Hormones and Behavior 176 (2025) 105836

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Hormonal lockdown: How mole-rat societies enforce infertility in helpers

A.K. Janse van Vuuren [a,*], T. Süess [a], Kyle Finn [a,c], N. Hagenah [a,b], A. Ganswindt [a,b], D.W. Hart [a,*,1], N.C. Bennett [a,b,1]

[a] Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield, 0028, South Africa
[b] Mammal Research Institute, University of Pretoria, Private Bag X20, Hatfield, 0028, South Africa
[c] Linnaeus University, Department of Biology and Environmental Science, 391 82 Kalmar, Sweden

ARTICLE INFO

Keywords:
Reproductive suppression
Prolactin
Androgen-mediated aggression
Cooperative breeding
Social dominance

ABSTRACT

Reproductive suppression is a defining feature of cooperative breeding, yet the hormonal mechanisms regulating infertility in non-breeding individuals remain poorly understood. This study examines associations between circulating prolactin (PRL) and faecal metabolites of androgens (fAM), glucocorticoids (fGCM), and progesterone (fPM) in relation to socially induced infertility in two closely related cooperatively breeding mole-rat subspecies: the highveld (*Cryptomys hottentotus pretoriae*) and Natal mole-rat (*C. hottentotus natalensis*). Our results reveal a fundamental dichotomy in reproductive suppression strategies. Highveld mole-rats exhibited seasonally elevated circulating PRL in non-breeders during the dry season, coinciding with reduced pituitary responsiveness to exogenous gonadotropin-releasing hormone (GnRH) and lower gonadal steroid metabolites. These associations are consistent with PRL involvement in physiological suppression of the hypothalamic-pituitary-gonadal (HPG) axis, but causality cannot currently be inferred. In stark contrast, Natal mole-rats showed relatively low and stable PRL levels across reproductive groups and seasons, with breeders exhibiting higher androgen and progesterone metabolites compared to non-breeders, a pattern consistent with behavioural suppression through incest avoidance, aggression or social dominance. These findings highlight the adaptive flexibility of cooperatively breeding systems and provide new insights into the hormonal architecture of reproductive suppression. Although the present findings do not establish causality, they delineate key hormonal and behavioural pathways that warrant future investigation.

1. Introduction

Mammals exhibit a wide variety of group-living strategies and social structures, with cooperative breeding being one of the most intriguing (Clutton-Brock, 2021). In this social system, individuals apart from the parents help raise offspring, and this complex social behaviour is shaped by specific behavioural and physiological adaptations (Solomon and French, 1997). While its exact definition has been widely debated, three widely accepted criteria classify a species as a cooperative breeder, namely, delayed dispersal of offspring beyond reproductive maturity, socially induced infertility in subordinate adults (helpers), and the provisioning of alloparental care and other cooperative behaviours by helpers (Solomon and French, 1997). Only a small subset of mammalian species meet these criteria, resulting in diverse social systems that range from solitary living through to highly cooperative groups, with some even resembling the eusocial organisation seen in certain insect species (Ben Mocha et al., 2023; Bennett and Faulkes, 2000; Clutton-Brock, 2021; Jarvis and Bennett, 1993; Sherman et al., 1995).

While delayed dispersal (Koenig et al., 1992; Nelson-Flower et al., 2018) and cooperative care (Gachomba et al., 2024; Griesser et al., 2025) are relatively common in social mammals, the defining feature of cooperative breeding, is socially induced infertility in subordinate adults (Bennett et al., 1996; Dunbar and Shultz, 2021; Lukas and Clutton-Brock, 2012), which poses an apparent paradox in light of natural selection (Darwin's, 1859). In these systems, reproduction is monopolised by a dominant breeding pair, leading to significant reproductive skew (Bennett and Faulkes, 2000; Saltzman et al., 2009). Helpers perform essential group maintenance tasks and provide alloparental care, but do not reproduce (Bennett and Faulkes, 2000; Saltzman et al., 2009). This reproductive suppression occurs primary through two mechanisms: behavioural suppression, which includes inbreeding avoidance and socially enforced mating inhibition (often associated with androgen-

* Corresponding authors.
E-mail addresses: andriesvanvuuren10@gmail.com (A.K.J. van Vuuren), daniel.hart@up.ac.za (D.W. Hart).
[1] **Equal contributions.**

https://doi.org/10.1016/j.yhbeh.2025.105836
Received 2 April 2025; Received in revised form 22 August 2025; Accepted 23 September 2025
Available online 1 October 2025

mediated aggression but not exclusively so), and physiological suppression, where disruption of the hypothalamic-pituitary-gonadal (HPG) axis prevents gametogenesis and ovulation (Abbott, 1984; Bennett and Faulkes, 2000; Wasser and Barash, 1983). These mechanisms are not mutually exclusive, and their relative importance varies across taxa. For example, in meerkats (*Suricata suricatta*), dominant females frequently suppress subordinate behaviourally through aggression, eviction, and infanticide, yet subordinate females also show reduced fertility associated with stress-axis activation and impaired gonadal hormone production (Drea and Davies, 2022; Thavarajah et al., 2014; Young and Clutton-Brock, 2006). In marmosets and tamarins (Callitrichidae), suppression is primarily physiological: subordinate females often fail to ovulate due to disruption of gonadotropin secretion, a mechanism mediated by social cues and possibly pheromonal signals (Abbott et al., 1988; Mustoe, 2023; Saltzman et al., 2009). In contrast, in some cooperatively breeding carnivores, such as dwarf mongooses (*Helogale parvula*) and wild dogs (*Lycaon pictus*), reproductive skew is maintained largely through behavioural mechanisms, including aggression and mate guarding by dominants, though endocrine correlates of suppression are also evident (Creel, 2005; Creel and Waser, 1991; Milewski et al., 2022; Spiering et al., 2010). The African mole-rat family (Bathyergidae) provides an exceptional model for investigating the various forms of reproductive suppression in cooperative mammals (Barker et al., 2021; Bégay et al., 2022; Bennett and Faulkes, 2000; Faulkes and Bennett, 2001; Hart and Bennett, 2022; Wallace et al., 2023a, 2023b). These subterranean rodents exhibit a continuum of sociality, ranging from strictly solitary species through to highly social, eusocial-like species (Bennett and Faulkes, 2000; Hart et al., 2022a). In social African mole-rat species, reproduction is typically restricted to a single breeding pair, while the remaining adult group members function as non-reproductive helpers (Bennett and Faulkes, 2000). The degree and mechanism of reproductive suppression varies between species, with some exhibiting lifelong prepubescent states and others maintaining reproductive capability, but refraining from breeding due to social constraints (Bennett and Faulkes, 2000). One of the primary tools for assessing reproductive suppression in African mole-rats has been the measurement of pituitary sensitivity to gonadotropin-releasing hormone (GnRH) (Bennett et al., 2000, Bennett et al., 1997, Bennett et al., 1996, Bennett et al., 1993; Faulkes et al., 1990; Oosthuizen et al., 2008; Spinks et al., 2000; Van der Walt et al., 2001). The administration of exogenous GnRH and subsequent changes in circulating luteinizing hormone (LH) provide insights into whether reproductive suppression is physiological or behavioural (Bennett et al., 2000, Bennett et al., 1997, Bennett et al., 1996, Bennett et al., 1993; Faulkes et al., 1990; Oosthuizen et al., 2008; Spinks et al., 2000; Van der Walt et al., 2001). In cases of physiological suppression, helpers exhibited a reduced LH response, indicative of an inhibited HPG axis, whereas behaviourally suppressed individuals maintained a functional response comparable to breeders (Bennett et al., 2000, Bennett et al., 1997, Bennett et al., 1996, Bennett et al., 1993; Faulkes et al., 1990; Oosthuizen et al., 2008; Spinks et al., 2000; Van der Walt et al., 2001).

Despite extensive research on the HPG axis in African mole-rats, the precise hormonal drivers of physiological and behavioural reproductive suppression remain unclear. Previous studies have largely discounted stress-related mechanisms, such as activation of the hypothalamic-pituitary-adrenal (HPA) axis or opioid-mediated inhibition, as primary factors (Edwards et al., 2020; Edwards, 2022; Hart et al., 2022b, 2022c, 2024; Majelantle et al., 2023; Majelantle et al., 2024; Molteno and Bennett, 2002a, 2002b; Du Toit et al., 2006a, 2006b; Toor et al., 2022; Wallace et al., 2023a, 2023b). Instead, variations in glucocorticoids appear to be more closely linked to metabolic demands rather than chronic stress or reproductive suppression (Hart et al., 2023; Jimeno and Verhulst, 2023; Majelantle et al., 2024; Vullioud et al., 2021). Recent attention has shifted to prolactin (PRL) as a key modulator of socially induced infertility. Typically associated with lactation and parental care, PRL is also capable of suppressing the HPG axis when elevated, as seen in conditions such as hyperprolactinemia (>20.0 ng/ml) (Bennett et al., 2018; Hart et al., 2022b; Medger et al., 2019).

