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Original article

Physiological effects of repeated transport in pregnant goats and their offspring

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Abstract — Although pregnant farm animals can encounter different stressors on numerous occasions, studies on the physiological effects of repeated stress during gestation on females and their offspring are quite recent and still scarce. The present experiment was performed to study the effects of repeated transportations on some physiological parameters in pregnant goats and to determine whether repeated exposure to prenatal stress resulted in alterations in the hypothalamic-pituitary-adrenocortical (HPA) axis and the sympatho-adrenomedullary (SAM) system of their offspring. Twenty-six goats were assigned to one of two treatments during the last five weeks of gestation: 9 series of 55 min of transport (n = 13) or no transport (n = 13). During transport, the goats were physically and visually isolated from their congeners. Transport in isolation induced a large increase in plasma concentrations of cortisol, glucose and non-esterified fatty acids, which confirms that it is a very stressful situation for goats. Moreover, the goats did not become accustomed to the stressor. Gestation length, birth weight, litter weight and growth of the kids were not modified by treatment. Cortisol concentrations tended to be higher in prenatally stressed kids than in control kids 1 h after birth ($P < 0.10$) and the opposite was observed at 48 h of age ($P < 0.10$). Indeed, the decrease in cortisol concentrations between 1 and 48 h was greater in prenatally stressed kids than in control kids (time × treatment interaction, $P < 0.01$). The effect of prenatal stress on the HPA axis did not persist, since in older kids cortisol concentrations were not modified by treatment. At one month of age, prenatally stressed kids showed a higher medulla weight ($P < 0.05$) and tended to show a higher phenylethanolamine N-methyl transferase activity ($P < 0.10$) than control kids. Therefore, repeated transport in isolation is an important stressor in pregnant goats and can affect the HPA axis and the SAM system of their offspring.

transport / goat / prenatal stress / cortisol / catecholamine

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Résumé — Effets physiologiques de transports répétés pendant la gestation chez la chèvre et le jeune chevreau. Alors que les animaux d'élevage rencontrent fréquemment des agents stressants pendant leur gestation, ce sujet a reçu peu d'attention. Nous avons étudié les effets de transports répétés sur certains paramètres physiologiques de chèvres gestantes et tenté de déterminer si l'exposition répétée à un stress prénatal entraîne des altérations de l'axe corticotrope et du système catécholaminergique de leurs chevreaux. Vingt-six chèvres ont été réparties dans deux traitements pendant les 5 dernières semaines de gestation : 9 transports de 55 min en étant physiquement et visuellement isolées de leurs congénères (n = 13) ou absence de transport (n = 13). Le transport en isolement a induit une augmentation importante des concentrations plasmatiques en cortisol, glucose et acides gras non-estérifiés, ce qui confirme que c'est un agent stressant important pour la chèvre. De plus, les chèvres ne se sont pas habituées au traitement. La durée de gestation, le poids de naissance et de portée et la croissance des chevreaux n'ont pas été modifiés. Les concentrations de cortisol ont eu tendance à être plus élevées chez les chevreaux stressés prénatalement (SPN) que chez les témoins 1 h après la naissance ($P < 0,10$) et l'inverse a été observé chez les chevreaux âgés de 48 h ($P < 0,10$). La diminution des concentrations de cortisol entre 1 h et 48 h a été plus importante chez les chevreaux SPN que chez les témoins (interaction temps × traitement : $P < 0,01$). L'effet du stress prénatal sur l'axe corticotrope n'a pas persisté chez les chevreaux plus âgés. À l'âge d'un mois, le poids de la médullo-surrénale a été plus élevé ($P < 0,05$) et l'activité de la phényléthanolamine N-méthyl transférase a eu tendance à être augmentée ($P < 0,10$) chez les chevreaux SPN par rapport aux témoins. Par conséquent, le transport répété en isolement est un agent stressant important chez la chèvre gestante et peut modifier l'axe corticotrope et le système catécholaminergique des chevreaux.

transport / caprin / stress prénatal / cortisol / catécholamine

1. INTRODUCTION

Common livestock production practices cause activation of the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol concentrations increase after transport in cattle [12, 20] and goats [18, 27], after restraint and isolation in sheep [1, 25] and cattle [6] and after social isolation in goats [17]. Stressors can also activate the sympatho-adrenomedullary (SAM) system through the release of catecholamines as observed in goats [27] or the increase in heart-rate as shown in sheep [3].

