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Original article

The effect of a mixed-management system on the release of oxytocin, prolactin, and cortisol in ewes during suckling and machine milking

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Abstract — Milk yield and plasma oxytocin (OT), prolactin (PRL), and cortisol (CORT) during suckling and machine milking were measured in multiparous ewes subjected to a mixed management system of 3 sucklings and two daily milkings. Peak hormones were significantly increased and were similar during suckling and milking for PRL (181 vs. 163.3 ng·mL^{-1}) and CORT (12.5 vs. 11.5 ng·mL^{-1}). During the period of exclusive suckling, OT was always significantly released (90.3 pg·mL^{-1}); however, during the period of mixed management, OT concentrations only increased during suckling compared to milking (91.7 vs. 13.1 pg·mL^{-1}). The mean volume of milk obtained during suckling (632 mL) was significantly higher than during milking (255 mL). Thus, during a mixed management system, oxytocin and prolactin releases are not under similar central regulation. A mixed system, without OT release during milking, does not contribute to accelerate the conditioning of ewes for machine milking.

oxytocin / prolactin / cortisol / milking / suckling / ewes

Résumé — **Effet de la conduite mixte sur la décharge d'ocytocine, de prolactine et de cortisol lors des traites et tétées journalières chez la brebis.** La production laitière et les concentrations en ocytocine (OT), prolactine (PRL) et cortisol (CORT) ont été mesurées chez 12 brebis multipares lors d'une conduite mixte d'allaitement/traite (3 tétées et 2 traites journalières). La quantité moyenne de lait obtenue à la tétée (632 mL) est supérieure à celle obtenue lors des traites (255 mL). Entre traites et tétées, des décharges significatives mais non différentes ont été enregistrées pour PRL et CORT (181 vs. 163.3 ng·mL^{-1} et 12.5 vs. 11.5 ng·mL^{-1} respectivement). La décharge d'ocytocine n'est significative que durant les tétées et est inexistante durant les traites (91.7 vs. 13.1 pg·mL^{-1}) alors qu'elle est présente aux mêmes horaires lors de la tétée exclusive. Les décharges de PRL, CORT et OT peuvent donc être découplées prouvant l'existence d'un contrôle central différent. L'absence de décharge d'ocytocine à la traite fait que la conduite mixte n'est pas efficace pour adapter physiologiquement les brebis à la traite mécanique avant le sevrage.

ocytocine / prolactine / cortisol / traite / tétée / brebis

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1. INTRODUCTION

When dairy ewes were subjected to machine milking within 8 h post-partum, OT release is limited but increases rapidly during the first 5 days in response to mechanical stimulation, thereby facilitating alveolar milk ejection and an increase in milk yield [23, 25]. Conversely, when ewes are not exclusively machine milked immediately post-partum, the longer they remain in contact with their lambs during the suckling period, the more difficult it is for them to adapt to exclusive machine milking following weaning [7, 16, 19]. Several authors have observed a marked drop (30 to 40%) in total milk yield at the time of weaning [7, 17, 19, 31]. The decreased milk yield following weaning can only partially be explained by less frequent udder evacuation [18] because total milk yield obtained by lamb suckling was higher than that obtained by machine milking with the same frequency [19]. Labussière et al. [19] and Gargouri et al. [8] have therefore tried to habituate ewes to machine milking and prevent some of the loss in milk yield at weaning by using a mixed management system. In this system, ewes are allowed to nurse their lambs for part of the day, and then are separated for machine milking once daily the following morning. However, despite increases in total milk yield with the mixed system because of more complete udder evacuation, they still found a significant drop in milk production at the time of complete weaning (20%). These results suggested that the suckling stimulus of a ewe's own lamb was more efficient in stimulating milk ejection, and therefore milk yield, than the stimulus generated by a milking machine [19]. Furthermore, when cows or ewes nursed offspring other than their own, milk yields were consistently lower compared to nursing of their own offspring, yet similar to milk yields obtained by machine milking [19, 29].

