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Seasonal changes in cortisol and progesterone secretion in Common hamsters

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Abstract

In this study, we investigated endocrine factors and behaviour in free-living Common hamsters (*Cricetus cricetus*) during reproductive and non-reproductive periods of the annual cycle. We applied a non-invasive method to gain information on seasonal changes in adrenocortical activity in male and female hamsters by analysing faecal glucocorticoid metabolite concentrations (FCM). In addition, plasma progesterone concentrations were monitored in females throughout the non-hibernation season. The animals were live-trapped from spring emergence until the onset of hibernation in autumn. Reproductive status was determined at capture and blood and faecal samples were collected. During behavioural observations, agonistic and sexual interactions were recorded. FCM concentrations were significantly higher in males than in females during the reproductive period. In males, a pronounced increase in FCM during the reproductive period coincided with high frequencies of intrasexual aggression. In females, FCM levels remained relatively constant. Aggressive behaviour in females increased during the reproductive period, but was much less frequent than in males. Females, which successfully raised a second litter after a postpartum oestrus and concurrent lactation and gestation had lower FCM levels than individuals, which lost their second litter after parturition. As expected, plasma progesterone concentrations were low before and after the reproductive period. During gestation, levels peaked and remained elevated during lactation. The results of this field study provide insight in critical periods associated with reproduction in male and female Common hamsters.

Keywords: *Cricetus cricetus*; Glucocorticoids; Reproduction; Aggression; Progesterone

1. Introduction

Common hamsters (*Cricetus cricetus*) are small mammals with a pronounced annual cycle sequestered into different seasonal activities including reproduction, moult and hibernation (Backbier et al., 1998). In early April, hamsters leave their hibernacula and start above-ground activity, usually males emerging some days before females (Franceschini and Millesi, 2005). Shortly after female emergence the reproductive period with first matings starts in mid April. Compared to other hibernating small mammals, Common hamsters have a relatively long reproductive period, lasting for 4–5 months (Seluga, 1996). In our study population, the last matings were observed in mid August, coincident with the onset of testes regression in adult males (Lebl, 2005). The first litters in the season emerged from the natal burrows during May (Franceschini and Millesi, 2005). At our study site, most females (66%) had two litters per season and a few individuals even managed to successfully raise three litters. The last litters were weaned during September in our study population (Franceschini and Millesi, 2005). We found no evidence for male parental behaviour. Adult males entered hibernation in September followed by females and juveniles, which started to hibernate in October.

As in many other seasonal breeding mammals, the reproductive period in Common hamsters is characterised

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by intense intrasexual competition among males (Franceschini, 2002). In ground squirrels this is assumed to negatively affect male condition due to high energetic costs and injuries and causes a high stress load during the mating period (Michener, 1983). The increased plasma concentration of glucocorticoids is widely used as an indicator of stress (von Holst, 1998; Sapolsky et al., 2000). Glucocorticoids mobilise energy reserves via, for example, hepatic gluconeogenesis (Munck and Náray-Fejes-Tóth, 1995), needed to cope with environmental challenges (Sapolsky et al., 2000). In recent years, non-invasive methods using faecal glucocorticoid metabolite analyses have been established to avoid the stress effect of handling the animals and facilitate the sample collection in field studies (rev. in Palme et al., 2005).

Common hamsters are solitary animals, which are known to exhibit offensive behaviour in conflict situations (Niethammer, 1982). In males, agonistic interactions have been shown to occur mainly during the reproductive period (Franceschini, 2002; Adlassnig, 2005). This period can cause a major increase in circulating concentrations of glucocorticoids in reproductively active males. In female mammals, pregnancy and lactation are the two most demanding periods in life histories (Wade and Schneider, 1992). Thus, these time periods cause a major increase in concentrations of corticosteroids and corticosteroid-binding globulin in a variety of small mammals (e.g. Rosenthal et al., 1969; McDonald et al., 1988). In female hamsters, stressful situations might be more frequent during late lactation as a result of high energetic demands and an increased predation risk when the offspring begin to emerge above ground.

