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Environmental enrichment for pregnant sows modulates HPA-axis and behavior in the offspring

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ABSTRACT

Our goal was to assess the effects of environmental enrichment during gestation on the hypothalamic–pituitary–adrenal axis (HPA) and behavior of offspring. In order to test our hypothesis, we kept 18 sows (*Sus scrofa domesticus*) in straw during the final third of gestation (from 90 to 114 days) and 18 sows without straw (control). On the piglets born (one pair per sow), we performed an analysis of behavior, cortisol levels in saliva and performed fear tests to assess resilience, emotional reactivity, responsiveness to stressors, and cognition. The environmental enrichment used during gestation reduced aggressiveness ($p < 0.02$) and nosing in piglets ($p < 0.08$). In addition, salivary cortisol was higher in piglets from sows in barren environments ($p < 0.03$). Salivary cortisol was higher in piglets from sows in environmentally enriched conditions only on the day of weaning ($p < 0.00001$). There was no difference in the piglets' emotionality when we compared groups with both sexes together. However, there was a sex-specific difference, in which females born from sows kept with environmental enrichment explored more (novel object exploration, $p = 0.02$; time pushing the object, $p = 0.03$) and showed less fear at the novel object test. Environmental enrichment in the end of gestation influenced the offspring's HPA-axis activity and behavior, improving their welfare.

1. Introduction

Environmental enrichment is the creation of a dynamic, complex and interactive environment that allows physical and cognitive challenges similar to those an animal would experience in nature. It has been widely discussed as a tool to improve animal welfare. Environmental enrichment allows the expression of natural behaviors, modulates aggressiveness (Kadry and Barreto, 2010), increases brain plasticity (Ebbesson and Braithwaite, 2012; Williams et al., 2001), alters HPA-axis activity (Gro et al., 2013; Kotrschal and Taborsky, 2010; Larsson et al., 2002), and reduces methylation in hippocampal and frontal cortex genes (Mychasiuk et al., 2012). Furthermore, affects brain weight, promotes an increase in arborization and density of dendritic spines (Leggio et al., 2005), modulates neurogenesis in the hippocampus (Segovia et al., 2006), and makes the cognitive bias positive (Douglas et al., 2012). Also, it affects the expression of genes in the brain, especially those involved in neuronal structure, synaptic signaling, and plasticity (Baroncelli et al., 2009). Some of those genes have been known to be associated with learning and memory (Rampon et al., 2000). However, in captivity, animals are usually kept in barren environments. The lack of stimuli and prevention of animals' engagement in species-typical behaviors for which they appear to have a "behavioral need" is an important source of stress in captivity. Behavioral needs can be broadly defined as behaviors that appear to be largely internally motivated, since they may occur even in the absence of appropriate trigger stimuli. Preventing animals from performing such behaviors is thought to be detrimental to their well being, which can be indicated by the manifestation of abnormal and stereotypic behaviors. There is a wide range of studies demonstrating barren environments as a trigger of stereotypic behaviors expression (Morgan and Tromborg, 2007; Wemelsfelder et al., 2000), which is considered a welfare indicator.

The effects of environmental enrichment in animals are scientifically proven to be beneficial, but it is still unclear how it affects fetal programming. In mammals, pregnancy has an important role in shaping the organism. The mother's experiences and the womb environment

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may have effects on the offspring. This concept is derived from the "thrifty phenotype hypothesis", in which neurodevelopment programming induces alterations that predict the postnatal environment (Hales and Barker, 2001). In other words, the prenatal environment has the potential to adjust the offspring phenotype, preparing individuals for the environment that they will experience based on what the mother was exposed to. Ergo, the environment in which an animal is maintained during gestation may result in changes in several offspring parameters (Baxter et al., 2016; Braastad, 1998; Meyer et al., 2009; Rutherford et al., 2014; Urakubo et al., 2001). For example, hunger experienced by sows during gestation increases aggressive behavior in the offspring (Bernardino et al., 2016), possibly inferring that the postnatal environment would involve dispute over resources, where aggressiveness is a desirable phenotype. Through this mechanism, factors such as emotional reactivity, responsiveness to stressors, and cognition can be modulated by challenges in the prenatal and neonatal periods (Poletto et al., 2006; Rutherford et al., 2014; Weinstock, 2008).

Prenatal stress can generate changes that are not necessarily pathological (Braastad, 1998), but one important consequence is the excess of glucocorticoids that the offspring receives in womb. Glucocorticoids are important stress hormones in adult animals and are highly related to stress, but the functions range more widely in the fetuses, in which the effects are completely different depending on gestational age, severity, and duration of exposure (Fowden et al., 2016). By the end of gestation the fetus' brain has functional glucocorticoids receptors, which could be affected by the stress experienced by the mother, shaping important brain structures and generating negative effects (Baxter et al., 2016; Coulon et al., 2013; Rutherford et al., 2014).

