The development of individual differences in cooperative behaviour: maternal glucocorticoid hormones alter helping behaviour of offspring in wild meerkats

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Abstract

The phenotype of parents can have long-lasting effects on the development of offspring as well as on their behaviour, physiology, and morphology as adults. In some cases, these changes may increase offspring fitness but, in others, they can elevate parental fitness at a cost to the fitness of their offspring. We show that in Kalahari meerkats (*Suricata suricatta*), the circulating glucocorticoid (GC) hormones of pregnant females affect the growth and cooperative behaviour of their offspring. We performed a 3-year experiment in wild meerkats to test the hypothesis that GC-mediated maternal effects reduce the potential for offspring to reproduce directly and therefore cause them to exhibit more cooperative behaviour. Daughters (but not sons) born to mothers treated with cortisol during pregnancy grew more slowly early in life and exhibited significantly more of two types of cooperative behaviour (pup rearing and feeding) once they were adults compared to offspring from control mothers. They also had lower measures of GCs as they aged, which could explain the observed increases in cooperative behaviour. Because early life growth is a crucial determinant of fitness in female meerkats, our results indicate that GC-mediated maternal effects may reduce the fitness of offspring, but may elevate parental fitness as a consequence of increasing the cooperative behaviour of their daughters.

Keywords: Cooperation, Early life adversity, Glucocorticoids, Growth, Maternal stress

Introduction

Parental effects are a mechanism of trans-generational phenotypic plasticity that occurs when the parental phenotype or parental environment modifies offspring characteristics (1). Parental effects can increase the survival or reproduction of offspring, thereby elevating the direct fitness of both offspring and parents (2-6). Alternatively, parental effects can increase parental fitness, but at some cost to the fitness of their offspring (7-8) – a process regarded as a type of parental manipulation (9-12) or 'selfish parental effect' (13). For example, in mammals, the optimal birth weight or litter size often differs between mothers and offspring (14) and pregnant females experiencing stressful environments may reallocate resources away from offspring and towards themselves, so that their offspring are smaller or grow more slowly before weaning (15). Despite these observations, it has been suggested that selfish parental effects may be rare and unstable because selection would be expected to favour the evolution of resistance mechanisms in offspring (7, 11, 13, 16, 17).

Selfish parental effects may in fact be more likely in cooperatively breeding species where philopatric offspring (subordinates) help to rear the subsequent offspring of their parents or other close relatives. This could be especially likely under low food or high stress conditions as parents may gain substantial direct fitness benefits from delaying the development of their offspring if this causes them to invest in alloparental care directed at the parent's subsequent offspring (9-10). In addition, the costs of selfish parental effects to offspring could be reduced in these circumstances, as offspring will gain indirect fitness benefits by contributing to raising the subsequent offspring of their parents (18). For example, laboratory studies of eusocial insects suggest the possibility

65 that selection will favour the evolution of alleles that enable mothers to increase the
66 helping behaviour of their offspring while simultaneously reducing their capabilities of
67 reproducing on their own (19-20; but see 21).

68 To date, empirical field tests of how parental effects shape the helping behaviour
69 of offspring are rare (23) and studies of selfish parental effects have mostly focused on
70 non-social species (13, 24). Here, we report the results of experiments designed to test
71 the hypothesis that elevated maternal glucocorticoid levels (GCs) reduce the potential
72 for offspring to have direct reproductive opportunities and causes them to exhibit more
73 cooperative behaviour. In a 3-year field study, we experimentally elevated maternal
74 GCs by treating pregnant dominant female meerkats with cortisol and tracking the
75 growth, stress physiology, and cooperative behaviour of their offspring from birth until
76 ~18 months of age, compared to those from control litters. We manipulated maternal
77 GCs because they are known to cause mothers to reallocate energy away from
78 offspring and towards themselves (15), indicating that they may function as a mediator
79 of selfish maternal effects. Changes in maternal GCs have also previously been shown
80 to delay the dispersal of offspring as well as influence the parental care behaviour of
81 offspring (25-26), both traits that are important in cooperative breeders where philopatric
82 offspring exhibit alloparental care behaviour towards juveniles.

83 To identify if the exposure of mothers to heightened GCs reduced reproductive
84 success of their offspring, we examined if offspring from mothers treated with cortisol
85 during pregnancy grew more slowly early in life. In meerkats, the rate of early life growth
86 and body mass is closely linked to future direct fitness through its effects on survival,
87 foraging success, adult body mass (27-29), as well as the probability of acquiring

88 dominance (30-31) and other direct reproductive opportunities (32). As elevated
89 exposure to maternal GCs in some mammals may reduce offspring size and growth
90 early in life (15), we predicted that offspring from mothers treated with cortisol during
91 pregnancy would be smaller or grow more slowly early in life. Because the rate of early
92 life growth is predictive of future direct fitness in meerkats (27-32), we predicted that if
93 offspring from mothers treated with cortisol did grow more slowly, they would
94 consequently invest more in indirect fitness opportunities by contributing more to
95 cooperative activities than controls.

96 Secondly, we determined if offspring from mothers treated with cortisol during
97 pregnancy subsequently increased their contributions to two types of cooperative
98 behaviours: pup rearing ("babysitting": 33) and food provisioning during the period when
99 the pups are foraging with their natal group, but are not yet nutritionally independent
100 ("pup feeding": 34). We chose these two behaviours as they appear to be most costly
101 from an energetic perspective (35) and are most closely tied to the probability of parents
102 successfully rearing offspring. If offspring from mothers treated with cortisol during
103 pregnancy exhibit more of either of these two types of alloparental care, this should
104 increase both parental direct fitness (the number of offspring that they subsequently
105 produce) and the indirect fitness of offspring, because subsequent offspring that receive
106 more alloparental care should grow faster or have higher early life survival (27, 32, 36,
107 37). Previous studies in meerkats show that offspring with more helpers or those that
108 receive more alloparental care grow faster or have early life survival (27, 32, 36, 37).

109 To assess the mechanism by which elevated exposure to maternal stress may
110 affect the alloparental care behaviour of offspring, we repeatedly measured plasma

111 cortisol and faecal glucocorticoid metabolite (fGCM) concentrations of offspring from
112 when they were approximately 1 to 18 months of age to identify how our manipulations
113 affected their neuroendocrine stress axes (GC output). Elevated maternal GCs can
114 cause long-term changes in the neuroendocrine stress axis of offspring (38) and
115 elevated activity of the neuroendocrine stress axis in meerkats can reduce their
116 contributions to alloparental care (39). We therefore predicted that if offspring born to
117 mothers treated with cortisol during pregnancy exhibited more alloparental care
118 behaviour compared to controls, they would also have reduced plasma cortisol and
119 fGCM concentrations.
120

121 **Methods**

122 *Study site & basic data collection*

123 We studied free-living meerkats at the Kuruman River Reserve (26° 58' S, 21°
124 49' E) in the Northern Cape, South Africa from 2014-2017. Individuals were marked
125 uniquely with PIT tags (Identipet®, Johannesburg, South Africa) as well as dye marks so
126 that they could be identified. Groups were visited for ~4-8 hours per day ~4-6 times per
127 week throughout each year of study and sometimes more frequently such as when
128 there were pups being babysat. Groups were visited at sunrise before meerkats
129 emerged from their sleeping burrow. After all the meerkats had emerged, but prior to
130 when they started going foraging, we counted the total number of meerkats in the group
131 (to get estimates of group size) and recorded which individuals were present (using their
132 unique combinations of dye marks). We recorded their body mass on a portable
133 balance each morning before foraging, 2-4 hrs after foraging was initiated, and

134 immediately prior to when foraging ended (40). These measures of body mass provided
135 our estimates of growth, body mass, and foraging success that are used in our analyses
136 described below.

137 *Experimental manipulations of dominant females*

138 Dominant females in each group were identified via behavioural observations
139 (41). The pregnancy status of dominant females was determined visually (distended
140 abdomen) as well as noting a constant increase in their body mass. Dominant females
141 were treated with either a cortisol solution or a control oil vehicle when they were
142 pregnant by feeding them food containing one of these two treatments. We initially
143 offered experimental animals hard boiled eggs with added cortisol but found that they
144 rejected all foods that contained added cortisol with the exception of scorpions. We
145 consequently fed experimental females with cortisol (10 mg/kg of hydrocortisone, Sigma
146 H4126), that were dissolved in 100 μl of 100% coconut oil and injected into a dead
147 scorpion (*Opistophthalmus* spp.). Control females were fed a dead scorpion that was
148 injected with 100 μl of 100% coconut oil. A previous study using the same protocol
149 showed that meerkats that were fed cortisol had significantly higher plasma cortisol and
150 fGCM concentrations than control animals and these increases were within a
151 biologically relevant range (39). This indicated that our treatment causes the exogenous
152 glucocorticoids that we feed the meerkats to enter their bloodstream and leads to
153 sustained increases in their circulating glucocorticoid concentrations.

