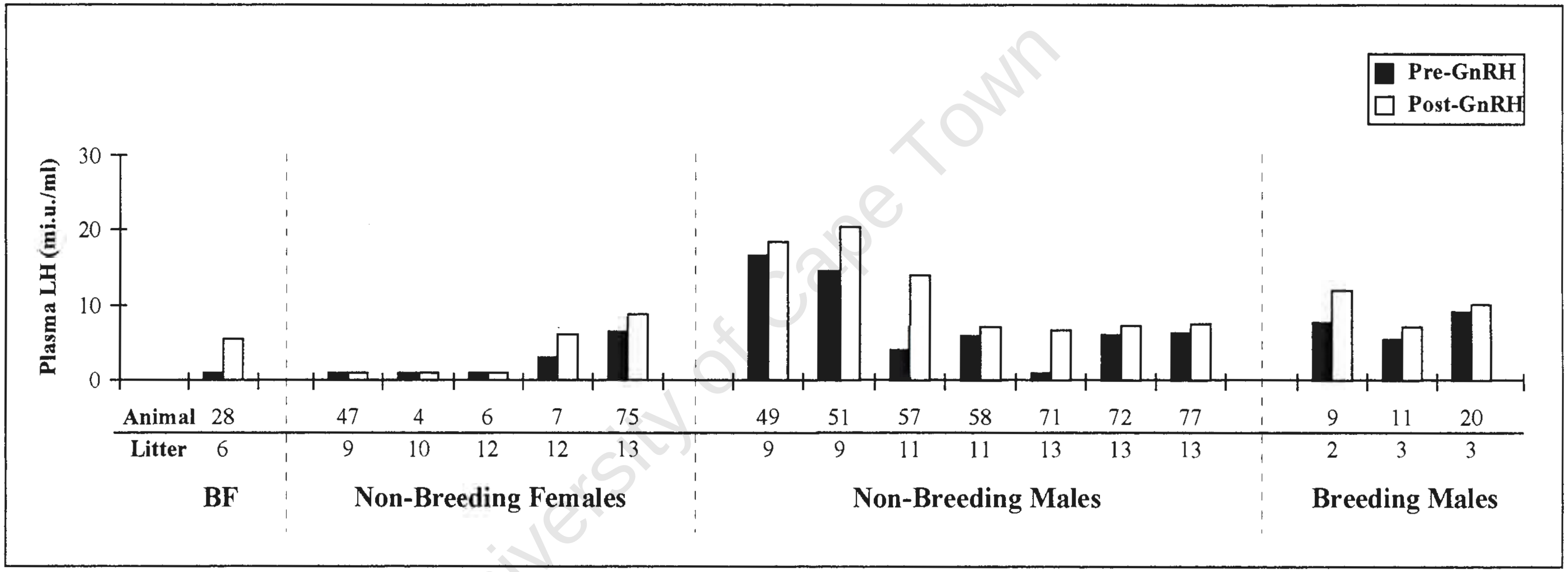
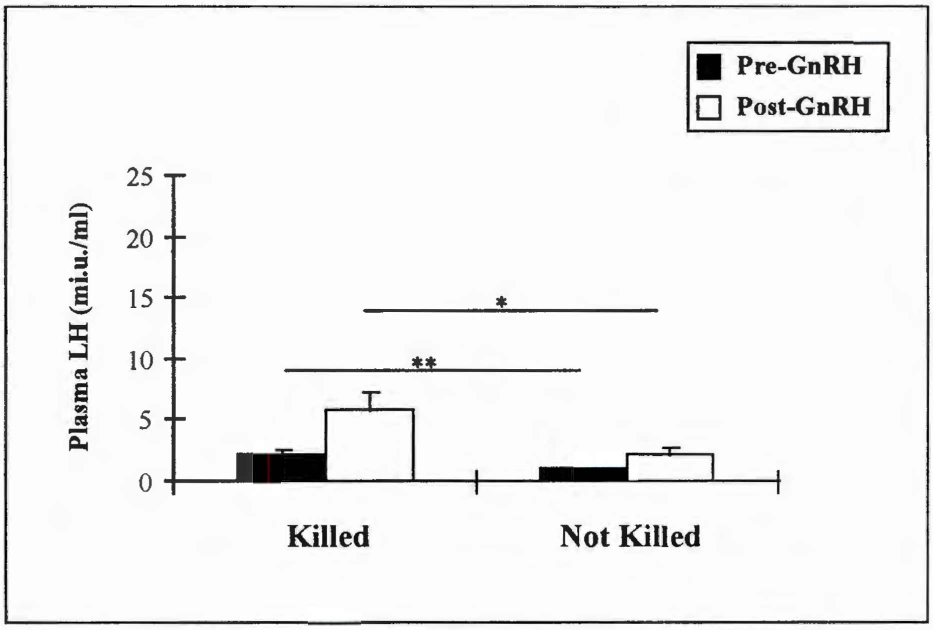


FIGURE 4.3: Plasma LH concentrations of the individuals of colony 7400 following the take-over event, before (Pre-GnRH) and 20 min after (Post-GnRH) a single s.c. injection of 0.1 μg GnRH.

Females

Figure 4.4 shows the differences in the mean plasma LH concentrations, prior to the take-over event, of those non-breeding females that were subsequently killed and those that survived. The basal LH concentrations of those that were killed (2.17 ± 0.34 mi.u./ml, n = 6), were significantly greater than of those that were not killed (1.00 ± 0.00 mi.u./ml, n = 6) ($U = 33$, $P = 0.01$) as were the post-GnRH LH concentrations, (5.84 ± 1.48 mi.u./ml and 2.18 ± 0.56 mi.u./ml respectively) ($U = 29$, $P = 0.05$) (Mann-Whitney *U*-test).

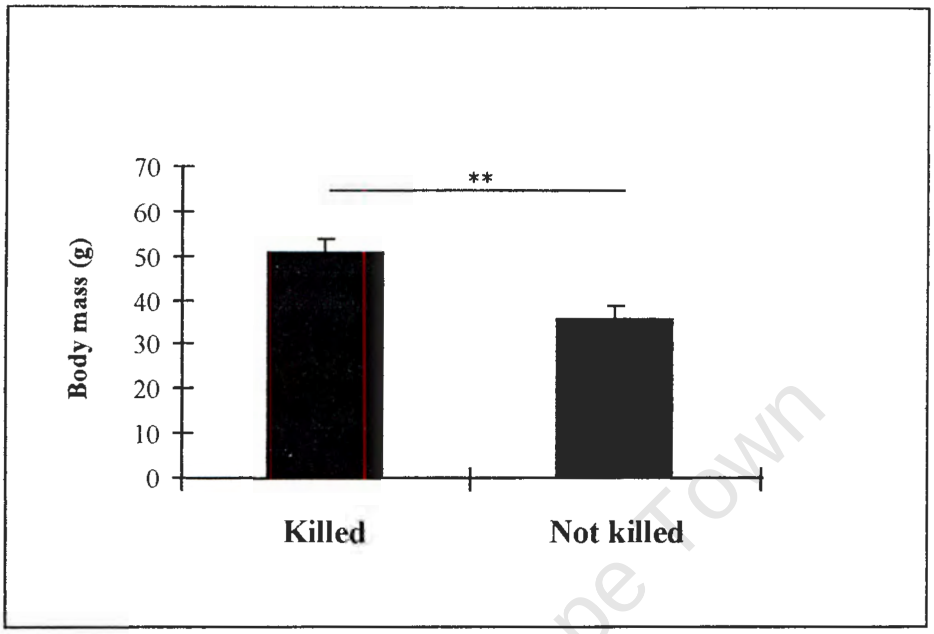


* $P = 0.05$
** $P = 0.01$

FIGURE 4.4: Plasma LH concentrations (mean ± s.e.m.), prior to the take-over event, of those non-breeding females that were subsequently killed (n = 6) and those that were not killed (n = 6), before (Pre-GnRH) and 20 min after a single s.c. injection of 0.1 μg GnRH.

The mean body masses of those females that were killed and those that were not, are presented in Figure 4.5. Those females that were killed were significantly heavier (50.95 ± 2.72 g, n = 6) than those that were not killed (35.98 ± 3.01 g, n = 11) ($U = 58$, $0.005 < P < 0.01$) (Mann-Whitney *U*-test).

Although as a group the females that were killed during the take-over event were significantly heavier than those that survived, the heaviest non-breeding female in the colony prior to the take-over event (#28, 61.1 g) did not get involved in the initial fighting that broke out in the colony, but she finally emerged as the new breeding female. Prior to the take-over event both the basal and post-GnRH plasma LH concentrations of female #28 were below the sensitivity of the assay.

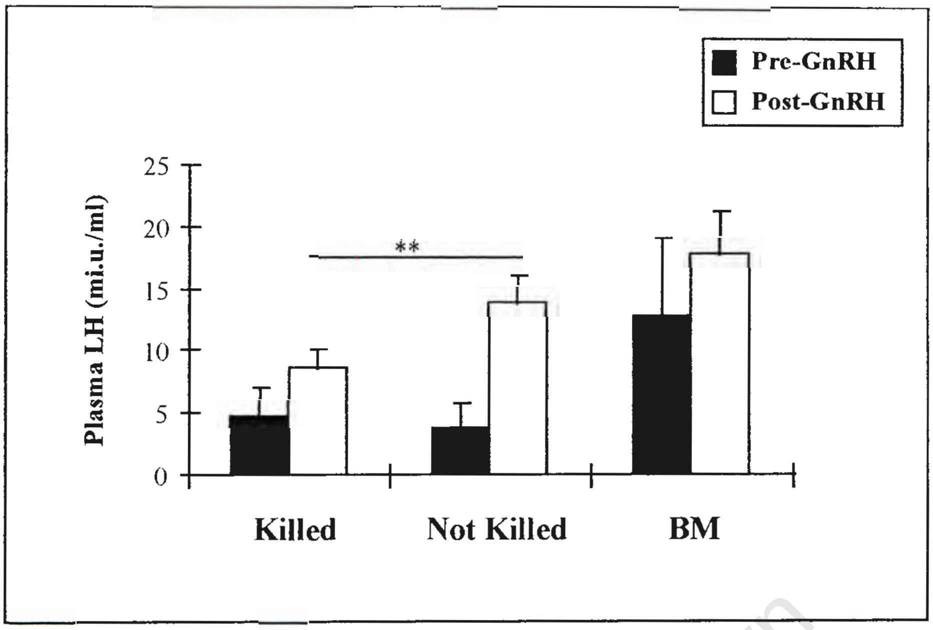


** 0.005 < *P* < 0.01

FIGURE 4.5: Body masses (mean ± s.e.m.), prior to the take-over event, of those non-breeding females that were subsequently killed (n = 6) and those that were not killed (n = 6).

