J Comp Physiol B (2011) 181:1089–1100
DOI 10.1007/s00360-011-0591-1

ORIGINAL PAPER

Chronic stress in pregnant guinea pigs (*Cavia aperea f. porcellus*) attenuates long-term stress hormone levels and body weight gain, but not reproductive output

Hanna Schöpper · Rupert Palme · Thomas Ruf · Susanne Huber

Received: 15 March 2011 / Revised: 11 May 2011 / Accepted: 16 May 2011 / Published online: 7 June 2011

Abstract Stress, when extreme or chronic, can have a negative impact on health and survival of mammals. This is especially true for females during reproduction when self-maintenance and investment in offspring simultaneously challenge energy turnover. Therefore, we investigated the effects of repeated stress during early- and mid-gestation on the maternal stress axis, body weight gain and reproductive output. Female guinea pigs (*Cavia aperea f. porcellus, n* = 14) were either stressed (treatment: exposure to strobe light in an unfamiliar environment on gestational day -7, 0, 7, 14, 21, 28, 35, 42) or left completely undisturbed (control) throughout pregnancy. Females of both groups received the same respective diets, and reproductive parameters were evaluated upon parturition. Additionally, hormonal data were obtained from blood and feces. The stress exposure induced a significant increase in plasma cortisol concentrations during the afternoon. In contrast to this short-term response in plasma cortisol concentrations, we found no significant differences in the levels of cortisol metabolites in feces collected after stress exposure between groups and even significantly decreased levels of fecal cortisol metabolites on non-stress days over time in treatment females. Among treatment females, gain in body weight was attenuated over gestation and body weight was lower compared to control females during lactation, especially in cases of large litter sizes. No differences could be seen in the reproductive parameters. We conclude that repeated stress exposure with strobe light during early- and mid-gestation results in a down-regulation of the hypothalamic–pituitary–adrenal axis and lower weight gain in treatment females, but has no effect on reproductive output.

Keywords Body mass · Cortisol · Gestation · Guinea pig (*Cavia aperea f. porcellus*) · Hypothalamic–pituitary–adrenal (HPA) axis · Reproduction

Abbreviations

ACTH	Adrenocorticotropic hormone
BMR	Basal metabolic rate
EIA	Enzyme immunoassay
FCM	Fecal cortisol metabolites
GD	Gestational day
HPA axis	Hypothalamus–pituitary–adrenal axis
L	Lactational day
SEM	Standard error of the mean

Introduction

Animals and humans face challenging situations during their lifetime. Prominent examples for animals are harsh weather conditions, low food availability, high population, and predator density, while for humans heavy workload may exemplify a stressful situation.

Communicated by G. Heldmaier.

H. Schöpper (✉) · T. Ruf · S. Huber
Department of Integrative Biology and Evolution, Research Institute of Wildlife Ecology, University of Veterinary Medicine, Savoyenstrasse 1, 1160 Vienna, Austria
e-mail: Hanna.Schoepper@fiwi.at

R. Palme
Department of Biomedical Sciences, Institute of Biochemistry, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria

S. Huber
Department of Anthropology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

Independent of their nature, all these challenges lead to comparable stress reactions providing the individual with the physical prerequisites to cope and finally overcome the demanding situation.

When stressful events occur during gestation, however, maternal stress physiology can influence the fetus and "program" its development (for review see de Weerth and Buitelaar 2005). This prenatal programming can have long-term consequences on the offspring's survival, health and reproduction (Ross and Desai 2005; Vehaskari 2010; Rhind et al. 2001). As a potential underlying mechanism, an increase of glucocorticoids has been suggested to transfer external cues from the environment via the placenta to the otherwise well-protected fetus (for review see Seckl 2004).

During the last few decades, many studies have been undertaken to identify the effects of maternal stress exposure on fetal development in a variety of species (e.g., fish: Alsop and Vijayan 2009; rats: Götz et al. 2008; pigs: Otten et al. 2010). In experimental studies, various physical and psychological stressors have been used (for review see Reynolds et al. 2010). In guinea pigs, for instance, strobe light exposure during pregnancy, resulting in an increase of plasma cortisol levels (Cadet et al. 1986), was shown to affect behavior, brain development and stress response of the offspring (Kapoor and Matthews 2005). Most of these studies were primarily concerned with the examination of short-term responses in pregnant animals and long-term effects in the offspring. Therefore, usually no further evaluation of maternal status during the remaining gestation period was conducted. Studies of the long-term effects of stress on mothers are limited (e.g., Meek et al. 2001; Patin et al. 2002; Léonhardt et al. 2007). As pregnancy is a time of high energy demand, however, the additional burden of stress might in the long run be detrimental both to the mother and offspring (Darnaudéry et al. 2004). This may be particularly true in precocial species because of the long gestation time and high degree of development at birth. Also when stress occurs during early pregnancy, the potential consequences for maternal physiology may persist for possibly quite a lengthy period.

We used a precocial species, the domestic guinea pig (*Cavia aperea f. porcellus*), to examine the effects of stress exposure to strobe light in an unfamiliar environment applied during early- to mid-gestation on stress hormone levels, body weight gain and reproductive performance of pregnant and lactating females. Guinea pigs may be particularly prone to the effects of stress exposure during pregnancy because of their long gestation period and high investment in the development of unborn offspring. Female guinea pigs gain as much as 60% of pre-pregnancy weight (Sparks et al. 1981) during a long gestation (duration of gestation $\sim$68 days) and give birth to three to four extremely precocial offspring. Strobe light has been shown to induce a cortisol increase in guinea pigs (Dauprat et al. 1984). We therefore predicted that glucocorticoid levels should be elevated after stress exposure. As we applied the stress exposure repeatedly, we further predicted that gain in body weight should be attenuated in treatment compared to control females. However, strobe light is a relatively mild stressor, so effects on reproductive performance were not necessarily expected.

Materials and methods

All husbandry and experimental procedures were approved by the institutional ethics committee and the Austrian Federal Ministry of Science and Research (GZ 68.205/0211-II/10b/2008).

Animals and housing conditions

Fourteen female guinea pigs (*Cavia aperea f. porcellus*) aged between 3 and 6 months were used for this study. Additionally, seven male guinea pigs within the same age range were used for breeding. Animals originated from a multicolored stock and could be identified clearly by individual differences in natural coat pattern. None of the females were pregnant or lactating at the onset of the study.

Animals were randomly assigned to the treatment (stress procedure: for detailed description see below) or control group. Body weight did not differ significantly between groups (mean ± SEM, ♀ treatment: 721.08 g ± 25.98 g, $n = 7$, ♀ control: 719.14 g ± 34.07 g, $n = 7$, Student's t test: $t_{10.67} = -0.05$, $p = 0.965$; ♂ treatment: 911.79 g ± 62.29 g, $n = 3$, ♂ control: 961.50 g ± 55.27 g, $n = 4$, Student's t test: $t_{4.52} = 0.60$, $p = 0.579$). Prior to the experiment, in a pilot study, we used a subsample of these females ($n = 10$) to determine the stress-inducing potential of a single strobe light exposure in non-pregnant females.

Conditions in the animal housing facility were unchanged throughout the experiment: 12:12-h light–dark cycle, (lights on at 07:00 hour), mean ambient temperature of 22.7°C (SEM 0.1°C) and mean relative humidity of 50.2% (SEM 0.7%).

