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Social stress and reproductive success in the female Syrian hamster: Endocrine and behavioral correlates

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ABSTRACT

In many mammal species, reproduction is not shared equally among the members of a social unit. Even though reproductive skew seems unlikely in females of solitary species, this phenomenon could result from environmental factors. Although solitary in the wild, captive Syrian hamsters (*Mesocricetus auratus*) are generally housed in groups. We investigated whether social stress produces some degree of reproductive skew in this solitary species and whether female reproductive success varies as a function of social rank. To assess the physiological relationship between social stress and fertility, we monitored reproductive hormones and glucocorticoids of solitary and pair-housed females during pregnancy by means of recently established non-invasive methods for measuring hormone metabolites in the feces. The patterns of fecal progesterone, estrogen and glucocorticoid metabolites were similar to those found in blood and reported in the literature for pregnant hamsters. As expected, dominant females had higher breeding success than subordinate females. However the rate of reproductive failure was also very high among the singly housed females of our control group. The number of pups per litter, the average sex-ratio in each group, and the mean weight of pups did not differ significantly among groups. Glucocorticoid concentrations were unaffected by housing and social rank and the few differences between the endocrine profiles of singly- and pair-housed females are not sufficient to explain the observed difference in breeding success. It is likely that social isolation impairs reproduction in the same manner as subordination. Our findings suggest that social isolation of animals accustomed to group living was equally as disturbing as cohabitation with an unknown conspecific.

1. Introduction

In many mammal species, reproduction is not shared equally among the members of a social unit. Known as reproductive skew, this phenomenon is ubiquitous among social, cooperatively breeding animals. Typically, a dominant individual monopolizes most (e.g. common marmosets [1]), if not all (e.g. naked mole-rats, *Heterocephalus glaber* [2]) of the group's reproductive output.

While high reproductive skew is relatively common among females of cooperatively breeding species, only low skew, if any, occurs in females that do not breed cooperatively [3]. A fortiori, reproductive skew seems unlikely in females of solitary species, and to the best of our knowledge, has only been reported in one such species. In a study of group-housed wolverines (*Gulo gulo*), a solitary species in the wild, Dalerum et al. [4] observed reproductive failures probably related to social rank, although no single female monopolized reproduction.

Social organization is affected by environmental factors such as density or resource distribution, and intraspecies variation in social systems related to environmental variability has been observed in a number of species. For example, studies conducted on populations of Bamboo rats (*Kannabateomys amblyonyx*) in southeastern Brazil found traits corresponding to either polygyny or monogamy depending on the region [5]. Similarly, African striped mice (*Rhabdomys pumilio)* live in groups in a semi-desert region but are solitary in the moist grasslands of South Africa [6]. Moreover, Sachser [7] showed that individual guinea-pigs interact in ways affected by previous social experiences. Finally, in the Dalerum et al. [4] study, group housed wolverines displayed social interactions similar to those of many obligate group-living species. If ecological factors affect sociability, we might expect that many behavioral and physiological mechanisms responsible for reproductive suppression are present as latent traits, even in species usually considered as solitary.

In several models for the social suppression of reproduction which have been described and used to study the evolution of social group formation [8] both behavioral and physiological mechanisms may account for the observed suppression. Lower reproductive success of subordinate females has been considered conventionally as an effect of subordination stress and related high levels of glucocorticoids.

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However, in many species, dominant individuals show higher glucocorticoid levels than subordinates [3], and sexual behavior as well as reproductive hormone levels seems modulated by still unclear non-glucocorticoid-mediated mechanisms.

Although solitary in the wild [9]; captive Syrian hamsters (*Mesocricetus auratus*) are generally housed in groups. Grouping of adult hamsters forcing social interaction has been shown to produce symptoms of stress [10]. For this reason, the Syrian hamster has been considered as an ideal model for studying effects of social stress on reproduction [11]. Several authors reported the establishment of a stable social hierarchy in group-housed hamsters as well as some degree of reproduction suppression, including reduction in litter size and the percentage of male pups born from subordinate females [11–13].

On the other hand, more recent studies suggested that the exposure to social stress causes no long-lasting effects on the agonistic behavior of the female hamster [14,15]. Specifically, the group-housing-induced dominance hierarchy – stable in males – is unstable among females [16].

Potential endocrine influences on aggressive and submissive behavior have been examined with ambiguous results. Pratt and Lisk [12] reported a significant reduction in circulating progesterone concentrations in female hamsters exposed to social subordination early in pregnancy. Although they did not provide any physiological measure of the hypothalamic–pituitary–adrenal (HPA) axis activity, they suggested that the stress-related activation of HPA is responsible for this decrease. In contrast, Fritzsche et al. [16] found higher levels of progesterone in both dominant and subordinate group-housed cycling females when compared with those housed individually. Females treated with an estradiol or testosterone implant displayed less submissive behavior than females receiving progesterone or no hormone [17]. The exact role of adrenal glucocorticoids in the modulation of agonistic behavior and their potential effects on the reproductive success of female hamsters is still unclear.

The aim of our study was to examine the effects of social grouping on the female Syrian hamster, both before and at the beginning of gestation. We investigated whether social stress produces some degree of reproductive skew in this solitary species and whether female reproductive success varies as a function of social rank. In order to assess the physiological relationship between social stress and fertility, we monitored reproductive hormones and glucocorticoids of solitary and pair-housed females during pregnancy by means of recently established non-invasive methods [18,19] for measuring the respective hormone metabolites in the feces.

We hypothesized that: 1) the fertility of the dominant females would be similar to those in isolation; 2) reproductive skew would occur in pair-housed females; 3) there would be more reproductive failure among subordinate females than among dominant or isolated females; 4) subordinate females would deliver smaller litters than dominant or isolated females; 5) a female bias in the sex ratio would only occur in subordinate females, and finally; 6) endocrine profiles would differ between groups.