Considering the impact of prenatal stress in fetal programming and the beneficial outcomes of stress reducing with stimulating environments, we aimed to investigate the effects of environmental enrichment during gestation on offspring's welfare indicators. Through the mechanism of the "thrifty hypothesis", we believe that improving the mother's welfare during pregnancy, with the use of environmental enrichment, can lead to positive changes in the offspring. Therefore, our hypothesis is that environmental enrichment during gestation affects the offspring development. In this study, we addressed how the prenatal environment acts on offspring development at the final third of gestation, in which the placenta can serve as a buffer and mediate the effects on the offspring phenotype.

2. Material and methods

2.1. Animals and holding condition

The Ethics Committee on Animal Use of the Faculty of Veterinary Medicine and Animal Science, University of São Paulo (CEUA / FMVZ - protocol number 6,157,201,114) approved this study. The study was performed at the Araporanga Farm - Jaguariaíva, Paraná, Brazil.

For this study, 58 pregnant sows were used at first (swine lineage TopGen Afrodite®). The animals were distributed by body condition score (subcutaneous fat and estimated weight, standardized by the farm they were kept) in six gestation pens with ten animals per pen (except two pens with 9 sows each). From this total, 36 sows (six for pen) were studied and ranging from 2nd to 7th parturition in the treatment and control (*t*-test, $p > 0.05$). Three pens had straw (hay) as substrate from day 90 of gestation, while control pens (without straw) were maintained with concrete floors (conventional pens). Thus, half of the animals had access to straw, which was replaced daily between 8 h and 11 h. Sows were divided into three blocks, one week apart in relation to the gestational period. In each block, there was a treatment and a control pen, in order to maintain balance.

Each pen was 6 m long and 3.86 m wide, with a solid/slatted concrete floor area of 3.97 m in length and walls that were 0.85 m high. The feeder was 5 m long and 0.37 m wide. However, the slatted floor area was covered with plywood boards in order to avoid the straw from becoming trapped and harming the manure management system. The control pens also received the plywood boards, to avoid any differences in relation to the microclimate inside the pens. Food was provided twice daily, at 0700 h and 1140 h. The animals had access to water *ad libitum*.

2.2. Experimental design

In order to assess the effect of straw at the final third of gestation, 18 pregnant sows were submitted to an environment with substrate. Their behavior was sampled two days before the beginning of the treatment (basal collections: Days 88 and 89 of gestation), at the beginning (Days 91–92) and at the end of the third trimester (Days 106 and 107 of gestation) prior to transfer to farrowing crates. The control treatment had the same routine behavioral observations. Saliva was collected on the same days as the behavioral samples, at 6 h and 18 h for cortisol analysis. From each sow, one pair of piglets (one male and one female) were chosen based on the placenta collection. To measure the effects of environmental enrichment at the final third of gestation, these piglets were evaluated regarding aggressiveness and piglet-directed behavior, emotionality, and salivary cortisol levels. In addition, during farrowing, placental tissues were collected.

2.3. Sow behavioral data

In order to collect behavioral data, an ethogram was adapted (Zonderland et al., 2004). Behavioral measures of sows were obtained by direct observation on Days 88, 89 (basal collection, before environmental enrichment), 91, 92, 106, and 107, at the final third of gestational period. The behavioral assessments were performed by direct observation during the two feeding times, one hour before and one hour after, and a final one, at 1730 h, adding up to five observations each day. Two observers were previously standardized to avoid bias in data collection. In order to randomize the two observers, by each collection they were changing the pens and then, treatment. Observations were carried out using a combination of methods for behavioral measures, which started with a sample scan, followed by continuous observation of the focal animal. For each observation, each sow was observed three times for uninterrupted 120 s, adding up to 6 min per animal per observation time (before, after feeding, and at the end of the day), with a total of 30 min per observation per day. The collection periods spanned over two consecutive days to avoid possible interference from stressful events (e.g. Days 88 and 89 in the evaluation of the basal behavior).

2.4. Salivary collection and cortisol analysis

Saliva was collected on the same days as the behavioral evaluation, that is, Days 88, 89, 91, 92, 106, and 107. On all of these days, two samples were collected per animal, at 06:00 h and 18:00 h in order to assess cortisol in respect to the circadian rhythm and to assess the effect of enrichment on HPA-axis activity. Saliva was collected using hydrophilic cotton in two roller-shaped units tied to a dental floss with long tips and presented to each animal. The animal chewed the cotton until it was saturated with saliva. The first sample collected was discarded and the protocol repeated, to collect only recently produced saliva. After the second sample was collected, it was placed in a 15-milliliter falcon tube. Subsequently, the tube was stored in ice until the end of the collection, then frozen at -20 °C until processing. Samples were thawed on ice. After complete thawing, the sample was centrifuged (10 min., 1000 x g) and the supernatant was aliquoted into microtubes and again frozen at − 20 °C until analysis. This process removes mucins and other components that may interfere with the analysis. For sample analysis, 50 µl of saliva were analyzed with a cortisol enzyme immunoassay (EIA – based on Cooper et al., 1989; Palme and Möstl, 1997) in duplicate for

each sow, with a pool of each gestation period, without mixing the morning and afternoon collections (e.g. with samples from 88 and 89 gestation days in the morning collection). The sensitivity of the EIA was 0.2 pg/well.

