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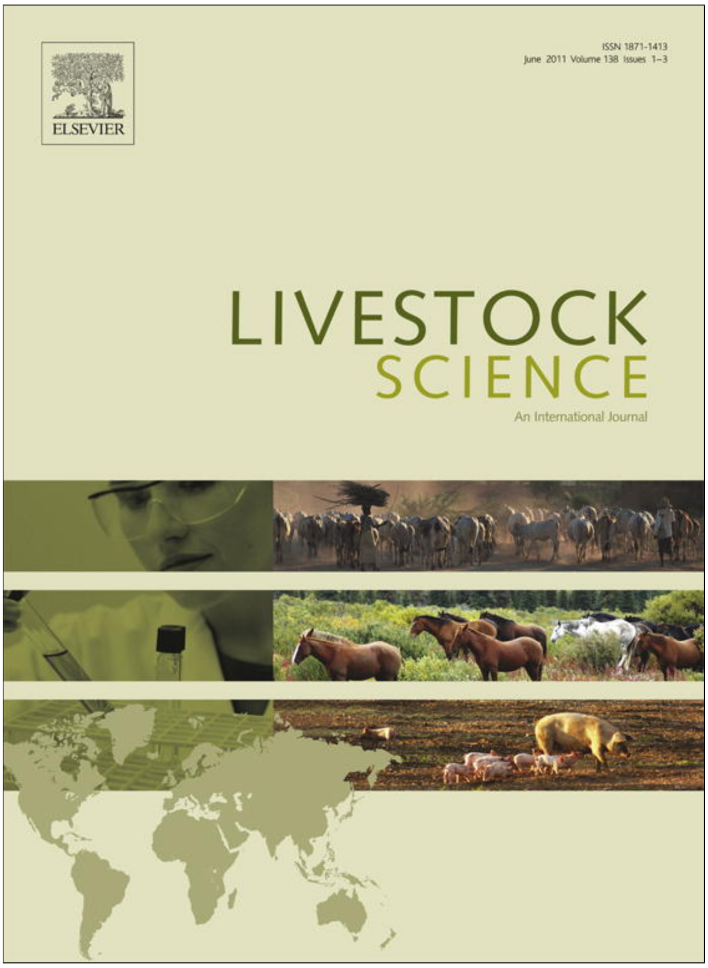

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Ovarian function in cows submitted to acute stress during proestrus

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ABSTRACT

Studies in which ACTH was administrated in heifers after the occurrence of luteolysis showed a delay in the onset of estrus and the estrus duration was shortened. This study evaluated the effect of acute stress by road transportation on estrous behavior and ovulation, monitored by serum progesterone and cortisol concentrations in cows at the periovulatory period, using a crossover design. Eleven crossbred cows, divided into Control and Transport groups had their estrus cycle synchronized with GnRH, an intravaginal progesterone device, and cloprostenol. Thirty hours after withdrawal of the device, the animals of the Transport group were transported for 60 min by truck and those from the Control group remained at pasture. Ovarian ultrasound examination was performed every 12 h from device withdrawal until ovulation in every cow. From the day after removal of the device until ovulation estrous behavior was monitored 24 h a day. Blood samples for serum cortisol and progesterone concentrations were taken at −90, −60, 0, 30, 60 and 180 min in relation to the end of transportation. Transportation during the estrous period induced stress in cows as reflected by changes in serum concentrations of progesterone and cortisol. However, we did not detect impairment in estrus expression, estrus duration or ovulation ($P>0.05$).

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1. Introduction

Many physiological changes in animals occur due to stress, and one of them that can be highlighted is subfertility. Hence, stress influence upon reproductive performance is of great importance when it comes to inappropriate handling practices, especially under intensive farm management.

The periovulatory period is a very sensitive period which depends upon a series of actions of the hypothalamus–pituitary–gonads axis (HPG). Moreover, there are neuroendocrine connections between the HPG and the hypothalamus–pituitary–adrenal axis (HPA) that promote inhibitory effects upon the reproductive system (Bage et al., 2000).