Females were given at least 4 weeks for acclimatization in the housing facility while being kept as same-sex pairs of either treatment or control females. For breeding, a male was introduced into each cage between day -7 and day 0 (varying days resulted from housing of 1 male with 2 females, whose cycles were not synchronized pharmacologically and therefore varied between individuals but not between groups), so that stable social groups of three individuals were established and maintained throughout gestation. About 3 days prior to expected parturition, each female was transferred to a littering cage to prevent

postpartum pregnancies and disturbance by the other female. During this phase, all animals were housed individually, however, with visible and audible contact to their former cage mates. If short-term isolation had any influence on the animals, it would have affected both groups in equal measure. After weaning at an age of 21 days, offspring were housed in same-sex groups of two to four individuals and mothers were kept in the same pairs as at the start of the experiment.

A daily quantity of 15 g guinea pig standard feed (Altromin 3013, Altromin GmbH, Lage, Germany) was supplemented with 40 g of fresh fruit or vegetables and a handful of hay ($\sim$35 g) per individual. Animals were fed in the morning between 08:30 and 09:30 hour, and water was available ad libitum. During the last trimester of gestation as well as during lactation, females received an additional 20 g pellets to meet the increased energy demand. Offspring were offered 10 g of pelletized feed plus 10 g of fresh food until weaning.

Stress procedure

Treatment females were stressed via exposure to a high-frequency strobe light (Mini-Flash DK-011, China, distributed by Conrad Electronics, Austria) operated in an unfamiliar dark room. The apparatus was placed approximately 1 m above the animal, which was housed individually during exposure between 09:00 and 11:00 hour. Afterward, females were transferred back to their housing facility. In addition to strobe light, treatment females were therefore exposed to handling, isolation and an unfamiliar environment. Control females were left completely undisturbed in their home cages during the entire time.

During the pilot study, non-pregnant females ($n = 6$) were treated with a single strobe light exposure for 2 h between 09:00 and 11:00 hour to determine the stress-inducing potential of this procedure. Four undisturbed, non-pregnant females served as a control. Due to the limited number of animals in the respective range of age, we had to use the same animals for both, the pilot study and the experiment. To minimize the potential influences of previous stress experience, at least 2 weeks passed before the onset of the experiment. In addition, females used as treatment animals during the pilot study were distributed equally between the two groups of the experiment. During the experiment, treatment females were exposed to stress once per week during the first two-thirds of pregnancy, starting 1 week before conception. Guinea pigs show a distinct biphasic diurnal rhythm of glucocorticoids with lowest measurements in the morning and peaks at 16:00 hour and during night at 01:00 hour (Sachser 1994). Therefore, strobe light exposure was applied both in the morning and the afternoon to meet times of both low and high levels of cortisol. In detail, treatment females were exposed to strobe light from 09:00 to 11:00 hour and from 16:00 to 17:00 hour on gestational day (gd)-7 (1 week prior to conception), gd0 (conception), gd7, gd14, gd21, gd28, gd35 and gd42.

Measurements of physiological data

The stage of estrus cycle was determined by daily visual inspection of the vaginal membrane around 09:00 hour. We specified estrus as the first day of fully ruptured vaginal membrane, which according to Young (1937) correlates with physiological estrus. During the following 1 to 6 days, the vaginal membrane recovers and stays closed until the next estrus (Weir 1970). Thus, this process can be used as a marker for the stage of cycle, with a normal cycle length of 16 days (Stockard and Papanicolaou 1917). Day 9 after the preceding estrus was therefore assigned day -7 before estrus/conception. In reality, duration of cycles varied, so that first stress application of treatment females ranged from 9 to 2 days before conception (referred to as gd-7).

Conception (gd0) was ascertained when mating or a copulatory plug was observed. The number of days between conception and birth was recorded as duration of gestation.

All but one parturition were directly observed and we counted, sexed and weighed the offspring to the nearest 0.01 g immediately after birth. The pups born unobserved were detected within 2 h of delivery, as indicated by fresh umbilical cords and the coat being still wet, and immediately body weight was recorded and included in the analysis of birth weight. When delivery of placentae was observed, organs were freed from adjacent amnion and weighed to the nearest 0.01 g within 5 min.

The body weights of the females were recorded to the nearest 0.5 g before feeding at 09:00 hour on a weekly basis during gestation and every day during the lactation period. Offspring were also weighed on a daily basis.

Blood sampling and endocrine analysis

Blood samples were taken to determine the effects of stress exposure on plasma cortisol levels. In the pilot study, blood samples were taken from non-pregnant females prior to stress exposure at 09:00 hour, directly after termination of strobe light exposure at 11:00 and 2 h after their return to the home environment at 13:00 hour. To control for the effects of the blood sampling procedure, blood was also obtained from otherwise undisturbed non-pregnant control females at the same time points.

During the experiment, strobe light was applied to treatment females on gd 14 and gd 35, and blood samples were taken immediately after stress exposure at 11:00 and

17:00 hour. To obtain the basal levels, we collected blood samples at the same times 1 day prior to stress exposure (i.e., gd13 and gd34) to avoid influences of diurnal variation and minimize burden to the pregnant females during the day of stress. We did not take any blood samples from control females during the experiment.

Collection of blood (100 µl) was performed by punctuating the marginal ear veins with a sterile lancet without anesthesia following the protocol described by Sachser and Pröve (1984). The whole procedure took less than 3 min per female, including removing the animal from the cage and returning it afterward. Samples were collected with a heparinized capillary tubes, centrifuged and the plasma stored at −20°C until further analysis.

For the analysis of blood samples, we extracted plasma (diluted in assay buffer to a total of 500 µl) with 5 ml of diethyl ether. Cortisol, the principal glucocorticoid in guinea pigs (Malinowska and Nathanielsz 1974), was measured with an enzyme immunoassay (EIA) and all samples were run in duplicate. Details of the EIA, including cross-reactions of the antibody, are given by Palme and Möstl (1997). The intra- and interassay coefficients of variation were 8.9 and 11.1%, respectively.

Feces collection and endocrine analysis

During the experiment, we collected feces from both, treatment and control females. For this purpose, females were briefly (usually <5 min) separated from their partners between 09:00 and 10:00 hour four times a week and transferred to a cage with fresh bedding of chipped wood. After defecation, each female was returned to its home cage and feces were collected from the bedding. Measuring fecal samples takes into account the time lag between hormone secretion and excretion in the feces of between 14 and 20 h (Bauer et al. 2008). So, fecal samples were collected from treatment females in the morning preceding stress exposure, thus reflecting a non-stress situation, and in the morning of the following day, thus reflecting stress exposure during the afternoon of the previous day. After sample collection, feces were immediately frozen and stored at −20°C until further analysis. Urine-contaminated feces were not used in the analysis. One sample per week and all samples collected before and after stress treatment were processed for the analysis of fecal cortisol metabolites (FCM).

Extraction of fecal samples followed the procedure described by Palme and Möstl (1997) with slight modifications. Samples were dried at 85°C for 2 h and homogenized. A total of 0.1 g dried feces was suspended in 1.8 ml of 80% methanol, vortexed for 30 min and centrifuged at 2,500*g* for 15 min. An aliquot of the supernatant was diluted (1:10) with assay buffer and stored at −20°C until further analysis. We used a group-specific 11-oxoetiocholanolone-EIA measuring FCM with a 3α-OH-11-one structure (Möstl et al. 2002), which was previously successfully validated for measuring adrenocortical activity in guinea pigs (Bauer et al. 2008). The intra- and interassay coefficient of variation were 9.5 and 11.3%, respectively.