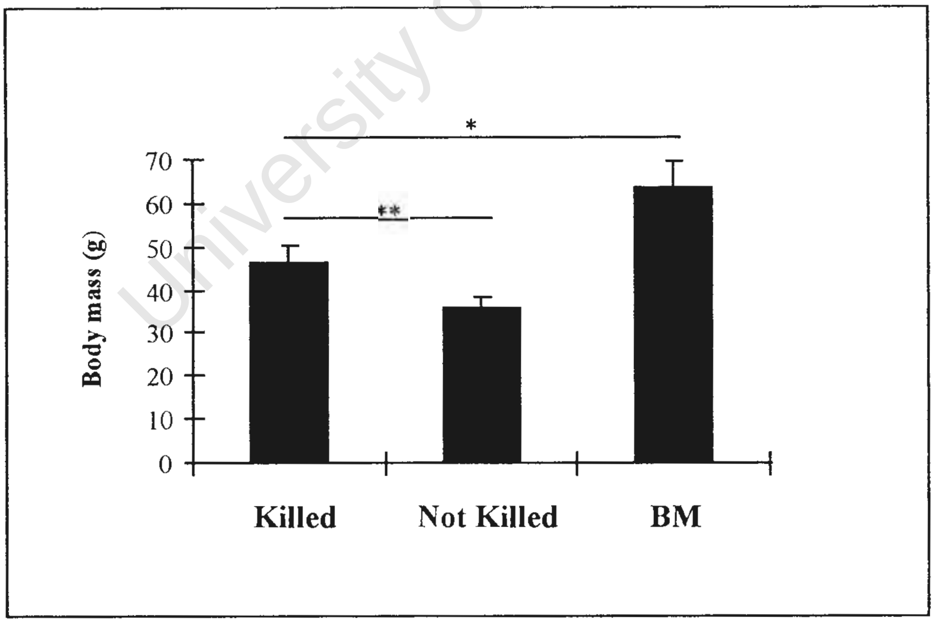
Males

Figure 4.6 shows the differences in the mean plasma LH concentrations, prior to the take-over event, of those non-breeding males that were subsequently killed, the surviving non-breeding males and the putative breeding males. The basal LH concentrations of the males that were killed during the take-over event (4.78 ± 2.32 mi.u./ml, n = 5) were not significantly different from those of the non-breeding males that were not killed (3.85 ± 1.93 mi.u./ml, n = 7) ($U = 18$, $P > 0.1$) nor from those of the putative breeding males (12.71 ± 6.34 mi.u./ml, n = 3) ($U = 11.5$, $P > 0.1$). The post-GnRH plasma LH concentrations were, however, significantly lower in those males that were killed (8.57 ± 1.55 mi.u./ml, n = 5) than in the non-breeding males that were not killed (14.02 ± 1.92 mi.u./ml, n = 7) ($U = 29$, $P = 0.05$), and although the post-GnRH concentrations appeared to be higher in the breeding males (18.02 ± 3.17 mi.u./ml, n = 3) than in the non-breeding males that were killed, the difference was not significant ($U = 13$, $P = 0.10$) (Mann-Whitney *U*-test).



** $P = 0.05$

FIGURE 4.6: Plasma LH concentrations (mean ± s.e.m.), prior to the take-over event, of those non-breeding males that were subsequently killed (n = 5), those that were not killed (n = 7) and the putative breeding males (n = 3), before (Pre-GnRH) and 20 min after a single s.c. injection of 0.1 µg GnRH.



* $P = 0.05$
** $P = 0.025$

FIGURE 4.7: Body masses (mean ± s.e.m.), prior to the take-over event, of those non-breeding males that were subsequently killed (n = 5), those that were not killed (n = 7) and the putative breeding males.

The mean body masses of those non-breeding males that were killed, those that survived, and those of the putative breeding males, are presented in Figure 4.7. Although the masses of the males that were killed (46.34 ± 3.85 g, n = 5) were significantly lower that those of the putative breeding males (63.23 ± 6.27 g, n = 3) ($U = 14$, $P = 0.05$), they were significantly higher than those of the non breeding males that were not killed during the take-over (35.74 ± 2.59 g, n = 5) ($U = 30$, $P = 0.025$) (Mann-Whitney *U*-test).

Interactions initiated by the breeding female

Females

Prior to the take-over event, four of the non-breeding females (#31, #30, #27, #28) received agonistic interactions from the breeding female. Three of these females (#31, #30, #27) were subsequently killed in the take-over event, while #28 went on to become the new breeding female. No agonistic interactions were observed between the breeding female and any of the other non-breeding females. Females #31, #30 and #28 received 11.11%, 3.70% and 7.40% respectively, of the total agonistic interactions (n = 27) initiated by the breeding female towards the non-breeding females. The primary recipient of the breeding females' agonistic interactions was #27, who received 77.78% of the total agonistic interactions initiated by the breeding female towards the non-breeding females, and 52.5% of the all the breeding female's agonistic interactions towards both the males and females (n = 40). The only non-breeding female to initiate any agonistic interactions towards the breeding female was #7, who initiated a single tooth-fencing interaction.

The breeding female initiated no sexual interactions towards any of the non-breeding females, and received a single genital nuzzle from female #23, one of the females subsequently killed in the take-over.

Males

The breeding female was seen to initiate agonistic interactions towards all three of the putative breeding males (#9, #11, #20) and three of the non-breeding males (#49, #26, #29). All except #26 received <10% of the breeding females agonistic interactions (n = 13) initiated towards males. Male #26 received 61.54% of the total agonistic interactions directed at the males and 20% of all the breeding female's agonistic interactions (n = 40), and was the secondary recipient of the breeding female's aggression. Both #26 and #29 were subsequently killed in the take-over event.

The only males to initiate agonistic interactions towards the breeding female were two of the putative breeding males, #11 and #20.

The primary recipients of the sexual interactions initiated by the breeding female (n = 52) were the three putative breeding males, #9, #11 and #20 who received 28.85%, 34.62% and 32.69% respectively. The sexual interactions typically included mutual ano-genital nuzzling, as well as genital sniffing and nuzzling. The breeding female was observed nuzzling the genitals of two of the non-breeding males (#57, #77) although only once each.

The only non-breeding male to initiate any sexual interactions towards the breeding female was #26 who was observed nuzzling her genitals once, and the only males to regularly initiate sexual interactions towards the breeding female were the putative breeding males #11 and #20, who were responsible for 58.02% and 29.03% respectively, of the total sexual interactions initiated towards the breeding female (n = 31). Male #9 too initiated sexual interactions towards the breeding female but was responsible for only 3.22% of the total.

ii) During the take-over event

Qualitative description of the take-over event

Prior to the take-over event, during late pregnancy, the breeding female began to display heightened aggression towards female #27, the third largest non-breeding female in the colony. This individual spent a great deal of time resting at the bottom of the pile of animals in the nest. The breeding female would enter the nest, actively seek out #27 from beneath the other animals, and initiate attacks against her. Female #27 did not retaliate when attacked, but would assume a submissive posture, accompanied by squeaks, keeping her head tucked down away from the attacker.

After parturition, the attacks on female #27 became subjectively more vicious, and the frequency of the attacks increased from 2.1 attacks/h before parturition (10 hrs of observation) to 8 attacks/h after parturition (3 h of observation). On the third day after parturition, both # 27 and the breeding female had been badly bitten. The breeding female had several bite marks on her face and a deep wound under her armpit. This type of wound has previously been observed in animals which have been killed by the breeding female of a colony (O'Riain, pers. comm.). The wounds of #27 were not as severe as those sustained by the breeding female.

It was unfortunate that #27's retaliation was not actually observed, as it occurred at night when the colony was not under observation. Furthermore, although agonistic encounters were recorded between the individuals of colony 7400 during the course of the take-over event, none of the actual killings were observed as they invariably took place outside of the hours of observation.

Prior to the fighting, female #27 was not seen engaging in any interactions with the other colony members, apart from the breeding female, and was only rarely seen leaving the nest. However, during the days following the fight with the breeding female, #27 appeared to become far more active, both within and outside the nest. Whereas previously she would commonly rest at the bottom of the pile of animals in the nest, she was now seen lying at the top of the pile. She also appeared to leave the nest more frequently, and spent time basking under the lamp, a behaviour she had not previously performed. She engaged in several interactions with other colony members, possibly as a consequence of her heightened activity outside the nest, which included mainly tail-pulling and tugging of the skin in an attempt to pass an animal obstructing the tunnel system

The other animal whose behaviour appeared to have changed following the fight between the breeding female and #27 was male #26. On the day following the fight #26 was involved in several agonistic encounters with #11 and #20, two of the putative breeding males. Prior to the fight, #26 had been one of the main targets of the breeding female's aggression, although several times the breeding female had retreated after encountering #26 in the tunnel system.

By 1 week after the initial fighting had broken out, female #27 appeared to be the most dominant animal in the colony. She was involved in far more interactions with the other colony members than previously, and had begun to exhibit behaviours characteristic of a breeding-female. She regularly left the nest to patrol the burrow system and initiated frequent attacks against the breeding female who, although very weak, was still alive. A new bite mark had appeared on the face of #27, and was likely to have been inflicted by female #14, the second largest non-breeding female in the colony, who had begun to initiate attacks against #27. Females #14 and #27 were responsible for 30.19% and 35.85% respectively of the total agonistic interactions initiated (n = 53, 10 h), and although four of the females (#4, #28, #31, #36) and five of the males (#9, #11, #20,#26, #29) were also seen to initiate agonistic interactions, each was responsible for <10% of the total. The primary recipients of the agonistic interactions were females #14, #27 and #31 who each received 18.86%, 24.53% and 11.32% respectively. Four other females (#7, #23, #28, #47) and four males (#9, #11, #29, #49) each received <10% of the total agonistic interactions.

Before the breeding female was injured, sexual interactions had been rare amongst the non-breeding animals, but during the days 7-10 after the injury of the breeding female, these interactions were observed in several of the non-breeding animals. Many of the animals were observed sniffing and nuzzling their own genitals as well as those of others. Mutual ano-genital nuzzling, a behaviour which typically occurs between the breeding animals, was observed amongst several of the non-breeding animals. The majority of the sexual interactions were initiated by three of the non-breeding females (#14, #27, #31) who were responsible for 14.02%, 30.84% and 15.89% respectively of the total sexual interactions initiated (n = 107, 10 h). Although several other non-breeding males and females, as well as the putative breeding males, were seen to initiate sexual interactions, none initiated >10%. The majority of the sexual interactions were received by four of the non-breeding females (#14 = 16.82%, #23 = 17.76%, #27 = 19.63%, and #31 = 14.95%) and one of the putative breeding males (#9 = 11.21%). Those animals that were observed nuzzling and sniffing their own genitals were the males #9, #11, #20, #25, #26, #34, #49, #51 and the females #14, #23, #27, #30, #31. Of these animals, males #25, #26, #34, and all four of the females, were subsequently killed. The details of the animals that were killed during the take-over event, and the dates of their respective deaths, are given in Table 4.3.

TABLE 4.3: Details of the animals that were killed, and the dates of their respective deaths.

