Prenatal transportation stress alters temperament and serum cortisol concentrations in suckling Brahman calves1,2


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ABSTRACT: This experiment examined the relationship between prenatal stress and subsequent calf temperament through weaning. The prenatal stressor used was repeated transportation of pregnant Brahman cows for 2 h at 60 ± 5, 80 ± 5, 100 ± 5, 120 ± 5, and 140 ± 5 d of gestation. Prenatally stressed calves (n = 41) were compared with controls (n = 44; dams did not undergo transportation during pregnancy) from 2 wk of age until weaning (average age at weaning = 174.8 ± 1.3 d). Temperament was defined by pen score (PS; 1 = calm and 5 = excitable), exit velocity (EV; m/sec), and temperament score (TS; (PS + EV)/2) and was recorded for each calf on d –168, –140, –112, –84, –56, –28, and 0 relative to weaning (d 0 = weaning). Cortisol concentrations were determined in serum samples obtained on d –168, –140, –28, and 0 relative to weaning. Birth weight and weaning weight were not different between treatment groups (P > 0.1). Exit velocity was greater (P = 0.03) in prenatally stressed calves (2.84 ± 0.21) relative to controls (2.31 ± 0.21). Exit velocity was greater (P < 0.01) in prenatally stressed calves (2.1 ± 0.14 m/sec) than in controls (1.61 ± 0.14 m/sec). Exit velocity was affected by a treatment × calf sex interaction (P = 0.04) and was greater in prenatally stressed females. Exit velocity was also affected by day (P < 0.0001). Temperament score was greater (P = 0.01) in prenatally stressed calves (2.45 ± 0.16) than in controls (1.95 ± 0.16). Temperament score was affected by day (P < 0.01). Basal cortisol concentrations were greater (P = 0.04) in prenatally stressed calves (15.87 ± 1.04 ng/mL) than in controls (13.42 ± 1.03 ng/mL). Basal cortisol concentrations were greater (P < 0.01) in females (16.61 ± 1.06 ng/mL) than in males (12.68 ± 1.02 ng/mL). Cortisol concentrations were positively correlated (P < 0.01) with PS (r = 0.55, P < 0.01), EV (r = 0.4, P < 0.01), and TS (r = 0.55, P < 0.01). Overall, suckling Brahman calves that were prenatally stressed were more temperamental and had greater circulating serum concentrations of cortisol than control calves.

Key words: calves, cortisol, prenatal stress, temperament


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INTRODUCTION

Current concepts of stress biology were derived from the foundational writings of Cannon (1929) and Selye (1936), which define the roles of the sympathomedullary system and hypothalamic–pituitary–adrenal (HPA) axis in regulating homeostasis in growing and mature animals. Scientists have focused on potential adverse effects of weaning and transportation on health and performance of livestock for various scientific, societal, and economic reasons. Recognition that one’s health begins in utero led to assessment of the impact of prenatal stress on postnatal behavior, health, reproduction, growth, and performance. Transportation during gestation is a stressor for pregnant cows as evidenced by increased serum cortisol (Lay et al., 1996; Price et al., 2015). Furthermore, adrenocortical function of suckling calves was altered due to prenatal transportation stress (Lay et al., 1997a,b). Elevated maternal cortisol may alter the fetal environment, thereby affecting fetal programming (the fetal response to a specific insult during a critical period of prenatal development; Zambrano et al., 2014). Alterations in the fetal environment program the fetal HPA axis to prepare the neonate to survive in a stressful postnatal environment (Matthews, 2002). Such programming may include mechanisms that lead to alterations in temperament (aggressiveness, behavioral reactivity to humans and novel environments, willingness to take risks, exploration, and sociality; Fordyce et al., 1988; Réale et al., 2007). It has been reported that temperamental cattle have increased circulating concentrations of catecholamines and cortisol (Stahringer et al., 1990; Curley et al., 2006, 2008; Burdick et al., 2009, 2010). Because temperament and the HPA axis are interrelated, the objective of this study was to determine if prenatal transportation stress altered temperament as well as adrenocortical function in suckling Brahman calves.

MATERIALS AND METHODS

All experimental procedures were in compliance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) and were approved by the Texas A&M University Animal Care and Use Committee.