Due to the limited time available for reproduction, some short-lived and seasonally breeding species like the Common hamster have developed time saving strategies facilitating multiple breeding in one season, as found for instance in Djungarian hamsters (Newkirk et al., 1995) and in rabbits (e.g. Fortun-Lamothe et al., 1999; Martínez-Gómez et al., 2004). Females can have a postpartum ovulation and conceive shortly after parturition, resulting in an overlap of lactation and gestation (Franceschini and Millesi, 2005). Very little is known about the endocrine interactions of a concurrent pregnancy and lactation. During gestation, progesterone concentrations are known to increase (Leavitt and Blaha, 1970) as a result of luteal and placental activity, followed by a sharp decrease shortly before parturition. This abrupt decline of progesterone preceding parturition is essential not only to allow uterine contractility (Djiane and Durand, 1977), but also to initiate lactogenesis through the action of prolactin (rev. in Tucker, 1994). As a consequence of potential interferences between progesterone and prolactin, lactation is characterised by low progesterone levels (Leavitt and Blaha, 1970; Roy and Wynne-Edwards, 1995).

In this study, we aimed at investigating changes in adrenocortical activity, as reflected in concentrations of faecal cortisol metabolites (FCM) before, during and after the reproductive period in free-ranging Common hamsters. Potential relationships between FCM concentrations and agonistic as well as sexual behaviour were investigated in both sexes. In females, we further analysed plasma progesterone secretion throughout the active season. Relationships between FCM and progesterone concentrations and individual reproductive output per season were examined.

2. Methods

2.1. Field techniques

The study was carried out in an urban area in southern Vienna, Austria. Free-living Common hamsters inhabited the green areas (4.6 ha) surrounding apartment complexes. Animals were captured using Tomahawk live traps baited with peanut butter. The hamsters were released from the traps into cone-shaped, black cotton sacks immediately after capture. This method minimised stress for the animals during the investigation and enabled us to handle the hamsters and take blood and faecal samples without anaesthesia. The cotton sacks were equipped with Velcro fasteners, which allowed us to open parts of the sack and to investigate the abdominal region of the hamsters and to take blood from the femoral vein. The procedure lasted for about 5–10 min and thereafter the hamsters were released near their burrows. The manipulations did not seem to negatively affect the animals as they did not avoid the traps and we found no differences in behaviour, body mass or survival between frequently and less frequently trapped individuals (Franceschini, unpubl. data). All animal manipulations were approved by the Austrian Ministry, the City of Vienna and the Ethical Committee for Animal Welfare (GZ 68.210/12-BrGT/2003 and MA22-2605/02). At first capture, we implanted a transponder chip (PIT tag, data mars) subcutaneously in the dorsal region. The implantation was done using sterile applicators. At each capture the transponder and the implantation area were checked. None of the animals had lost the transponder or showed any signs of infection. For distant recognition, the animals were marked with a commercial hair dye in individually recognisable patterns. Parameters recorded at capture were location, body mass (±1 g) and reproductive status defined by teat and vulval development in females and testes size in males. Size and pigmentation of teats were classified using a 3-point-scale from small (1) to swollen with milk excretion (3). Vulval development was categorised on a 4-point-scale: vagina completely closed (0), small opening (1), wide opening (2), wide opening with bloody mucus (3). At each capture, vaginal smears were collected and stained (Papanicolaou, 1954). Vaginal cytology was classified under a Biovar microscope based on the proportion of cell components, nucleated epithelial cells, cornified cells and leucocytes. Vaginal oestrus was defined by a predominance of cornified cells in the smear (Weissbach, 1996). Testes width was measured in males with scrotal testes using a calliper. At the onset and the end of the non-hibernation season the males had regressed, abdominal testes which could not be measured.

At capture, 100 μl blood were taken from the femoral vein. Having fixed the hamster in the handling sack, the Velcro fastener was partly opened to get access to one of the hind legs. The femoral area was shaved and the femoral vein was punctually penetrated with a sterile needle (Terumo 0.6×25 mm). Blood was collected in heparinised capillaries. The blood sample was centrifugated immediately after collection in the field (3000 rpm/min). After centrifugation, the plasma was stored at −20 °C until analysis. Faecal samples were collected at capture and stored at −20 °C until analysis.

2.2. Reproductive and non-reproductive phases

According to the reproductive stages of the individual hamsters, different phases were defined:

(a) *Males.* Phases were assigned on an individual level because the course of testes development differed among individual males (Lebl, 2005; Siutz, unpubl. data).

Premating phase. The time from spring emergence until individual testes width had reached the maximal level (late March to mid April; Lebl, 2005). In this period no matings were observed.

Reproductive phase. started when males had fully developed testes and lasted until the individual onset of testes regression.

Postreproductive phase. From the onset of testes regression until the individual onset of hibernation (mid August until early October). Our classification was supported by data on calculated conceptions on the basis of parturition and litter emergence.