2.5. Farrowing

The deliveries were monitored and occurred in conventional farrowing crates. At birth, each piglet had its umbilical cord tied with string kept in antiseptic solution and dipped in iodine (10%) for 5 s. The piglets were then cleaned with paper towels, assigned a number for the order of birth on the back with a stick marker, and were passed through antiseptic powder to reduce body moisture. After this initial management, the piglets were placed with their mother to ingest colostrum. On the first day of life, the piglets had their teeth grinded, the tail cut, the ears notched, and individual weight recorded. The dextran iron application was performed the day after delivery. All these procedures are routine in Brazilian commercial pig farms.

2.6. Placenta collection and glucocorticoid extraction

The placenta was collected from four piglets per sow; a standardized (size and location) piece for each placenta was cut and frozen in a –20 °C freezer. All placentas from each sow were macerated together in order to prepare a pool. Once the placenta was macerated, 0.1 g of the powder was placed in a 1.5-ml microtube. About 200 µl of ultrapure water were added and the mixture was homogenized by vortexing for 15 s. 20 µl of this mixture were placed in another similar tube, for total protein analysis (performed in triplicate for each sample, following the Bradford protocol (Bradford, 1976)). Ethyl acetate (1 ml) was added to this tube with water and placenta, vortexed for 15 s, and centrifuged for 15 min at 4 °C. About 400 µl of supernatant was transferred to a new 1.5 ml microtube; the second (duplicate) was transferred to another tube. All samples were dried overnight in a hood until dry. For glucocorticoid analysis, all samples were re-suspended with the same volume, using assay buffer. The analysis was performed using the same EIA protocol as for salivary cortisol (item 2.4), and cortisone was analyzed by an EIA first described by Rettenbacher et al., 2004.

2.7. Weaning and emotionality tests on piglets

The piglets were weaned at 28 days of age, vaccinated (vaccines against Porcine circovirus, *Streptococcus suis*, *Haemophilus parasuis*, *Mycoplasma hyopneumoniae*), and transported from Fazenda Araporanga in Jaguariaíva-PR (where the first stage of the experiment was carried out) to the Fernando Costa Campus of the University of São Paulo in Pirassununga-SP, with an approximately eight-hour travel. One pair of piglets per sow was used for the second part of the experiment. During the transportation, each pair was placed in a box (73.5 cm long, 53 cm wide, 21 cm high) lined with straw (hay).

After weaning, the animals were kept in suspended pens, with six litters kept in the same pen. Each pen consisted of 12 animals: a pair from each sow, grouped according to their mothers during gestation treatment, totaling six pens. The piglets had access to water and food *ad libitum*. The piglets were weighted on Days 28 and 42. Behavioral analysis was performed based on the videos recorded by a video camera installed in the roof. The piglets were observed during six consecutive days, from 4h00 to 7h00, assessing five uninterrupted minutes per piglet, adding up to 20 min per piglet, per day. The behaviors observed were piglet directed behavior (nosing), and aggressive behavior.

For fear tests, a combination of open field and novel object tests (Zupan et al., 2016) was performed to assess fear levels and exploratory motivations of each animal. The tests were conducted at 41 days of age. The piglets were tested one by one, returned to the pen immediately after the test, and withdrawn one at a time sequentially between the pens, so that the absence of one individual from the group would be balanced over time. The combination of the tests allowed a previous habituation of the piglets in the arena test, in which the open field test preceded the novel object test. The animals were individually tested in the arena (243 cm x 194.5 cm), which contained demarcations on the ground, forming quadrants. Each test lasted five minutes, for a total of 10 min. Each piglet was gently placed in a predetermined location in the arena and recorded during the test period. From the recording, the behaviors were analyzed and the latency to walk was quantified, as well as the number of central and lateral quadrants accessed, walking time, freezing time, and vocalizations (events). After this test, a novel object (traffic cone) was inserted by a pulley system in the center of the pen. We recorded subsequent behaviors for five minutes. In this test, the latency for walking, time near the object (quadrants surrounding the object), time exploring the object (close to the object with the head facing the object or physically interacting with it), freezing time, and vocalizations (events) were evaluated. After each animal was tested, the pen was washed with water to reduce possible chemical cues, as well as to remove feces and urine from the pen. The analyzer was blind to treatment.

2.8. Skin lesions on piglets

To evaluate aggressiveness, the number of skin lesions was counted using photographs, (Guy et al., 2009) performed on weaning day (Day 28), and later on Days 29, 35, 36, 42, and 43. Each piglet was restrained and individually photographed, in which photos of the body, back, face, ears, and neck were recorded on both sides, totaling six photos per animal per day of registration. The analyzer was blinded to treatment.