Pregnant farm animals can encounter these stressors on numerous occasions during gestation. However, studies on the physiological effects of stress during gestation on females and on their offspring are quite recent and still scarce. The situations studied involved transportation of Brahman cows [21, 22], handling of blue foxes [7], isolation and restraint of sheep [34] and pigs [29], restraint of pigs with ACTH injection [14] but to date no experiments have been performed on dairy goats, a production system which is of increasing importance. Moreover, the results are often contradictory. The physiological effects of prenatal stress on the offspring are either an increase in basal cortisol concentrations as shown in lambs [34], no modification of basal cortisol concentrations as observed in calves [14, 21, 22] or even a decrease in basal cortisol concentrations as shown in piglets [29]. Prenatal stress has also been observed to induce a higher adrenal cortex-to-medulla area ratio in pigs [14] while the ratio was not modified in calves [22]. Adrenals were found to be lighter in weight in blue foxes [7, 28] while no modification was observed in pigs [29]. The birth weight of the litter was either not affected [7], increased [34], tended to be decreased [14, 29] or tended to be increased [22] by prenatal stress.

The aims of this study were to evaluate the effects of repeated stress on some physiological parameters in pregnant goats and to determine whether repeated exposure to

prenatal stress resulted in alterations in the HPA axis and SAM system of their offspring. Transportation and isolation are known to be very stressful situations for farm animals. Therefore, they were used as stressors in this study. Indeed, in the goat, it has been observed that there was an immediate increase in plasma concentrations of epinephrine after the start of transport. This was accompanied by a slower rise in cortisol [27]. Moreover, cortisol and epinephrine concentrations remained high throughout transport [27]. In this study, we used indices which have previously been developed to measure the effects of chronic stress such as increased cortisol release after ACTH administration, modification of adrenal weight and activity of catecholamine-synthesizing enzymes [37, 38].

2. MATERIALS AND METHODS

2.1. Goats

2.1.1. Animals and stress treatment

Sixteen multiparous and ten primiparous goats of Saanen and Alpine breeds were used in this experiment. They were synchronised prior to mating in order to give birth within a period of 7 days. Two months before parturition goats were assigned to one of two treatments according to breed, milk yield at the previous lactation, physiological status (five primiparous and eight multiparous goats in each treatment) and the buck used for mating. The two treatments were: transport in isolation (TRANS, n = 13, including six Alpine and seven Saanen goats) or no transport (CONTROL, n = 13, including seven Alpine and six Saanen goats). From the 3rd month of gestation all the goats were housed in the same pen. Throughout gestation they were offered a complete diet containing dehydrated alfalfa, sugar-beet pulp, maize silage, chopped hay and concentrate. The diet was adapted to cover requirements [16]. Water and minerals were supplied ad libitum.

Starting five weeks before parturition TRANS goats were transported twice a week with a total of 9 transports per goat. Four to five TRANS goats were loaded onto a lorry modified to transport each goat so that it was visually and physically isolated from the others. The lorry was divided into five 60 × 100 cm compartments using wooden panels 150 cm high. The goats were transported for 20 km each time, always by the same driver and on the same itinerary, between 08.00 and 14.00 h. The transportations were performed in January and February. T0 was defined as the start of transportation. After 20 min of transport the lorry was stopped for 15 min and the goats remained in the lorry. Transport was then continued for another 20 min. Afterwards, the goats were returned to their original pen.

2.1.2. Measurements

Body weight was recorded before and after transport.

On the 1st, 5th and 9th days of transport, a blood sample was taken 15 min (T-15) before the start of transport. A second blood sample was taken 10 min after the start of the pause in transport (T30) and a third just after unloading (T55). Blood samples were taken simultaneously from CONTROL goats in order to minimize the effect of circadian rhythm on cortisol secretion [30]. Every effort was made not to agitate the animals during blood sampling. The samples were drawn as quickly as possible after catching the animals to avoid as much as possible the influence of blood sampling on cortisol release. Jugular blood samples were collected into heparinised tubes by venipuncture and were immediately centrifuged at 3000 × *g* for 10 min at 4 °C. Plasma was stored at –20 °C until analysis of

cortisol, glucose and non-esterified fatty acids (NEFA).

2.2. Goat kids

2.2.1. Animals and rearing procedures

The kids were separated from their dams within one hour after birth and they were put in a heated pen for 24 h. After 24 h, a maximum of two kids per dam were included in the two experimental groups. Each treatment group (TRANS kids and CONTROL kids) was balanced for kid body weight and sex. The kids were thereafter housed in two pens. The two treatments were equally represented in each pen. One hour, three hours and seven hours after parturition each kid was bottle fed with 30 $g \cdot kg^{-1}$ body weight of its dam's colostrum from the first milking. Twelve and twenty-four hours after birth all the kids received a milk replacer by bottle (30 $g \cdot kg^{-1}$ body weight). Thereafter, they were fed twice daily with a milk replacer using a bucket equipped with rubber teats. The kids were sacrificed at 35 days of age by exsanguination after electro-narcosis.

2.2.2. Measurements

Body weight was recorded at birth and at 1, 2, 3 and 4 weeks of age.

Blood samples were collected at 1, 12 and 48 h of age in order to measure cortisol concentrations. At 1, 2 and 4 weeks of age three blood samples, separated by 30 min, were taken in order to estimate basal cortisol concentrations by calculating the average value of the 3 blood samples.