(b) *Females.* As in males, the phases were defined for each individual female. The premating phase was defined as the time period from spring emergence of a female until its onset of first gestation in the season. The reproductive period included gestation and lactation. These were determined on the basis of individual teat development, body mass changes and litter emergence (Franceschini and Millesi, submitted). Observed sexual interactions with males during vaginal oestrus or 1–2 days thereafter were used as indicators for conception. Gestation could be clearly identified by subsequent mass increases and cumulative teat swelling. Parturition and lactation onset were detected on the basis of a rapid body mass loss in combination with increased teat size and sometimes milk rests on the teats as a consequence of milk secretion. The lactation period lasted until weaning, defined as the date when the juveniles emerged from the natal burrow for the first time. At this time, teat size of the mothers decreased and the pups were observed to feed above ground. The postreproductive phase was defined as the period from weaning of the last litter in the season until individual hibernation onset.

Postpartum litters. Signs of concurrent lactation and gestation after a postpartum oestrus, were increasing body mass during lactation, bloody and mucous vaginal status and rapid body mass loss at the expected end of the postpartum pregnancy. In addition, persisting lactation (swollen teats, milk secretion) after weaning of the first litter was a clear indicator for having conceived postpartum. In some cases we were able to observe sexual interactions with males during postpartum oestrus (Franceschini and Millesi, submitted). A female was defined as having successfully produced a postpartum litter, if the second litter emerged from the breeding burrow at the expected time, about 40–45 days after postpartum oestrus. When no young could be observed, although concurrent gestation and lactation were detected, a female was considered to have lost this second litter. Earlier studies had shown that in our study population about 53% of the females showed a postpartum pregnancy (Franceschini and Millesi, submitted).

2.3. Behaviour

Behavioural observations were carried out 5 days/week throughout the active season. The hamsters were observed during their daily activity periods in the morning and evening hours (Schmelzer and Millesi, 2005). The study site was divided into six sectors. In each sector, we chose an observation point, which enabled us to completely overlook this section of the study area. Each sector was subdivided by a 16×16 m grid. Behavioural observations were carried out 2–3 h/d, alternately in each sector in 2- to 3-d intervals. One observation session lasted for 1 h/sector. Social interactions that could be observed during an observation session (event-sampling) were recorded and classified into aggressive (chases and fights) and sexual (copulations, mate chases; Adlassnig, 2005) interactions. To control for potential recipients, all individuals within 15 m distance were recorded. This distance was used because earlier observations had shown that animals at larger distances were never attacked (Franceschini, unpubl. data). In addition, the initiator and receiver of the dyadic interaction were noted. For analysis the data of all sectors were pooled. We calculated the mean number of aggressive and sexual interactions/h.

To investigate relationships between individual aggressive and sexual behaviour and hormone concentrations we analysed the mean number of initiated and received interactions/individual/hour. We only used observation sessions in which potential recipients were present within 15 m distance. To calculate the divergence between initiated and received aggression of individuals, we subtracted the mean number of received aggression/h from the mean number of initiated encounters/h. By that we yielded a delta value for initiated/received aggressive interactions for each individual.

To determine sexual interactions of individual hamsters only observation sessions in which at least one female was observed above ground simultaneously with a male were used for analysis.

2.4. Faecal cortisol metabolites (FCM)

We extracted 0.2 g of each faecal sample with 4.0 ml of 80% methanol. After shaking (40 min) and centrifugation (3600 rpm/min, 15 min), FCM were determined in an aliquot of the supernatant (10 μl diluted 1:10) using an 11-oxoaetiocholanolone enzyme immunoassay (EIA). Details of this group specific EIA (measuring glucocorticoid metabolites with 3α,11-oxo structure) are described in Möstl et al. (2002). Intra- and inter-assay coefficients of variation (CV) were 10.5% and 9.0%, respectively. No significant differences between the years 2004 and 2005, both in males ($p = 0.74$, $Z = -0.33$, 2004: $n = 30$, 2005: $n = 24$) and in females ($p = 0.94$, $Z = -0.076$, 2004: $n = 24$, 2005: $n = 18$) were found. Mean FCM values per individual per phase were used for analysis.