Across mole-rat species, a clear pattern emerges: in species where physiological suppression is dominant, such as in female Damaraland (*Fukomys damarensis*) and Mahali mole-rats (*Cryptomys hottentotus mahali*), as well as both sexes of naked mole-rats (*Heterocephalus glaber*), PRL-mediated inhibition of the HPG axis appears to be the primary mechanism (Hart et al., 2022c; Medger et al., 2019; Medger et al., 2018; Voigt and Bennett, 2018; Bennett et al., 2018). However, the specific form of PRL-mediated suppression varies by species. For example, in the well-studied naked mole-rat and the lesser-studied Mahali mole-rat, PRL levels are significantly elevated in physiologically suppressed helpers, likely reinforcing its role in infertility in these species (Bennett et al., 2018; Hart et al., 2022b; Medger et al., 2019; Medger et al., 2018; Voigt and Bennett, 2018). In contrast, in the Damaraland mole-rat, physiologically suppressed female helpers do not exhibit hyperprolactinemia (Bennett et al., 2018), but instead show increased PRL receptor expression in key brain regions, suggesting localised PRL effects despite normal circulating levels of PRL (Voigt and Bennett, 2018). This mechanism likely operates by inhibiting kisspeptin neurons, reducing GnRH activation, and thereby suppressing reproduction (Hackwell et al., 2025; Voigt and Bennett, 2018). These findings suggest that both central and peripheral pathways may mediate PRL effects, but the exact mechanisms remain unresolved. Meanwhile, in species where behavioural suppression is the primary mechanism, such as male Damaraland and Mahali mole-rats and female common mole-rats (*C. h. hottentotus*), reproductive inhibition is primarily behaviourally driven. In these species, aggression (often androgens, such as testosterone, mediated) and incest avoidance inhibits reproduction in the absence of PRL mediation (Bennett et al., 2018; Hart et al., 2021a, 2021b, 2022b; Lutermann et al., 2013; Spinks et al., 1999; Wallace et al., 2023a, 2023b).

To better understand the hormonal links to the mode of reproductive suppression this study examines two closely related cooperatively breeding mole-rat subspecies, the highveld mole-rat (*C. h. pretoriae*) and the Natal mole-rat (*C. h. natalensis*). Previous research, involving the administration of exogenous GnRH to individuals across different reproductive classes, sexes, and seasons (Du Toit et al., 2006a, 2006b; Oosthuizen et al., 2008; Van der Walt, 2003; Van der Walt et al., 2001), revealed distinct patterns of the mechanism of reproductive suppression orchestrated on the subordinate non-breeding individuals. Namely, in highveld mole-rats, non-breeding males and females exhibited a significantly reduced LH response during the dry season, indicating a pattern consistent with physiological suppression (Du Toit et al., 2006a, 2006b; Oosthuizen et al., 2008; Van der Walt, 2003; Van der Walt et al., 2001). This suppression was alleviated during the wet season, suggesting a seasonal relaxation of reproductive inhibition (Du Toit et al., 2006a, 2006b; Oosthuizen et al., 2008; Van der Walt, 2003; Van der Walt et al., 2001). In contrast, Natal mole-rats showed a consistent LH response across all seasons, indicating a functionally intact HPG axis in non-breeders of both sexes throughout the year (Du Toit et al., 2006a, 2006b; Oosthuizen et al., 2008; Van der Walt, 2003; Van der Walt et al., 2001). This pattern aligns with a behavioural suppression model, where reproduction is inhibited primarily through incest avoidance and social dominance and aggression rather than endocrine disruption. These findings highlight a fundamental divergence in reproductive suppression strategies: highveld mole-rats exhibit seasonally regulated physiological suppression, whereas Natal mole-rats rely on year-round behavioural suppression through social interactions.

Building on this framework, the present study investigates whether PRL-driven suppression coincides with physiological infertility in highveld mole-rats, in contrast to the potential behavioural suppression (potentially mediated through androgens) in Natal mole-rats. By examining plasma PRL levels, and faecal metabolites of androgens (fAM), glucocorticoids (fGCM), and progesterone (fPM) across reproductive classes and seasons, we aimed to: (1) determine whether PRL levels correlate with physiological reproductive suppression in highveld

mole-rats, (2) assess whether androgens are elevated in the dominant breeding males and female Natal mole-rats, and (3) and finally evaluate how seasonal changes influence the expression of these hormonal mechanisms.

2. Methods

2.1. Animal capture

A total of 154 Natal mole-rats (breeding females [BF]: $n = 25$; non-breeding females [NBF]: $n = 50$; breeding males [BM]: $n = 26$; non-breeding males [NBM]: $n = 53$; Table S1) were captured at Glengarry Holiday Farm, Kamberg Valley, KwaZulu-Natal, South Africa (29°19′24.3"S, 29°42′32.8″E)(Süess et al., 2024). In addition, 134 highveld mole-rats (BF: $n = 24$, NBF: $n = 49$, BM: $n = 33$, NBM: n = 26; Table S1) were captured in the National Botanical Garden of Pretoria, Gauteng, South Africa (25°44′13.92"S, 28°16′24.24″E) between January and December 2020, in two distinct seasons (the wet and dry)(Süess et al., 2024). Mole-rats were caught using Hickman live traps (Hickman, 1979) baited with sweet potatoes, which were placed at the entrances of excavated tunnels and checked every two hours.

Upon capture, animals were weighed (±1 g), sexed, and categorised as either reproductive (breeders) or non-reproductive (non-breeders). Natal mole-rats were transported to an on-site field laboratory, while highveld mole-rats were taken to the Department of Zoology and Entomology, University of Pretoria (Süess et al., 2024). Entire colonies were housed together in plastic containers with sweet potatoes, apples, wood shavings, and paper towelling for nesting material (Süess et al., 2024). The food provides all water requirements, as mole-rats obtain their water exclusively from their food (Bennett and Jarvis, 1995; Hart et al., 2022a). Animals remained in captivity until all colony members were captured and sampled (Natal mole-rats: 5.1 ± 2.5 days; highveld mole-rats: 4.2 ± 2.7 days), after which they were released back into their burrows (Süess et al., 2024). A colony was considered fully captured when no further trapping activity occurred for at least 48 h (Süess et al., 2024).

2.2. Determination of reproductive status

Juveniles (< 40 g body mass) and solitary individuals were excluded from this study (Hart et al., 2021a, 2021b; Süess et al., 2024). Breeding males were generally the largest males within their colonies and were distinguished from NBMs by their large, descended inguinal testes and yellow staining around their mouths (Hart et al., 2021a, 2021b; Süess et al., 2024). Breeding females were identified by their prominent auxiliary teats and perforated vaginas, features absent in NBFs (Hart et al., 2021a, 2021b; Süess et al., 2024).

2.3. Blood collection

Blood samples were taken from each individual upon capture by pricking the dorsal pedal tarsal vein of the hindfoot with a sterile 23G needle. Blood was collected using a heparin-coated microhaematocrit capillary tube and transferred to sterile vials containing heparin. Blood volumes collected did not exceed 1 % of body mass (0.3–0.5 ml per animal). After sampling, the puncture site was stemmed with tissue paper, and animals were returned to their colonies. Whole blood was centrifuged at 500*g* for 15 min to separate the plasma, which was stored at −80 °C until hormone analysis. Blood samples were collected between 09:00 and 12:00. Given that mole-rats inhabit a subterranean environment devoid of light and do not exhibit circadian entrainment to photoperiod, it is unlikely that the timing of sampling introduced systematic variation in circulating hormone concentrations (Bennett et al., 2025; Finn et al., 2022; Grenfell et al., 2024; Hart et al., 2021a, 2021b; van Jaarsveld et al., 2019).

2.4. Faecal sample collection and steroid extraction

After capture, individuals were isolated for faecal sample collection. To minimise the influence of capture-induced stress and to ensure that fGCM concentrations reflected pre-capture endocrine status, all samples were collected within 20 h of capture (Hart et al., 2022c). Faecal samples were collected using sterilized tweezers, frozen, and stored in Eppendorf tubes at −20 °C until further analysis at the Endocrine Research Laboratory of the University of Pretoria.

Faecal samples were lyophilised, pulverised, and sieved through a wire-mesh strainer. Between 0.05 and 0.06 g of faecal powder was extracted with 1.5 ml of 80 % ethanol. Suspensions were shaken for 15 min on a multi-vortexer and then centrifuged for 10 min at 1500*g*. The supernatant was transferred to a clean Eppendorf tube and stored at −20 °C until analysis.

Faecal steroid measurements are widely used in mammals as they provide an integrated measure of hormonal output over time, rather than a single time-point snapshot. This approach is particularly valuable in our wild populations as it reflects endocrine activity while animals are in their natural environment and avoids stress-induced alterations in circulating hormone levels that may occur during capture and handling. Previous studies in rodents and other mammals have demonstrated strong correlations between faecal and circulating concentrations of testosterone, progesterone, and cortisol (Capezzuto et al., 2008; Mohan et al., 2020; Yimer, 2010), supporting the reliability and validity of this method in our study species.

2.5. Hormone analysis

Plasma PRL concentrations were quantified using an enzyme immunoassay (EIA) (Elabscience guinea pig PRL Elisa kit E-EL-GP0358, batch number: AK0017JUL11012) following the manufacturer's guidelines and successfully used previously for mole-rats by Bennett et al. (2018). Intra- and Inter-assay coefficients of variation (CV) of high-, medium-, and low-concentration controls were 5.54 %, 4.88 % and 4.05 % (Intra-assay), and 6.56 %, 4.39 %, and 3.04 % (Inter-assay), respectively. Sample dilutions differed from neat to 1/50, and the sensitivity of the assay was 0.1 ng/ml. According to the manufacturer's user manual, no significant cross-reactivity or interference between Guinea pig PRL and analogues has been observed. Samples were randomised prior to analysis and measured in duplicates, if sufficient volume was available, using a total of 15 plates. The standard curve is provided in the supplementary material (Fig. S2 and S3).