Statistical analysis

We used R 2.9.1 (R Developmental Core Team 2007) for statistical analysis. To analyze body weight and concentrations of plasma cortisol and FCM, we used linear mixed effects models (LME: function lme in package nlme; Pinheiro et al. 2007) to adjust for repeated measurements. Group (treatment versus control) and time (used as a second-order polynomial when the quadratic term was significant and as factor in the pilot study) were entered as fixed factors and different intercepts per animal as random factor. Litter size was included as covariate in the analysis of body weight. Analysis of plasma cortisol and FCM levels before and after stress exposure in treatment females was also analyzed by a LME model, with stress exposure and time as fixed factors and animal as random factor. Response variables were log-transformed for the analyses of hormonal levels to ensure that residuals were normally distributed. The results of these models are presented as F values with degrees of freedom and corresponding p value. Note that the numerator degrees of freedom of 2 for the time effect indicate the use of a quadratic polynomial. Comparison of plasma cortisol values between different stages of gestation were done using paired Student's t test; comparison of FCM at gd-8 and at conception and body weight at conception were done using unpaired Student's t test.

Depending on the distribution of the data, we compared the reproductive parameters using Mann–Whitney U tests and Fisher exact test. Comparison of birth weight was performed with Student's t test. Gestational effort was calculated as follows: total litter weight (g)/maternal body weight at conception (g) × 100 (Laurien-Kehnen and Trillmich 2004). Observed differences were considered significant at p values < 0.05.

Results

Reproductive parameters

All 14 females became pregnant. None of the measured parameters (i.e., duration of gestation, sex ratio, litter size, birth weight, total litter weight, total placental weight and gestational effort) was significantly different between treatment and control group (Table 1).

Table 1 Statistical analysis of reproductive parameters of female guinea pigs

Reproductive parameters	Treatment	Control	*n*	W/*t*	*p*
Duration of gestation[a] (days)	69 (67–69)	68 (66–69)	7/7	21	0.679
Litter size[a]	3 (1–4)	4 (2–5)	7/7	29.5	0.540
Total litter weight[a] (g)	372 (123–387)	397 (266–407)	7/7	34	0.259
Total placental weight[a] (g)	15.8 (5.7–17.3)	15.4 (11.2–18.0)	6/7	19.5	0.886
Gestational effort[a]	48.6 (15.7–61.2)	51.9 (42.5–62.9)	6/7	26	0.710
Sex ratio	♂13:♀9	♂13:♀12			0.770
Birth weight[b] (g)	105.4 ± 3.5	103.5 ± 3.8	22/25	−0.364	0.718

[a] Data given as median (range)

[b] Data given as mean ± SEM

Body weight

The mean body weight did not differ significantly between the treatment and control females on the day of conception (mean ± SEM in treatment: 734.29 g ± 29.11 g and control 719.21 g ± 31.41 g, $n = 7/7$, t test: $t_{11.93} = -0.35$, $p = 0.731$).

From day -7 before gestation until parturition, the gain in body weight over time was significantly lower in treatment compared to control females (Fig. 1). In addition, there was a significant influence of litter size on body weight over time (LME: time: $F_{2/130} = 33.72$, $p < 0.001$; litter size: $F_{1/11} = 1.70$, $p = 0.219$; group: $F_{1/11} = 0.01$, $p = 0.978$; time:litter size: $F_{2/130} = 58.67$, $p < 0.001$; time:group: $F_{2/130} = 7.92$, $p < 0.001$).

The time course of changes in body weight during lactation was affected by litter size, group and interactions of these factors (Fig. 1; LME: time: $F_{1/274} = 74.99$, $p < 0.001$; litter size: $F_{1/10} = 17.26$, $p = 0.002$; group: $F_{1/10} = 10.79$, $p = 0.008$; time:litter size: $F_{1/274} = 23.01$, $p < 0.001$; time:group: $F_{1/274} = 18.71$, $p < 0.001$; litter size:group: $F_{1/10} = 9.08$, $p = 0.013$; time:litter size:group: $F_{1/274} = 18.72$, $p < 0.001$). Treatment females started with a lower mean body weight compared to control females

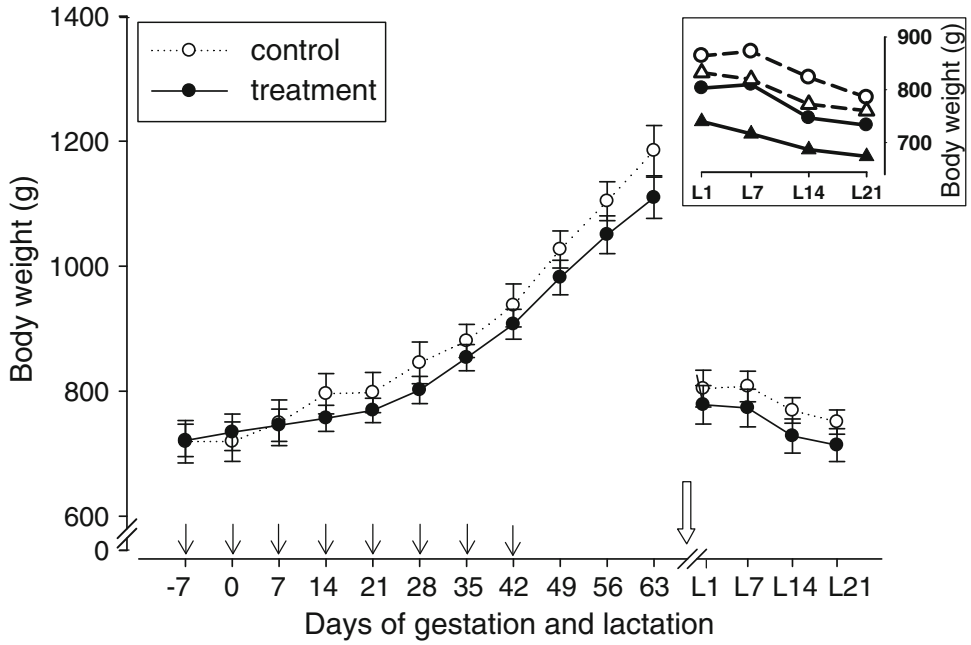

Fig. 1 Body weight throughout gestation and lactation according to group (main graph, mean ± SEM) and group–litter size interaction (*inset graph*, mean). Main graph: Body weight of treatment (*closed circles*, $n = 7$) and control females (*open circles*, $n = 7$ except gestational day (gd)7, gd14, gd28: $n = 6$) from 1 week prior to conception (gd0) to the last recorded body weight before parturition as well as during lactation. The *small arrows* mark days of stress exposure of treatment females, the *white arrow* marks day of birth. Weaning of pups took place at lactation day (L)21. *Inset graph* body weight of treatment (*closed circles* 3 pups, *closed triangles* 4 pups) and control females (*open circles* 3 pups, *open triangles* 4 pups) during lactation. Litter sizes of one, two and five animals occurred only once and are not included in this graph to maintain clarity