In cattle, the separation of dams from their calves after a suckling period longer than 24 h, induces mammary involution [20, 24]. Additionally, plasma PRL concentrations during suckling indicate that the release pattern of PRL is higher in cows that are exclusively nursed compared to exclusively machine milked following weaning [1, 2, 30]. Release patterns for plasma OT concentrations were not different for cows during exclusive suckling compared to exclusive machine milking [1]. During the first few machine-milkings following complete weaning, Tancin et al. [36] demonstrated a sharp increase in plasma CORT concentrations which suggests a significant level of stress associated with complete dam-offspring separation or a change in environment (i.e. the milking parlor) [4, 36].

Given the above experimental results in cattle, it seems therefore necessary to evaluate the hormone release pattern in ewes for OT, PRL, and CORT, the later potentially serving as an important indicator of stress. The objective of the present experiment was to estimate the effects of a mixed-management weaning system on accelerating the adaptation of ewes to machine milking, by measuring the release and peak plasma concentrations of OT, and to a lesser extent of PRL and CORT, and by comparing their concentrations during suckling and machine milking.

2. MATERIALS AND METHODS

2.1. Animals

Twelve multiparous Lacaune ewes (parity 2 to 5) of similar body weight, size, and udder morphology were managed by either exclusive suckling, mixed suckling/machine milking and exclusive milking after weaning (6 ewes only), from parturition through the first 7 weeks of lactation. Five of the ewes gave birth to twins and seven of the ewes gave birth to one lamb. During the interval between sucklings or machine milkings, ewes were physically separated from their lambs to bar udder access yet were allowed to maintain some contact (visual, olfactory, auditory, and occasionally nose contact) with their lambs. For the first three weeks of lactation, ewes were not machine milked and were suckled five times per day (7:00, 10:00, 13:00, 16:00, and 19:00). During weeks four to six, instead of suckling, ewes were machine milked at 10:00 and 16:00. After day 42, weaning occurred for six of the ewes entering a normal period of exclusive milking two times per day at 10:00 and 16:00. The six other ewes continued in the same mixed system as before. Ewes consumed a basal diet of forage (hay) supplemented with concentrate that was balanced in energy and protein for their respective milk yield. Perennial rye-grass hay as well as vitamin and mineral supplements were offered to the ewes ad libitum.

At the time of suckling, a brief moment was necessary for lamb recognition, after which a stopwatch was engaged to measure the length of time that the lamb(s) suckled. In order not to disturb the ewe and her lamb(s) during nursing, suckling was considered over when it had been voluntarily interrupted by the lambs for more than 30 s. The volume of milk consumed by the lambs was measured by weighing them immediately before and after suckling. During machine milking, all ewes were milked for two minutes and then massaged to achieve machine stripping. The milking machine parameters were set at a vacuum of 40 kPa, a pulsation ratio of 50:50, and a rate of 180 pulses per minute (Alfa-Laval® teatcup #9610361 and silicon liner #10000080). Milk yield was measured in graduated electronic milk collection jars placed between the claw and the milk line.

2.2. Blood sampling and hormone assay

To avoid disrupting the ewes during sample collection, an intravenous catheter was placed in the jugular vein one week prior to collection. Venous blood samples were collected during all episodes of suckling and milking on the 15th and 16th day during period of exclusive suckling, on the 41st and 42nd day of lactation during the period of mixed system and on the 46th to 55th day during the early post weaning period. Blood was collected at –0.5, 0.5, 1, 4, 10, 15 and 30 min (relative to placement of the teatcups) into heparinized tubes previously cooled in crushed ice and kept at 4 °C following collection. The tubes were centrifuged at 3 000 *g* for 15 min at 4 °C and the plasma removed and stored at –20 °C until the time of assay. Plasma OT was measured by EIA [22] during the three periods (limit of detection 1.5 $pg \cdot mL^{-1}$, CV inter and intra-assay respectively of 13% and 8.6% for 4 $pg \cdot mL^{-1}$). PRL (limit of detection 1.24 $ng \cdot mL^{-1}$; CV inter and intra-assay respectively of 4.5 and 12.6% for 1.36 $ng \cdot mL^{-1}$) and CORT (limit of detection 98 $pg \cdot mL^{-1}$; CV inter and intra-assay respectively of 5.3% and 15.3% for 2 $ng \cdot mL^{-1}$) were measured by RIA [12] and [9], only during the period of mixed management.

