Chapter 4

Ovulation and Postnatal Growth

Abstract

In those eutherian species which need either the physical presence of a male (induced ovulators) or his pheromones (spontaneous ovulators) to ovulate, female activation of oestrous is the result of hypothalamic-pituitary stimulation of oestrogen production. Spontaneous ovulation could be an advantage in harsh environments (particularly with low mating success/skewed sex ratios) but is an energy expensive activity. Induced ovulation could also be advantagous in harsh environments (particularly if seasonal) where resources (food) are low and could be an energy saving mechanism. Bathyergids and other hystricomorphs studied to date appear to be spontaneous ovulators with a suppression of reproduction at high densities being enforced by a breeding female or male (either behaviourally, chemically or both). This study investigated whether the female highveld mole-rat is an induced or spontaneous ovulator (when suppression is removed); both quantitatively (progesterone profiles) and qualitatively (histologically). The female highveld mole-rat appears to be an induced ovulator shown by increased urinary progesterone as well as the presence of corpora lutea, while in the physical presence of a novel male. Copulatory behaviour and postnatal growth and development were also investigated. The highveld mole-rat has similar copulatory behaviour to other loosely social bathyergids and pups have a slow mean maximum growth rate which fits the species position in the bathyergid sociality continuum.

Introduction

In some mammals, male pheromonal cues activate oestrous in females (Fadem 1989) and female pheromones may play a role in the suppression of reproduction in social groups. In others, the physical presence of males may activate oestrous and ovulation (Fadem 1985). Furthermore, male presence as well as male-related pheromones can stimulate oestrous and ovulation in females (Hinds *et al.* 1992). It has been hypothesized that the pheromonal activation of oestrous in eutherian mammals is the result of hypothalamic-pituitary stimulation (a neuroendocrine response) of ovarian oestrogen production (Fadem 1989, Flint *et al.* 1997). The mechanism by which the male pheromones cause ovulation in the female is by alteration of gonadotrophin secretion and subsequent oestradiol release in the female (Fadem 1985). It has been shown that both spontaneous and induced ovulators are responsive to the oestrous-activating properties of male pheromone(s) (Fadem 1985).

Ovulation in eutherian species can be either spontaneous or induced; there are advantages and disadvantages to each method of ovulation. Spontaneous ovulators show a continuous cycling of reproductive hormones and subsequent ovulation with and without the physical and/or chemical cue(s) of a male, whereas induced ovulators only show the cycling of reproductive hormones and ultimately ovulation in the physical presence of males. This physical presence usually involves coitus. Induced ovulators fail to undergo hormonal cyclicity in the absence of a male as a result of the lack of coitus.

In eutherian species that show major fluctuations in population density, the stimulation of oestrous by males is a characteristic (Fadem 1985). Whitten (1966) and Milligan (1980) state that induced ovulation is relatively common among eutherian species. Conaway (1971) found that physiological responsiveness to social cues is closely related to the ecology of a species. In harsh environments where males are difficult to locate and/or the chance of successful mating is low, spontaneous ovulation would be a great advantage.

Spontaneous ovulation can also have significant advantages where mates are aggressive towards each other or, for other reasons, do not stay in contact for extended periods, or where sex ratios are skewed in favour of females and where males have to move on to other females in order to ensure reproductive success. By ovulating continuously (except while gravid) females can ensure reproductive success with mating occurring quickly as priming of female reproductive hormones by a male is unnecessary. This would obviously be an advantage in aseasonal breeders. However, this places continuous stress on the female that, in a resource limited and/or seasonal breeding niche, can not be afforded. Whitten and Bronson (1970), as well as Stoddart (1980), concluded that for solitary species where males and females are separated physically, activation of oestrous by close contact with males would not be advantageous. This is in contrast to the theory outlined above for spontaneous ovulators.

The highveld mole-rat (*Cryptomys hottentotus pretoriae*) occurs in a fossorial niche that is limited in terms of food availability and the opportunity to forage. Foraging can only occur when the soil is soft enough to permit burrowing and hence the obtaining of food reserves (typically geophytes). Food reserves are unevenly distributed and if the soil is not workable may not even be found (burrowing is achieved using extra-buccal incisors which are themselves restricted by their growth rate – the incisors being eroded much faster when soil is less workable). A female in the colony that continuously ovulates would be using energetically demanding resources that have been acquired. In this seasonally breeding mole-rat (Janse van Rensburg 2000), spontaneous ovulation would be impractical and place an unnecessary demand on stored energy reserves.

Induced ovulators require priming by the physical contact of a male prior to ovulation taking place. It would therefore be expected that a successful mating would take relatively longer to achieve. It is evident that males of induced ovulating species are also paired with the female for an extended period.

The naked mole-rat (*Heterocephalus glaber*; Bathyergidae) (Faulkes *et al.* 1990) as well as many other hystricomorph rodents are spontaneous ovulators (e.g. the guinea pig and

chinchilla) (Weir and Rowlands 1974). Indeed, most hystricomorph rodents studied to date have been shown to ovulate spontaneously (Weir 1974). However, the naked mole-rat is an aseasonal breeder that produces several litters per year. Because of increasing population densities in regions with limiting constraints, there is often a suppression of reproduction in many rodent species, which may be the resultant effect of urinary semio-chemicals or primer pheromones. Social interactions affect reproductive function in a wide range of species (Flint *et al.* 1997). Within group living species such as *C. h. pretoriae* an optimal group size should exist, since, if too many animals were to reproduce it could negatively affect survival, especially in a fossorial environment where food resources are limiting. A colony is founded by an unrelated pair of mole-rats that procreate, all other colony members being the offspring of the reproductive pair. Thus, suppression of reproduction in these colonies is important to ensure heterozygozity since close relatives should not produce offspring due to the possible negative deleterious effects of inbreeding depression. A dominance hierarchy is thought to exist in eusocial colonies where dominant animals are the breeding animals. Thus, ovulation in non-breeding animals is suppressed by the dominant, breeding female. A suppression of female reproduction can be manifested in several ways; through puberty delay, suppression of oestrous, suppression of ovulation or a block to embryo implantation (Faulkes *et al.* 1990).

Subterranean rodents are usually solitary in nature and thus xenophobic towards conspecifics. These rodents typically communicate via seismic signalling initially in order to announce their presence and intention (Bennett & Faulkes 2000). It is in this way that two solitary individuals could come together for breeding purposes without fatal mutual aggression.

Seismic communication has been reported in the Geomyidae as well as in five species of the Bathyergidae (Bennett & Jarvis 1988a, b, Jarvis & Bennett 1990, 1991). These species appear to use their hind limbs in order to drum on the substrate. In other species such as *Tachyoryctes splendens* (Rhizomyidae) and *Spalax ehrenbergi* (Spalacidae), the

incisors or head are used to tap on the burrow system in order to communicate (Jarvis 1969a; Heth *et al.* 1987; Rado *et al.* 1987 cited in Bennett & Faulkes 2000).

After the two individuals have accepted each other, copulation may take place. It is evident that solitary subterranean rodent species exhibit very similar copulatory behaviour that includes thrusting during copulation, multiple intromission and multiple ejaculation (Bennett & Faulkes 2000). Copulation is usually followed by grooming behaviour.

Limited data suggests that subterranean rodents have great variability in terms of their gestation period. Gestation period can range from as little as 18 days in the pocket gopher (*Thomomys talpoides*) to 120 days in the tuco-tuco (*Ctenomys talarum*) (Schramm 1961 and Weir 1974 respectively). Social bathyergids as well as ctenomyid species tend to have longer gestation periods than solitary bathyergids, geomyids or spalacids (Bennett & Faulkes 2000).

To date, studies of bathyergid gestation periods reveal that solitary species can have gestation lengths ranging from 44 days (Cape mole-rat) to 52 days (Cape dune mole-rat). Whereas in the social species such as the Mashona mole-rat and common mole-rat, gestation period can fall between 56 to 66 days (Bennett 1989 and Bennett *et al.* 1994a). Interestingly, the social giant Zambian mole-rat has a very long gestation period of 111 days (Bennett & Aguilar 1995 and Burda 1989).

Litter sizes of subterranean rodents are generally very small (Bennett *et al.* 1991 and Malizia & Busch 1991). In geomyids, litter size can range from 1- 13 (Hanson 1960, Miller 1946 and Howard & Childs 1959). In ctenomyids litters can range from 1-5 (Weir 1974) while in spalacids 1-5 (Shanas *et al.* 1995 and Gazit *et al.* 1996). In the bathyergids the solitary species may have larger litters (1-10) (Bennett & Faulkes 2000) compared to social species (1-5) (Bennett & Faulkes 2000).

There is limited data available documenting the postnatal development of bathyergids. (Bennett & Jarvis 1988a, b, Bennett 1989, Burda 1989). Jarvis (1978) showed that the eusocial mole-rat, *Heterocephalus glaber*, had a very long period of postnatal development. Thereafter, a further two articles were published on various species of bathyergids; both solitary and social species (Bennett *et al.* 1991 and Bennett & Navarro 1997). These studies generally found that the social bathyergids have a much slower rate of postnatal growth than solitary species (Bennett *et al.* 1991). Members of the genus *Cryptomys* have extremely low mean maximum growth rates compared to solitary and non-bathyergid solitary subterranean and fossorial rodents (Bennett *et al.* 1991). The long postnatal development of social bathyergids appears to be characteristic of the Bathyergidae. It has been hypothesized that, because the pups of social species are more altricial when born than those of solitary species, and due to the fact that these pups are incorporated into the colony (do not disperse as quickly as pups of solitary species), pups of social species tend to grow slower than pups of solitary species (Bennett *et al.* 1991).

This study was undertaken in order to elucidate aspects of the reproductive biology and development of the highveld mole-rat; concentrating on ovulation method and postnatal development (growth). It was hypothesized that non-reproductive females, in the absence of other colony females, would be induced to ovulate and that postnatal development would fall within ranges expected for social bathyergids.