To demonstrate the biological relevance of the measured FCM in hamsters, we performed a validation experiment (Palme, 2005; Touma and Palme, 2005). Five male and five female hamsters were exposed to a stressful situation. Animals were captured in the field, kept in cages over 24 h and were exposed to conspecifics in an adjacent cage. In 30-min intervals, the cages were checked and all faecal samples in the cages were collected. We were unable to collect samples from all individuals in each interval, therefore the intervals between the samples varied among the individuals. The analysis showed that the experimental situation led to a 1.5- to 5-fold increase (2.7 ± 1.2) in FCM excretion. Maximal levels were reached after 13.7 h (± 7.6).

In the field, trapping was done mainly in the morning and sometimes in the evening hours according to the daily activity patterns of Common hamsters. We compared FCM levels between samples collected in the morning and the evening to test for potential circadian patterns in cortisol secretion. We found no significant differences between faecal samples collected in the morning and in the evening (males: Mann–Whitney *U*-test: $p = 0.22$, morning: $n = 97$, evening: $n = 33$; females: Mann–Whitney *U*-test: $p = 0.47$, morning: $n = 210$, evening: $n = 48$).

2.5. Progesterone

Following extraction with diethyl ether, plasma progesterone concentrations were determined using a progesterone EIA described previously (for details see Schwarzenberger et al., 1996). The intra- and inter-assay CV were 11.2 and 16.9%, respectively. No significant interyear differences were found.

2.6. Statistics

Data distributions were tested for normality by Wilk-Shapiro tests (SPSS 11.5 for Windows). Correlation statistics were done with Pearson correlations in case of normally distributed samples, otherwise Spearman rank correlations were performed. To compare two independent samples, Mann–Whitney *U*-tests were applied. In case of a homogenic data distribution, one-way ANOVA was applied to test for phase differences. Kruskal–Wallis tests were applied for heterogenic data. For post-hoc comparisons we used Mann–Whitney *U*-tests (significance levels were Bonferroni corrected). Common hamsters showed high fluctuations in individuals per phase (Franceschini and Millesi, in press). Therefore we were unable to capture a sufficient number of individuals going through each phase during the season. Hence, the samples were treated as independent and, if possible, we additionally included intraindividual comparisons. To test for intraindividual differences, we performed Wilcoxon

tests for paired samples or Friedman tests (in case of more than two samples). Significance values were obtained from two-tailed statistics. If not stated otherwise means ± SD are shown.

3. Results

3.1. Behaviour

Agonistic behaviour initiated by male and female hamsters was compared before, during and after the reproductive phase. During premating no conflict behaviour could be observed. This conspicuously changed at the onset of the reproductive phase. Aggressive behaviour was observed in both sexes, being significantly higher in males than in females (Fig. 1). During the postreproductive phase, only two agonistic interactions of adult males, directed against juveniles, were observed. In total, 73% of all observed aggressive encounters were initiated by adult males and only 12% by adult females, the remaining 15% were conflicts among juveniles. Aggression initiated by adult males was mainly directed towards other males (92%) and very rarely towards females (5%) and juveniles (3%). In contrast to males, conflict behaviour among adult females was never observed. Female aggression occurred exclusively during the reproductive period and was directed mainly against juveniles (60%) and less frequent against adult males (40%).

3.2. Faecal cortisol metabolites (FCM)

Cortisol excretion in adult Common hamsters differed significantly between the sexes. Concentrations of faecal cortisol metabolites (FCM) in males were nearly twice as high as that of females (males: 1011 ± 837, $n = 54$; females: 558 ± 385, $n = 42$; $p = 0.001$, $Z = -3.21$). To investigate these differences in more detail, we compared cortisol excretion before, during and after the reproductive period. Concentrations of FCM in the premating and postreproductive period were similar in both sexes (Fig. 2). However, FCM concentrations differed clearly during the reproductive phase. Males had significantly higher FCM levels than females (Fig. 2).

In males, FCM levels differed between the phases (Kruskal–Wallis test, $p = 0.000$). Cortisol excretion increased

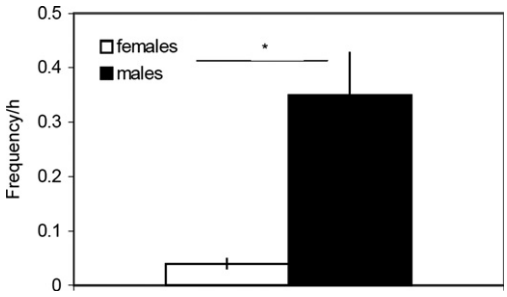

Fig. 1. Initiated agonistic interactions (mean frequency/h ± SE) in adult male and female Common hamsters during the reproductive period (Mann–Whitney U-test, $p = 0.03$, $n = 175$ observation sessions for both males and females). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