2.3. Statistical analysis

Analysis of variance was conducted with the GLM procedure of SAS [33]. The Student's t-test and Newman-Keul's test were used for comparison of the means at a significance level of 0.05. The model used in the analyses for hormone release accounted for the main effect of suckling or machine milking, the sampling, animals or the litter size effects and the two-way interactions. The relationships between milk yield and hormone concentrations were analyzed using correlation matrices. Data are presented as mean ± standard error (SEM).

3. RESULTS

3.1. Oxytocin

Baseline OT concentrations were similar during suckling and machine milking during the entire experiment (6.2 ± 1.8 and

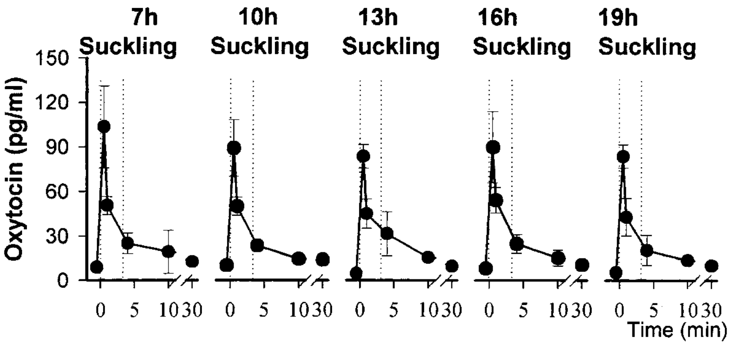

Figure 1. Daily pattern of oxytocin during period of exclusive suckling (7, 10, 13, 16, 19 h). The vertical dotted lines indicate the beginning and the end of suckling episodes (data are means ± SEM for 12 ewes and 2 days, n = 24).

7.4 ± 2.4 pg·mL^{-1}, respectively). Before application of the mixed system, plasma OT concentrations increased significantly during suckling (90.3 ± 23.4 pg·mL^{-1} for peak OT concentration) and did not differ between the episodes of suckling and between days (Fig. 1). During suckling, OT concentrations peaked at approximately 0.5 min after the beginning of nursing; by 4 min, OT concentrations were no longer different from baseline controls.

During the mixed system, plasma OT concentrations (Fig. 2A) were consistently and significantly higher during suckling compared to machine milking (91.7 ± 26.1 vs. 13.1 ± 1.8 pg·mL^{-1} for peak OT concentrations, respectively). OT pattern of release was similar during the exclusive suckling period and during mixed management. However, machine milking was only able to induce low, yet significant, OT release compared to baseline levels. During machine milking, a sharp increase in plasma OT concentration was noted following teat cup attachment, however OT levels dropped to non-significant levels between 1 and 4 min. Plasma OT concentrations were similar between sucklings and between machine milkings episodes and between the succeeding two days. This pattern was maintained as long as the mixed system continued (i.e. 55 days).

At the first milking immediately after weaning (beginning of the exclusive milking period), 3 of the 6 ewes presented a significant increase in the release of oxytocin. At the second milking 5 of the 6 ewes responded to milking and after 3 milkings all ewes presented a significant increase in OT concentration at milking. The concentration increased for 3 days to finally reach the normal level of OT recorded in ewes within the flock that are exclusively machine-milked immediately after parturition (Fig. 3).

Total or peak OT concentrations during suckling never significantly differed between ewes with twins or single lambs. However, there was a clear tendency for OT to be higher during milking and suckling in ewes nursing twins. For ewes nursing twins, during suckling, the shape of the mean curve was different and was characterized by a longer release compensated by a lesser peak concentration.

3.2. Prolactin

There was little variation between baseline plasma PRL concentrations, however PRL tended to be slightly higher during the 13:00 and 16:00 samplings. PRL concentrations during either suckling or machine milking (Fig. 2B) were consistently and significantly higher than baseline levels, however there were no significant differ-