A Cortisol (highveld mole-rats) and Oxoaetiocholanolone I (Natal mole-rats) EIA were used to quantify fGCMs, and concentrations reported in ng/g of faecal dry weight (DW) (Janse van Vuuren, 2022). Faecal androgen metabolites (fAM) were quantified using an Epiandrosterone EIA (Janse van Vuuren, 2022), and concentrations were reported in μg/g DW. Faecal progesterone metabolite (fPM) concentrations were measured using a 5a-Tetrahydroprogesterone EIA (Janse van Vuuren, 2022) and reported as μg/g DW. Detailed assay characteristics, including full descriptions of the assay components and cross-reactivities, have been provided by Palme and Möstl (1997) for the cortisol and 11-oxoetiocholanolone EIA, by Palme and Möstl (1993) for the Epiandrosterone EIA, and by Szdzuy et al. (2006) for the 5a-Tetrahydroprogesterone EIA. Sample dilutions differ from 1/5 to 1/100 for fGCM and 1/10–1/1000 for fAM and fPM analyses. The sensitivity of the EIAs was 0.75 ng/g dry weight (DW) (cortisol), 1.2 ng/g DW (11-oxoetiocholanolone I); 7.2 ng/g DW (Epiandrosterone), and 6.0 ng/g DW (5a-Tetrahydroprogesterone EIA). Intra-assay CV of high-concentration and low-concentration controls were 4.42 % and 6.24 % (cortisol), 5.33 % and 6.14 % (11-oxoetiocholanolone I), 4.96 % and 5.09 % (Epiandrosterone), and 4.36 % and 6.67 % (5a-Tetrahydroprogesterone), respectively. Inter-assay CV of high-concentration and low-concentration controls were 8.62 % and 12.39 % (cortisol), 4.26 % and 7.98 % (11-oxoetiocholanolone I), 13.45 % and 13.52 %

(Epiandrosterone), and 11.82 % and 13.64 % (5a-Tetrahydroprogesterone), respectively. Samples were randomised prior to analysis and measured in duplicate, using a total of 33 plates for all analyses. All assays were performed at the Endocrine Research Laboratory, University of Pretoria, as described by Ganswindt et al. (2012, 2014).

2.6. Statistical analysis

All statistical analyses and visualisations were performed using R version 4.4.0 and GraphPad Prism (version 8.4.3), with statistical significance set at $p \leq 0.05$. Data are presented as mean ± standard error (SE). Truncated violin plots were used to visualise the distribution of the data over the observed range of data. The width of each violin indicates the density of observations at a given value. The bold dashed line represents the median, and the thin dashed lines represent the interquartile range.

The assumptions of normality and homogeneity of variance for the dependent variables, including plasma PRL, fGCM, fAM, and fPM concentrations, were tested using Shapiro–Wilk and Levene's tests, respectively. Even after log-transformation, the dependent variables did not meet assumptions of normality or homogeneity of variance. Accordingly, we employed generalised linear models (GLMs) fitted with gamma distributions and link-identity functions using the *lme4* package.

A GLM was employed to assess how plasma PRL concentrations were affected by species (Natal and highveld mole-rats), season (wet and dry), sex (male and female), reproductive class (breeder and non-breeder), and their two-, three-, and four-way interactions. As species differences emerged as the primary driver of PRL variation (see Table 1), subsequent analyses were performed separately for each sex within each species. This approach allowed us to account for potential sex-specific variation and species-specific suppression mechanisms, thereby providing a clearer interpretation of the hormonal patterns observed. As such, separate GLMs were performed to examine the effect of season and reproductive class on plasma PRL, fAM, and fPM concentrations within male and female Natal and highveld mole-rats, respectively. Body mass was included in all GLMs. Linear regression analyses were used to investigate correlations between fAM and fPM with plasma PRL concentrations in male and female individuals of both species.

Two separate GLMs, one for Natal mole-rats and one for highveld mole-rats, were conducted to assess the effects of season, sex, reproductive class, and their two- and three-way interactions on fAM concentrations. Similarly, separate GLMs were used to evaluate the same variables' effects on fGCM concentrations for both mole-rat species separately. Body mass was included in all GLMs. Species were analysed separately to account for species-specific differences in fAM and fGCM production.

Post hoc comparisons were carried out using Tukey's Honestly Significant Difference (HSD) tests to identify significant pairwise differences. Effect sizes were estimated using partial eta squared (η^2) for GLMs and R^2 values for linear regression analyses, using the *effectsize* package in R.

Table 1
The statistical outputs of the effects of species [highveld (*Cryptomys hottentotus pretoriae*) and Natal (*Cryptomys hottentotus natalensis*) mole-rat], sex [male and female], season [wet and dry] and reproductive class season [breeding and non-breeding], their two, three- and four-way interactions and body mass on plasma prolactin concentrations.

Fixed term	η^2	t value	*p*-Value
Body Mass	0.01	−2.27	**0.03***
Species	0.20	5.36	**<0.0001***
Sex	1.70e^{-06}	−0.24	0.81
Season	0.01	0.16	0.88
Class	8.83e^{-03}	0.53	0.59
Species*Sex	8.69e^{-05}	0.09	0.93
Species*Season	0.01	−0.35	0.73
Sex*Season	3.49e^{-04}	1.00	0.32
Species*Class	9.59e^{-03}	−0.80	0.42
Sex*Class	1.20e^{-03}	0.01	0.99
Season*Class	0.02	0.02	0.99
Species*Sex*Season	2.06e^{-05}	−0.55	0.58
Species*Sex*Class	4.93e^{-05}	0.31	0.76
Species*Season*Class	0.02	−0.87	0.38
Sex*Season*Class	1.96e^{-03}	−1.19	0.24
Species*Season*Class*Sex	9.50e^{-04}	0.42	0.68

Significant relationships ($p \leq 0.05$) are indicated with '*'.

3. Results

3.1. Prolactin defines physiological-based socially induced infertility

Highveld mole-rats exhibited significantly higher plasma PRL levels than Natal mole-rats (Fig. 1, Table 1). While some highveld mole-rats displayed PRL levels indicative of hyperprolactinemia, all Natal mole-rats maintained comparatively lower PRL levels (Fig. 1). Species differences emerged as the primary driver of PRL variation (Table 1). Additionally, an inverse relationship between body mass and PRL levels was observed, with smaller individuals exhibiting higher plasma PRL concentrations (Table 1). No other significant effects were detected (Table 1).

In male highveld mole-rats, a significant season*class interaction affected PRL levels ($\eta^2 = 0.05$, $t = -2.36$, $p = 0.03$). Non-breeding males captured in the wet season had significantly lower plasma PRL levels than those captured in the dry season ($p = 0.006$) and breeders from both seasons ($p \leq 0.006$, for both) (Fig. 1). Similarly, in female highveld mole-rats, PRL levels were significantly affected by the season*class interaction ($\eta^2 = 0.03$, $t = -2.05$, $p = 0.04$). Non-breeding females in the wet season exhibited lower PRL levels than those in the dry season ($p = 0.05$) (Fig. 1). All remaining *post-hoc* comparisons were not significant ($p > 0.05$) (Fig. 1). During the wet season, no NBM or NBF highveld mole-rats exhibited hyperprolactinemia, whereas several individuals did so during the dry season (Fig. 1). In contrast, breeding females in both wet and dry seasons consistently exhibited high PRL levels (Fig. 1). Body mass had opposing effects on PRL levels in male and female highveld mole-rats, with a significant inverse relationship observed in males (η^2

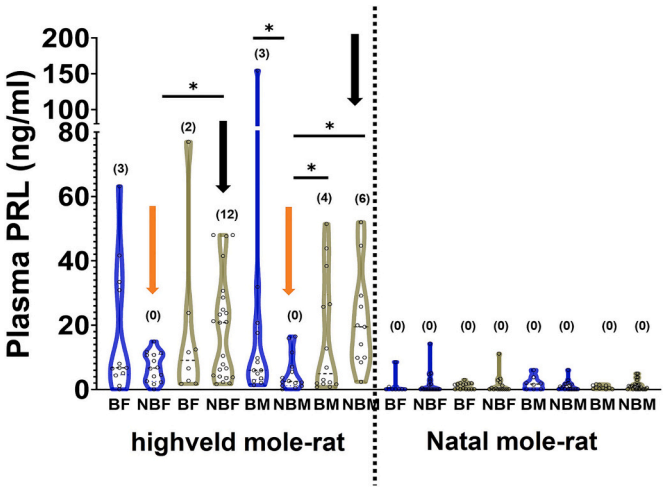

Fig. 1. Circulating plasma prolactin (PRL) levels (ng/ml) in highveld (*C. h. pretoriae*) and Natal (*C. h. natalensis*) mole-rats across reproductive classes: breeding males (BM), breeding females (BF), non-breeding males (NBM), and non-breeding females (NBF). Numbers in brackets indicate individuals with clinical hyperprolactinemia (≥ 20.0 ng/ml). Brown bars represent dry-season samples; blue bars represent wet-season samples. Black arrows indicate physiological suppression, while orange arrows indicate its relaxation. Significant relationships (Tukey's HSD post-hoc comparisons: $p \leq 0.05$) are marked with '*'.

= 0.03, $t = -2.91$, $p = 0.001$) and a significant positive relationship in females ($\eta^2 = 0.07$, $t = 1.96$, p = 0.05). However, neither sex showed significant independent effects of season ($\eta^2 \leq 0.04$, 0.02, $t \leq 0.58$, $p \geq 0.57$) or reproductive class ($\eta^2 \leq 0.05$, $t \leq 1.52$, p ≥ 0.57).

In male Natal mole-rats, no significant differences in circulating PRL levels were detected with respect to season ($\eta^2 = 0.002$, $t = 1.83$, $p = 0.07$) or reproductive class ($\eta^2 = 0.02$, $t = 0.23$, $p = 0.82$). However, the season*class interaction ($\eta^2 = 0.06$, $t = -2.04$, p = 0.05) did influence variations in circulating PRL levels. But, no significant pairwise comparisons were found upon further analysis ($p \geq 0.13$, for all). Although an inverse relationship between PRL and body mass was noted, it did not reach statistical significance ($\eta^2 = 0.03$, $t = -1.80$, $p = 0.08$). Similarly, in female Natal mole-rats, PRL levels did not differ significantly with respect to season ($\eta^2 = 0.0009$, $t = 0.11$, $p = 0.91$), reproductive class ($\eta^2 = 0.003$, $t = 0.36$, $p = 0.72$), or their interaction ($\eta^2 = 0.00005$, $t = -0.01$, $p = 0.99$) (Fig. 1). While an inverse relationship between PRL and body mass was observed, it was not statistically significant ($\eta^2 = 0.007$, $t = -0.60$, $p = 0.55$).