An ACTH challenge is often used to assess the functioning of the HPA axis and to detect states of chronic activation of the HPA axis due to external stressors [37]. Therefore, ACTH challenges were performed at 8 days and 1month of age. In the evening of the day before the test, the kids were given an intramuscular injection of 0.02 $mg \cdot kg^{-1}$ body weight of dexamethasone (Dectancyl ND, Roussel, Paris, France) to induce intense negative feedback on the HPA axis. The following morning a single intravenous injection of 1 $IU \cdot kg^{-1}$ metabolic weight of ACTH (Synacthene, ND, Novartis-Pharma, Rueil-Malmaison, France) was given to assess the capacity of the adrenal cortex to produce cortisol in response to ACTH. Dexamethasone and ACTH were diluted in saline (0.9% sodium chloride) up to a total volume of 1 mL before use. Blood samples were obtained before injection and at 30, 60, 90, 120 and 180 min after injection to measure the increase in cortisol concentrations. The kids were separated into two groups, with one kid from each pair being tested at 8 days of age and the other kid being tested at 1 month of age. The single kids were randomly assigned between the test days.

All blood samples were collected and treated as previously described for the goats.

The activities of tyrosine hydroxylase (TH) and phenylethanolamine N-methyl transferase (PNMT) were measured in the adrenal glands, which were recovered immediately after slaughter. They were dissected clear of surrounding tissue, weighed, cut in half and frozen in liquid nitrogen and stored at –80 °C until determination of catecholamine-synthesizing enzymes. The mean length of time between slaughter and freezing of the adrenal gland in liquid nitrogen was less than 12 min per kid (11.2 ± 0.34 min and 11.2 ± 0.31 min for CONTROL and TRANS kids, respectively; $P > 0.05$; means ± SEM).

2.3. Biological analyses

2.3.1. Cortisol analysis

Plasma concentrations of cortisol were measured by ELISA using an automated method (Elecsys, Roche Diagnostics, Meylan, France). The sensitivity of the

cortisol assay was 1 nmol·L^{-1}. The inter-assay coefficient of variation was 4.5% at 344 nmol·L^{-1}.

2.3.2. Glucose and non-esterified fatty acid (NEFA) analyses

Plasma was assayed for glucose by a modified hexokinase-glucose-6-phosphate dehydrogenase procedure [8] and for NEFA by an adaptation of an enzymatic procedure [5]. Both methods were adapted for a Coulter semi-automatic instrument (Chemical Profile Analyzer (CPA), Coultronics, Magency, France) and used commercial kits (Gluco-quant®, Roche Diagnostics GmbH, Mannheim, Germany and NEFA C, Wako Chemicals GmbH, Neuss, Germany).

2.3.3. Tyrosine hydroxylase (TH) and phenylethanolamine N-methyl transferase (PNMT) analyses

TH and PNMT activities were determined in the medulla of adrenals by methods adapted from Waymire et al. [39] and Axelrod [2] by Veissier et al. [38].

2.4. Calculations and statistical analysis

The integrated response to transport was determined by calculating the area under the curve described by cortisol, glucose and NEFA concentrations from 0 to 55 min. The integrated response to exogenous ACTH was determined by calculating the area under the curve described by cortisol values from 0 to 180 min. The area under the curve (C) was calculated using the following formula [38]:

$$C = \Sigma (C_t + C_{t+1})/2 \times dt$$

where C_t is the concentration at the time t and dt is the time in minutes between samples taken at t and t+1.

The same calculations were performed for CONTROL goats.

The goat responses to transport were analysed by the MIXED model procedure of SAS® (Statistical Analysis Systems Institute, 1996) which used the repeated factor time. Gestation length, litter weight and litter size were analysed by analysis of variance using the GLM procedure of SAS®. The model to analyse litter weight data used the number of kids born per goat as a covariate.

The integrated and maximal cortisol responses of the kids after ACTH challenge were analysed by analysis of variance using the GLM procedure of SAS® with a statistical model including the factors: treatment (TRANS vs. CONTROL), sex and treatment × sex. Adrenal data, body weights, weight gain of the kids and their cortisol concentrations were analysed by the MIXED model procedure of SAS®. The model included the factors treatment, sex, treatment × sex and goat nested within treatment as a random variable. For kid cortisol concentrations the model also included the repeated factor age.

For kid data the number of kids born per goat was used as a covariate except for adrenal and cortisol data since it did not have a significant effect on the results.

Simple linear coefficients of correlation between goat cortisol, glucose and NEFA concentrations were calculated.

Kid cortisol concentrations of more than 3 standard deviations from the mean value were considered to be outliers and omitted from the statistical analysis (between 4 to 5 values were discarded for a given week of sampling). For cortisol concentrations from birth to 12 hours after birth, 2 TRANS kids and 4 CONTROL kids were omitted from the analysis because the blood samples could not be collected on time.