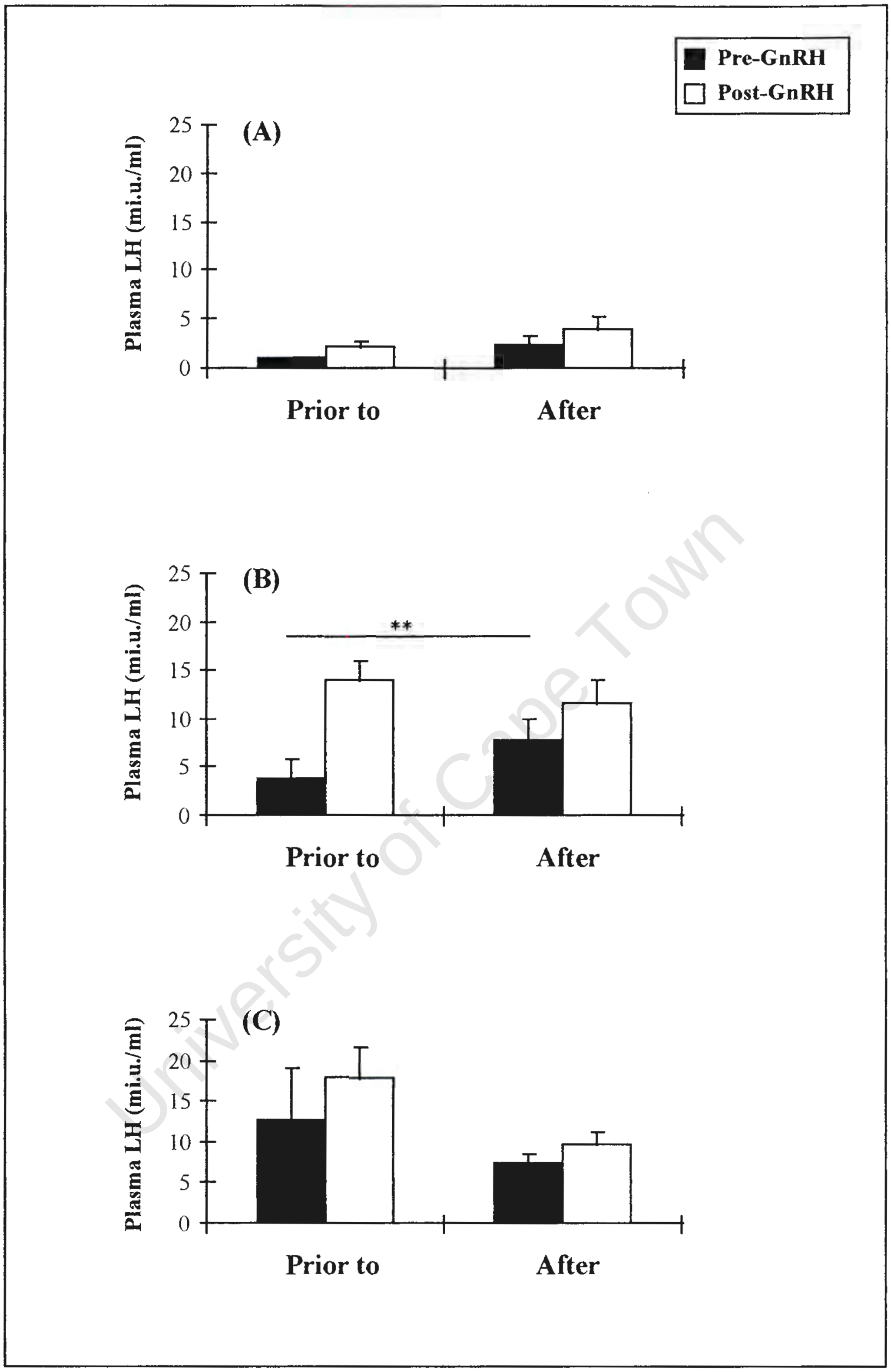
Animal	Sex	Litter	Date Died	Days after initial fighting
14	F	3	1/9/95	16
23	F	5	10/9/95	25
25	M	5	14/9/95	29
26	M	5	16/9/95	30
4	BF	1	19/9/95	34
27	F	5	19/9/95	34
31	F	6	20/9/95	35
36	F	7	27/9/97	42
29	M	6	2/10/95	47
30	F	6	3/10/95	48
35	M	7	18/10/95	63
34	M	7	25/10/95	70

Sixteen days after the fight between the breeding female and female #27, female #14 was killed, presumably by #27 with whom she had engaged in frequent agonistic encounters. Nine days later female #23 was killed, but it was uncertain whether #27 or female #31 was responsible, as both had sustained fresh wounds over this time. In the following 2 weeks female #28 emerged as the most dominant animal in the colony and began to initiate frequent attacks on the three oldest, and among the largest, non-breeding females in the colony (#30, #31, #36). The five non-breeding males (#25, #26, #29, #34 #35) which were killed during the take-over event, and two males which were not killed (#49, #51) were seen to engage in frequent sexual encounters with each other and in particular with female #31. In addition #25, #26 and #29 engaged in agonistic encounters with the putative male #20. Males #25 and #26 were killed 29 and 31 days after the initial fighting. However it was not certain that #20 had been responsible for their deaths.

The breeding female and female #27 both died 34 days after the initial fighting had broken out between them, the breeding female from septic wounds and #27 from new wounds inflicted either by #31, with whom she had engaged in agonistic interactions, or female #28 who had become increasingly more aggressive. The following day, female #31 was found dead and had presumably been killed by #28. At this stage only two of the non-breeding females (#30, #36) which were eventually killed in the take-over fighting, were still alive, and it was towards these two females, and male #29, that #28 now directed her agonistic interactions. Females # 30 and #36 continued to receive sexual interactions from the other colony members, but did not appear to be particularly aggressive. They were not seen to initiate agonistic interactions, and when attacked by #28 they assumed submissive postures, as female #27 had done when attacked by the breeding female, prior to the outbreak of fighting. Following the death of female #36, 42 days after the initial fighting had broken out, female #28 continued to initiate attacks against male #29 and female #30, and both were killed within 7 days. After this no more females were killed, and #28 subsequently became the new breeding female. It therefore took female #28, the heaviest female in the colony at the start of the study, less than 50 days to attain the position of breeding female and she gave birth to her first litter 84 days after the death of her last rival (female #30). Although no more females were killed, a further two non-breeding males (#34, #35) were killed within the next month. It was uncertain whether or not they had been killed by #28 because although she displayed aggression towards both, the attacks appeared to be far less frequent and vicious than those accompanying the other killings.

iii) After the take-over event

Figure 4.8 shows the basal and post-GnRH plasma LH concentrations prior to and following the take-over event, in the non-breeding females and non-breeding males which were not killed, and in the putative breeding males.



** $P = 0.05$

FIGURE 4.8: Plasma LH concentrations (mean ± s.e.m) in (A) the surviving non-breeding females (n = 5), (B) the surviving non-breeding males (n = 7) and (C) the breeding males (n = 3), before (Pre-GnRH) and 20 min after a single s.c. injection of 0.1 μg GnRH, prior to and following the take-over event.

There was no significant change in either the basal LH concentrations (1.00 ± 0.0 mi.u./ml to 2.50 ± 1.07 mi.u./ml) (T = 6, $P > 0.25$) or post-GnRH LH concentrations (2.42 ± 0.62 mi.u./ml to 3.57 ± 1.63 mi.u./ml) (T = 6, $P > 0.25$) of the non-breeding females (excluding #28) (n = 5) prior to and following the take-over event (Wilcoxon paired-sample test).

In the non-breeding males (n = 7) there was a significant increase in the basal plasma LH concentrations following the take-over event (3.85 ± 1.93 mi.u./ml to 7.80 ± 2.15 mi.u./ml) (T = 3, $P = 0.05$), but no significant difference in the post-GnRH LH concentrations (14.02 ±.1.92 mi.u./ml to 11.62 ±.2.26 mi.u./ml) (T = 7, $0.1 < P < 0.25$) (Wilcoxon paired-sample test).

Although there appeared to be a decrease in both the basal plasma LH concentrations (n = 3) (12.71 ± 6.32 mi.u./ml to 7.43 ± 1.09 mi.u./ml) and post-GnRH LH concentrations (18.02 ± 3.62 mi.u./ml to 9.73 ± 1.40 mi.u./ml) of the breeding males following the take-over event, the changes were not significant (L = 0.207, $P > 0.05$ and L = 0.498, $P > 0.05$ respectively) (Lord's Range Test).

Figures 4.9 and 4.10 show the relationship between body mass and plasma LH concentrations prior to and following the take-over event in the non-breeding females and non-breeding males. Prior to the take-over event there was a significant positive correlation between body mass and basal plasma LH concentration ($r_s = 0.610$, $0.02 < P < 0.05$) in the non-breeding females, although not between body mass and post-GnRH LH concentration ($r_s = 0.290$, $0.2 < P < 0.5$). After the take-over event there was no correlation between body mass and either basal LH or post-GnRH LH concentration ($r_s = 0.415$, $0.2 < P < 0.5$ for both). In the non-breeding males, however, the opposite pattern was observed, and prior to the take-over event there was no significant correlation between body mass and basal plasma LH concentration ($r_s = 0.412$, $0.1 < P < 0.2$) or post-GnRH LH concentration ($r_s = 0.161$, $P > 0.5$). Following the take-over event there was a strong positive correlation between body mass and both basal plasma LH concentration ($r_s = 0.742$, $0.02 < P < 0.05$) and post-GnRH ($r_s = 0.905$, $P = 0.0025$) LH concentration in the surviving non-breeding males (Spearman's rank correlation coefficient).

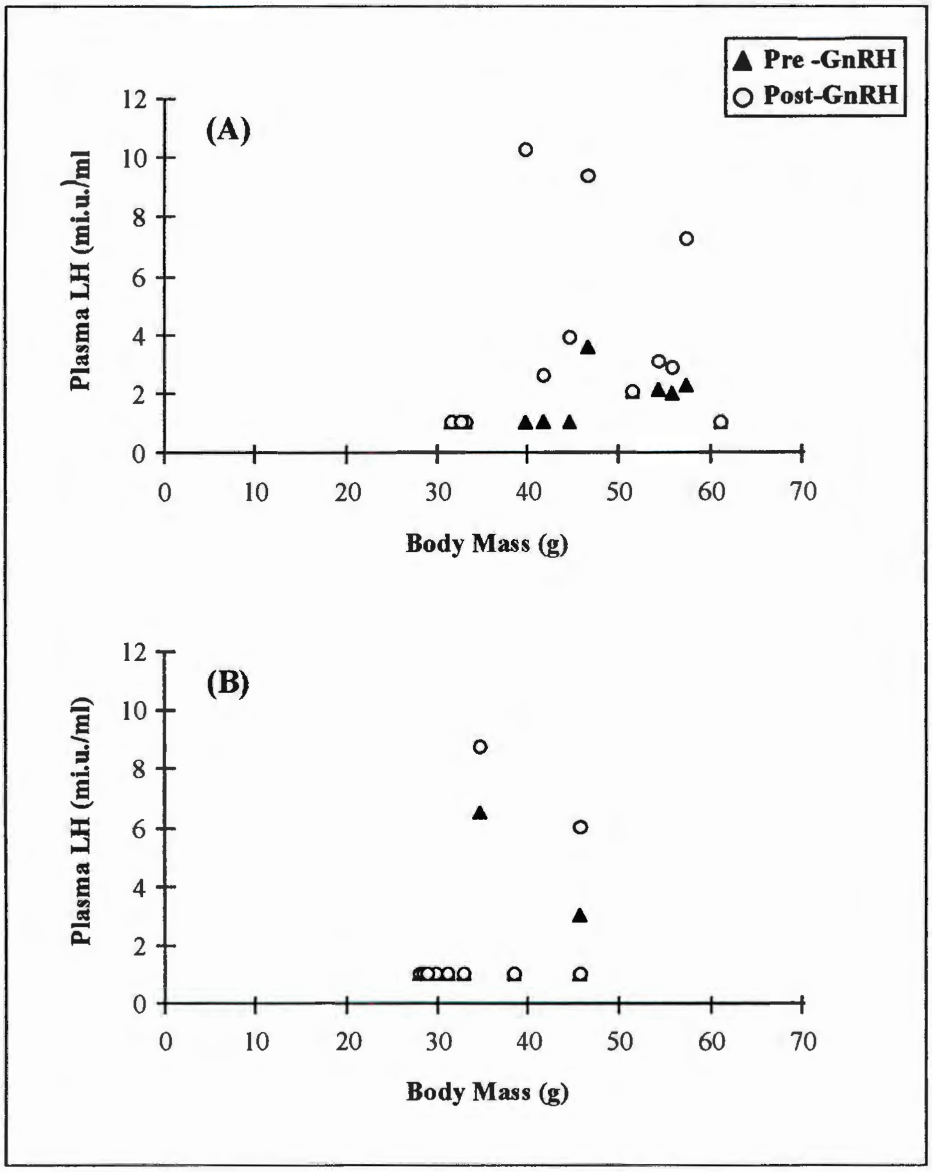


FIGURE 4.9: Plasma LH concentrations of the non-breeding females before (Pre-GnRH) and 20 min after a single s.c. injection of 0.1 µg GnRH, expressed as a function of body mass, (A) prior to (n = 12) and (B) after (n = 10) the take-over event.