154 Females were randomly allocated to the treatments. Across the three years of
155 this study, we produced a total of 13 cortisol-treated litters from 10 females and 7
156 control litters produced by 6 females (Table 1). Three of the females experienced both

157 the control and cortisol treatments at different time points of the experiment, whereas
158 one female experienced the control treatment once and the cortisol treatments twice.
159 For these latter females treated twice, the order of treatments was randomly selected.
160 We conducted these experiments over the course of three years: 13 litters from 10
161 females in 2014 (April-December 2014, 3 litters aborted), 5 litters from 5 females in
162 2015 (February-July 2015, 1 litter aborted), and 3 litters from 3 females in 2016 (July
163 2016). Three cortisol-treated mothers and one control mother aborted their offspring
164 prior to birth and were excluded from any analyses except for assessing differences in
165 the frequency of abortion between control and cortisol-treated mothers (Table 2). This
166 provided final sample sizes of 31 pups from 10 litters from 9 cortisol-treated females
167 and 25 pups from 6 litters from 6 control females (Table 1).

168 We aimed to experimentally increase the glucocorticoid concentrations of
169 pregnant dominant females from when they were first confirmed to be pregnant (second
170 half of gestation) until parturition. Gestation in meerkats is ~70 days so we aimed to
171 treat them with glucocorticoids from approximately 35-70 d during gestation. In reality,
172 females that successfully produced a litter where pups emerged from the natal burrow
173 were treated with cortisol for 12-36 days prior to birth (n=10 litters from 9 females, mean
174 = 23.7 d, median = 23.5 d), whereas controls were fed for 12-58 d prior to birth (n=6
175 litters from 6 females, mean = 30 d, median = 20.5 d). Although controls were treated
176 for slightly longer, there was no significant difference in treatment duration between
177 control and cortisol-treated females (general linear model, $t = 1.05$, $P = 0.31$).

178 To provide an additional comparison group to investigate how our treatments (fed
179 during pregnancy or fed cortisol during pregnancy) affected offspring survival, growth,

180 and cooperative behaviour, we also monitored these traits in offspring produced by
181 dominant females that were untreated during pregnancy (n = 52 litters from 21 dominant
182 females, Table 1). These females were not fed or treated with cortisol (hereafter,
183 "untreated mothers"). For our analyses of how the treatments affected offspring survival
184 and growth, the untreated offspring were those from litters produced by dominant
185 females in other meerkat groups in our same study area and were born during our
186 study. We assessed the contributions of offspring from mothers treated with cortisol
187 during pregnancy to two cooperative behaviours (babysitting and pup feeding)
188 compared to those from control mothers, but also to other group members from
189 untreated mothers. We did not have data from offspring from untreated mothers when
190 we assessed how our treatments affected their plasma cortisol or fGCM concentrations.

191 *Quantifying early life growth of offspring*

192 Meerkat pups typically first emerge from their natal burrow approximately 21-30 d
193 after birth. Meerkat groups and dominant females were monitored daily around the
194 estimated date of parturition and birth dates were estimated according to the change in
195 the physical appearance of the dominant female, a large drop in body mass overnight,
196 and group members exhibiting babysitting behaviour at the sleeping burrow. Burrows
197 containing pups were monitored each day and, when pups emerged, they were uniquely
198 marked by trimming small sections of hair before permanent PIT tags could be applied.
199 Pups were weighed each time we visited the groups on a portable balance in the
200 morning after group members emerged from their sleeping burrow (as above).

201 *Quantifying cooperative behaviour of offspring*

202 We measured the babysitting (controls: 195-655 d; cortisol: 184-655 d; untreated:
203 155-655 d) and pup feeding (controls: 220-635 d; cortisol: 184-655 d; untreated: 155-
204 626 d) contributions of offspring from cortisol-treated and control mothers when they
205 were >6 months of age until death or disappearance. We visited sleeping burrows
206 containing pups every day in the morning and recorded the identity of the attending
207 babysitters. As we have done previously (33, 36, 39, 42), we calculated relative
208 babysitting contributions of each individual meerkat for each litter by dividing the total
209 number of days an individual babysat a litter over the total number of days that this
210 specific litter had a babysitter. Pup feeding behaviour for each pup produced by the
211 dominant females in the different treatment groups was estimated using *ad libitum*
212 sampling (34, 39). When the social group contained pups (up to 90 d of age), we
213 recorded all pup-feeding events from all individuals, which are visually and acoustically
214 conspicuous to observers (43). We then used these data to estimate the proportion of
215 pup-feeding events exhibited by an individual relative to all others in the group (i.e.,
216 relative pup feeding). Because the total amount of time devoted to the *ad libitum*
217 recording sessions varied, we corrected for variation in observation time (see below).

218 *Quantifying plasma cortisol concentrations from offspring*

219 We obtained plasma samples from offspring from cortisol-treated and control
220 mothers approximately every 3 months from first emergence from the burrow (~1
221 month) until ~18 months of age (controls: 20-548 d; cortisol-treated: 25-559 d). Capture
222 and blood processing procedures are described elsewhere (44-45). The amount of time
223 it took to obtain the blood samples varied (median = 10.6 min, SD = 7.2 min), but we
224 included co-variates for sampling time and sampling time^2 to control for effects of

225 sampling time (described in 45). We measured total plasma cortisol concentrations
226 using a previously validated assay (Coat-a-Count, Siemens Diagnostic Products
227 Corporation, Los Angeles, USA: validation described in 44). The sensitivity of the assay
228 was 1.9 ng/ml and cross-reactivity to other hormones was 76% with prednisolone,
229 11.4% with 11-deoxycortisol, 2.3% with prednisone and <1% with aldosterone,
230 corticosterone, cortisone, oestriol, estrone and pregnenolone. Intra-assay coefficient of
231 variation (CV) was 7% (n = 20 samples). Inter-assay CV for a low control (78.5 ± 6.3
232 ng/ml n = 5 assays) was 8% and 2.8% for a high control (187 ± 5.3 ng/ml, n = 5
233 assays).

234 *Quantifying fGCM concentrations from offspring*

235 We collected faecal samples from offspring of cortisol-treated and control
236 mothers opportunistically during behavioural observations over the course of the study
237 (controls: 25-356 d; cortisol-treated: 32-326 d). Faecal samples were processed as
238 described previously using a methanol solution to extract fGCMs for analysis (46-47).
239 Immunoreactive fGCM concentrations were determined using a group-specific enzyme
240 immunoassay measuring cortisol metabolites with a 5β-3α,11β-diol-structure (11β-
241 hydroxyetiocholanolone), already validated and established for monitoring fGCM
242 alterations in meerkats (47). Faecal GCMs measured reflect average adrenal cortisol
243 production over the previous ~24 to 48 hr period (47). Detailed assay characteristics,
244 including full descriptions of the assay components and cross-reactivities, are found
245 elsewhere (48). The sensitivity of the assay was 1.2 ng/g dry weight and intra-assay CV
246 determined by repeated measurements of high and low value quality controls were

247 6.9% and 7.4% and inter-assay CV values were 11.5% and 15.9% (n = 29 assays),
248 respectively.

249 *Statistical analyses*

250 We used generalized (binomial errors) or linear mixed-effects models (LMMs) to
251 examine how our treatments affected the probability that the litter was aborted, litter size
252 and sex ratio at emergence from the burrow, and the proportion of the litter that survived
253 to emergence from the burrow, independence (~90 d of age: 29), and 6 or 12 months of
254 age. We focused on addressing whether the offspring from cortisol-treated mothers
255 differed from control or untreated mothers. These models included a fixed effect for date
256 of birth of the litter and random intercept terms for dominant female identity and year (as
257 the experiments were conducted over 3 years). None of the GLMMs were
258 overdispersed (Table 2).

259 We used a LMM to investigate how the maternal treatments affected offspring
260 growth from first emergence from their natal burrow (~1 month) to 3 months of age
261 when the pups are typically foraging independently (29, 34). Morning body mass (in
262 grams) was the response variable with the fixed effects of maternal treatment (cortisol-
263 treated, control, or untreated), pup sex, pup age, litter size at burrow emergence, first
264 measure of body mass when the pups first emerged from the burrow (to control for
265 possible differences in age or development when they entered our study population),
266 group size, group size^2, total rainfall in the previous 60 days, two measures of
267 seasonality (sine and co-sine functions of day of weight measure: see 40), and two
268 three-way interactions between sex, treatment, age or age^2. Group size was defined as
269 the average number of subordinate meerkats >6 months of age in the group during the

entire period of offspring growth. Random intercept terms for year and the identity of the individual nested in litter, nested in dominant female identity, nested in group were also included in this model. Fixed and random effects included in these models were based upon previous studies investigating meerkat body mass and/or growth from 1-3 months (28, 31, 40). To prevent any issues associated with selective disappearance of specific individuals, only individuals that survived to 90 d were included in these analyses.