All data are presented as least square means ± standard errors except when otherwise stated.

3. RESULTS

3.1. Goats

Neither goat body weight, gestation length, litter size nor litter weight differed between treatments (Tab. I).

On average, transport in isolation decreased goat body weight by 1.06 ± 0.106 kg ($P < 0.01$, mean ± SEM), which corresponded to a decrease of 1.35 ± 0.093% of initial body weight (percent shrink). There was no effect of the day of transport on the decrease in body weight or the percent shrink ($P > 0.10$). Cortisol, glucose and NEFA concentrations were higher in TRANS goats compared to CONTROL goats on all days of transport as shown by the increase in the areas under the curve (Fig. 1; $P < 0.001$). The concentrations of glucose, cortisol and NEFA at the different times and days of sampling are shown in Figure 2. An effect of day of transport was observed on the glucose response ($P < 0.0001$) with an increase from day 1 to day 9, this was not so for the cortisol and NEFA responses. A significant interaction day × treatment was also found for the glucose response ($P < 0.01$). Indeed, the glucose response increased with time in TRANS goats (Fig. 1; $P < 0.05$) while a significant increase in glucose concentrations was only observed between the 5th and the 9th transport for the CONTROL goats ($P < 0.05$).

When the glucose and cortisol data obtained at T55 from the 3 days of transport in TRANS goats (n = 39) were pooled, a positive relationship was observed between cortisol and glucose concentrations (r = 0.458, $P < 0.01$). No correlation was observed between cortisol and NEFA concentrations.

3.2. Kids

3.2.1. Body weight and weight gain

Body weights and growth rate from 1 to 35 days did not differ between treatments (Tab. II).

3.2.2. Cortisol concentrations around birth and at 1, 2 and 4 weeks of age

Cortisol concentrations were not influenced by treatment from 1 to 48 h after birth ($P > 0.10$). However, during this period cortisol concentrations decreased with time ($P < 0.001$) and there was a significant interaction between time and treatment ($P < 0.01$). Indeed, in TRANS kids cortisol concentrations were affected by age (1 h > 12 h > 48 h, at least $P < 0.05$) while in CONTROL kids cortisol concentrations only tended to be affected by time (1 h > 12 h; $P < 0.10$) and did not vary

Table I. Production data in goats either exposed to 9 series of 55 min of transport in isolation during the last third of gestation (TRANS) or not transported (CONTROL) (lsmeans ± SE).

	CONTROL (n = 13)	TRANS (n = 13)	Probability
Body weight before the 1st series of transport (kg)	70.7 ± 3.61	69.5 ± 3.71	$P = 0.82$
Body weight before the 9th series of transport (kg)	77.8 ± 3.47	77.3 ± 3.61	$P = 0.91$
Gestation length (d)	151.4 ± 0.92	152.7 ± 0.92	$P = 0.32$
Litter weight (kg)	8.2 ± 0.46	8.6 ± 0.46	$P = 0.52$
Litter size	2.3 ± 0.23	1.8 ± 0.23	$P = 0.12$