Methods and Materials

Ovulation

Experimental design

In order to determine whether females were induced or spontaneous ovulators, nine females were used in this study. The study was carried out from the middle of May 2000 (which is within the breeding season, Janse van Rensburg 2000). Nine non-reproductive females were placed in isolation from possible chemical and behavioural stimulation of males for a period of five weeks. This time period was chosen for all experiments, since bathyergid oestrous cycles are thought to be around one month in duration (see Faulkes *et al.* 1990). The time period for a complete oestrous cycle in *Heterocephalus glaber* is around 34 days (Faulkes *et al.* 1990). Six large males (three of which had been vasectomized) were also placed in isolation. After the five-week isolation period, the nine females were left in isolation for a further five weeks and urine samples were collected from them every second day. These samples constituted the control samples. After this five-week period, three of the females ('alone-separated' females coded AS) were placed in chemical contact with large, unvasectomized males and urine was collected every second day as before. Also, three females ('alone-contact' females coded AC) were placed in chemical and behavioural contact with vasectomized males; urine being collected every second day. The final three females were left in isolation as control animals (`alone' females coded A). Data thus consisted of five weeks of control data for six females (control period) and 10 weeks of control data for two females (control period and experimental period). One female died during the study, thus limiting the control data during the experimental period to two females. The amount by which data would be limited by the death of this female was thought to be insignificant.

Sample collection

Urine samples were collected throughout the sampling day (09h00 – 14h00 every second calendar day) whenever urine was observed to be present in the collecting dish of the experimental chambers. The chambers had a smooth mesh floor to allow urine to fall through to the collecting dish whilst not allowing faecal contamination. Urine in the collecting dish was removed and frozen at –40 °C. All urine collected for a single female was combined for the sampling day. Each female was fed the same amount of food at the same time each day (11h00). Urine volume that was collected during each sampling day was variable (due to variable indirect fluid intake) but usually in excess of that required for analysis (240 μl).

Creatinine determination

All urine samples were analysed for progesterone concentration. These concentrations had to be corrected, since the urine concentration was variable due to varied fluid intake. The correction was undertaken by analysing each urine sample for creatinine concentration. Creatinine is used to correct for urine concentration as it is a breakdown product of tissue proteins and is excreted at a relatively constant rate. Final results are therefore not expressed as ng progesterone/ml urine but rather as ng progesterone/mg creatinine. This method therefore gives an index by which progesterone concentration can be measured; standardising all progesterone concentrations to that of 1 mg creatinine.

The creatinine concentration of each sample was determined using a modified Jaffe reaction (Folin 1914). Creatinine standards were made up at concentrations of 0, 0.05, 0.1, 0.5, 1.0, 1.5, 2.0 and 2.5 mg creatinine/ml distilled-deionized water (DDIW) from a stock solution of 3 mg creatinine/ml DDIW. Ten microlitres of standard or sample was added to the wells of a microtitre plate (in duplicate) leaving two wells empty as a duplicate blank. To each well (including the blanks) 300µl of picrate reagent was added. Picrate reagent was made up fresh and consisted of saturated picric acid solution, alkaline triton and DDIW (1:1:10). The alkaline triton was, in turn, made up of 4.2 ml triton X-

100, 12.5 ml 1N NaOH and 66.0 ml DDIW. Alkaline triton was only used when homogenous by continuous stirring. The microplate was then left in the dark, at room temperature, to allow colour development for a period of 1.5 hours. The absorbance of the standards, controls, blanks and samples were measured at an optical density of 492 nm using a microplate reader.

A straight line was plotted (absorbance vs creatinine concentration) for the means of the standards. Using simple linear regression, the equation of the straight line was determined. Rearranging the equation for the standard curve and substituting absorbance values for y-values, independent values were interpolated (creatinine concentration) for each sample. Descriptive statistics (mean ± SD) were calculated.

Progesterone determination

Using a plasma-based solid phase ^{125}I RIA (radioimmunoassay) technique designed for the direct, quantitative measurement of progesterone in serum or plasma, (Diagnostic Products Corporation 1998), progesterone concentration in each urine sample was determined. The validity of using the test kit for the detection of progesterone in urine samples was determined by Molteno (1999) who found serial dilutions of urine and plasma to parallel the standard curve

^{125}I-labelled progesterone competes for a fixed time (3 hour incubation period at room temperature; Basic Procedure) with progesterone in the sample for antibody sites. The antibody is immobilized to the supplied polypropylene tubes (1 per sample) and therefore, by decanting the supernatant, competition can be terminated thus isolating the antibody-bound fraction of the radiolabelled progesterone of the tubes. Placing the polypropylene tubes in a gamma counter and counting for a period of 60 s results in a number (counts/min) which by means of a calibration curve could be converted to progesterone concentration (uncorrected for creatinine).

An advantage of this progesterone kit is that decanting the supernatant from the polypropylene tubes can be done vigorously without the loss of antibody-bound material. This results in negligible non-specific binding (NSB) (Coast-a-Count Progesterone Manual, Diagnostic Products, California). During in-house testing of the Basic Procedure (500 tubes) it was found that coefficients of variation (CVs) were low and uniform, the assay being able to detect progesterone concentrations as low as 0.02 ng/ml. The antiserum (rabbit) has a high specificity for progesterone as well as very low cross-reactivity to other naturally occurring steroids present in the samples. Other in-house tests conclude that the assay is accurate over a broad range of progesterone values (evident in extensive spiking recovery and linearity under dilution experiments). Due to the high specificity of the tracer, total counts approximate 70 000 cpm (counts/min) at iodination; maximum binding is approximately 50% (35 000 cpm) for the Basic Procedure.

Four uncoated tubes were labelled in duplicate for NSB and Total Count determination. Fourteen coated tubes were used for seven duplicated standards used to construct the standard curve from which results were interpolated. Standard solutions consisted of 0, 0.1, 0.5, 2.0, 10.0, 20.0 and 40.0 ng/ml. A volume of 100 µl of each standard solution was pipetted into each of the respective tubes. A further 100 µl of 0 ng/ml standard was pipetted into each of the duplicate NSB tubes. A volume of 100 µl of sample was pipetted into each labelled sample tube. A volume of 1.0 ml of ^{125}I Progesterone was added to every tube. The tubes were incubated at room temperature for a period of 3 hours. Following decantation of the tubes, the tubes were placed in a gamma counter for a period of 60 s.

Results of NSB and Total Counts were compared to that given in the kit manual. Using the following calculations, the binding of each pair of tubes could be determined as a percent of maximum binding:

Net count = average count (cpm) – average NSB (cpm)

Percent bound = (net count/net maximum binding count) * 100

Taking the NSB-corrected counts for the 0 ng/ml standard as having a 100% binding, the percent bound of each of the other standards could be determined.

By plotting log (concentration of standard) against the percentage bound, a standard curve was fitted to all the standard concentrations except for the 0 ng/ml as log(0) = undefined. As three assays were undertaken, three standard curves were fitted to each set of points; yielding three standard curves which were in turn used in interpolation for respective samples. An equation was determined for the straight line fitted using simple linear regression with correlation coefficients exceeding 94%.

Corrected progesterone determination

Using the results of the progesterone and creatinine determinations it is possible to correct the progesterone concentrations for the dilution of the sample. Progesterone concentrations (ng/ml) were divided by creatinine concentrations (mg/ml) for each sample. This results in a final, corrected progesterone concentration expressed as ng progesterone/mg creatinine.

Data analysis

Descriptive statistics that were employed in data reduction were the mean ± SD. These statistics were calculated without the outlier data values (<3 % of data eliminated). Means ± SD were calculated for all animals separately for each stage of the experiment therefore giving mean ± SD for eight animals both before (control) and after (experiment) experimental manipulation. Means of each female that were in chemical contact with males and those in contact with males were then compared to the data of females being entirely separated from males; using a series of 1-sample t-tests.

Histology

Qualitative observations were made of the ovaries of the females used in the study. One sample could not be sectioned as preservation had been unsuccessful. Ovaries were removed from the females directly after the last urine sample had been taken. The ovaries were placed in Bouins fixative for preservation. Standard histological techniques were employed to dehydrate, section, stain and mount the ovarian samples (Drury and Wallington 1967). Ovaries were stored in Bouins solution for 2 months prior to being transferred into 70 % ethanol before sequential dehydration in increasing ethanol concentrations. Entire ovaries were then embedded in paraffin wax. Sections of 7 μm thickness were then mounted on slides and stained with Ehrlich's haemotoxylin. Counter-staining was then achieved using eosin. The qualitative analysis was primarily concerned with the absence or presence of follicular development within the ovaries and the presence (or absence) of corpora lutea. Of the sections that were successfully prepared, the clearest of sections were observed, thus the nature of the qualitative approach used in this study.

The process of successful ovulation involves the development and maturation of follicles that produce and support the oocyte prior to an egg (mature oocyte) being released for possible fertilisation. If the egg is fertilised, the corpus luteum will develop further to support pregnancy. If ovulation is unsuccessful, various stages of follicular development are still evident in the ovary but the corpus luteum will degenerate. The presence of primordial follicles indicates early follicular development, these structures being interior to the tunic albuginea in the Highveld mole-rat ovary (Janse van Rensburg 2000). Primordial follicles then proceed through various stages of development. These stages include the development of primordial follicles into primary, secondary and Graafian follicles. The primary follicle is differentiated from primordial follicles by an increase in size as well as a cellular shape change (squamous cells become cuboidal) (Janse van Rensburg 2000). An oocyte will usually be evident within the structure of a primary follicle. Secondary follicles result from further development of primary follicles. The

secondary follicles, although even larger than the primary follicles, undergo a morphological change that makes them clearly identifiable. Areas filled with liquor folliculi appear in the stratum granulosum of the secondary follicle (Janse van Rensburg 2000). Once this fluid-filled space increases in size, the oocyte becomes forced to one side of the follicle. It is at this stage that the follicle can be recognised as being Graafian (Janse van Rensburg 2000). If the follicle, with its oocyte matures further, ovulation will occur - the mature oocyte being released for possible fertilization. A corpus luteum is formed from the mature follicle following successful ovulation. If successful ovulation does not occur, the Graafian follicle, secondary follicle or primary follicle will degenerate – evident by the presence of atretic follicles (Janse van Rensburg 2000).

Gestation period and development

Gestation period was estimated from two pairs of individuals and was considered to be from the time of observed coitus of each of the pairs to birth (days). Two animals were paired on the 12th October, the male separated on the 15th October. A further two animals were paired on the 17th October.

