Pergamon

Pharmacology Biochemistry and Behavior, Vol. 52, No. 4, pp. 743–748, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00168-9

Naltrexone Delays the Onset of Maternal Behavior in Primiparous Parturient Ewes

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Received 30 December 1993

CABA, M., P. POINDRON, D. KREHBIEL, F. LÉVY, A. ROMEYER AND G. VÉNIER. *Naltrexone delays the onset of maternal behavior in primiparous parturient ewes.* PHARMACOL BIOCHEM BEHAV **52**(4) 743–748. – Previous studies indicate that morphine facilitates the induction of maternal behavior in nonpregnant multiparous ewes but has no effect in nulliparous females. Naltrexone (NAL) has the opposite effect. The aim of the present experiment was to investigate whether this also applied at parturition in ewes lambing for the first time. We studied the behavior of parturient ewes that received either saline (n = 9) or 50 mg (n = 7) or 150 mg (n = 8) of naltrexone, intravenously, at the first signs of lambing. Either dose of naltrexone was found to reduce significantly the duration of maternal licking of the neonate and the emission of low-pitched bleats during the first 30 min following parturition ($p < 0.005$). The proportion of mothers failing to display an immediate onset of maternal care (in < 5 min) was also significantly higher in NAL-treated ewes (six of 15 vs. none of nine; $p = 0.05$). On the other hand, there was no indication that NAL affected the establishment of selectivity or that it facilitated the manifestation of postpartum estrus. Our results therefore tend to confirm that opiates have a role in the facilitation of maternal behavior at parturition in the ewe.

Sheep Maternal behavior Opioids Naltrexone Maternal selectivity Primiparous Parturition

THE ONSET of maternal behavior in sheep depends on the physiologic events of parturition. This process begins some hours before expulsion when the ewe becomes strongly attracted toward amniotic fluids (18). Immediately after birth, the ewe avidly licks the neonate, emits numerous low-pitched bleats, and accepts the young at the udder as soon as it is able to stand (26). At the same time, an exclusive bond develops rapidly between the mother and her young. Once this bond is established, mothers actively reject alien lambs that try to suck, and react very strongly to separation from their lamb, displaying frequent high-pitched bleats, intense motor activity, and frequent eliminations (27).

The neuropeptide oxytocin (OT) has an important role in this onset of maternal behavior. During parturition and following vaginocervical stimulation (VCS), there is an increase in cerebrospinal (CSF) concentrations of OT (9). Intracerebroventricular (ICV) administration of OT to ovariectomized, estrogen-treated ewes induces rapid maternal responses in multiparous ewes (8). Moreover, the inhibition of maternal behavior observed in parturient primiparous ewes following peridural anesthesia (13) is reversed by the ICV infusion of OT (20). Subsequent studies have shown that this effect of OT may be mediated, at least in part, by endogenous opioids. Naltrexone (NAL), an opioid antagonist of the μ and δ systems, prevents the CSF increase in oxytocin after VCS and significantly reduces the ability of VCS to stimulate maternal behavior in multiparous nonpregnant ewes (6). On the other hand, morphine enhances the central release of oxytocin in such ewes and facilitates the activation of maternal behavior by VCS. However, a similar effect was not found in nulliparous inexperienced females (11). These results suggest that the activation of the β-endorphin systems, reported both peripherally (3) and centrally (32), may facilitate maternal behavior in sheep [see (10) for an extensive review of this point]. In addition, the experience achieved by successive parturitions may be necessary for opiate release to be significant and have a role in this process. This possibility cannot be ruled out, especially because it has been recently demonstrated that maternal experience is associated with differences in neurobiologic processes occurring in the olfactory bulbs of parturient ewes [i.e., re-

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lease of norepinephrine and acetylcholine (21)]. Also, studies in the rat indicate that sensitivity to opiates can vary depending on parity (12). Finally, the results indicating a facilitatory effect of opiates on the onset of maternal behavior have been obtained only in nonpregnant ewes receiving vaginocervical stimulation, and their validity in the normal process of parturition has not been previously established.

Therefore, in the present experiment we investigated the possible involvement of opioids in the facilitation of maternal behavior in inexperienced ewes by studying whether the opiate antagonist naltrexone could disrupt the onset of maternal behavior in ewes giving birth for the first time. A second purpose of this experiment was to examine the possibility that the absence of estrus behavior in periparturient ewes is due to inhibition by β-endorphin, as proposed by Keverne (10). This possibility is also suggested by the fact that opiates inhibit sexual receptivity in the rat (31). The lack of postpartum estrus in ewes has been puzzling, because the pattern of ovarian hormone secretion (high estradiol levels preceded by a decline in progesterone levels) which occurs at parturition is similar to that which induces estrus in other circumstances. Therefore, we also tested the animals for estrus behavior to see whether the proposed inhibition by β-endorphin could be blocked by naltrexone.