Under stress the body secretes glucocorticoids and progesterone by the adrenal glands. This response is triggered by the HPA axis through the corticotropin releasing hormone (CRH), which is secreted by the hypothalamus. Consequently, the adenohypophysis releases of adrenocorticotropic hormone occurs (ACTH; Chrousos et al., 1998). The adrenal gland secretion of hormones in response to stress conditions under the CRH control affects reproduction since it inhibits the release of gonadotropins by the pituitary gland. Some studies have shown that the negative effects caused by stress may inhibit the preovulatory peak of LH or even inhibit ovulation (Hein and Allrich, 1992; Bage et al., 2000; Dobson and Smith, 2000; Hollenstein et al., 2006). Other studies have shown that ACTH exogenous administration produces similar effects to the ones caused by acute stress leading to increased blood concentrations of cortisol and progesterone (Hein and Allrich, 1992; Bage et al., 2000; Yoshida and Nakao, 2005; Hollenstein et al., 2006).

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Davidge et al. (1987) reported that administration of ACTH could inhibit estrus in cows due to lower estrogen secretion. Hence, the LH peak was delayed and ovulation happened somewhat later. Such low concentration of estradiol led to a lower estrus intensity and frequency (Dobson et al., 2001).

Dobson and Smith (2000) mentioned that road transportation is one of the most efficient ways to provoke stress to ruminants. According to Buckham Sporer et al. (2008) classical acute increases in plasma cortisol and progesterone concentrations have occurred by 4.5 h after the onset of truck transportation in young beef bulls. Likewise, healthy Japanese Black calves transported by truck had a significant increase in circulating cortisol between 1 and 2 h relative to start of transport. Moreover, this increase in circulating cortisol was more pronounced in calves that were moved around in a mountainous area (Ishizaki and Kariya, 2010). Road transportation may influence the release of hypothalamic and pituitary reproductive hormones, thus interfering with the reproductive performance of transported animals. Therefore, this experiment aimed to evaluate the effect of stress induced by a 60-min road transportation during proestrus upon estrous behavior and ovulation in cows.

2. Material and methods

Eleven non-lactating zebu cows (Nellore or Nellore X Simmental crossbreds) aging between 4 and 7 years-old were used. They were kept in a *Brachiaria decumbens* paddock, with mineral supplementation and *ad libitum* water. Nellore and crossbred cows were divided and equally distributed to two treatment groups: Control and Transport, following a crossover design. The experiment was held at the Experimental Farm of Embrapa Genetic Resources and Biotechnology, Brasília, DF, Brazil on March, 2006.

All animals had their estrous cycles synchronized according to the following protocol: D0: administration of 50 μg GnRH (i.m., Gestran Plus, ARSA S.L.R., Argentina) and an intravaginal device containing progesterone was inserted (DIB, Syntex S.A., Argentina) and kept for 7 d. On D6 and D7, 0.50 mg and 0.25 mg of Cloprostenol (Sincrosin, Vallée S.A., São Paulo, Brazil) was injected i.m., respectively.

Thirty hours after DIB withdrawal, animals of the Transport group were transported by truck for 60 min on a paved busy road, whereas the animals from the Control group were kept in the pasture. For both replicates, cows were submitted to transport from 10:00 a.m. to 11:00 a.m. under ambient temperatures between 22 and 26 °C. During transport cows were kept loose, water was not available and the spatial allowance per animal on the transporter was 1.3 m^2.

Transrectal ovarian ultrasound evaluation was done every 12 h from DIB withdrawal until ovulation of each cow using an Aloka SSD 500 with a linear 7.5 MHz transducer (Aloka Co., Tokyo, Japan). Estrus signs were visually observed round the clock after DIB withdrawal until ovulation. All cows were kept together, in the same paddock. Signs of estrus were considered as being mounted and standing for more than 2 s. The onset of estrus was defined as the time of the first acceptance of mounting by another female and the end, when this behavior ceased. During estrus observation several variables were evaluated, such as, the time span between DIB withdrawal and onset of estrus, estrus duration (time between the first and the last acceptance of mounting), number of times each cow accepted mounting, time between onset of estrus and ovulation, and time between the end of estrus and ovulation.