We assessed how the treatments affected the relative babysitting and pup feeding contributions of subordinates when they were >6 months (as they rarely do alloparental care behaviour when <6 months: 36) from cortisol-treated, control, and untreated mothers. Relative babysitting and pup feeding contributions are defined as the proportion of babysitting or pup feeding contributions exhibited by a specific individual compared to the total number of babysitting or pup feeding contributions for that litter exhibited by all individuals in the group that were >6 months of age at the time of the birth of the litter (36, 39, 42). In these generalized linear mixed-effects models (GLMM, binomial errors), we included a three-way interaction between treatment, sex, and age of the subordinate to assess if the effects of the treatments on babysitting or pup feeding varied according to the sex or age of the subordinate, as contributions to cooperative behaviour in meerkats are known to vary according to subordinate sex and age (36). To account for differences in observation time, we included a co-variate for the number of days the litter was babysat (babysitting length) and the number of days the subordinate was observed in the group during babysitting as well as the total time spent observing the group during pup feeding (observation time). We included a range of co-variates (see Tables 4-6) that have been previously documented to affect relative

contributions to babysitting and pup feeding, including age, foraging success, body mass, and group size (34-36, 42, 49; 50). Group size was defined as the average number of subordinate meerkats >6 months of age in the group while the litter was being babysat or pup fed. Foraging success was defined as the average weight gained per hour estimated as the change in body mass from morning weight to evening weight over the total number of hours that had elapsed since those two weights (45). Relatedness between the subordinate and the litter being babysat was not included as it has not been shown to impact babysitting or pup feeding contributions (27, 42) and nearly all of the litters in our dataset were produced by the mother or full sibling of the subordinate. Random intercept terms for year and the identity of the individual, and litter being babysat or pup fed were nested within the group where the litter was being babysat or pup fed. Overdispersion was not an issue for our GLMM for babysitting as indicated by the goodness of fit test (Pearson χ^2 = 147.1, df = 154, P = 0.64, using package aods3: 51) but our GLMM for pup feeding was initially overdispersed (Pearson χ^2 = 310, df=165, P < 0.0001) so we included an observation level random intercept term.

We used two separate LMMs to assess how our manipulations affected plasma cortisol and fGCMs in offspring from cortisol-treated and control mothers (we did not have these data from offspring from untreated mothers). Each model included fixed effects for maternal treatment, pup sex and age, time of day and year that the sample was acquired (2014 or 2015), and random intercept terms for identity of individual nested in their birth litter and group. In the model for plasma cortisol concentrations, we also included a linear and second order fixed effect for the time it took to acquire the

316 blood sample to control for any variation in plasma cortisol concentrations due to
317 restraint stress (45). Year was included as a fixed effect because we only had samples
318 from two separate years. We included covariates associated with the individual meerkat
319 and weather or social group characteristics that are known to affect plasma cortisol (45)
320 or fGCM (47) concentrations (see Tables 6-7).

321 We used R (version 3.4.3: 52) for all of our statistical analyses. R package lme4
322 (version 1.1-14: 53) was used for LMMs and P values were estimated using lmerTest
323 (version 2.0-33: 54). A graphical approach was used to confirm normality and
324 homoscedasticity of residuals and to confirm there were no observations with high
325 leverage (55). Collinearity among predictor variables included in our models was
326 assessed by calculating variance inflation factors (55) or generalized variance inflation
327 factors (for variables that had a second order term or those included in an interaction:
328 56). Collinearity was not a problem as indicated by our variance inflation factors (VIFs)
329 as VIFs or generalized VIFs were less than ~4 for all variables. In our model for how our
330 treatments affected offspring growth (Table 3), the generalized VIF for the two
331 measures of seasonality (sine and co-sine functions of day of weight measure) were <6
332 but these two variables were included a priori given their previously documented effects
333 on body mass and growth in meerkats (40). All continuous variables were standardized
334 to a mean of 0 and SD of 1.

335

336 **Results**

337 *Effects of treatments on litter characteristics and offspring survival*

There was no evidence that the treatment of pregnant females with cortisol affected their ability to maintain litters to term or the survival of their pups prior to emergence from the natal burrow (Tables 1-2). The number of pups surviving to emergence from the natal burrow or 3, 6, or 12 months of age and the litter sex ratio were not different among litters from cortisol-treated, control, or untreated females (Tables 1-2).

Effects of treatments on offspring early life growth

The effects of the treatments on offspring growth from initial emergence to nutritional independence (1-3 months) differed between daughters and sons, as reflected in the significant three-way interaction between treatment, sex, and age (Table 3). Daughters (but not sons) from cortisol treated mothers grew more slowly from 1-3 months compared to those from control (fed) mothers (daughters: age x treatment, $t = -4.17$, $P < 0.0001$; sons: $t = -1.48$, $P = 0.14$), but exhibited similar growth compared to those from untreated (unfed) mothers (daughters: age x treatment, $t = 0.65$, $P = 0.51$; sons: $t = -0.52$, $P = 0.6$, Table 3, Fig. 1). Daughters, but not sons, from control (fed) mothers grew faster than those from untreated mothers (daughters: age x treatment, $t = -4.24$, $P < 0.0001$; sons: $t = 1.35$, $P = 0.18$).

Effects of treatments on offspring cooperative behaviour

The effects of the maternal treatments on babysitting behaviour of offspring depended upon the age and sex of the offspring (Table 4). Babysitting contributions in daughters from mothers treated with cortisol during pregnancy were slightly, but significantly higher with increasing age of the babysitter compared to those from control mothers (age x treatment, $z = -2.89$, $P = 0.0039$) but not untreated mothers (age x

361 treatment, z = 1.88, P = 0.06; Table 4, Fig. 2). Babysitting contributions in sons from
362 mothers treated with cortisol during pregnancy showed a similar tendency to slightly
363 increase with age compared to those from control mothers, but this difference was not
364 significant (age x treatment, z = -1.92, P = 0.055). Further, age-related increases in
365 babysitting contributions between males from mothers treated with cortisol during
366 pregnancy and untreated mothers did not differ (age x treatment, z = -0.03, P = 0.97;
367 Table 4, Fig. 2). Comparisons of the magnitude of effect sizes showed that the
368 interaction between age and maternal treatment had a larger effect on babysitting
369 contributions in daughters but not sons than other variables known to impact babysitting
370 contributions, such as foraging success, age-related body mass, or group size (Table
371 4).

372 The effects of the maternal treatments on pup feeding depended upon the sex of
373 the offspring, but not their age (Table 5). Daughters, but not sons from mothers treated
374 with cortisol during pregnancy exhibited significantly more pup feeding contributions
375 than those from control mothers (females: z = -3.12, P = 0.00018; males: z = -1.14, P =
376 0.25) or untreated mothers (females: z = -3.49, P = 0.0005, sons: z = -1.03, P = 0.3,
377 Table 5, Fig. 3). Notably, the magnitude of effect size of maternal treatment for
378 daughters was much larger than other variables known to impact babysitting
379 contributions such as foraging success, age-related body mass, and group size (Table
380 5).

381 *Effects of treatments on offspring stress physiology*

382 Daughters from mothers treated with cortisol during pregnancy had lower
383 plasma cortisol concentrations (age x treatment, t = -1.76, P = 0.08, Table 6, Fig. 4A)

384 and lower fGCM concentrations (age x treatment, t = -2.9, P = 0 .004, Table 7, Fig. 5A)
385 as they became older compared to those from control mothers but these differences
386 were only significant for fGCM concentrations. Sons from mothers treated with cortisol
387 during pregnancy had significantly lower plasma cortisol concentrations as they became
388 older compared to those from control mothers (age x treatment t = -2.68, P = 0.008,
389 Table 6, Fig. 4B) but similar fGCM concentrations compared to those from control
390 mothers as they became older (age x treatment, t = -0.1, P = 0.49, Table 7, Fig. 5B).
391

392 **Discussion**

393 We found some support for our hypothesis that elevated maternal GCs would
394 reduce the potential for offspring to have direct reproductive opportunities and would
395 therefore shift them towards exhibiting more cooperative behaviour that could increase
396 their indirect fitness. Daughters, but not sons, from mothers treated with cortisol during
397 pregnancy grew more slowly early in life and exhibited more babysitting and pup
398 feeding behaviour as they became older compared to controls. Other than offspring
399 survival (Table 2), we were unable to quantify the direct and indirect fitness of offspring
400 from control or cortisol-treated mothers, but early life growth or body mass (which we
401 measured here) is closely linked to direct fitness opportunities in daughters (27-32).
402 Previous studies in meerkats show that female, but not male, offspring that grow faster
403 from 1-3 months are more likely to acquire the dominant breeding position (31), perhaps
404 because offspring that grow faster in their first 3 months of life are heavier later in life
405 (32, 57, 58), and heavier females are more likely to acquire a vacant dominant breeding
406 position (30, 32). As such, daughters, but not sons, from mothers treated with cortisol

407 levels during pregnancy should have reduced future direct fitness opportunities and
408 therefore increase their investment in behaviours that elevate their indirect fitness. Our
409 results are consistent with studies in other taxa that suggest that individuals adjust their
410 contributions to cooperative behaviour according to their future reproductive potential.
411 For example, in cooperatively breeding birds, when the chances of direct reproduction
412 are elevated, subordinates often stop helping at the nest (59). Studies of social wasps
413 show that individuals whose probability of acquiring the dominant breeding position was
414 experimentally increased exhibited significantly less helping behaviour (60, 61). Finally,
415 in cooperatively breeding fish, subordinates will reduce their helping investment
416 immediately prior to dispersal from their natal group where they attempt to reproduce on
417 their own rather than stay in their natal group and queue for dominance (62).