3.2. Steroid hormones and reproductive activity throughout the year

In highveld mole-rats, fPM concentrations in females were significantly affected by reproductive class ($\eta^2 = 0.15$, $t = -3.61$, p = 0.001) and the season*class interaction ($\eta^2 = 0.18$, $t = 3.17$, $p = 0.004$). In particular, BFs possessed higher fPM than NBFs, this was most clear in NBFs captured in the dry season which possessed lower fPM concentrations compared to NBF captured in the wet season (p = 0.004), and BFs from both seasons ($p \leq 0.05$, for both) (Fig. 2a). Larger females had lower fPM levels ($\eta^2 = 0.11$, $t = -2.34$, $p = 0.03$). However, season alone did not affect fPM concentrations ($\eta^2 = 0.09$, $t = -1.00$, $p = 0.33$). Similarly, fAM levels in highveld mole-rat males were affected by class ($\eta^2 = 0.004$, $t = -2.61$, $p = 0.01$) and the season*class interaction ($\eta^2 = 0.04$, $t = 2.70$, $p = 0.009$). In particular, BMs possessed higher fAM concentrations than NBMs, this was most clear in NBMs captured in the dry season which possessed lower fAM levels compared to NBMs captured in the wet season (p = 0.004), and BMs from both seasons ($p \leq 0.05$, for both) (Fig. 2b). Larger males had higher fAM levels ($\eta^2 = 0.11$, $t = 2.63$, p = 0.01). Season alone did not affect fAM levels in highveld mole-rat males ($\eta^2 = 0.10$, $t = 0.52$, $p = 0.61$).

In female Natal mole-rats, only reproductive class affected fPM levels, with BFs showing higher fPM concentrations than NBFs ($\eta^2 = 0.23$, $t = -2.30$, p = 0.03) (Fig. 2c), indicting pregnancy and ovulation. While in female Natal mole-rats, fPM levels were unaffected by body mass ($\eta^2 = 0.04$, $t = -0.35$, $p = 0.73$), season ($\eta^2 = 0.009$, $t = 0.76$, $p = 0.46$) and the two-way interaction ($\eta^2 = 0.01$, $t = 0.33$, $p = 0.75$). In contrast, fAM levels in male Natal mole-rats were unaffected by body mass ($\eta^2 = 0.06$, $t = 1.51$, $p = 0.14$), season ($\eta^2 = 0.02$, $t = 0.66$, $p = 0.51$), class ($\eta^2 = 0.02$, $t = 0.56$, $p = 0.58$), or the season*class interaction ($\eta^2 = 0.000007$, $t = -0.11$, p = 0.91) (Fig. 2d).

3.3. PRL disruption of reproductive hormone production in highveld, but not natal mole-rats

In highveld mole-rats, higher PRL levels were associated with lower fPM concentrations in NBFs ($R^2 = 0.33$, F = 5.96, p = 0.03) and lower fAM levels in NBMs ($R^2 = 0.24$, F = 7.37, p = 0.01) (Figs. 3a & b). However, no significant relationship between PRL and fPM or fAM levels were observed in highveld mole-rat breeders ($R^2 \leq 0.22$, F ≤ 2.81, $p \geq 0.12$) (Figs. 3a & b). In contrast, no significant associations between PRL and either fPM or fAM concentrations were detected in Natal mole-rats, irrespective of reproductive class ($R^2 \leq 0.09$, F ≤ 1.03, $p \geq 0.33$) (Fig. S3).

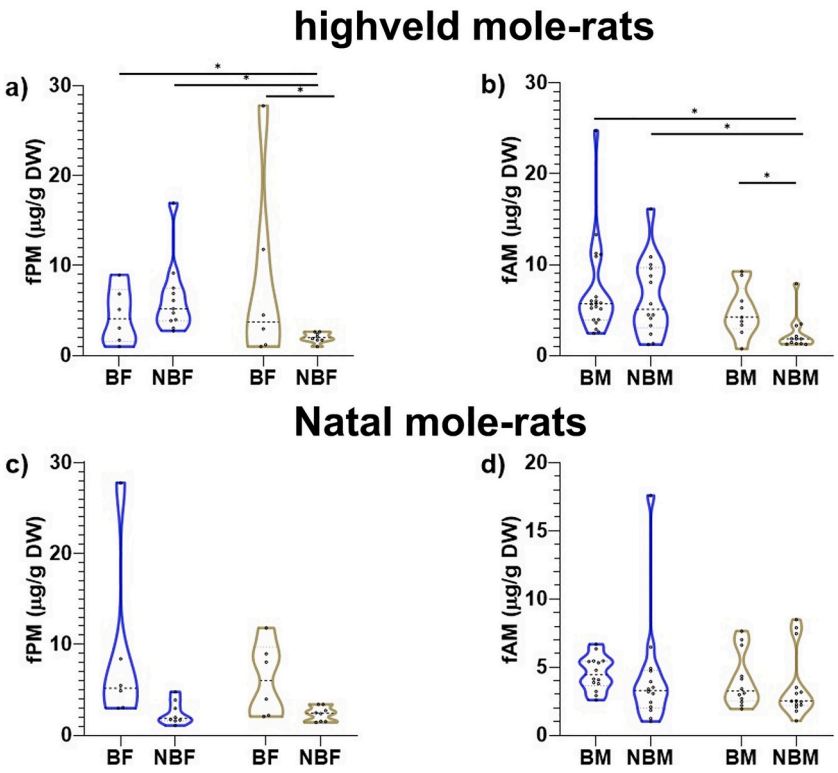

Fig. 2. Seasonal variation in faecal progesterone metabolite (fPM) and faecal androgen metabolite (fAM) concentrations in highveld mole-rats (*Cryptomys hottentotus pretoriae*) and Natal mole-rats (*C. h. natalensis*). (a) fPM concentrations in female highveld mole-rats. (b) fAM concentrations in male highveld mole-rats. (c) fPM concentrations in female Natal mole-rats. (d) fAM concentrations in male Natal mole-rats. Brown bars indicate dry-season samples, while blue bars represent wet-season samples. Asterisks (*) denote statistically significant differences (Tukey's HSD post-hoc comparisons: p ≤ 0.05).

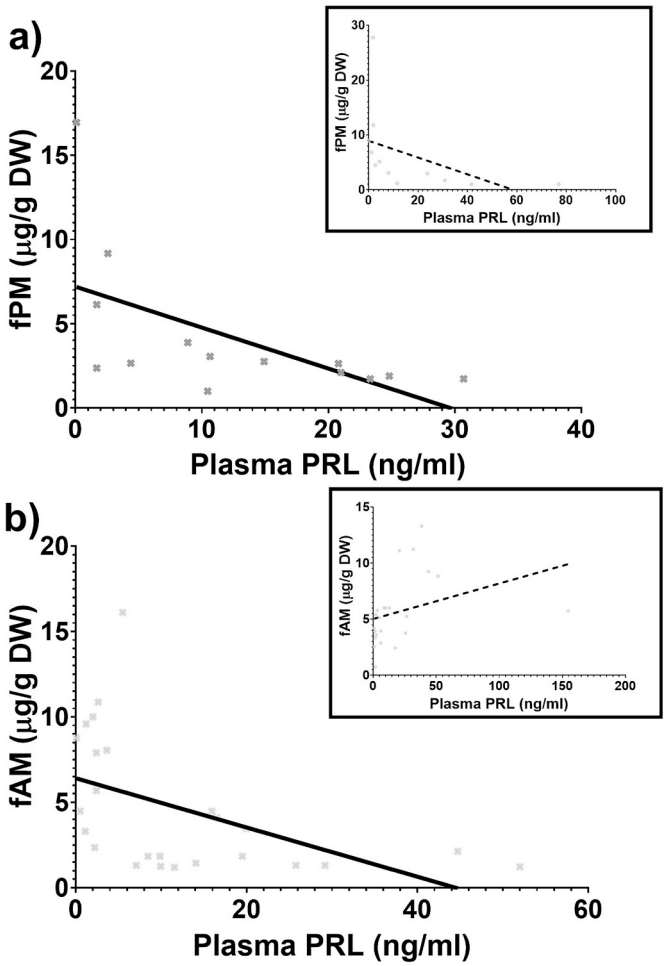

Fig. 3. Linear relationships between faecal progesterone metabolite (fPM) and faecal androgen metabolite (fAM) concentrations and plasma prolactin (PRL) levels in non-breeding female (a) and non-breeding male (b) highveld mole-rats (*Cryptomys hottentotus pretoriae*). Inserts show the corresponding relationships in breeding females and males. Solid lines represent significant relationships ($p \leq 0.05$) and dashed lines represent non-significant relationships ($p < 0.05$).

3.4. Testosterone defines behavioural-based socially induced infertility

In highveld mole-rats, males (5.55 ± 0.59 µg/g DW) possessed significantly greater fAM concentrations than females (4.09 ± 0.38 µg/g DW) (Fig. 4, Table 2). The interaction between sex and class significantly affected highveld mole-rat fAM, but no significant *post-hoc* comparisons were found ($p > 0.05$). Faecal androgen metabolite levels did not vary with any other predictor (Table 2).