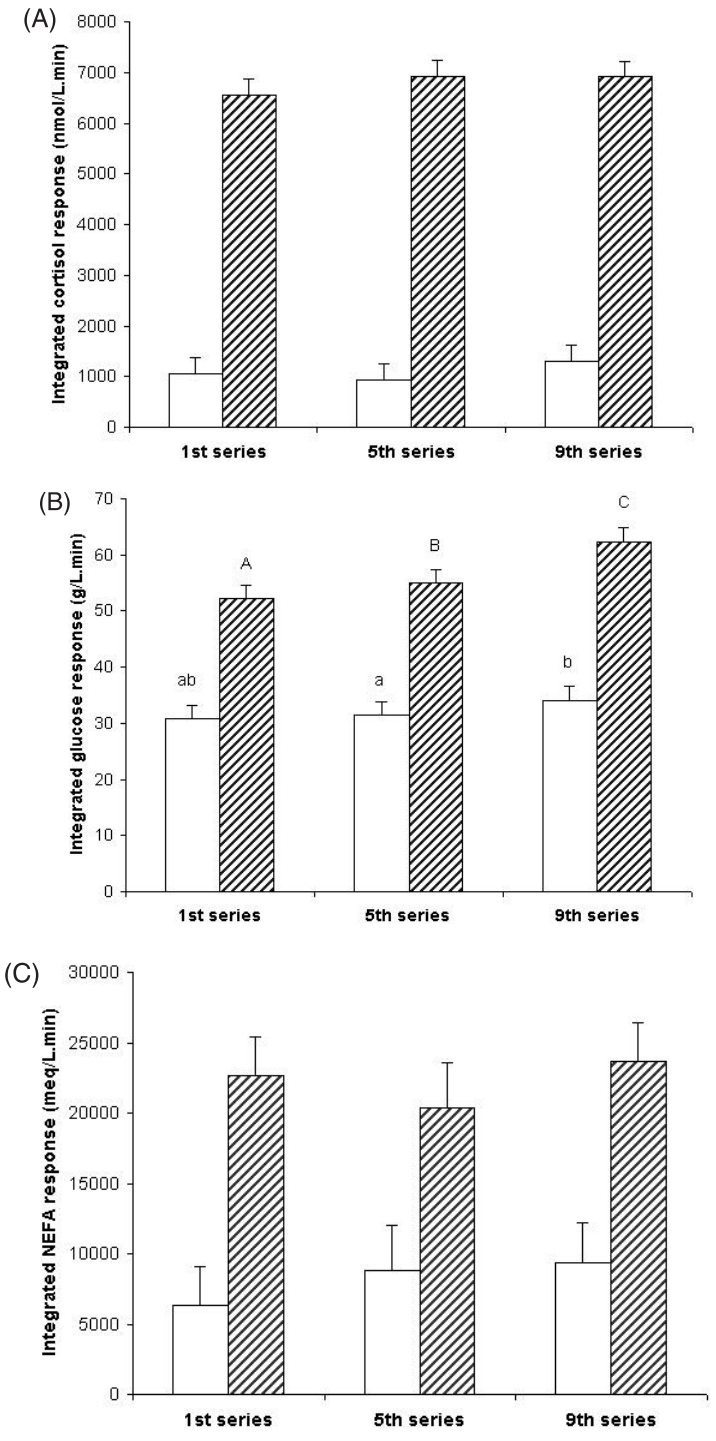

Figure 1. Integrated cortisol (A), glucose (B) and NEFA (C) responses of goats either exposed to 9 series of 55 min of transport in isolation during the last third of gestation (TRANS, hatched columns, n = 13) or not transported (CONTROL, open columns, n = 13) during the 1st, 5th and 9th series of stress treatments (lsmeans ± SE). Treatment effect: $P < 0.001$ for cortisol, glucose and NEFA response; Day effect: $P < 0.0001$ for glucose response; Treatment × day effect: $P < 0.01$ for glucose response; a, b: CONTROL values with different superscripts differ ($P < 0.05$); A, B, C: TRANS values with different superscripts differ ($P < 0.05$).

significantly after 12 h. One hour after birth there was a trend for TRANS kids to have higher cortisol concentrations than CONTROL kids ($P < 0.10$) and the opposite was observed at 48 h of age ($P < 0.10$) (Fig. 3).