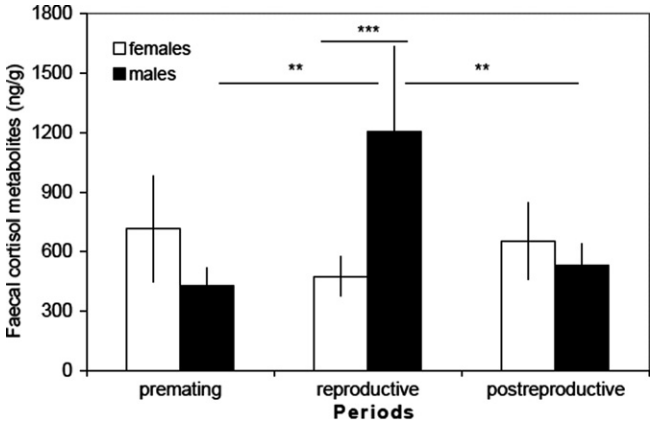

Fig. 2. Concentration of faecal cortisol metabolites (mean ± SD) in adult Common hamsters before, during and after the reproductive period. Sex differences: Mann–Whitney U-tests, premating period: $p = 0.71$, females $n = 10$, males $n = 10$; reproductive period: $p = 0.000$, females $n = 25$, males $n = 35$; postreproductive period: $p = 0.97$, females $n = 9$, males $n = 9$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

from the premating to the reproductive period (Mann–Whitney U-test, Bonferroni corrected, $p = 0.002$), and decreased again in the postreproductive period (Mann–Whitney U-test, Bonferroni corrected, $p = 0.002$). No significant differences were found between the premating and postreproductive phase (Mann–Whitney U-test, Bonferroni corrected, $p = 0.3$). Five individual males were captured during premating and reproduction and seven males during reproduction and the postreproductive period. None of the male hamsters could be captured in all three phases. Intraindividual comparisons of FCM levels showed a similar pattern, however, the increase from the premating to the reproductive phase was not significant (Wilcoxon test, $p = 0.14$, $n = 5$). We found a significant decrease in FCM concentrations from the reproductive to the postreproductive period in individual males (Wilcoxon test, $p = 0.018$, $n = 7$). During the reproductive period some of the males disappeared and new individuals were trapped. To control for a potential difference in resident and immigrated individuals during the reproductive period we compared FCM levels in both groups. Cortisol excretion did not differ between males that had hibernated in the study area and individuals that had immigrated during the reproductive period (residents: 1148 ± 917 ng/g, $n = 16$, immigrants: 1259 ± 819 ng/g, $n = 16$, Mann–Whitney U-test, $p = 0.65$).

When we compared different reproductive phases of females we found no significant differences between gestation (508 ± 195 ng/g), lactation (499 ± 331 ng/g) and lactation with concurrent gestation (211 ± 65 ng/g), (one-way ANOVA: $p = 0.11$, gestation: $n = 20$, lactation: $n = 19$, lactation with concurrent gestation: $n = 4$). Therefore, FCM values of all reproductive phases in females were pooled. Cortisol excretion in adult females did not differ between the premating, reproductive and postreproductive period (Fig. 2, Kruskal–Wallis test, $p = 0.20$, premating period:

$n = 10$, reproductive period: $n = 25$, postreproductive period: $n = 9$).

In some females, it was possible to do the phase comparisons on an intraindividual level. We were able to compare samples of 6 individuals during the premating and reproductive phase and 9 individuals during the reproductive and postreproductive phase. No significant changes in FCM concentrations were found (Wilcoxon tests, premating vs. reproductive period: $p = 0.91$, $n = 6$; reproductive vs. postreproductive period: $p = 0.13$, $n = 9$). We were unable to perform intraindividual comparisons between the pre- and postreproductive period because of the low sample size ($n = 2$).

Potential relationships between initiated and received aggression and FCM concentrations during the reproductive period were investigated in male and female hamsters. In addition, we compared the mean frequency of individual sexual interactions (mate chases, copulations) with aggressive behaviour and cortisol excretion. In males, we found no significant relationships between both initiated and received aggression and FCM levels (initiated aggression: $r_s = 0.20$, $p = 0.52$, $n = 12$, received aggression: $r_s = -0.44$, $p = 0.14$, $n = 12$). When we correlated FCM levels with the delta values for initiated/received aggression, we found a significant relationship ($r_s = 0.66$, $p = 0.01$, $n = 12$). Individuals that initiated more aggression than they received had higher FCM levels than those that mainly received aggression from other males. We found no significant relationships between FCM and observed sexual interactions ($r_s = 0.24$, $p = 0.44$, $n = 12$) and between sexual and aggressive behaviour in individual males ($r_s = 0.13$, $p = 0.67$, $n = 12$).