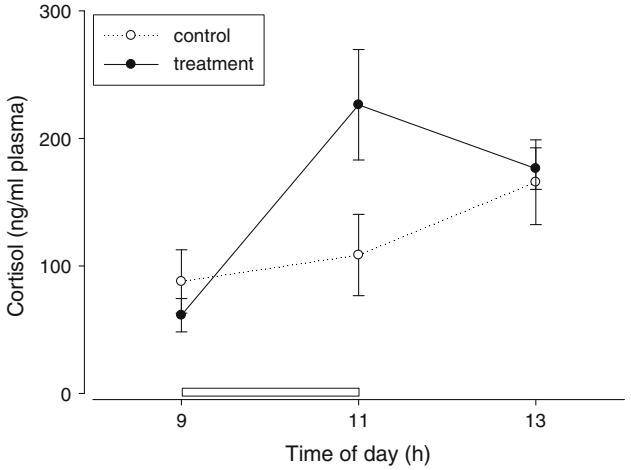

Fig. 2 Plasma cortisol concentrations (mean ± SEM) at 09:00, 11:00 and 13:00 hour in treatment (*closed circles* $n = 6$) and control females (*open circles* $n = 4$) exposed to strobe light between 09:00 and 11:00 hour. The *bar* marks time of strobe light exposure

right after parturition, with the difference persisting throughout lactation. There was a mean reduction in body weight of about 65 g in treatment and 55 g in control females during the 21 days of lactation, which was affected by litter size. Treatment females lost more weight than control females, especially in the case of large litters (Fig. 1, inset graph).

Stress effects on glucocorticoid concentrations

Plasma cortisol after single stress exposure

In the pilot study using non-pregnant females, concentration of plasma cortisol differed significantly between groups depending on the point of time ($n = 6/4$, LME: time:group: $F_{2/16} = 4.00$, $p = 0.039$). Prior to the single strobe light exposure at 09:00 hour, plasma cortisol was at similar levels, while during the time of stress exposure plasma cortisol levels increased in treatment females and at 11:00 hour showed higher concentrations than control females. Back in the familiar environment at 13:00 hour, plasma cortisol concentrations were at a similar baseline level in both groups again (Fig. 2).

Plasma cortisol concentration after repeated stress exposure in treatment females

In the experiment, plasma cortisol concentrations in treatment females during the second week of gestation remained at a level similar to non-pregnant females. At gd34/35, overall cortisol concentrations were significantly higher than at gd13/14, both in the morning (11:00 hour: paired *t* test: $t_6 = -12.02$, $p < 0.001$) and in the afternoon

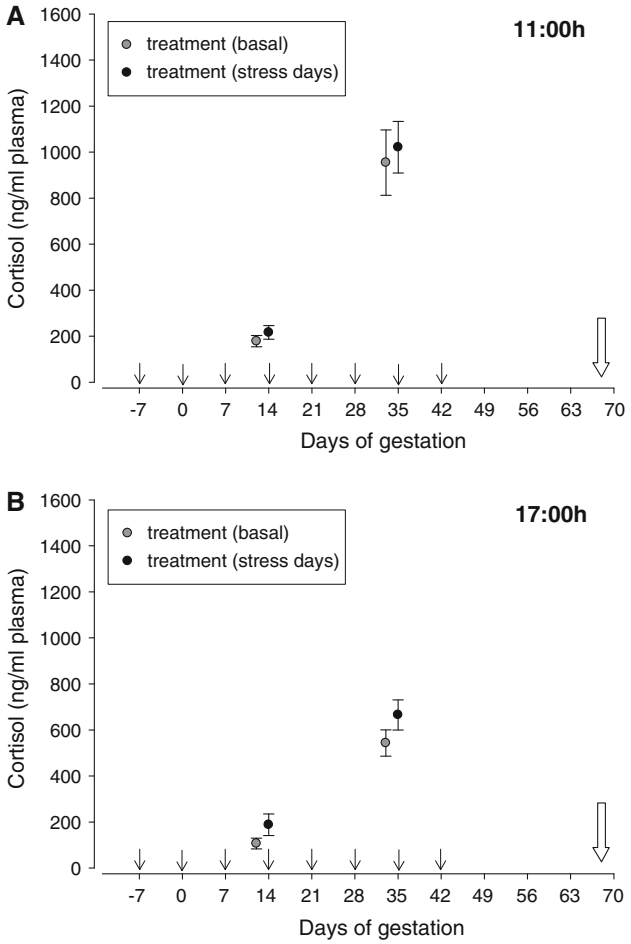

Fig. 3 Plasma cortisol concentrations (mean ± SEM) of treatment females ($n = 7$ except for gd-7, $n = 6$) at 11:00 hour (**a**) and 17:00 hour (**b**) on non-stress days gd13 and gd34 (*gray circles*) and after stress exposure on gd14 and gd35 (*closed circles*). The *small arrows* mark days of stress procedure during gestation, and the *white arrow* marks expected day of birth

(17:00 hour: paired *t* test: $t_6 = -14.52$, $p < 0.001$; Fig. 3a, b).

Range of measurements was more than five times higher in the morning samples of gd34/35 than the morning samples of gd13/14 (1,135 vs. 205 ng/ml). The range of measurements in the afternoon was only 1.7 times higher in the samples collected at gd34/35 compared to gd13/14 (698 vs. 413 ng/ml).

Comparing cortisol concentrations from the morning of non-stress days with those after stress exposure showed no significant difference (Fig. 3a; LME: time: $F_{1/19} = 158.69$, $p < 0.001$; stress exposure: $F_{1/19} = 1.09$, $p = 0.310$), whereas in the afternoon samples, mean concentrations of plasma cortisol were significantly lower on non-stress compared to stress days (Fig. 3b; LME: time: $F_{1/19} = 84.24$, $p < 0.001$; stress exposure: $F_{1/19} = 4.85$, $p = 0.040$).

