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Evidence for contrasting roles for prolactin in eusocial naked mole-rats, *Heterocephalus glaber* and Damaraland mole-rats, *Fukomys damarensis*

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1 Evidence for contrasting roles for prolactin in eusocial naked mole-rats, *Heterocephalus glaber* and
2 Damaraland mole-rats, *Fukomys damarensis*

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17 Prolactin, African mole-rats, reproductive suppression, cooperative breeding
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Abstract

Elevated prolactin (PRL) has been associated with the expression of social and cooperative behaviours in a number of vertebrate species, as well as suppression of reproduction. As social mole-rats exhibit both of these traits, PRL is a prime candidate in mediating their social phenotype. While naked and Damaraland mole-rats (NMRs and DMRs) have evolved eusociality independently within their family, both species exhibit an extreme skew in lifetime reproductive success, with breeding restricted to a single female and one or two males. Non-breeding NMRs of both sexes are physiologically inhibited from reproducing, while in DMRs only the non-breeding females are physiologically suppressed. Newly emerging work has implicated the dopamine system and PRL as a component in socially-induced reproductive suppression and eusociality in NMR, but the DMR remains unstudied in this context. To investigate evolutionary convergence in the role of PRL in shaping African mole-rat eusociality, we determined plasma PRL concentrations in breeders and non-breeders of both sexes, comparing DMRs with NMRs. Among samples from non-breeding NMRs 80% had detectable plasma PRL concentrations. As a benchmark, these often (37%) exceeding those considered clinically hyperprolactinaemic (25 ng/ml) in humans: mean ± SEM: 34.81 ± 5.87 ng/ml; range 0.00-330.30 ng/ml. Conversely, 85% of non-breeding DMR samples had undetectable values and none had concentrations above 25 ng/ml: 0.71 ± 0.38 ng/ml; 0.00-23.87 ng/ml. Breeders in both species had the expected variance in plasma PRL concentrations as part of normal reproductive function, with lactating queens having significantly higher values. These results suggest that while elevated PRL in non-breeders is implicated in NMR eusociality, this may not be the case in DMRs, and suggests a lack of evolutionary convergence in the proximate control of the social phenotype in these mole-rats.

Introduction

Prolactin has the potential to play a key role in a number of components of mammalian sociality and cooperative breeding. Elevated PRL has been associated with the expression of social and cooperative behaviours in both sexes in a number of vertebrates [1,2,3,4]. A principal feature of cooperative breeding strategies is a reproductive division of labour, in some cases maintained by a socially induced suppression of fertility [5]. Elevated circulating PRL (hyperprolactinemia) is well known to be a major cause of infertility in mammals (males and females), and mediates natural suppression of reproduction that occurs during lactation [6,7]. Due to medical implications, elevated prolactin has been well studied in humans, where PRL concentrations above 25 ng/ml for women and 20 ng/ml for men are considered as clinical hyperprolactinemia [8].

NMRs and DMRs are divergent species within the African mole-rat clade, and have convergently evolved highly social cooperative breeding systems similar to those seen in eusocial insects [5]. A characteristic of these societies is an extreme reproductive division of labour and skew in lifetime reproductive success, with breeding restricted to a single female and one or two males, with more than 90% of individuals never having the opportunity to reproduce [8]. While non-breeding NMRs of both sexes are physiologically inhibited from reproducing, in DMRs only non-breeding females are physiologically suppressed. Differences between species in the underlying mechanism of female suppression are also evident, as the ovaries of non-breeding NMRs are prepubescent, while those of DMRs are fully developed [5,9,10,11,12]. In both species, socially-induced physiological suppression is mediated centrally via the hypothalamic GnRH system, with GnIH (RF amide related protein 3) and kisspeptin also implicated in the mechanism in NMRs [13,14,15]. Newly emerging work has implicated the dopamine system and raised levels of PRL in non-breeders as another potential component in reproductive suppression and eusociality in NMRs [16]. Despite the potential for PRL in playing a part in cooperative breeding in African mole-rats, either in suppression of reproduction, or in expression of alloparental behaviour, its possible role has been largely unexplored. This study aims to compare and contrast plasma PRL concentrations in eusocial DMRs with NMRs,

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68 examining breeders and non-breeders of both sexes. We predict that if the proximate control of
69 sociality in these species has, together with a eusocial lifestyle, convergently evolved in both species,
70 then plasma PRL should be elevated in non-breeders in both NMRs and DMRs.

71

Materials and methods

Sampling

74 Blood samples were obtained from 112 NMRs (2 lactating and 10 non-lactating queens, 7 breeding
75 males, 51 non-breeding females, 42 non-breeding males) from among 13 captive colonies from the
76 University of Pretoria and Queen Mary University of London. 132 DMRs were sampled (6 lactating
77 and 18 non-lactating queens, 20 breeding males, 41 non-breeding females, 47 non-breeding males),
78 from among 31 colonies from Winton and the Kalahari Research Centre, South Africa. The blood was
79 centrifuged at 500g and the resulting plasma decanted and stored at -80°C until hormone analysis.
80 Further details are given in the Electronic Supplementary Materials and tables S1 and S2.