Postnatal Growth

Three pups (two males and a female) were used in this study. Pups were weighed daily prior to feeding (between 11h00 ad 13h00) until individuals reached a relatively constant mass. This stage was evident when the curve of mass vs age reached a plateau. Attempts were made to weigh pups from day of birth (day 1) but this usually resulted in a decline of pup health and an increase in mother-pup disturbance. The weighing of pups could therefore only be started when pups were not constantly with the mother. Pups were always kept with the same animals in the holding chamber as at birth. Mass was rounded to the nearest gram as daily variation in weight due to feeding time was evident.

Several different growth models have been employed to analyse the growth data of bathyergid species. These techniques include the use of the Gompertz, Logistic and von

Bertalanffy mathematical models. To date, the most successful model has been the Gompertz equation due to the least residual sum of squares for bathyergid species comparison (Bennett *et al.* 1991, Bennett & Navarro 1997). From the Gompertz equation, it is possible to estimate maximum growth rate, age at maximum growth rate, mass at maximum growth rate and maximum mass. Maximum growth rate allows various taxa to be compared to one another (Bennett *et al.* 1991). Zullinger *et al.* (1984) found the Gompertz model to be the best compromise; since there is minimal difference in the models except at early and late growth periods where the Gompertz model is superior. In one study (Zullinger *et al.* 1984) the von Bertallanfy model was shown to be superior, intraspecifically, while the Gompertz model was shown to be the superior model when comparing species. As it was a goal of the study to compare the growth rate of the study species with other bathyergids, the Gompertz model was chosen.

Due to the difficulty of breeding subterranean, fossorial rodents successfully in the laboratory very few complete sets of individual growth data could be obtained. Although in the time allocated for the study there were numerous births in the laboratory, a large proportion of the pups did not survive. This study concentrates on the postnatal development (growth) of the loosely social mole-rat *Cryptomys hottentotus pretoriae* (Moolman *et al.* 1998). It was predicted that, due to the social status of the species, pups would show slow postnatal development evident in a slow maximum growth rate.

As two of the three pups were only first weighed when they were three days old, the mass at birth could not be accurately calculated. Another important advantage of using the Gompertz function is the reliability when the y-intercept values (day 0=day of birth) is unknown (Zullinger *et al.* 1984).

Parameters that could be estimated from the Gompertz function were: maximum mass (A), the maximum potential for growth (B) and immediate growth rate (R). The Gompertz function, for this study, is represented as:

$$Y(T) = A * e^{-B * R^{T}}$$

Other parameters which could be estimated from the data, using the Gompertz function, were: maximum growth rate (GR), age at maximum growth rate (TM) and mass at maximum growth rate (YTM). All of these parameters that were estimated could be statistically analysed for significant sexual dimorphisms, even though there were only three growth curves. These statistical analyses were undertaken by means of bootstrapping.

Bootstrapping

The technique of bootstrapping is considered to be a useful, valid and an important tool in statistical analyses of data sets that fall short in terms of sample size for other statistical procedures to be employed. In the case of growth data, bootstrapping uses each of the original data sets in order to allow descriptive statistics to be estimated for that particular data set. For each of the three data sets, 400 points were chosen at random around the area of the curve that showed the highest growth rate. Each of these points was subjected to an analysis that estimated the rest of the curve based on that point. This resulted in 400 'curves' being estimated from 400 randomly chosen points of the original curve. Descriptive statistics could then be calculated based on these 400 curves therefore allowing comparisons between pups, and sexes, to be made. Thus, for each of the three original growth curves, parameters were estimated from 400 curves obtained from bootstrapping of the original curve.

Data Analysis

Two of the three curves had data for a period of longer than one year. These two curves were therefore constructed and analysed for data of a 365 day period. Anomalous variation in one of these curves after 365 days prohibited analysis of data beyond the 365 day period. All three curves had data for a period of 318 days. Therefore, a second,

separate analysis was undertaken which included all three data sets. The results of these two separate analyses were then compared to each other in order to determine whether the 365 day data set could be eliminated so that all results could be based on the 318 day data sets.

Comparisons were also made pertaining to the six variable parameters listed above. Comparisons were made between all three sets of data as well as between sexes.

Bathyergid species comparisons were made on mean maximum growth rates only. Where mean maximum growth rates were not reported, calculations were made to estimate this parameter. Providing that the maximum mass (A in g) and the growth rate constant (K in $days^{-1}$) were reported, the mean maximum growth rate (MGR in $gday^{-1}$) could be calculated as follows:

$$\mathbf{MGR = K * A * e^{-1}}$$

In order to use mean maximum growth rate as an interspecies comparative tool, the assumption is made that this rate of growth is constant over the animal's entire growth period until it has reached its adult mass (Bennett *et al.* 1991).

Results

Ovulation

Control

The control data (before experimental manipulation) revealed that non-reproductive females had a basal corrected progesterone concentration below 32 ng progesterone/mg creatinine. Mean basal concentrations ranged from 13.5 to 31.1 ng progesterone/mg creatinine.

Experimental manipulation

Females that were only in chemical (pheromonal) contact with novel males showed no visually significant increase in corrected progesterone concentration compared to basal concentration (that when alone). Each mean for the female placed in chemical contact was statistically tested against the mean basal concentration. No significant differences were evident. See Table 2 for a summary of the statistical outputs.

Females that were in physical contact with novel males all showed a highly significant result when comparing mean basal concentration (that where alone) Mean progesterone values measured for these females ranged between 47.5 ng progesterone/mg creatinine to 57.5 ng progesterone/mg creatinine. However, these values are means over the entire experimental period for each of the females. These values provide no indication of how the progesterone varies and the data could be seen as being a straight line along these mean values. With the use of descriptive statistics, standard deviations from the mean were calculated for each of the females placed in physical contact with a male. From these standard deviations a range for progesterone concentration were obtained showing that progesterone concentration is indeed highly variable and the variance may be an order of magnitude within a single individual during a relatively short period of time. The standard deviations associated with each of the means are reported in Table 1.

Table 1: Descriptive statistics calculated for each of the females that were placed in physical contact with novel males.

Individual	Highest Concentration	Lowest Concentration	Mean ± SD	2*SD = Range
AC1	82.31	26.29	54.30 ± 28.01	56.02
AC2	80.79	34.29	57.54 ± 23.25	46.50
AC3	70.70	24.35	47.53 ± 23.17	46.35

Table 2: Results of 1-sample t-tests concerning corrected progesterone concentration. Mean corrected basal concentration (n=10) of separately housed non-breeding females compared to experimental data for each experimentally manipulating non-breeding female. The arithmetic mean of the basal concentration was 19.30 ng progesterone/mg creatinine. The critical t-statistic for each test was 2.26. *=significant rejection of Ho.

Statistical Test	Test Statistic |Tcalc|	Result (Reject/ Do not reject Ho)	Associated probability
AS1 EXPT VS MEAN BASAL CONC	0.32	Do not reject	0.753
AS2 EXPT VS MEAN BASAL CONC	1.01	Do not reject	0.324
AS3 EXPT VS MEAN BASAL CONC	1.05	Do not reject	0.307
AC1 EXPT VS MEAN BASAL CONC	18.19	Reject	< 0.001*
AC2 EXPT VS MEAN BASAL CONC	19.87	Reject	< 0.001*
AC3 EXPT VS MEAN BASAL CONC	14.67	Reject	< 0.001*

From individually constructed progesterone profiles (Figures 1 – 3), it is evident that changes in corrected progesterone concentration only occurred in those females who were placed in physical contact with vasectomized novel males. The changes in corrected progesterone concentration were also only evident after experimental manipulation. This gives further credibility to the results.

If females were spontaneous ovulators, it would be apparent that they would be cycling to some continuous, regular and predictable degree in the corrected progesterone concentrations while the females were alone. From the data that were obtained there appears to be little, if any, cycling pattern evident that would be conclusive to mapping an oestrous cycle. This appears to be the case in individuals that were housed alone and those in chemical contact with males. Females that were in physical contact with males

exhibit a cycle. However, it was not possible, from this data, to map an oestrous cycle accurately.

Table 3: Results of the qualitative histological analysis showing the presence or absence of various stages of follicular development and whether ovulation has occurred (shown by the presence of corpora lutea).

Female ID	Primordial Follicles	Primary Follicles	Seondary Follicles	Graafian Follicles	Lutenized Unruptured Follicles	Atretic Follicles	Corpus Lutea
A2	Present	Present	Absent	Absent	Present	Present	**Absent**
A3	Present	Present	Present	Absent	Present	Present	**Absent**
AS2	Present	Present	Absent	Present	Present	Present	**Absent**
AS3	Present	Present	Absent	Present	Absent	Present	**Absent**
AC1	Present	Absent	Absent	Absent	Present	Present	**Present**
AC2	Present	Present	Absent	Present	Absent	Present	**Present**
AC3	Present	Present	Absent	Absent	Present	Present	**Present**

All individuals (except AC1) had primordial as well as primary follicles present. Secondary follicles were generally absent. Graafian follicles were only found in AS females as well as a single AC female. They were not present in A animals. Luteinized unruptured follicles were present in most samples, except AS3 and AC2. All females showed evidence of follicular regression as there were atretic follicles present in all samples. Most importantly, corpora lutea were only found in AC females.

Courtship and copulation

Precopulatory behaviour is initiated by a head-to-head confrontation between the two reproductive animals. The male then sniffs and nuzzles the female around her flanks and the ano-genital area and whilst doing so he emits a chip chip chip call. The female raises her tail and emits a series of high pitched squeals resembling iz-iz-iz, kicks her hind feet backwards into the face of the male. The male grooms the rump of the female by gently nibbling her fur with his incisors. Courtship can last between 60 and 180 seconds. Following the foreplay and if the female is receptive, the male slides over the back of the female and intromission proceeds. During a successful intromission, the female remains

stationary, ceases vocalising, lifts and thrusts her posterior into the face of the male and adopts a lordodis-like posture with her tail upturned against her back. The female braces herself against the burrow using her forepaws. The male grips her lumbar region with his forepaws and thrusts at a rate of between 3-4 thrusts per second. Thrusting becomes slower, but deeper towards the end of copulation that terminates with the female emitting a high pitched prolonged squeal. At the end of copulation the male and female separate and groom their genitalia with their forefeet and mouth. Mating almost always takes place in the burrow.

Gestation and Litter Size

Few copulation events were observed in the laboratory. Two animals were paired on the 12th October and the male separated from the female on the 15th October 2000. A pup was born on the 18th December, 2000 providing a gestation period of between 63 and 66 days. A further two animals were paired on the 17th October, 2000 producing a litter of two pups on the 16th December 2000, providing a maximum gestation period of 64 days.

