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Gestational immaturity has a bilateral dysregulatory effect on the HPA axis in adult horses

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Human medical research shows that gestational immaturity can have a developmental impact on health, with studies involving adults reporting a link between extremely low birthweight and ongoing HPA axis dysregulation. A bilateral effect has been observed amongst affected groups, with both elevated and depressed basal cortisol levels reported, as well as altered pituitary-adrenal or adrenocortical responses to stressors amongst adults. Similar studies involving pigs, sheep and cattle have mostly reported elevated cortisol responses, while limited equine studies have reported an elevated pituitary-adrenal response due to neonatal stress. We speculated that an adapted endocrine stress response would be present in mature horses with a history of gestational immaturity, and that this would be identifiable through an ACTH stimulation and measurement of free cortisol in saliva. We assembled 10 horses aged 3-12 y known to have been small for gestational age at birth due to prematurity or dysmaturity, plus 7 control horses of similar age living in the same herds. All horses received low-dose exogenous synthetic ACTH by intramuscular injection (0.1 µg/kg bwt; Tetracosactrin 250 mg/ml, Synacthen®). Five saliva samples from each horse were collected between 0 and 2.5 h post-stimulus. These were later assayed using a salivary cortisol enzyme immuno-assay kit (Salimetrics). Data were analysed using R Studio. Mean basal salivary cortisol concentration (SCC) for all horses was within normal ranges (1.4±0.18 nmol/l). Mean peak SCC values (corrected for baseline) for affected horses fell into 2 distinct groups, comprising low cortisol responders (mean 5.18±1.42 nmol/l) and high cortisol responders (13.99±1.61 nmol/l), both significantly different to controls (9.89±1.51 nmol/l; ANOVA $P<0.001$). These results suggest that although basal cortisol levels remain unaffected, gestational immaturity affects the adrenocortical stress response, leading to a blunted or elevated cortisol response to stress in adult horses. It appears that, like other species, gestationally immature horses can experience adrenocortical dysregulation in later life.

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Consequences of stress during the pregnancy period can affect the normal development of the offspring. In this study, the effects of heat stress were studied in Murciano-Granadina goats (n=30; 41.8±5.70 kg) which were exposed to 2 treatments: thermal-neutral (TN; n=15; 15 to 20 °C.); and heat stress (HS; n=15; 30 to 37 °C), and in their offspring. The TN and HS goats were maintained under the ambient treatment conditions from 15 d before mating until 45 d of pregnancy. Female kids born in TN group (n=16) and HS group (n=10) 30±15 day-old were subjected to a novel arena test (NAT) and a novel object test (NOT). In both tests, goat kids were entered a 4×4 m^2 arena. For the NAT, distance travel, number of squares entered, number of jumps and numbers of sniffs of the arena were recorded. The same parameters were recorded in NOT also including the latency to sniff the object and the number of object sniffs. For NAT and NOT count data, repeated measures and a simple linear model were used under a Poisson or negative binomial distribution. Compared to TN, HS goats had 3-d shorter pregnancy duration ($P<0.01$) and their offspring tended to show a reduction of 7% of the birth weight ($P<0.13$). In NAT, HS kids displayed a lower number of sniffs ($P<0.01$) and vocalizations ($P<0.10$). In NOT, HS kids also tended to show a lower number of sniffs ($P<0.10$). In conclusion, heat stress during the first third of pregnancy reduced the duration of gestation with probable effects on the weight of the offspring. In addition, behavioural tests suggested an altered emotional reactivity during the postnatal life of the goat kids after the heat stress suffered in utero.

Prenatal heat stress on emotional reactivity and behavioral reaction of female goats kids

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INTRODUCTION

Consequences of stress during the pregnancy period can affect the normal development of the offspring.