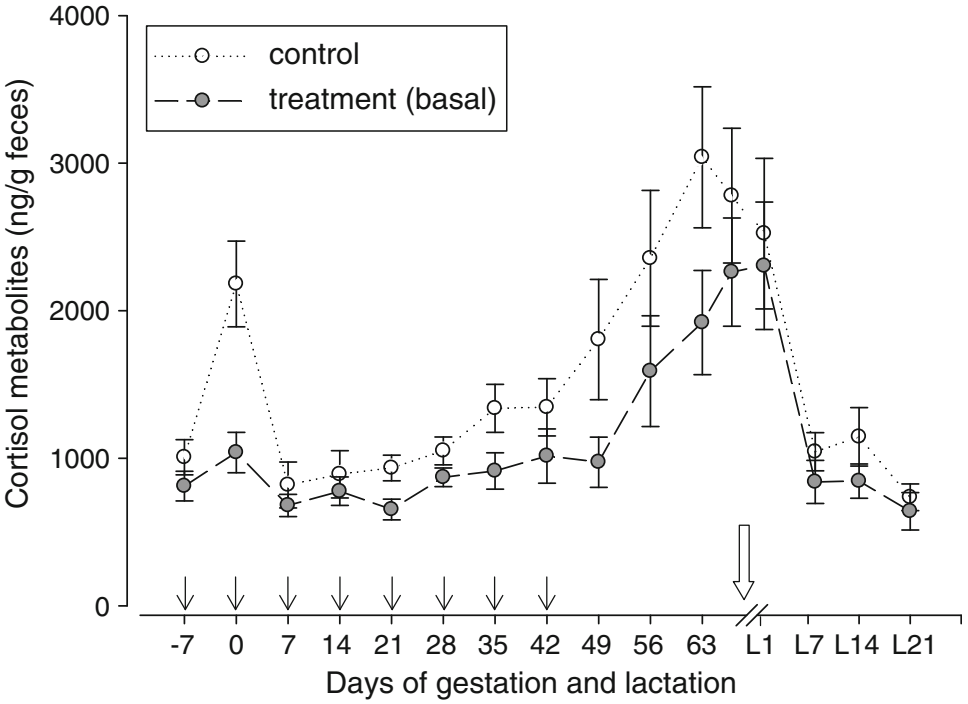

Fig. 4 Fecal cortisol metabolite concentrations (mean ± SEM) of treatment (*gray circles* $n = 7$ except for gd-7, gd28 and L3: $n = 6$) and control females (*open circles* $n = 7$ except for gd28, gd67 and L2: $n = 6$) before and during gestation as well as throughout lactation, measured on non-stress days. The *small arrows* mark days of stress exposure of treatment animals, and the *white arrow* marks day of birth. Weaning of pups took place at lactation day (L)21

Concentrations of FCM in treatment versus control females

At the onset of the experiment, on day -8 before conception, basal FCM concentrations did not differ significantly between control and treatment females (*t* test: $t_{10.32} = 1.26$, $p = 0.235$). On gd0 (conception), control females had significantly higher concentrations of FCM compared to treatment females (*t* test: $t_{7.17} = 3.55$, $p = 0.009$). Concentrations in treatment females remained at levels comparable to those 1 week earlier. During the following 4 weeks, concentrations in both groups returned to levels similar to those prior to gestation. From around gd35, there was an increase in FCM concentrations, as well as variation, in control females, whereas treatment females remained at baseline levels for another 2 weeks before their FCM increased (Fig. 4). Samples taken only a few days before parturition showed a decrease in control animals, while FCM concentrations in treatment females still increased.

Analyzing feces samples collected on non-stress days, FCM concentrations during gestation were significantly higher in control than in treatment females, the difference between groups remaining apparent even after termination of stress administration at gd42 (LME: time: $F_{2/145} = 72.03$, $p < 0.001$; group: $F_{1/12} = 8.48$, $p = 0.013$). Feces samples taken the morning following stress procedure (thus reflecting the stress response of the previous day) were also

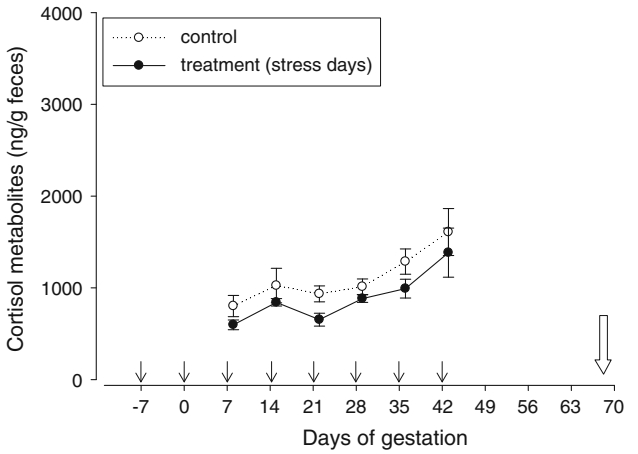

Fig. 5 Fecal cortisol metabolite concentrations (mean ± SEM) of treatment (*closed circles* $n = 7$) and control females (*open circles* $n = 7$ except for gd8, gd15 and gd29: $n = 6$) during gestation, measured on days following stress exposure (representing stress days) of treatment females. The *small arrows* mark days of stress exposure of treatment animals, and the *white arrow* marks day of birth

lower in treatment females; however, the difference between groups was not significant (Fig. 5; LME: time: $F_{2/65} = 33.00$, $p < 0.001$; group: $F_{1/12} = 4.20$, $p = 0.063$). Comparison of the concentrations of FCM representing non-stress versus stress days between gd7 and gd42, however, revealed no significant influence of stress

exposure in treatment females (LME: time: $F_{2/73} = 20.41$, $p < 0.001$, stress exposure: $F_{1/73} = 2.07$, $p = 0.155$).

During lactation, mean concentrations of FCM remained higher in control compared to treatment females, but the difference was not significant (Fig. 4; LME: time: $F_{2/64} = 9.31$, $p < 0.001$, group: $F_{1/12} = 1.13$, $p = 0.309$, time: group: $F_{2/64} = 0.11$, $p = 0.897$).

Discussion

We demonstrated that stress exposure during the first two-thirds of gestation affected the hypothalamic–pituitary–adrenal (HPA) axis and the development of body weight, but not reproductive parameters, in pregnant guinea pigs.

Reproductive parameters

No significant differences were found in any measured parameter of reproductive performance between treatment and control females. A vast amount of literature is available about the effects of stress and glucocorticoids on reproductive parameters. The results of studies conducted in many species vary from study to study, dependent on time, dosage and type of stressor (Fowden and Forhead 2009; Diego et al. 2006; Roussel et al. 2005; Braastad 1998; Götz et al. 2008). Kapoor and Matthews (2005), for instance, have previously used strobe light exposure in guinea pigs during late gestation and also did not find any significant effects on birth weight, sex ratio or litter size. During early gestation, future investment in offspring can be reduced at times of challenging situations by adjusting litter size or sex ratio (Wiebold et al. 1986; Trivers and Willard 1973). This may especially be true for precocial species, as they have a relatively high energy demand during gestation due to their advanced development at birth and long gestation time.

Yet, in our study, treatment females were apparently able to adjust to the early gestational stress procedure without impairment of reproductive performance. As gestational stress may also affect offspring development via fetal programming, (Welberg and Seckl 2001), we analyzed the potential effects of gestational stress on offspring physiology and behavior at the present.

Body weight

In line with our prediction, gain in body weight of treatment females was attenuated throughout gestation. This result is in accordance with findings of Sparks et al. (1981), who reported that stressful events caused by surgery and anesthesia during gestation reduced weight gain in guinea pigs. According to Darnaudéry et al. (2004), gestation is a period of high vulnerability to stress, which in rats can impair growth up to 4 weeks after termination of stress. Léonhardt et al. (2007), however, found no effect of repeated strobe light exposure during early gestation on gain in maternal body weight in rats. In addition to environmental conditions, the number of pups is a major factor influencing maternal weight gain during gestation. The effect of litter size is large in guinea pigs, where total fetal weight accounts for up to 60% of maternal weight before conception (Sparks et al. 1981). Yet in our study, the average litter size did not differ significantly between treatment and control females and therefore cannot adequately explain differences in female body weight.

Other more plausible causes of group differences in weight gain are food intake, energy uptake and energy expenditure. Harris et al. (2002) reported that chronic stress can result in temporary hypophagia as demonstrated in rats. However, reduced food intake is an unlikely reason for the observed difference in body weight of treatment and control females in our study, as all animals received the same amount of pellets and fresh food, which was always completely consumed. Although differences in hay consumption cannot be ruled out as hay rations were not always fully consumed, owing to its low energy content ($\sim$6 MJ/kg), different hay consumption is unlikely to strongly affect body weight. Since we did not control for individual food intake within breeding groups, reduced food intake of one female could pass undetected, because of possible higher food consumption by the partner animal. However, those females were assigned to the same group, either treatment or control. Thus, potentially uneven food intake between individuals would always occur within the same group. In addition, no difference in weight gain could be observed in male partner animals (data not shown). As both consumption of food and reproductive output were comparable between treatment and control females, increased energy expenditure seems the most likely explanation for the reduced weight gain in treatment females. In addition, a reduction of energy uptake in terms of nutrient assimilation might also play a role, although we do not have data or any indication of differences in energy uptake between groups.