Prolactin assay

82 Plasma PRL concentrations were validated for mole-rats and determined using a commercial enzyme-
83 linked immunosorbent assay (Elabscience© Guinea pig prolactin ELISA kit, Catalogue No: E-EL-
84 GP0358) according to the Manufacturer's instructions. Data were analysed using a General Linear
85 Model and results plotted using R statistical software [17,18] (see Electronic Supplementary
86 Materials).

87

Results

89   The mean, 95% Highest Density Intervals (HDI) values and a density plot of plasma PRL
90   concentrations for status groups in both species are displayed in figure 1. For DMRs, only 13 of 88
91   non-breeders of both sexes (15%) had detectable concentrations of PRL, and of these none had values
92   exceeding 25 ng/ml (that would be considered as clinically hyperprolactinemic; see Supplementary
93   Material for justification of the use of this value as a benchmark): non-breeding females (mean ±
94   SEM), 0.65 ± 0.58 ng/ml; n=41; range, <0.03-23.87 ng/ml; non-breeding males, 0.77 ± 0.49 ng/ml;
95   n=47; range, <0.03-20.22 ng/ml. For breeding queens (six of which were lactating), 13 out of the 24
96   samples had detectable PRL values: 4.98 ± 2.20 ng/ml; range <0.03-47.65 ng/ml. In breeding males
97   only 3 out of 20 samples had detectable concentrations of PRL: 0.12 ± 0.07 ng/ml; range <0.03-1.19
98   ng/ml. When the DMR data were analysed separately to the NMRs, the difference between breeders
99   and non-breeders was significant (F= 4.530; d.f.= 1, 124; p= 0.0353; figure 1; Supplementary
100 Material).

101   In contrast, 75 of 93 non-breeding NMRs (80%) had detectable concentrations of PRL, and
102 these often reached distinctly higher levels than DMRs (F= 50.325; d.f.= 1, 236; p= $1.51x10^{-11}$). None
103 of the interactions in the GLM were significant at α=0.05 (Supplementary Material). 37% exceeded
104 concentrations of >25 ng/ml: non-breeding females 32.64 ± 6.13 ng/ml; n=43; range, <0.03-23.87
105 ng/ml; non-breeding males 36.77 ± 9.81 ng/ml; n=4; range, <0.03-330.30 ng/ml. As with DMRs,
106 breeding queens had the expected variance in plasma PRL concentrations as part of normal
107 reproductive function (ovarian cyclicity, pregnancy and lactation): 33.02 ± 12.94 ng/ml; n=12; range,
108 3.60-160.80 ng/ml. Two values were obtained from lactating queens, 21.14 ng/ml (23 days post-
109 partum, at the end of the period of lactation) and 160.80 ng/ml (seven days post-partum), the latter
110 being the highest concentration recorded among the breeding female samples. Only a small number of
111 breeding males were sampled as they are often difficult to identify with certainty, and these had
112 comparatively low plasma PRL concentrations, 15.91 ± 6.21 ng/ml; n=7; range, 3.92-47.92 ng/ml.

113   Analysis of breeding queens revealed an overall significant increase in PRL in lactating
114 versus non-lactating females (F= 11.22; d.f.= 1, 32; p= 0.002), and a significant interaction (F= 7.33;
115 d.f.= 1, 32; p= 0.011), indicating a species difference (although only two samples were obtained from
116 lactating NMRs; see figure S2).

117

118 **Discussion**

119 Elevated PRL is well known to suppress fertility, and has also been implicated in many studies
120 (including birds and mammals) as a factor mediating both parental and alloparental care, a key feature
121 of cooperatively breeding societies, together with affiliative and other sociosexual behaviours
122 [e.g.1,2,3,4, and references therein]. For example, in a study of zebra finches, plasma PRL was found
123 to be positively correlated with parental behaviour, the number of chicks hatched, and chick survival
124 [19]. Further, in one of the classic examples of avian cooperative breeding, non-breeding Florida
125 scrub jay helpers (*Aphelocoma coerulescens*) have been shown to have PRL levels that positively
126 correlated with the rate of their nestling provisioning [20]. In one of the relatively few studies of
127 mammalian cooperative breeders, elevated levels of PRL in male meerkats preceded bouts of helping
128 behavior in the form of babysitting [4].

129   Patterns of plasma PRL differed markedly between Damaraland and naked mole-rats. As
130 expected for a reproductively active mammal, circulating PRL was detected in breeding queens in
131 both species, with high values recorded during pregnancy and lactation (tables S1 and S2; figure S2).
132 However, most non-breeding NMRs (80%) of both sexes had detectable plasma PRL concentrations,
133 often exceeding those considered clinically hyperprolactinaemic. Conversely, 85% of non-breeding
134 DMR samples of both sexes had undetectable values and none reached clinically high values. These
135 results suggest that while elevated PRL in non-breeders may be an important component in NMR
136 eusociality, this is apparently not the case in convergently eusocial DMRs.