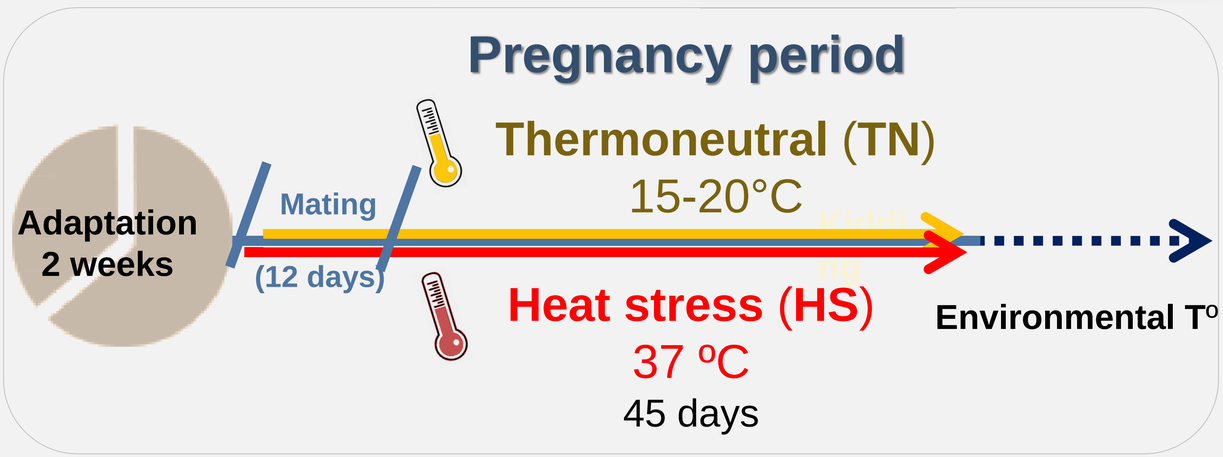
Aim: Evaluate the postnatal effects of heat stress during early pregnancy on dairy goats and on the reaction of offspring to emotional and behavioural stresses.

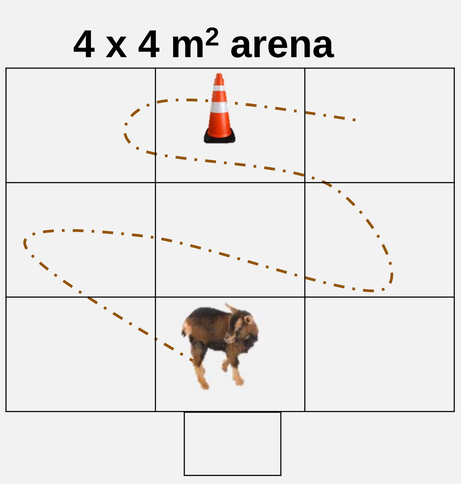
MATERIAL AND METHODS

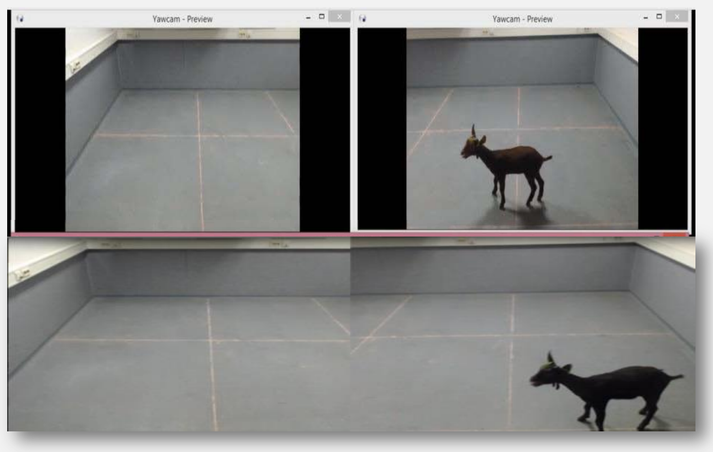
Goats

30 Murciano-Granadina dairy goats:
- Late lactation (41.8 ± 5.70 kg BW)
- Six pens 5 × 2.5 m^2
- 5 goats / pen

Treatments & design







Statistics

Productive variables evaluated followed the GLM procedure of SAS (v. 9.3), and for NAT and NOT count data, PROC GLIMMIX and GENMOD, respectively.

RESULTS

Heat stress effects on post-partum period

Heat stress during the early pregnancy shortened its duration, and consequently birth weight of the kids and litter weight tended to be lower in the HS group (Table 1).

Table 1. Post-partum performance of goats and offspring under thermoneutral (TN; n=15) and heat stress (HS; n= 15) condition.