Animal Procedures

Ninety-six pregnant Brahman cows (Bos indicus) were determined pregnant by rectal palpation 45 d after the last breeding date. At that time, each cow was assigned to 1 of 2 treatment groups (n = 48 prenatally stressed [PNS] and n = 48 control) based on age, parity, and temperament. Dam temperament was balanced (Table 1) between treatment groups using each cow’s pen score (PS), exit velocity (EV), and temperament score (TS) at weaning as well as each cow’s mature temperament classification (MTC). The stressor consisted of 5 transportation events at 60 ± 5, 80 ± 5, 100 ± 5, 120 ± 5, and 140 ± 5 d of gestation (Price et al., 2015). Immediately before and after each transportation event, blood samples were collected from the tailvein. During each transportation event, cows were transported in the same 3-section trailer (2.4 by 7.3 m) that was towed by a three-quarter ton truck on smooth highways for a total of 2 h. This treatment was modeled after a previous study that resulted in differences in the function of the HPA axis in calves born to cows transported at these stages of gestation (Lay et al., 1997b). Control cows were maintained in the same manner as stressed cows with the exception of being transported. The 2 groups were housed in the same pasture (at the Texas A&M AgriLife Research Center in Overton, TX) and fed the same diet. The diet consisted of ad libitum coastal bermudagrass in pastures during the summer and fall. The same pastures were overseeded with rye (Secale cereale) and ryegrass (Lolium multiflorum). Cows were supplemented with coastal bermudagrass (Cynodon dactylon) hay and a 3:1 corn:soybean meal mix as required depending on forage quality and availability. From these cows, controls gave birth to 26 males and 18 females and cows that were stressed by transportation during gestation gave birth to 20 males and 21 females. Male calves were maintained as bulls throughout the study. Birth weight was recorded within 24 h after birth, and weaning weight (WWW) was recorded on the day each calf was weaned. Data were recorded for temperament traits on d –168, –140, –112, –84, –56, –28, and 0 relative to weaning (d 0 = weaning; average age at weaning = 174.8 ± 1.3 d of age). Each calf was manually restrained on d –168 and –140 for less than 5 min, and each calf was restrained in a squeeze chute on d –112 through 0 for less than 5 min. Whole blood samples (2 × 10 mL) were collected via jugular venipuncture into 10-mL uncoated vacuum tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) with no additives using a sterile 18-gauge needle on d –168, –140, –28, and 0 relative to weaning. Blood samples were refrigerated over-

| Table 1. Temperament variables of pregnant dams within control and transport treatment groups1 |
|----------------------------------|----------------|------------------|------------------|
| Item2                           | Control (n = 44) | Transport (n = 41) | P-value |
| Weaning PS                       | 2.74 (0.17)     | 2.66 (0.17)      | 0.74          |
| Weaning EV                       | 2.54 (0.17)     | 2.51 (0.18)      | 0.90          |
| Weaning TS                       | 2.64 (0.14)     | 2.59 (0.15)      | 0.79          |
| MTC                             | 2.02 (0.10)     | 2.0 (0.10)       | 0.87          |

1Data compare control and transported dam treatment groups and are presented as least squares means (SE).
2Data are represented for dams’ weaning pen score (PS; scale of 1 to 5), weaning exit velocity (EV; m/s), weaning temperament score (TS; PS + EV)/2, and mature temperament classification (MTC; scale of 1–3).
night at 4°C before being centrifuged at 1,700 × g for 25 min at 6°C and processed to yield serum. The serum was stored in aliquots at −20°C until determination of cortisol concentrations.

