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Seasonal variations in plasma cortisol, testosterone, progesterone and leukocyte profiles in a wild population of tuco-tucos

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Abstract

Studies addressing seasonal changes in hormone levels are important in order to understand the interplays between ecology and physiology. In this study, we evaluated seasonal variations in cortisol, testosterone, and progesterone plasma levels in males and females of the subterranean rodent *Ctenomys talarum*. For the case of females, we also aimed to evaluate their capacity to increase their plasma cortisol concentrations in response to capture and restraint during reproductive and non-reproductive seasons. In addition, we registered concomitant seasonal variations in the neutrophil to lymphocyte ratio (N:L) aimed to discriminate between basal and stress-induced seasonal changes in cortisol levels in both males and females. Both basal and stressed-induced cortisol levels were significantly higher in reproductive than non-reproductive females. For the case of males, cortisol levels were also higher during the reproductive season, though values were two- to threefold lower than in females. The N:L ratios attained low values, typical of unstressed animals, in both males and females, indicating that the animals were not facing acute or chronic stressors at the moment of their capture. Testosterone levels in males were significantly elevated in relation to other mammals reaching up to 486 ng mL^{-1}, with significantly higher levels during the reproductive season (mean: 209.45 $\pm$ 177.76 ng mL^{-1}) and a remarkable inter-individual variation. On the other hand, progesterone levels in females captured during reproductive and non-reproductive seasons were not significantly different. This study shows that seasonal modulation in testosterone and also in baseline and stress-induced cortisol levels occurs in tuco-tucos associated with reproduction.

Introduction

Free-living animals show seasonal modulation of hormone secretion associated with changes in the physical and social environment, reproductive activity and energetic requirements. Glucocorticoids (GCs, cortisol and/or corticosterone, depending on the species) and reproductive hormones (e.g. androgens and progestagens) are key components of the endocrine responses to these seasonal environmental changes (Place & Kenagy, 2000; Romero, 2002; Boonstra, 2005; Reeder & Kramer, 2005; Soto-Gamboa, Villalón & Bozinovic, 2005; Schradin, 2008). GCs are regulated by the activity of the hypothalamic-pituitary-adrenal (HPA) axis and modulate multiple physiological and behavioural responses (Sapolsky, Romero & Munck, 2000). For instance, these hormones exhibit regulatory effects on energy balance (Dallman *et al*., 2007) and daily activity patterns (Malisch *et al*., 2008) under basal concentrations. In addition, this axis is responsive to a great diversity of extrinsic and intrinsic factors, or stressors (e.g. food deprivation, change in social status, fear), resulting in increased secretion of GCs from the adrenal glands a few minutes after the perception of the stressor (Sapolsky *et al*., 2000; Boonstra, 2005).

Testosterone and progesterone levels are regulated by the activity of the hypothalamic-pituitary-gonadal (HPG) axis. Testosterone secretion can be stimulated by social stimuli during the breeding season (Wingfield, Jacobs & Hillgarth, 1997; Soto-Gamboa *et al*., 2005; Schradin *et al*., 2009), producing direct effects in mating activities such as competition among males and attractiveness to females (Wingfield *et al*., 1990; Sinervo *et al*., 2000). Nonetheless, agonistic social interactions may function as stressors, increasing concentrations of GCs, which ultimately may inhibit testosterone secretion (Soto-Gamboa *et al*., 2005). Indeed, the HPA and HPG axes

do not function separately but, on the contrary, complex interactions occur at different levels (e.g. Handa *et al*., 1994; Kirby *et al*., 2009). In the females, progesterone participates in the regulation of sexual receptivity and it is involved in the oestrous cycle and required for the maintenance of pregnancy (Lisk & Reuter, 1980).