During lactation, the mean body weight of treatment females was also lower compared to control females. This group effect on the mother's body weight during lactation was dependant on litter size, which was visible even when looking at only those females with medium-sized litters of three or four pups (see Fig. 1, inset graph). Our results differ from the findings of Léonhardt et al. (2007) who exposed pregnant rats to strobe light repeatedly for 1 h, but found no effect on maternal weight gain during lactation. The difference between body weights during lactation of mothers with three and four offspring per litter suggest that treatment females, who start with lower body weights after

parturition, are more affected by large litters and subsequently higher energy demands than control females.

Glucocorticoid concentrations

In accordance with the findings of Dauprat et al. (1984) and Kapoor and Matthews (2005), as well as in line with our prediction, plasma cortisol concentrations were elevated after both a single and repeated stress exposure. The elevation of cortisol levels after repeated stress exposure, however, was significant only in the afternoon and not in the morning samples. One possible reason for this discrepancy could be that the stress response in the afternoon was facilitated by the stress experience of the previous morning. Léonhardt et al. (2007) also showed a diurnal variation in glucocorticoid response to strobe light exposure in rats, with an increase in glucocorticoid levels only in the afternoon that is consistent with our results. In addition, we found that around gd35 variation and overall levels in glucocorticoids were higher in the morning than in the afternoon samples, both before and after stress exposure. Such a diurnal variation in plasma cortisol concentration, with highest levels during the morning, has also been reported by Kapoor and Matthews (2005). Thus, it seems also possible that the ability to respond adequately to the stressor may have been impaired during the morning, either by poor physiological capacity itself or by high baseline levels, indicating a potential desensitization of the HPA axis. Another possible reason for the significant increase in cortisol concentrations in the afternoon but not the morning samples might be methodological in origin. The interval between strobe light exposure and blood sampling was 2 h in the morning but only 1 h in the afternoon and, therefore, we may have missed peak cortisol levels after stress exposure in the morning but not in the afternoon. This is supported by Kapoor and Matthews (2005), who found that plasma cortisol concentrations peaked 60 min after an ACTH challenge and were back at baseline levels after 2 h. In contrast, Sachser (1994) showed elevated cortisol levels after ACTH injection for more than 4 h. One reason for the discrepancy between both studies may be that in Sachser (1994), ACTH was administered intramuscularly, which results in slower uptake and adrenal response compared to intravenous application as carried out by Kapoor and Matthews (2005). Though one should be cautious when comparing different stressors, there are two reasons for our interpretation: On the one hand, exposure to strobe light elicited an immediate stress response, which may be rather comparable with intravenous ACTH application. On the other hand, the response to our stress procedure is much smaller and thus likely also shorter than the response to pharmacological ACTH treatment. Therefore, cortisol levels might have been back to basal levels at the time we took blood samples 2 h after onset of stress exposure during the morning.

FCM concentrations from samples on gd0 represent the social and reproductive situation characterized by the presence of males that are highly interested in receptive females and harassing them continuously. Interestingly, concentrations of FCM increased markedly in control females only, while in treatment females, pre-exposed to stress on gd-7, concentrations remained close to baseline levels. Martí et al. (2001) showed that a single immobilization stress in rats enhanced termination of response to the same stressor days later. Hence, it is reasonable to assume that the treatment females in our study likewise down-regulated the release of glucocorticoids. Although animals were used to handling and strobe light is supposed to be a relatively mild stressor and was applied only twice (i.e., once in the morning and once in the afternoon) a week before on gd-7, stress exposure was obviously effective in inhibiting the physiological stress response to the new mating situation, seen in control females. This suggests a desensitization of the HPA axis in guinea pigs, which may last for at least several days and is not limited to the same stressor previously experienced.

Plasma cortisol levels of treatment females increased almost threefold between gd13 and gd34 within samples collected both in the morning and afternoon. FCM levels also started to increase from around gd35 in control females, reaching peak levels during the third trimester of pregnancy. Similar elevations during gestation were also found in other studies on guinea pigs, rats and primates, including humans (Whipp et al. 1976; Léonhardt et al. 2007; Bales et al. 2005; Allolio et al. 1990). This increase in cortisol concentration is associated with elevated levels of corticotrophin-releasing hormone of placental origin (Mastorakos and Ilias 2003) and increased production of adrenal steroids by the fetus (Coulter and Jaffe 1998). Elevation of glucocorticoids during normal gestation is thought to be necessary to suit the increased maternal, as well as fetal metabolic requirements (Lazinski et al. 2008), and is mandatory for fetal organ maturation (Fowden et al. 1998). Interestingly, in treatment females, FCM stayed around baseline levels until at least 1 week after termination of the stress procedure at gd42 before any major elevation of FCM was measured. This indicates that in treatment females, the physiological increase was delayed by at least 2 weeks, compared to control females.

Thus, in contrast to our prediction, mean FCM concentrations from both stress and non-stress days were consistently lower in treatment than in control females during gestation, the difference between groups actually increasing with the progressing pregnancy. Lower FCM

levels in treatment than in control females were also found during lactation, though the difference between the groups was no longer significant. As glucocorticoids facilitate fetal development (Venihaki et al. 2000), this reduction and retardation of steroid increase in treatment females may also have implications for the unborn offspring.

What might be the reason for the lower FCM levels found in treatment females? Measurement of FCM is a suitable way of evaluating adrenocortical activity in guinea pigs, reflecting the hormonal status at about 14 to 20 h earlier (Bauer et al. 2008). Even though an increase in cortisol was apparent in blood samples, it was probably only short-term, and could have been dampened in fecal samples reflecting the overall status rather than a specific event. In addition, negative feedback mechanisms may have led to a down-regulation of the HPA axis and thus reduced levels of FCM in treatment females. When a stressful stimulus remains, cortisol concentrations may decline after an acute rise, even though other response parameters such as behavior or body weight may still indicate the presence of a stressful situation (reviewed in Mormède et al. 2007). Studies in rats and heifers showed reduced glucocorticoid concentrations after chronic stress (Harris et al. 2002; Darnaudéry et al. 2004; Fisher et al. 1997). Nevertheless, the decline of glucocorticoids alone does not imply tolerance to the stressor, as according to Cyr and Romero (2009) hormonal habituation is contradicted if body weight decreases or the baseline of stress mediators is affected, as occurred in our study. Accordingly, a desensitization of the physiological stress response with accompanying down-regulation of the HPA axis may account for the lower FCM levels, together with the lower body weight found in the treatment females. Further research is needed to elucidate potentially underlying physiological mechanisms of both desensitization of the HPA axis and subsequent changes in physiology.