2. Material and methods

2.1. Animals and housing conditions

The subjects were 34 adult (12 weeks of age; mean body weight 170 ± 15 g) female Syrian hamsters (*Mesocricetus auratus*) kept in isosexual groups of four or five individuals from weaning. These hamsters were born and raised in the Laboratory Animal Facility of the Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Brazil, whose Ethics Committee approved the experimental design. The animals were housed in standard propylene cages in the same animal facility under conventional conditions (12:12-h light:dark, lights on at 03:00 h; room temperature: 22 ± 2 °C; 20 changes of air per hour; air pouch filters). Specific pelleted food (Nuvilab CR1, Nuvital, Curitiba, Brazil) and filtered bottled tap water were supplied ad libitum. The animals were free from any common pathogens according to the FELASA Health Monitoring Recommendations [20]. In order to facilitate feces collection for endocrine analysis, absorbent paper pads were used in place of wood-shaving bedding.

2.2. Procedure

At least two consecutive 4-day cycles per female were monitored before the beginning of the trial by detection of the characteristic post-ovulatory discharge in the morning following ovulation. One animal was discarded because it was not regularly cycling. Among the others we selected 11 pairs of non-relative females (PH = pair housed) with similar body weight (± 5 g). As the expression of aggressive behavior is estrus-cycle dependent, females in a pair had synchronized estrus cycles [16,21]. Each female was marked with a commercial hair dye in a recognizable pattern to facilitate behavioral recording. These animals remained housed in pairs for 10 days. The remaining 11 females, forming the control group (ISOL = isolated), were housed singly.

To determine the social rank of pair-kept females, the behavior of each pair was observed continuously during three 10-min sessions with 4-day intervals between sessions. Before these observations each pair was separated for 20 min during the routine cage exchange and then regrouped in a clean cage containing 7 food pellets scattered on the floor. Aggressive (upright/side offense, chase, bite, attack) and defensive (upright/side defense, flee, full submissive posture) behaviors regarded as specific markers or indices of social stress [22] were recorded, as well as how many food pellets each animal secured. Huck et al. [13] showed that dominant females successfully removed food from the subordinate females. In each encounter one point was given to the female which displayed more aggressive behavior and one point to the female that secured more food pellets. The female who had the higher score in at least two sessions was considered dominant (DOM) and the other one was subordinate (SUB). All sessions were completed during the first 2 h of the dark phase of the LD cycle under dim red light.

On the first estrus (D0) following the first behavioral observation, all females were mated with proven breeders. Each female was housed with a male for 24 h. Afterwards the former female pairs were regrouped until completing 10 days. All the females were then transferred to individual cages and stayed singly housed until parturition (D16). All cages were daily monitored and parturitions registered on the exact day when they occurred. However, to avoid cannibalism, common in hamsters when females are disturbed after delivery [23], it was only on Day 4 after parturition (D20) that pups were counted and sexed (by measurement of anogenital distance), and dams and litters were weighed.

2.3. Collection of fecal samples

To habituate the females to the sampling procedure, feces collection started 10 days before pair forming. It proceeded daily until the day before parturition to avoid cannibalism. Sample collection took place between 14:00 and 15:00 h at the end of the light period. As hamsters void frequently a small but quite variable number of fecal pellets, samples were easily obtained by transferring each animal into a separate cage until it defecated (approximately 15 to 20 min). After defecation, it was immediately returned to its home cage and fecal samples were collected directly from the cage floor.

2.4. Steroid analyses

Fecal steroids were extracted using the methanol-based procedure described by Palme [24]. Because hamster feces are very dry, lyophilization was not necessary. After the homogenization of each fecal sample, we shook an aliquot of 0.5 g (dry weight) or the whole sample for 20 min on a multivortex with 5 ml of 80% methanol for

Table 1
Reproductive data (mean ± sd) of female Syrian hamsters kept isolated or pair-housed during 10 days before mating and in the beginning of gestation. Housing condition and social rank in the pairs did not affect these results.

	Isolated	Pair-housed	Dominant	Subordinate	Indeterminate
n	11	22	10	10	2
Breeding success	6 (54.5%)	15 (68.2%)	9 (90.0%)	5 (50%)	1 (50%)
Total of pups	51	143	81	57	5
Pups per liter	8.5 ± 3.2	9.5 ± 3.3	9.0 ± 3.6	11.4 ± 1.1	5
Weight of pups on day 4 post partum (g)	5.3 ± 1.2	5.0 ± 0.9	5.0 ± 1.1	4.7 ± 0.6	5.1
Offspring sex-ratio (% of male pups)	39.4 ± 24.5	50.3 ± 16.8	53.3 ± 19.2	44.8 ± 11.3	20.0

samples heavier than 0.25 g, 2.5 ml for samples whose weight was between 0.1 and 0.25 g and 1 ml for samples lighter than 0.1 g. The suspension was then centrifuged at 500×*g* for 10 min. The supernatant was stored at −20 °C until assayed.