METHODS

Subjects

The study was carried out at the experimental INRA farm of Brouessy (Yvelines, France). We used 24 pregnant ewes of the Prealpes-du-Sud breed in this study. Animals were kept permanently indoors. They were fed dehydrated lucern and maize, together with hay and peas, and water ad lib. Reproduction was fully controlled by vaginal sponges and PMSG to synchronize estrus. Animals that had never given birth before were divided into three groups. In group 1 (controls; $n = 9$), females received saline; in group 2 (NAL 50; $n = 7$), they received 50 mg of naltrexone (Sigma Chemical Co., St. Louis, MO.) and in group 3 (NAL 150; $n = 8$), 150 mg of naltrexone.

Procedure

On day 144 of pregnancy, ewes were injected with dexamethasone (16 mg Dexadreson; Intervet, France) to induce lambing. When a ewe showed signs of impending birth (i.e., observation of the lamb's hooves or rupturing of the amniotic sac), she was separated from the group and placed into an individual pen (2 × 1 m). Ewes were assigned to one of the three mentioned groups and received an intravenous injection of the vehicle or drug (dissolved in 10 ml saline). After parturition the lamb was left with her dam, and the behavior of the ewe toward her lamb was recorded for the first 30 min after parturition.

Behavioral Records

Behavior at parturition. Behavior was recorded using a preestablished checklist. The various components taken into account were:

- Low- and high-pitched bleats (mouth closed and open respectively)
- Total length of time that the mother licked the neonate
- Number of acceptances at the udder (the lamb's head engaged under the inguinal region and the mother not moving away for at least 10 s)
- Number of rejections at the udder (mother moving away < 10 s after the lamb had engaged under the inguinal region, or mother avoiding the lamb when it approached the udder, or mother circling around the neonate as it tried to reach the inguinal region)
- Total suckling time (time with nipple in the mouth)
- Aggressive behavior (number of head threats and butts from the mother to the lamb)

Immediately after this period of observation, the lamb was withdrawn from the pen and isolated in another building. Maternal responses to lamb separation were then studied by recording the vocalizations of the ewe for a further 5 min. This allowed further assessment of maternal responsiveness, because maternal ewes react very strongly to the removal of their lamb (27).

Establishment of Maternal Selectivity

The primary aim of the study was to investigate the possible effects of naltrexone on the onset of maternal behavior. However, we also tried whenever possible to verify whether naltrexone could also disturb the dynamics of maternal selectivity, which normally develops within 2–4 h postpartum (28). Thus, once the lamb was returned to its mother, the mother-young dyad was left undisturbed until the lamb was 2–4 h old. Whenever possible, ewes (controls: six ewes; group NAL50: seven ewes; group NAL150: six ewes) were then subjected to a qualitative test of maternal selectivity. The ewe's own lamb was removed, an alien lamb of about the same age (± 1 h) was introduced, and maternal responses were observed for 5 min. Ewes were considered to reject the alien lamb if displaying aggressive behavior and/or rejection at the udder or, in the absence of these behaviors, if > 50% of vocalizations were high bleats, together with < 10 s of licking and no acceptance at the udder or suckling of the alien lamb.

Sexual Receptivity

At a time ranging from 6–12 h postpartum, mothers were put in the presence of a ram and tested for sexual receptivity (i.e., becoming immobile when a ram mounted). The test was stopped after the ram had displayed five mounting attempts. Several rams were used as needed for these tests.

Statistics

Differences of behavioral scores between groups over the 30 min of observation were analyzed using the Kruskall-Wallis and Mann-Whitney tests for independent samples. In addition, analyses were also carried out by 5-min blocks to verify whether the possible differences in behavior were still present at the end of the observation time.

As the direction of the differences between control and treated animals was predicted, unilateral probability values were used (30). The analyses were performed with the statistical package Systat 5.03 (Evanston, IL). In addition, the proportions of mothers displaying the various components of maternal behavior, or that failed to show any sign of maternal behavior at 5 and 30 min postpartum, were compared using the Fisher exact probability test (one-tailed) (4).