Variables	TN	HS	RMSE	*P* value
Litter size (No. kids)	2.3	2.2	0.79	0.806
Litter weight (kg)	5.4	4.7	0.71	0.061
Pregnancy length (day)	146	143	2.3	0.006
Birth weight of kids (kg)	2.3	2.2	0.38	0.122
BW 35-days-old kids (kg)	7.9	7.6	1.34	0.520

Heat stress effects on goat kids behaviour

In **NAT**, HS kids displayed a lower number of sniffs and vocalizations (P < 0.10; Figure 1). The number of jumps and distance travel were not affected by the HS (P > 0.05).

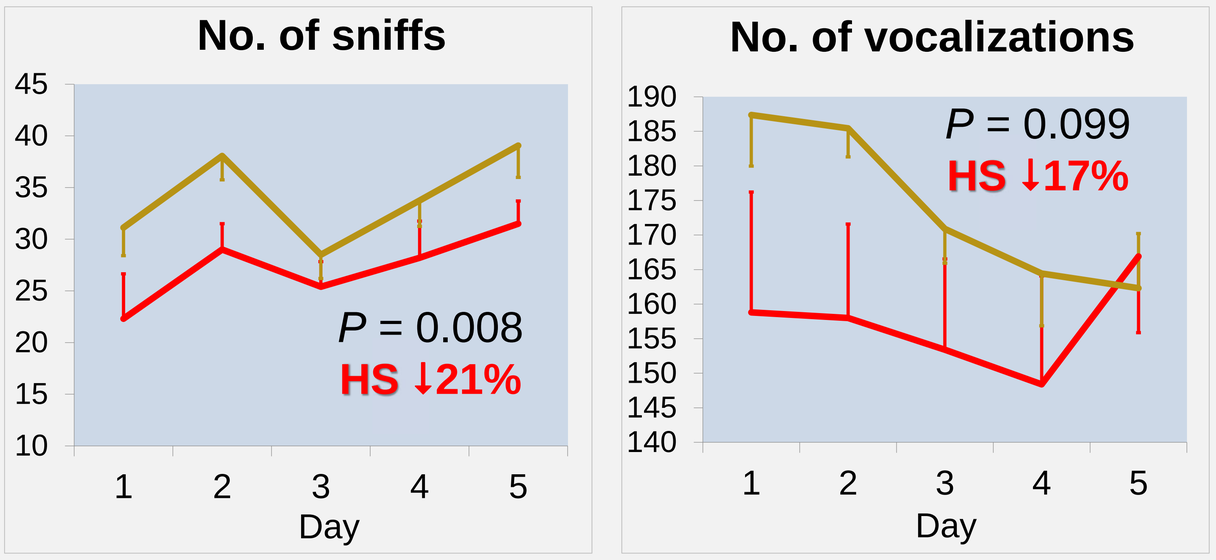


Figure 1. Behavioural responses to novel arena test (NAT) in dairy goat kids born in TN (●) and HS (●) group of dams.

In **NOT**, HS kids also tended to lower the number of sniffs of the arena (P < 0.10; Figure 2), and numerically to the object.