Litters born in the laboratory ranged from a single pup to three pups; usually two pups were born to a female (1.6 ± 0.8, n=6).

Postnatal Development

New born pups are 2-2.5cm long, hairless and pink, except for purple pigmentation around the cheeks. The eyes and auditory meatus are closed, the digits of the feet are well formed and clawed and the incisors have erupted. If placed on their backs, the pups can right themselves with difficulty. The pups produce high frequency cries and mewing sounds when suckling.

By day 8 the pups have a slatey grey fur that is darker around the saddle and head region. Solid foods are consumed on day 14. Sibling-sibling sparring occurs around day 40. By

day 14 the pelage is shaggy, slatey grey to charcoal in colour and has a whorled appearance. The pups were fully weaned around day 32.

The bootstrapping technique allowed descriptive statistics to be calculated for the parameters of each growth data set. Two of these parameters (growth potential; B and immediate growth; R) were not analysed statistically as they revealed little information. Descriptive statistics for these parameters were however calculated and are presented in Table 4. Data collected for the 365 day period are not used further in this study.

Table 4: Mean ± standard deviation from bootstrapping analyses of parameters from the Gompertz equation not shown in Figures 5 - 6; B=growth potential and R=immediate growth.

Data Set	Sex	Data up to (days)	N	B ± SD (ratio)	R ± SD (ratio)
1*	Female	318	295	2.12 ± 0.07	0.996 ± 0
2**	Male	318	295	2.38 ± 0.04	0.996 ± 0
3	Male	318	299	2.83 ± 0.08	0.996 ± 0
4*	Female	365	341	2.13 ± 0.03	0.996 ± 0
5**	Male	365	341	2.30 ± 0.02	0.995 ± 0

*Data set 1 and 4 are from the same individual.

**Data set 2 and 5 are from the same individual.

Table 5: Results of statistical comparisons of relevant data sets. Values indicated are probabilities. The null hypothesis was rejected at $p<0.05$.

Data Sets Compared	**MGR**	TM	YTM
1* and 2**	0.000***	0.747	0.001***
1 and 3	0.000***	0.021***	0.000***
2 and 3	0.013***	0.016***	0.092
4* and 5**	0.000***	0.229	0.000***

*Data set 1 and 4 are from the same individual.

**Data set 2 and 5 are from the same individual.

***The null hypothesis is rejected.

Maximum growth rates ranged from 0.23 gday-1 to 0.39 $gday^{-1}$. Males had a maximum growth rate of 0.36 ± 0.00 $gday^{-1}$ and 0.39 ± 0.01 $gday^{-1}$ (Figure 6). The maximum

growth rate of the female was lower (0.23 ± 0.00 $gday^{-1}$). These results were based upon between 295 and 299 data points that were calculated for each curve using bootstrapping techniques. Maximum growth rates calculated using 341 data points do not significantly differ from using the slightly shorter data sets. The maximum growth rates from males and females using 341 data points are 0.23 ± 0.00 $gday^{-1}$and 0.35 ± 0.00 $gday^{-1}$ for the female and male respectively (Figure 6).

It is evident that there are significant differences in maximum growth rates for all relationships investigated. The maximum growth rates of males appear to be higher than that of females. However, when comparing data sets 2 and 3 (both males) it would be expected that the null hypothesis would be accepted as the maximum growth rates for the males are very similar. When statistically comparing these means and their standard deviations, it is evident that there is a significant difference between the data sets of the males in terms of maximum growth rate. This result should be treated with caution due to the very low standard errors inherent in using 400 randomly chosen data points for bootstrapping analyses.

Males and females reached their maximum growth rates at different masses. For males, maximum growth rate was achieved at a mass of 88.7 g (318 day data) and 107.8 g (365 day data). These results were not statistically different from one another. When the mass at maximum growth rate for the female is compared to that of the males (for 318 and 365 day data respectively) it is evident that the mass at which a female attains her maximum growth rate (61.6 g or 62.1 g) is statistically different from that of males.

The age at which males and females reach their maximum growth rates does not appear to be strictly attributed to sex. Males reached their maximum growth rate at ages of 211.1 ± 15.5 days old and 287.9 ± 27.98 days old (for 318 days of data) and 184.0 ± 7.0 days old (for 365 days of data). This gives a range of ages where males can reach their maximum growth rate of between 184.0 ± 70 days old and 287.9 ± 27.9 days old. The female reached her maximum growth rate at 201.6 ± 25.11 days old (318 days old) and 204.2 ± 15.3 days old (for 365 days of data). Although there was a significant difference

between the age at which males and females reached their maximum growth rates (for the 318 day data), there was also a significant difference between the age at which males reached their maximum growth rate (for 318 days of data). Also, there was no significant difference between age of maximum growth rate when comparing males and female data for 365 days.

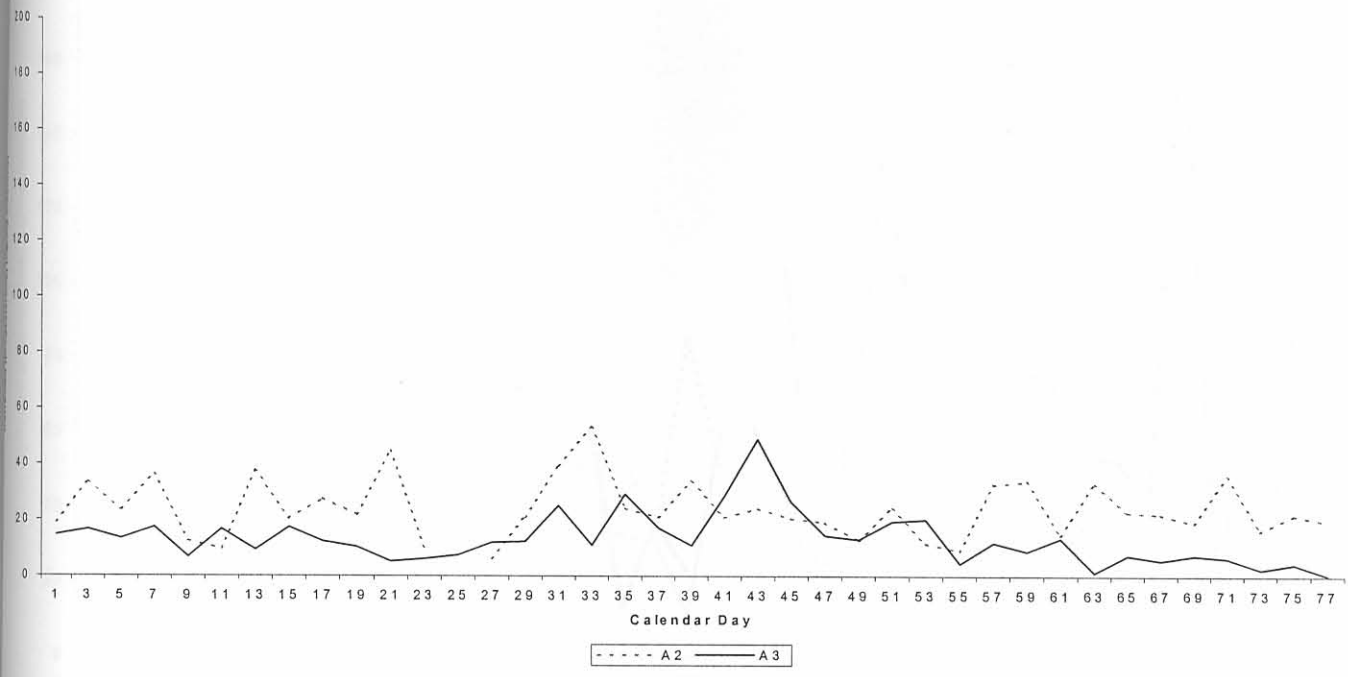

Figure 1: Progesterone profiles for non-reproductive females (n=2) while housed completely on their own for the entire duration of the study.

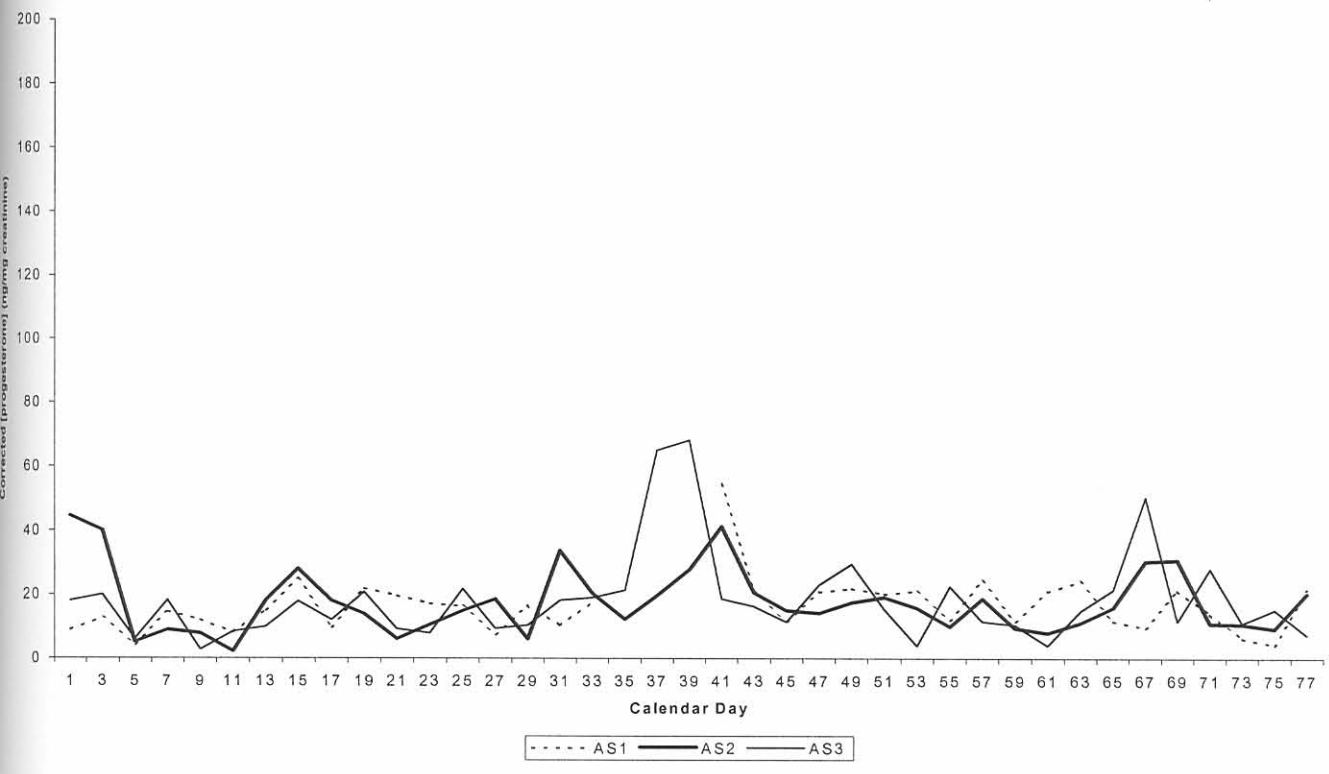

Figure 2: Progesterone profiles for non-reproductive females (n=3) while housed completely on their own (day 1 – 40) and then physically separated from a novel male (day 41 – 77).