RESULTS

Onset of Maternal Behavior

An initial comparison of the three groups revealed significant differences for licking and emission of low-pitched bleats

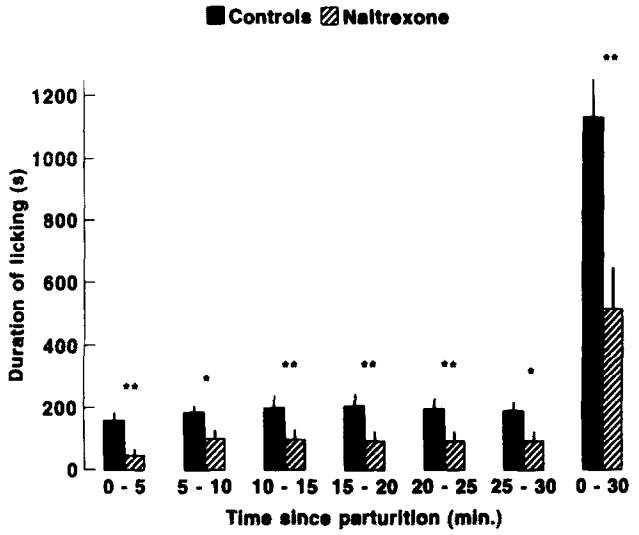

FIG. 1. Mean durations of licking of the neonate (±SEM) in controls (n = 9) and naltrexone-treated ewes (n = 15), over the first 30 min following the birth of the lamb. *0.03 ≤ p ≤ 0.01; **p ≤ 0.007 (between controls and naltrexone-treated ewes, Mann-Whitney test, one-tailed).

(Kruskall-Wallis tests). Whereas the two NAL groups differed from controls for these variables, analyses of ewes receiving 50 and 150 mg of naltrexone, carried out using Mann-Whitney tests, showed no significant differences between the two treatments for any variables. Therefore, to simplify the presentation of the results, the data of these two groups were pooled (NAL) and compared with those of the controls for further analyses.

A significant reduction in licking duration was found in NAL ewes, both when considering the mean licking time over the 30 min of observation (518 s vs. 1135 s, p = 0.003, Mann-Whitney test, U = 20) and when analyzing each 5-min period individually (Fig. 1). Low-pitched bleats were also significantly reduced in NAL-treated females (49 vs. 144, p = 0.003, Mann-Whitney test, U = 20.5) (Fig. 2). In fact, five of the NAL-treated ewes emitted fewer than 10 and one emitted none during the observations. A significant effect of the drug was observed not only for the total period of observation, but also for each 5-min period, indicating that the effect was still present at the end of the observations (Fig. 2). Finally, when deprived of their lamb at the end of the observation time, control ewes tended to respond more strongly to the separation than did NAL-treated ewes, with the difference just reaching significance (47.0 vs. 34.1 high bleats/5 min, p = 0.05, Mann-Whitney test, U = 40).

The effects of NAL could also be seen in the proportion of ewes displaying maternal behavior (Fig. 3). Significantly more NAL ewes failed to lick their lamb (eight of 15 vs. one of nine in controls) or to emit low-pitched bleats (nine of 15 vs. none of nine) in the first 5 min following birth (p = 0.048 and 0.004, respectively, Fisher exact probability test). The proportion of mothers that totally failed to display any signs of maternal behavior during the first 5 min of observations was also higher in NAL-treated females (six of 15 vs. none of nine, p = 0.037). Three of these six mothers had still not shown interest in their young 30 min after birth. However, at this time the difference was no longer statistically significant (three of 15 vs. none of nine, p > 0.05).

On the other hand, there was no clear effect of the treatment on acceptance at the udder, as in each of the two groups some lambs were accepted (four of nine in controls vs. six of 15 in NAL). However, in many cases the lambs had not succeeded in reaching the udder (four of nine in controls vs. nine of 15 in NAL). Also, the proportion of mothers in each group rejecting their lamb at the udder did not differ (two of 15 vs. two of nine, respectively), nor did the frequencies of this behavior. Similarly, no differences were observed for the emission of high-pitched bleats during the 30 min of observation. Finally there was a slight although not significant trend toward an increase of aggressive behavior in NAL-treated females (0.7 vs. 0; Mann-Whitney U-test, p = 0.08).