Fecal cortisol metabolites (FCM) were quantified in an aliquot of the extract (50 µl further diluted 1:10 with assay buffer) using a group specific 11-oxoetiocholanolone enzyme immunoassay (EIA measuring glucocorticoid metabolites with a 5β-3α-ol-11-one structure) successfully validated for the Syrian hamster [19]. Details of this assay were described by Möstl et al. [25]. All samples were assayed in duplicate. Intra- and interassay coefficients of variation were 8.6 and 9.7, respectively. Concentrations of glucocorticoid metabolites were expressed as nanograms per gram fecal dry matter. Fecal estradiol (FEM) and progesterone metabolites (FPM) were quantified in 40-fold dilutions of the fecal extracts using commercial solid-phase radioimmunoassays with antibody-coated tubes previously validated for the female Syrian hamster [18] following the manufacturer's protocol (Coat-A-Count estradiol, and progesterone, Siemens, Los Angeles, CA, EUA). Respective cross-reactivities are provided by the manufacturer (http://www.medicalsystems.it/MetodicheSiemens/RIA/pitke2-8_siemens.pdf and http://www.medicalsystems.it/MetodicheSiemens/RIA/pitkpg-7_siemens.pdf, respectively). Mean sensitivity was 2.14 pg/mL for both the estradiol and progesterone assays. Intra- and interassay coefficients of variation for all assays were <12%.

2.5. Statistics

We used chi-square analysis to compare the proportions of breeding success between isolated and pair-housed females and between isolated, dominant, and subordinate females. Mean number of pups per litter, pup weight, and sex ratio were compared between groups using ANOVA. The effects of time, pregnancy, housing, and social status on concentrations of steroid metabolites were assessed using GLM for repeated measures (rmGLM). Correlations between mean hormone concentrations were calculated. Means are given with standard deviations and significance level for all tests was set at $p<0.05$ unless otherwise noted. We performed statistical analysis with the software SPSS 13.0 for Windows.

3. Results

3.1. Social rank

Pair-housing led to social hierarchy in most pairs, established in a few minutes after pairs were formed in eight of 11 pairs. In six of these eight pairs, agonistic behavior was intense on the first encounter but only sporadic during the subsequent observation sessions. In two pairs we did not observe any manifestation of aggression, but from the beginning one female secured all seven food pellets, a behavior indicating dominance [13]. In another pair, one female was very aggressive on the first encounter but did not display any aggressive behavior during the other encounters. During all the observation sessions its partner immediately took possession of all the food pellets, claiming its dominant status without fighting. In one pair, social rank was inverted on the third session with the former subordinate attacking and chasing the former dominant and securing all the food pellets. In the last pair the females displayed similar amounts of agonistic and hoarding behavior, so we were unable to derive relative social rank between these individuals (IN = indeterminate).

3.2. Reproductive success

Of the 33 mated females, 21 delivered a litter, and a total of 194 live pups was counted on Day 4 after parturition (Table 1). The number of delivered litters was not affected by group-housing ($\chi^2=0.589$, 1df, $p=0.35$). However, dominant females delivered more litters than subordinate females ($\chi^2=3.810$, 1df, $p=0.05$). The number of pups per litter on Day 4 after delivery (ISOL x PH, *t*-test, $t=0.654$, 19df, $p=0.52$; ISOL x DOM x SUB, ANOVA, $F=1.371$, 2df, $p=0.28$), the average sex-ratio in each group (ISOL x PH, *t*-test, $t=0.816$, 19df, $p=0.42$; ISOL x DOM x SUB, ANOVA, $F=0.710$, 2df, $p=0.51$), and the mean weight of pups on Day 4 post-partum (ISOL x PH, *t*-test, $t=0.752$, 19df, $p=0.46$; ISOL x DOM x SUB, ANOVA, $F=0.437$, 2df, $p=0.65$) did not differ among groups.

3.3. Concentrations of fecal cortisol metabolites (FCM)

Concentrations of FCM varied between individuals and over time within a wide range (6 to 1060 ng/g feces). We observed a significant within-subject effect of time ($F=4.103$, $p<0.001$) and of the interaction [time × pregnancy] ($F=3.309$, $p=0.001$) on FCM concentrations. FCM were also affected by pregnancy ($F=1079.8$, $p<0.001$) as between-subject factor. Neither housing ($F=1.128$, $p=0.30$), nor social rank ($F=0.693$ $p=0.51$) had a significant effect on FCM in both pregnant and non-pregnant females (housing: $F=1.021$, $p=0.34$). In pair-housed female concentrations of FCM were not affected by pair formation (113 ± 47 ng/g feces on the day of pair formation and 123 ± 62 ng/g feces one day after; paired samples *t*-test $t=0.698$, 21df, $p=0.49$). Among pair-housed but not singly-housed females,

Table 2
FCM concentrations (mean ± sem; ng/g feces) before and after pair formation and mating.

	Isolated	Pair-housed	Dominant	Subordinate	Indeterminate
n	11	22	10	10	2
Baseline	135 ± 10^{a}	151 ± 9^{a}	139 ± 10^{a}	149 ± 10^{a}	217 ± 10
Day of pair formation	75 ± 9^{b}	113 ± 10^{b}	104 ± 8^{b}	118 ± 20	132 ± 35
Day after pair formation	83 ± 11^{b}	123 ± 13^{b}	105 ± 10	137 ± 27	146 ± 1
Day of mating (D0)	119 ± 18	$108 \pm 11^{b,c}$	109 ± 22	112 ± 12^{b}	87 ± 11
Day after of mating (D1)	116 ± 21	215 ± 40^{d}	193 ± 28	263 ± 83	148 ± 26

[a, b,c, d] Different letters in the same column indicated significant difference (*t*-test for paired samples, $p<0.05$).