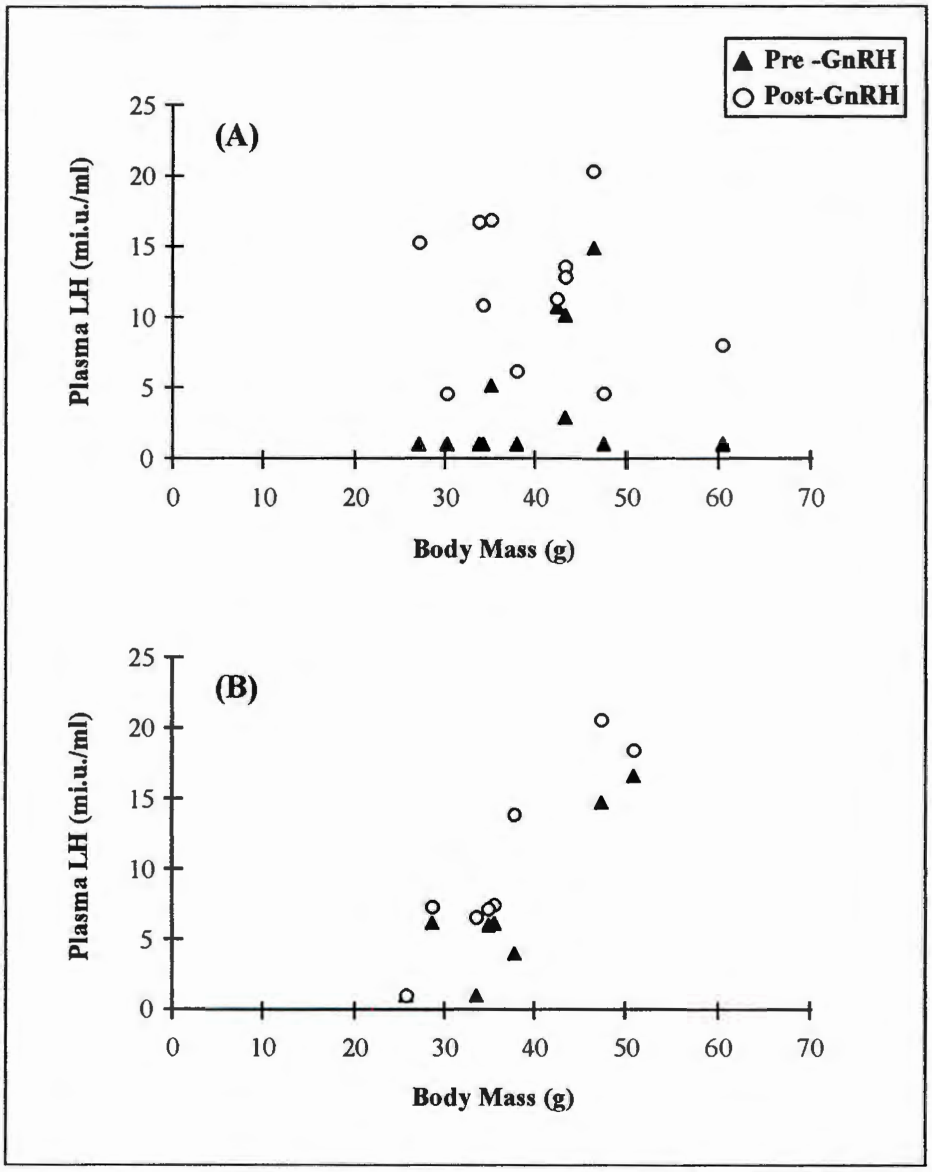


FIGURE 4.10: Plasma LH concentrations of the non-breeding males before (Pre-GnRH) and 20 min after a single s.c. injection of 0.1 µg GnRH, expressed as a function of body mass, (A) prior to (n = 12) and (B) after (n = 8) the take-over event.

4.4 Discussion

The factors which trigger take-over events in naked mole-rat colonies, where a subordinate non-breeding female challenges the supremacy of the breeding female's reproductive status, are unknown. In colony 7400 it is possible that the breeding female (#4) may have been ill or weakened at the time, however, she did not appear to be so. Nevertheless, the high level of aggression that she directed towards female #27 during late pregnancy, should have provided a clue that something was wrong. The level of aggression displayed by breeding females is variable (Jarvis, 1991a). Assuming that breeding females do in fact use aggressive agonistic interactions to maintain reproductive suppression in non-breeders, it is possible that, if a breeding female displays a high frequency of aggressive behaviour, it may indicate that she has less control over her colony than more placid breeding females have over their colonies, and therefore needs to continuously exert her dominance in order to maintain control.

It is possible that the death of the breeding male may sometimes be a trigger for such a take-over event. Indeed, the breeding male (#FO) had died 48 days prior to the commencement of the take-over event, when the initial fighting broke out between the breeding female (#4) and female #27. Moreover, the previous take-over in colony 7400, in which female #4 challenged the original breeding female and successfully became the new breeding female, had also been preceded by the death of the original breeding male, 7 months earlier (O'Riain, pers com.; 1996). Previously reported instances of "natural" take-over events in captive colonies have not considered the role that the death of the breeding male may play in initiating a take-over event, probably because breeding males are not always easy to identify. One study (Margulis *et al.*, 1995) has even removed both the breeding female and breeding male from a colony in order to investigate breeding female succession, and then assumed that the removal of the breeding female was the overriding factor governing the subsequent events, without really considering the effects that removal of the breeding male may have played. At this stage, the role that the death of the breeding male may play in initiating a take-over event is unclear, but it is possible that it may be far greater than previously considered, and is a factor which should be examined more critically in the future.

The death of the breeding male (#FO) was the result of injuries sustained during the inter-colonial fighting which took place after a neighbouring colony had managed to escape from their system and break into colony 7400. This break-in had occurred 46 days prior to the commencement of the study, and the breeding male (#FO) had died 13 days later. In addition, several other mole-rats (females #23, #30, #1, #6, #75 and males #9, #35, #49, #77) had been bitten, and the breeding female had also been very slightly bitten. It is interesting to note that many of the individuals killed during the take-over event were not amongst those injured in the inter-colonial fighting. Moreover, it is strange that both breeders were injured, as breeding females in particular do not usually become involved in inter-colonial fighting.(O'Riain & Jarvis, 1997).

It is possible that this invasion may have precipitated the take-over event that occurred following parturition, either as a consequence of the death of the breeding male or by the possible sexual activation of several non-breeding females as a result of contact with the foreign males which had entered the colony. Interestingly, Jarvis (1991a) has recorded a once-off pregnancy of a previously non-breeding female following such an invasion event. In *C. damarensis* colonies, the introduction of a foreign male into a colony, after the breeding female has been removed or died, induces sexual activity in previously reproductively quiescent non-breeding females, whereas the mere removal of the breeding female results in a sexually inactive colony (Rickard & Bennett, 1997). However, *C. damarensis* is an obligate outbreeder and colony succession does not occur (Jarvis & Bennett, 1993). Therefore a different control mechanisms may operate to keep non-breeders reproductively suppressed, and there is evidence to suggest that incest avoidance is one of the main causes of reproductive suppression in *C. damarensis* (Bennett *et al.*, 1997).

Whatever the trigger for the take-over event that occurred in colony 7400, an unresolved question is why it did not commence at a time when previous authors have suggested that the breeding female appears to have the least control over reproduction, *i.e.* when she is highly gravid just prior to parturition (Jarvis, 1991a; Westlin *et al.*, 1994). It is interesting that the take-over event began a few days after parturition when the queen began to display highly aggressive behaviour towards female #27.

Removal experiments have shown that non-breeding naked mole-rats are capable of becoming reproductively active within a relatively short time when the social cues bringing about reproductive suppression are removed (Faulkes, 1990; Faulkes *et al.*, 1990a). However, since all non-breeding animals are potential reproductives, the question is what determines whether or not an individual becomes involved in the fighting during succession and who eventually becomes the new breeder within a colony situation?

In most instances of "natural" succession in the lab following the death of the breeding female where the ages of the colony members are known, it is one of the oldest and largest females in the colony that eventually becomes the new reproductive (Jarvis, 1991a; O'Riain, 1996). Under experimental conditions, Clarke & Faulkes (1997) have demonstrated that those females that succeed as the new reproductives, in addition to being the oldest and heaviest, are also the most dominant non-breeding females in their colonies, and O'Riain (1996) has shown that replacement reproductives tend to be the individuals that are most involved in initiating and receiving agonistic interactions. Margulis *et al.* (1995) found that, following the removal of the breeding pair, the female that eventually emerged as the new breeding female was the not the largest, but the oldest, non-breeding female in the colony. However, Lacey & Sherman (1991) have found that breeders are not necessarily the oldest animals in the colony.

In the present study, the non-breeding females that were killed in the take-over event were, as a group, older and heavier than those that were not killed. However, one of the females that was killed (#36) had a lower body mass than two of the females that were not killed (#4 and #7), although she was older than both of these females (Table 4.1). Moreover, female #28, who eventually became the new breeding female, was the heaviest, but not the oldest female in the colony at the commencement of the take-over. Nevertheless, by the time the fighting had ended, she was the oldest survivor, and, apart from her, all the females of the six oldest litters had been killed. It would therefore appear that in this take-over event, it was a combination of age and mass that determined which animals participated in the succession struggle. It is also interesting to note that female #28 only became involved in the fighting once the three next heaviest females had been killed (#14 and #23) or severely injured (#27).

Prior to the take-over event, all those females that survived, including female #28, had basal plasma LH concentrations which were below the sensitivity of the assay. Of those females that were subsequently killed, however, all but one, the smallest of those killed (#36), had detectable basal plasma LH concentrations. The mean basal plasma LH concentrations of those females that were killed (2.17 ± 0.34 mi.u./ml, n = 6) were higher than previously measured mean plasma LH concentrations for non-breeding females (1.6 ± 0.1 mi.u./ml, n = 57 samples from 44 females; Faulkes *et al.* 1990a) and than those measured for non-breeding females in Chapter 3 (1.55 ± 0.15 mi.u./ml, n = 22 and 1.65 ± 0.20 mi.u./ml, n = 10). Furthermore, the mean post-GnRH plasma LH concentrations of the non-breeding females that were subsequently killed (5.84 ± 1.48 mi.u./ml; n = 6), in addition to being higher than those of the non-breeding females that survived, were also higher than previously measured values for non-breeding females (2.9 ± 0.5 mi.u./ml, n = 5; Faulkes *et al.*, 1990b) and than those measured for non-breeding females in Chapter 3 (3.06 ±

0.33 mi.u./ml, n = 22 and 4.19 ± 1.00 mi.u./ml, n = 10). However, although the basal and post-GnRH plasma LH concentrations of the non-breeding females that were subsequently killed were higher than the mean values previously recorded for non-breeding females, they were still lower than those which have been recorded for breeding females (3.4 ± 0.8 mi.u./ml and 9.6 ± 2.0 mi.u./ml respectively, n = 6; Faulkes *et al.*, 1990b). Nevertheless, the elevated basal plasma LH concentrations and greater pituitary sensitivity to exogenous GnRH in the non-breeding females that were killed, seem to indicate that there was a degree of relaxation of reproductive suppression in these females, prior to the take-over event.

Prior to the take-over event there was a significant positive correlation between body mass and both basal and post-GnRH plasma LH concentrations in the non-breeding females. This pattern was not found in a stable colony (See colony 100, Chapter 3), and could perhaps have provided a clue that colony 7400 was unstable. Perhaps the breeding female was losing control over the non-breeding females just prior to the take-over event. After the take-over, once the new breeding female had begun to reproduce, this relationship disappeared. This could have been due to the fact that most of the larger females had been killed, or may reflect that the colony had restabilised under the control of the new breeding female.