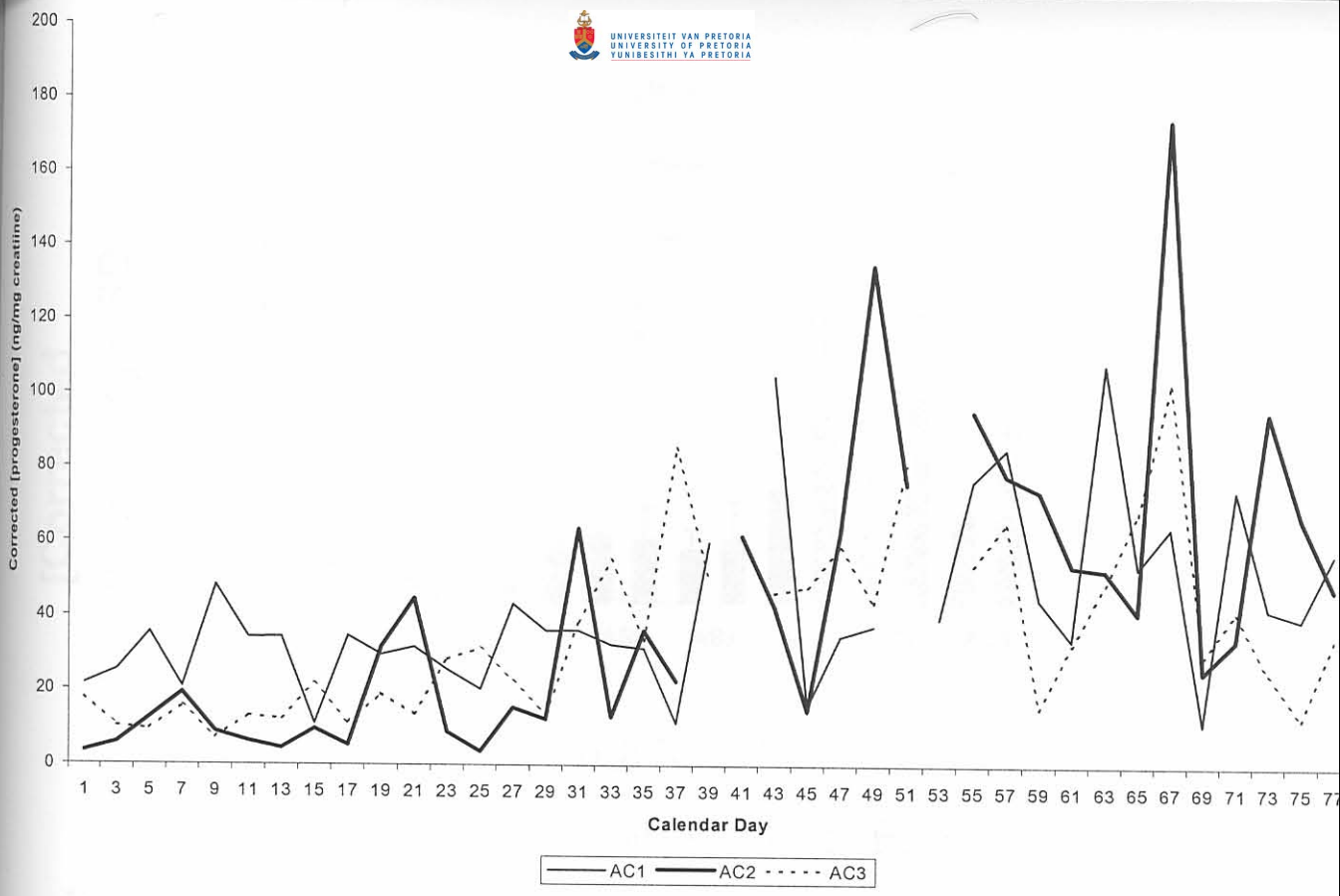

Figure 3: Progesterone profiles for non-reproductive females (n=3) while housed completely on their own (day 1 – 40) and then placed in physical contact with a novel, vasectomised male (day 41 – 77).

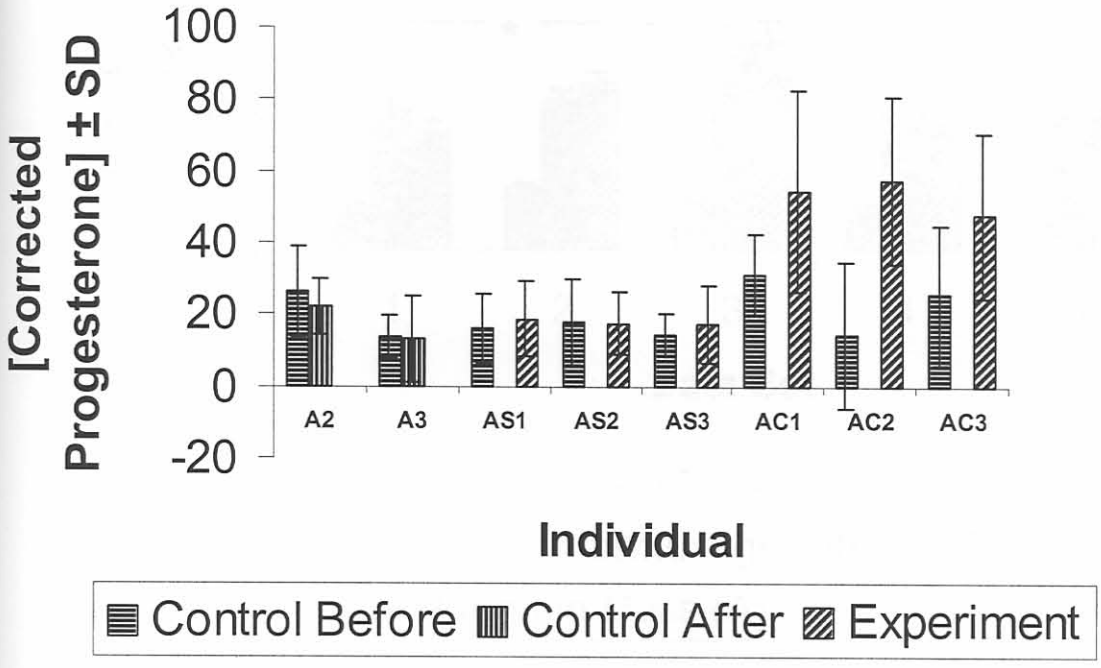

Figure 4: Corrected progesterone concentrations for each non-reproductive female for each part of the study; while totally alone (control before and control after) and while physically separated or in contact with novel males (experiment).

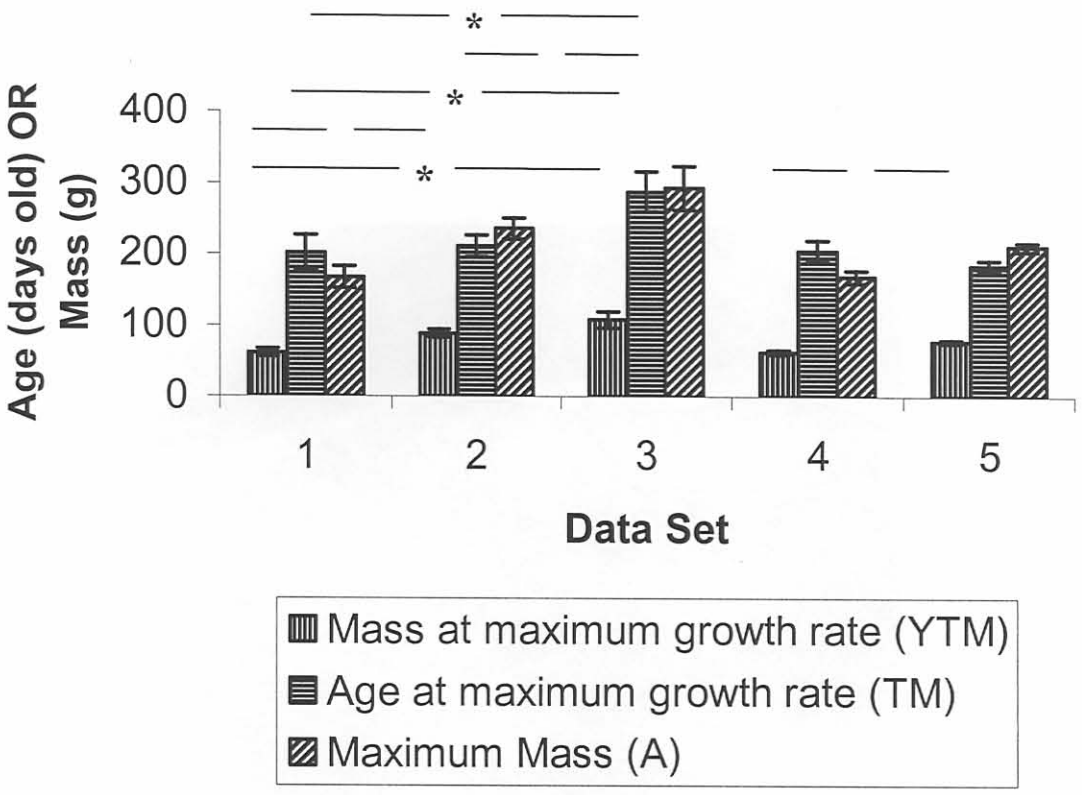

Figure 5: A comparison of results obtained from the different data sets. Data sets varied in terms of the amount of data used in the analyses. Parameters that are compared are mass at maximum growth rate (YTM), age at maximum growth rate (TM) as well as maximum mass (A).