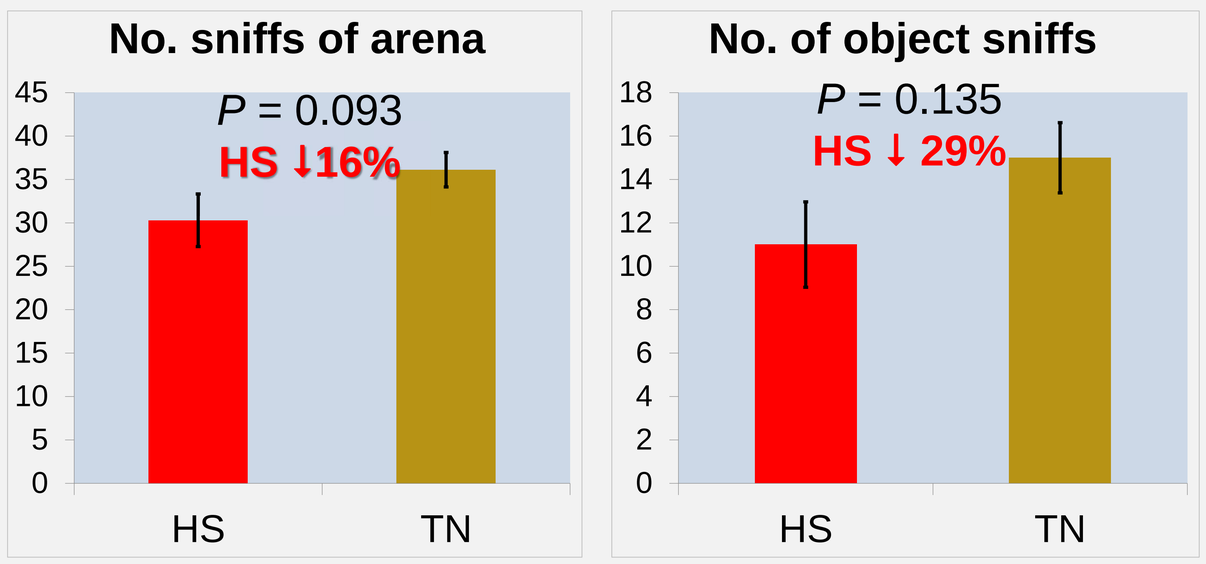


Figure 2. Behavioural responses to novel object test (NOT) in dairy goat kids.

CONCLUSIONS

- Heat stress during the early pregnancy reduced the duration of the pregnancy.
- Behavioural tests suggested an altered emotional reactivity during the postnatal life of the goat kids after the heat stress suffered *in utero.*

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Prenatal heat stress on emotional reactivity and behavioral reaction of female goats kids

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ABSTRACT:

Consequences of stress during the pregnancy period can affect the normal development of the offspring. In this study, the effects of heat stress were studied in Murciano-Granadina goats (n=30; 41.8 ± 5.70 kg) which were exposed to 2 treatments: thermal-neutral (TN; n=15; 15 to 20°C.); and heat stress (HS; n=15; 30 to 37°C). The TN and HS goats were maintained under the ambient treatment conditions from 15 d before mating until 45 d of pregnancy. Female kids born in TN group (n=16) and HS group (n=10) 30±15 day-old were subjected to a novel arena test (NAT) and a novel object test (NOT), which was also performed four months after the birth. In both tests, goat kids were entered a 4x4 m^2 arena. For the NAT, distance travel, number of squares entered, number of jumps and numbers of sniffs of the arena were recorded. The same parameters were recorded in NOT also including the latency until the first object sniff observed and the number of object sniffs. For NAT and NOT count data, repeated measures and a simple linear model were used under a Poisson or negative binomial distribution. Compared to TN, HS goats had 3-d shorter pregnancy duration (P<0.01) and their offspring tended to show a reduced birth weight of 7% (P<0.13). In NAT, HS kids displayed a lower number of sniffs (P<0.01) and vocalizations (P<0.10). In NOT, HS kids also tended to show a lower number of sniffs (P<0.10). In conclusion, heat stress during the first third of pregnancy reduced the duration of gestation with probable effects on the weight of the offspring. In addition, behavioral tests suggested an altered emotional reactivity during the postnatal life of the goat kids after the heat stress suffered *in utero*.

Keywords: Heat stress, fetal programming, emotional reactivity, goat kids

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Introduction

There is evidence that the environmental conditions affecting mothers during gestation modify fetal programming through physiological and epigenetic changes (Viltart & Vanbesien-Mailliot 2007). As a consequence, the behavior, health and productivity of their offspring are permanently conditioned. Studies in animals have shown that episodes of stress during pregnancy have negative effects on the postnatal life of the offspring. These include reduction of the duration of pregnancy and the birth weight (Seckl 2001; Schneider et al. 2002; Schroeder & Weller 2009), alteration of the immune system (Tuchscherer et al. 2002), reduction of neuromotor capacities (locomotion), exploration and learning (Valle et al. 1997; Schneider et al. 2002), alteration of sexual behavior (Ordian et al. 2006) and reduction of social interaction with episodes consistent with depression (Winstock 2001).

Although the behavioral and neuroendocrine effects of prenatal stress have been studied extensively, little is known about the effects of heat stress on goats and their development at later stages. It is also unknown if a specific type of stress during fetal life could condition the response to other types of stress during the subsequent postnatal life.

The objective of this study was to investigate the effect of heat stress on goats at the beginning of pregnancy and on the reaction of offspring to emotional and behavioral stresses.