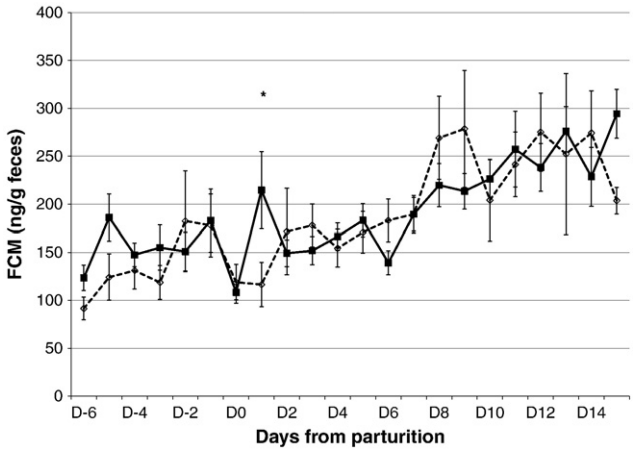

Fig. 1. Fecal cortisol metabolites (FCM; mean ± sem) in pair-housed (■) and isolated (··◊··) females hamster from Day 6 (D-6) before mating (D 0) to one day before parturition (D 15). Asterisk indicates significantly ($p<0.05$) different concentrations between the groups.

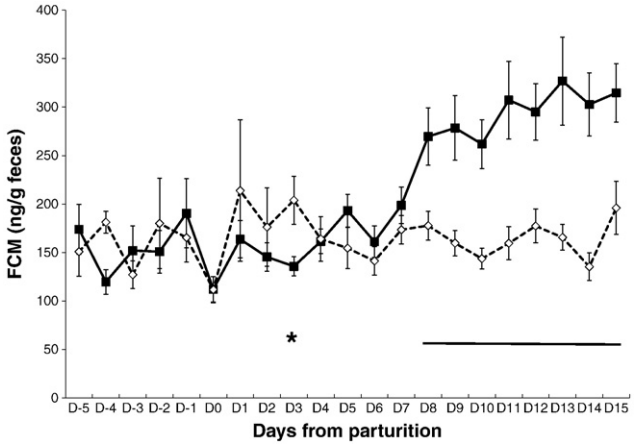

Fig. 2. Fecal cortisol metabolites (FCM; mean ± sem) in pregnant (■) and non-pregnant (··◊··) females hamster from Day 5 (D-5) before mating (D 0) to one day before parturition (D 15). Asterisk and black bar indicate significantly ($p<0.05$) different concentrations between the groups.

FCM increased significantly as a result of mating (Table 2, Fig. 1). For unclear reasons one subordinate female showed a very high FCM concentration (984 ng/g feces) on the day after mating. This female did not give birth to any pup.

On Days 1 to 3 after mating FCM concentrations were higher in non-pregnant females than in pregnant ones (though statistically significant only Day 3: 204 ± 86 ng/g feces versus 135 ± 46 ng/g feces; *t*-test for independent samples $t=2.556$, $p=0.02$). However, from Day 8 after mating until parturition, pregnant females had increased FCM levels, which were significantly higher than those in non-pregnant animals whose levels remained baseline (*t*-test for independent samples $p<0.05$; Fig. 2).

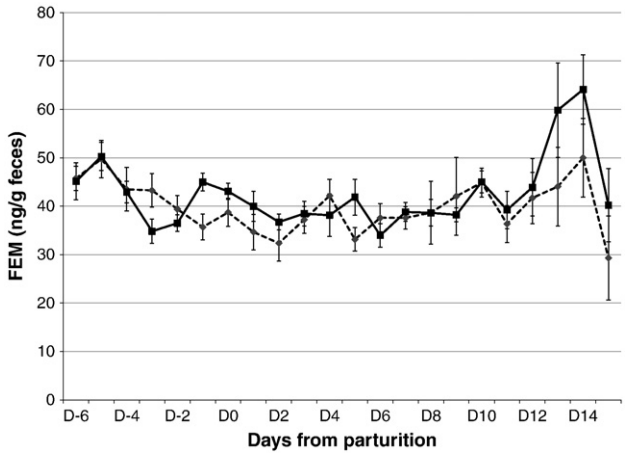

Fig. 3. Fecal estradiol metabolites (FEM; mean ± sem) in pair-housed (■) and isolated (··◊··) females hamster from Day 6 (D-6) before mating (D 0) to one day before parturition (D 15).

3.4. Concentrations of fecal estradiol metabolites (FEM)

Concentrations of FEM varied from 4.6 ng/g feces to 238.8 ng/g feces. The rmGLM analysis showed significant effects of time ($F=5.481$, $p<0.001$) and of the interaction [time × pregnancy] as within-subject factors as well as of pregnancy as a between-subject factor ($F=6.071$, $p=0.02$). Only among pregnant female concentrations of FEM were affected by housing but not by social ranking as between subject factors (housing: $F=7.588$, $p=0.014$; social rank: $F=0.194$, $p=0.67$) (Table 3). More precisely, it was only on the day before mating (proestrus) that FEM concentrations differed between groups, with pair-housed females having higher levels than isolated females (46.1 ± 79.2 ng/g feces and 35.7 ± 88.6 ng/g feces respectively; *t*-test for independent samples $t=3.368$, $p=0.002$; Fig. 3). FEM concentrations of pregnant females increased from Day 13 of pregnancy, reaching significantly higher concentrations on Day 14 compared to non-pregnant females (75.9 ± 26.1 ng/g feces versus 38.0 ± 16.6 ng/g feces, respectively; *t*-test for independent samples $t=4.146$, $p<0.001$). They decreased again on Day 15, 1 day before parturition (Fig. 4).

3.5. Concentrations of fecal progesterone metabolites (FPM)

FPM concentrations varied on a 100-fold range, from 0.10 μg/g feces to 1.605 μg/g feces. There was a significant effect of time ($F=6.926$, $p<0.001$) and of the interaction time × pregnancy ($F=2.834$, $p=0.005$) as within-subject factors and of pregnancy as a between subject factor ($F=28{,}524$, $p<0.001$) on FPM levels. Fig. 6 shows that FPM increased progressively in pregnant females after mating and were significantly higher in comparison with non pregnant females from Day 7 to Day 15 (*t*-test for independent samples, $p<0.05$). Among pregnant females, FPM were affected by housing ($F=9.371$, $p=0.007$) but not by social rank ($F=0.387$, $p=0.55$) as between subject factors. Specially, pair-housed females

Table 3
FEM concentrations (mean ± sem; ng/g feces) before and after pair formation and mating.