Conversely, in Natal mole-rats, fAM concentrations were significantly affected by body mass (Table 2), with larger animals possessing higher fAM concentrations. Sex also had an effect, with males (4.12 ± 0.36 µg/g DW) having higher fAM levels than females (3.98 ± 0.58 µg/g DW). Reproductive class differences were also significant (Table 2), with breeders (5.12 ± 0.57 µg/g DW) having higher fAM concentrations than non-breeders (3.19 ± 0.36 µg/g DW). Particularly, BFs showed higher fAM concentrations than NBFs ($p = 0.05$), but their levels were similar to both NBMs ($p = 0.75$) and BMs ($p = 0.29$), regardless of season (Fig. 4). No further significant pairwise comparison was observed ($p > 0.05$).

3.5. No apparent effect of glucocorticoids on socially induced infertility

As expected, fGCM levels in highveld mole-rats did not differ significantly between sexes and reproductive classes across seasons (Table 3; Fig. S4). In contrast, statistical models indicated significant variation in fGCM concentrations in Natal mole-rats across several variables (Table 3). However, upon closer examination, no significant interactions were found in *post-hoc* analyses ($p > 0.05$) (Fig. S4).

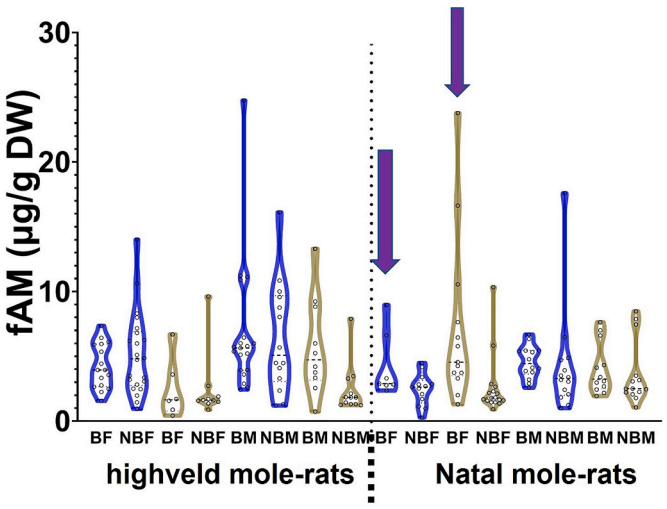

Fig. 4. Faecal androgen metabolite (fAM) concentrations (µg/g dry weight, DW) in breeding males (BM), breeding females (BF), non-breeding males (NBM), and non-breeding females (NBF) of highveld (*Cryptomys hottentotus pretoriae*) and Natal (*Cryptomys hottentotus natalensis*) mole-rats. Brown bars represent animals sampled during the dry season, while blue bars indicate those sampled in the wet season. Purple arrows indicate increased fAM levels likely associated with testosterone-mediated aggression.

Table 2
The statistical outputs of the effects of sex [male and female], season [wet and dry] and reproductive class season [breeding and non-breeding], their two and three-way interactions and body mass on faecal androgens faecal concentrations in highveld (*Cryptomys hottentotus pretoriae*) and Natal (*Cryptomys hottentotus natalensis*) mole-rats.

Species	Fixed term	η^2	t value	p-Value
Highveld mole-rats	Body Mass	0.08	1.68	0.10
	Sex	0.01	2.23	**0.03***
	Season	0.11	1.93	0.06
	Class	$4.52e^{-04}$	0.63	0.53
	Sex*Season	$5.34e^{-04}$	−1.16	0.25
	Sex*Class	0.02	−2.18	**0.04***
	Season*Class	0.02	0.08	0.94
	Sex*Season*Class	$7.78e^{-03}$	1.48	0.14
Natal mole-rats	Body Mass	0.11	2.07	**0.04***
	Sex	$8.16e^{-03}$	−2.39	**0.02***
	Season	$3.83e^{-03}$	−1.19	0.24
	Class	0.01	−2.50	**0.01***
	Sex*Season	0.03	1.33	0.19
	Sex*Class	0.08	2.44	**0.02***
	Season*Class	$5.35e^{-03}$	0.60	0.55
	Season*Class*Sex	$7.23e^{-03}$	−0.54	0.59

Significant relationships ($p \leq 0.05$) are indicated with '*'.

4. Discussion

This study provides novel insights into the endocrine mechanisms underlying socially induced infertility in cooperatively breeding mole-rats. In particular, it implicates physiological suppression via PRL and its modulation of the HPG axis in highveld mole-rats, together with behavioural suppression likely mediated by androgen-dependent processes, including aggression, sexual behaviour, and incest avoidance in Natal mole-rats. Although the present findings do not establish causality, they delineate key hormonal and behavioural pathways that warrant future investigation.

Our findings align with the hypothesis that elevated PRL levels may

Table 3
The statistical outputs of the effects of sex [male and female], season [wet and dry] and reproductive class season [breeding and non-breeding], their two and three-way interactions and body mass on faecal glucocorticoid metabolite concentrations in highveld (*Cryptomys hottentotus pretoriae*) and Natal (*Cryptomys hottentotus natalensis*) mole-rats.

Species	Fixed term	η^2	t value	p-Value
Highveld mole-rats	Body Mass	$6.80e^{-03}$	0.02	0.99
	Sex	$3.12e^{-03}$	0.30	0.76
	Season	0.02	0.65	0.52
	Class	$7.56e^{-03}$	0.22	0.83
	Sex*Season	$8.23e^{-04}$	−0.64	0.52
	Sex*Class	$2.34e^{-05}$	−0.55	0.58
	Season*Class	0.01	0.18	0.86
	Sex*Season*Class	$4.16e^{-03}$	0.73	0.47
Natal mole-rats	Body Mass	0.05	1.74	0.09
	Sex	$5.05e^{-04}$	−1.23	0.22
	Season	$8.73e^{-03}$	−1.55	0.12
	Class	$2.50e^{-03}$	−0.85	0.40
	Sex*Season	$2.06e^{-03}$	2.22	**0.03***
	Sex*Class	$1.57e^{-03}$	1.35	0.18
	Season*Class	$1.95e^{-03}$	2.13	**0.04***
	Season*Class*Sex	0.06	−2.43	**0.02***

Significant relationships ($p \leq 0.05$) are indicated with '*'.

play a central role in physiological suppression (Al-Fahham and Al-Nowainy, 2016; Gangwar et al., 2020), as seen in highveld mole-rats. Non-breeding males and females exhibited significantly higher PRL levels during the dry season compared to the wet season. This seasonal increase in PRL levels corresponds with decreased pituitary responsiveness to exogenous GnRH, pointing to HPG axis dysfunction (Van der Walt et al., 2001). The correlation between elevated PRL levels and reduced reproductive activity in non-breeders supports the notion that PRL-induced suppression is a potential mechanism of infertility (Bennett et al., 2018). The lower levels of circulating plasma PRL and lack of hyperprolactinemia in non-breeding highveld mole-rats during the wet season implies a temporary relaxation of physiological suppression, potentially allowing these individuals to regain reproductive function when environmental conditions are more favourable and potentially promoting dispersal and the establishment of new colonies. This is likely advantageous during periods when breeding success is higher, such as in the wet season when dispersal opportunities increase due to reduced soil hardness and greater food availability (Molteno and Bennett, 2002b). In highveld mole-rats, non-breeders had significantly lower levels of fPM in females and fAM in males during the dry season compared to both breeders and non-breeders in the wet season. This reduction in steroid hormone levels likely reflects the downstream effects of PRL-mediated suppression of the HPG axis, as evidenced by the inverse relationship between PRL and fPM/fAM levels, further supporting the potential direct inhibition of gonadal function in non-breeders. Similar findings in naked and female Mahali mole-rats also implicate PRL in reproductive suppression among helpers, suggesting a common physiological strategy in these species (Bennett et al., 2018; Hart et al., 2022b, 2022c). At the mechanistic level, PRL may suppress reproduction centrally, by inhibiting hypothalamic GnRH neurons and thereby reducing gonadotropin release, or peripherally, by modifying pituitary and/or gonadal receptor density and sensitivity (Hackwell et al., 2025; Szukiewicz, 2024). Differences in PRL receptor expression between breeders and non-breeders, already documented in other mole-rat species (Voigt and Bennett, 2018), suggests that similar processes could contribute to reproductive suppression in highveld mole-rats. However, targeted studies are needed to confirm this possibility. Notably, PRL levels among non-breeders varied substantially, with some individuals showing hyperprolactinemia and others resembling breeders. Such heterogeneity suggests suppression is not uniform, echoing observations in naked mole-rats where non-breeders differ in pituitary responsiveness to GnRH (Hart et al., 2024; Van Der Westhuizen et al., 2002). Furthermore, some of the highest PRL concentrations were detected in BMs, an unusual, but previously documented phenomenon in naked mole-rats (Bennett et al., 2018; Majelantle et al., 2024). Highveld mole-rats have the smallest colony sizes of all social mole-rat species (Süess et al., 2024), and therefore cooperative behaviour, whether it be in the form of protecting the nest or digging tunnels or caring for offspring, is divided between a smaller number of individuals compared to the other cooperative mole-rat species. Breeding male highveld mole-rats may therefore be required to invest more time in offspring care and other cooperative behaviours when compared to other species, which may explain their higher levels of plasma prolactin. These increased PRL levels should impair their breeding abilities; however, in adult sexually, i.e. post- pubescent, mature men (*Homo sapiens*), PRL has been observed to be a pituitary hormone that stimulates testosterone secretion from the Leydig cell of the testes (Rubin et al., 1976), but also see (Rubin et al., 1978). Consequently, once a highveld mole-rat male attains reproductive dominance in a colony or escapes the social suppression of their colony, circulating PRL may take on a stimulatory role of both reproduction and parental care. However, additional research is required to unravel this phenomenon in this species.