In females, neither initiated nor received aggressive behaviour were significantly related to FCM concentrations (initiated aggression: $r_s = 0.20$, $p = 0.57$, $n = 10$, received aggression: $r_s = -0.52$, $p = 0.11$, $n = 10$). In contrast to males, we did not find a significant relationship between delta values for initiated/received aggression and FCM levels ($r_s = 0.60$, $p = 0.20$, $n = 10$). However, in females, sexual interactions were positively correlated with FCM concentrations ($r_s = 0.68$, $p = 0.02$, $n = 10$).

We further investigated relationships between cortisol excretion and reproductive success in individual females. No significant relationships between reproductive output and cortisol excretion were found (number of litters per season: $r_S = -0.31$, $p = 0.30$, $n = 13$; offspring per season: $r_P = -0.44$, $p = 0.16$, $n = 12$).

Some female hamsters showed a postpartum oestrus and mated shortly after parturition. In only four out of eight females that gave birth to a postpartum litter, were the juveniles of the second litter observed surviving until weaning. When we compared females, which successfully produced a postpartum litter after an overlap of lactation and gestation, and individuals which lost their second litter after parturition, we found significant differences in FCM concentrations during the reproductive period: Successful individuals had significantly lower FCM levels (333 ± 122 ng/g, $n = 4$) than unsuccessful ones (547 ± 53 ng/g, $n = 4$; $p = 0.021$).

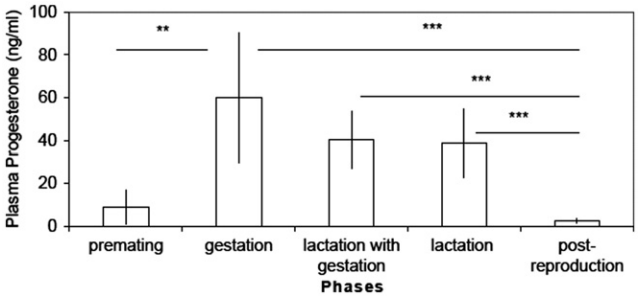

Fig. 3. Changes in plasma progesterone concentrations (ng/ml) in female Common hamsters (mean ± SD) during the five phases of the annual cycle (Kruskal–Wallis test: $p = 0.000$, df = 4, premating: $n = 5$, gestation: $n = 26$, lactation with gestation: $n = 16$, lactation: $n = 21$, postreproduction: $n = 14$, post hoc comparisons: Mann–Whitney U-tests, Bonferroni corrected, premating/gestation $p = 0.001$, gestation/postreproduction $p = 0.000$, lactation with gestation/postreproduction $p < 0.001$, lactation/postreproduction $p = 0.000$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3.3. Progesterone

Plasma progesterone concentrations differed in the course of the season (Fig. 3). Progesterone levels at spring emergence were found to be quite low. As soon as reproductive activity started, progesterone levels increased significantly and peaked during gestation. Progesterone levels remained elevated in lactating females and in individuals with overlapping lactation and gestation after a postpartum oestrus. During the postreproductive period, progesterone secretion declined again to baseline levels (Fig. 3). Intraindividual comparisons supported these results (Wilcoxon test: premating/gestation: $p = 0.06$, $n = 4$, gestation/lactation: $p = 0.21$, $n = 11$, lactation/postreproduction: $p = 0.003$, $n = 11$, lactation with gestation/postreproduction: $p = 0.018$, $n = 7$).

No significant correlations were found between progesterone secretion during the reproductive period and individual reproductive output (number of litters: $r_S = 0.22$, $p = 0.57$, $n = 9$; offspring number per season: $r_S = 0.24$, $p = 0.53$, $n = 9$). We also found no correlations between FCM and progesterone concentrations during the reproductive period ($r_S = 0.07$, $p = 0.75$, $n = 22$). When we tested for differences in progesterone concentrations during the reproductive period between females having successfully weaned a postpartum litter and females which did not, we found no significant differences (successful females: 55 ng/ml ± 20 ng/ml, $n = 7$; unsuccessful females: 94 ng/ml ± 124 ng/ml, $n = 7$; Mann–Whitney U-test: $p = 0.71$).