	Isolated	Pair-housed	Dominant	Subordinate	Indeterminate
n	11	22	10	10	2
Baseline	$45.8^{a} \pm 1.0^{a}$	42.6 ± 1.2	42.9 ± 1.6	42.7 ± 2.1	40.5 ± 3.3
Day of pair formation	$40.4^{b} \pm 1.6$	46.3 ± 2.9	49.5 ± 5.4	43.8 ± 3.3	42.5 ± 2.7
Day after pair formation	$38.8^{b} \pm 2.7$	$37.6^{b} \pm 2.2$	38.4 ± 1.8	39.7 ± 3.5	22.5 ± 13.7
Day of mating (D0)	$38.7^{b} \pm 2.9$	43.1 ± 1.7	42.9 ± 2.5	42.8 ± 2.8	44.9 ± 1.8
Day after of mating (D1)	$34.6^{b} \pm 3.7$	39.9 ± 3.1	40.7 ± 5.5	41.1 ± 4.2	30.5 ± 5.3

[a, b] Different letters in the same column indicated significant difference (*t*-test for paired samples, $p<0.05$).

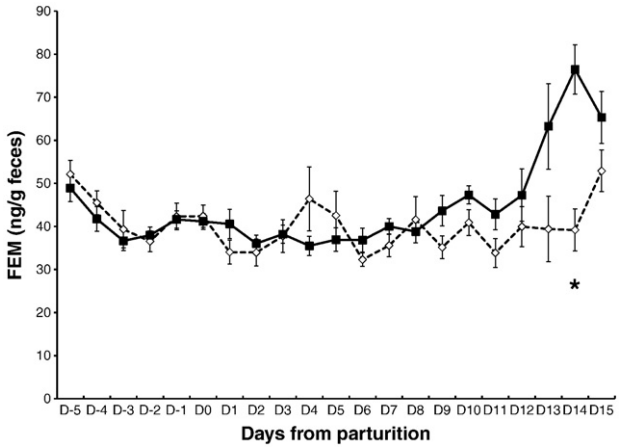

Fig. 4. Variation of fecal estrogen metabolites (FEM; mean ± sem) in pregnant (■) and non-pregnant (··◊··) females hamster from Day 5 (D-5) before mating (D 0) to one day before parturition (D 15). Asterisk indicates a significantly ($p<0.001$) different concentration between the groups.

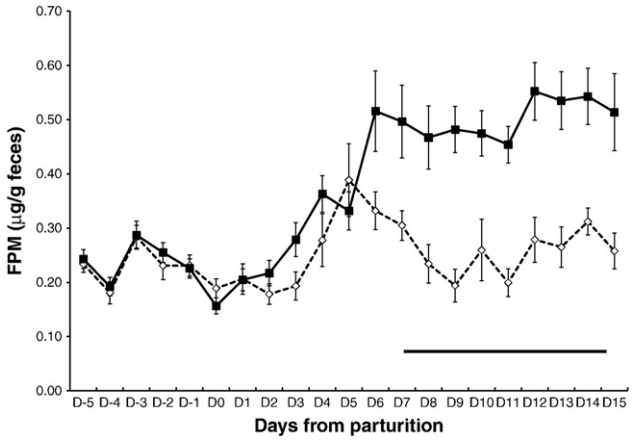

Fig. 6. – Fecal progesterone metabolites (FPM; mean ± sem) in pregnant (■) and non-pregnant (··◊··) females hamster from Day 5 (D-5) before mating (D 0) to one day before parturition (D 15). Black bar indicates significantly ($p<0.05$) different concentrations between the groups.

showed higher FPM levels than isolated ones on the day of pair formation (Table 4), and on Day 6 (0.52 ± 0.32 µg/g feces and 0.30 ± 0.12 µg/g feces, respectively, *t*-test for independent samples, $t = 2.247$, $p = 0.039$) and Day 11 of pregnancy (0.50 ± 0.11 µg/g feces and 0.33 ± 0.16 µg/g feces, respectively, *t*-test for independent samples, $t = 2.696$, $p = 0.015$, Fig. 5). The same did not occur among non-pregnant females (housing: $F = 1.295$, $p = 0.29$; social rank: $F = 2.248$, $p = 0.19$). On Day 3 after mating FPM concentrations were negatively correlated with FCM concentrations (Pearson coefficient $r = -0.346$, $p = 0.048$).

4. Discussion

Our data confirm that adult hamsters are able to interact socially with other adults of the same sex and to establish social hierarchies when kept in groups. This conclusion is consistent with findings regarding wolverines, another solitary species studied by Dalerum et al. [4]. We also show that social environment may create a reproductive skew, which is another feature of group-living species. We provide evidence that, although glucocorticoids participate in the mechanism for reproductive failure, increased glucocorticoid concentrations due to the social stress are not the main proximal cause of the lower reproductive success of subordinate females.

The changes of steroid hormones in the blood during the pregnancy of the Syrian hamster were described more than 20 years ago [26,27]. To our knowledge this is the first study on this species monitoring patterns of estradiol, progesterone, and glucocorticoid concentrations through gestation by quantifying their fecal metabolites. Our results are similar to blood profiles reported in the literature, with the same timing of changes but less expressed which may result from the method used. Unlike blood levels fecal hormone metabolites reflect the cumulative secretion and excretion of hormones over long time periods, and fluctuations in the blood are smoothed in the feces [28]. These results further confirm the validity of non-invasive methods to assess the endocrine status.