Material and Methods

Characteristics, treatments and management of goats

Thirty lactating Murciano-Granadina dairy goats of 41.8 ± 5.7 kg body weight (BW) coming from the herd of the experimental farm of the Universitat Autònoma de Barcelona in Bellaterra were used. Goats were housed in 6 pens (5 × 2.5 m) at 5 goats per pen. Three pens were in one room and three in another adjoining room in the same building. Goats were synchronized at two-day intervals to ensure that births occur within a 2-d period. The synchronization procedure was by intravaginal sponges (progesterone) during 12 d plus application of equine chorionic gonadotropin (eCG) at the time of sponge extraction (d 12) and at d 13 natural mating.

After two weeks of adaptation, to pens at environmental temperature, goats were distributed in 2 weight-balanced groups and assigned to two climatic conditions: Thermo-Neutral (TN; 15 - 20°C; n = 15); and Heat Stress (HS; 12-h day at 37.0 ± 0.5°C and 12-h night at 30 ± 0.5°C; n = 15). TN goats were kept indoor and maintained at room temperature and HS goats were kept indoor by achieving the temperature with four electric heaters equipped with a thermostat. Photoperiod was maintained

constant at 12-12 h light-dark (9 to 21 h). The experiment was divided in two periods: before the mating (d 1-12) and early gestation (d 13-57 after mating). The experiment was carried out during the spring (March to June). The environmental temperature and humidity were recorded every 10 min throughout the experiment by data loggers.

Feed was provided as a total mixed ration consisting of 70% alfalfa hay and 30% concentrate. Mineralized salt blocks were freely available for each pen and the water was freely available at room temperature, according to the treatment.

Rectal temperature and respiration ratio from mothers were recorded three times a week at 8, 12 and 17 h.

At 21 and 45 d after the natural mating a diagnosis of pregnancy was made by trans-rectal ultrasound. From d 45 of gestation all the goats were united in a single lot and followed the same management in semi-intensive conditions, with grazing (6 h/d) on pasture and complementation indoors. Two weeks before the expected date of birth, the goats were weighed and moved to farrowing pens under permanent vigilance.

Characteristics, treatments and management of the kids

Immediately after the birth, the goat kids were separated from their mothers, weighed, disinfected their navels with an iodine solution and identified with official plastic ear tag. Goat kids received the colostrum from their own mothers and were bred together with artificial milk in two pens with straw bed and environmentally controlled area by heat lamps (20ºC).

Due to farm management, male goat kids were slaughtered at 9 - 10 kg body weight (BW). Therefore the exploratory and reactivity to a completely novel environment was assessed on female goat kids (TN, n = 16; HS, n = 10) at 30 ± 15 d of age (end of the breeding period) and assessed in two behavioral tests, according to the methodology of Roussel et al. (2005) consisting of a novel arena test (NAT) repeated during five consecutive days and a novel object test (NOT) 48 h thereafter. Kids were chosen at random for each test among the two treatments. The test was performed into an artificial climatic chamber that allowed sound isolation and the same temperature of nursery. All tests were video-recorded for subsequent analysis.

NAT was carried out in a 4 × 4 × 2.3 m arena ($w \times l \times h$), with 9 squares of 1.3 × 1.3 m painted on the ground. The access to the arena was through a guillotine door connected to a start box of 50 × 50 × 60 cm ($w \times l \times h$), where each kid was placed individually. After 30 s, the start box door was opened to allow the goat kid to enter the arena. The duration of the test was 8 min and the time started to run when the goats were completely inside of arena. The following behavioral parameters were measured: distance travel (forward movement), number of squares entered, number of jumps and numbers of sniffs of the arena.

NOT procedure was similar to NAT except for the addition of a novel object, consisting of a red road hazard cone placed in front of the wall opposite to the start box. The same behavioral measures as in

NAT were analyzed, and the latency of the first sniff to the object and the number of times the object was sniffed were also recorded.

Statistical analysis

The duration of pregnancy, litter size, prolificity, and birth weight were analyzed by the GLM procedure of SAS (version 9.1.3; SAS Institute Inc., Cary. NC). Behavioral data were analyzed as repeated measures using the GLIMMIX for NAT and PROC GENMOD for NOT, under a Poisson or negative binomial distribution for count data or PROC MIXED for the latency of the first sniff to the object variable. The models included treatment (TN vs. HS) as a fixed effect and the interaction with the day in the case of repeated measures. Significance was declared at $P < 0.05$ unless otherwise indicated.