Blood samples for circulating cortisol and progesterone concentrations were taken at −90, −60, 0, 30, 60 and 180 min in relation to the end of transportation. Blood samples were centrifuged at 1500×g for 15 min and serum was kept in polypropylene tubes at −20 °C until radioimmunoassay. One assay for progesterone and one assay for cortisol were performed using commercial kits (Coat-a-Count, DPC–Medlab) to determine the level and duration of stress induced by the transportation. Assays were conducted at the Endocrinology Laboratory of the Department of Animal Reproduction and Radiology, School of Veterinary Medicine and Animal Science, UNESP, Botucatu. Intra-assay coefficients of variation were 4.4 and 6.1% for cortisol and progesterone, respectively.

Comparison between groups for the continuous variables related to estrus and ovulation was done using paired t test. ANOVA was used for the repeated measurements regarding circulating hormones using the MIXED procedure with the REPEATED command of SAS (Littell et al., 1996). The Pearson Correlation test for blood cortisol and progesterone concentrations was done using the PROC CORR from SAS.

3. Results and discussion

At the beginning of the experiment, serum cortisol concentrations in both Control and Transport groups were between 25 and 35 ng/mL (Fig. 1). Post-transport circulating concentration of cortisol had increased, reaching a mean of 56.6 ± 6.6 (mean ± standard error; range of 17.0 to 71.8) ng/mL (89.3% increase), whereas animals from the Control group, which remained at pasture, had no change in cortisol concentration. Thirty min after leaving the truck, circulating cortisol had already reduced and by 180 min cortisol was similar between Transport and Control groups (Fig. 1).

Blood concentration of progesterone in the Control group was around 0.3 and 0.4 (0.32 ± 0.03) ng/mL at the blood sampling collection time-points (Fig. 2). On the other hand, circulating progesterone concentration from the Transport group has increased from 0.4 ± 0.1 to 0.7 ± 0.1 (range of 0.2 to 1.3) ng/mL (84.2% increase) soon after transport ($P < 0.05$). Circulating progesterone was similar to Control values at 180 min after end of transportation (Fig. 2).

Despite the increase in blood progesterone and cortisol concentrations after road transportation, there was no difference between groups in the variables related to estrous

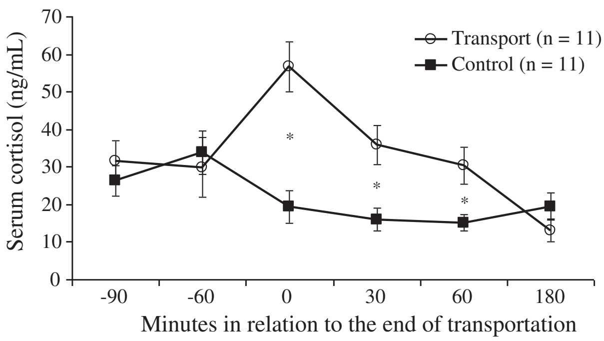

Fig. 1. Serum cortisol concentration (ng/mL; mean ± SE) in both Control cows and cows submitted to acute stress (Transport) during proestrus. *$P < 0.05$.

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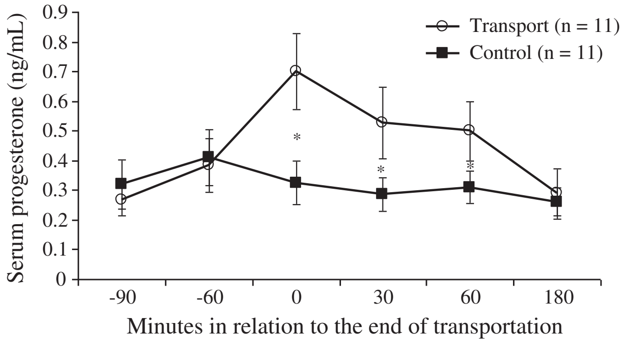

Fig. 2. Serum progesterone concentration (ng/mL; mean ± SE) in both Control cows and cows submitted to acute stress (Transport) during proestrus. *P < 0.05.

behavior and ovulation (P > 0.10; Table 1). There was also no difference between treatments for maximum ovulatory follicle diameter (Table 1).