418 Our results show that increases in maternal GCs can increase the cooperative
419 behaviour of daughters, which should lead to substantial direct fitness benefits to
420 mothers. Daughters from mothers treated with cortisol during pregnancy exhibited more
421 alloparental care compared to controls, such that subsequent offspring produced in
422 groups with offspring from cortisol-treated mothers should have received more
423 alloparental care. Because offspring that receive more alloparental care grow faster
424 early in life or are larger later in life (32, 57), the presence of offspring from cortisol-
425 treated mothers should increase the direct fitness of dominant breeders and the indirect
426 fitness of the offspring from cortisol-treated mothers. Taken together, our results
427 suggest that this GC-mediated maternal effect reduced the direct fitness opportunities of
428 daughters by reducing their early life growth, but they compensated by increasing their
429 investment in indirect fitness opportunities (helping to rear non-descendent offspring).

430 This is in line with theoretical predictions that parental manipulation of the cooperative
431 behaviour of offspring can evolve if the costs of resisting the parental effect are high and
432 inclusive fitness benefits of helping rear subsequent offspring are increased (18), as is
433 the case in cooperative breeders.

434 Control females that were fed during pregnancy produced daughters that grew
435 faster during early development (1-3 months) compared to daughters from cortisol-
436 treated or untreated mothers. Although mothers that were treated with cortisol during
437 pregnancy received the same amount of supplemental food as controls, daughters and
438 sons from mothers fed cortisol during pregnancy did not differ in early life growth
439 compared to those from untreated mothers. This indicates that the additional food
440 provided to dominant females during pregnancy had the potential to increase growth,
441 but the added cortisol prevented those gains in body mass. This has implications for
442 understanding the fitness consequences of maternal stress on offspring growth
443 trajectories (15, 63) because our results show that elevated circulating GC levels in
444 pregnant females in the absence of energetic constraints induced reductions in the early
445 life growth of offspring. This supports the hypothesis that maternal GC levels during
446 offspring development act as a cue that induces plasticity in offspring growth rather than
447 simply mediating the effects of energetic constraints. Alternatively, elevated maternal
448 GCs could alter patterns of maternal investment in offspring. Identifying whether
449 offspring or mothers are driving these effects is a major challenge in studies of maternal
450 stress effects in wild animals.

451 The reductions in the activity of the neuroendocrine stress axis of daughters may
452 have potentiated the increased alloparental care behaviour that we observed.

453 Compared to daughters from control mothers, daughters from mothers treated with
454 cortisol during pregnancy exhibited more babysitting as they became older, more overall
455 pup feeding, and they also had lower plasma cortisol and fGCM concentrations. Males
456 from mothers treated with cortisol during pregnancy had significantly lower plasma
457 cortisol concentrations, but not fGCM concentrations as they got older and also tended
458 to exhibit more babysitting as they aged. The activity of the neuroendocrine stress axis
459 is closely linked to an array of social behaviours (64) and our recent work shows that
460 elevated activity of the neuroendocrine stress axis reduces babysitting in both females
461 and males and decreases pup feeding in females (39). Together, this supports the
462 hypothesis that the mechanism by which early life stress increases the cooperative
463 behaviour of daughters is by dampening the activity of their neuroendocrine stress axis.
464 Our results show that the effects of maternal GCs on offspring growth,
465 physiology, and behaviour were greater in daughters than in sons, which adds to
466 biomedical (65-66) and ecological (67-69) studies that highlight how early life conditions
467 or maternal GC levels can have sex-specific consequences for offspring. In meerkats,
468 there may be added benefits for the dominant female for altering the cooperative
469 behaviour of daughters compared to sons; daughters exhibit more cooperative
470 behaviour than sons (36) and are more responsive to the begging calls of subsequent
471 offspring that they provision with food (70). More broadly, sex-differences in natal
472 dispersal may cause these differential responses to parental effects. In meerkats,
473 subordinate males voluntarily disperse from their natal group to look for receptive
474 females but can return to their natal group whereas subordinate females rarely
475 voluntarily disperse from their natal group (71). In our case and in others (63), the more

476 philopatric sex (females) is more sensitive to early life conditions, which may be due to
477 differential costs of parental modification between the philopatric and dispersing sex. If
478 parental effects have long-term consequences on offspring characteristics, as we show
479 here, there may be an increased degree of mismatch between the phenotype of the
480 dispersing sex and the postnatal environment where individuals eventually settle. If this
481 mismatch has fitness costs, this should select for individuals from the dispersing sex to
482 be less responsive to cues from the parental phenotype or environment.

483 Our results provide some support for the hypothesis that parents may alter the
484 cooperative tendencies of their offspring by manipulating the characteristics of their
485 offspring (9-10), though we note that it is uncertain if the transfer of maternal GCs to
486 offspring was passive or active. Explanations regarding the evolutionary origins of
487 cooperative behaviour involve nepotism or kin selection (72), mutualisms or reciprocity
488 (73), but few studies have tested the "parental manipulation" hypothesis proposed by
489 Alexander (9). Some studies show that alleles that increase maternal fitness at the
490 expense of the direct fitness of offspring can evolve (19) and that cooperative breeders
491 may bias investment towards offspring that exhibit more cooperative behaviour (74).
492 Our study supports the hypothesis that environmental stressors may induce a parental
493 effect that can modify the cooperative tendencies of their offspring.

494 Finally, our results have two implications for theoretical models examining the
495 evolution of parental effects. First, given the sex-specificity of parental effects, our
496 results challenge the conclusions of models examining the evolution of parental effects
497 that assume that all offspring are equally sensitive to the parental effect (16), or those
498 that assume that the benefits of exhibiting the phenotype resulting from the parental

499 effect are equal for all offspring (18). Second, selfish parental effects are thought to be
500 relatively rare (8, 13) and theory (16-17) and empirical studies showing sex-specific
501 responses to early life stress (65-66) indicate that offspring can become resistant to
502 such selfish parental effects. However, some models indicate that the evolution of
503 selfish parental effects may be dependent upon the social environment (24), especially
504 if the selfish parental effect influences the expression of alloparental care behaviour of
505 offspring and therefore increases the indirect fitness of offspring. Our results provide an
506 example whereby a GC-mediated maternal effect should decrease the direct fitness of
507 daughters (by reducing their early life growth), but should increase the direct fitness of
508 mothers and indirect fitness of daughters by elevating their cooperative behaviour.
509
510 **Ethics:** All protocols used in our experiments were approved by the Animal Ethics
511 Committee at the University of Pretoria (Pretoria, South Africa: #EC031-13, #EC047-16)
512 and the Northern Cape Department of Environment and Nature Conservation Research
513 (South Africa: FAUNA 050/2013, FAUNA 192/2014, FAUNA 1020/2016).
514
515 **Acknowledgements:** We thank M. Manser for her contributions to maintain the long-
516 term Kalahari Meerkat Project (KMP) and her contributions to this specific project.
517 Thanks to P. Roth, S. Bischoff-Mattson, L. Howell, J. Samson, N. Thavarajah, and
518 numerous KMP volunteers who contributed to data collection. Thanks to M. Manser and
519 S. Guindre-Parker for helpful comments. Thanks to the Northern Cape Conservation
520 Authority for permission to conduct research and the Kotze family for allowing us to
521 study meerkats on their property.

522

523 **Authors' Contributions:** B.D. and T.H.C-B planned the study, B.D. designed the
524 experiments, B.D., C. Dubuc, D.L.C, T.H.C-B, D.G., I.BG., N.B., M.H., A.G., and C.
525 Duncan coordinated and collected data, B.D. conducted analyses and produced figures,
526 B.D. and T.H.C-B authored manuscript with contributions from all authors.

527

528 **Date, code and materials:** All data will be archived on Data Dryad. All code for
529 statistical analyses is available from first author.

530

531 **Competing interests:** We have no competing interests.

532

533 **Funding:** Research was supported by grants from National Environment Research
534 Council (RG53472) and the European Research Council (294494) to T.H.C-B. I.B.G.
535 was funded by the Swiss National Science Foundation (grant no. 31003A_13676 to M.
536 Manser). NCB was supported by a DST-NRF SARCHI chair of Mammal Behavioural
537 Ecology and Physiology. The KMP has also been financed by the University of
538 Cambridge (T.H.C.-B) and University of Zurich (M. Manser).