Ctenomys talarum (talas tuco-tuco) is a solitary and highly territorial rodent that inhabits individual galleries parallel to the surface in southern parts of South America (Reig *et al*., 1990; Antinuchi & Busch, 1992). The species presents a polygynous mating system in which some males monopolize the access to multiple females (Zenuto, Lacey & Busch, 1999; Zenuto, Vassallo & Busch, 2002). The natural breeding season extends over 9 months starting in late autumn (June; Malizia & Busch, 1991) and, since pregnancy extends over 95 days, most births occur during spring (mean litter size is 4.55 ± 1.25 standard deviation (SD); Zenuto, Vassallo & Busch, 2001). They show high levels of aggression towards conspecifics, particularly the males (Zenuto *et al*., 2002), suggesting important roles for testosterone in the seasonal regulation of aggressive behaviours. *C. talarum* females are induced ovulators and develop a post-partum oestrus during the reproductive season, resulting in the overlapping of gestation and lactation (Malizia & Busch, 1991; Fanjul, Zenuto & Busch, 2006), which could generate a trade-off in the secretion of progesterone, as has been reported for hamsters *Cricetus cricetus* (see Franceschini *et al*., 2007). Both cortisol and corticosterone are present in plasma but the relative proportions of both hormones may show marked variation among sexes and sampling years (Vera, Antenucci & Zenuto, 2011*a*; Vera, Zenuto & Antenucci, 2012). However, only cortisol is responsive to acute stressors and adrenocorticotropic hormone (Vera *et al*., 2011*a*, 2012).

In order to have a better understanding of the seasonal changes in GC levels in natural populations, other physiological parameters known to be affected by stressors should be determined simultaneously. Particularly, the neutrophil to lymphocyte ratio (N:L) is known to increase rapidly in response to a wide variety of stressors (Davis, Maney & Maerz, 2008). For *C. talarum*, this ratio varies between 0.1 and 1.5 in undisturbed animals kept in captivity, once they have acclimated to the laboratory conditions. When animals are subjected to acute stressors, N:L ratios increase up to 2.5–3, reaching values up to 5 when they are subjected to chronic stressors (e.g. acclimation to captivity, food restriction, repeated blood sampling; Vera, Zenuto & Antenucci, 2008; Vera *et al*., 2011*a*; F. Vera, unpublished data). Thus, the simultaneous determination of the N:L ratios with plasma GC levels can be used to discern if variations in GC levels are triggered by naturally occurring stressors or represent seasonal changes in basal levels of these hormones (Vera *et al*., 2011*a*).

The goals of the present study were to: (1) evaluate variations in the plasma levels of cortisol (both sexes), testosterone (males) and progesterone (females) in free-living tuco-tucos during reproductive and non-reproductive seasons; (2) evaluate, for the case of females, their capacity to increase their cortisol levels in response to acute stress (capture and restraint) during reproductive and non-reproductive seasons; (3) assess concomitant seasonal variations in leukocyte profiles to discriminate if variations in cortisol levels can be attributed to naturally occurring stressors or represent changes in basal levels in both males and females. We hypothesized that cortisol and reproductive hormones show seasonal variation with increased levels during the reproductive season in both males and females.

Materials and methods

Study site and animals

We captured 71 individuals of *C. talarum* during 2009 in a population located at Mar de Cobo (Buenos Aires Province, Argentina; 37°45′S, 57°26′W). The animals were cared for in accordance with the guidelines for the use of animals in research and teaching (ASAB/ABS, 2003). All the animals were in good health after blood sampling.

Determination of the sampling seasons

To evaluate seasonal variation in hormone levels, we defined three sampling seasons following the work of Malizia & Busch (1991) and Fanjul *et al*. (2006). The seasons were defined as follows: (1) non-reproductive season (hereafter NRS, April–May): no reproductive activity is observed in captured females (closed vagina; Fanjul *et al*., 2006); (b) beginning of reproductive season (hereafter BRS, July): the first pregnancies of the year are observed; (c) peak of reproductive season (hereafter PRS, November–December): females usually show advanced pregnancies and/or enlarged and hairless nipples, indicating that lactation is occurring (Fanjul *et al*., 2006). The distinction among seasons was made according to the reproductive activity of females, since males after attaining reproductive maturity do not undergo regression of their testes and contain sperm in their epididymes year round (Malizia & Busch, 1991). For the case of males, we obtained blood samples during the three defined sampling seasons, whereas for females data were obtained during NRS and PRS in order to compare between seasons when their reproductive activity is clearly different (Malizia & Busch, 1991). Samplings were conducted between 10:00 AM and 3:00 PM during all sampling dates.