137   Recent transcriptome profiling of the NMR brain has shown that breeding animals have
138 increased expression of genes involved in dopamine metabolism, (dopamine inhibits PRL secretion),
139 compared to non-breeders. This is consistent with the observed high levels of PRL in non-breeding
140 NMRs and strongly suggests a role for hyperprolactinaemia as a component in socially-induced
141 reproductive suppression [16]. The highly elevated levels of plasma PRL observed in non-breeding
142 NMRs could function to inhibit the release of GnRH, and thus LH, FSH, oestrogen, and testosterone,
143 leading to the well-characterised block to follicular development (in females) and spermatogenesis (in
144 males). In addition to a possible role in suppressing reproduction, it is tempting to speculate that
145 elevated PRL in NMRs may also play a part in the mechanisms eliciting cooperative behaviour. It is
146 therefore intriguing that apart from the breeding queen, almost all monitored DMRs in this study had
147 little or no detectable PRL. This may reflect the fact that unlike NMRs, the ovaries of female
148 DMRs have varying levels of follicular development, although they also do not ovulate.

149   In this first major cross-species study of PRL in African mole-rats, our data suggests that
150 divergent mechanisms have evolved in a role for this hormone in convergently eusocial mole-rats. At
151 present the results, although highly persuasive, are of an associative nature and functional studies on
152 both species are now needed to confirm cause and effect, and to establish the mechanistic details.

153

154   Figure 1: Pirate plot showing plasma prolactin (PRL) concentrations for Damaraland and naked mole-
155   rats respectively, by reproductive status: BM, breeding male; NBF, non-breeding female; NBM, non-
156   breeding male; Q, breeding queen. Raw data for each group are displayed as an open circle with the
157   points randomly jittered horizontally for ease of viewing, together with the densities (how crowded or
158   sparse the data are at every possible value) shaded in colour. The mean is shown as a dark horizontal
159   line, with boxes showing the 95% Highest Density Intervals (HDI) of the mean of each group (the
160   interval spanning 95% of the distribution [18]).

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Author contributions

NCB and CGF conceptualised the project. NCB, JUMJ, MZ and CGF collected samples. SG, AG and CGF undertook the assays. CGF analysed the data and CGF and NCB refined several drafts of the manuscript. All authors contributed towards revision of the article, approve the final version of the manuscript, and agree to be held accountable for the content therein.

Data accessibility

All raw data are supplied in the Supplementary Material.

Funding

Competing interests

The authors have no competing interests to declare.

Ethical statement

All procedures involving live animals and sample collection described in this manuscript were conducted in accordance with appropriate national and provincial guidelines, permits and regulations. The protocol used in this study was approved by the animal ethics committee of the University of Pretoria EC0 84-15.
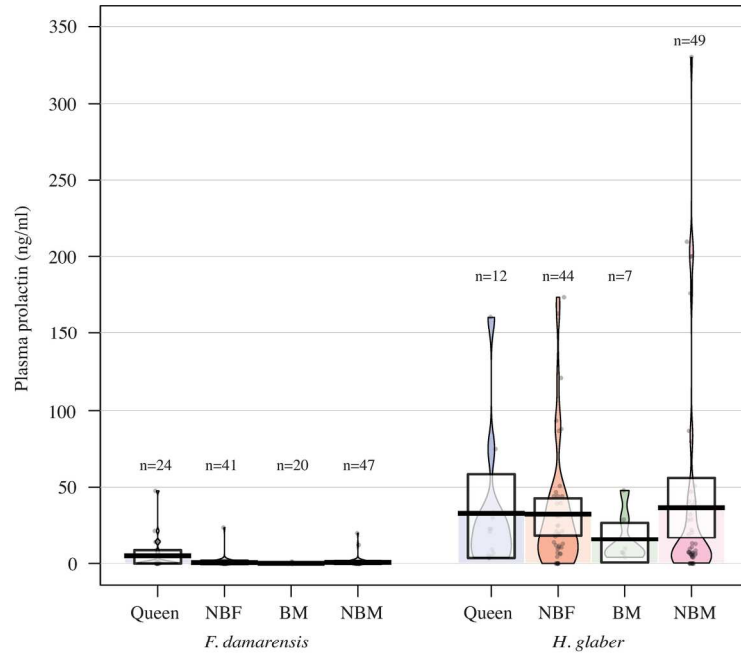
Pirate plot showing plasma prolactin (PRL) concentrations for Damaraland and naked mole-rats respectively, by reproductive status: BM, breeding male; NBF, non-breeding female; NBM, non-breeding male; Q, breeding queen. Raw data for each group are displayed as an open circle with the points randomly jittered horizontally for ease of viewing, together with the densities (how crowded or sparse the data are at every possible value) shaded in colour. The mean is shown as a dark horizontal line, with boxes showing the 95% Highest Density Intervals (HDI) of the mean of each group (the interval spanning 95% of the distribution [18]).