The deaths of the non-breeding males during the take-over event were unexpected, and it seems unlikely that they were killed as a result of fighting for breeding status. The breeding male (#FO) had indeed been killed approximately 1 month prior to the commencement of the study, but the breeding female (#4) had begun engaging in mutual ano-genital nuzzling with the three putative breeding males (#9, #11 and #20) with whom she had previously engaged in pre-copulatory behaviour prior to the introduction of #FO into the colony (O'Riain, 1996). It therefore appeared that these three males would inevitably become the breeding males. Indeed, at least one of them fathered the litter born to #28 after the take-over was complete. As with female succession there seems to be variability with regard to male succession. Jarvis (1991a) found that the succession of breeding males appears to be achieved without intense male-male fighting, and suggested that this could perhaps be because the breeding female selects her own mates. Alternatively, it has been suggested that there may be a higher turnover of male breeders than female breeders, and males may thus have less incentive than females to fight for reproductive status, as the probability of their obtaining the opportunity to breed may be greater (Brett, 1991a). Although female-female and female-male conflicts occur, male-male conflict is uncommon (Lacey & Sherman, 1991; Jarvis, 1991a). Males that are killed in intra-colonial fights are usually killed by the breeding female, but why they are killed is unknown (Jarvis, 1991a). Jarvis (1991a) has suggested that when males are killed by the breeding female, it may be because they are showing increased sexuality but are not closely bonded to the breeding female. If this is the case then they might constitute a threat to the breeding female by mating with perforate non-breeding females when she is highly gravid and unable to exert her dominance effectively via behavioural interactions (Jarvis, 1991a). Clarke and Faulkes (1997) have reported the deaths of four non-breeding males following the removal of the breeding female from a colony. These males had been four of the most dominant males in the colony prior to the planned removal, and died as a result of fighting with the new breeding female 67, 120, 185 days, and 11 months after the removal of the previous breeding female. Post mortem examination revealed that their reproductive tracts were full of sperm (Clarke & Faulkes, 1997).

During the present study, five non-breeding males were killed during the course of the take-over event. These five males were the among the heaviest non-breeding males and, apart from the three putative breeding males, they were also the oldest males in the colony. In this sense there is a similar pattern to that seen in the females that were killed and to the findings of (Clarke & Faulkes, 1997). However, it is unclear from hormonal data, why these particular five non-breeding males were killed. Unlike the females that were killed, their basal plasma LH concentrations were no greater than those of the surviving males. In addition, they displayed reduced pituitary sensitivity to a single exogenous dose of 0.1 μg GnRH in comparison with the survivors, indicating a greater degree of reproductive suppression. If these males were more suppressed, then why were they killed? They were the largest, oldest, non-breeding males in the colony and therefore, according to the results of previous studies, most likely to become reproductive. There are no clear answers to this question, but it is possible that there may have been unidentified factors keeping them suppressed, prior to the take-over. Alternatively, perhaps if they had presented the greatest threat to the previous breeding female, they might have been subject to long-term aggression, prior to the commencement of this study, and her death may thus have resulted in changes in their behaviour.

It is possible that the factors triggering the death of one of the males could have been the accidental introduction, by a temporary laboratory assistant, of two foreign males into the colony on 9/9/95. They were only discovered on 15/9/95 and it is possible that they may have been instrumental in killing male #25. However, his behaviour prior to the introduction of the foreign males, negates this suggestion. He had engaged in frequent sexual encounters with other colony members, and had also engaged in agonistic encounters with the putative breeding male #20. Males #26 and #29 had also engaged in agonistic encounters with #20, but it was unclear whether or not #20 had been responsible for the deaths of these three animals. It is likely that #29, at least, was killed by female #28, as she had shown much aggression towards him. Whatever the reason for the deaths, it is clear that succession in males, as well as the role of males in breeding female succession, merits further study.

In the non-breeding males there was a significant positive correlation between body mass and both basal and post-GnRH plasma LH concentrations following the take-over, but not prior to the event. This could simply reflect the fact that the heavier males that were killed in the take-over event had lower basal and post-GnRH plasma LH concentrations than those that survived.

It is in the best interests of the breeding female to actively maintain reproductive suppression of the non-breeding animals, even during a period of apparent stability in a colony, in order to retain reproductive supremacy. It is believed that she does this by preferentially directing dominant agonistic behaviour, such as aggressive shoving, towards those animals that pose the greatest threat. It appears that the breeding female directs her aggression towards the older colony members (Chapter 3). Indeed, O'Riain (1996) has found that the oldest colony members are the primary recipients and instigators of dominance interactions such as shoving, and that the younger members and pups are never shoved, but are the main recipients of pup-pushing by the older members. Pup-pushing occurs when a juvenile or adult places its nose against the body of a young animal or pup, and shoves or rams it for a short distance (O'Riain, 1996). One of the theories that Lacey *et al.* (1991) have put forward as to the functional significance of pup-pushing is that it may help to enforce social dominance among colony members. O'Riain (1996) has found support for this theory, and has suggested that pup-pushing by older siblings may serve to establish a dominant/subordinate relationship at a time when individuals are physically incapable of retaliating. This would facilitate the formation of a dominance hierarchy amongst non-reproductives which may determine the possibility of future reproductive success. O'Riain has found that those individuals that are frequently pup-pushed (*i.e.* of low social rank) but never shoved, show no signs of sexual activity following the death or removal of the breeders, whereas those individuals that are most frequently shoved are usually the first to show external signs of reproductive activity (*e.g.* a perforate vagina in females) (O'Riain, 1996). Similar results have been obtained by Margulis *et al.* (1995) who found that the breeding female preferentially directed aggressive shoving towards the non-breeding female who subsequently showed the first signs of reproductive activation.

The results of the present study compliment the results of previous studies. Margulis *et al.* (1995) found signs of ovarian activity, as evidenced by elevated urinary progesterone levels, in three non-breeding females, prior to the planned removal of the breeding female. Moreover, one of these females, the heaviest non-breeding female in the colony, had been the primary target of shoving by the breeding female, and was the first to exhibit characteristic breeding female-like behaviours shortly after removal of the breeding female from the colony. The same pattern was seen in colony 7400 in the present study. Female #27, the second heaviest female in the colony, had been the primary recipient of the breeding female's aggression during late pregnancy and for a few days after parturition, and it was her retaliation and the accompanying fight between her and the breeding female, that sparked the subsequent take-over event. Like the non-breeding female of the study by Margulis *et al.* (1995), female #27 was the first female to exhibit breeding female-like behaviour, and appeared to be the most dominant animal in the colony within 1 week after the initial fight. Prior to the fight, during late pregnancy, female #27 had had basal and post-GnRH plasma LH concentrations of 1.99 mi.u./ml and 2.89 mi.u./ml respectively. These were by no means the highest values of the females that were subsequently killed, but it is possible that, had she received no agonistic interactions from the breeding female, this non-breeding female may have exhibited a greater degree of relaxation of reproductive suppression prior to the commencement of the take-over event.

Apart from #27, the only other non-breeding females who had received agonistic interactions from the breeding female were two who were subsequently killed (#30 and #31) and #28 who became the new breeding female. Of the five non-breeding males that were killed, only two (#26 and #29) had received agonistic interactions from the breeding female prior to the take-over, with #26 being the secondary recipient of the breeding female's aggression, superseded only by female #27.

Sexual interactions such as ano-genital nuzzling and sniffing usually occur exclusively between the breeders, and, unless there is colony strife, only occasionally between the breeders and a few non-breeders (of both sexes), and among these non-breeders. However, when there is colony strife, such as during colony succession, the number of sexual interactions involving non-breeders increases (Jarvis, 1991a). This is exactly what was observed in colony 7400 during the take-over event. Prior to the initial fighting, sexual interactions occurred almost exclusively between the breeding female and the three putative breeding males (#9, #11 & # 20). However, two non-breeding animals (female #23 and male #26) were observed nuzzling the genitals of the breeding female, and both were subsequently killed. During the take-over event, sexual interactions among non-breeders, and between non-breeders of both sexes and the putative breeding males, became frequent. These sexual interactions were mainly male-female, with the primary recipients being those non-breeding females most actively involved in the fighting at the time. Female-female sexual interactions were also common, particularly between the fighting females, often prior to an agonistic encounter, but male-male sexual interactions although they occurred, were less common. Many of the animals, including three of the males and five of the females that were subsequently killed, were observed sniffing their own genitals, particularly after an agonistic encounter with another animal. All five of the non-breeding males that were killed, engaged in frequent sexual encounters.

In addition to the five that were killed, two other non-breeding males (#49 and #51), also engaged in sexual encounters, although not agonistic encounters, with other males and females, but were not killed. It is very interesting that prior to the take-over, #49 had the highest post-GnRH plasma LH concentration of all the non-breeding males, and after the take-over event #49 and #51 had the highest basal plasma LH concentrations and highest post-GnRH plasma LH concentrations of all the animals in the colony and were therefore the least suppressed. Why were they not killed? During the take-over, both these males began to display traits of the disperser morph (O'Riain *et al*, 1996), namely increased fat deposits around the neck region and a high incidence of escape from the colony. Dispersers have previously been shown to have higher basal plasma LH concentrations and greater responses to exogenous GnRH than other non-breeding males (O'Riain, 1996) but nevertheless are rarely victims of breeding female aggression (O'Riain, pers com.).

The common factor to all the animals, both male and female, that were killed during the course of the take-over event, is that they were all the offspring of the original breeding pair, and therefore the oldest colony members. Following the take-over, the only offspring of the original breeding pair that survived were the new breeding female (#28 - litter 6, Figure 4.1) and the three putative breeding males (#9 - litter 2, # 11 and # 20 - both litter 3). The remaining animals (litters 9-14) were all the offspring of the breeding female (#4) and the foreign male (#FO) that had been accidentally introduced into the colony in 1993. The animals from litters 9-14 were considerably younger than the others because the breeding female (#4) had only begun to successfully rear pups more than a year after the birth of litter 7. Therefore, in the present study, it was not possible to separate the effects of relatedness from those of age.

There is a great deal of variability in both the time taken for colony succession to be completed, and in the levels of aggression and violence accompanying successions. Jarvis (1991a) has reported that when colony structure has been severely disrupted, for example by the removal of the breeding pair and some of the larger mole-rats, colony succession is almost always accompanied by fierce fighting, leading to the deaths of some or even all of the fighting animals. This is what was seen in colony 7400 in which the death of the breeding male, followed by a challenge to the breeding female, resulted in the deaths of seven females, including the breeding female, and six males. However, despite the fact that this particular take-over was violent, it occurred relatively quickly, and #28 became pregnant within 10 days after the death of her last female rival, and had given birth to her first litter only 98 days after the death of the breeding female. In the study conducted by Margulis *et al.* (1995), it took 67 days before another non-breeding female challenged the non-breeding female which had become dominant after removal of the breeding female. In the study by Clarke and Faulkes (1997) two colonies had each had one aggressive female which had become the new breeding female, one of which began breeding 54-65 days after removal of the breeding female, while in a third colony a single breeding female had not emerged 20 months following removal of the breeding female.

The relative rapidity with which the natural take-over event observed in colony 7400 was completed, together with the fact that prior to the initial fighting, those non-breeding females that were subsequently killed appeared, from hormonal data, to be less suppressed than the others, indicates that the breeding female had lost some control and that colony was to some extent primed for the event. As mentioned earlier, the trigger may have been the death of the breeding male, or possibly her injury or disruption of the colony as a result of the invasion of the colony by foreign animals a month earlier. The take-over event in colony 7400 occurred spontaneously, so the factors governing it may have been different to those controlling succession in colonies from which the breeding female has been artificially removed. In a natural take-over one would expect that some of the colony members have been partially released from reproductive suppression, to enables them to challenge the breeding female. In an artificial situation, these early cues for release from reproductive suppression may be missing.