2.9. Piglet saliva collection

As in sows, the piglet's saliva collections aimed to assess the activity of the HPA-axis. The samples were collected when the piglets were 28, 29, 35, and 36 days old, with samples collected individually at 0600 h and 1800 h. The collection material and methods were the same as in the sows (see 2.4). The animal chewed the cotton until saliva saturation. Piglets do not accumulate significant amounts of saliva, so the first cotton was used and each sample was placed in a 15-ml falcon tube, placed in a box with ice, and then frozen at -20 °C until processing. The cortisol analysis, using EIA, followed the same protocol as described before (item 2.4).

2.10. Data analysis

For analysis, we first tested the normality of the data using the Shapiro-wilk test. The statistical tests were performed using the software R studio, using the scripts for each test, specified in the respective figures. We compared both groups (enrichment and barren conditions), by *t*-test when the data was normal based on Shapiro-wilk outcomes, or Mann-Whitney U Test, when there was no normality. We considered each individual as the statistical unit. The significance level adopted was $p < 0.05$ and the data are presented by mean (± SEM). Data that did not show normality were tested using a corresponding non-parametric test. The data is presented in mean and standard error.

3. Results

The environmental enrichment changed positively behavior and HPA axis in the sows, as well as in their offspring. There was difference in the stereotypic behavior expressed between treatments (Fig. 1). There was no difference in the duration of lying down laterally ($p > 0.05$ in all days). There was no difference in the number of piglets born alive (T test; $p = 0.77$; $F = 1.11$), total number of piglets born (Mann-Whitney U Test; $p = 0.40$; $Z = 0.83$), and farrowing duration (Mann-Whitney U Test; $p = 0.66$; $Z = 0.42$). Saliva cortisol concentrations were the same at each time point, during gestation before