539

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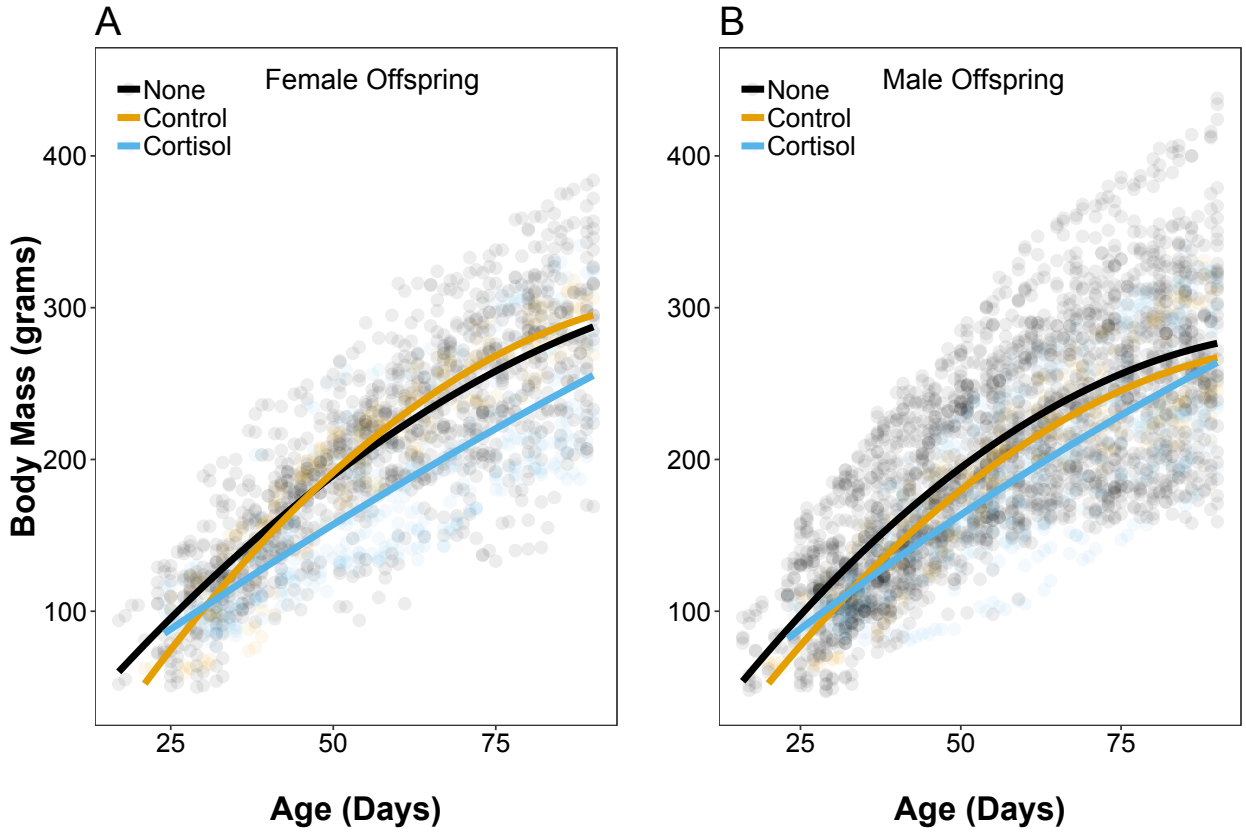
741
742

743 **Figure 1.** (A) Daughters but not (B) sons from mothers treated with cortisol during
744 pregnancy were significantly smaller from initial emergence from their natal burrow to
745 nutritional independence (~1-3 months) compared to those from control mothers
746 (daughters: age x treatment, t = -4.17, P < 0.0001; sons: t = -1.48, P = 0.14), but not
747 untreated mothers (daughters: age x treatment, t = 0.65, P = 0.51; sons: t = -0.52, P =
748 0.6, Table 3). Data are body mass measures from offspring from cortisol-treated
749 (females: n = 373 estimates; males: n = 488), control (females: n = 215; males: n =
750 241), and untreated mothers (females: n = 1121; males: n = 2238). Raw data and
751 regression lines are shown (full results in Table 3).



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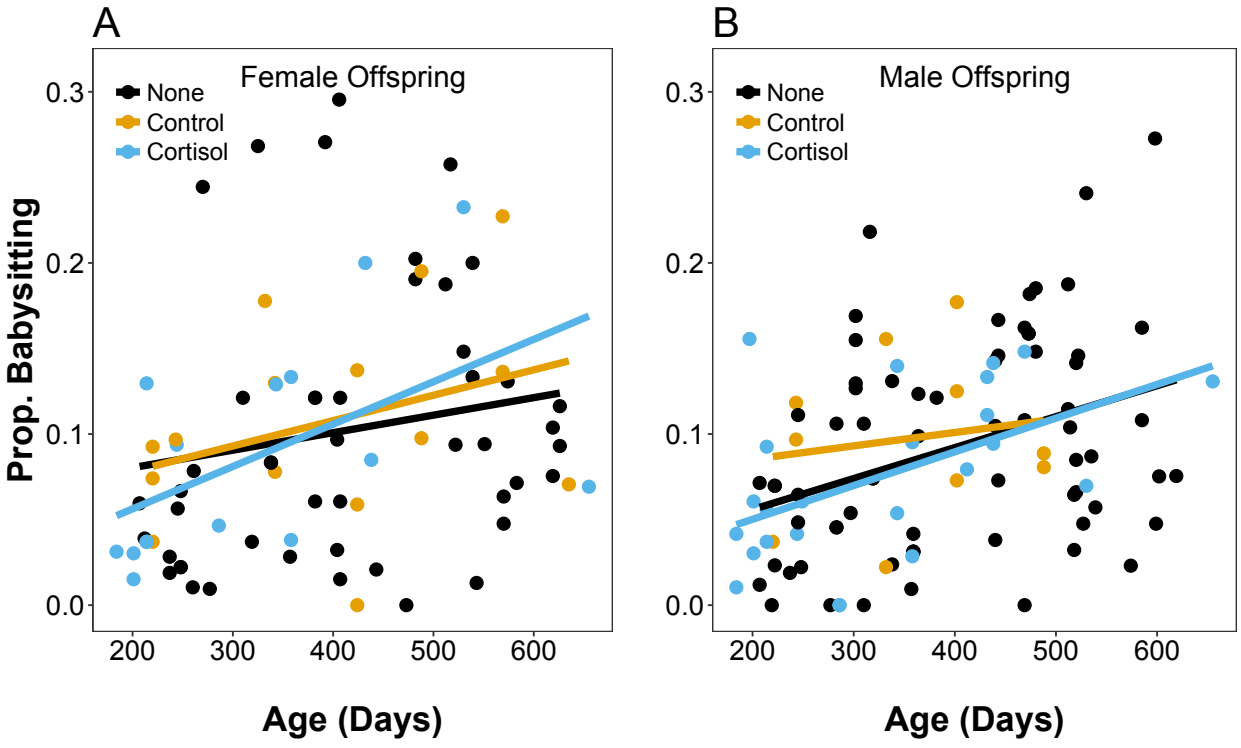
754 **Figure 2.** Babysitting contributions of (A) daughters and (B) sons from mothers treated
755 with cortisol during pregnancy increased with age at a faster rate than those from
756 control (females: z = -2.89, P = 0.0039; males: z = -1.92, P = 0.055), but not untreated
757 ("None") mothers (females: z = 1.88, P = 0.06; males: z = -0.03, P = 0.97, Table 4).
758 Data are relative babysitting contributions from offspring from cortisol-treated (females:
759 n = 15 estimates; males: n = 24), control (females: n = 15; males: n = 10), and untreated
760 mothers (females: n = 49; males: n = 69). Raw data and regression lines are shown (full
761 results in Table 4).



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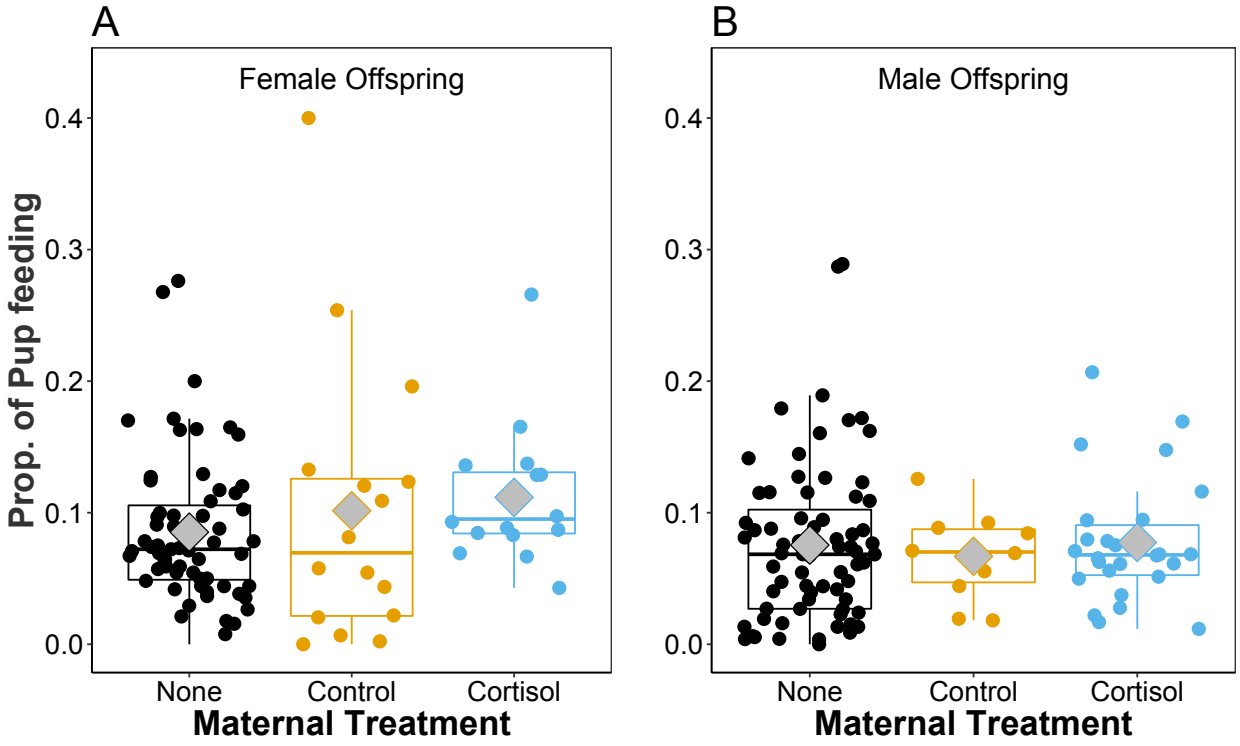
764 **Figure 3.** Pup feeding contributions of (A) daughters, but not (B) sons, from mothers
765 treated with cortisol during pregnancy were significantly higher compared to from control
766 (females: z = -3.09, P = 0.0004; males: z = -1.15, P = 0.25) or untreated ("None")
767 mothers (females: z = -3.47, P = 0.0005; males: z = -0.89, P = 0.37, Table 5). Data are
768 relative pup feeding contributions from offspring from cortisol-treated (females: n = 16
769 estimates; males: n = 26), control (females: n = 16; males: n = 10), and untreated
770 mothers (females: n = 64; males: n = 71). Raw data are shown (full results in Table 5).
771 Boxplots show median (solid horizontal line), mean (grey diamonds), and first (25%)
772 and third (75%) quartiles.