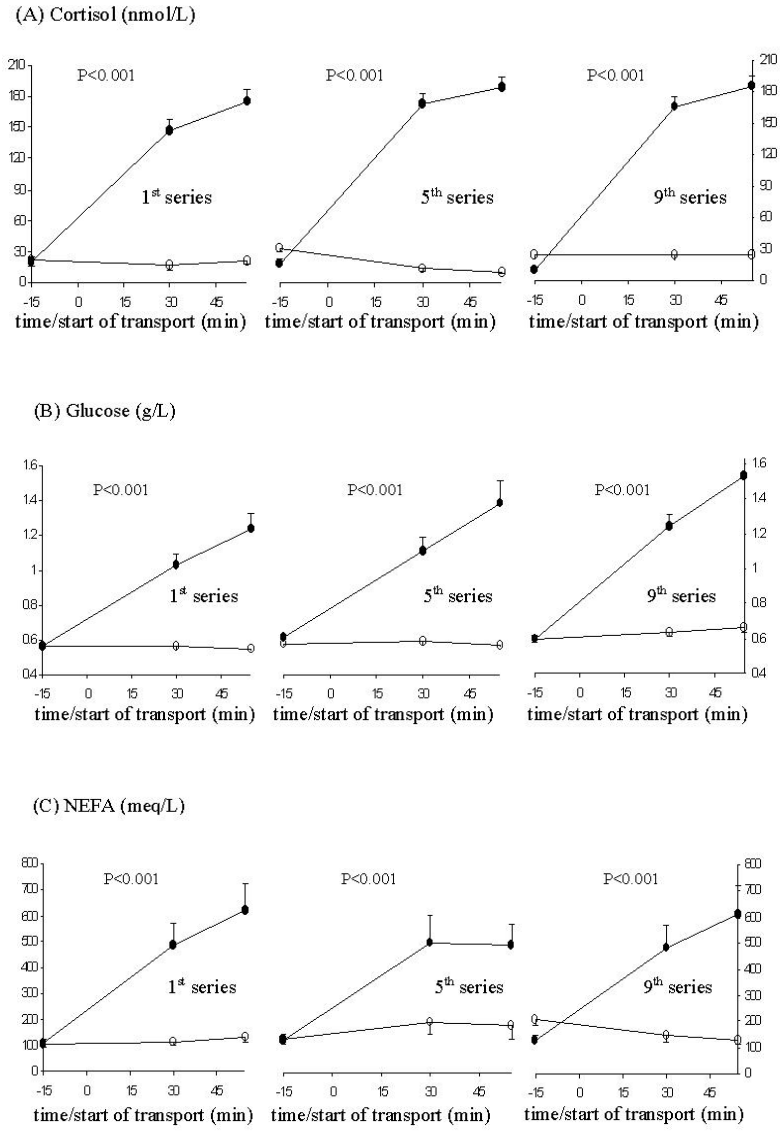

Figure 2. (A) Cortisol, (B) glucose and (C) NEFA responses of goats either exposed to 9 series of 55 min of transport in isolation during the last third of gestation (TRANS, full circles, n = 13) or not transported (CONTROL, empty circles, n = 13) during the 1st, 5th and 9th series of stress treatments (means ± SEM).

Basal cortisol concentrations decreased with age ($P < 0.0001$). However, no significant differences between treatments were found for basal cortisol concentrations at 1, 2 and 4 weeks of age ($P > 0.10$; Tab. III).

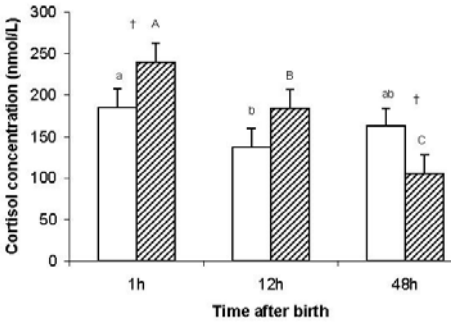

Figure 3. Cortisol concentrations (nmol·L^{-1}) in kids born from goats either exposed to 9 series of 55 min of transport in isolation during the last third of gestation (TRANS, hatched columns) or not transported (CONTROL, open columns) (lsmeans ± SE). †: $P < 0.10$ for the level of significance for the difference between CONTROL and TRANS kids at a given time; a, b: CONTROL values with different superscripts differ ($P < 0.10$); A, B, C: TRANS values with different superscripts differ ($P < 0.05$).

3.2.3. ACTH challenge

The integrated cortisol response to ACTH challenge was unaffected by treatment at 8 and 30 days of age ($P > 0.10$; Tab. IV). However, at 30 days of age, the response tended to be higher in females than in males ($P < 0.10$; Tab. V). At both ages, sampling time influenced plasma cortisol concentrations ($P < 0.001$). Cortisol concentrations reached a maximum between 30 and 60 min after the challenge and returned to basal levels by 180 min. At 30 days of age the maximum cortisol concentrations were higher for females than for males ($P < 0.05$; Tab. V) but were not influenced by treatment ($P > 0.10$).

3.2.4. Tyrosine hydroxylase (TH) and phenylethanolamine N-methyl transferase (PNMT)

No difference between treatments was found for adrenal gland weight. The adrenal medullas were heavier in TRANS compared to CONTROL kids ($P < 0.05$; Tab. VI) and PNMT activity tended to be higher in TRANS compared to CONTROL

Table II. Production data in kids born from goats either exposed to 9 series of 55 min of transport in isolation during the last third of gestation (TRANS) or kids born from goats not transported (CONTROL) (lsmeans ± SE).

	CONTROL (n = 23)	TRANS (n = 21)	Probability
Birth weight (kg)	4.2 ± 0.19	4.2 ± 0.20	$P = 0.98$
Body weight at slaughter (kg)	9.7 ± 0.31	10.0 ± 0.33	$P = 0.54$
Weight gain from day 1 to day 35 (g·d^{-1})	157 ± 7.9	164 ± 8.3	$P = 0.55$

Table III. Basal concentrations of cortisol (nmol·L^{-1}) in 1-, 2- and 4-week-old kids born from goats either exposed to 9 series of 55 min of transport in isolation during the last third of gestation (TRANS) or kids born from goats not transported (CONTROL) (lsmeans ± SE).

	CONTROL (n = 23)	TRANS (n = 21)	Probability
1-week-old	30.2 ± 3.27	31.5 ± 3.39	$P = 0.77$
2-week-old	13.9 ± 3.27	7.5 ± 3.39	$P = 0.18$
4-week-old	9.5 ± 3.27	10.4 ± 3.39	$P = 0.85$

Table IV. Integrated cortisol response (C_{ACTH}) and cortisol concentrations 2 minutes before, 30, 60, 90, 120 and 180 minutes after ACTH challenge in 8-day-old and 30-day-old kids born from goats either exposed to 9 series of 55 min of transport in isolation during the last third of gestation (TRANS) or kids born from goats not transported (CONTROL).