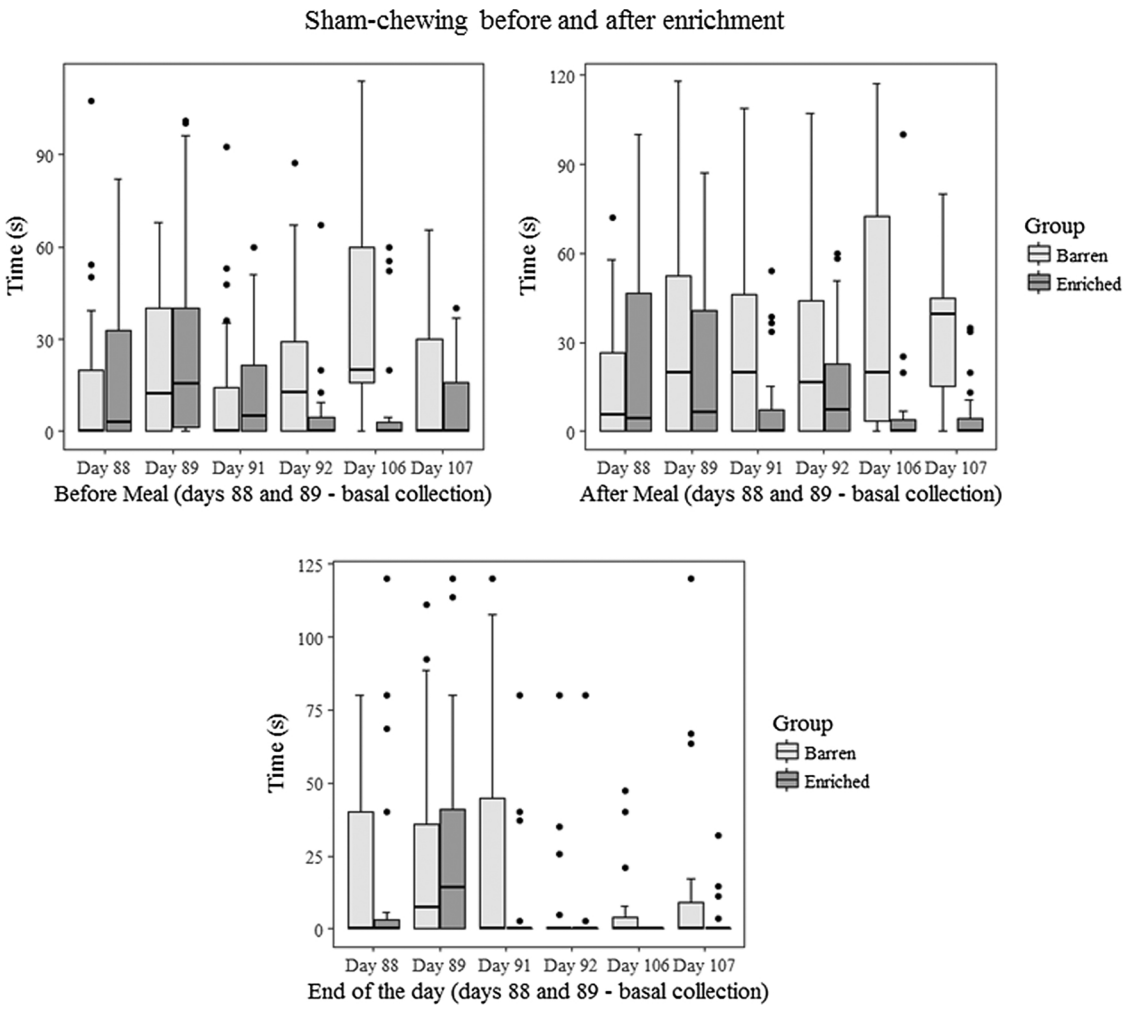

Fig. 1. Stereotypic behavior before (basal Days: 88 and 89) and after enrichment (Days 91, 92, 106 and 107). The barren treatment expressed more sham-chewing before meals on Days 92 (Mann-Whitney U Test; p = 0.001; Z = 3.14) and 106 (Mann-Whitney U Test; p = 0.00006; Z = 4.01). After meals, they expressed more sham-chewing on Days 91 (Mann-Whitney U Test; p = 0.004; Z = 2.81), 106 (Mann-Whitney U Test; p = 0.001; Z = 3.20), and 107 (Mann-Whitney U Test; p = 0.00006; Z = 3.99). There was no difference in sham-chewing in the afternoon (performed in the end of each day of samples collection).

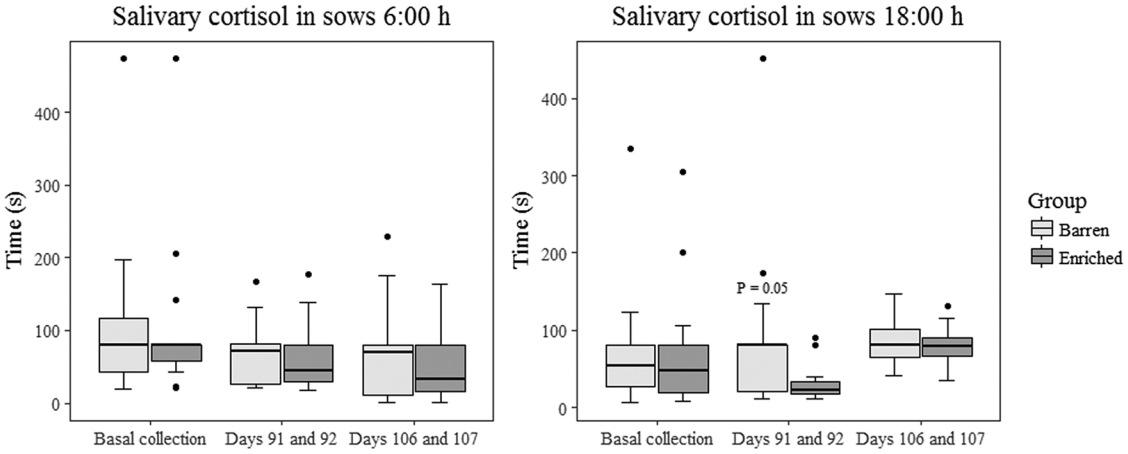

Fig. 2. The control group showed higher saliva cortisol concentrations on Days 91 and 92 (Mann-Whitney U Test; p = 0.05; Z = 1.92) at 6:00 pm. There was no difference in the morning on Days 88 and 89 (Mann-Whitney U Test; p = 0.75; Z = 0.31), Days 91 and 92 (Mann-Whitney U Test; p = 0.77; Z = 0.28), or Days 106 and 107 (Mann-Whitney U Test; p = 0.83; Z = 0.20). There was no difference at 6:00 pm on Days 88 and 89 (*t*-test; p = 0.80; F = 1.05) or Days 106 and 107 (*t*-test; p = 0.37; F = 1.53).

enrichment and in the basal sampling (Days 88 and 89), showing that the sows had similar HPA-axis activity. The only difference between groups was a higher cortisol concentration in the afternoon in the control group (Days 91 and 92; Fig. 2). There was no difference in the glucocorticoid concentrations in the placenta tissue (Fig. 3).

The piglets born from enriched sows had higher cortisol

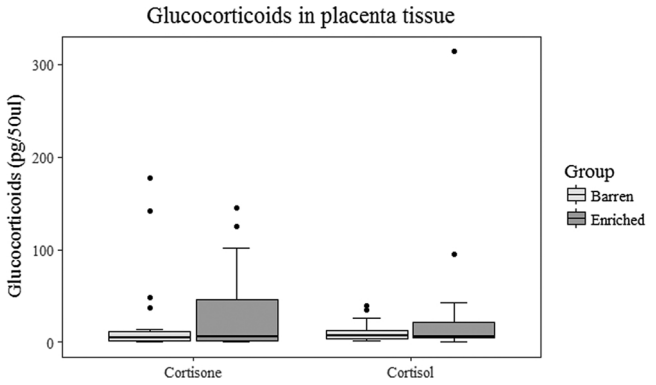

Fig. 3. Mean (± SEM) concentrations of 19 sows in the barren and 15 sows in the enriched treatment. There was neither a difference in the cortisol (Mann-Whitney U Test; p = 0.84; Z = -0.19) nor in cortisone concentrations (Mann-Whitney U Test; p = 0.61; Z = -0.50).