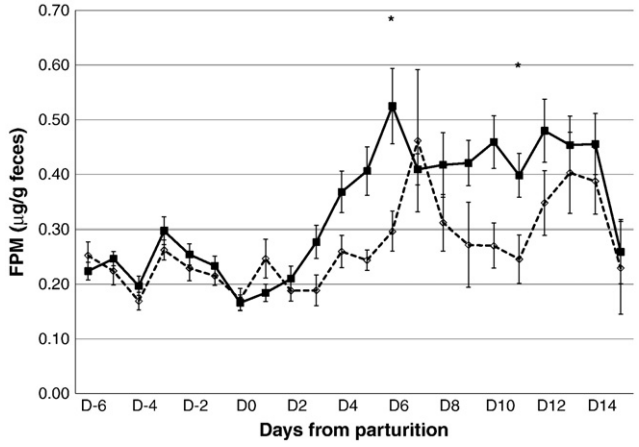

Fig. 5. Fecal progesterone metabolites (FPM; mean ± sem) in pair-housed (■) and isolated (··◊··) females hamster from Day 6 (D-6) before mating (D 0) to one day before parturition (D 15). Asterisk indicates significantly ($p<0.05$) different concentrations between the groups.

Overall, of the 33 females that we mated, only 21 (63.6%) gave birth. The 90% reproductive success of dominant females was similar to the percentage usually reached in our animal facility [29]. In contrast, only 50% of the subordinate females delivered live pups. This result is in accordance with data of previous studies [12,13]. However, in contrast to these authors, we did not find any significant difference between pair-housed and isolated females regarding the number of pups per litter, the litter sex-ratio, or the mean weight of the pups. Curiously, the index of breeding success (54%) of isolated females was very low when compared to the percentage (more than 90%) usually reached in our animal facility. Therefore, it seems that reproductive failure was not related to housing and was an all-or-nothing issue. Besides, the high breeding success (90%) of dominant females does not support the hypothesis that the handling involved in fecal sampling might have disturbed the females and caused reproductive failure.

Our results showed that concentrations of FCM did not change as a result of pair formation despite the agonistic behavior observed in most pairs. As previously explained, it is possible that the signal of a short rise in circulating glucocorticoids had been dampened in the feces, or that we missed it as a result of our collection schedule. However, FCM concentrations remained similar over time in isolated, dominant and subordinate females suggesting that cohabitation did not result in chronic stress. This result is inconsistent with the finding of Jian-Xu Zhang et al. [30] of a significant increase in cortisol baseline as a result of cohabitation. It is worth noting that although agonistic behavior was relatively intense in most pairs on the first encounter of the females, we observed few attacks (one attack in six of nine pairs in which social rank was clear) and almost no submissive or defensive behaviors on the subsequent encounters. The low level of submissive behavior displayed by subordinate females may be related with the higher concentrations of estradiol in pair-housed females on the day before mating [17] and is consistent with the solitary habit of female hamsters in the wild. Submission may have a very high cost for a female: besides severe wounding the female could lose its food hoard,

Table 4
FPM concentrations (mean ± sem; μg/g feces) before and after pair formation and mating.

	Isolated	Pair-housed	Dominant	Subordinate	Indeterminate
n	11	22	10	10	2
Baseline	0.25 ± 0.02^{a}	0.23 ± 0.004^{a}	0.23 ± 0.01^{a}	0.23 ± 0.01^{a}	0.24 ± 0.01
Day of pair formation	0.24 ± 0.01^{a}	0.31 ± 0.01^{b}	0.31 ± 0.02^{b}	0.31 ± 0.02^{b}	0.27 ± 0.01
Day after pair formation	0.25 ± 0.02^{a}	0.28 ± 0.01^{b}	0.30 ± 0.02^{b}	0.27 ± 0.02^{b}	0.24 ± 0.06
Day of mating (D0)	0.17 ± 0.02^{b}	0.17 ± 0.01^{c}	0.17 ± 0.02	0.17 ± 0.02^{c}	0.13 ± 0.01
Day after of mating (D1)	0.25 ± 0.04^{a}	0.18 ± 0.01^{c}	0.18 ± 0.02^{c}	0.20 ± 0.02^{c}	0.23 ± 0.05

$^{(a, b, c)}$ Different letters in the same column indicated significant difference (t-test for paired samples, $p<0.05$).

its litter, and even its burrow. Male hamsters have less to lose in displaying submissive behavior instead of fighting with a bigger or more aggressive male. In six of nine female pairs in our study, the only dominant behavior displayed on the third encounter was securing more food-pellets. The importance of hoarding behavior for the successful reproduction of female hamsters was demonstrated by Huck et al. [13]. In conditions of food restriction, dominant females removed food from the subordinate females, and reproduced more successfully even compared to singly-housed females.

In contrast with the attenuation by estrogens of the glucocorticoid responses to stress observed in sheep [31], and despite their higher FEM levels on the precedent day, mating induced a significant FCM increase in pair-housed females, but not in singly-housed females. No relationship between FCM levels on D0 or D1 and reproductive success was found. Independently of the group, all females were in estrus and receptive to male on the mating day, and this encounter with an unknown male did not seem stressful for singly-housed females. It is possible that the previous cohabitation experience had sensibilized the females to social interaction and had enhanced their adrenal response to social stress.