Similar hormonal changes to those that occurred in colony 7400 following the take-over event, have been found in other studies. The increase in the basal LH levels of the non-breeding males following the take-over event, but not in the breeding males or non-breeding females, supports the findings of Clarke & Faulkes (1997) who found that, following a colony succession, there was a significant increase in the urinary testosterone levels of the non-breeding males, but not of the breeding males or non-breeding females. The lack of change in the post-GnRH responses in both the non-breeding females and non-breeding males implies that the animals that were not killed in the take-over were equally suppressed prior to and following the event. From the individual profiles this appears to be true for the females, however there was more variability amongst the males, with some males appearing to be more suppressed, and others less suppressed, following the take-over event.

In conclusion, the results of this study show that non-breeding females do in fact differ in their likelihood of actively competing for breeding status. With hindsight, there were clear indications that the colony was not stable and that the possibility of a take-over existed. In addition, predictions could have been made as to some, but not all, of the animals that would become involved in the fighting that ensued. All the non-breeding animals of the oldest six litters were killed, except for female #28. At the start of the study, female #28 was the heaviest female in the colony, and despite the fact that she received negligible aggression from the breeding female prior to the take-over event, and displayed little hormonal evidence of reproductive activity, she was the non-breeding female that finally emerged as the new breeding female. In conclusion therefore, although it is not possible to predict which female will become the new breeding female, it is likely that she will be among the oldest and heaviest females in the colony. It is therefore possible, to a certain extent, to predict which group of females, and to a lesser degree which males, are the most likely to become involved in the intra-colonial fighting accompanying colony succession. Finally, this study also indicates that the death of the breeding male in a colony may have important consequences, in terms of colony succession, which have previously been overlooked.

CONCLUSIONS

1. This was the first study to investigate reproductive suppression in the individuals of entire colonies of naked mole-rats over the reproductive cycle of the breeding female. The mean basal and post-GnRH plasma LH concentrations of the non-breeding females and non-breeding males of the two study colonies were comparable with values measured in previous studies. Nevertheless, there was a great deal of individual variation over the study period, and no animal could be identified as having consistently higher basal or post-GnRH concentrations than all the other individuals of its colony. However, there were certain individuals that had mean basal or post GnRH plasma LH concentrations that were higher than the other non-breeding animals of their respective genders, and there appeared to be far more variation in terms of individual plasma LH concentrations amongst the non-breeding males than the non-breeding females. Moreover, the non-breeding males had higher basal and post-GnRH plasma LH concentrations than the non-breeding females, thus the results of the present study are consistent with previous findings that reproductive suppression is less clear-cut in non-breeding males than in non-breeding females.

2. In the well established colony 100, the non-breeding females could be divided into two groups; those that had basal plasma LH concentrations that were consistently below the sensitivity of the assay (Group 1) and those that had detectable basal plasma LH concentrations at some time during the study period (Group 2). These two groups of non-breeding females differed not only in terms of plasma LH concentrations, but also in terms of age and the amount of aggression received from the breeding female. The females of Group 2 had significantly higher post-GnRH plasma LH concentrations, and were thus less reproductively suppressed, than those of Group 1. Moreover, the breeding female directed most of her female-directed aggression towards Group 2, and there was a significant positive correlation between the percentage of the total agonistic interactions directed towards the non-breeding females and basal plasma LH concentration, although not post-GnRH plasma LH concentration. In colony 7400, which had recently undergone a take-over event, the non-breeding females could also be divided into two groups based on the criterion of detectable basal plasma LH concentrations. However, these two groups could not be distinguished in terms of body mass, age or the amount of agonism received from the breeding female.

Unlike the non-breeding females, the non-breeding males of the two study colonies could not be divided into distinct groups on the basis of detectable basal plasma LH concentrations, as only one male in colony 100 and two in colony 7400 had consistently undetectable basal plasma LH concentrations.

3. It is generally believed that the control of the breeding female over the other colony members is weakest during late pregnancy, when she is highly gravid and unable to patrol the burrow system as effectively as at other times of the cycle. However, in the present study, several non-breeding animals, particularly males, showed their greatest LH response to exogenous GnRH during mid pregnancy, and there appeared be a slight relaxation of reproductive suppression at this time as evidenced by the elevated mean post-GnRH plasma LH concentrations in the non-breeding animals of both sexes. Although this was not statistically significant, it supported an observation from a previous study in which there was an unexpected synchronous peak in urinary testosterone in two non-breeding males, during mid-pregnancy of the breeding female. Although the frequency of agonistic interactions by the breeding females of the two study colonies did not appear to be lower during mid-pregnancy than at other times of the cycle, they did spend far more time basking during mid pregnancy than during either early or late pregnancy. This increased frequency in basking behaviour during mid-pregnancy may mean that the breeding female has less contact with the other colony members than at other times in the cycle, with the result that some are released from suppression at this time.

4.. It was not possible to determine, at an individual level, the relationship between plasma LH concentration and the behaviour of the breeding female towards certain non-breeding males and non-breeding females. moreover, relatively few interactions occur between the breeding female and the majority of the colony members, and in both colonies there was no clear relationship between basal or post-GnRH plasma LH concentration and the percentage of the total agonistic interactions initiated by the breeding female towards the non-breeding males, or towards the non-breeding females of colony 7400. However, in the well-established colony 100, the there was a significant positive correlation between basal plasma LH and the percentage of the total agonistic interactions initiated by the breeding female towards the non-breeding females, and the breeding female of this colony preferentially directed her aggression towards the non-breeding females of

Group 1, which had higher basal and post-GnRH plasma LH concentrations than those of Group 2.

5. Age appears to be more important than mass in determining which animals pose the greatest threat to the reproductive status of the breeding female. The older colony members have higher plasma LH concentrations than younger animals, and most of the breeding female's aggression is directed towards the older animals.

6. During the take-over event which took place in colony 7400, seven females, including the breeding female, and five males were killed in the accompanying fighting. The female that finally became the new breeding female was the heaviest female at the start of the study. Nevertheless, age appears to be more important than body mass in determining which animals become involved in the fighting accompanying succession because although not all the largest animals were killed, all the offspring of the original breeding pair, *i.e.* the oldest colony members, except for the new breeding female and the three breeding males, were killed. It is thus evident that certain predictions can be made as to which non-breeding females, and, to a lesser extent which breeding males, are most likely to become involved in the fighting that usually accompanies colony succession and these animals are most likely to be among the oldest, heaviest animals in the colony.

7. It is interesting that the take-over event was preceded by the death of the breeding male 48 days earlier, and although it has not previously been considered, it appears that the death of the breeding male may play an important role in initiating a take-over events.

REFERENCES

ABBOTT, D.H. 1984, Behavioural and physiological suppression of fertility in subordinate marmoset monkeys. *Am. J. Primatol.* 6: 169-186.

ABBOTT, D.H. 1988. Natural suppression of fertility. *Symp. Zool. Soc. Lond.* No. 60: 7-28.

ABBOTT, D.H. 1989. Social suppression of reproduction in primates. In: *Comparative Socioecology: The Behavioural Ecology of Humans and Other Mammals.* Eds. V. Standen & R.A. Foley. Blackwell Scientific Publications, London. pp.285-304.

ABBOTT, D.H., BARRETT, J., FAULKES, C.G. & GEORGE, L.M. 1989. Social contraception in naked mole-rats and marmoset monkeys. *J. Zool. Lond.* 219: 703-710.

ABBOTT, D.H., HODGES, J.K. & GEORGE, L.M. 1988. Social status controls LH secretion and ovulation in female marmoset monkeys (*Callithrix jacchus*). *J. Endocr.* 117: 329-339.

ABBOTT, D.H., MCNEILLY, A.S., LUNN, S.F., HULME, M.J. and BURDEN, F.J. 1981. Inhibition of ovarian function in subordinate female marmoset monkeys (*Callithrix jacchus jacchus*). *J. Reprod. Fert.* 68: 335-345.

BENNETT, N.C. 1989. The social structure and reproductive biology of the common mole-rat, *Cryptomys h. hottentotus* and remarks on the trends in reproduction and sociality in the family Bathyergidae. *J. Zool. Lond.* 219: 45-59.

BENNETT, N.C. 1994. Reproductive suppression in social *Cryptomys damarensis* colonies - a lifetime of socially-induced sterility in males and females (Rodentia: Bathyergidae). *J. Zool. Lond.* 234: 25-39.

BENNETT, N.C., FAULKES, C.G. & MOLTENO, A.J. 1996. Reproductive suppression in subordinate, non-breeding female Damaraland mole-rats: two components to a lifetime of socially induced infertility. *Proc. R. Soc. Lond.* B 263: 1599-1603.

BENNETT, N.C., FAULKES, C.G. & SPINKS, A.C. 1997. LH responses to single doses of exogenous GnRH by social Mashona mole-rats. (In press).

BENNETT, N.C. & JARVIS, J.U.M. 1988. The social structure and reproductive biology of colonies of the mole-rat *Cryptomys damarensis* (Rodentia, Bathyergidae). *J. Mammal.* 69: 293-302.

BENNETT, N.C., JARVIS, J.U.M. & COTTERILL, F.P.D. 1994. The colony structure and reproductive biology of the Afrotropical Mashona mole-rat, *Cryptomys darlingi*. *J. Zool. Lond.* 234: 477-487

BENNETT, N.C., JARVIS, J.U.M., FAULKES, C.G. and MILLAR, R.P. 1993. LH responses to single doses of exogenous GnRH by freshly captured Damaraland mole-rats, *Cryptomys damarensis*. *J. Reprod. Fert.* 99: 81-96.

BENNETT, N.C., JARVIS, J.U.M., MILLAR, R.P., SASANO, H. & NTSHINGA, K.V. 1994. Reproductive suppression in eusocial *Cryptomys damarensis* colonies: socially-induced infertility in females. *J. Zool. Lond.* 233: 617-630.

BRETT, R.A. 1986. The ecology and behaviour of the Naked mole-rat (*Heterocephalus glaber* Rüppell) (Rodentia: Bathyergidae) Unpublished PhD thesis, University of London.

BRETT, R.A. 1991a. The population structure of naked mole-rat colonies. In: *The Biology of The Naked Mole-Rat*. Eds. P.W. Sherman, J.U.M. Jarvis & R. Alexander. Princeton University Press, New York. pp. 97-136.

BRETT, R.A. 1991b. The ecology of naked mole-rat colonies: burrowing, food and limiting factors. In: *The Biology of The Naked Mole-Rat*. Eds. P.W. Sherman, J.U.M. Jarvis & R. Alexander. Princeton University Press, New York. pp. 137-184.

BRONSON, F.H. & DESJARDENS, C. 1974. Circulating concentrations of FSH, LH, estradiol and progesterone associated with acute, male-induced puberty in female mice. *Endocrinology*. 94: 1658-1668.

BRONSON, F.H. & HEIDEMAN, P.D. 1994. Seasonal regulation of reproduction in mammals. In: *The Physiology of Reproduction* (Vol 2). Eds. E. Knobil & J.D. Neill. Raven Press, New York. pp. 541-583.

BRUCE, 1965. The effects of castration on the reproductive pheromones of male mice. *J. Reprod. Fertil.* 10: 141-143.

BUFFENSTEIN, R., URISON, N., WOODLEY, R., VAN DER WESTHUIZEN, L.A. & JARVIS, J.U.M. 1996. Temperature changes during pregnancy in the subterranean naked mole-rat, (*Heterocephalus glaber*); the role of altered body composition and basking behaviour. *Mammalia* 60(4): 619-628.