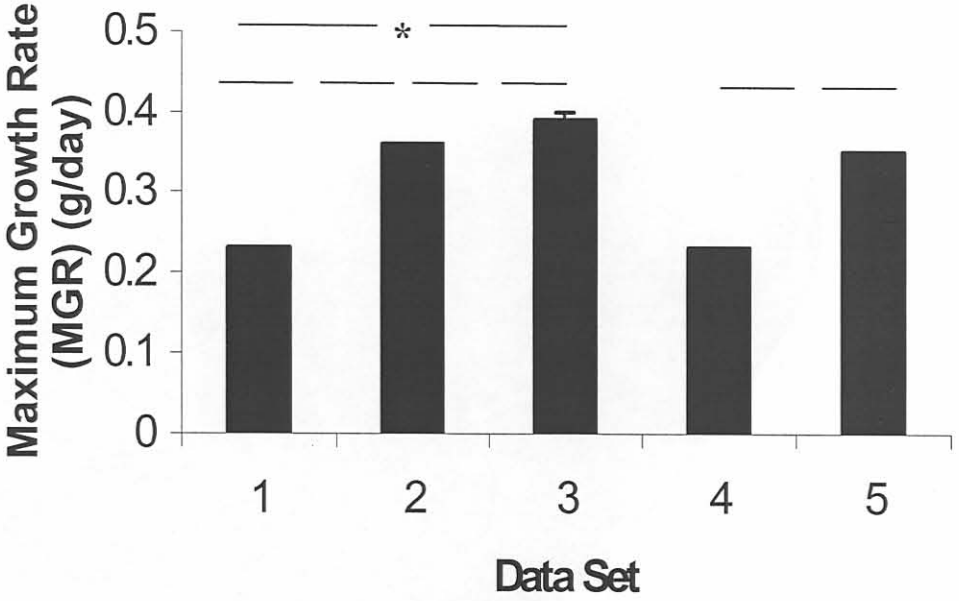

Figure 6: A comparison of results obtained from the different data sets. Data sets varied in terms of the amount of data used in the analyses. The parameter that was compared is maximum growth rate (MGR).

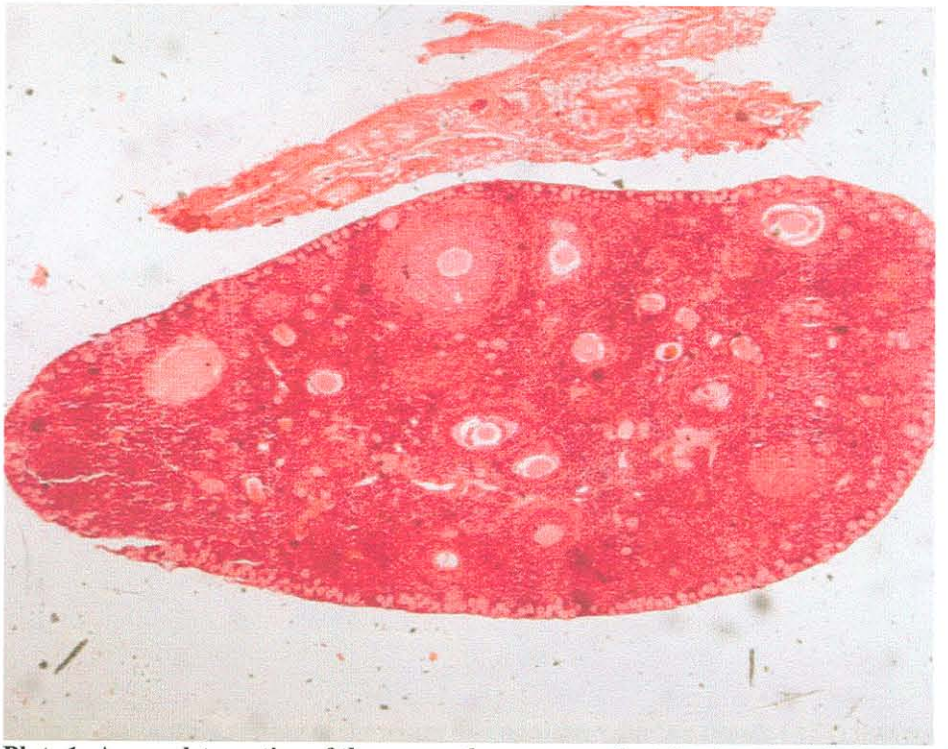

Plate 1: A complete section of the ovary of a non-reproductrive female (A1) that was housed alone for the entire duration of the study. Note the absence of a corpus luteum. Magnification = 550X.

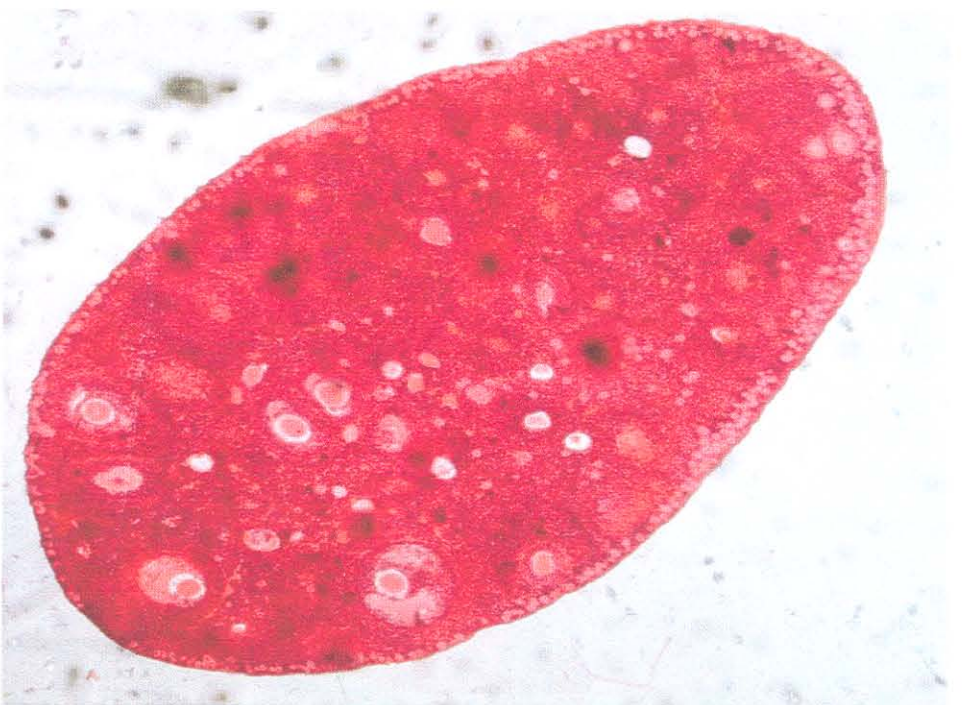

Plate 2: A complete section of the ovary of a non-reproductive female (A2) that was housed alone for the entire duration of the study. Note the absence of corpus luteum. Magnification = 550X.

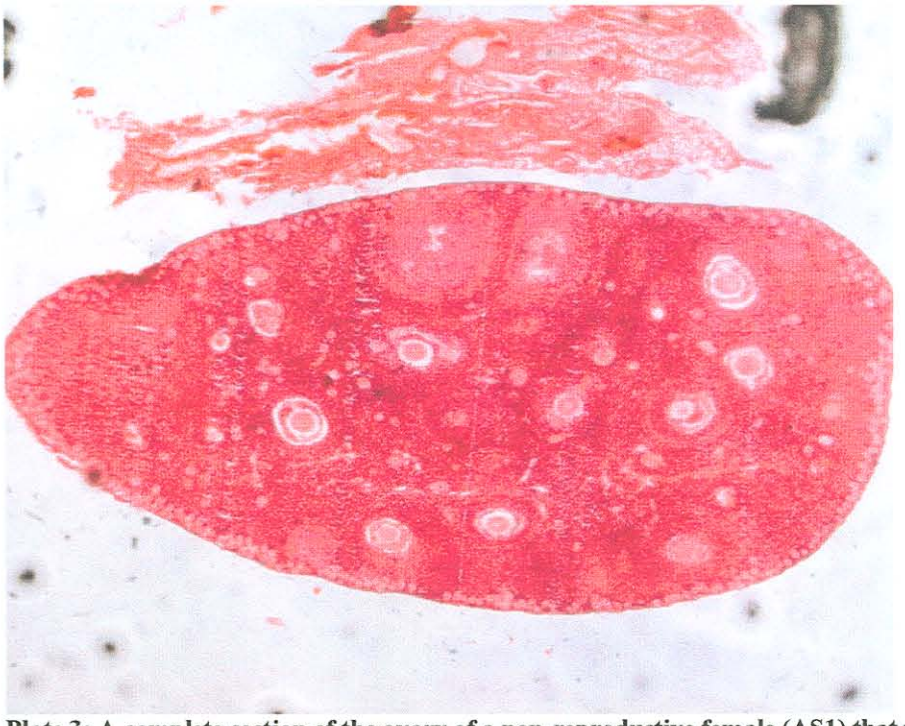

Plate 3: A complete section of the ovary of a non-reproductive female (AS1) that was physically separated from a novel male for the latter half of the study. Note the absence of corpus luteum. Magnification = 550X.