concentrations on the weaning day (Fig. 4). However, on day 35, piglets from sows with environmental enrichment showed lower cortisol concentrations (Fig. 4). In the afternoon, representing lower cortisol levels due to the circadian rhythm, piglets born from sows kept in the enriched environment had lower cortisol levels (Fig. 4). In addition, piglets born from sows kept in the enriched environment spent less time performing nosing on Days 4 and 5 (Fig. 5) and had less aggressive behavior (Fig. 6). Piglets born from sows in the enriched environment had lower skin lesion scores on day 42 (Fig. 7). There was no difference in the piglets' emotionality when we compared males and females. However, when the data were divided based on sex, there was a difference in the females' exploratory behavior (Fig. 8).

4. Discussion

We showed that environmental enrichment can change the offspring`s HPA-axis activity and behavior. Providing straw at the end of gestation in sows affected piglet's behaviors, such as aggression and nosing, which were both higher in the offspring from sows kept in the barren environment. Regarding emotionality, there was no difference when we compared groups with both sexes together. However, there was a sex-specific difference, in which females born from sows kept with environmental enrichment explored more and showed less fear. We also demonstrated that environmental enrichment during the end of gestation could change the HPA-axis in the offspring; higher salivary cortisol concentrations were observed in piglets born from sows kept in

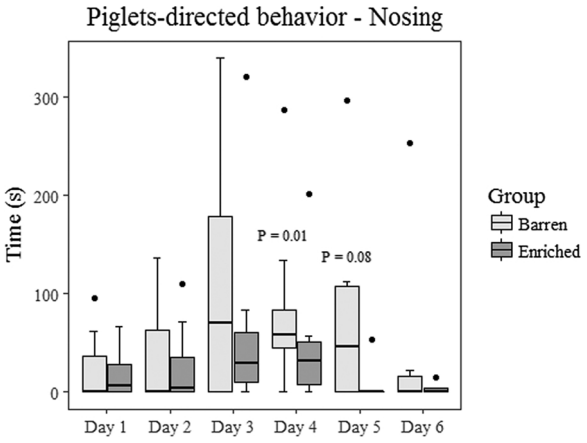

Fig. 5. The offspring from barren sows spent more time nosing on Days 4 (Mann-Whitney U Test; p = 0.01; Z = 2.51) and 5 (Mann-Whitney U Test; p = 0.08; Z = 1.70).

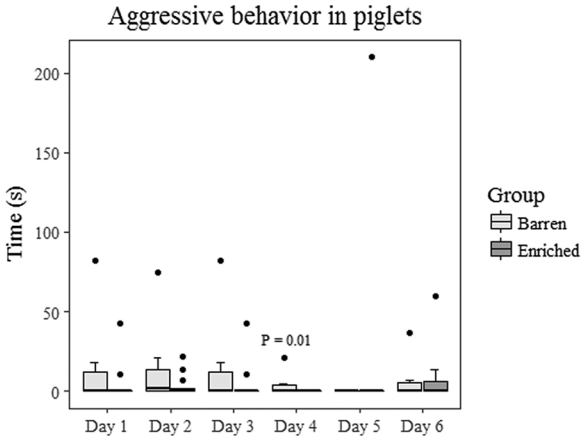

Fig. 6. In Day 4 the offspring from barren sows spent more time showing aggressive behavior (Mann-Whitney U Test; p = 0.01; Z = 2.42).

the barren environment. In the sows, salivary cortisol was higher only at the afternoon collection in sows maintained in the barren environment and there was no difference in glucocorticoid concentrations of placental tissue.