**Temperament Traits**

Three measures of calf temperament—PS, EV, and an overall TS—were assessed (Burdick et al., 2009). Before weighing, blood sampling, and recording EV, PS was recorded to assess behavior (recorded by the same experienced evaluator throughout the study). Calves were separated from their dams (dams were in a separate uncovered pen that was 100 m away from the uncovered pens containing the calves), herded into a large uncovered pen (18 by 36 m), and 45 min ± 15 min after that they were herded into a confined neighboring uncovered pen (18 by 18 m) in groups of 3 to 5. Pen scores were individually assigned on a subjective scale of 1 to 5 according to the following classifications: 1 = calves walked slowly and were not excited by the evaluator; 2 = calves ran along fences and kept their distance from the evaluator; 3 = calves’ heads were high and they avoided the evaluator and ran when approached by the evaluator but stopped before hitting fences; 4 = calves stayed at the back of the group with their heads high, were very aware of humans, and often ran into fences; and 5 = calves were very excited or aggressive, ran into fences, and ran over anything in their path (Hammond et al., 1996). After PS was evaluated in each group of 3 to 5 calves, that group of calves was herded into a separate neighboring uncovered pen, where they remained until PS was recorded for each individual calf. Exit velocity was recorded after pen scoring, weighing, and blood sampling. Exit velocity was defined as the rate, measured in meters per second, at which an animal traversed 1.83 m on exiting a squeeze chute (Burrow et al., 1988; Curley et al., 2006) using an infrared beam sensor system (FarmTek Inc., North Wylie, TX). Temperament score ((PS + EV)/2) was determined as previously reported (Curley et al., 2006; King et al., 2006). Mature temperament classification (a subjective measurement) was 1 of 3 variables used to assign pregnant cows to either the control or the transport treatment group. Before pregnancy testing, MTC was assigned when there was no calf at the cow’s side. Each cow’s MTC was assigned by a single experienced evaluator (the same individual assigned all MTC in the present study). In the presence of the evaluator, MTC was assigned to each individual animal on a scale of 1 to 3 according to the following classifications: 1 = calm (cows did not rapidly move away from the evaluator and were not aggressive toward the evaluator), 2 = intermediate (cows moved away from the evaluator but were not excited by the evaluator’s presence), and 3 = temperamental (cows rapidly avoided the evaluator, were easily excited by the presence of the evaluator, and may be aggressive toward the evaluator; Stahringer et al., 1990). Of the 96 Brahman cows assigned to the study, 85 cows gave birth and raised a calf. Therefore, the subsequent least squares means and SE for temperament measures between transported and control dam treatment groups are represented in Table 1. These temperament measures include the dams’ PS, EV, and TS when they themselves were weaned as well as the dams’ MTC, all of which were similar between treatment groups.

**Cortisol**

Serum concentrations of cortisol were determined using a solid phase RIA (DSL-2100; Diagnostic Systems Labs, Webster, TX) using antiserum-coated tubes according to the manufacturer’s directions (Burdick et al., 2009). Serum cortisol concentrations were determined by the average of duplicate unknown samples compared with a standard curve generated from known concentrations of cortisol using Assay Zap software (Biosoft, Cambridge, UK). The minimum detectable cortisol concentration for this assay was 1.2 ng/mL, and the intra- and interassay CV were 6.4 and 5.6%, respectively. Data are presented as concentration in nanograms per milliliter.

**Statistical Analysis**

Dam temperament variables were analyzed using Mixed Models procedures of SAS (SAS Inst. Inc., Cary, NC) with treatment (control or transport) as a fixed effect. Birth weight and WW data were analyzed using Mixed Models procedures of SAS with treatment (control or PNS) and calf sex as fixed effects and sire as a random effect. Age (d) at weaning was a covariate in the model for WW. Temperament and cortisol data were analyzed using Mixed Models procedures of SAS specific for repeated measures. The model included treatment, calf sex, day, and their interactions as fixed effects and sire as a random effect. Data are reported as least squares means (±SE). To compare PS, EV, and TS, Spearman correlation coefficients were obtained using SAS.

**RESULTS**

**Birth Weight and Weaning Weight**

The birth weight and WW were not affected by any interaction or the main effect of treatment (P >
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Temperament Traits

Calf PS was not affected by a treatment × calf sex interaction (Fig. 1), any other interactions, calf sex, or day (P > 0.1). However, PS was greater (P = 0.03) in PNS calves than in control calves (Table 2). Calf EV was affected by a treatment × calf sex interaction and was greater (P = 0.04) in PNS females (2.34 ± 0.17 m/s) than in control females (1.52 ± 0.19 m/s) but was similar in PNS males (1.85 ± 0.18 m/s) and control males (1.7 ± 0.16 m/s; Fig. 2). There were no other significant interactions for EV. However, EV was greater (P < 0.01) in PNS calves than in control calves (Table 2). Exit velocity was also affected by day (P < 0.01) and increased from d –168 until –112 (Fig. 3). Calf TS was not affected by a treatment × calf sex interaction (Fig. 3), any other interactions, or calf sex (P > 0.1). Temperament score was affected by day (P < 0.01; Fig. 4) and increased from d –168 until –112. Temperament score was greater (P = 0.01) in PNS calves compared with control calves (Table 2).