As the glucocorticoid profile was similar in our three groups before mating, we could not conclude that differences in adrenocortical activity and thus stress level was responsible for reproductive failure. The sole indicator of a role for glucocorticoids in reproductive failure was a higher FCM concentration on Days 1 to 3 (only significant on Day 3) after mating in females that did not gave birth, associated with a negative correlation between FCM and FPM on those days. Nepomnaschy et al. [32] reported that, in women, pregnancies characterized by increased maternal cortisol during the first 3 weeks after conception (placentation period) were more likely to result in spontaneous abortion. After implantation the embryo signaling results in increasing progesterone levels and decreased risk of miscarriage. As embryo implantation occurs in hamsters on Day 4 of pregnancy, it is likely that the high FCM on Day 3 were related to reproductive failure. The increased production of cortisol may indicate a stressful environment and may have signified poor reproductive conditions in which the cost of reproductive failure would be lower than the cost of a pregnancy with diminished chances of success. However, we cannot discard the possibility that the increase in cortisol concentration was a consequence and not the cause of pregnancy loss.

Pair-housing related changes in progesterone levels were also reported by several authors, but in an inconsistent way. Whereas Pratt and Lisk [11] reported a significant reduction in circulating progesterone concentrations in female hamsters exposed to social subordination early in pregnancy, Jian-Xu Zhang et al. [30] measured similar progesterone concentrations in singly and pair-housed females and Fritzsche et al. [16] found higher levels of progesterone in both dominant and subordinate group-housed cycling females when compared with individually-housed females. Our data support the findings of the latter with higher FPM levels in pair-housed pregnant females in the second half of pregnancy. Again, the physiological meaning of this difference is not clear. The percentage of reproductive failure was similar in subordinate (pair-housed) and isolated females. Moreover, at this time of pregnancy most of the circulating progesterone is produced by the placenta [26]. Therefore, the higher concentration in pair-housed females is not likely to result from a higher number of corpora lutea and does not seem associated with higher fertility.

Although reproduction was not monopolized by dominant females, social environment seems to have determined reproductive failure in subordinate ones. However, the percentage of reproductive failure was also very high among the singly housed females of our control group. The conventional explanation that high glucocorticoid levels related with subordination stress may constitute the proximal mechanism of reproductive failure in subordinate females does not fit our results as we did not find social-rank nor housing-related differences in FCM concentrations. This is perhaps the most intriguing point of our results. All females were normally cycling and all of them were mated with proven breeders. Thus, all of them were theoretically capable of becoming pregnant. Isolated females had the same possibility of hoarding than dominant ones and did not suffer any kind of aggression or intimidation. Moreover, the few differences between the endocrine profiles of singly and pair-housed females do not provide adequate ground for such a difference in breeding success.

Hamsters in our animal facility are kept in groups of five animals of the same sex from weaning and so were our females when we received them. When adult, two or three females from the same group are mated with an unfamiliar male and remain together until a few days before parturition. Unlike this normal mating procedure, our control animals were isolated from the beginning of the experiment, approximately 2 weeks before mating. The anxiogenicity of social isolation had been previously reported in laboratory rodents and primates [33], but not in the hamster. In young rats social isolation induces behavioral, morphological and neurochemical abnormalities [34]. Syrian hamsters are exclusively solitary in the wild as confirmed by Gatermann et al. [9] in Syria, who never found more than one adult per burrow. Moreover, the closest observed distance between occupied hamster burrows was 118 m. Therefore, we hypothesized that cohabitation would be stressful and isolation would not. However, our results suggest that social isolation was as disturbing for animals used to group-living as cohabitation with an unknown conspecific. Further studies involving different rearing conditions will confirm that, as in other rodents [6], sociability is flexible in the Syrian hamster.

There are some limitations in this study leaving us to interpret the data cautiously. As cannibalism is frequent among hamster dams when they are disturbed during the first days after delivery, pups were only counted on Day 4 after birth. Starting at the day before the expected parturition, females were daily and discreetly monitored and deliveries, indicated by the presence of pups or blood stains on the bedding, were registered. In our experience, cannibalism of the whole litter on the first hours after birth is relatively rare so we have a reasonable certainty of the number of delivered litters. However the number of born pups may be higher than counted on Day 4 post-partum and result from differential cannibalism among groups. In fact, stress may result in cannibalism [26] and the more stressed dams may have killed more pups than did unstressed females. Although not significantly different, the mean numbers of pups per litter do not support this assumption. In contrast, they suggest that subordinate females delivered more pups (11.4 ± 1.1) than dominant dams (9.0 ± 3.6) or that more pups were killed by dominant mothers supposed to be less stressed. Actually, even though hamsters can deliver up to 16 pups in one litter, our data are in

the range of litter sizes observed in previous studies [11,12,29,30] and cannot suggest a strong effect of cannibalism.

In conclusion, we found evidence for some features typically associated with solitary species, but also some others found in group-living species. It is likely that captivity and group-housing until adult age wake up latent physiological and behavioral mechanisms of social interaction. Our findings support the idea that social interactions may be a function of ecological conditions.