187x156mm (300 x 300 DPI)

Evidence for contrasting roles for prolactin in eusocial naked mole-rats, *Heterocephalus glaber* and Damaraland mole-rats, *Fukomys damarensis*

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Supplementary Material

Methods
Animal housing
Animals were briefly housed in their natal colonies in large plastic crates (1m x 0.5m x 0.5m) prior to sampling in the case of DMRs collected from Winton, before being taken to the University of Pretoria (UP). DMRs from the Kalahari Research Centre (KRC) and NMRs at UP and Queen Mary University of London (QMUL) were kept long-term in interconnecting tubing with communal nest, food and toilet chambers - see Tables S1 and S2. Wood shavings, sand and paper towelling served as nesting material. The room temperature ranged between 20 and 28ºC (DMRs) and 29 and 30ºC (NMRs) with relative humidity around 50-60% (Bennett & Jarvis, 1988; Faulkes *et al*., 1990, Zöttl *et al*., 2016). Animal rooms in Pretoria were maintained on a 12L:12D photoperiod, while the KRC and QMUL colonies were kept in the dark, with lights-on while daily husbandry and sampling was conducted. Photoperiod has not been shown to affect DMR and NMR behaviour (C.G. Faulkes and N.C. Bennett, unpublished data; Oosthuizen *et al*., 2003). Potential effects of the differing photoperiod regimes were tested for in this study, and ruled it out as a significant variable (see results below; F= 0.560; d.f.= 1, 124; p= 0.4558). Animals were fed on a variety of chopped vegetables and drank no free water. For both species, reproductive females were identified by the presence of prominent axillary and inguinal teats, well developed external genitalia with a perforate vagina, and/or pregnancy-related changes in girth/body size. Reproductive males were identified on the basis of observations of copulation with the reproductive female (NMRs), while in DMRs a dark stain around the periphery of the mouth and bulging testes which project from abdominal pockets discern dominant breeders. At the KRC records of dyadic behavioural interactions were used to identify socially dominant males.

Blood sampling
For NMRs, blood samples were collected between 08h00 and 15h00 as follows: The animals were hand held and venous blood samples collected from the hind foot. Approximately 300-500ul of blood was collected into heparinised micro-haematocrit tubes (University of Pretoria samples) or into a heparinised 1 ml syringe from trunk blood following euthanasia (QMUL) prior to tissue collection for other studies. Similarly, in DMRs, blood was either collected from the foot or trunk blood. All blood samples collected at the University of Pretoria and the KRC were obtained with University of Pretoria ethics committee clearance and at QMUL followed institutional guidelines.

Prolactin assay and validation
In brief, 100 μl of reference standard and diluted samples (1/2 to 1/50 in sample diluent) were transferred into coated wells of a 96-well micro-ELISA plate, respectively, and incubated for 90 min at 37°C. Subsequently, all supernatant was removed, and the plate patted dry. Immediately, 100 μl of biotinylated detection antibody was added, and incubated for 60 min at 37°C. The plate was washed 3 times, patted dry, and 100 μl of horse radish peroxide (HRP) conjugate were added and incubated for 30 min at 37°C. Subsequently, the plate was washed 5 times with wash-buffer, and patted dry. 90 μl of substrate reagent were added, and incubated for 15 min at 37°C. To terminate the enzymatic reaction, 50 μl of stop solution were added. Optical density was determined at 450 nm and results

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calculated using a best fit curve. The sensitivity of the assay was 0.1 ng/ml, the detection range 0.16-10 ng/ml, and coefficient of variation for repeatability was < 10%. Serial dilutions of spiked plasma pools for DMR and NMR gave displacement curves that were parallel to the respective standard curve of the PRL assay, validating the assay for use with both species (relative variation (%) of the slope of respective trend lines <2%; figure S1).
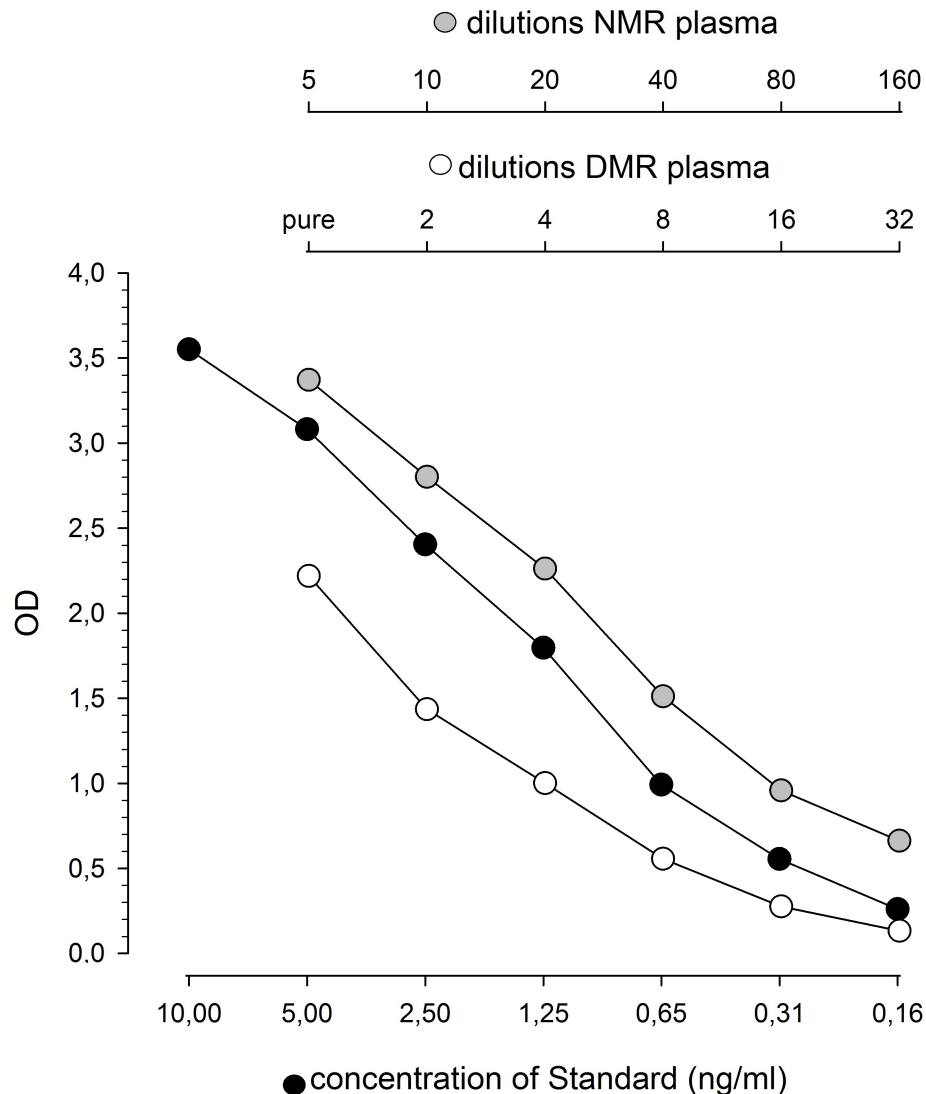
Figure S1: Assay validation revealed by parallelism of serial dilutions of spiked plasma pools for DMR and NMR, with standard for the PRL ELISA assay. Relative variation (%) of the slope of respective trendlines were <2% for DMR and NMR plasma, respectively. OD is the optical density measures the ELISA colour change, which is proportional to the concentration of PRL.