	C_{ACTH} (min·nmol·L^{-1}) (lsmeans ± SE)	Cortisol concentration (nmol·L^{-1}) (means ± SEM)					
		t_{-2}	t_{30}	t_{60}	t_{90}	t_{120}	t_{180}
8-day-old							
CONTROL (n = 10)	14 882 ± 1405.9	18 ± 5.7	187 ± 12.4	173 ± 15.3	85 ± 15.3	27 ± 6.0	6 ± 1.2
TRANS (n = 9)	14 201 ± 1461.0	5 ± 5.6	166 ± 13.1	159 ± 16.1	93 ± 16.2	26 ± 6.3	4 ± 1.3
30-day-old							
CONTROL (n = 10)	10 279 ± 988.5	5 ± 3.1	121 ± 11.2	128 ± 9.5	63 ± 8.8	17 ± 3.5	2 ± 0.6
TRANS (n = 10)	10 292 ± 968.5	3 ± 3.1	129 ± 11.0	132 ± 9.3	54 ± 8.6	15 ± 3.4	3 ± 0.6

Table V. Integrated cortisol response (C_{ACTH}) and maximal cortisol response after ACTH challenge in 30-day-old kids (lsmeans ± SE).

	Female (n = 9)	Male (n = 11)	Probability
C_{ACTH} (min·nmol·L^{-1})	11 539 ± 1027.3	9 032 ± 927.3	$P = 0.09$
Maximal cortisol response to ACTH (nmol·L^{-1})	150 ± 11.4	117 ± 10.3	$P = 0.05$

Table VI. Adrenal weight, medulla weight, tyrosine hydroxylase (TH) and phenylethanolamine N-methyl transferase (PNMT) activity in 35-day-old kids born from goats either exposed to 9 series of 55 min of transport in isolation during the last third of gestation (TRANS) or kids born from goats not transported (CONTROL) (lsmeans ± SE).

	CONTROL (n = 23)	TRANS (n = 21)	Probability
Total adrenal weight (g)	0.77 ± 0.029	0.80 ± 0.030	$P = 0.51$
Total medulla weight (mg)	176.2 ± 25.3	249.5 ± 26.0	$P = 0.05$
TH activity (nmol·h^{-1}·animal^{-1})	48 ± 6.6	62 ± 6.7	$P = 0.14$
PNMT activity (nmol·h^{-1}·animal^{-1})	2.0 ± 0.40	3.1 ± 0.41	$P = 0.06$

kids ($P < 0.10$; Tab. VI). TH activity was unaffected by treatment ($P = 0.14$; Tab. VI). However, there was a large goat effect ($P < 0.05$).

4. DISCUSSION

In the present experiment, less than one hour of transport induced an increase in cortisol concentrations 7 fold above baseline values, while Nwe et al. [27] observed that cortisol concentrations were multiplied by 4 in goats after 6 h of transport. This large increase in glucocorticoids is probably due to the physical as well as the psychological effects of transportation [10]. In addition, our data show that the goats did not become accustomed to the stressor.

This is in contradiction with the observations of Lay et al. [20] in transported Brahman cows, since they showed decreasing cortisol responses during 3 series of 24 km transport. In their experiment the cows were transported in trailer loads of four to six animals, while in our study goats were transported visually and physically isolated from one another which could explain this discrepancy. Indeed, temporary interruption of social contact has been shown to elicit an immediate increase in behavioural and physiological signs of arousal in farm animals which are highly gregarious [6, 33]. Moreover, isolation of goats from their social group causes a greater elevation of cortisol concentrations if the animals are not able to maintain visual contact with other animals [17]. Several authors have also shown no habituation of sheep to isolation on the basis of cortisol concentrations [26, 31]. Therefore, in our experiment, the goats might have been able to adapt to transportation but not to isolation.

Transport in isolation also induced an increase in glucose and NEFA concentrations. Moreover, glucose concentrations increased with time irrespective of treatment. This increase was more pronounced in TRANS goats and confirms that the goats did not become accustomed to the stressor. The maximum glucose concentration of only 1.53 $g \cdot L^{-1}$ observed in the present experiment is lower than the value of 2.64 $g \cdot L^{-1}$ obtained in goats by Nwe et al. [27] during 6 h of transport. This discrepancy could be explained by the shorter period of transport used in our experiment. Indeed, our glucose values are similar to those obtained in goats after a 2.5 h transport by Kannan et al. [18].

Cortisol and glucose concentrations follow a similar pattern during transport, which is related to adrenal function. In stressful conditions plasma glucocorticosteroids increase and the rate of glucose synthesis accelerates [24]. At the same time, glycogenolysis, stimulated by catecholamines, increases blood glucose concentrations [11]. In the present experiment a significant correlation was found between cortisol and glucose concentrations during transport. Both catecholamines and cortisol increase after the beginning of transport in goats [27]. Although transport in isolation considerably increased NEFA concentrations in the present experiment, we were not able to show a correlation between cortisol and NEFA, therefore not supporting the lipolytic effect of ACTH or the stimulatory effect of NEFA on the HPA axis [25]. However, the increase in NEFA could be due to a direct effect of epinephrine on lipolysis [24].