In marked contrast, Natal mole-rats showed significantly lower PRL than the physiologically suppressed highveld mole-rats and did not show significant variation in PRL levels across reproductive classes or seasons, suggesting that PRL-induced physiological suppression is not a mechanism operational in this subspecies. The functional HPG axis in non-breeding Natal mole-rats, demonstrated by their similar LH responses to exogenous GnRH to those of breeders (Oosthuizen et al., 2008), suggests that behavioural suppression mechanisms, such as androgen-driven aggression and incest avoidance (Finn, 2022; Lutermann et al., 2013), may play a more prominent role in regulating reproductive skew in this species. Breeding Natal mole-rats showed elevated fPM and fAM levels, compared to non-breeders, but these differences were not correlated with PRL. This pattern is expected given reproductive activity, but we also note that female breeders showed unusually high fAM levels, sometimes exceeding those of males. Such elevations may point to a dual role for androgens in reproduction and in supporting dominance-related aggression. Previous studies in naked mole-rats (Clarke and Faulkes, 2001; Clarke and Faulkes, 1998; Clarke and Faulkes, 1997) have shown that elevated androgens are associated with dominance and aggressive behaviour, suggesting a possible parallel in Natal mole-rats, but also see (Toor et al., 2022). Nonetheless, targeted experimentation is required to establish a direct association between increased androgen levels and aggressive behaviour in this species. While androgen-mediated behavioural suppression appears central in Natal mole-rats, the role of incest avoidance currently remains underexplored and should be a focus of future research (Bennett et al., 1996; Burland et al., 2004; Greeff and Bennett, 2000; Herbst and Bennett, 2001). Incest avoidance is likely to act as an alternative or complementary mechanism of reproductive suppression in Natal mole-rats, especially as obligate outbreeders (K. Finn unpublished results). The lack of significant seasonal variation in reproductive steroid hormone levels in Natal mole-rats further suggests that behavioural suppression mechanisms remain consistent throughout the year, contrasting with the seasonally dependent physiological suppression seen in highveld mole-rats.

Glucocorticoids do not appear to play a significant role in socially induced infertility in either of the subspecies. No clear significant differences in fGCM levels were observed between reproductive classes or across seasons in either highveld or Natal mole-rats, indicating that chronic stress is unlikely to drive reproductive suppression. This finding aligns with recent studies in other mole-rat species, which have shown that GC levels are more closely related to metabolic activity than to stress-induced infertility (Hart et al., 2023; Jimeno and Verhulst, 2023; Majelantle et al., 2024; Vullioud et al., 2021). However, our analyses of fGCMs revealed some significant interaction effects in Natal mole-rats, though clear *post-hoc* patterns were not evident. While this prevents firm conclusions, the results suggest that glucocorticoids may play an

indirect role in behaviour. One possibility is that variation in fGCM reflects metabolic demands rather than stress per se, consistent with the findings in other mole-rats where glucocorticoids align with energetic requirements (Vullioud et al., 2021). This interpretation is further supported by a non-significant trend for higher fGCM levels in larger individuals, which are shown to have higher energetic costs (D.M. Scantlebury, unpublished results). These results highlight the need for further work that disentangles metabolic and stress-related contributions to endocrine patterns in cooperative breeders.

The contrasting mechanisms of reproductive suppression in highveld and Natal mole-rats underscore the flexibility of social systems, even among closely related subspecies. Whereas highveld mole-rats rely primarily on PRL-mediated physiological suppression, Natal mole-rats appear to depend more on behavioural mechanisms, including, but not limited to, androgen-driven aggression and incest taboos. This divergence raises important questions about the selective pressures that favour physiological versus behavioural strategies.

Future research within the genus *Cryptomys* and other cooperative breeders should investigate the genetic and ecological drivers that shape PRL and androgen pathways, as well as their interactions with social structure. While our findings are correlational, and thus require cautious interpretation, experimental manipulations, such as PRL antagonism, androgen modulation, and assays of receptor expression in the brain and gonads, will be crucial to identify causal mechanisms. Equally, incorporating direct behavioural observations of aggression, dominance, and affiliative interactions will help clarify the role of androgens in regulating reproductive skew. Finally, examining variation among non-breeders within colonies may reveal why some individuals display endocrine profiles more similar to breeders, providing new insights into the dynamics of suppression and the maintenance of social hierarchies in cooperative mammals.

CRediT authorship contribution statement

A.K. Janse van Vuuren: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **T. Süess:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Kyle Finn:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **N. Hagenah:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **A. Ganswindt:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation. **D.W. Hart:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **N.C. Bennett:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Ethics

Permissions to capture animals were obtained from all relevant landowners, and a collecting permit was granted by the appropriate conservation authorities (Permit number: CPF6-0127—Gauteng & OP1545/2021—KwaZulu-Natal). The experimental protocol was approved by the Animal Use and Care Committee of the University of Pretoria (ethics clearance number: NAS128-2020), and the Department of Agriculture, Land Reform and Rural Development (DALRRD) section 20 approval (SDAH-Epi-20070806200, SDAH-Epi-12/11/1/1/8).

Funding

The project was funded by the SARChI Chair of Mammalian Behavioural Ecology and Physiology from the DST–NRF South Africa, the National Research Foundation (grant no. 64756), and the University of Pretoria to N.C.B.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful for funding from the SARChI Chair of Mammalian Behavioural Ecology and Physiology from the DST–NRF South Africa, the National Research Foundation (grant no. 64756), and the University of Pretoria to N.C.B. D.W.H reports financial support was provided by National Research Foundation. We thank the three anonymous reviewers for their constructive comments and valuable suggestions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2025.105836.

Data availability

Data will be made available on request.

References

Abbott, D.H., 1984. Behavioral and physiological suppression of fertility in subordinate marmoset monkeys. Am. J. Primatol. 6, 169–186. https://doi.org/10.1002/AJP.1350060305.

Abbott, D.H., Hodges, J.K., George, L.M., 1988. Social status controls LH secretion and ovulation in female marmoset monkeys (*Callithrix jacchus*). J. Endocrinol. 117, 329–339.

Al-Fahham, A.A., Al-Nowainy, H.Q., 2016. The role of FSH, LH, and prolactin hormones in female infertility. Int. J. PharmTech. Res. 6, 110–118.

Barker, A.J., Veviurko, G., Bennett, N.C., Hart, D.W., Mograby, L., Lewin, G.R., 2021. Cultural transmission of vocal dialect in the naked mole-rat. Science 1979 (371), 503–507. https://doi.org/10.1126/science.abc6588.

Bégay, V., Cirovic, B., Barker, A.J., Klopfleisch, R., Hart, D.W., Bennett, N.C., Lewin, G. R., 2022. Immune competence and spleen size scale with colony status in the naked mole-rat. Open Biol. 12, 210292.

Ben Mocha, Y., Scemama de Gialluly, S., Griesser, M., Markman, S., 2023. What is cooperative breeding in mammals and birds? Removing definitional barriers for comparative research. Biol. Rev. 98, 1845–1861. https://doi.org/10.1111/BRV.12986.

Bennett, N.C., Faulkes, C.G., 2000. African Mole-Rats: Ecology and Eusociality. Cambridge University Press, Cambridge.

Bennett, N.C., Jarvis, J.U.M., 1995. Coefficients of digestibility and nutritional values of geophytes and tubers eaten by southern African mole-rats (Rodentia: Bathyergidae). J. Zool. 236, 189–198.

Bennett, N.C., Jarvis, J.U.M., Faulkes, C.G., Millar, R.P., 1993. LH responses to single doses of exogenous GnRH by freshly captured Damaraland mole-rats, *Cryptomys damarensis*. J. Reprod. Fertil. 99, 81–86. https://doi.org/10.1530/jrf.0.0990081.

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. B 263, 1599–1603. https://doi.org/10.1098/RSPB.1996.0234.

Bennett, N.C., Faulkes, C.G., Spinks, A.C., 1997. LH responses to single doses of exogenous GnRH by social Mashona mole-rats: a continuum of socially induced infertility in the family Bathyergidae. Proc. R. Soc. B 264, 1001–1006. https://doi.org/10.1098/rspb.1997.0138.

Bennett, N.C., Molteno, A.J., Spinks, A.C., 2000. Pituitary sensitivity to exogenous GnRH in giant Zambian mole-rats, Cryptomys mechowi (Rodentia: Bathyergidae): support for the "socially-induced infertility continuum". J. Zool. 252, 447–452.

Bennett, N.C., Ganswindt, A., Ganswindt, S.B., Jarvis, J.U.M., Zöttl, M., Faulkes, C.G., 2018. Evidence for contrasting roles for prolactin in eusocial naked mole-rats, *Heterocephalus glaber* and Damaraland mole-rats, *Fukomys damarensis*. Biol. Lett. 14, 10–13. https://doi.org/10.1098/rsbl.2018.0150.

Bennett, N.C., Grenfell, K.L., van Jaarsveld, B., Hart, D.W., 2025. Thermoregulation meets chronobiology: how temperature, not light, governs circadian rhythms in subterranean mole-rats. Neuroscience 580, 14–15.