Captures and baseline samples

The animals were trapped using live-capture wire mesh traps set at fresh surface sandy mounds and baseline blood samples were obtained within 5 min after the animals entered the traps (see Vera *et al*., 2012 for trapping details). For *C. talarum*, 5 min is enough to guarantee that cortisol levels in plasma are not affected by the capture and extraction procedures (Vera *et al*., 2011*a*). These blood samples were used to determine baseline plasma levels of cortisol (both sexes), testosterone (males) and progesterone (females). Sample sizes were as follows: NRS: 14 females and 7 males, BRS: 8 males, PRS: 7

females and 10 males. All animals were weighted at their moment of their capture using a Pesola™ balance (Pesola AG, Baar, Switzerland).

Restraint

In order to evaluate the capacity of females to respond to acute stressors, an additional group was subjected to 2 min of restraint immediately after capture during both reproductive and non-reproductive seasons (7 and 10 individuals for NRS and PRS, respectively). Restraint was performed by firmly holding the animals against the soil, after removing them from the traps. Thirty minutes later, a blood sample was obtained (within 3 min) for the determination of cortisol levels in response to acute stress. This goal was restricted to females because we have previously evaluated in males their responses to acute stress using the same methodology (Vera *et al.*, 2011*a*).

Sample collection

All blood samples were obtained immediately after capture from the suborbital sinus as described previously (e.g. Vera *et al.*, 2011*a*). After blood collection, the samples were kept in a refrigerated cooler (for 1–4 h). Once in the laboratory, the samples were centrifuged (660×***g***, 15 min) to isolate the plasma, which was stored at –20°C until analysis.

Determination of N:L ratios

The N:L ratio was also determined from blood smears prepared in the field immediately after obtaining the blood samples (fixed with methanol for 10 min). Once in the laboratory, the smears were stained with May-Grunwald Giemsa solution and examined at magnification 1000×. All cell types (lymphocytes, neutrophils, eosinophils, basophils and monocytes) were counted until the cumulative total was 200 cells and the N:L ratio was then calculated.

Assessment of reproductive condition of females

Females captured during the PRS were externally examined (abdomen and mammary glands) to evaluate if they were pregnant and/or if lactation was occurring at the moment of their capture. We recorded the presence or absence of palpable embryos and enlarged and hairless nipples. Except for three juvenile females (that were excluded from all the analyses), all females captured during the reproductive season presented palpable embryos and/or enlarged nipples. However, data from reproductive females were pooled because (1) the number of captures was not large enough to allow an adequate comparison among different categories (i.e. 'only pregnant', 'only lactating', and 'both pregnant and lactating'); (2) based only on the external examination, the distinction among the categories 'only lactating' and 'both pregnant and lactating' was not obvious, because lactating females could present early pregnancies that might have passed unnoticed.

Hormone analysis

Cortisol, testosterone and progesterone were measured using the Coat-A-Count procedures (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Both the cortisol and testosterone kits have been extensively validated for their use with plasma samples of *C. talarum* and the characteristics of these assays were detailed in previous publications (Vera *et al.*, 2011*a*,*b*). Before testosterone determination, plasma samples were heated (56°C, 30 min) and then diluted (1:6 to 1:40) using phosphate buffered saline buffer (pH = 7) to eliminate interference of plasma components and measure testosterone levels near the optimal range of the assay (i.e. 50% binding), following the methodology of Vera *et al.* (2011*b*). Intra-assay coefficients of variation (CVs) were 8 and 8.5% for cortisol and testosterone, respectively. Given that all plasma samples were run in a single assay, the inter-assay CVs were not calculated.

We validated the progesterone assay to be used in plasma samples of *C. talarum* (see Supporting Information Appendix S1). Very briefly, we evaluated parallelism, accuracy and precision of the assay and also assessed the biological response to a chronic stress period. The outcomes of these validation steps indicated that progesterone can be measured directly in plasma samples of *C. talarum* with acceptable efficiency. Progesterone was determined only in 9 of the 14 samples obtained during NRS because we gave priority to the measurement of cortisol when the volume of the samples was not enough for the measurement of both hormones. The antibodies used in all the assays are highly specific for their respective targets, as reported by the manufacturer.