The HPA axis is involved in both stress reaction and metabolic pathways. Reduced glucocorticoid levels, possibly in combination with a higher metabolic rate (note, however, that as we do not have any data on basal metabolic rate (BMR) in this study and the following interpretation is speculative), may thus be potential candidates to explain the lower body weight increase in treatment females. Elevated levels of glucocorticoids have appetizing and metabolic effects that increase feeding efficiency in sheep and the gain in daily body weight in steers (Knott et al. 2010; Montanholi et al. 2010). In humans, elevated glucocorticoids are associated with decreased energy expenditure, which could also influence weight gain (Rohner-Jeanrenaud 1999). Decreased levels, however, as present in Addison's disease, a state of chronic hypocortisolism, are accompanied by loss of body weight, in humans and various animal species (for review see Ten et al. 2001; Klein and Peterson 2010). Damjanovic et al. (2009) showed that in pregnant women there is a negative correlation between plasma cortisol and BMR; women with lower cortisol levels have higher BMR. An increase in BMR, in turn, could negatively affect body weight. Arguably then, decreased levels of glucocorticoids due to chronic stress could potentially lead to higher BMR and ensuing lower weight gain. Certainly, this is just a hypothesis that needs experimental verification. Further research is warranted in this field, especially with regard to pregnancy as a time of challenged metabolism.

Taken together, our results suggest that even though females could not habituate to the stress procedure as indicated by their attenuated body weight gain (see Cyr and Romero 2009), it did not directly affect conception rate or reproductive parameters. This finding is surprising as it is widely believed that severe stress can act to antagonize the hypothalamus-pituitary-gonadal axis, which may lower the rate of conception and reproductive parameters including offspring quality (Turner et al. 2005; Wolfenson and Blum 1988). While the degree of stress may have been too mild to affect reproduction, our results indicate that, in times of chronic stress, pregnant females favor investment in offspring over self-maintenance, which is in contrast to the central dogma (Ots and Horak 1996; Breuner et al. 2008). Maybe, this concept might not apply to the same degree in precocial species, where maternal investment is generally high during gestation but limited thereafter due to the independence of the offspring shortly after birth. So, in species like the guinea pig, it may be particularly important to invest in offspring who are in good condition at birth. Therefore, in times of challenge, when pregnant females are facing a trade-off between self-maintenance and investment in offspring, they apparently decide in favor of reproduction.

Additionally, our results shed new light on the interpretation of stress effect studies, especially during the time of gestation. It is possible that a short-term increase of cortisol after stress exposure is insubstantial in producing direct effects, when actually it is the subsequent down-regulation that will dominate maternal physiology. If only a short-term analysis of cortisol increase following stress exposure is conducted, the adjacent down-regulation might be missed and the subsequent conclusions based on the false assumption of increased cortisol. Therefore, it is necessary to monitor the physiology of stressed animals on a long-term scale, and at close intervals, to be able to decode on the overall stress response. As glucocorticoids are crucial for fetal development (Bolt et al. 2001), an analysis of potential effects of attenuated glucocorticoid levels following chronic stress in pregnant females on the offspring is warranted.

Acknowledgments We are grateful to the two anonymous reviewers for their constructive and helpful comments on the manuscript. Furthermore, we thank Teresa Klaus for excellent assistance in animal keeping, Edith Klobetz-Rassam for performance of hormone assays, Barbara Bauer and Teresa Valencak for great encouragement throughout realization and writing of the study. We appreciate the help of Rebecca Drury in terms of linguistics. Animal facility was kindly provided by the Institute of Virology, and desk space by the Institute of Population Genetics, both located at the University of Veterinary Medicine Vienna. This work was funded by the PhD Initiative Program BIOREC of the University of Veterinary Medicine Vienna.

Conflict of interest The authors have no financial disclosures or conflicts of interest to report.

References

Allolio B, Hoffmann J, Linton EA, Winkelmann W, Kusche M, Schulte HM (1990) Diurnal salivary cortisol patterns during pregnancy and after delivery: relationship to plasma corticotrophin-releasing hormone. Clin Endocrinol (Oxf) 33:279–289

Alsop D, Vijayan MM (2009) Molecular programming of corticosteroid stress axis during zebrafish development. Comp Biochem Physiol A Physiol 153:49–54

Bales KL, French JA, Hostetler CM, Dietz JM (2005) Social and reproductive factors affecting cortisol levels in wild female golden lion tamarins (*Leontopithecus rosalia*). Am J Primatol 67:25–35

Bauer B, Palme R, Machatschke IH, Dittami J, Huber S (2008) Non-invasive measurement of adrenocortical and gonadal activity in male and female guinea pigs (*Cavia aperea f. porcellus*). Gen Comp Endocrinol 256:482–489

Bolt RJ, van Weissenbruch MM, Lafeber HN, Delemarre-van de Waal HA (2001) Glucocorticoids and lung development in the fetus and preterm infant. Pediatr Pulmonol 32:76–91

Braastad BO (1998) Effects of prenatal stress on behaviour of offspring of laboratory and farmed mammals. Appl Anim Behav Sci 61:159–180

Breuner CW, Patterson SH, Hahn TP (2008) In search of relationships between the acute adrenocortical response and fitness. Gen Comp Endocrinol 157:288–295

Cadet R, Pradier P, Dalle M, Delost P (1986) Effects of prenatal maternal stress on the pituitary adrenocortical reactivity in guinea-pig pups. J Dev Physiol 8:467–475

Coulter CL, Jaffe RB (1998) Functional maturation of the primate fetal adrenal in vivo: 3.Specific zonal localization and developmental regulation of CYP21A2 (P450c21) and CYP11B1/CYP11B2 (P450c11/aldosterone synthase) lead to integrated concept of zonal and temporal steroid biosynthesis. Endocrinology 139:5144–5150

Cyr NE, Romero LM (2009) Identifying hormonal habituation in field studies of stress. Gen Comp Endocriol 161:295–303

Damjanovic SS, Stojic RV, Lalic NM, Jotic AZ, Macut DP, Ognjanovic SI, Petakov MS, Popovic BM (2009) Relationship between basal metabolic rate and cortisol secretion throughout pregnancy. Endocrine 35:262–268

Darnaudéry M, Dutriez I, Viltart O, Morley-Fletcher S, Maccari S (2004) Stress during gestation induces lasting effects on emotional reactivity of the dam rat. Behav Brain Res 153:211–216

Dauprat P, Monin G, Dalle M, Delost P (1984) The effects of psychosomatic stress at the end of pregnancy on maternal and fetal plasma cortisol levels and liver glycogen in guinea-pigs. Reprod Nutr Dev 24:45–51

De Weerth C, Buitelaar JK (2005) Physiological stress reactivity in human pregnancy—a review. Neurosci Biobehav Rev 29:295–312

Diego MA, Jones NA, Field T, Hernandez-Reif M, Schanberg S, Kuhn C, Gonzalez-Garcia A (2006) Maternal psychological distress, prenatal cortisol, and fetal weight. Psychosom Med 68:747–753

Fisher AD, Crowe MA, Prendiville DJ, Enright WJ (1997) Indoor space allowance: effects on growth, behaviour, adrenal and immune responses of finishing beef heifers. Anim Sci 64:53–62

Fowden AL, Forhead AJ (2009) Endocrine regulation of feto-placental growth. Horm Res 72:257–265

Fowden AL, Li J, Forhead AJ (1998) Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? Proc Nutr Soc 57:113–122

Götz AA, Wolf M, Stefanski V (2008) Psychosocial maternal stress during pregnancy: effects on reproduction for F0 and F1 generation laboratory rats. Physiol Behav 93:1055–1060