Because this is the first time PRL has been measured in mole-rats, we do not know if measures of hyperprolactinaemia in humans correspond to measures of hyperprolactinaemia in mole rats. We have chosen to include the clinical values in humans as a relevant mammalian benchmark. Normal levels of PRL are by and large similar in other mammals, and less than the benchmark values for hyperprolactinaemia in humans of 25 ng/ml that we use for context. This includes rodents, e.g. rats < 10 ng/ml (controls in Fig 3B in Egli *et al*. 2010), Chinese hamsters < 10 ng/ml, Syrian hamsters < 14 ng/ml (Parkening *et al*., 1980) and meerkats <12 ng/ml (Carlson *et al*. 2006).

Statistical analysis

Because of the large number of animals in which prolactin levels were undetectable, and the resulting zeroes in the dataset, we used a Tweedie distribution (Tweedie, 1984) to describe the response distribution within a generalised linear model framework with prolactin levels as the response and species, sex and reproductive status (breeding or non-breeding) as predictors, with all interactions included. In separate analyses, PRL concentrations were compared in lactating and non-lactating queens for both species, and within DMRs for the effects of 12L:12D cycle versus dark/intermittent light (see methods above). The model was implemented using the R packages statmod (Giner and Smith, 2016) and tweedie (Dunn and Smyth, 2008).

Results

Summary of anova statistics from between species GLM, with prolactin levels as the response and species, sex and reproductive status (breeding or non-breeding) as predictors (spec, species; d.f., degrees of freedom):

	d.f.	Sum Sq	Mean Sq	F value	Pr(>F)
spec	1	56799	56799	50.325	1.51e-11 ***
sex	1	139	139	0.123	0.726
status	1	52	52	0.046	0.831
spec:sex	1	2	2	0.001	0.970
spec:status	1	762	762	0.675	0.412
sex:status	1	965	965	0.855	0.356
spec:sex:status	1	417	417	0.370	0.544
Residuals	236	266362	1129		

Summary of anova statistics from GLM, with prolactin levels as the response and lactating/non-lactating queens as predictors.

	d.f.	Sum Sq	Mean Sq	F value	Pr(>F)
queens$spec	1	6290	6290	12.83	0.00112 **
queens$lactating	1	5501	5501	11.22	0.00209 **
queens$spec:queens$lactating	1	3595	3595	7.33	0.01079 *
Residuals	32	15692	490		

Summary of anova statistics from within DMR GLM, with prolactin levels as the response and Light:Dark cycle (cycle), sex and reproductive status (breeding or non-breeding) as predictors:

	d.f.	Sum Sq	Mean Sq	F value	Pr(>F)
cycle	1	16	16.24	0.560	0.4558
sex	1	94	93.93	3.238	0.0744
status	1	90	90.28	3.112	0.0802
cycle:sex	1	65	64.88	2.236	0.1373
cycle:status	1	97	97.11	3.347	0.0697
sex:status	1	131	131.43	4.530	0.0353 *
cycle:sex:status	1	40	39.63	1.366	0.2447
Residuals	124	3598	29.01		