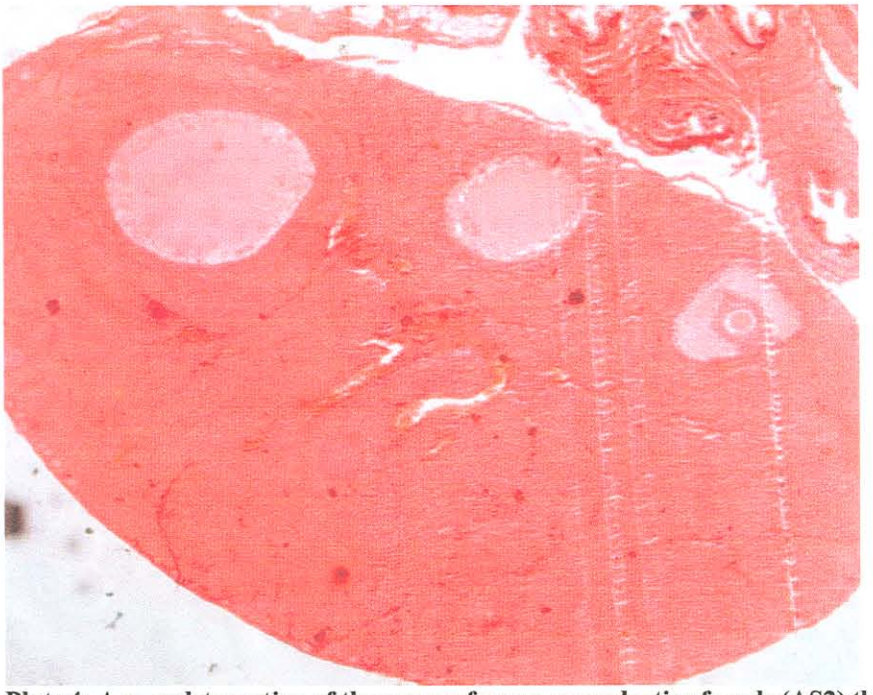

Plate 4: A complete section of the ovary of a non-reproductive female (AS2) that was physically separated from a novel male for the latter half of the study. Note the absence of corpus luteum. Magnification = 550X.

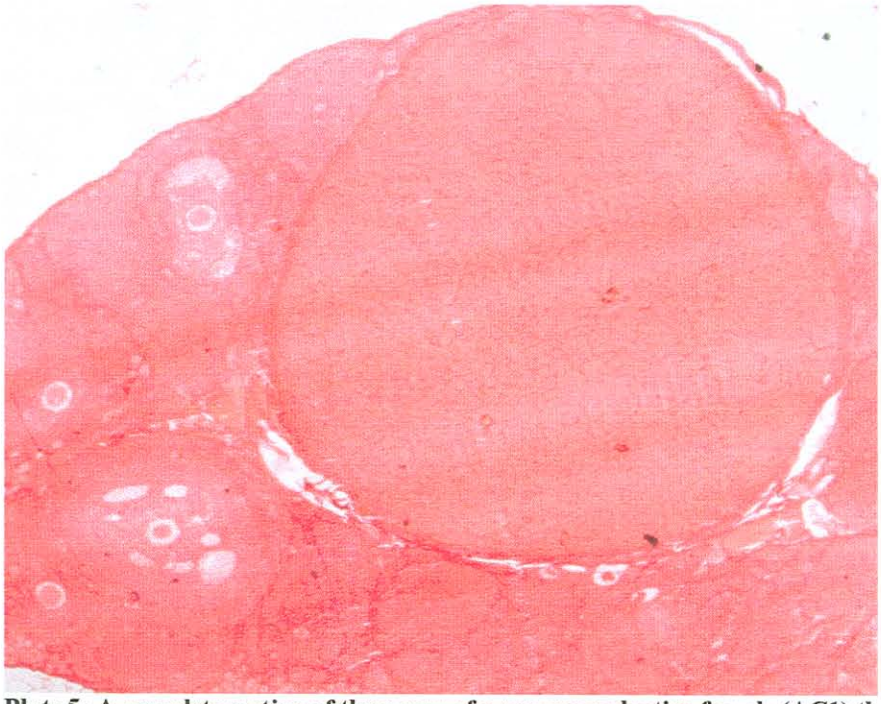

Plate 5: A complete section of the ovary of a non-reproductive female (AC1) that was placed in physical contact with a novel, vasectomised male for the latter half of the study. Note the presence of corpus luteum. Magnification = 550X.

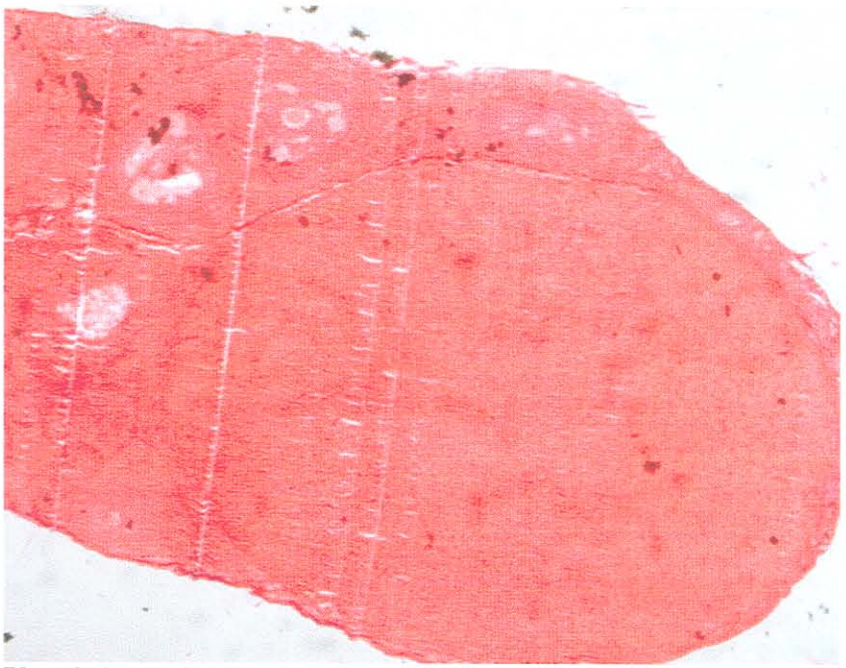

Plate 6: A complete section of the ovary of a non-reproductive female (AC2) that was placed in physical contact with a novel, vasectomised male for the latter half of the study. Note the presence of corpus luteum. Magnification = 550X.

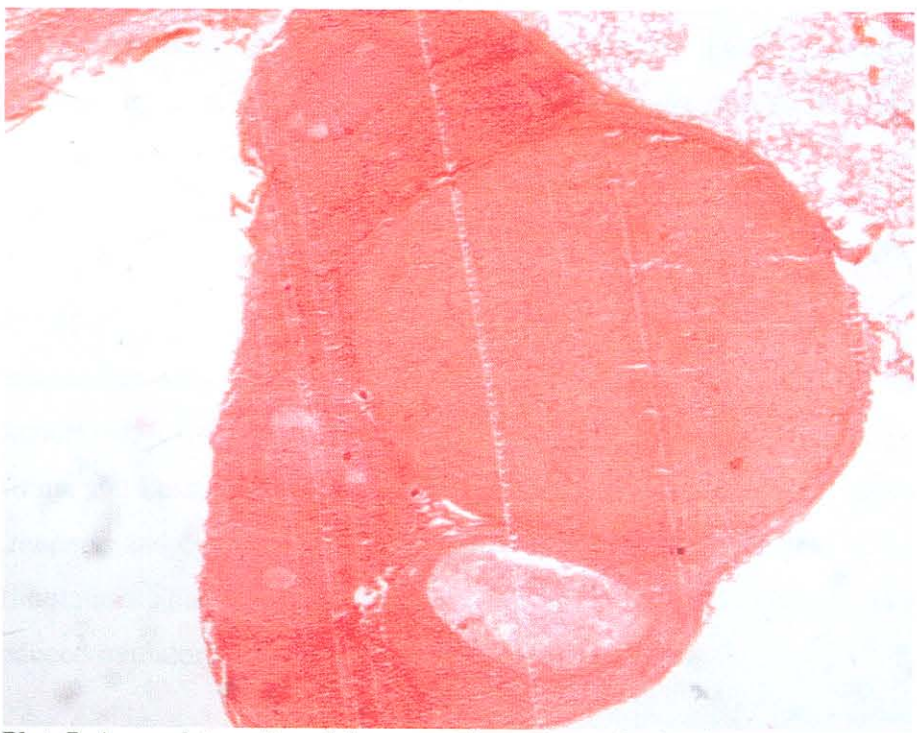

Plate 7: A complete section of the ovary of a non-reproductive female (AC3) that was placed in physical contact with a novel, vasectomised male for the latter half of the study. Note the presence of corpus luteum. Magnification = 550X.

Discussion

The family Bathyergidae comprises a range of species that exhibit a broad spectrum of social systems. Indeed, they are one of the few truly subterranean mammals that exhibit species that are social (Jarvis & Bennett 1990). Similarly, they exhibit a wide range of reproductive patterns that span the realms of strict incest avoidance as is the case in the Mashona mole-rat through to complete physiological suppression as is evidenced in the eusocial naked mole-rat (Bennett & Faulkes 2000).

Many solitary subterranean rodents appear to be induced ovulators. It would seem that induced ovulation is the rule in solitary mammals (Zarrow & Clark, 1968). Among the Ctenomyidae, *Ctenomys talarum* is known to be an induced ovulator (Weir, 1974). Altuna and Lessa (1985) studied the penile morphology of a Uruguayan species of *Ctenomys* and concluded that the spines on the glans provided cervico-vaginal stimulation. Shanas *et al.* (1995) has suggested that *Spalax ehrenbergi* may also be an induced ovulator.

In the female brush-tailed bettong (*Bettongia penicillata*) ovulation is induced by the male. This is evident from the absence of corpora lutea in the ovaries of separated females (Hinds & Smith 1992). The male is thus important as a stimulus for follicular growth and subsequent ovulation (Hinds & Smith 1992).

McClintock (1983), working on rats, reported that the number of intromissions could be correlated with successful pregnancy. Copulation acts on the neuroendocrine reflex in the female with a resultant surge in prolactin and increased progesterone levels required to maintain pregnancy (McClintock 1983).

To date, in social bathyergids, there are few data pertaining to the method of ovulation. Faulkes, Abbott & Jarvis (1990) and Faulkes *et al.* (1990) found the naked mole-rat to exhibit spontaneous ovulation, since if a non-breeding female was removed from a

colony and housed singly, she underwent a normal oestrous cycle. It appears from this study that the seasonally breeding social highveld mole-rat is an induced ovulator. This method of ovulation probably has evolved as a consequence of living in an environment with a marked seasonality to it.

The progesterone data revealed that, unlike many hystricomorph rodents outside of the bathyergid family (such as the guinea pig and chinchilla) and the naked mole-rat (*Heterocephalus glaber*), the highveld mole-rat shows elevated progesterone concentrations (relative to creatinine) only when females are in direct, physical contact with males.