Maternal Selectivity and Estrus Behavior

By contrast to what was found with respect of the onset of maternal behavior, there was no indication that naltrexone influenced maternal selectivity. Six control mothers and 13 NAL mothers were tested for selective behavior. Of these, one NAL female was still not maternal. Of the 12 remaining ewes, 10 were clearly selective, one gave some signs of acceptance of the alien lamb together with rejection behaviors, and one clearly accepted the alien. Among the controls, five mothers were clearly selective and one showed an ambiguous response.

As for estrus behavior, none of the 15 NAL and nine control females tested with a ram showed any sign of sexual receptivity, regardless of the treatment.

DISCUSSION

Our results indicate that naltrexone treatment delayed the onset of maternal behavior in ewes lambing for the first time, whereas it had no noticeable influence on the establishment of maternal selectivity or estrus behavior. The disruption of maternal behavior was evidenced both by a failure of mothers to display immediate maternal responses and a reduction in these responses for at least a half hour after the birth of the

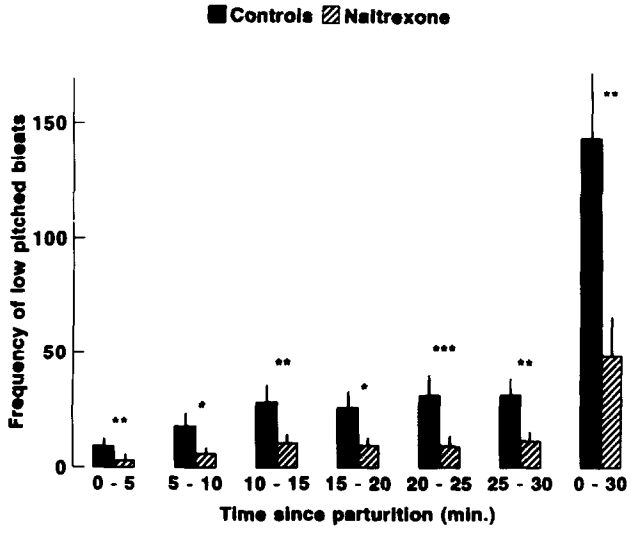

FIG. 2. Mean frequencies of low pitched-bleats (±SEM) in controls (n = 9) and naltrexone-treated ewes (n = 15), over the first 30 min following the birth of the lamb. *p = 0.02; **0.01 ≤ p ≤ 0.005; ***p = 0.001 (between controls and naltrexone-treated ewes, Mann-Whitney test, one-tailed).

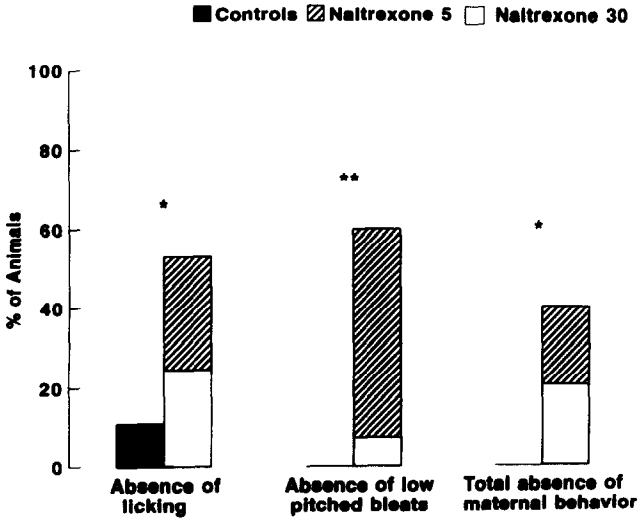

FIG. 3. Proportion of ewes failing to show any signs of maternal behavior following naltrexone injection at the first signs of labor. Controls: percent of control ewes that were not maternal 5 min after lambing (n = 9). Naltrexone 5: percent of naltrexone-treated ewes that were not maternal 5 min after lambing (n = 15). Naltrexone 30: percent of naltrexone-treated ewes that were not maternal 30 min after lambing (n = 15). All controls were maternal 30 min after lambing. *p = 0.048 and 0.037 for licking and absence of maternal behavior during the first 5 min, respectively; **p = 0.004 for absence of low-pitched bleats during the first 5 min (Fisher exact probabilities, one-tailed). No significant differences were found at 30 min for any of these three variables.

lamb. These findings lead to two main conclusions. First, it appears that opiates are likely to play some role in the onset of maternal behavior in parturient ewes, as suggested (6,11) in nonpregnant steroid-primed females. Second, our results indicate that this facilitatory action of opiates is not limited to multiparous mothers, but also exists in females giving birth for the first time. The latter finding does not necessarily conflict with an earlier report of no facilitation of maternal behavior by morphine in inexperienced nonpregnant females (11). Opiates may have a role in both experienced and inexperienced ewes, but the neurobiologic substrates of maternal behavior are probably less easily stimulated in the latter. This view is supported by other studies on the effects of sensory or physiologic manipulations in inexperienced ewes (i.e., olfactory, VCS, or steroid priming) (7,25). Thus, morphine may fail to stimulate maternal behavior in inexperienced females if the experimental situation does not fully replicate the physiologic context of parturition.