Signif. codes: '***' 0.001 '**' 0.01 '*' 0.05
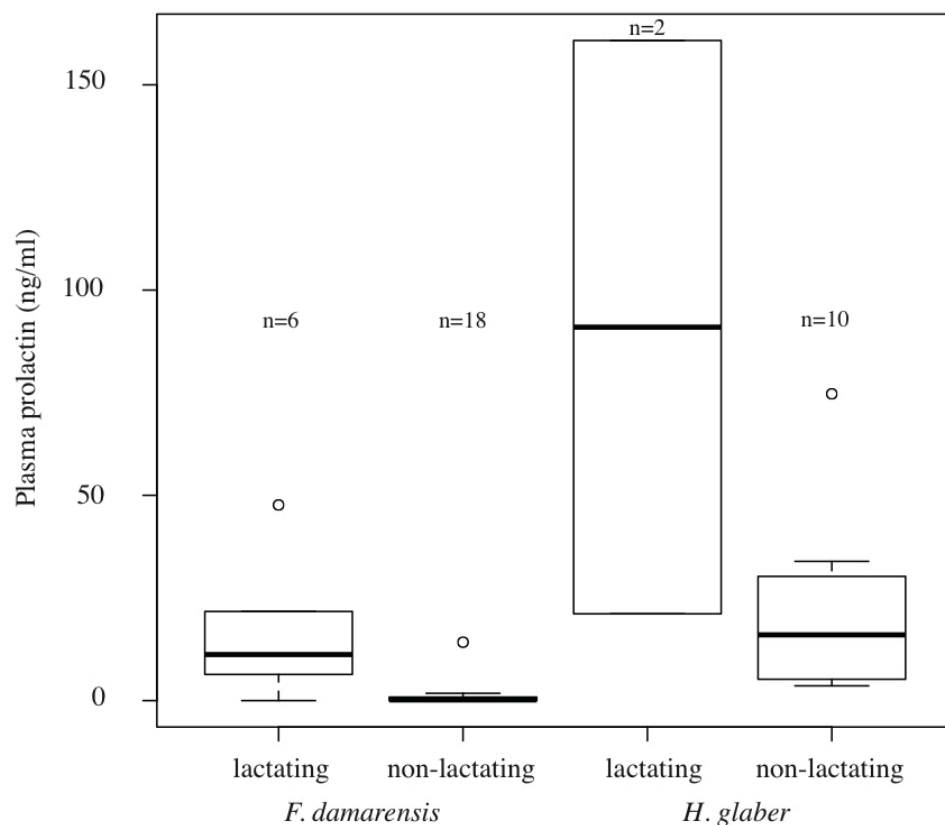
Figure S2: Plasma prolactin (PRL) concentrations for lactating and non-lactating Damaraland and naked mole-rats respectively. The box-plot midline is the median, with the upper and lower limits of the box being the third and first quartile (75th and 25th percentile) respectively. Whiskers extend up to 1.5 times the interquartile range from the top or bottom of the box to the furthest data point within that distance (beyond that distance, they are represented individually as points/'outliers').

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Supplementary Table S1 Sample details and respective prolactin values (ng/mg plasma) for naked mole-rats. NBF: non-breeding females, NBM: non-breeding males, BM: breeding males.

Animal/ sample No.	Sex/status	Body mass (g)	Prolactin (ng/ml)	Colony	Notes
1	NBM	45	19.50	1	
2	NBM	49	10.62	1	
3	NBF	72	19.34	1	
4	NBF	60	17.50	1	
5	NBF	40	6.50	1	
6	NBM	54	8.36	1	
7	NBF	53	11.78	1	
8	NBF	67	121.00	1	
9	NBF	40	93.56	1	
10	NBF	34	19.24	1	
11	NBM	55	38.30	1	
12	NBM	43	22.32	1	
13	NBM	40	47.09	1	
14	NBM	53	12.68	1	
15	NBF	47	88.26	1	
16	NBM	52	9.42	1	
17	NBM	38	330.30	1	
18	NBM	38	176.10	1	
19	NBM	37	209.50	1	
20	NBF	75	173.60	1	
21	NBF	63	163.10	1	
22	NBF	67	44.10	1	
23	NBM	66	20.48	1	
24	NBM	59	28.62	1	
25	NBM	46	32.34	1	
26	NBF	41	46.72	1	
27	NBF	44	39.72	1	
28	NBF	36	39.34	1	
29	NBF	49	32.72	1	
30	NBM	40	29.06	1	
31	NBF	44	11.44	2	
32	NBF	57	31.48	2	
33	Queen	73	9.02	2	44/72 days pregnant
34	NBM	56	4.74	2	
35	NBF	45	13.70	2	
36	NBF	46	7.06	2	
37	NBF	43	12.74	2	
38	NBF	47	6.02	2	
39	NBM	52	79.54	2	
40	NBM	49	5.96	2	
41	NBM	46	13.07	2	
42	NBF	40	9.46	2	