Harris RBS, Mitchell TD, Simpson J, Redmann SM Jr, Youngblood BD, Ryan DH (2002) Weight loss in rats exposed to repeated acute restraint stress is independent of energy or leptin status. Am J Physiol Regul Integr Comp Physiol 282:R77–R88

Kapoor A, Matthews SG (2005) Short periods of prenatal stress affect growth, behaviour and hypothalamo–pituitary–adrenal axis activity in male guinea pig offspring. J Physiol (Lond) 566:967–977

Klein SC, Peterson ME (2010) Canine hypoadrenocorticism: Part I Can Vet J 51:63–69

Knott SA, Cummins LJ, Dunshea FR, Leury BJ (2010) Feed efficiency and body composition are related to cortisol response to adrenocorticotropin hormone and insulin-induced hypoglycemia in rams. Domest Anim Endocrinol 39:137–146

Laurien-Kehnen C, Trillmich F (2004) Maternal food restriction delays weaning in the guinea pig, *Cavia porcellus*. Anim Behav 68:303–312

Lazinski MJ, Shea AK, Steiner M (2008) Effects of maternal prenatal stress on offspring development: a commentary. Arch Womens Ment Health 11:363–375

Léonhardt M, Matthews SG, Meaney MJ, Walker CD (2007) Psychological stressors as a model of maternal adversity: diurnal modulation of corticosterone responses and changes in maternal behavior. Horm Behav 51:77–88

Malinowska KW, Nathanielsz PW (1974) Plasma aldosterone, cortisol and corticosterone concentrations in the new born guinea-pig. J Physiol (Lond) 236:83–93

Martí O, García A, Vellès A, Harbuz MS, Armario A (2001) Evidence that a single exposure to aversive stimuli triggers long-lasting effects in the hypothalamus–pituitary–adrenal axis that consolidate with time. Eur J Neurosci 13:129–136

Mastorakos G, Ilias I (2003) Maternal and fetal hypothalamic–pituitary–adrenal axes during pregnancy and postpartum. Ann N Y Acad Sci 997:136–149

Meek LR, Dittel PL, Sheehan MC, Chan JY, Kjolhaug SR (2001) Effects of stress during pregnancy on maternal behavior in mice. Physiol Behav 72:473–479

Montanholi YR, Swanson KC, Palme R, Schenkel FS, McBride BW, Lu D, Miller SP (2010) Assessing feed efficiency in beef steers through feeding behavior, infrared thermography and glucocorticoids. Animal 4:692–701

Mormède P, Andanson S, Aupérin B, Beerda B, Guémené D, Malmkvist J, Manteca X, Manteuffel G, Prunet P, van Reenen CG, Richard S, Veissier I (2007) Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. Physiol Behav 92:317–339

Möstl E, Maggs JL, Schrötter G, Besenfelder U, Palme R (2002) Measurement of cortisol metabolites in faeces of ruminants. Vet Res Commun 26:127–139

Ots I, Horak P (1996) Great tits Parus major trade health for reproduction. Proc R Soc Lond B 263:1443–1447

Otten W, Kanitz E, Couret D, Veissier I, Prunier A, Merlot E (2010) Maternal social stress during late pregnancy affects hypothalamic–pituitary–adrenal function and brain neurotransmitter systems in pig offspring. Domest Anim Endocrinol 38:146–156

Palme R, Möstl E (1997) Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Int J Mamm Biol 62:192–197

Patin V, Lordi B, Vincent A, Thoumas JL, Vaudry H, Caston J (2002) Effects of prenatal stress on maternal behavior in the rat. Brain Res Dev Brain Res 139:1–8

Pinheiro J, Bates D, DebRoy S, Sarkar D, the R core team (2007) Nlme: linear and nonlinear mixed effects models. R package version 3.1-89. http://CRAN.R-project.org/package=nlme

R Development Core Team (2007) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org

Reynolds LP, Borowitz PP, Caton JS, Vonnahme KA, Luther JS, Hammer CJ, Maddock Carlin KR, Grazul-Bilska AT, Redmer DA (2010) Developmental programming: the concept, large animal models, and the key role of uteroplacental vascular development. J Anim Sci 88:E61–E72

Rhind SM, Rae MT, Brooks AN (2001) Effects of nutrition and environmental factors on the fetal programming of the reproductive axis. Reproduction 122:205–214

Rohner-Jeanrenaud F (1999) Neuroendocrine regulation of nutrient partitioning. Ann NY Acad Sci USA 892:261–271

Ross MG, Desai M (2005) Gestational programming: population survival effects of drought and famine during pregnancy. Am J Physiol Regul Integr Comp Physiol 288:R25–R33

Roussel S, Boissy A, Montigny D, Hemsworth PH, Duvaux-Ponter C (2005) Gender-specific effects of prenatal stress on emotional reactivity and stress physiology of goat kids. Horm Behav 47:256–266

Sachser N (1994) Sozialphysiologische Untersuchungen an Hausmeerschweinchen Gruppenstrukturen soziale Situation und Endokrinium Wohlergehen. Verlag Paul Parey, Hamburg

Sachser N, Pröve E (1984) Short-term effects of residence on the testosterone responses to fighting in alpha male guinea pigs. Aggress Behav 10:285–292

Seckl JR (2004) Prenatal glucocorticoids and long-term programming. Eur J Endocrinol 151:U49–U62

Sparks JW, Pegorier JP, Girard J, Battaglia FC (1981) Substrate concentration changes during pregnancy in the guinea pig studied under unstressed steady state conditions. Pediatr Res 15:1340–1344

Stockard CR, Papanicolaou GN (1917) A rhythmical "heat period" in the guinea pig. Sci, New Ser 46:42–44

Ten S, New M, MacLaren N (2001) Clinical review 130: Addison's disease. J Clin Endocrinol Metab 86:2909–2922

Trivers RL, Willard DE (1973) Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90–92

Turner AI, Hemsworth PH, Tilbrook AJ (2005) Susceptibility of reproduction in female pigs to impairment by stress or elevation of cortisol. Domest Anim Endocrinol 29:398–410

Vehaskari VM (2010) Prenatal programming of kidney disease. Curr Opin Pediatr 22:176–182

Venihaki M, Carrigan A, Dikkes P, Majzoub JA (2000) Circadian rise in maternal glucocorticoid prevents pulmonary dysplasia in fetal mice with adrenal insufficiency. Proc Natl Acad Sci USA 97:7336–7341

Weir BJ (1970) The management and breeding of some more hystricomorph rodents. Lab Anim 4:83–97

Welberg LAM, Seckl JR (2001) Prenatal stress, glucocorticoids and the programming of the brain. J Neuroendocrinol 13:113–128

Whipp GT, Wintour EM, Coghlan JP, Scoggins BA (1976) Regulation of aldosterone in the guinea-pig—effect of oestrus cycle, pregnancy and sodium status. Aust J Exp Biol Med Sci 54:71–78

Wiebold JL, Stanfield PH, Becker WC, Hillers JK (1986) The effect of restraint stress in early pregnancy in mice. J Reprod Fertil 78:185–192

Wolfenson D, Blum O (1988) Embryonic development, conception rate, ovarian function and structure in pregnant rabbits heat-stressed before or during implantation. Anim Reprod Sci 17:259–270

Young WC (1937) The vaginal smear picture, sexual receptivity and the time of ovulation in the guinea pig. Anat Rec 67:305–325