On the other hand, whereas naltrexone treatment significantly delayed the onset of maternal behavior, the effect did not appear to be as dramatic and long lasting as that of blocking other control factors (e.g., blockade of VCS by peridural anesthesia) (13). Indeed, recovery was rapid (< 30 min) in half of the animals affected by the treatment. This finding remains to be confirmed with a larger number of animals, but it may suggest that opiates have a modulatory role in the onset of maternal behavior rather than a primary one. Alternatively, it is also possible that a single injection of naltrexone at the first signs of lambing was not sufficient to antagonize the activation of the endogenous opiate systems totally, which occurs several days before parturition, at least in rats and humans (3,32).

In some respects, our results are similar to those of Mayer et al. (23), who found that opiate antagonists affected ingestive behaviors associated with parturition (i.e., placentophagia and pup cleaning in the rat). We obtained effects on licking of the neonate, probably the equivalent of pup cleaning. Interestingly, we also found effects on the emission of low-pitched bleats, another very specific indicator of maternal responsiveness in sheep, but without an ingestive component. This result suggests that the effects of naltrexone in the present experiment were not mediated through modulation of ingestive behavior or taste (16,29). There are several other possible modes of action of naltrexone. By blocking the action of β-endorphins that are normally released during parturition, naltrexone may reduce the release of oxytocin and thereby inhibit maternal behavior. Studies in sheep (6) indeed indicate that morphine facilitates the ICV release of oxytocin. Naltrexone might also block opiate stimulation of the attraction toward amniotic fluids occurring at parturition. This blockade would hamper the onset of maternal behavior, especially because it has been shown that amniotic fluids are essential for the normal development of maternal responses in primiparous ewes (17). The absence of low-pitched bleats found in our study could thus be a consequence of the effect of naltrexone on amniotic fluid ingestion.

A further possibility is that naltrexone affects pain thresholds at parturition, as has been proposed for the rat (23). In the rat, there is an increase of β-endorphins levels in the brain during the last 1-2 days of pregnancy (32). These changes are accompanied by an abrupt increase in jump thresholds on a hot plate, which is blocked by naltrexone treatment (2). In addition, this pregnancy-mediated analgesia is normally reinforced by amniotic fluid ingestion (14,15). Because naltrexone may block these analgesic effects, we cannot exclude the possibility that the disturbances found in our study arise from a more painful birth process, even though no such evidence was suggested from the behavior of the animals during labor. Further studies on the possible existence of analgesia caused by the process of parturition in sheep would be necessary to clarify this point.

Finally, the possibility that intravenous administration of naltrexone induced illness in our animals must also be considered. However, several facts argue against this proposal. None

TABLE 1

BEHAVIORAL SCORES (FREQUENCIES ± SEM) OF PARTURIENT EWES RECEIVING 50 OR 150 mg OF NALTREXONE, IV, AT FIRST SIGNS OF LAMBING

	Naltrexone 50	Naltrexone 150
LB/30 min	39.1 ± 15.8	57.1 ± 24.6
HB/30 min	5.4 ± 4.5	0.25 ± 0.3
LI/30 min	322.4 ± 100.7	688.4 ± 204.1
HB WL/5 min	29.6 ± 8.8	39.7 ± 10.9

LB: low bleats in presence of the lamb; HB: high bleats in presence of the lamb; LI: licking of the lamb; HB WL: high bleats on withdrawal of the lamb. The two groups do not differ significantly for any of the behaviors recorded. However, all behaviors indicative of maternal responsiveness (LB, LI, and HB WL) had a lower score in the NAL50 group, which was the opposite for HB. This does not support the hypothesis of unspecific effects of NAL.

of the animals showed observable signs of nausea (e.g., vomiting or prostration). It can also be pointed out that even though the animals receiving 50 and 150 mg of drug did not differ significantly in their behavior, the effect of naltrexone was more marked for all components of maternal behavior in the NAL50 group (Table 1). This finding does not agree with the hypothesis of nonspecific effects (either pain or illness), which would be more likely to occur at the higher dose. Also, in an experiment still in progress we have investigated the possible effects of naltrexone (20 mg, IV) on the maternal behavior of ewes at 5-10 days of lactation. No effects were found either on the maternal responses to separation from the lamb for 5 min or on their behavior on reunion (Table 2). Our results are consistent with those obtained in nonpregnant ewes receiving intracerebral injections of morphine or naltrexone (6,11), thus suggesting that the effects reported here are probably via a central rather than a peripheral action.