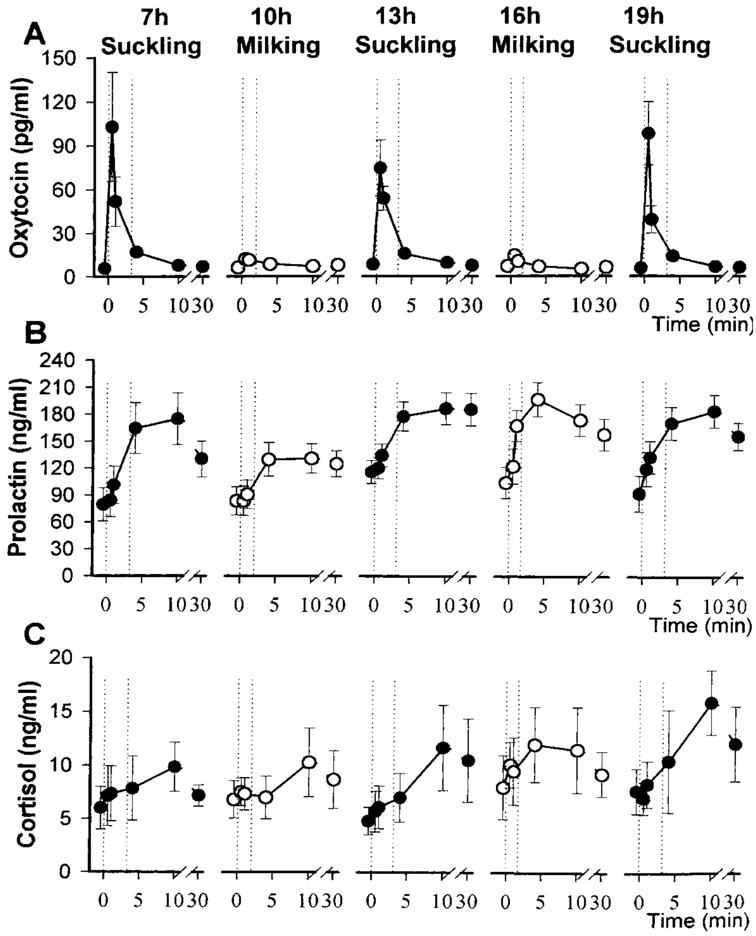

Figure 2. Daily pattern of oxytocin, prolactin and cortisol release during a mixed system of suckling (7, 13 and 19 h) and milking (10 and 16 h). The vertical dotted lines indicate the beginning and the end of suckling and milking episodes (data are means ± SEM for 12 ewes and 2 days, $n = 24$).

ences between suckling- and machine milking-peak PRL concentrations (181 ± 21.4 and 163.3 ± 17.5 ng·mL^{-1}, respectively). PRL concentrations were lowest ($P < 0.05$) during the 10:00 sampling taken during milking, whereas PRL at the 16:00 milking was as high as that measured during suckling. Regardless of either suckling or milking, PRL tended to peak at the 5-min sampling, and by the 15-min sampling, PRL was still higher ($P < 0.05$) than baseline levels. Plasma OT and PRL release patterns were slightly higher in ewes suckling twins compared to ewes suckling only one lamb. However, due to the small number of ewes in the experiment, these differences were not significant. The patterns of PRL release during milking and suckling and throughout the day were not different between the two succeeding days.

Total PRL release or peak concentrations during suckling never significantly differed between twins and single lambs despite the difference in suckling time.

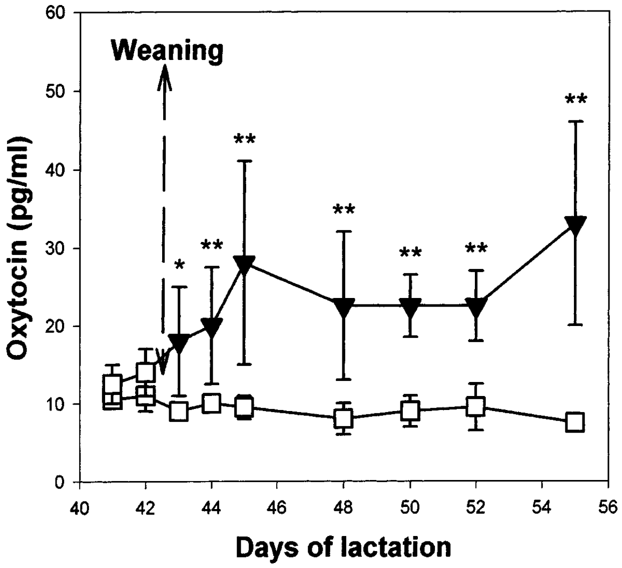

Figure 3. Thirteen days post weaning evolution of mean OT concentrations (over 10 min) at milking (10 and 16 h) for weaned animals subjected to exclusive milking —▼— (n = 6) after day 42 compared to animals continuing in a mixed system —□— (n = 6).