BUFFENSTEIN, R & YAHAV, S. 1991. Is the naked mole-rat *Heterocephalus glaber* an endothermic yet poikilothermic mammal? *J. Therm. Biol.* 16: 227-232.

CLARKE, F.M. & FAULKES, C.G. 1997. Dominance and queen succession in captive colonies of the eusocial naked mole-rat, *Heterocephalus glaber*. (In press).

COWLEY, J.J. & WISE, O.R. 1972. Some effects of mouse urine on neonatal growth and reproduction. *Anim. Behav.* 20: 499-506.

CREEL, S., CREEL, N, WILDT, D.E. & MONFORT, S.L. Behavioural and endocrine mechanisms of reproductive suppression in Serengeti dwarf mongooses. *Anim. Behav.* 43: 231-245.

DAVIS-WALTON, J. & SHERMAN, P.W. 1994. Sleep arrhythmia in the eusocial naked mole-rat.*Naturwissenschaften* 81: 272-275.

DUDLEY, R.A., EDWARDS, P., EKINS, R.P., FINNEY, D.J., MCKENZIE, I.G.M., RAAB, G.M., RODBARD, D. & RODGERS, R.P.C. 1985. Guidelines for immunoassay data processing. *Clin. Chem.* 31(8): 1264-1271.

DUNBAR, R.I.M. 1980. Determinants and evolutionary consequences of dominance among female Gelada baboons. *Behav. Ecol. Sociobiol.* 7: 253-265.

DUNBAR, R. 1985. Stress is a good contraceptive. *New Scientist* 1439: 16-18.

DUNBAR, R.I.M. 1989. Reproductive strategies of female gelada baboons. In: *The Sociobiology of sexual and Reproductive Strategies*. Eds. A.E. Rasa, C. Vogel & E. Voland. Cambridge University Press, Cambridge. pp. 74-92.

FAULKES, C.G. 1990. Social suppression of reproduction in the naked mole-rat, *Heterocephalus glaber*. Unpublished PhD thesis, University of London.

FAULKES, C.G. & ABBOTT, D.H. 1991. Social control of reproduction in both breeding and non-breeding male naked mole-rats, *Heterocephalus glaber*. *J. Reprod. Fert.* 93: 427-435.

FAULKES, C.G. & ABBOTT, D.H. 1993. Evidence that primer pheromones do not cause social suppression of reproduction in male and female naked mole-rats (*Heterocephalus glaber*). *J. Reprod. Fert.* 99: 225-230.

FAULKES, C.G., ABBOTT, D.H. & JARVIS, J.U.M. 1990a. Social suppression of ovarian cyclicity in captive and wild colonies of naked mole-rats, *Heterocephalus glaber*. *J. Reprod. Fert.* 88: 559-568.

FAULKES, C.G., ABBOTT, D.H. & JARVIS, J.U.M. 1991a. Social suppression of reproduction in male naked mole-rats, *Heterocephalus glaber*. *J. Reprod. Fert.* 91: 593-604.

FAULKES, C.G., ABBOTT, D.H., JARVIS, J.U.M. & SHERRIFF, F.E. 1990b. LH responses of female naked mole-rats, *Heterocephalus glaber*, to single and multiple doses of exogenous GnRH. *J. Reprod. Fert.* 89: 317-323.

FAULKES, C.G., ABBOTT, D.H., LIDDELL, C.E., GEORGE, L.M. & JARVIS, J.U.M. 1991b. Hormonal and behavioural aspects of reproductive suppression in female naked mole-rats. In: *The Biology of The Naked Mole-Rat*. Eds. P.W. Sherman, J.U.M. Jarvis & R. Alexander. Princeton University Press, New York. pp. 426-445.

FAULKES, C.G., ABBOTT, D.H. & MELLOR, A.L. 1990c. Investigation of genetic diversity in wild colonies of naked mole-rats (Heterocephalus glaber) by DNA fingerprinting. *J. Zool. Lond.* 221: 87-97.

FAULKES, C.G., TROWELL, S.N., JARVIS, J.U.M. & BENNETT, N.C. 1994. Investigation of sperm numbers and motility in reproductively active and socially suppressed males of two eusocial African mole-rats, the naked mole-rat (*Heterocephalus glaber*), and the Damaraland mole-rat (*Cryptomys damarensis*). *J. Rep. Fert.* 100: 411-416.

FOLLETT, B.K. 1985. The environment and reproduction. In: *Reproduction in mammals: 4 Reproductive fitness*. Eds. C.R. Austin & R.V. Short. Cambridge University Press, Cambridge. pp.103-132.

FRAME, L.H., MALCOM, J.R., FRAME, G.W. & VAN LAWICK, H. 1979. Social organization of African wild dogs (*Lycaon pictus*) on the Serengeti Plains, Tanzania 1967-1978. *Z. Tierpsychol.* 50: 225-249.

FRENCH, J.A., ABBOTT, D.H. & SNOWDON, C.S. 1984. The effect of social environment on oestrogen excretion, scent marking and sociosexual behaviour in tamarins (*Sanguinus oedipus*). *Am. J. Primatol.* 6: 155-167.

GANONG, W.F. 1991. Review of Medical Physiology (15th Ed.). Prentice Hall Inc., New Jersey.

GETZ, L.L. & CARTER, C.S. 1980. Social organization in *Microtus ochrogaster* populations. *The Biologist.* 62: 56-69.

GLASIER, A., MCNEILLY, A.S. & BAIRD, D.T. 1986. Induction of ovarian activity by pulsatile infusion of LHRH in women with lactational amenorrhoea. *Clin. Endocrinol.* 24: 243 - 252.

HARLOW, C.R., GEMS, S., HODGES, J.K. & HEARN, J.P. 1984. The relationship between plasma progesterone and the timing of ovulation and early embryonic development in the marmoset monkey (*Callithrix jacchus*). *J. Zool., Lond.* 201: 272-282.

HILL, W.C.O, PORTER, A, BLOOM, R.T., SEAGO, J. & SOUTHWICK, M.D. 1957. Field and laboratory studies on the naked mole-rat, *Heterocephalus glaber. Proc. Zool. Soc. Lond.* 128: 455-513.

HODGES, J.K., COTTINGHAM, P., SUMMERS, P.M. & YINGNAN, L. 1987. Controlled ovulation in the marmoset monkey, (*Callithrix jacchus*) with human chorionic gonadotrophin following prostaglandin induced luteal regression. *Fert. Steril.* 48: 299-305.

HONEYCUTT, R.L., ALLARD, M.W., EDWARDS, S.V. & SCHLITTER, D.A. 1991a. Systematics and evolution of the family Bathyergidae. In: *The Biology of The Naked Mole-Rat*. Eds. P.W. Sherman, J.U.M. Jarvis & R. Alexander. Princeton University Press, New York. pp. 45-65.

HONEYCUTT, R.L., NELSON, K., SCHLITTER, D.A. & SHERMAN, P.W. 1991b. Genetic variation within and among populations of the naked mole-rat: Evidence from nuclear and mitochondrial genomes. In: *The Biology of The Naked Mole-Rat*. Eds. P.W. Sherman, J.U.M. Jarvis & R. Alexander. Princeton University Press, New York. pp.195-208.

ISIL, S. 1983. A study of social behaviour in laboratory colonies of the Naked mole-rat (*Heterocephalus glaber* Rüppell, Rodentia: Bathyergidae). Unpublished M.Sc. thesis, University of Michigan.

JACOBS, S.S. & JARVIS, J.U.M. 1996. No evidence for the work-conflict hypothesis in the eusocial naked mole-rat (*Heterocephalus glaber*). *Behav. Ecol. Sociobiol.* 39: 401-409.

JARVIS, J.U.M. 1981. Eusociality in a mammal: Cooperative breeding in naked mole-rat colonies. *Science* 212: 571-573.

Jarvis, J.U.M. 1991a. Reproduction of naked mole-rats. In: *The Biology of The Naked Mole-Rat*. Eds. P.W. Sherman, J.U.M. Jarvis & R. Alexander. Princeton University Press, New York. pp. 384-425.

Jarvis, J.U.M. 1991b. Appendix: Methods for Capturing, Transporting and Maintaining Naked Mole-Rats in Captivity. In: *The Biology of The Naked Mole-Rat*. Eds. P.W. Sherman, J.U.M. Jarvis & R. Alexander. Princeton University Press, New York. pp. 467-483.

Jarvis, J.U.M. & Bennett, N.C. 1991. Ecology and behaviour of the family Bathyergidae. In: *The Biology of The Naked Mole-Rat*. Eds. P.W. Sherman, J.U.M. Jarvis & R. Alexander. Princeton University Press, New York. pp. 66-96.

Jarvis, J.U.M. & Bennett, N.C. 1993. Eusociality has evolved independently in two genera of bathyergid mole-rats - but occurs in no other subterranean mammal. *Behav. Ecol. Sociobiol.* 33: 253-260.

Jarvis, J.U.M., O'Riain, M.J. & McDaid, E. 1991. Growth and factors affecting body size in naked mole-rats. In: *The Biology of The Naked Mole-Rat*. Eds. P.W. Sherman, J.U.M. Jarvis & R. Alexander. Princeton University Press, New York. pp. 358-383.

Jarvis, J.U.M. & Sale, J.B. 1971. Burrowing and burrow patterns of East African mole-rats *Tachyoryctes*, *Heliophobius* and *Heterocephalus*. *J. Zool. Lond.* 163: 451-479.

Kann, G. & Martinet, J. 1975. Prolactin levels and duration of postpartum anoestrus lactating ewes. *Nature* 257: 63-63.

Karsch, F.J., Bittman, E.L., Foster, D.L., Goodman, R.L., Legan, S.J. & Robinson, J.E. 1984. Neuroendocrine basis of seasonal reproduction. *Rec. Prog. Horm. Res.* 40: 185-232.

Kayanja, F.I.B. & Jarvis, J.U.M. 1971. Histological observations of the ovary, oviduct and uterus of the naked mole-rat. *Z. Saugetierkd.* 36: 114-121.

Keverne, E.B. 1983. Pheromonal influences on the endocrine regulation of reproduction. *Trends Neurosci.* 6: 381-384.

Lacey, E.A., Alexander, R.D., Braude, S.H., Sherman, P.W. & Jarvis, J.U.M. 1991. An ethogram for the naked mole-rat: nonvocal behaviours. In: *The Biology of The Naked Mole-Rat*. Eds. P.W. Sherman, J.U.M. Jarvis & R. Alexander. Princeton University Press, New York. pp. 209-242.

Lacey, E.A. & Sherman, P.W. 1991. Social organization of naked mole-rat colonies: evidence for divisions of labor. In: *The Biology of The Naked Mole-Rat*. Eds. P.W. Sherman, J.U.M. Jarvis & R. Alexander. Princeton University Press, New York. pp. 275-336.

Lincoln, G.A. & Short, R.V. 1980. Seasonal breeding: nature's contraceptive. *Rec. Prog. Horm. Res.* 36: 1-52.

MARGULIS, S.W., SALTZMAN, M. & ABBOTT, D.H. 1995. Behavioural and hormonal changes in female naked mole-rats (*Heterocephalus glaber*) following removal of the breeding female from a colony. *Hormones and Behaviour*. 29: 227-247.

MATTIOLO, M., CONTE, F., GALEATI, G. & SEREN, E. 1986. Effect of naloxone on plasma concentrations of prolactin and LH in lactating sows. *J. Reprod. Fert.* 76: 167-173.

MCNAB, B.K. 1966. The metabolism of fossorial rodents: a study of convergence. *Ecology*. 47: 712-733.