Results and Discussion

Effects of heat stress during the pregnancy

Regarding the physiology of goats during the prenatal period, HS goats showed a higher rectal temperature compared to TN goats (+0.68 ºC; $P < 0.01$), as well as an increase in the respiration rate (+76 breaths/min; $P < 0.01$), accordingly with Hamzaoui et al. (2013). On the other hand, the feed intake was also affected by the heat stress that decreased in HS compared to TN goats (-15%; $P = 0.001$). These results show the effectiveness and the strong effect of heat stress treatment applied to pregnant goats.

Effects of the heat stress on postpartum period

The results of the different parturition and early postpartum period parameters are shown in Table 1. Duration of pregnancy was different between HS and TN animals ($P = 0.006$). HS goats reduced total gestation period in 3 days. Consequently, the birth weight of the kids tended to be a 7% lower in the HS group ($P = 0.06$). There was no effect of the treatment on litter size and weight of 35-days-old kids.

Table 1. Performance and reproductive parameters evaluated in goats and goat kids at parturition and early postpartum period.

Variables	TN	HS	RMSE[1]	P value	
				Treatment	Litter size
Litter size (no. kids)	2.31	2.23	0.793	0.806	-
Litter weight (kg)	5.40	4.71	0.712	0.061	<0.001
Duration of pregnancy (day)	146	143	2.267	0.006	0.915
Birth weight of kids (kg)	2.34	2.18	0.383	0.122	-
Weight of 35-days-old kids (kg)	7.88	7.64	1.340	0.520	-

[1] Root mean standard error.

Behavioral tests

The results of both behavioral tests are summarized in Table 2. In NAT, HS goat kids performed 21% less sniffs of the environment (P = 0.008) than the TN ones. This test is used to evaluate the expression of fear in goats and sheep (Forkman et al. 2007), suggesting that greater sniffs correspond to low levels of fear in goats (Ruiz-Miranda & Callard 1992). Our results agree with that indicated by Roussel et al. (2005) in kids born from goats exposed to transport stress at the end of gestation, also observing a decrease in sniffing when the mothers were treated with corticotrophin. A trend to reduce the numbers of squares entered (P = 0.101) and numbers of vocalizations (P = 0.099) observed in HS kids would indicate a less reactive behavior. Finally, no differences were observed between TN and HS goat kids in the number of jumps or the forward movement.

Regarding the NOT, HS kids also tended to reduce the number of sniffs of the arena compared to TN ones (-16%; P < 0.10). Despite the fact that only numerical differences were found, HS kids lowered the number of squares entered (P = 0.126) and the number of sniffs to the novel object (P = 0.135). The rest of the variables measured did not respond to HS treatment.

Table 2. Behavior of lactating goats kids born from mothers subjected to thermo-neutral conditions (TN) or heat stress (HS) during the first 45 days of gestation.

Variables	TN	HS	SED[1]	P value
Novel arena test (NAT)				
No. of squares entered	46.0	36.3	0.20	0.101
No. of jumps	3.2	2.2	0.49	0.442
No. of sniffs of the arena	32.8	26.2	0.17	0.008
No. of vocalizations	177	154	1.00	0.099
Distance travel (movement forward)	57.4	50.7	0.30	0.534
Novel object test (NOT)				
No. of squares entered	47.4	38.9	0.09	0.126
No. of jumps	4.8	2.3	0.47	0.412
No. of sniffs of the arena	36.1	30.0	0.09	0.093
No. of vocalizations	156	161	1.00	0.670
Distance travel (movement forward)	48.9	41.0	0.07	0.220
No. of sniffs of the object	14.8	10.5	0.10	0.135
Latency before 1st sniff of the object (s)	53.8	77.4	0.35	0.562

[1] Standard error of the mean.

Conclusions

Heat stress during the mating season until the first 45 days of gestation reduced the duration of pregnancy with probable effects on the weight of the offspring. In addition, behavioral tests suggested an altered emotional reactivity of the goat kids during the postnatal life after the heat stress suffered *in*

utero. It is especially interesting to observe how the exposure of the fetus to a type of stress in the uterine environment (i.e. heat stress) can modify its ability to respond to other types of stress (e.g. environmental stress) in postnatal life.

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