Burland, T.M., Bennett, N.C., Jarvis, J.U.M., Faulkes, C.G., 2004. Colony structure and parentage in wild colonies of co-operatively breeding Damaraland mole-rats suggest

incest avoidance alone may not maintain reproductive skew. Mol. Ecol. 13 (8), 2371–2379. https://doi.org/10.1111/j.1365-294X.2004.02233.x.
Capezzuto, A., Chelini, M.O.M., Felippe, E.C.G., Oliveira, C.A., 2008. Correlation between serum and fecal concentrations of reproductive steroids throughout gestation in goats. Anim. Reprod. Sci. 103, 78–86.
Clarke, F.M., Faulkes, C.G., 1997. Dominance and queen succession in captive colonies of the eusocial naked mole–rat, *Heterocephalus glaber*. Proc. R. Soc. Lond. B Biol. Sci. 264, 993–1000.
Clarke, F.M., Faulkes, C.G., 1998. Hormonal and behavioural correlates of male dominance and reproductive status in captive colonies of the naked mole–rat, *Heterocephalus glaber*. Proc. R. Soc. Lond. B Biol. Sci. 265, 1391–1399.
Clarke, F.M., Faulkes, C.G., 2001. Intracolony aggression in the eusocial naked mole-rat, *Heterocephalus glaber*. Anim. Behav. 61, 311–324.
Clutton-Brock, T.H., 2021. Social evolution in mammals. Science (1979) 373, eabc9699.
Creel, S., 2005. Dominance, aggression, and glucocorticoid levels in social carnivores. J. Mammal. 86, 255–264.
Creel, S.R., Waser, P.M., 1991. Failures of reproductive suppression in dwarf mongooses (*Helogale parvula*): accident or adaptation? Behav. Ecol. 2, 7–15.
Darwin, C., 1859. On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life. John Murray, London.
Drea, C.M., Davies, C.S., 2022. Meerkat manners: endocrine mediation of female dominance and reproductive control in a cooperative breeder. Horm. Behav. 145, 105245.
Du Toit, L., Bennett, N.C., Gutjahr, G.H., Coen, C.W., 2006a. Reproductive suppression in subordinate female highveld mole-rats (*Cryptomys hottentotus pretoriae*): no role for endogenous opioid peptides. Physiol. Behav. 87 (5), 897–902.
Du Toit, L., Bennett, N.C., Katz, A.A., Kalló, I., Coen, C.W., 2006b. Relations between social status and the gonadotrophin-releasing hormone system in females of two cooperatively breeding species of African mole-rats, *Cryptomys hottentotus hottentotus* and *Cryptomys hottentotus pretoriae*: neuroanatomical and neuroendocrinological studies. J. Comp. Neurol. 494 (2), 303–313.
Dunbar, R.I.M., Shultz, S., 2021. The infertility trap: the fertility costs of group-living in mammalian social evolution. Front. Ecol. Evol. 9, 634664. https://doi.org/10.3389/FEVO.2021.634664/BIBTEX.
Edwards, P.D., 2022. For the physiology of cooperative breeding, it's time to move beyond stress: a comment on:'stress in an underground empire'(2022) by Medger. Biol. Lett. 18 (10), 20220375. https://doi.org/10.1098/rsbl.2022.0375.
Edwards, P.D., Mooney, S.J., Bosson, C.O., Toor, I., Palme, R., Holmes, M.M., Boonstra, R., 2020. The stress of being alone: removal from the colony, but not social subordination, increases fecal cortisol metabolite levels in eusocial naked mole-rats. Horm. Behav. 121, 104720.
Faulkes, C.G., Bennett, N.C., 2001. Family values: group dynamics and social control of reproduction in African mole-rats. TREE 16 (4), 184–190.
Faulkes, C.G., Abbott, D.H., Jarvis, J.U.M., Sherriff, F.E., 1990. LH responses of female naked mole-rats, *Heterocephalus glaber*, to single and multiple doses of exogenous GnRH. Reproduction 89, 317–323.
Finn, K.T., 2022. Sociality in African Mole-Rats: Exploring how Rainfall Affects Dispersal and Genetic Exchange in the Natal Mole-Rat (*Cryptomys hottentotus natalensis*) (Doctoral Dissertation). University of Pretoria, Pretoria.
Finn, K.T., Janse van Vuuren, A.K., Hart, D.W., Suess, T., Zottl, M., Bennett, N.C., 2022. Seasonal changes in locomotor activity patterns of wild social Natal mole-rats (*Cryptomys hottentotus natalensis*). Front. Ecol. Evol. 10, 819393.
Gachomba, M.J.M., Esteve-Agraz, J., Márquez, C., 2024. Prosocial behaviors in rodents. Neurosci. Biobehav. Rev. 163, 105776. https://doi.org/10.1016/j.neubiorev.2024.105776.
Gangwar, P.K., Sankhwar, S.N., Pant, S., Krishna, A., Singh, B.P., Mahdi, A.A., Singh, R., 2020. Increased gonadotropins and prolactin are linked to infertility in males. Bioinformation 16 (2), 176.
Ganswindt, A., Tordiffe, A.S.W., Stam, E., Howitt, M.J., Jori, F., 2012. Determining adrenocortical activity as a measure of stress in African buffalo (*Syncerus caffer*) based on faecal analysis. Afr. Zool. 47, 261–269.
Ganswindt, S.B., Myburgh, J.G., Cameron, E.Z., Ganswindt, A., 2014. Non-invasive assessment of adrenocortical function in captive Nile crocodiles (*Crocodylus niloticus*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 177, 11–17.
Greeff, J.M., Bennett, N.C., 2000. Causes and consequences of incest avoidance in the cooperatively breeding mole-rat, *Cryptomys darlingi* (Bathyergidae). Ecol. Lett. 3 (4), 318–328.
Grenfell, K.L., Jacobs, P.J., Bennett, N.C., Hart, D.W., 2024. The role of ambient temperature and light as cues in the control of circadian rhythms of Damaraland mole-rat. Chronobiol. Int. 1–13.
Griesser, M., Bennett, N.C., Burkart, J.M., Hart, D.W., Uomini, N., Warrington, M.H., 2025. The power of caring touch: from survival to prosocial cooperation. TREE 40 (4), 346–355.
Hackwell, E.C.R., Ladyman, S.R., Clarkson, J., McQullian, H.J., Boehm, U., Herbison, A. E., Brown, R.S.E., Grattan, D.R., 2025. Prolactin-mediates a lactation-induced suppression of arcuate kisspeptin neuronal activity necessary for lactational infertility in mice. Elife 13, RP94570.
Hart, D.W., Bennett, N.C., 2022. Seasonality of reproduction in African mole-rats (Rodentia: Bathyergidae) is a function of group size: a novel hypothesis. Lynx, series nova 53, 53–65.
Hart, D.W., Medger, K., Van Jaarsveld, B., Bennett, N.C., 2021a. Filling in the holes: the reproductive biology of the understudied mahali mole-rat (*Cryptomys hottentotus mahali*). Can. J. Zool. 99, 801–811. https://doi.org/10.1139/cjz-2020-0158.
Hart, Daniel W., van Jaarsveld, B., Lasch, K.G., Grenfell, K.L., Oosthuizen, M.K., Bennett, N.C., 2021b. Ambient temperature as a strong Zeitgeber of circadian rhythms in response to temperature sensitivity and poor heat dissipation abilities in subterranean African mole-rats. J. Biol. Rhythm. 36 (5), 461–469.
Hart, D.W., Bennett, N.C., Oosthuizen, M.K., Waterman, J.M., Scantlebury, D.M., 2022a. Energetics and water flux in the subterranean rodent family Bathyergidae. Front. Ecol. Evol. 10, 867350.
Hart, D.W., van Vuuren, A.K.J., Erasmus, A., Süess, T., Hagenah, N., Ganswindt, A., Bennett, N.C., 2022b. The endocrine control of reproductive suppression in an aseasonally breeding social subterranean rodent, the Mahali mole-rat (*Cryptomys hottentotus mahali*). Horm. Behav. 142, 105155. https://doi.org/10.1016/j.yhbeh.2022.105155.
Hart, D.W., Bennett, N.C., Voigt, C., 2022c. Social stress is unlikely to play a major role in reproductive suppression of female subordinate naked mole-rats and Damaraland mole-rats. Biol. Lett. 18, 20220292.
Hart, D.W., Bennett, N.C., Best, C., van Jaarsveld, B., Cheng, H., Ivy, C.M., Kirby, A.M., Munro, D., Sprenger, R.J., Storey, K.B., Milsom, W.K., Pamenter, M.E., 2023. The relationship between hypoxia exposure and circulating cortisol levels in social and solitary African mole-rats: an initial report. Gen. Comp. Endocrinol. 339, 114294. https://doi.org/10.1016/j.ygcen.2023.114294.
Hart, D.W., Roberts, E., O'Riain, M.J., Millar, R.P., Bennett, N.C., 2024. The curious case of the hypothalamic–pituitary–gonadal axis dysfunction in subordinate female naked mole-rats (*Heterocephalus glaber*): no apparent role of opioids and glucocorticoids. J. Neuroendocrinol. 36 (10), 13444. https://doi.org/10.1111/JNE.13444.
Herbst, M., Bennett, N.C., 2001. Recrudescence of sexual activity in a colony of the Mashona mole-rat (*Cryptomys darlingi*): an apparent case of incest avoidance. J. Zool. 254 (2), 163–175. https://doi.org/10.1017/S095283690100067X.
Hickman, G.C., 1979. A live-trap and trapping technique for fossorial mammals. S. Afr. J. Zool. 9–12. https://doi.org/10.1080/02541858.1979.11447641.
Janse van Vuuren, A.K., 2022. Endocrine Control of Reproductive Suppression in Three *Cryptomys hottentotus* Subspecies (MSc). University of Pretoria, Pretoria.
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.
Jimeno, B., Verhulst, S., 2023. Meta-analysis reveals glucocorticoid levels reflect variation in metabolic rate, not 'stress'. Elife 12, RP88205.
Koenig, W.D., Pitelka, F.A., Carmen, W.J., Mumme, R.L., Stanback, M.T., 1992. The evolution of delayed dispersal in cooperative breeders. Q. Rev. Biol. 67, 111–150. https://doi.org/10.1086/417552.
Lukas, D., Clutton-Brock, T., 2012. Cooperative breeding and monogamy in mammalian societies. Proc. R. Soc. Lond. B Biol. Sci. 279, 2151–2156. https://doi.org/10.1098/RSPB.2011.2468.
Lutermann, H., Young, A.J., Bennett, N.C., 2013. Reproductive status and testosterone among females in cooperative mole-rat societies. Gen. Comp. Endocrinol. 187, 60–65. https://doi.org/10.1016/j.ygcen.2013.03.026.
Majelantle, T.L., Bennett, N.C., Ganswindt, S.B., Hart, D.W., Ganswindt, A., 2023. Validation of enzyme immunoassays via an adrenocorticotrophic stimulation test for the non-invasive quantification of stress-related hormone metabolites in naked mole-rats. Animals 13, 1424.
Majelantle, T.L., Ganswindt, A., Hart, D.W., Hagenah, N., Ganswindt, S.B., Bennett, N.C., 2024. The dissection of a despotic society: exploration, dominance and hormonal traits. Proc. R. Soc. Lond. B Biol. Sci. 291 (2022), 20240371. https://doi.org/10.1098/RSPB.2024.0371.
Medger, K., Bennett, N.C., Lutermann, H., Ganswindt, A., 2018. Non-invasive assessment of glucocorticoid and androgen metabolite levels in cooperatively breeding Damaraland mole-rats (*Fukomys damarensis*). Gen. Comp. Endocrinol. 266, 202–210. https://doi.org/10.1016/j.ygcen.2018.05.018.
Medger, K., Bennett, N.C., Ganswindt, S.B., Ganswindt, A., Hart, D.W., 2019. Changes in prolactin, cortisol and testosterone concentrations during queen succession in a colony of naked mole-rats (*Heterocephalus glaber*): a case study. Science of Nature 106, 26. https://doi.org/10.1007/s00114-019-1621-1.
Milewski, T.M., Lee, W., Champagne, F.A., Curley, J.P., 2022. Behavioural and physiological plasticity in social hierarchies. Philos. Trans. R. Soc. B 377, 20200443.
Mohan, N.H., Nath, A., Thomas, R., Kumar, S., Banik, S., Das, A.K., Das, R.K., Sarma, D. K., 2020. Relationship between plasma, saliva, urinary and faecal cortisol levels in pigs. Indian J. Anim. Sci. 90, 768–772.
Molteno, A.J., Bennett, N.C., 2002a. Rainfall, dispersal and reproductive inhibition in eusocial Damaraland mole-rats (*Cryptomys damarensis*). J. Zool. 256 (4), 445–448.
Molteno, A.J., Bennett, N.C., 2002b. Social suppression in nonreproductive female Damaraland mole-rats, *Cryptomys damarensis*: no apparent role for endogenous opioid peptides. Horm. Behav. 41, 115–125. https://doi.org/10.1006/hbeh.2001.1743.
Mustoe, A., 2023. A tale of two hierarchies: hormonal and behavioral factors underlying sex differences in social dominance in cooperative breeding callitrichids. Horm. Behav. 147, 105293.
Nelson-Flower, M.J., Wiley, E.M., Flower, T.P., Ridley, A.R., 2018. Individual dispersal delays in a cooperative breeder: ecological constraints, the benefits of philopatry and the social queue for dominance. J. Anim. Ecol. 87, 1227–1238. https://doi.org/10.1111/1365-2656.12814.
Oosthuizen, M.K., Bennett, N.C., Lutermann, H., Coen, C.W., 2008. Reproductive suppression and the seasonality of reproduction in the social Natal mole-rat (*Cryptomys hottentotus natalensis*). Gen. Comp. Endocrinol. 159, 236–240. https://doi.org/10.1016/j.ygcen.2008.09.004.
Palme, R., Möstl, E., 1993. Biotin-streptavidin enzyme immunoassay for the determination of oestrogens and androgens in boar faeces. Advances of steroid analysis 111–117.
Palme, R., Möstl, E., 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Inst J Mammal Biol 62, 192–197.