Cortisol

Serum concentrations of cortisol were not affected by any interactions nor were they affected by day (P > 0.1). Serum concentrations of cortisol were greater (P = 0.04) in PNS calves relative to control calves (Table 2) and were greater (P < 0.01) in females (16.61 ± 1.06 ng/mL) compared with males (12.68 ± 1.02 ng/mL).

Table 2. Main effects of treatment on pen score, exit velocity, temperament score, and serum cortisol

<table>
<thead>
<tr>
<th>Item²</th>
<th>Control (n = 44)</th>
<th>PNS (n = 41)</th>
<th>P-value³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pen score</td>
<td>2.31 (0.21)</td>
<td>2.84 (0.21)</td>
<td>0.03</td>
</tr>
<tr>
<td>Exit velocity, m/s</td>
<td>1.61 (0.14)</td>
<td>2.10 (0.14)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Temperament score</td>
<td>1.95 (0.16)</td>
<td>2.45 (0.16)</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum cortisol, ng/mL</td>
<td>13.42 (1.03)</td>
<td>15.87 (1.04)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

¹Data compare control and prenatal stressed (PNS) treatment groups and are presented as least squares means (SE).

²Data for pen score (scale of 1 to 5), exit velocity (m/s), and temperament score ((pen score + exit velocity)/2) consists of records for each calf on d –168, –140, –112, –84, –56, –28, and 0 relative to weaning (d 0 = weaning). Data for serum cortisol concentrations (ng/mL) consist of records for d –168, –140, –28, and 0 relative to weaning.

Correlations

Pen score with EV (r = 0.56), PS with TS (r = 0.93), and EV with TS (r = 0.82) were positively correlated (P < 0.01). Serum cortisol concentrations were positively correlated (P < 0.01) with PS (r = 0.55), EV (r = 0.40), and TS (r = 0.55).

DISCUSSION

The present study demonstrated that prenatal transportation stress did not alter calf birth weight or WW. Because all PNS dams were stressed at 5 stages of gestation, this lab group was unable to determine if effects of prenatal stress were derived from an accumulation of the 5 transportation events or if one or more of these stressful events caused the effects of prenatal stress reported in this study. As previously reported by Price et al. (2015), pregnant Brahman cows in this study had increased circulating concentrations of cortisol and glucose after each transportation event and tended to exhibit increased vaginal temperatures during the transportation process. These measures indicate that the transported cows experienced a stress response, as increased cortisol (Lefcourt and Elsasser, 1995), glucose (Price et al., 2015), and vaginal temperature (Price et al., 2015) are indicators of a stress response in cattle. Stress incurred by transported dams during gestation altered their calves’ temperament as measured by PS, EV, and TS as well as adrenocortical function as measured by serum concentration of cortisol. Prenatal stressors (electrical shocks) encountered by rats have been associated with increased plasma concentrations of maternal and fetal glucocorticoids (Takahashi et al., 1998). Additionally, various prenatal stressors and resultant increased exposure to stress hormones altered fetal development and led to adverse health issues. Examples of these issues include hypertension (Benediktsson et al., 1993) and glucose intolerance (Nyirenda et al., 1998) in rats and altered sexual development in rats, sheep,
and nonhuman primates (Zambrano et al., 2014) as well as neurological and behavioral disorders such as autism, schizophrenia, anxiety, and depression in humans (O’Donnell et al., 2009). The subsequent discussion describes potential effects of prenatal stress on postnatal BW, temperament, and adrenocortical function.