Basal progesterone concentrations were generally low in non-reproductive females housed alone. The females that remained alone during the experimental manipulation as well as those that were in chemical contact with males revealed little evidence of any change in progesterone concentration.

Histological examinations revealed that successful ovulation had occurred in the females that had been placed in direct, physical contact with males. This was evident by the presence of a single, large corpus luteum in each ovary examined. In females where no corpora lutea were observed, there was evidence of follicular development. Follicular development was characterized by the presence of primordial and primary follicles. Graafian follicles were observed in females that were placed in chemical contact with males (AS females). Atretic follicles resulting from incomplete follicular development were evident in all females. Hence follicular development occurred in all females removed from the colony, yet ovulation was blocked by the lack of coitus.

It is interesting that females placed in chemical contact with males showed increased follicular development when compared to females housed alone. All of these females (AS females) had an ovarian histology that was more developed than that of females housed alone. This was evident from the presence of Graafian follicles. It appears as though an olfactory stimulation of the female by the male is enough to stimulate the female to

produce further stages of follicular development but the stimulation is not sufficient to cause successful ovulation, since corpora lutea were absent in the ovaries of these females.

The naked mole-rat and Damaraland mole-rat are the only bathyergids currently found to be spontaneous ovulators. (Faulkes *et a.l.* 1990 and Molteno 1999). Spontaneous ovulation is expected in an aseasonal breeder since females must be fertile all year round. In a seasonal breeder such as the highveld mole-rat, induced ovulation is the predicted method of ovulation. The Bathyergidae is an interesting taxon as member species appear to exhibit different methods of ovulation.

The restriction of reproduction to a single female in a colony is a feature common to all social bathyergids (Bennett & Faulkes 2000). Courtship and pre-copulatory behaviours at the onset of the breeding season shows a trend within the Bathyergidae. In the solitary *G. capensis,* precopulatory behaviours at the onset of the breeding season may possibly be directed at breaking down the strong aggression normally exhibited towards conspecifics. Courtship is initiated by the male signalling to the female through the soil substratum separating their burrows by drumming with the hindfeet. (Bennett & Jarvis 1988a). In the common mole-rat, *C. h. hottentotus*, foreplay is again initiated by the male, however, since the animals occupy the same burrow system for the whole year there is low intensity interaction by both animals. These interactions include vocalizations and the smelling of the genitalia prior to copulation (Bennett 1989). In the Damaraland mole-rat, *Cryptomys damarensis*, the situation is reversed, and the female initiates courtship (Bennett & Jarvis 1988b). While in the naked mole-rat once again it is the female who is the solicitor (Jarvis 1981). When copulation was observed during this study, it was evident that courtship is initiated by the male highveld mole-rat consisting of sniffing, nuzzling, vocalization as well as precopulatory grooming. This display of behaviour by the highveld mole-rat fits the trend of males of loosely social and solitary bathyergid species initiating courtship, while in the more social species, courtship is initiated by the reproductive female.

The relatively long gestation period of the highveld mole-rat of approximately 66 days is comparable to that of the phylogenetically closely related common mole-rat (Bennett 1989). The gestation period of *G. capensis* is relatively short 45-50 days (Bennett & Jarvis 1988a), when compared to that of the naked mole-rat 66-74 days (Jarvis 1991); Damaraland mole-rat 78-92 days (Bennett & Jarvis 1988b) or Giant mole-rat 111 days (Bennett & Aguilar, 1995). It is unusual for small mammals to have such long gestation periods and supports the close taxonomic affinity that the bathyergids have with the hystricomorphs (Bennett & Jarvis 1988a).

The litter size of the highveld mole-rat is within that expected for a social bathyergid (and subterranean rodent) with litter size being approximately 2 pups. Litter sizes of subterranean rodents are generally very small (Bennett *et al.* 1991, Malizia & Busch 1991); ranging from 1 –13. Solitary bathyergid species may have larger litters (1-10) (Bennett & Faulkes 2000) compared to social species (1-5) (Bennett & Faulkes 2000).

There appears to be no overall pattern in the development of pups of the bathyergids. The young are all relatively altricial. They move out of the nest within a few days of birth and begin to supplement their food intake with solids from day 14 (Bennett *et al.* 1991). Although pups of eusocial bathyergids seem to eat solids at a relatively early age (when compared to social and solitary bathyergids), there is no clear trend evident. Pups of the highveld mole-rat begin consuming solids at approximately day 14; which is similar to that of the eusocial *H. glaber* (day 14), the social *C. darlingi* (day 14) and the solitary *B. janetta* (day 13) (Bennett & Faulkes 2000). Weaning in the highveld mole-rat occurs at about day 32, which is similar to *C. darlingi* and *G. capensis* (solitary).

Trends can be seen in the growth rates of pups within the Bathyergidae (as well as between bathyergids and other subterranean mammals and hystricomorph rodents). The pups of solitary species channel energy into rapid growth. They disperse at an early age and for this it is advantageous to be large and strong so as to be able to dig effectively and defend their own burrow. The pups of social species do not have such selection

pressures operating upon them, instead the pups must fit into the colony hierarchy and eventually join the colony work force.

Subterranean mammals adaptively converge on a variety of traits (Nevo 1979). However, contrasting lifestyles caused by different constraints lead to disparate patterns of development of young and associated growth rates (Bennett *et al.* 1991). Most subterranean mammals (including hystricomorphs) have long breeding cycles (Burda 1989); bathyergids having long periods of postnatal development (Bennett & Faulkes, 2000). Young of mammalian species can be altricial (under developed at birth) or precocial (fully developed at birth other than size) and nidifugous (feed own once weaned) or nidicolous (parentally fed). One or more of these traits can accurately describe the way that a pup is likely to behave. Solitary species usually have precocial and nidifugous young, while social species tend to have altricial young. Altricial species nest almost exclusively below ground, in burrows, caves or tree holes (Bennett *et al.* 1991).

Pups of solitary bathyergid species are strong, large and ready to disperse and establish themselves within several months of birth, whereas pups of social species are usually altricial and remain in their natal colony indefinitely (Bennett *et al.* 1991). Pups of social species thus rely more on their close relatives for foraging and care of themselves. These factors have implications on growth (Bennett *et al.* 1991; Bennett & Navarro, 1997).

Mammalian species have two main mechanisms that cause a variation in growth rates; a physiological mechanism (which can be limiting to growth or allowing an increase in growth) as well as the mechanism of the selective values of differential growth rates (Case 1978).

There are two ways that growth rates can be adjusted metabolically, namely through rates of energy assimilation and the efficiency at which assimilated nutrients are converted into living tissue (Case 1978). It has been hypothesized that the slowest growth rates should be found in precocial, nidifugous young as a greater proportion of their energy

requirements are invested in foraging and temperature regulation (Case 1978). This is because they usually have larger birth weights and thus more tissue to support metabolically.

Body size and growth rates have been found to be highly heritable traits; thought to be a function of the potency of growth hormone from the anterior pituitary. Species specific growth rates are genetically determined and are adjusted adaptively in order to meet the forces of the environment (Case 1978). Selection pressures acting on the mother usually favour altricial offspring particularly when the nest is a safer environment for a pup to complete development in, than when compared to the womb (Case 1978). Another case where altricial young would be favoured occurs when foraging requires so much skill and experience that no matter how precocial the pup is, the pup could not effectively compete with adults (Case 1978).

There are several factors which could lead to the selection of a, usually, faster growth rate. For example, faster growth rates could be selected for in order to reduce the risk of infants to predation (Williams 1966 and Lack 1968).

All *Cryptomys* species are social and have a slower mean growth rate than their solitary bathyergid counterparts (Bennett *et al.* 1991). Social bathyergids have a lower resting metabolic rate than solitary species (Bennett *et al.* 1991) and this may result in a lower postnatal growth rate. Several socio-biological factors are thought to act as forces on the selection of a slower postnatal growth rate in social bathyergids. Young are not forced to take full responsibility for their own well-being from birth as pups are incorporated into a hierarchy which cooperate in meeting the foraging needs of the colony (Bennett *et al.* 1991).

Table 6: Summary of maximum growth rates for several bathyergid species.

Species	Social Status	Mean Maximum Growth Rate ($gday^{-1}$)	Reference
Bathyergus suillus	Solitary	3.3	Bennett *et al.* 1991
Bathyergus janetta	Solitary	1.68	Bennett *et al.* 1991
Georychus capensis	Solitary	1.22	Bennett *et al.* 1991
Cryptomys sp. (anselli)	Social	0.08 – 0.63*	Burda 1989
Cryptomys hottentotus pretoriae	Social	0.23 – 0.39**	This study
Cryptomys hottentotus hottentotus	Social	0.229	Bennett *et al.* 1991
Cryptomys damarensis	Eusocial	0.233	Bennett *et al.* 1991

*dependent on sex, colony environment (whether with or without parents) and stage of development (of which three stages were identified).

**dependent on sex.

Table 7: Summary of maximum growth rate for other subterranean mammals.

Species	Mean Maximum Growth Rate ($gday^{-1}$)	Reference
Tachyoryctes ruandae	1.35	Zullinger *et al.* 1984
Cannomys badius	2.01	Zullinger *et al.* 1984
Thomomys talpoides	2.21	Zullinger *et al.* 1984

Table 8: Summary of growth rates of other hystricomorph rodents (cited in Case 1978).

Species	Growth Rate ($gday^{-1}$)	Reference
Cavia sp.	3.2 – 4.5*	Brody 1945, Rood 1972
Proechimys semispinosus	2.1 – 2.5**	Tesh 1970
Hoplomys gymnurus	2.4	Tesh 1970, Weir 1974
Microcavia australis	1.9	Rood 1970, 1972
Erethizon dorsatum	21.2	Walker 1964, Weir 1974
Galea musteloides	2.4	Rood 1972

*dependant on species.

**dependant on sex.

It is clear that social bathyergids have a much slower maximum growth rate compared to solitary bathyergids, hystricomorph rodents and subterranean rodents (non-hystricomorphs). The study species, *C. h. pretoriae*, exhibits the trend found for social bathyergid species in which an increase in sociality is met with a decrease in maximum growth rate. With a maximum growth rate of 0.23 – 0.39 $gday^{-1}$, it is apparent that the altricial pups of this species have a very low maximum growth rate like other social bathyergids; which is much lower than that estimated for solitary bathyergids, other hystricomorph rodents as well as other subterranean rodents (non-hystricomorphs).

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