Animal/ sample No.	Sex/status	Body mass (g)	Prolactin (ng/ml)	Colony	Notes
1	Queen	55	21.14	3	23 days post-partum (lactating)
2	NBF	76.86	44.2	3	
3	BM	65.31	7.24	3	
4	NBF	73.3	31.36	3	
5	NBF	70.9	10.4	3	
6	NBF	49.1	21.64	3	
7	NBM	62.3	7.36	3	
8	NBM	45.75	40.68	3	
9	NBF	45.5	0	3	
10	NBM	43.3	0	3	
11	NBM	46.4	6.48	3	
12	NBF	43.3	25.12	3	
13	NBM	36.1	87.04	3	
14	NBF	36.9	50.78	3	
15	NBF	41	0	3	
16	NBM	36.3	4.34	3	
17	NBM	36	6.94	3	
18	NBM	35.6	5.84	3	
19	NBF	37.1	0	3	
20	NBM	37.4	0	3	
21	NBM	40.9	0	3	
22	NBM	28.9	0	3	
23	NBM	35.3	4.04	3	
24	NBF	35.2	0	3	
25	NBM	32.7	5.6	3	
26	NBM	28.2	0	3	
1	BM	64	6.44	3a	
2	Queen	60	5.2	3a	45/72 days pregnant
3	NBM	55.2	6.04	3a	
4	NBF	37	9.36	3a	
5	NBM	58	15.62	3a	
6	NBM	49	9.3	3a	
7	NBF	37	8.54	3a	
8	NBM	43	0	3a	
9	NBF	37	18.76	3a	
10	NBF	45	7.36	3a	
11	NBF	33	5.26	3a	
12	NBM	42	18.34	3a	
1	BM	65.9	47.92	3a	
2	Queen	55.2	160.8	3a	lactating
3	NBM	64.7	16.14	3a	
4	NBF	51	27.58	3a	

Animal/ sample No.	Sex/status	Body mass (g)	Prolactin (ng/ml)	Colony	Notes
5	NBM	44.4	25.5	3a	
6	NBM	52.2	61.84	3a	
7	NBF	40.3	68.58	3a	
8	NBF	38.1	16.14	3a	
9	NBM	52.4	26.2	3a	
10	NBM	52.2	21.4	3a	
11	NBF	38.1	6.52	3a	
12	NBM	37.3	53.28	3a	
13	NBM	40.6	10.36	3a	
14	NBF	40.3	9.52	3a	
15	NBM	41.4	8.98	3a	
16	NBM	39.3	7.78	3a	
17	NBM	38.2	15.12	3a	
18	NBM	30.2	10.4	3a	
19	NBM	29.3	45.12	3a	
5.01	Queen	61.7	23.02	5	pregnant
5.02	BM	48.9	29.1	5	
628	Queen	45.5	4.66	11a	Not pregnant
827	Queen	57.2	6.72	Omega	Not pregnant
527	Queen	44.8	30.26	11c	Not pregnant
881	Queen	57.5	33.92	B	Not pregnant
297	Queen	59.1	23.20	G	Not pregnant
52	Queen	52	74.72	11b	Not pregnant
555	BM	36.1	9.74	11b	
578	BM	46.6	3.92	Omega	
107	Queen	32	3.60	17a	Not pregnant
118	BM	31	7.04	17a	
33	NBM	56	90.94	B	

Supplementary Table S2 Sample details and respective prolactin values (ng/mg plasma) for Damaraland mole-rats. NBF: non-breeding females, NBM: non-breeding males, BM: breeding males. Colony names in green text signify animals housed on a 12L:12D photoperiod, while those in blue were from the KRC where colonies were kept in the dark, with lights-on while daily husbandry and sampling was conducted.

Animal/ sample No.	Sex/status	Body mass (g)	Prolactin (ng/ml)	Colony	Notes
1.1	Queen	119	*0.856*	Winton1	non pregnant
1.2	BM	194	0.389	Winton1	
1.3	NBF	59	0	Winton1	
2.1	Queen	116	0	Winton2	non pregnant
2.2	NBF	84	0.878	Winton2	
2.4	BM	133	1.188	Winton2	
2.5	NBF	102	0	Winton2	
2.6	NBF	120	0	Winton2	
2.7	NBM	106	0	Winton2	
3.1	BM	143	0	Winton3	
3.2	Queen	95	0.569	Winton3	non pregnant
3.3	NBF	80	0	Winton3	
3.4	NBM	88	0.59	Winton3	
5.1	NBM	98	0	Winton5	
5.2	NBM	70	0	Winton5	
5.3	BM	190	0	Winton5	
5.4	Queen	136	0	Winton5	non pregnant
5.5	NBF	65	0	Winton5	
6.1	Queen	129	0	Winton6	non pregnant
6.2	BM	149	0	Winton6	
7.1	BM	186	0	Winton7	
7.2	Queen	145	0.483	Winton7	non pregnant
8.1	NBF	120	0	Winton8	
8.2	NBM	128	0.474	Winton8	
8.3	NBM	90	0	Winton8	
8.4	BM	154	0	Winton8	
8.5	BM	188	0	Winton8	
8.6	NBF	81	0	Winton8	
8.7	Queen	112	0.332	Winton8	non pregnant
8.8	NBM	118	0	Winton8	
9.1	NBM	138	0	Winton9	
9.2	NBF		0	Winton9	
9.3	NBM	81	0.336	Winton9	
9.4	NBF	119	0	Winton9	
9.5	NBM	61	0	Winton9	
9.6	NBM	47	0.378	Winton9	
9.7	NBM	144	0	Winton9	
9.8	BM	172	0	Winton9	
9.9	NBM	80	0.396	Winton9	
9.11	NBF	84	0	Winton9	