In the present study, the data collected during estrus observation showed that all animals from both groups (Control and Transport groups) were detected in standing estrus after the second administration of PGF2α and progesterone intravaginal device withdrawal. Studies on zebu cattle reported that 46% of the females were detected in estrus after the use of two injections of PGF2α (Pinheiro et al., 1998) or between 75–86% after one injection of PGF2α combined with one or two injections of a GnRH agonist (Barros et al., 2000). In *Bos taurus*, studies have shown that, after estrous synchronization with PFG2α, 90% of the animals presented estrus (Tanabe and Hann, 1983). Additionally, in dairy heifers the use of GnRH analog combined with PGF2α resulted in 76.9% of animals in estrus (Yoshida et al., 2009).

Cattle that had a simulated stress by the administration of ACTH (320 IU) 30 h after the second injection of PFG2α presented 65% (13/20) of heat whereas Control group presented 80% (16/20; Hein and Allrich, 1992). Hence, these authors reported that the administration of ACTH and the consequent increase of circulating cortisol were responsible for either diminishment or inhibition of estrus. Nevertheless, there was no statistical difference between groups. In the study by Hein and Allrich (1992) and ours, an acute response to stress was obtained, but the first study employed an exogenous treatment with a pharmacological dose of ACTH, which presumably has taken shorter to have its effect when compared with the present study in which stress was obtained by transportation and its effect was delayed. Stoebel and Moberg (1982) reported that multiple injections of ACTH inhibited the pre-ovulatory LH peak and changed ovary function of cyclic cows. In the present study, the induced stress caused by road transportation did not alter estrus signs (Table 1). Moreover, the synchronization protocol that was applied has shown to be efficient since all animals presented estrus.

The duration of estrus was similar in all groups, but it was longer than the duration reported by Pinheiro et al. (1998) for zebu females. These authors observed that Nelore heifers and cows presented an estrus average duration of 10.9 ± 1.4 h. Other studies have shown that both *Bos indicus* × *Bos taurus* crossbred females and *Bos taurus* purebred cattle presented a similar estrus duration to what we have found in the present study (revised by Bó et al., 2003 and Sartori and Barros, in press).

The maximum diameter of the ovulatory follicles did not differ between groups and it was the same or even bigger than the ones found for *Bos indicus* females in the literature and inferior to the ones found for *Bos taurus* (Sartori and Barros, in press). These results were similar to the results described by Martins et al. (2008) that used a similar group of cows.

The interval between intravaginal progesterone device withdrawal and the beginning of estrus, as well as the time between the beginning of estrus and ovulation were not different between groups. When comparing to the literature, the interval between the second administration of PFG2α and the onset of estrus observed in our study was longer than the interval described by Hein and Allrich (1992). Such experiment reported that the animals began estrus at 43.7 ± 2.2 h in the Control group and 62.9 ± 2.6 h in the ACTH-treated group after the administration of PFG2α. This might have happened due to a delayed LH peak caused by the maintenance of circulating progesterone concentrations via the adrenal gland as observed by Bage et al. (2000) in repeat breeder heifers. The interval between the beginning of estrus signs and ovulation was longer than the intervals reported in the literature (Pinheiro et al., 1998; Bó et al., 2003 and Sartori and Barros, in press) probably due to the low frequency of ultrasound evaluations (twice a day) if compared to other studies that have scanned ovaries up to 12 times a day.

Despite the fact that the road transportation associated with a 60 min feed restriction during transport did not express any influence upon both estrus behavior and ovulation, the increases in circulating cortisol and progesterone concentrations have proved that stress was effectively induced by this procedure. Serum cortisol concentrations in the Control and Transport groups were equal or higher than 20 ng/mL, and the basal values determined for the bovine are reported to be between 5 and 10 ng/mL (Yoshida and Nakao, 2005). These high values for cortisol suggest that some regular procedures such as animal handling in the chute, blood collection by venipuncture and transrectal palpation are sufficient to trigger stress in the cows; taking into consideration the fact that cortisol is the most important indicative hormone of stress (Knights and Smith, 2007). For the Control group the average cortisol values were around 20 ng/mL. These values were higher than the ones found in experiment 1 (12.4 ng/mL), but not in experiment 2 (39.0 ng/mL) by Hein and Allrich (1992) for the animals from the Control group that were submitted to blood sampling and administration of intravenous aqueous vehicle. Other authors reported that the basal levels were

Table 1
Results (mean ± SE) from variables related to estrus and ovulation in either Control cows or cows submitted to acute stress (Transport) during proestrus.