In the first part of our study, we showed that environmental

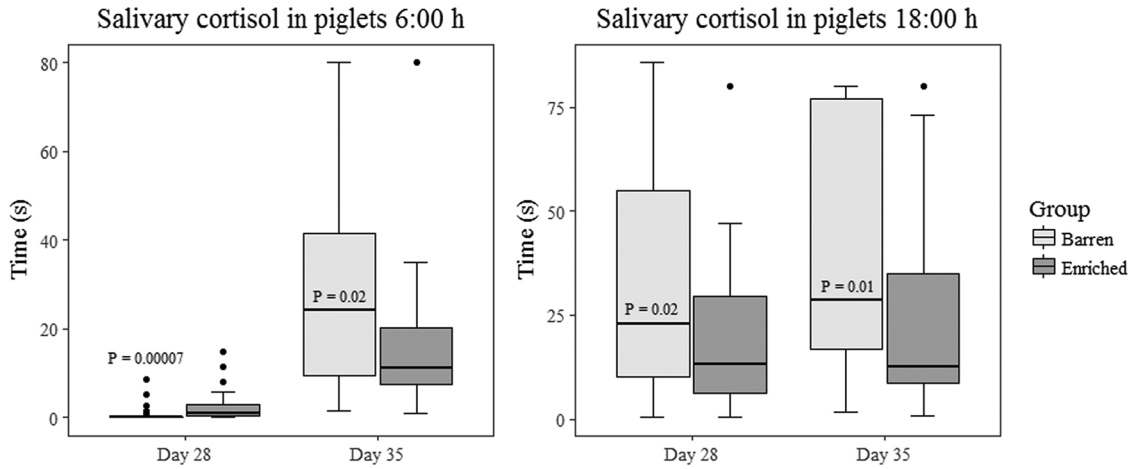

Fig. 4. In the morning offspring from enriched sows had higher cortisol concentrations on the weaning day (Day 28; Mann-Whitney U Test; p = 0.00007; Z = -3.37). On day 35, offspring from barren sows had higher cortisol concentrations (Mann-Whitney U Test; p = 0.02; Z = 2.21). In the afternoon, offspring from barren sows had higher cortisol concentrations on day 28 (Mann-Whitney U Test; p = 0.02; Z = 2.28) and on day 35 (Mann-Whitney U Test; p = 0.01; Z = 2.37).

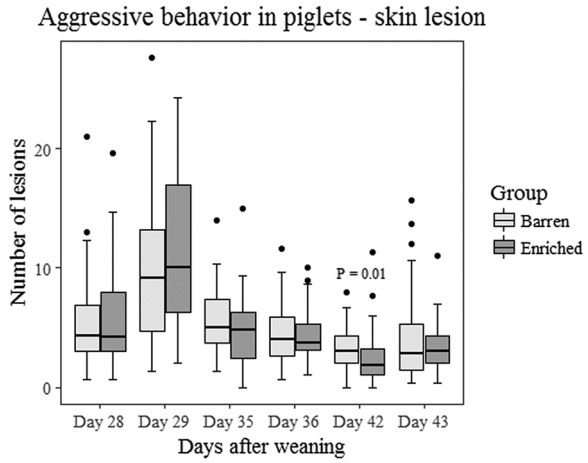

Fig. 7. Piglets from enriched sows had lower skin lesion scores on day 42 after weaning (Mann-Whitney U Test; p = 0.01; Z = -2.48).

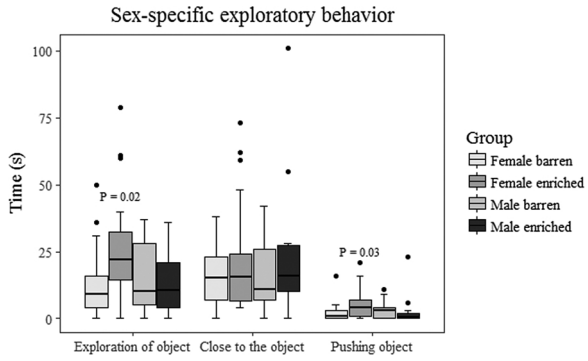

Fig. 8. The female offspring from enriched sows expressed higher object exploration (Mann-Whitney U Test; p = 0.02; Z = -2.22), and spent more time pushing the object (Mann-Whitney U Test; p = 0.03; Z = -2.08).

enrichment changed sow behavior by reducing stereotypic behavior (sham-chewing). We expected the oral stereotypic behavior reduction from the addition of straw, which is in agreement with other studies (Fraser, 1975; Spoolder et al., 1995). For salivary cortisol concentrations, there was only a difference in one measure (Days 91 and 92 of gestation) in the afternoon, with higher levels in sows kept in barren environment. The morning peak in the cortisol curve is similar between sows, because it plays a role in the circadian rhythm and there was no straw effect. However, in the afternoon, cortisol concentrations were lower in sows subjected to environmental enrichment. As expected, there was no difference in the basal measures (Days 88 and 89 of gestation). This result also suggests that the effect we showed was a result of the treatment, not to a possible difference in the individuals or groups (e.g. high stress levels in a pen associated with the social context). The absence of differences on Days 106 and 107 of gestation could be due to the physiology in the sows changing to prepare them for delivery, overlapping with the straw effect.

In the placental tissue there was no difference in the glucocorticoid concentrations between sows kept in barren or enriched environments. The placenta protects the fetus during gestation. In placenta physiology, the 11β-hydroxysteroid dehydrogenase inactivates cortisol by converting it into cortisone (and vice versa), thus regulating glucocorticoid availability and their effects on the neural receptors. During development, the amount of glucocorticoids that the fetus receives from the mother has the potential to completely change the trajectory of the offspring (Abe et al., 2007; Fowden et al., 2016; Nolvi et al., 2016; Rutherford et al., 2014). The alterations in the offspring HPA-axis activity in our results, as a reflex of the stress response, indicate that the glucocorticoid-associated mechanisms are altered.

For the piglets, Day 28 represented a challenge because it was the weaning day. In the morning, salivary cortisol concentrations were higher in piglets born from sows kept in environmental enrichment. It is possible that their responsiveness to stressful events was more functional and efficient. The combination of weaning and transportation was a huge stressor, which raises HPA-axis activity in order to overcome it. In other words, they were coping properly with stressful events. However, in the afternoon for both measures (Day 28 and Day 35), during which time cortisol levels are reduced as part of the circadian rhythm, piglets born from sows in the barren environment showed higher cortisol concentrations. In this case when they were not dealing with an acute stressor, the cortisol concentration was an indicator that the HPA-axis in these piglets was more active.