Statistical analyses

Baseline and stress-induced cortisol levels of reproductive and non-reproductive females were compared using two-way analysis of variance (ANOVA). Factors were: 'season' (levels: reproductive and non-reproductive) and 'treatment' ('baseline' and 'stress-induced'). Data were log transformed to meet normality and homoscedasticity. For the case of males, cortisol and testosterone concentrations and N:L ratios during NRS, BRS and PRS were compared using the non-parametric Kruskal–Wallis test, followed by multiple comparisons using Dunn's method, when significant differences were detected. Baseline cortisol levels in males and females captured during NRS and PRS were compared with two-way ANOVA with sex and season as factors (log-transformed data). The variances of testosterone levels during the different sampling seasons were compared using the *F*-test for homogeneity of variances. Progesterone levels, body mass, and the N:L ratios of reproductive and non-reproductive females were compared using Mann–Whitney *U*-tests. Seasonal differences in body mass of males were evaluated using ANOVA, followed by a multiple comparison with the Holm–Sidak method. Correlations among variables were assessed by calculating the Spearman's correlation coefficient (ρ) (Zar, 1984). All data are presented as mean ± SD.

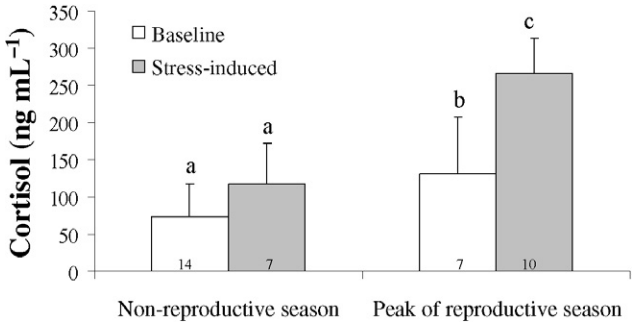

Figure 1 Baseline and stress-induced plasma cortisol concentrations in females of *Ctenomys talarum* captured during non-reproductive season and the peak of the reproductive season (mean + standard deviation). Different letters indicate statistically significant differences ($P < 0.05$). Sample size is provided at the base of each bar.

Results

Variations in body mass

Males captured during the NRS presented lower mean body mass than those captured during BRS and PRS, with values lower than 120 g in four out of the seven individuals (NRS: 122.04 ± 32.90 g, BRS: 170.06 ± 25.73 g, PRS = 156.81 ± 19.55 g, ANOVA: $F_{20,22} = 6.55$, $P = 0.006$, Holm–Sidak: $P < 0.05$). Males captured during BRS and PRS did not show statistically significant differences in body mass. Body mass of females captured during reproductive and non-reproductive seasons was not statistically different (NRS: 125.74 ± 10.97 g, PRS: 131.44 ± 14.35 g; Mann–Whitney: Z = 1.21, $P = 0.24$). Only 7 of the 17 captured females during the reproductive season (both baseline and stressed captures) presented obvious palpable embryos. The other captured females were lactating, as evidenced by the presence of enlarged and hairless nipples (see Methods).

Seasonal variations in plasma cortisol levels

Reproductive females presented significantly higher baseline and stress-induced cortisol levels than non-reproductive females (two-way ANOVA: $F_{31,36} = 33.11$, $P < 0.001$, Fig. 1), indicating that both baseline and stress levels are seasonally modulated in females. The restraint treatment produced significant increases in cortisol levels in reproductive females though not in non-reproductive females (two-way ANOVA: $F_{31,36} = 24.94$, $P < 0.001$, see Fig. 1 for the multiple comparison). Season and treatment showed a significant interaction ($P = 0.017$).

Males also showed a seasonal pattern with increased cortisol levels during the reproductive season [Kruskal–Wallis: H = 13.51, degrees of freedom (d.f.) = 2, $P = 0.001$, Dunn: $P < 0.05$, Fig. 2A]. Plasma cortisol concentrations also differed between sexes. Baseline cortisol levels were significantly higher in females than in males during both NRS and PRS (two-way ANOVA: $F_{34,39} = 10.32$, $P < 0.001$).