3.3. Cortisol

Plasma CORT concentrations did not differ between suckling and milking (12.5 ± 3.1 and 11.5 ± 3.35 $ng \cdot mL^{-1}$, respectively), however CORT release was consistently above baseline levels measured before stimulus (Fig. 2C). Plasma CORT concentrations tended to be lower in the morning compared to evening. It should be noted that inter-animal variation in CORT was large during both suckling and machine milking. These patterns of release during milking and suckling and throughout the day were not different between the two succeeding days.

Total CORT release or peak concentrations during suckling never significantly differed between twins and single lambs despite the difference in suckling time.

3.4. Milk yield

Mean suckling time for twin lambs was longer than that for single lambs (5.5 vs. 3.5 min, respectively). Mean daily milk yield during the experiment was 2 424 ± 55 mL. Figure 4 summarizes the mean milk production from either suckling or milking times. Mean milk yield during suckling (632 ± 71 mL) was significantly higher than that obtained during milking (256 ± 24 mL). The largest amount of milk was obtained during the 7:00 suckling (1 172 ± 110 mL) which was significantly more than at any other suckling or milking time. Suckled milk yields during the 13:00 and 19:00 times (378 ± 28 and 422 ± 29 mL respectively) were consistently higher than machine milk yields during the 10:00 and 16:00 time (259 ± 42 and 193 ± 23 mL, respectively). However these differences were only significant between the 19:00 suckling and 16:00 milking. Less milk was obtained during machine milking from ewes with twin lambs compared to single lambs (192 ± 35 vs. 260 ± 32 mL, respectively). However during suckling, ewes with twin lambs produced significantly more milk than those nursing singles (764 ± 113 vs. 551 ± 86 mL, respectively). Lamb live-weights during the experiment (4.00 ± 0.35 and 19.1 ± 1.5 kg at birth and 42 days, respectively) were comparable to non-experimental animals in this flock.

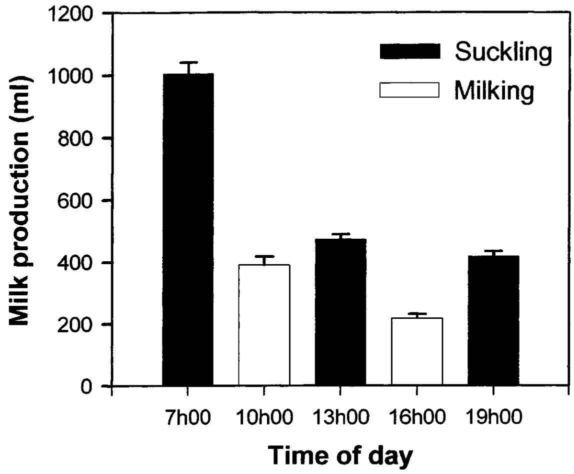

Figure 4. Mean milk production during each episode of suckling (7, 13 and 19 h) and milking (10 and 16 h) per day. Lambs were not allowed to suckle during the night (from 23 h to 7 h). (Data are means ± SEM for 12 ewes and 6 days, $n = 72$).

4. DISCUSSION

This experiment demonstrates that suckling results in significant increases in plasma OT concentrations above baseline levels, and more importantly, the increases in OT are clearly higher during suckling compared to machine milking during application of the mixed system. This is in agreement with other authors who have studied dairy cattle during milking and suckling [1, 2] and during mixed management systems [2]. Although the trends between these experiments and the present one appeared to be similar, quantitative comparisons are unfortunately not possible because blood samples in the later experiments were collected at approximately 10 min after the end of stimulation and the results were presented as means of samples collected every half-hour.