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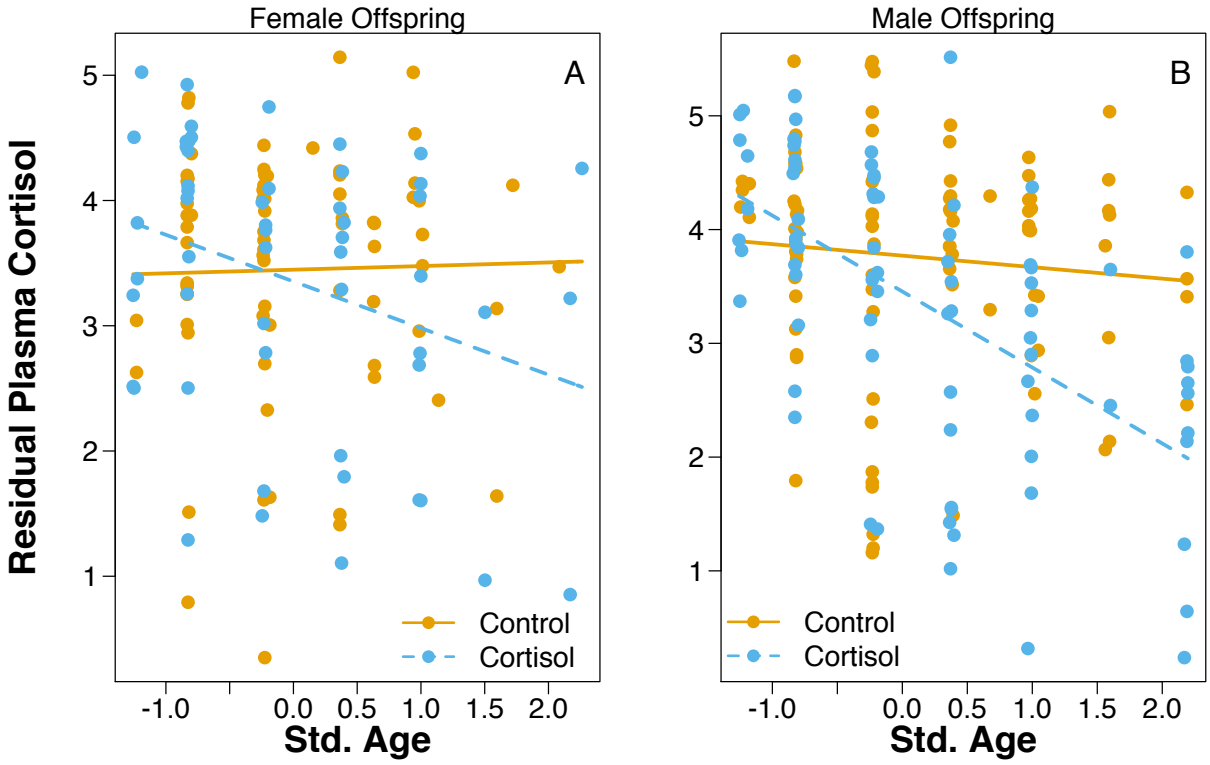
775 **Figure 4.** (A) Daughters and (B) sons from mothers treated with cortisol during
776 pregnancy had lower plasma cortisol concentrations as they became older compared to
777 those from control mothers, though the difference was only significant in males
778 (daughters: age x treatment, t = -1.76, P = 0.08; sons: age x treatment t = -2.68, P =
779 0.008, Table 6). Data are residual plasma cortisol concentrations from offspring from
780 cortisol-treated (females: n = 64 samples; males: n = 92) and control (females: n = 89;
781 males: n = 104) mothers. Residuals from a linear mixed-effects model (Table 6) are
782 shown on y-axis.



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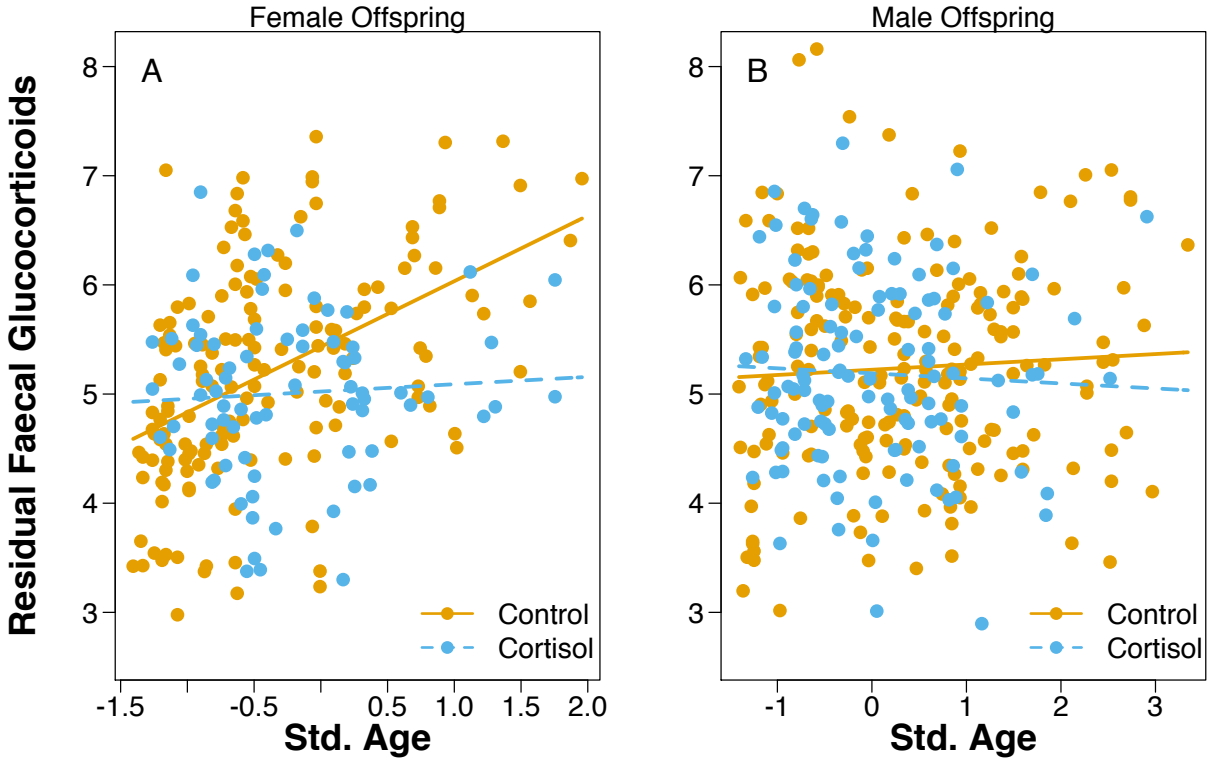
785 **Figure 5.** Faecal glucocorticoid metabolite (fGCM) concentrations in (A) daughters but
786 not (B) sons from mothers treated with cortisol during pregnancy were significantly
787 lower than those from control mothers as they became older (daughters: age x
788 treatment, t = -2.9, P = 0.004; sons: age x treatment, t = -0.1, P = 0.49, Table 7. Data
789 are residual fGCM concentrations from offspring from cortisol-treated (females: n = 79
790 samples; males: n = 118) and control (females: n = 154; males: n = 201) mothers.
791 Residuals from a linear mixed-effects model (Table 7) are shown on y-axis.