Thus, our results confirm that opiates play, directly or indirectly, some facilitatory role in the normal onset of maternal behavior at parturition in sheep, as already suggested by previous studies in nonpregnant ewes. Although they are also in agreement with those of Mayer et al. (23) discussed earlier, it must be stressed that they are at variance with the findings that μ-receptor agonists delay the onset of maternal behavior in rats whose pregnancies were surgically terminated on day 17, whereas naltrexone suppresses this effect (1,22). Whether these discrepancies reflect a different role of opiates in rats and sheep is not clear. Even in the rat there is some variation in the effects encountered in the various studies, perhaps depending on the type of experimental models used (parturition vs. pregnancy-terminated models).

On the other hand, there was no indication that naltrexone disturbed the establishment of selective suckling in the parturient ewe. Although the present study provides only preliminary results on selectivity, they are consistent with other evidence concerning the different mechanisms controlling the onset of maternal behavior and the memorization of the lamb's odor. For example, vaginocervical stimulation influences both maternal responsiveness and selective bonding (10), but norepinephrine release in the olfactory bulbs influences only maternal selectivity (19,24). This differential effect of naltrexone on maternal responsiveness and selective behavior is also a further indication that the action of the drug was specifically on maternal responsiveness and not due to general discomfort or nausea.

Finally, we were unable to find any enhancement by naltrexone treatment of postpartum estrus behavior. Therefore, it would appear that the high level of β-endorphins at parturition is not responsible for inhibiting estrus behavior, which one might expect because of the very high levels of estradiol preceded by sustained levels of progesterone. On the other hand, β-endorphins might have been released well before we treated the animals, and this may have been sufficient to mask the effects of naltrexone on sexual receptivity. It is also possible that estrus behavior would have appeared later had we tested our animals at longer intervals after parturition. Although for practical reasons it was impossible to prolong testing in the present experiment, this possibility merits further study. Finally, recent studies obtained in cycling ewes indicate that the central release of oxytocin itself can reduce sexual receptivity (5). Because there is significant release of this peptide at the time of parturition (9), oxytocin is likely to have some role in inhibiting sexual receptivity at this time, even though this possibility does not exclude some participation of opiates in this process. It is clear that further investigations are still required to understand fully the role of endorphins in regulating the behavior of the parturient ewe.

TABLE 2

EFFECTS OF NALTREXONE TREATMENT (20 mg, IV) ON MATERNAL RESPONSES (FREQUENCY/5 min) OF EWES IN PRESENCE AND IN ABSENCE OF THEIR LAMB AT 5-10 DAYS OF LACTATION

	Controls		Naltrexone	
	With Lamb	Without Lamb	With Lamb	Without Lamb
LB	3.2 ± 1.6	4.6 ± 1.3	2.3 ± 1.1	4.8 ± 1.4
HB	1.7 ± 0.7 *	51.1 ± 5.0	2.2 ± 0.9 *	50.5 ± 5.3
SQ	1.4 ± 0.6 *	21.8 ± 4.8	0.8 ± 0.4 *	17.0 ± 2.3
AU	0.3 ± 0.1	–	0.4 ± 0.1	–
NO	4.3 ± 0.9	–	4.6 ± 1.6	–

LB: low bleats; HB: high bleats; SQ: number of crossed squares; AU: acceptance at the udder; NO: nosing of the lamb by the ewe. For further details of testing conditions, see (27).

*$p < 0.05$ between the two testing situations (Wilcoxon test). No significant differences were found between the two groups for any of the variables studied (Mann-Whitney test). [From Poindron et al. (unpublished).]

ACKNOWLEDGEMENTS

M. Caba was supported by a traveling grant from CIRA, CINVESTAV-UAT, and the Universidad Veracruzana. The authors are grateful to V. Piketty for help during the experiment, R. H. Porter for help and comments, and the staff of Brouessy for allowing this study to be carried out on the experimental INRA farm of Magny les Hameaux.

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