Glucocorticoids are important stress hormones in adult animals, but the functions range more widely in the fetus (Fowden et al., 2016; Moisiadis and Matthews, 2014). Glucocorticoids have a non-linear "U-shape function", in which low or high concentrations can cause negative effects on emotionality and learning (Lupien and Lepage, 2001), and the effects are completely different depending on the gestational age, severity, and duration of the exposure (Fowden et al., 2016). Later in gestation when the fetal HPA-axis has functionally developed, fetal glucocorticoid concentrations can also work independently of maternal levels through cortisol secretion from the fetal adrenal glands. This can occur through HPA-axis activation in response to adverse intrauterine conditions, such as hypoxia and hypoglycemia (Fowden et al., 2016).

There was a difference in the behavior of piglets with regard to nosing and aggressiveness. Nosing was higher in the offspring born from sows in the barren environment on day four and showed a tendency on day five. Nosing is an undesirable piglet-directed behavior expressed in piglets after weaning that can sometimes be a trigger for aggressiveness (personal observation) and cause skin lesions in the receiver when persistently performed, as well as belly-nosing (Gardner et al., 2001). When this behavior pattern occurs before suckling and milk intake, it may be associated with hunger or feeding (Gonyou et al., 1998). However, there is a negative correlation between suckling behavior on the sow and nosing after weaning (Torrey and Widowski, 2006). There are differences between nosing (as a piglet-directed behavior) and belly-nosing; the levels in nosing remain much more consistent over time and are seen from the first day after weaning. We consider the nosing we observed to be a stereotypic behavior, since it agrees with the definition of a repetitive behavior without obvious function (Mason, 1991).

Aggressiveness was also higher in the piglets born from sows kept in the barren environment on day four of behavioral analysis, and the same result was at day 42 in skin lesion. The stress experienced by the mother could have changed the development of the amygdala, a part of the limbic system. The amygdala is a brain structure that mediates fear, anxiety, aggressiveness, and emotional learning (Balleine and Killcross, 2006; Chiba, 1996).

Emotionality, assessed by fear tests, was not different when we compared males and females in both treatments. However, our results showed that females from sows in enriched conditions explored the novel object more, indicating that they are less afraid and have more exploratory motivations. The placenta works in a different way with respect to sex and the metabolism of glucocorticoids by 11β-hydroxysteroid dehydrogenase type 2, which sometimes auto regulates in females but not in males (Stark et al., 2009). This difference has sex-specific consequences (Mukhopadhyay et al., 2016), in which the placenta can protect the female fetus from excess glucocorticoid exposure, enabling appropriate adrenal responses to physiological stressors (Stark et al., 2009). In our data, we cannot assess the effects of placental glucocorticoids because we pooled samples without regard to sex.

We also have to consider that the effects we found may not be a result of the prenatal environment, since the effects from the mother-infant relationship have been widely demonstrated (Mogi et al., 2011).

Environmental enrichment during gestation could change the mothers' behavior, or even alter traits like anxiety; then the effects that we saw in the piglets could be related to the mothers' behavior during lactation instead of during the prenatal period. An alternative explanation for the effects in the offspring is the early postnatal period, in which the HPA-axis altered during gestation releases high concentrations of cortisol, and as a consequence changes the brain. In both cases, our outcomes would not be related to the prenatal period and we have to consider these possibilities.

In addition, in our study, it was difficult to collect saliva from the animals kept in the enriched pens, especially in the collection performed at 1800 h, because they were apparently in a deeper sleep than the control animals. These observations may be because the animals were more active during the day, interacting with the straw and entering deeper sleep states. Some studies have shown that environmental enrichment may alter the circadian rhythm (De Groot et al., 2000; Mirmiran et al., 2003; Ruis et al., 1997), and may possibly alter the quality of sleep states. Sleep states can be related to metabolism pathways and can change the cortisol circadian rhythm.

Regarding the way that the animals lay down, we did not observe an effect of the substrate in the choice between lying down ventrally or laterally. Pregnant sows fed a higher volume (fiber-rich diets) are more satiated and spend more time lying laterally (Zonderland et al., 2004). These observations are relevant because a possible undesirable interfering variable would be the difference in satiety, since the substrate was consumed. However, we consider the straw to be extremely important because it is biologically relevant to pigs. Straw consumption may be an important factor for animal welfare, which suggests the possibility of controlling some variables in relation to their state. The possibility to interact with the environment in which the animal is placed increases the likelihood of adjusting to the environment.

5. Conclusions

In this study, we have shown that environmental enrichment during end of gestation changed the offspring phenotype, making them more adjusted to their environment. The behavioral and physiological indicators were consistent, corroborating our hypothesis. Swine was the model used in this study and we suggest that environmental enrichment should be used during gestation in animals for avoiding or reducing stress.

Authors contribution

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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