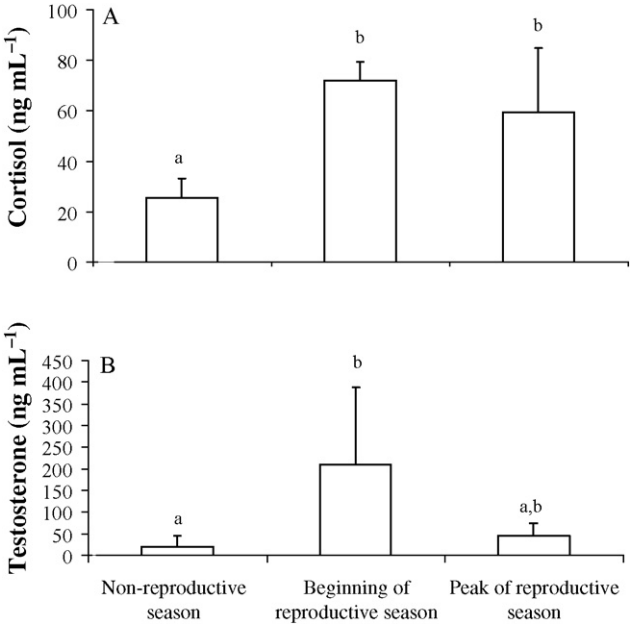

Figure 2 Seasonal variations in plasma cortisol (A) and testosterone (B) levels in males of *Ctenomys talarum* (mean + standard deviation). Different letters indicate statistically significant differences ($P < 0.05$). Sample sizes are 7, 8 and 10 for non-reproductive season, beginning of the reproductive season and peak of this season, respectively.

Table 1 Seasonal variations in the neutrophil to lymphocyte ratio in males and females of *Ctenomys talarum*

	Non-reproductive season	Beginning of reproductive season	Peak of the reprocuctive season
Males	0.21 ± 0.24	0.2 ± 0.19	0.29 ± 0.25
Females	0.28 ± 0.26	–	0.25 ± 0.22

Data are shown as mean ± standard deviation. There are no significant differences among seasons for neither sex.

Seasonal variations in N:L ratio

The N:L ratio attained low values during the whole reproductive cycle and there were no significant differences among seasons neither for males (Kruskal–Wallis: H = 0.32, $P = 0.88$) nor females (Mann–Whitney: Z = 0.22, $P = 0.86$, Table 1). We found no significant correlations between N:L ratios and cortisol, testosterone or progesterone levels.

Seasonal variations in plasma testosterone levels

Plasma testosterone concentrations in free-living males were elevated compared with other mammals (up to 486 ng mL^{-1}) with higher values during the reproductive season (Kruskal–Wallis: H = 10.79, d.f. = 2, $P = 0.005$, Dunn: $P < 0.05$, Fig. 2B). Mean testosterone levels during BRS were 11.4 and 4.7 times

higher than in NRS and PRS, respectively. In addition, we found a remarkable inter-individual variation in testosterone levels. We registered during all seasons animals with very low levels (even below the detection limit of the assay, 0.08 ng mL^{-1}) and individuals with extremely elevated concentrations (e.g. range of concentrations for BRS was 5.38–406.8 ng mL^{-1}). The variance of testosterone levels was significantly higher for BRS in relation to NRS ($F_{7,6} = 46.88$, $P < 0.001$) and PRS ($F_{7,9} = 38.90$, $P < 0.001$). Testosterone concentrations were not correlated with cortisol levels for neither of the sampling seasons (NRS: Spearman's $\rho = 0.15$, $P = 0.72$; BRS: Spearman's $\rho = 0.43$, $P = 0.26$; PRS: Spearman's $\rho = -0.19$, $P = 0.58$). Testosterone was neither correlated with body mass during NRS (Spearman's $\rho = 0.18$, $P = 0.66$), BRS (Spearman's $\rho = 0.52$, $P = 0.16$) or PRS (Spearman's $\rho = 0.29$, $P = 0.46$).