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796 **Table 1. Summary of effects of dominant female treatments on litter characteristics and**
797 **offspring survival.** Number of pups emerged correspond to those that emerged from the natal
798 burrow and in some cases these pups died before their sex could be determined (shown as
799 "Unk"). Three of the 13 litters treated with cortisol and one of the 7 control (fed) litters were
800 aborted prior to birth.

Treatment	Total # litters & females treated	Total # pups emerged (F, M, Unk)	Avg. Pups emerged	Avg. Pups Surviving to 3 months	Avg. Pups Surviving to 6 months	Avg. Pups Surviving to 12 months
Untreated	52 (21 females)	49 F, 84 M, 52 Unk	3.78 ± 1.23	2.68 ± 1.58	2.1 ± 1.63	1.62 ± 1.54
Control	7 (6 females)	12 F, 13 M	4.17 ± 0.98	3.83 ± 1.17	3.83 ± 1.17	2.83 ± 1.94
Cortisol	13 (10 females)	13 F, 18 M	3.87 ± 0.83	3.25 ± 1.03	2.75 ± 1.28	1.75 ± 1.75

801
802

Table 2. Effects of dominant female treatments (cortisol or control) on litter characteristics and pup survival. Results are from a linear mixed-effects model (# pups emerged) or generalized linear mixed-effects models (GLMMs, all other response variables) that each contained random intercept terms for dominant female identity and year. No GLMM was overdispersed as indicated by goodness of fit tests (R package aods3, P-values from Pearson χ^2 tests ranged from 0.13 to 1). The number of litters aborted by untreated females was not known so we only assessed the effects of cortisol vs. control treatments on the number of litters aborted. Litter sex ratio is the proportion of males in the litter.

Response variable	Fixed effect	b	SE	t or z	P-value
# Litters aborted					
	Intercept	-1.21	0.66	-1.83	0.07
	Birthdate	-0.17	0.59	-0.29	0.77
	Treatment				
	Control	-0.59	1.27	-0.46	0.64
# Pups emerged					
	Intercept	**3.9**	**0.42**	**9.36**	**<0.0001**
	Birthdate	0.02	0.16	0.11	0.91
	Treatment				
	Control	0.3	0.62	0.49	0.63
	Untreated	-0.11	0.45	-0.25	0.8
Litter sex ratio					
	Intercept	-0.54	0.3	-1.84	0.066
	Birthdate	0.02	0.12	0.15	0.88
	Treatment				
	Control	-0.11	0.45	-0.23	0.81
	Untreated	0.09	0.33	0.27	0.78
Prop. litter surviving to 3 months					
	Intercept	-0.16	0.27	-0.62	0.53
	Birthdate	-0.12	0.11	-1.09	0.27
	Treatment				
	Control	0.06	0.39	0.16	0.87
	Untreated	-0.19	0.3	-0.64	0.52
Prop. litter surviving to 6 months					
	Intercept	-0.32	0.28	-1.16	0.24
	Birthdate	-0.19	0.11	-1.65	0.099
	Treatment				
	Control	0.21	0.4	0.52	0.6
	Untreated	-0.28	0.31	-0.91	0.36
Prop. litter surviving to 12 months					
	Intercept	**-0.91**	**0.46**	**-1.999**	**0.046**
	Birthdate	-0.22	0.14	-1.58	0.11
	Treatment				
	Control	0.36	0.46	0.79	0.43
	Untreated	0.096	0.38	0.25	0.8

Reference for Treatment was cortisol-treated mothers. Data other than # litters aborted are based upon an initial sample size of offspring from untreated (195 pups from 52 litters produced by 21 dominant females), control (25 pups from 6 litters produced by 6 females), or cortisol-treated (31 pups from 10 litters produced by 9 females) litters that produced pups that emerged from the burrow.

Table 3. Effect of dominant female treatments on offspring growth from emergence to nutritional independence (1-3 months of age). Data are from a linear mixed-effects model where the response variable was morning body mass that contained random intercept terms for individual identity nested in birth litter nested in mother nested in natal group ($\sigma^2 = 116.7$) and year ($\sigma^2 = 0$). If fixed effects by themselves were involved in significant higher order interactions with other variables, only parameter estimates are shown.

Fixed Effect	b	SE	t	df	P-value
Intercept					
Females	**184.6**	**9.75**	**18.93**	**53**	**<0.0001**
Males	**189.1**	**9.5**	**19.8**	**49**	**<0.0001**
Litter size	-5.31	3.9	-1.37	48	0.18
First weight	**15.96**	**2.52**	**6.344**	**166**	**<0.0001**
Sex (M)	4.54	4.32			
Age	53.3	1.06			
Age^2	-4.76	1.15			
Rainfall	**2.81**	**0.61**	**4.57**	**4550**	**<0.0001**
Season (Sine)	**-67.2**	**3.3**	**-20.6**	**4238**	**<0.0001**
Season (Co-sine)	**66.22**	**3.4**	**19.2**	**4191**	**<0.0001**
Group size	2.3	2.66	0.86	109	0.39
Group size^2	-0.09	2.21	-0.04	178	0.97
Treatment (Control)					
Females	22.6	15.6			
Males	12.9	15.5			
Treatment (None)					
Females	23.6	10.6			
Males	22.9	10.4			
Age x Sex (M)	1.64	1.37	1.19	4567	0.23
Age^2 x Sex (M)	1.02	1.53	0.67	4543	0.5
Sex (M) x Treatment (Control)	-9.73	7.7	-1.27	164	0.21
Sex (M) x Treatment (None)	-0.63	5.1	-0.12	172	0.9
Age x Treatment (Control)					
Females	**7.6**	**1.8**	**4.17**	**4595**	**<0.0001**
Males	-2.3	1.5	-1.48	4548	0.14
Age x Treatment (None)					
Females	0.79	1.2	0.65	4574	0.51
Males	-0.53	1.01	-0.52	4580	0.6
Age^2 x Treatment (Control)					
Females	-0.13	1.93	-0.07	4521	0.94
Males	-1.05	1.6	-0.65	4547	0.52
Age^2 x Treatment (None)					
Females	-2.6	1.3	-1.96	4534	0.051
Males	**-6.9**	**1.1**	**-6.23**	**4556**	**<0.0001**
Age x Treatment (Control) x Sex (M)	**-9.9**	**2.3**	**-4.23**	**4574**	**<0.0001**
Age x Treatment (None) x Sex (M)	-1.3	1.6	-0.84	4581	0.4
Age^2 x Treatment (Control) x Sex (M)	-0.93	2.5	-0.37	4552	0.71
Age^2 x Treatment (None) x Sex (M)	**-4.3**	**1.7**	**-2.5**	**4552**	**0.01**

Data used in these analyses were 4676 measures of body mass from 195 meerkats produced by 21 dominant females across 53 different litters in 16 different social groups in three different years. Only offspring that survived to 90 days of age were included in these analyses.

Table 4. Effect of dominant female treatments on relative babysitting contributions. Data are from a generalized linear mixed-effects model where the response variable is the proportion of babysitting exhibited by the subordinate meerkat relative to the total babysitting contributions the litter received. The model contained random intercept terms for individual ($\sigma^2 = 0.12$), litter nested within group ($\sigma^2 = < 0.0001$), and year ($\sigma^2 = 0.000$). If fixed effects by themselves were involved in significant higher order interactions with other variables, only parameter estimates are shown.

Fixed Effect	b	SE	z	P-value
Intercept				
Females	**-2.1**	**0.22**	**-9.37**	**< 0.0001**
Males	**-2.14**	**0.19**	**-11.01**	**< 0.0001**
Babysitting length	**0.24**	**0.08**	**2.77**	**0.0056**
Observation time	**-0.28**	**0.08**	**-3.4**	**0.0007**
Litter size	0.015	0.05	0.32	0.75
Mixed Litter?	-0.04	0.12	-0.31	0.76
Sex (M)	-0.03	0.27	-0.13	0.9
Age				
Females	0.13	0.17		
Males	0.4	0.19		
Foraging success	-0.05	0.05	-0.99	0.32
Mass				
Females	-0.32	0.12		
Males	-0.098	0.14		
Group size				
Females	**-0.35**	**0.09**	**-3.97**	**< 0.0001**
Males	**-0.27**	**0.08**	**-3.28**	**0.001**
Treatment (Control)				
Females	-0.67	0.31		
Males	-0.12	0.28		
Treatment (None)				
Females	-0.06	0.23		
Males	-0.16	0.18		
Foraging success x Mass	0.036	0.06	0.57	0.57
Age x Mass				
Females	**-0.47**	**0.09**	**-5.08**	**< 0.0001**
Males	**-0.29**	**0.08**	**-3.79**	**0.0001**
Age x Sex (M)	0.28	0.23	1.18	0.24
Mass x Sex (M)	0.22	0.17	1.26	0.21
Group size x Sex (M)	0.07	0.1	0.77	0.44
Treatment (Control) x Sex (M)	0.55	0.42	1.31	0.19
Treatment (None) x Sex (M)	-0.1	0.28	-0.36	0.72
Age x Treatment (Control)				
Females	**-0.62**	**0.21**	**-2.89**	**0.0039**
Males	-0.52	0.27	-1.92	0.055
Age x Treatment (None)				
Females	0.34	0.18	1.88	0.059
Males	-0.006	0.16	-0.03	0.97
Age x Mass x Sex	0.18	0.12	1.5	0.13
Age x Treatment (Control) x Sex (M)	0.09	0.34	0.28	0.78
Age x Treatment (None) x Sex (M)	-0.34	0.24	-1.45	0.14

Data used in these analyses were 182 observations of relative babysitting contributions to 28 litters produced in 9 groups across 3 years recorded from 105 subordinate meerkats.