The absence of an effect of prenatal stress on birth weight in the present experiment could be explained by the large variation in litter size which drastically influences the birth weight of offspring. In addition, the results published in the literature are often contradictory. This could be explained by differences in the type of stressor used, the species involved and the duration of stress [4, 13].

One hour after birth, cortisol concentrations tended to be higher in TRANS kids than in CONTROL kids. Moreover, a significant interaction was observed between time and treatment with a larger decrease in cortisol concentrations between 1 and 48 h in TRANS than in CONTROL kids. At 48 h the decrease in cortisol concentrations in TRANS kids was so large that cortisol concentrations tended to be lower in TRANS than in CONTROL kids. This could result from a more intense negative feed-back on the HPA axis of the TRANS kids which may take place straight after birth since it is known that the HPA axis is already functional in fetal sheep [41].

The effect of prenatal stress on the HPA axis of the offspring in the present experiment did not persist, since in older kids cortisol concentrations were not modified by treatment. This is the opposite of what has been observed for rats [40] and for sheep

[34]. Despite a higher concentration of basal cortisol at birth, we did not find any difference between treatments after the ACTH challenge. This is in accordance with results obtained in Brahman calves [21]. The number of times the goats were transported or the total duration for which the stressor was applied may not have been sufficient to modify durably the HPA axis of their offspring, although no sign of goats becoming accustomed to the stressor was detected.

An interesting result from our study was the increase in medulla weight and PNMT activity observed in prenatally stressed kids compared to control kids. This could be the consequence of an over-reactivity of the SAM system. Indeed, in rats, prenatal stress induces an over-reactivity in the brain catecholamine system [36] and a higher concentration of norepinephrine after a foot shock stimulus [40]. With regard to the activity of TH measured in our study, the higher concentration observed in stressed compared to control kids was not significant. This lack of effect could be due to the strong goat effect observed in this experiment. PNMT is an enzyme that catalyzes the N-methylation of norepinephrine to epinephrine in the medulla of adrenals, while TH controls the rate of synthesis of norepinephrine. Studies in rats show that prenatally stressed rats have significant alterations in hypothalamic concentrations of norepinephrine and serotonin [32] as well as an increase in the turnover rates of norepinephrine and dopamine [36]. To our knowledge, the effects of prenatal stress on the activity of the TH and PNMT enzymes in the medulla of adrenals have not been studied.

The discrepancies between the physiological effects of prenatal stress could be due to species differences but also to stage of gestation when the stressor was applied. Even if in most experiments, including ours, the stressor was applied during the last third of gestation as in rats [23, 35], foxes [28] or sheep [34], this was not always the case. Indeed, in other experiments, rats were stressed throughout gestation [36, 40] or during the last two thirds of gestation [32] and in Brahman cows the stressor was applied during the first half of gestation [20–22].

The mechanism by which prenatal stress affects the HPA axis and the SAM system is not clear. It could involve transplacental transport of maternal glucocortocoids, as observed in rats [43], pigs [19] and sheep [41]. In addition, corticosteroids that cross the placenta, have been shown to be able to bind to the fetal hypothalamus [43]. Placental 11β-hydroxysteroid dehydrogenase partially inactivates the maternal cortisol which crosses the placenta. The extent of inactivation depends on the species and the stage of gestation [42], however it can be assumed that a significant amount of maternal cortisol can be found in fetal circulation (see review by Dalle [9]). Indeed, 2–3 weeks before lambing 37% of fetal cortisol has been shown to be of maternal origin [15]. Moreover, in blue foxes, a significant correlation has been observed between cortisol concentrations of dams which had been stressed and their fetuses [28]. The repeated increases in cortisol in fetal blood due to the HPA axis of the dam being stimulated could have sensitized the fetal HPA axis. Therefore, a more intense negative feedback on cortisol secretion in the newborn kid could explain the more pronounced decrease in cortisol concentrations seen after birth in TRANS compared to CONTROL kids.

We were not able to confirm the results obtained by McCormick et al. [23] and Szuran et al. [35] in rats, which showed that the female HPA axis was more susceptible to prenatal stress induced modifications than the male axis. The only sex difference observed in kids in the present experiment was the greater cortisol response to the ACTH challenge in females compared to males at 1 month of age as previously shown in vitro in female foxes [28].

This finding is particularly interesting and warrants further work since females represent the majority of farm animals and they are usually kept for several reproductive cycles, therefore an effect of prenatal stress could have long term implications on animal husbandry.

In conclusion, transport in isolation induced stress in pregnant goats. In addition, prenatal stress can affect the HPA axis of kids at birth, since cortisol concentrations are modified. Moreover, the SAM system is also affected as shown by the increase in catecholamine-synthesizing enzyme activity in prenatally stressed kids. Therefore management practices should avoid stressful situations for pregnant females. The mechanism by which prenatal stress affects the HPA axis and the SAM system remains to be studied.

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