Seasonal variations in plasma progesterone levels

Progesterone levels did not differ between NRS and PRS (NRS: 10.26 ± 14.08 ng mL^{-1}; PRS: 9.29 ± 12.55 ng mL^{-1}, Mann–Whitney: $Z = 0.22$, $P = 0.86$). It is worth mentioning that four of the seven females captured during PRS for progesterone determination (i.e. 'baseline samples') presented palpable embryos, while the other three presented external indicators of lactation.

Discussion

Variations in plasma cortisol levels

The present study shows that both females and males of *C. talarum* presented higher cortisol levels during the reproductive season. Elevated GC levels are usually interpreted as indicating that the animals are in an energetically demanding situation (Romero, 2002). For our study species, resting metabolic rates were higher in lactating than in non-reproductive females, although no seasonal differences were found for males (Zenuto, Antinuchi & Busch, 2002). GCs have orexigenic effects in vertebrates and higher concentrations may stimulate food consumption if energetic demands are increased (see Dallman *et al*., 2007). Increased GC levels may also serve to modulate the priming of stress pathways during periods with higher potential exposures to stressors. Interestingly, this may result in higher GC levels during those periods, even though individuals are not actually confronted with stressors (see Romero, 2002). In our study, N:L ratios were low (mean values below 0.3) and typical of unstressed animals during the whole reproductive period in both males and females (see Vera *et al*., 2008), indicating that the animals were not stressed during the reproductive season in spite of having higher cortisol concentrations. Therefore, cortisol data obtained within 5 min can be interpreted as representing strictly basal levels (see Romero, 2004).

Our present data show that reproductive females exhibit a greater capacity of secreting cortisol in spite of having higher baseline levels, suggesting that their ability of coping with environmental stressors is not compromised at this critical period of the year (Fig. 1). It could be expected that physiological and behavioural needs (such as lactation, sexual behaviour or immune challenges) may place constraints on the ability of an animal to mount a stress response or reduce the importance of doing so (Reeder & Kramer, 2005). Indeed, the HPA axis typically shows blunted responsiveness to acute stressors during gestation and lactation in many mammals (e.g. laboratory Wistar rats: Neumann *et al*., 1998; flying foxes *Pteropus hypomelanus*: Reeder, Kunz & Widmaier, 2004; ground squirrels *Spermophilus columbianus*: Hubbs, Millar & Wiebe, 2000), although this is not always the case (e.g. marmoset monkeys *Callithrix jacchus*: Saltzman & Abbott, 2011). The HPA responsiveness to stressors might be down-regulated during these periods to protect against stress-induced disruption of reproductive effort (Wingfield & Sapolsky, 2003). The increased response reported here for reproductive females could be related to regulatory interactions between the HPA axis and oestrogens (Handa *et al*., 1994). Mated females of *C. talarum* showed higher oestradiol levels than females that were indifferent to males under conditions of captivity (Fanjul & Zenuto, 2012). If oestradiol levels are also increased in reproductive females under field conditions that may account for higher cortisol secretion (see Handa *et al*., 1994).

The higher cortisol levels in females compared with males suggest that higher rates of cortisol secretion in females are required for survival and reproduction and/or that interactions with productive hormones differently affect the activity of the HPA axis in both sexes (see Handa *et al*., 1994). Inversely to the stimulatory effects of oestrogens on GC secretion, androgens are known to negatively affect the activity of the HPA axis (Handa *et al*., 1994). Previous studies in rodents and other mammals have found differences between sexes in the levels of GCs, although whether males or females have the higher levels (or show similar levels) markedly depends on the species (e.g. Boonstra *et al*., 2001: arctic ground squirrels *Spermophilus parryii*; Reeder *et al*., 2004: flying foxes *P. hypomelanus*; Kenagy & Place, 2000; Place & Kenagy, 2000: chipmunks *Tamias amoenus*; *Romero et al*., 2008: lemmings *Lemmus trimucronatus*; Schradin, 2008: striped mice *Rhabdomys pumilio*).