Table 5. Effect of dominant female treatments on relative pup feeding contributions. Data are from a generalized linear mixed-effects model where the response variable is the proportion of pup feeds exhibited by the subordinate meerkat relative to the total pup feeds the litter received. The model contained random intercept terms for individual ($\sigma^2 = 0.000$), litter nested within group ($\sigma^2 = 0.2$), year ($\sigma^2 = 0.08$), and an observational level random intercept term to control for overdispersion ($\sigma^2 = 0.19$). If fixed effects by themselves were involved in significant higher order interactions with other variables, only parameter estimates are shown.

Fixed Effect	b	SE	z	P-value
Intercept				
Females	**-2.27**	**0.28**	**-8.2**	**<0.0001**
Males	**-2.66**	**0.25**	**-10.45**	**<0.0001**
Observation time	**0.59**	**0.06**	**9.41**	**<0.0001**
Litter size	-0.055	0.1	-0.55	0.58
Mixed litter (Y)	-0.03	0.29	-0.1	0.92
Sex (M)	-0.39	0.2		
Age				
Females	-0.37	0.16		
Males	0.29	0.14		
Foraging success	**0.14**	**0.06**	**2.12**	**0.033**
Mass				
Females	0.1	0.11		
Males	-0.37	0.11		
Group size				
Females	**-0.48**	**0.13**	**-3.57**	**0.0003**
Males	**-0.32**	**0.13**	**-2.46**	**0.014**
Treatment (Control)				
Females	**-0.73**	**0.23**	**-3.12**	**0.0018**
Males	-0.24	0.21	-1.14	0.25
Treatment (None)				
Females	**-0.64**	**0.18**	**-3.49**	**0.0005**
Males	-0.14	0.13	-1.03	0.3
Foraging success x Mass	0.06	0.06	0.97	0.33
Age x Mass				
Females	-0.04	0.07	-0.62	0.54
Males	**-0.19**	**0.07**	**-2.68**	**0.007**
Age x Sex (M)	**0.66**	**0.19**	**3.49**	**0.00048**
Mass x Sex (M)	**-0.47**	**0.13**	**-3.63**	**0.0003**
Group size x Sex (M)	0.16	0.08	1.89	0.059
Treatment (Control) x Sex (M)	0.49	0.31	1.58	0.11
Treatment (None) x Sex (M)	**0.5**	**0.22**	**2.23**	**0.023**
Age x Treatment (Control)				
Females	-0.11	0.22	-0.5	0.61
Males	0.03	0.29	0.11	0.91
Age x Treatment (None)				
Females	0.17	0.16	1.03	0.3
Males	-0.02	0.14	-0.12	0.9
Age x Mass x Sex	-0.14	0.09	-1.51	0.13
Age x Treatment (Control) x Sex (M)	0.14	0.34	0.42	0.67
Age x Treatment (None) x Sex (M)	-0.19	0.21	-0.91	0.36

Data used in these analyses were 192 observations of relative pup feeding contributions to 26 litters produced in 7 groups across 3 years recorded from 101 subordinate meerkats.

Table 6. Effect of dominant female treatments on plasma cortisol concentrations. Data are from a linear mixed-effects model where the response variable is plasma cortisol concentrations (ln transformed) of the subordinate meerkat. The model contained random intercept terms for individual nested within their birth litter ($\sigma^2 = 0.034$), and capture group ($\sigma^2 = 0.000$). If fixed effects by themselves were involved in significant higher order interactions with other variables, only parameter estimates are shown.

Fixed Effect	b	SE	t	df	P-value
Intercept					
Females	**3.18**	**0.29**	**11.31**	**128**	**<0.0001**
Males	**3.37**	**0.25**	**13.37**	**113**	**<0.0001**
Sampling time	**1.16**	**0.10**	**11.95**	**259**	**<0.0001**
Sampling time^2	**-0.24**	**0.03**	**-7.24**	**269**	**<0.0001**
Time of day	-0.2	0.14	-1.38	276	0.17
Sample year (2015)	0.41	0.24	1.72	228	0.09
Sex (M)	0.2	0.2	0.97	39	0.34
Age					
Females	-0.24	0.18			
Males	-0.01	0.14			
Foraging success					
Females	0.13	0.11	-1.22	231	0.22
Males	-0.15	0.1	-1.42	269	0.16
Group size	0.07	0.11	0.62	242	0.54
Group size^2	0.05	0.08	0.63	211	0.53
Pups in group	-0.31	0.18	-1.79	276	0.075
Group sex ratio	**0.2**	**0.08**	**2.53**	**180**	**0.01**
Relatedness	-0.09	0.18	-0.5	40	0.62
Weather (PC1)	-0.08	0.1	-0.75	276	0.45
Treatment (Cortisol)					
Females	-0.02	0.24			
Males	-0.32	0.19			
Sex (M) x Age	0.22	0.22	1.04	272	0.3
Sex (M) x Foraging success	-0.013	0.14	-0.09	257	0.93
Sex (M) x Treatment (Cortisol)	-0.3	0.3	-1.01	35	0.32
Age x Treatment (Cortisol)					
Females	-0.42	0.24	-1.76	276	0.08
Males	**-0.5**	**0.19**	**-2.68**	**274**	**0.008**
Group size x Pups Present (Yes)	-0.05	0.15	-0.36	216	0.72
Group size^2 x Pups Present (Yes)	0.07	0.12	0.6	263	0.55
Group size x Weather (PC1)	-0.02	0.08	-0.30	273	0.76
Age x Sex (M) x Treatment (Cortisol)	-0.08	0.31	-0.26	275	0.8

Data used in these analyses were 299 measures of plasma cortisol concentrations from 49 subordinate meerkats produced in 14 litters from 10 different groups. Reference levels (intercept) for "Sex" was female, "Pups in group" was Yes, and for Relatedness was "No parent was dominant".

Table 7. Effect of dominant female treatments on faecal glucocorticoid metabolite (fGCM) concentrations. Data are from a linear mixed-effects model where the response variable fGCM concentrations (ln+1 transformed) of the subordinate meerkat. The model contained random intercept terms for individual nested within their birth litter ($\sigma^2 = 0.094$) and collection group ($\sigma^2 = 0.000$). If fixed effects by themselves were involved in significant higher order interactions with other variables, only parameter estimates are shown.

Fixed Effect	b	SE	t	df	P-value
Intercept					
Females	**5.4**	**0.25**	**21.36**	**50**	**<0.0001**
Males	**5.33**	**0.23**	**23.4**	**23**	**<0.0001**
Time of day	**-0.14**	**0.04**	**-3.29**	**516**	**0.001**
Sample year (2015)	0.07	0.17	0.43	120	0.67
Sex (M)	-0.06	0.2	-0.31	22	0.76
Age					
Females	0.48	0.16			
Males	0.06	0.1			
Foraging success					
Females	0.16	0.13	1.29	355	0.2
Males	0.04	0.06	0.81	520	0.42
Group size	**0.41**	**0.12**	**3.52**	**96**	**0.0007**
Group size^2	-0.05	0.11	-0.78	267	0.43
Pups in group	-0.13	0.16	-0.78	267	0.43
Group sex ratio	**0.16**	**0.07**	**2.27**	**158**	**0.024**
Relatedness	0.28	0.25	1.1	14	0.29
Weather (PC1)	0.09	0.07	1.4	203	0.16
Treatment (Cortisol)					
Females	-0.12	0.29			
Males	-0.03	0.24			
Sex (M) x Age	**-0.42**	**0.17**	**-2.47**	**221**	**0.014**
Sex (M) x Foraging success	-0.12	0.13	-0.87	375	0.38
Sex (M) x Treatment (Cortisol)	0.1	0.33	0.29	18	0.77
Age x Treatment (Cortisol)					
Females	**-0.63**	**0.22**	**-2.9**	**447**	**0.004**
Males	-0.1	0.14	-0.69	491	0.49
Group size x Pups Present (Yes)	-0.17	0.12	-1.46	216	0.14
Group size^2 x Pups Present (Yes)	0.11	0.12	456	0.97	0.33
Group size x Weather (PC1)	0.04	0.05	0.69	129	0.49
Age x Sex (M) x Treatment (Cortisol)	**0.53**	**0.25**	**2.15**	**413**	**0.032**

Data used in these analyses were 542 faecal samples (n= 355 from controls, n = 187 from cortisol treated litters) from 34 subordinate meerkats (control: n = 12 females, n = 11 males; cortisol-treated: n = 5 females, n = 6 males) produced in 10 litters from 7 different groups. Reference levels (intercept) for "Sex" was female, "Pups in group" was Yes, and for Relatedness was "No parent was dominant".