Rubin, R.T., Poland, R.E., Tower, B.B., 1976. Prolactin-related testosterone secretion in normal adult men. J. Clin. Endocrinol. Metab. 42 (1), 112–116.
Rubin, R.T., Poland, R.E., Sobel, I., Tower, B.B., Odell, W.D., 1978. Effects of prolactin and prolactin plus luteinizing hormone on plasma testosterone levels in normal adult men. J. Clin. Endocrinol. Metab. 47, 447–452.
Saltzman, W., Digby, L.J., Abbott, D.H., 2009. Reproductive skew in female common marmosets: what can proximate mechanisms tell us about ultimate causes? Proc. R. Soc. Lond. B Biol. Sci. 276, 389–399. https://doi.org/10.1098/RSPB.2008.1374.
Sherman, P.W., Lacey, E.A., Reeve, H.K., Keller, L., 1995. The eusociality continuum. Behav. Ecol. 6, 102–108.
Solomon, N.G., French, J.A., 1997. Cooperative Breeding in Mammals. Cambridge University Press.
Spiering, P.A., Somers, M.J., Maldonado, J.E., Wildt, D.E., Gunther, M.S., 2010. Reproductive sharing and proximate factors mediating cooperative breeding in the African wild dog (*Lycaon pictus*). Behav. Ecol. Sociobiol. 64, 583–592.
Spinks, A.C., Bennett, N.C., Jarvis, J.U.M., 1999. Regulation of reproduction in female common mole-rats (*Cryptomys hottentotus hottentotus*): the effects of breeding season and reproductive status. J. Zool. 248 (2), 161–168. https://doi.org/10.1111/j.1469-7998.1999.tb01192.x.
Spinks, A.C., Bennett, N.C., Faulkes, C.G., Jarvis, J.U.M., 2000. Circulating LH levels and the response to exogenous GnRH in the common mole-rat: implications for reproductive regulation in this social, seasonal breeding species. Horm. Behav. 37, 221–228.
Süess, T., Finn, K.T., Van Vuuren, A.K.J., Hart, D.W., Bennett, N.C., 2024. A comparison of the population ecology of 4 *Cryptomys hottentotus* subspecies. J. Mammal. 105, 26–39. https://doi.org/10.1093/JM/GYAD115.
Szdzuy, K., Dehnhard, M., Strauss, G., Eulenberger, K., Hofer, H., 2006. Behavioural and endocrinological parameters of female African and Asian elephants *Loxodonta africana* and *Elephas maximus* in the peripartal period. International Zoo Yearbook 40, 41–50.
Szukiewicz, D., 2024. Current insights in prolactin signaling and ovulatory function. Int. J. Mol. Sci. 25, 1976.
Thavarajah, N.K., Fenkes, M., Clutton-Brock, T.H., 2014. The determinants of dominance relationships among subordinate females in the cooperatively breeding meerkat. Behaviour 151, 89–102.
Toor, I., Faykoo-Martinez, M., Edwards, P.D., Boonstra, R., Holmes, M.M., 2022. Hormones do not maketh the mole-rat: no steroid hormone signatures of subordinate behavioral phenotypes. Horm. Behav. 145, 105236.
Van der Walt, L., 2003. Reproductive Inhibition in Female Common and Highveld Mole-Rats: Neuroanatomical and Neuroendocrine Perspectives. University of Pretoria (South Africa).
Van der Walt, L., Bennett, N.C., Zoology, S.S., 2001. Reproductive suppression and pituitary sensitivity to exogenous GnRH in the highveld mole-rat (*Cryptomys hottentotus pretoriae*). J. Zool. 254, 177–184. https://doi.org/10.1017/S0952836901000681.
Van Der Westhuizen, L.A., Bennett, N.C., Jarvis, J.U.M., 2002. Behavioural interactions, basal plasma luteinizing hormone concentrations and the differential pituitary responsiveness to exogenous gonadotrophin-releasing hormone in entire colonies of the naked mole-rat (*Heterocephalus glaber*). J. Zool. 256, 25–33.
van Jaarsveld, B., Bennett, N.C., Hart, D.W., Oosthuizen, M.K., 2019. Locomotor activity and body temperature rhythms in the Mahali mole-rat (*C. h. mahali*): the effect of light and ambient temperature variations. J. Therm. Biol. 79, 24–32. https://doi.org/10.1016/j.jtherbio.2018.11.013.
Voigt, C., Bennett, N.C., 2018. Reproductive status affects the expression of prolactin receptor mRNA in the brain of female Damaraland mole-rats. J. Chem. Neuroanat. 94, 1–7.
Vullioud, P., Mendonca, R., Glauser, G., Bennett, N.C., Zottl, M., Katlein, N., Leal, R., Fuerst, R., Clutton-Brock, T., 2021. Increases in glucocorticoids are sufficient but not necessary to increase cooperative burrowing in Damaraland mole-rats. Horm. Behav. 135, 105034.
Wallace, Kyra M.E., Hart, D.W., Hagenah, N., Ganswindt, A., Bennett, N.C., 2023a. A comprehensive profile of reproductive hormones in eusocial Damaraland mole-rats (*Fukomys damarensis*). Gen. Comp. Endocrinol. 333, 114194.
Wallace, K.M.E., Hart, D.W., Venter, F., Janse Van Vuuren, A.K., Bennett, N.C., 2023b. The best of both worlds: no apparent trade-off between immunity and reproduction in two group-living African mole-rat species. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 378 (1883), 20220310. https://doi.org/10.1098/rstb.2022.0310.
Wasser, S.K., Barash, D.P., 1983. Reproductive suppression among female mammals: implications for biomedicine and sexual selection theory. Q. Rev. Biol. 58, 513–538.
Yimer, N., 2010. Correlation between plasma progesterone concentrations and fecal Progestins during the estrus cycle of Kedah Kelantan cows. In: International Seminar on Tropical Animal Production (ISTAP), pp. 595–598.
Young, A.J., Clutton-Brock, T., 2006. Infanticide by subordinates influences reproductive sharing in cooperatively breeding meerkats. Biol. Lett. 2, 385–387.