**Birth and Weaning Weights**

As expected, birth weights were heavier for males than females in the present study, which is in agreement with Browning et al. (1995). However, the birth weights and WW of beef calves in the present study did not differ between the control and PNS groups. Previous literature on this subject is contradictory. For example, Drake et al. (2005) reported that prenatal dexamethasone exposure on d 15 through 21 of gestation was associated with decreased birth weight in rats. In contrast, Roussel et al. (2004) reported that prenatal stress (gestating dams were kept in isolation while a dog was present) was associated with increased birth weight in PNS lambs relative to control lambs. In pigs, ADG from birth until weaning was lower in pigs whose dams were stressed by restraint for 5 min/d from 85 to 110 d of gestation compared with control pigs (Reyna et al., 2006). Due to differences between species in gestation length and stage of development at birth, it is difficult to relate these conflicting results to the present study.

**Temperament and the Hypothalamic–Pituitary–Adrenal Axis**

Temperament is a heritable trait in Brahman cattle (Schmidt et al., 2014). In the present study, temperament was balanced across control and PNS groups of pregnant cows to avoid the influence of dam temperament on the effect of prenatal stress on calf temperament or adrenocortical function. Prenatally stressed calves were more temperamental than control calves through weaning in the present study. Rats whose dams were exposed to periodic sessions of bell noises and flashing lights throughout gestation were more anxious than control rats as demonstrated by less time spent in open areas when placed in a maze that was partially enclosed by walls and partially without walls at 6 mo of age (Fride and Weinstock, 1988). Prenatally stressed rats whose dams were exposed to electrical shock daily from 2 to 20 d of gestation exhibited a longer defense mechanism of freezing (remaining immobile) than control rats in response to foot shock (Takahashi et al., 1992). Furthermore, the effects of prenatal stress on temperament and behavior may be long lasting. At 4 yr of age, PNS monkeys (dams were placed in a small dark cage and exposed to loud noise 5 d/wk from 90 to 145 d of gestation) were less exploratory and more vocal (indicating distress) and exhibited greater locomotion and disturbance on separation from cage mates (Clarke et al., 1996). In the present study, preweaning EV and TS increased from d –168 until –112 before weaning (d 0 = weaning). Burdick et al. (2011) reported that EV increases over time (Burdick et al. recorded EV every 28 d until 56 d after weaning) in Brahman calves. Consistent with prior reports (Burdick et al., 2009), temperament measures such as EV were greater in calves that had greater circulating concentrations of cortisol. Prenatally stressed calves had greater PS, EV, and TS and greater serum concentrations of cortisol. Temperament is interrelated with HPA axis function in the bovine, where circulating concentrations of cortisol have been shown to be greater in temperamental Brahman calves relative to calmer herdmates (Curley et al., 2006, 2008). Therefore, temperamental calves may be more responsive to environmental stressors compared with calmer calves (Curley et al., 2006, 2008). As previously reported by this laboratory, prenatal transportation stress (2 h at 60 ± 5, 80 ± 5, 100 ± 5, 120 ± 5, and 140 ± 5 d of gestation) was associated with altered adrenal function in Brahman calves.
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Additionally, PNS calves maintained greater plasma cortisol concentrations for a longer duration of time compared with control calves in response to restraint stress (Lay et al., 1997b). Prenatally stressed pigs (dams were restrained by snout snare for 5 min daily from 84 to 112 d of gestation) had greater serum concentrations of cortisol relative to their nonstressed counterparts (Collier et al., 2011). Blue foxes whose dams were exposed to handling stress daily for the final 15 d of gestation were more active (crawling, fighting, yelping, or showing signs of aggression) than controls when held by a human, which indicates greater distress in response to handling stress (Braastad et al., 1998). Overall, prenatal stress appears to be associated with altered behavior and adrenocortical function in multiple species.