	Control (n = 11)	Transport (n = 11)	P
Time between DIB [1] withdrawal and onset of estrus; h	66.2 ± 8.9	73.1 ± 8.4	0.58
Estrus duration; h	14.8 ± 1.5	14.9 ± 1.8	0.97
Standing mounts received; n	30.3 ± 5.1	40.5 ± 6.6	0.24
Time between onset of estrus and ovulation; h	36.3 ± 3.7	34.2 ± 3.0	0.66
Time between end of estrus and ovulation; h	23.6 ± 2.7	18.4 ± 2.1	0.35
Ovulatory follicle; mm	12.7 ± 0.3	12.4 ± 0.5	0.62

[1] DIB: intravaginal device containing progesterone.

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between 3.8 and 4.4 ng/mL for animals submitted to only blood sampling (Yoshida and Nakao, 2005). It is important though, to remember that differences in the results of hormone concentrations between studies may occur due to different laboratorial techniques and not due to different animal physiology. The increase in blood cortisol concentrations of the animals that were submitted to the acute 60-min road transport showed the functional activity of the adrenal glands. Buckham Sporer et al. (2008) and Ishizaki and Kariya (2010) have also described an elevation in plasma cortisol concentration just after transport. Similarly to the present study, Yoshida and Nakao (2005) showed that the use of ACTH at 25 IU induced an average cortisol peak between 33.2 and 46.5 ng/mL. On the other hand, when Hein and Allrich (1992) used ACTH at 350 IU they observed an average cortisol peak of 91.0 ng/mL, and a basal concentration of 9.6 ng/mL. In another study, Hollenstein et al. (2006) reported average cortisol concentrations between 11.5 and 44.3 ng/mL in cyclic cows submitted to acute stress caused by a 2-h restraint. When assessing blood cortisol concentrations in repeat breeder cows and heifers, Bage et al. (2000) have also observed high cortisol concentrations with values ranging between 20 and 30 ng/mL.

The present study observed that the cows submitted to road transportation had an increase in circulating progesterone similar to the increase in cortisol. This circulating progesterone was very likely derived from the adrenal gland since the cows did not present corpus luteum. In another study, by injecting ACTH (350 IU), Hein and Allrich (1992) observed the diminishment of estrus signs along with an increase in progesterone blood concentrations that reached a peak of 4.1 ng/mL. Those values were higher than the concentrations found in our study. Yoshida and Nakao (2005) also reported that the animals treated with ACTH had an increase in blood progesterone concentration from 0.6 to 4.5 ng/mL. In that experiment, the animals underwent ovariectomy, having the adrenal glands as the sole source of progesterone. With this, they have proved that adrenal glands were able to produce high amounts of progesterone, reaching blood concentrations above 1 ng/mL. In the present study, the mean increase caused by transportation did not reach 1 ng/mL, and such values were possibly incapable of producing physiological changes on the HPG axis.

Is this study, circulating progesterone and cortisol in cows under stress presented a high and positive correlation ($r = 0.63$, $P < 0.001$). Other authors also reported that the increase in cortisol concentrations was accompanied by the increase in circulating progesterone ($r = 0.8$, $P < 0.05$; Bage et al., 2000; Yoshida and Nakao, 2005). These data show that the adrenal glands respond to stress producing simultaneously these two hormones that are indicators of stress.

4. Conclusion

Despite the fact that the 60-min transportation period induced an increase in serum cortisol and progesterone concentrations, this transient stress response was not of significant magnitude or of duration to lead to changes in the occurrence and duration of estrus, or time span between luteolysis and ovulation.

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