Animal/ sample No.	Sex/status	Body mass (g)	Prolactin (ng/ml)	Colony	Notes
9.12	Queen	126	0	Winton9	non pregnant
9.13	NBM	106	0	Winton9	
11.1	NBM	96	0	Winton11	
11.2	NBF	47	0	Winton11	
11.3	BM	169	0	Winton11	
11.4	NBM	155	0	Winton11	
11.5	Queen	121	1.43	Winton11	non pregnant
11.6	NBM	67	0	Winton11	
11.7	NBF	114	0	Winton11	
11.8	NBM	106	0	Winton11	
11.9	NBM	118	0.512	Winton11	
11.10	NBM	77	0	Winton11	
11.11	NBF	74	0	Winton11	
11.12	NBM	70	0	Winton11	
11.13	NBF	49	0	Winton11	
5	Queen	130	8.55	Tswalu1	5 days lactating
8	Queen	142	21.71	Tswalu2	lactating
9	Queen	138	13.89	Tswalu3	9 days lactating
10	Queen	119	6.375	Tswalu4	lactating
15	Queen	157	47.65	Tswalu5	7 days lactating
G3F009	Queen	170	14.23	Crick	No Lact
NOM003	NBM	85	0	Nowak	
NOM004	NBM	91	0	Nowak	
NOF002	NBF	82	0	Nowak	
G13F012	NBF	74	0	G13	
G5F004	Queen	90	0	Grunenthal	No Lact
JA5M002	BM	91	0	Grunenthal	
SAF001	NBF	93	23.875	Sappolsky	
SAM002	NBM	132	0	Sapplosky	
SAF004	NBF	53	0	Sappolsky	
L6M009	NBM	142	0	L6	
JRM001	NBM	154	0	Jarvis	
L7F007	Queen	163	0	Aristotle	early pregnant
G10M017	BM	173	0	Aristotle	
L7F009	NBF	135	0	Aristotle	
JA1M001	BM	140	0	Lorenz	
G1F002	Queen	126	0.835	Lorenz	non pregnant?
G1F012	Queen	151	0	Burda	non pregnant?
G7M008	BM	160	0	Burda	
KR7F010	Queen	119	1.785	Hermes	non pregnant?
BO50M005	BM	116	0	Hermes	
G10M003	NBM	163	20.225	G10	
G10M002	NBM	170	0	G10	
L2M002	NBM	214	0	L2	

Animal/ sample No.	Sex/status	Body mass (g)	Prolactin (ng/ml)	Colony	Notes
L2M001	NBM	156	0	L2	
L3M001	NBM	189	0	L2	
FAF001	NBF	63	0	Faulkes	
G4F010	Queen	109	0.88	Faulkes	
Z3M007	NBM	144	11.995	Faulkes	
F2F002	Queen	95	0	West	non pregnant?
JA1M003	BM	115	0.885	West	
L2F017	NBF	42	0	L2	
L2M016	NBM	54	0	L2	
G10F026	NBF	63	1.035	G10	
BUF001	NBF	74	0	Burda	
G4F019	NBF	55	0	G4	
G4M017	NBM	52	0	G4	
G4F020	NBF	58	0	G4	
G4M018	NBM	64	0	G4	
G4F016	NBF	89	0	G4	
CRF004	NBF	76	0	Crick	
CRF005	NBF	81	0	Crick	
G11F011	NBF	132	0	G11	
G11F010	NBF	117	0	G11	
G11M004	NBM	170	0	G11	
G11F015	NBF	109	0	G11	
G11F003	NBF	107	0	G11	
G11F017	NBF	77	0.84	G11	
G11M018	NBM	75	0	G11	
G11M012	NBM	130	0	G11	
G11M016	NBM	144	0	G11	
G11M008	NBM	165	1.08	G11	
G11F007	Queen	147	0	G11	lactating
L2F004	NBF	154	0	L2	
L3M001	NBM	207	0	L2	
L2M001	BM	165	0	L2	
L2F017	NBF	56	0	L2	
L2M002	BM	212	0	L2	
L3F0003	NBF	153	0	L2	
L2M016	NBM	65	0	L2	
L2M012	NBM	133	0	L2	
L2M003	NBM	186	0	L2	
G1M025	NBM	197	0	G1	
G1F010	Queen	156	0	G1	
G1F022	NBF	42	0	G1	
G1M008	BM	191	0	G1	

Animal/ sample No.	Sex/status	Body mass (g)	Prolactin (ng/ml)	Colony	Notes
G1F021	NBF	98	0	G1	
G1F014	NBF	150	0	G1	
G1M004	BM	181	0	G1	
G1F025	NBF	51	0	G1	
G1M017	NBM	168	0	G1	
G1M026	NBM	53	0	G1	