4. Discussion

In this study, we investigated endocrine and behavioural parameters in Common hamsters during reproductive and non-reproductive periods of the annual cycle. We applied a

non-invasive method to gain information on changes in adrenocortical activity in hamsters by analysing faecal glucocorticoid metabolites. The patterns of glucocorticoid excretion were found to differ between the sexes. During the reproductive period, male FCM levels increased significantly whereas FCM concentrations in females remained relatively constant throughout the active season. This result probably revealed the high stress load that males face during the reproductive phase. A common feature of mating behaviour is male-male competition to gain access to oestrous females (Anderson, 1994). In our study population, intrasexual aggression among adult males occurred exclusively during the reproductive phase and may have caused increased adrenal activity during this period. Cortisol is produced in the adrenal glands and is known to be released in response to stressors (Engler et al., 1989; Sapolsky et al., 2000). At our study site, Common hamsters have a promiscuous mating system (Franceschini and Millesi, submitted), males compete for access to receptive females and females have been observed to copulate with several males during one oestrous period (Adlassnig, 2005; Franceschini, unpubl. data). The high population density in our study area could increase conflict situations. Conflict rates varied among individual males. Some males were frequently observed to be involved in agonistic interactions, others had only a few conflicts. Earlier results indicated that high testosterone levels were associated with male aggression during the reproductive period (Lebl, 2005). This could lead to higher stress levels and increased adrenal activity in males with high conflict frequencies. However, we found no significant relationships between individual frequencies of initiated or received aggression and FCM levels. More detailed analyses showed that in individual males the ratio of initiated versus received encounters seemed to be related to cortisol excretion. Males that were more often initiators than receivers had higher FCM concentrations. Apparently, males that were more active in defending territories or oestrous females had a higher stress load than those that mainly received aggression. Intrasexual aggression was not the only factor determining mating effort. Males had to locate oestrous females leading to increased home range size during the mating phase (Lebl, 2005). Specific male-female interactions like frequent approaches or mate chases have been observed to precede copulations and could be energetically costly, particularly for males. In addition, mate guarding following copulations had frequently been observed (Adlassnig, 2005). All aspects of male behaviour during the reproductive period reflected adaptive strategies in a dense population with a high potential for sperm competition (Parker, 1984; Birkhead et al., 1988), but are time and energy consuming and may cause elevated tonic cortisol levels in reproductively active males.

In the premating period no aggressive encounters could be observed and male hamsters used most time above ground for foraging (Schmelzer, 2005). Consequently, they gained body mass to be prepared for the long and exhausting breeding season (Lebl, 2005). Although testosterone secretion during that period was similar to the mating phase (Lebl, 2005), FCM concentrations were low and males did not behave aggressively. During the postreproductive period, no aggressive interactions between adult hamsters were observed. Only one adult male was observed to chase a juvenile twice. The termination of aggressive behaviour is paralleled by testes regression and a decrease in FCM concentrations. During the postreproductive period, males had to recover from reproductive effort and accumulate fat reserves and food stores for hibernation.

In contrast to males, female FCM levels did not increase during the reproductive period. Although aggressive behaviour in both sexes was limited to the reproductive period, agonistic encounters occurred much less frequently in females than in males. Aggressive encounters between adult females were never observed and conflicts with males and juveniles were less intense than among adult males. Females stayed in close proximity to the burrow during lactation (Franceschini and Millesi, submitted) lowering the probability of intrasexual encounters. In our population, we found no evidence for infanticide by females (Franceschini and Millesi, submitted). Aggressive interactions between adult males and females occurred during the reproductive period and were mainly initiated by females. This could be due to rejected mating attempts. As some females entered oestrus shortly after parturition, mating temporally overlapped with lactation onset (Vohralik, 1974; Grulich, 1986; Franceschini and Millesi, 2005). Males tried to sexually approach females during this postpartum oestrus but had only a narrow time window to be successful. Sexual approaches by males might be stressful for females especially when they had newborn juveniles in the burrow. This may be reflected in the relationship between the frequency of sexual interactions and FCM levels in females.