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References

[1] Sousa MBC, Albuquerque ACSR, Albuquerque FS, Araújo A, Yamamoto ME, Arruda MF. Behavioral strategies and hormonal profile of dominant and subordinate common marmoset (*Callithrix jacchus*) females in wild monogamous groups. Am J Primatol 2005;67:37–50.
[2] Clarke FM, Faulkes CG. Hormonal and behavioural correlates of male dominance and reproductive status in captive colonies of the naked mole-rat, *Heterocephalus glaber*. Proc R Soc Lond B 1998;265:1391–9.
[3] Creel S. Social dominance and stress hormones. Trends Ecol Evol 2001;16:491–7.
[4] Dalerum F, Creel S, Hall SB. Behavioral and endocrine correlates of reproductive failure in social aggregations of captive wolverines (*Gulo gulo*). J Zool 2006;269: 527–36.
[5] Silva RB, Vieira EM, Izar P. Social monogamy and biparental care of the neotropical southern bamboo rat (*Kannabateomys amblyonyx*). J Mammal 2008;89:1464–72.
[6] Schradin C, Pillay N. Intraspecific variation in the spatial and social organization of the African striped mouse. J Mammal 2005;86:99–107.
[7] Sachser N. Of domestic and wild guinea pigs: studies in sociophysiology, domestication, and social evolution. Naturwissenschaften 1998;85:307–17.
[8] Kokko H. Are reproductive skew models evolutionarily stable? Proc R Soc London, Ser B 2003;270:265–70.
[9] Gattermann R, Fritzsche P, Neumann K, Al-Hussein I, Kayser A, Abiad M, et al. Notes on the current distribution and the ecology of wild golden hamsters (*Mesocricetus auratus*). J Zool 2001;254:359–65.
[10] Gattermann R, Weinandy R. Time of day and stress response to different stressors in experimental animals. Part I: golden hamster (*Mesocricetus auratus* Waterhouse, 1839). J Exp Anim Sci 1996–97;38:66–76.
[11] Pratt NC, Lisk RD. Effects of social stress during early pregnancy on litter size and sex ratio in the golden hamster (*Mesocricetus auratus*). J Reprod Fertil 1989;87: 763–9.
[12] Pratt NC, Lisk RD. Role of progesterone in mediating stress-related litter deficits in the golden hamster (*Mesocricetus auratus*). J Reprod Fertil 1991;92:139–46.
[13] Huck UW, Lisk RD, Miller KS, Bethel A. Progesterone levels and socially-induced implantation failure and fetal resorption in golden hamsters (*Mesocricetus auratus*). Physiol Behav 1988;44:321–6.
[14] Huhman KL, Solomon MB, Janicki M, Harmon AC, Lin SM, Israel JE, et al. Conditioned defeat in male and female Syrian hamsters. Horm Behav 2003;44: 293–9.
[15] Taravosh-Lahn K, Delville Y. Aggressive behavior in female golden hamsters: development and the effect of repeated stress. Horm Behav 2004;46:428–35.
[16] Fritzsche P, Riek M, Gattermann R. Effects of social stress on behavior and corpus luteum in female golden hamsters (*Mesocricetus auratus*). Physiol Behav 2000;68: 625–30.
[17] Faruzzi AN, Solomom MB, Demas GE, Huhman KL. Gonadal hormones modulate the display of submissive behavior in socially defeated female Syrian hamsters. Horm Behav 2005;47:569–75.
[18] Chelini MOM, Souza NL, Rocha M, Felippe ECG, Oliveira CA. Quantification of fecal estradiol and progesterone metabolites in Syrian hamster (*Mesocricetus auratus*). Braz J Med Biol Res 2005;38:1711–7.
[19] Chelini MOM, Otta E, Yamakita C, Palme R. Sex differences in the excretion of fecal glucocorticoid metabolites in the Syrian hamster (*Mesocricetus auratus*). J Comp Physiol B 2010;180:919–25.
[20] FELASA. Recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit breeding colonies. Lab Anim 2002;36:20–42.
[21] Solomon MB, Karom MC, Huhman KL. Sex and estrous cycle differences in the display of conditioned defeat in Syrian hamsters. Horm Behav 2007;52:211–9.
[22] Blanchard RJ, McKittrick CR, Blanchard DC. Animal models of social stress: effects on behavior and neurochemical systems. Physiol Behav 2001;73:261–71.
[23] Da Silva VA, Smart JL, Freire EM, Paumgarten FJR. Neurobehavioral development of the golden hamster. Neurotoxicol Teratol 1989;11:105–14.
[24] Palme R. Measuring fecal steroids: guidelines for practical application. Ann NY Acad Sci 2005;1046:75–80.
[25] Möstl E, Maggs JL, Schrötter G, Besenfelder U, Palme R. Measurement of cortisol metabolites in faeces of ruminants. Vet Res Commun 2002;26:127–39.
[26] Siegel H. The hamster. Reproduction and behavior. New York: Plenum Press; 1985.
[27] Soares MJ, Talamantes F. Placental and serum hormone changes during the second half of pregnancy in the hamster. Biol Reprod 1982;27:523–9.
[28] Touma C, Palme R. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of a biological validation. Ann NY Acad Sci 2005;1046: 54–74.
[29] Chelini MOM, Souza NL, Otta E. Breeding system can affect the offspring sex ratio in Syrian hamsters (*Mesocricetus auratus*). Lab Anim 2011;45:55–7.
[30] Zhang Jian-Xu, Rao Xiao-Ping, Sun Lixing, Wang Da-Wei, Liu Dingzhen, Zhao Chenghua. Cohabitation impaired physiology, fitness and sex-related chemosignals in golden hamsters. Physiol Behav 2008;93:1071–7.
[31] Komesaroff PA, Esler M, Clarke IJ, Fullerton MJ, Funder JW. Effects of estrogen and estrous cycle on glucocorticoid and catecholamine responses to stress in sheep. J Clin Endocrinol Metab 1998;84:4292–3.
[32] Nepomnaschy PA, Welch KB, McConnell DS, Low BS, Strassmann BI, England BG. Cortisol levels and very early pregnancy loss in humans. PNAS 2006;103:3938–42.
[33] Olssona IAS, Westlundb K. More than numbers matter: the effect of social factors on behaviour and welfare of laboratory rodents and non-human primates. Appl Anim Behav Sci 2007;103:229–54.
[34] Kone KC, Porkess MV. Behavioural and neurochemical effects of post-weaning social isolation in rodents-Relevance to developmental neuropsychiatric disorders. Neurosci Biobehav Rev 2008;32:1087–102.