MCNEILLY, A.S. 1994. Suckling and the control of gonadotrophin secretion. In: *The Physiology of Reproduction* (Vol 2). Eds. E. Knobil & J.D. Neill. Raven Press, New York. pp. 1179-1212.

MICHENER, C.D. 1969. Comparative social behaviour of bees. *Annu. Rev. Entomol.* 14: 299-342.

MILLAR, R.P., FLANAGAN, C.A., de L. MILTON, R.C & KING, J.A. 1989. Chimeric analogues of vertebrate gonadotrophin releasing hormones comprising substitutions of the variant amino acids in positions 5, 7 and 8. *J Biol. Chem.* 264: 21007-21013.

MOON, T.W., MUSTAFA, T. & JØRGENSEN, J.B. 1981. Metabolism, tissue metabolites and enzyme activities in the fossorial mole-rat, *Heterocephalus glaber*. *Molec. Physiol.* 1: 179-194.

NEVO, E. 1979. Adaptive convergence and divergence of subterranean mammals. *Ann. Rev. Ecol. Syst.* 10: 269-308.

O'RIAIN, M.J. 1996. Pup ontogeny and factors influencing behavioural and morphological variation in naked mole-rats, *Heterocephalus glaber* (Rodentia, Bathyergidae). Unpublished PhD thesis, University of Cape Town.

O'RIAIN, M.J. & JARVIS, J.U.M. 1997. Colony member recognition and xenophobia in the naked mole-rat. *Anim. Behav.* 53: 487-498.

O'RIAIN, M.J., JARVIS, J.U.M. & FAULKES, C.G. 1996. A dispersive morph in the naked mole-rat. *Nature* 380: 619-621.

QUAY, W.B. 1981. Pineal atrophy and other neuroendocrine and circumventricular features of the naked mole-rat, *Heterocephalus glaber* (Rüppell), a fossorial, equatorial rodent. *J. Neural Transm.* 52: 107-115.

RASA, A.O. 1973. Intra-familial sexual repression in the dwarf mongoose, *Helogale parvula*. *Die Naturwissenschaften*. 60: 303-304.

REEVE, H.K. 1992. Queen activation of lazy workers in colonies of the eusocial naked mole-rat. *Nature* 358: 147-149.

REEVE, H.K. & GAMBOA, G.J. 1983. Colony activity integration in primitively eusocial wasps: the role of the queen (*Polistes fuscatus*, Hymenoptera: Vespidae). *Behav. Ecol. Sociobiol.* 13: 63-74.

REEVE, H.K. & GAMBOA, G.J. 1987. Queen regulation of worker foraging in paper wasps: a social feedback control system (*Polistes fuscatus*, Hymenoptera: Vespidae). *Behaviour* 102: 147-167.

REEVE, H.K. & SHERMAN, P.W. 1991. Intracolonial aggression and nepotism by the breeding female naked mole-rat. In: *The Biology of The Naked Mole-Rat*. Eds. P.W. Sherman, J.U.M. Jarvis & R. Alexander. Princeton University Press, New York. pp. 337-357.

REEVE, H.K., WESTNEAT, D.F., NOON, W.A., SHERMAN, P.W. & AQUADRO, C.F. 1990. DNA "fingerprinting reveals high levels of inbreeding in colonies of the eusocial naked mole-rat. Proc. Natl. Acad. Sci. USA 87: 2496-2500.

RICKARD, C.A. & BENNETT, N.C. 1997. Recrudescence of sexual activity in a reproductively quiescent colony of the Damaraland mole-rat (Cryptomys damarensis), by the introduction of an unfamiliar and genetically unrelated male - a case of incest avoidance in 'queenless' colonies. *J. Zool. Lond.* 241: 185-202.

ROOD, J.P. 1980. Mating relations and breeding suppression in the dwarf mongoose. *Anim. Behav.* 28: 143-150.

RÜPPELL, E. 1842. *Heterocephalus* nov. gen. Uber Saugethiere aus der Ordnung der Nager, beobachtet in nordöstlichen Africa. *Museum Senckenbergianum Abhandlungen* 3: 91-116.

SANDOW, J. 1983. The regulation of LHRH action at the pituitary and gonadal receptor level: a review. *Psychoneuroendocrinology* 8(3): 277-297.

SHERMAN, P.W., JARVIS, J.U.M. & BRAUDE, S.H. 1992. Naked mole rats. *Sci. Am.* 267(2): 72-78.

SHORT, R.V. 1984. Oestrous and menstrual cycles. In: *Reproduction in mammals: 3 Hormonal control of reproduction* (2nd Ed.). Eds. C.R. Austin & R.V. Short. Cambridge University Press, Cambridge. pp.115-152.

SIRINATHSINGHJI, D.J.S. & MARTINI, L. 1984. Effects of bromocriptine and naloxone on plasma levels of prolactin, LH and FSH during suckling in the female rat: responses to gonadotrophin releasing hormone. *J. Endocr.* 100: 175-182.

TUCKER, R. 1981. Digging behaviour and skin differentiations in *Heterocephalus glaber*. *J. Morphol.* 168: 51-71.

TUREK, F.W. & VAN CAUTER, E. 1994. Rhythms in reproduction. In: *The Physiology of Reproduction* (Vol 2). Eds. E. Knobil & J.D. Neill. Raven Press, New York. pp. 487-540.

VAN DAMME, M.-P., ROBERTSON, D.M. & DICZFALUSY, E. 1974. An improved *in vitro* bioassay method for measuring luteinizing hormone (LH) activity using mouse Leydig cell preparations. *Acta endocr., Copenh.* 77: 655-671.

VANDENBERGH, J.G. 1969 Male odour accelerates female sexual maturation in mice. *Endocrinology.* 84: 658-660.

VANDENBERGH, J.G. 1994. Pheromones and mammalian reproduction. In: *The Physiology of Reproduction* (Vol 2). Eds. E. Knobil & J.D. Neill. Raven Press, New York. pp. 343-359.

WASSER, S.K. & BARASH, D.P. 1983. Reproductive suppression among female mammals: implications for biomedicine and sexual selection theory. *Quart. Rev. Biol.* 58: 513-538.

WESTLIN, L.M., BENNETT, N.C. & JARVIS, J.U.M. 1994. Relaxation of reproductive suppression in non-breeding female naked mole-rats, *Heterocephalus glaber. J. Zool. Lond.* 234: 177-188.

WHITTEN, W.K. 1956. Modification of the oestrous cycle of the mouse by external stimuli associated with the male. *J. Endocrinol.* 13: 399-404.

WHITTEN, W.K. 1959. Occurrence of anoestrous in mice caged in groups. *J. Endocrinol.* 18: 102-108.

WILSON, E.O. 1971. *The insect societies*. Belknap Press of Harvard University Press, Cambridge, Massachusetts.

WITHERS, P.C & JARVIS, J.U.M. 1980. The effect of huddling on thermoregulation and oxygen consumption for the naked mole-rat. *Comp. Biochem. Physiol. A, Comp. Physiol.* 66: 215-219.

ZAR, J.H. 1984. Biostatistical Analysis (2nd Ed.). Prentice-Hall, Inc., New Jersey.

APPENDIX I

An ethogram of the agonistic and sexual behaviours used in the data analyses.

Agonistic interactions

Attack - one animal bites or begins to spar with another, thus initiating an agonistic encounter. This behaviour was most commonly observed in the nest and was frequently initiated by the breeding female. It was the most common agonistic interaction initiated by the breeding females of the two study colonies, and commonly occurred between the breeding female and breeding male(s) usually before a sexual encounter

Bite - the teeth of one animal close over the body of another animal

Drag - one animal grasps the skin of another and drags it along

Shove - two animals face each other with their heads touching, and one animal pushes the other backwards

Spar - animals face each other and ward one another off with their incisors, which may become locked in the process.

Sexual interactions

Mouth genitals -one animal mouthes the genitals of another

Mutual ano-genital nuzzling - two animals lie head to tail and nuzzle and groom one anothers genitals

Nuzzle - one animal rubs the sides of its muzzle against the body of another animal

Nuzzle genitals -one animal rubs the sides of its muzzle against the genitals of another

Sniff genitals - one animal sniffs the genitals of another

APPENDIX II

TABLE 1: Results of the Mann-Whitney *U*-Tests used to determine whether there were any differences between the non-breeding females of Group 3 (n = 4) and Group 4 (n = 6) in terms of body mass, age or percentage of the total agonistic interactions received from the breeding female.

Criterion	*U*	*P*
Body mass	18	$P > 0.20$
Age	14.5	$P > 0.20$
Agonistic interactions	15.5	$P > 0.20$

TABLE 2: Results of the Kruskal Wallis tests used to determine whether there were any differences between early, mid and late-cycle in terms of the plasma LH concentrations of the non-breeding males and non-breeding females of colony 100 and colony 7400, before (Pre-GnRH) and 20 min after (Post-GnRH) a single s.c. injection of 0.1 µg GnRH. ($\chi^2_{0.05,2} = 5.991$).

Colony	*H*	*P*
100 **(Cycle 1)**		
Females		
Pre	$H = 2.094$	$0.25 < P < 0.50$
Post	$H = 4.299$	$0.10 < P < 0.25$
Males		
Pre	$H = 1.361$	$0.50 < P < 0.75$
Post	$H = 0.985$	$0.50 < P < 0.75$
100 **(Cycle 2)**		
Females		
Pre	$H = 0.505$	$0.75 < P < 0.90$
Post	$H = 0.231$	$0.75 < P < 0.90$
Males		
Pre	$H = 2.715$	$0.25 < P < 0.50$
Post	$H = 5.704$	$0.05 < P < 0.10$
7400		
Females		
Pre	$H = 0.352$	$0.75 < P < 0.90$
Post	$H = 2.355$	$0.25 < P < 0.50$
Males		
Pre	$H = 0.601$	$0.50 < P < 0.75$
Post	$H = 5.180$	$0.05 < P < 0.10$

TABLE 3: Results of the Spearman's rank correlation coefficient procedures used to determine whether there were any correlations between plasma LH concentrations and the percentage of the total agonistic interactions received from the breeding female, in three female and four male non-breeding mole-rats of colony 100, over two cycles (*i.e.* n = 6).

Animal		r_s	P
Females			
12	Pre	0.611	$0.20 < P < 0.50$
	Post	-0.182	$P > 0.50$
13	Pre	0.574	$0.20 < P < 0.50$
	Post	0.696	$0.10 < P < 0.20$
15	Pre	0.664	$0.10 < P < 0.20$
	Post	1.000	$P = 0.005$
Males			
16	Pre	0.086	$P > 0.50$
	Post	0.371	$P = 0.50$
17	Pre	-0.086	$P > 0.50$
	Post	-0.371	$P = 0.50$
30	Pre	-0.143	$P > 0.50$
	Post	-0.600	$0.20 < P < 0.50$
XX	Pre	0.845	$0.05 < P < 0.10$
	Post	0.371	$P = 0.50$

TABLE 4: The percentage time spent basking, by the breeding females of colony 100 and colony 7400, during early, mid and late pregnany of each of the cycles investigated. Data were recorded during each of the 5 h of focal study on the breeding females, that were conducted during each of the time periods.

Colony	Early	Mid	Late
100			
Cycle 1	5.82	57.08	13.30
Cycle 2	4.16	53.02	36.76
7400	5.12	60.83	27.89

TABLE 5: The frequency of agonistic interactions initiated by the breeding females of colony 100 and colony 7400, during early, mid and late pregnany of each of the cycles investigated. Values are expressed as the number of agonistic interactions initiated by the breeding female per hour.

Colony	Early	Mid	Late
100			
Cycle 1	2.8	2.6	2.3
Cycle 2	1.4	2.2	2.1
7400	2.9	1	0.7