It is remarkable that the seasonal variation pattern of cortisol reported for males in this study is different from the one reported for the same population during 2007 (i.e. NRS 2007: 41.5 ± 30.44 SD, $n = 12$; BRS 2007: 23.07 ± 13.54 SD, $n = 14$; PRS 2007: 45.08 ± 17.08 SD, $n = 13$; Vera *et al*., 2011*a*). This is interesting because, although seasonal patterns are variable among species, it is generally assumed that seasonal GC variations are a characteristic for a given species. In our view, the complexity of endocrine systems and the multiplicity of factors affecting hormone levels make it reasonable that seasonal variation patterns may also differ among years, as it is the case of our data in *C. talarum*. Our present data suggest that the factors underlying the seasonal variation in cortisol secretion (presumably the relative importance of social interactions, micro-environmental

conditions within the burrows and food availability) vary from year to year.

Levels of reproductive hormones

Plasma testosterone levels reported in this study for *C. talarum* males (up to 486 ng mL^{-1}) are, as far as we know, the highest reported for mammals. These concentrations are even higher than those of males captured during the BRS 2010 reported in a recent publication (Vera *et al*., 2011*b*). The implications of these extremely elevated testosterone levels are extensively discussed in our previous publication (Vera *et al*., 2011*b*). Very briefly, this may be related to (1) a low sensitivity of the target tissues to the testosterone signal; (2) elevated levels of sex hormone-binding globulin (SHBG) that could buffer males' tissues against total testosterone concentrations; (3) a reduced capacity to generate more potent androgens (such as dihydrotestosterone).

An interesting issue that presents data highlights is the large inter-individual variation in testosterone levels throughout the whole reproductive cycle. One possibility is that different animals could differentiate in their sensitivity to the testosterone signal, so that different levels of the hormone are required to sustain a physiological and behavioural function (Williams, 2008). In addition, the high variability in testosterone levels could be related to the establishment of dominant–subordinate relationships in the field. *C. talarum* males establish such relationships in semi-natural conditions (Zenuto *et al*., 2002). As proposed previously (Gleason *et al*., 2009), the dominant males (that presumably maintain larger levels of aggression towards other males and/or access to females) would maintain higher levels of testosterone, whereas those with lower testosterone levels may be subordinates with low access to females. The greater variability of data during the BRS could be related to the establishment of dominant–subordinate relationships among males at this time. Finally, it should be also considered that testosterone secretion is pulsatile and, thus, some of the variation observed for plasma samples may just be related to the timing of this pulse. Daily rhythms in hormone secretion might have little impact on these data because blood samples were collected within a restricted range of hours during all sampling dates (see Methods).

The peak of testosterone levels during the reproductive season coincided with increased cortisol concentrations (Fig. 2), and testosterone and cortisol levels were not correlated during neither of the sampling seasons. Therefore, the present data do not agree with a scenario in which cortisol and testosterone negatively affect the levels of each other, as reported for other species (e.g. Knapp & Moore, 1997), but show that GCs and reproductive hormones may peak concurrently during the reproductive period. Presumably, the lack of a negative relationship between both hormones occurs because the increase in cortisol levels during the reproductive season reflects a change in the basal levels of the hormone, as previously explained. It is possible that some critical level of GC must be reached in order to substantially influence circulating androgen concentrations.

Plasma progesterone levels reported here for females of *C. talarum* are low in comparison to values reported for other hystricomorph rodents during gestation (such as guinea pig *Cavia porcellus* and viscacha *Lagostomus maximus*; Heap, Ackland & Weir, 1981). The lack of differences between reproductive and non-reproductive females could suggest that there may be a trade-off in the secretion of progesterone, as reported for the common hamster *C. cricetus* (Franceschini *et al*., 2007). These authors reported elevated levels during the first pregnancy and somewhat lower values when gestation and lactation occurred simultaneously. However, further data are required to perform an adequate comparison between pregnant females, those with overlapping gestation and lactation, and those that are only lactating to fully address this issue in *C. talarum*.

Overall, this study shows that there are differences in the levels of GC between sexes during reproductive and non-reproductive seasons and it also highlights that testosterone levels in males are elevated in comparison to other mammals and show a remarkable variation. Future studies should address the seasonal variation in free testosterone levels (the fraction unbound to SHBG, see Rosner, 1990) and also compare free cortisol levels in males and females of the species. Indeed, the interactions between reproductive hormones and the HPA axis are worth of study in *C. talarum* (particularly in males), considering the elevated levels of testosterone reported here.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Validation of progesterone assay.