When we compared females, which successfully produced a second litter after a postpartum oestrus with others that failed to do so, we found significantly higher FCM levels in unsuccessful females during the reproductive period. One explanation could be that females which had lost their litters were exposed to stressful situations during gestation and/or lactation. High stress load during late lactation could affect the postpartum pregnancy shortly before parturition. This might lead to increased pre- or postnatal offspring mortality and pup rejection by the mother. On the other hand, this result may indicate better stress coping abilities in successful females throughout the reproductive period, which may positively affect reproductive performance. Further investigations are needed to get more insight into this critical period of female reproduction.

In contrast to males, female adrenocortical activity did not increase during the reproductive period although conflicts and mating attempts were frequently observed. This might be related to potential relationships between cortisol and gonadal steroid hormones like oestrogens and progesterone. Several studies have shown effects of oestradiol on stress responses. For example, in sheep

oestradiol significantly reduced ACTH and cortisol responses to stressors (Komesaroff et al., 1999). In ovariectomised rats, oestrogens lowered the ACTH and corticosterone levels (Redei et al., 1994). Unfortunately, we were unable to measure oestradiol changes in the hamsters because the concentrations were below the detection limits of our assays. Although it has been suggested that progesterone inhibits corticosterone secretion in the rat (e. g. Rodier and Kitay, 1974), little information on the effects of progesterone on the HPA activity exists so far. In our study, no significant relationships between FCM concentrations and progesterone were found.

Plasma progesterone concentrations were analysed in different time periods. After emergence from hibernation, in the premating period, concentrations were very low. Our results are similar to those of Hilfrich et al. (1977), who found no corpora lutea in ovaries of Common hamsters at the end of hibernation. As expected, progesterone levels peaked during gestation as progesterone is required for the maintenance of pregnancy (Leavitt and Blaha, 1970; McMillan and Wynne-Edwards, 1999). Progesterone concentrations in female hamsters with overlapping lactation and gestation after a postpartum oestrus were found to be at intermediate levels between gestation and lactation. Little is known about the endocrine interaction during concurrent gestation and lactation. There might be potential interferences among endocrine requirements during pregnancy and lactation. Studies on Djungarian hamsters compared serum progesterone of lactating females with those of lactating and simultaneously pregnant individuals. Concentrations in females with concurrent lactation and gestation were constantly lower during the first two thirds of lactation than in nonpregnant lactating females (Roy and Wynne-Edwards, 1995). It seemed though that during periods of concurrent lactation and gestation, a female must fulfil the endocrine premises supporting both, lactation and gestation. Progesterone is associated with gestation maintenance, while prolactin is essential for lactation. High progesterone levels downregulate prolactin receptors and interfere with growth of the mammary gland and lactogenesis (Tucker, 1994). Therefore, progesterone levels during concurrent lactation and gestation might serve as a compromise, delivering as much progesterone as necessary to maintain gestation on the one hand, and simultaneously minimising potential interfering effects on lactation. We found no significant differences in progesterone levels between females having successfully raised a postpartum litter and those that had lost their postpartum litter, during the reproductive period. This result indicated that the failure to wean the offspring was not related to increased progesterone secretion in the unsuccessful individuals.

During lactation progesterone concentrations were above baseline levels. As high progesterone levels were found to facilitate the expression of behavioural receptivity in dwarf hamsters (McMillan and Wynne-Edwards, 1999), elevated progesterone concentrations in nonpregnant, lactating female Common hamsters could be related to further matings. This assumption was supported by the analysis of vaginal smears collected from lactating females. We found a high proportion of nucleated epithelial cells, which is typical for vaginal prooestrus and often associated with behavioural oestrus (Nelson, 1995). In the postreproductive period, before hibernation, progesterone concentrations strongly declined. This corresponds well with the common pattern in hibernating species showing a decrease in reproductive activity before entering hibernation (e.g. Wollnik et al., 1991; Millesi et al., 2000).

In conclusion, our results indicate that elevated stress levels in male Common hamsters during the reproductive period seem to be related to intense intrasexual competition in a high-density population with an extended reproductive period and asynchronous oestrus phases in females (Franceschini and Millesi, 2005). Chronic stress can have profound effects on many aspects of an individual's life. It is known to negatively affect immune competence (rev. in Padgett and Glaser, 2003) and by that could lead to increased mortality rates (Wadhwa et al., 2001). This is supported by the fact that male hamsters had a shorter life span than females (Franceschini and Millesi, in press).

Females seemed to have lower and more constant levels of adrenal activity, which could be due to less intense conflict behaviour and/or interactions with other endocrine factors during gestation and lactation. Stressful situations in females might be caused by sexual harassments and could lead to offspring loss especially during the critical period of concurrent gestation and lactation.

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