Factors Affecting Temperament and Adrenocortical Function in Prenatally Stressed Calves

In the present study, PNS females had greater EV through weaning than control females, PNS males, or control males. Other investigators have reported female cattle to be more temperamental (Shrode and Hammack, 1971; Hoppe et al., 2010), with heifers having greater blood concentrations of cortisol compared with bulls (Lay et al., 1992; Hulbert et al., 2012) and steers having greater cortisol compared with bulls (Tennessee et al., 1984). In the present study, female calves had greater serum concentrations of cortisol relative to males. Effects of prenatal stress have been reported to be sexually dimorphic in species such as rats, pigs, foxes, and goats (McCormick et al., 1995; Braastad et al., 1998; Roussel et al., 2005; Rutherford et al., 2014). Female but not male rats whose dams were restrained in wire mesh daily from d 15 to 19 of gestation had increased plasma ACTH and corticosterone concentrations (McCormick et al., 1995). Additionally, 10-wk-old female but not male pigs whose mothers were exposed to a social stressor during gestation had greater corticotropin-releasing hormone receptors 1 and 2 in the amygdala and increased anxiety-like behavior as adolescents and as mothers (Rutherford et al., 2014). At 10 d of age, female but not male blue foxes whose dams were exposed to handling stress daily for the final 15 d of gestation had greater adrenal content of cortisol than controls (Braastad et al., 1998). Additionally, goats whose dams were repeatedly transported in isolation during the last trimester of gestation exhibited sexually dimorphic effects of prenatal stress (Roussel et al., 2005). Specifically, PNS female goats stopped drinking more often than control females when startled while drinking whereas PNS male goats stopped drinking less often than control males when startled (Roussel et al., 2005). Therefore, prenatal stress elicits sex-specific effects in species such as rats, pigs, foxes, and goats.

The stage of gestation in which a stressor affects the fetal environment may differentially affect postnatal function of male and female progeny. Developmental alterations are dependent on the timing and duration of the insult or stressor and likely act on distinct differentiating systems in the developing fetus. Mueller and Bale (2008) reported that prenatal stress incurred during the first 7 d of gestation by male mice resulted in maladaptive behavior in response to stress, increased HPA axis activity, modifications in glucocorticoid receptor (GR) and corticotropin-releasing factor (CRF) expression, and altered methylation of GR and CRF relative to control males. Stage of gestation in which a prenatal stressor is encountered affects alterations of the HPA axis in higher-order species such as sheep (Rakers et al., 2013). Pregnant sheep stressed by isolation (fully isolated from auditory, tactile, or visual contact with flock mates) twice each week for 3 h during gestation (30 to 100 d) had fetuses with greater cortisol concentrations in response to the stress of infusion of a vasodilator, sodium nitroprusside, relative to fetuses exposed to the same prenatal stressor later in gestation (100 to 120 d). Therefore, prenatal stress from d 30 to 100 had a greater effect on alterations of the fetal HPA axis in the ovine (Rakers et al., 2013). Contrasting effects of a different stressor were reported by Coulon et al. (2011), who stated that lambs whose dams were roughly handled daily during the last 35 d of gestation were slower to approach a human evaluator and fled a greater distance after they were startled. These data indicate that prenatal stress during late gestation was associated with increased fearfulness and altered behavior compared with lambs whose dams were gently handled. Further research is required to determine differential

Figure 4. Comparison of temperament score among control (white bars) and prenatally stressed (PNS; gray bars) male and female calves. Data are represented as least squares means (±SE) for 18 control females, 21 PNS females, 26 control males, and 20 PNS males. A,B Means for each variable with different superscripts differ (P < 0.05).

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alterations associated with prenatal stress due to timing of gestational stressors. This reveals that prenatal stress is complex and multifaceted, resulting in diverse effects that vary based on species, sex of the fetus, stage of gestation, and type of stressor encountered.

Conclusions

Increased PS, EV, TS, and serum cortisol concentrations of PNS Brahman calves indicate increased temperament or excitability as well as increased HPA axis activity. Prenatally stressed calves were more stress responsive relative to control calves due to their altered behavior and adrenocortical function. Increased temperament exhibited by PNS calves may prepare them to survive and thrive in a stressful postnatal environment as stated by Matthews (2002). However, elevated temperament or increased excitability in calves is not desirable from a managerial standpoint, as temperamental cattle have been reported to be more difficult to manage (Hassall, 1974).

In conclusion, prenatal stress was associated with increased temperament and serum cortisol concentrations through weaning. Because one’s health begins in utero, this model can provide insight into the genetic and/or epigenetic effects of prenatal stress on postnatal health and performance of animals and humans. Future studies should investigate transgenerational epigenetic effects of prenatal stress on temperament and adrenocortical function.

LITeRATURE CITED