OT release during suckling in the present mixed management system was similar to that observed during the exclusive suckling period and agrees with other reports in the literature of suckled [5] and exclusive machine-milked ewes [24, 25, 27, 28]. Conversely, OT release in the present experiment during machine milking was very low by comparison to that recorded for ewes during exclusive machine milking [24, 25, 27, 28]. The low plasma OT concentrations during machine milking might not be explained by an inability of the pituitary gland to respond to the machine stimulus within three hours. It is not the case with the same frequency of exclusive suckling and additionally, we have previously confirmed that the release of OT from the pituitary gland during very frequent machine milking (7 milkings per day) is not a limiting factor [26]. Because this specific inhibition disappeared just after mother-young bond rupture at weaning, by comparison to non-weaned ewes, it is more likely due to an effect of maternal behavior. The specificity component of maternal behavior appears to be operational not only when foreign lambs suckle but also during other sources of stimulation (the milking machine in the present experiment). Therefore the inhibition of the milk ejection reflex is probably not due to a signal that is disruptive, but instead due to a lack of the proper signal normally present during suckling by the ewe's own lambs which results in oxytocin release.

Our results indicate that inhibition of the milk ejection reflex during this mixed system is not related to further milkability of the animals after weaning.

PRL release in these experimental ewes was not different between suckling and machine milking. However, both stimuli resulted in significant increases in circulating PRL relative to baseline levels, which were consistent with previously reported values in the ewe [13, 27, 28]. Akers and Lefcourt [1] also demonstrated no significant difference in PRL between suckling and milking in the cows. However, their results may have been confounded by the fact that PRL was measured very early in lactation, the time when concentrations are normally highest. In cattle, PRL release during suckling compared with machine milking appears to be different. Bar-Peled et al. [2] found no significant release of PRL during milking either in a mixed management system or during exclusive machine milking, yet found significant PRL release during suckling. Perez et al. [30] also demonstrated that PRL release during exclusive suckling was clearly higher than during exclusive milking. A caveat to these two later reports is that the experimental design did not allow these authors to monitor suckling and milking within the same cow, and the cows were completely separated from their calves at calving. In the present experiment, ewes could see, smell and hear their lambs. Although it has been demonstrated that PRL release could be induced by the presence of offspring [1], PRL release during milking with contact with the calves is the same or even lower [10] than that during the milking of cows separated from their calves. The above inconsistencies in PRL concentrations during suckling and machine milking may in fact be due to the degree of mother-offspring bonding, influenced by species, breed, and number of days post-partum of the experimental period. In hardy breeds of cattle not selected for machine milking aptitude (i.e. the Salers in France), milking requires the presence of the calf on one teat to induce milk ejection. The interruption of the mother-offspring bond by the forced suckling of a foreign calf or by machine milking may induce rapid drying-off. This phenomenon could be a result of major inhibition of the release of all galactopoietic hormones during udder stimulation [15, 20]. Moreover, the pattern of PRL release in the present experiment may be an indication that Lacaune ewes are in fact being effectively selected for their adaptability to machine milking. Further experimental work is necessary to confirm that these observations are not merely coincidental with expected habituation to machine milking by 40 days in lactation.

Although non-significant because of high variability of response and of reduced number of animals, overall plasma concentrations of OT and PRL tended to be higher and more prolonged at suckling for ewes nursing twins compared to singles. Other authors have shown that the larger the number of teats, the higher the PRL release [14, 30]. Suckling duration may have contributed to slightly higher plasma concentrations, because ewes with twins suckled their lambs for approximately two minutes longer than ewes with singles. This point remains to be verified in the future in order to explain the function of non-nutritive suckling and to improve lactation persistency by means of longer stimulation at milking.

After suckling and machine milking, plasma CORT concentrations increased significantly over baseline levels, however, the differences at suckling and milking were not significant. Work done in cattle by Wagner and Oxenreider [38] agree with our observations. They found no significant differences in CORT concentrations at suckling and milking, and observed a peak in CORT, 15 min after the beginning of udder stimulation. CORT concentrations have also been shown to be markedly increased (peaks as high as 4 to 5 times baseline levels) when measured before, during, and after the first post-weaning milking, quite possibly due to mother-offspring separation and new environmental factors such as the machine milking routine [36]. Because CORT concentrations in the present experiment were similar at suckling and at machine milking, it is reasonable to assume that habituation to machine milking was probably effective, and is therefore probably not a result of untoward stress during udder stimulation. This is further supported by our observations of the willingness of the ewes to enter the milking parlor, the lack of cluster falls, and the presence of rumination during machine milking. The increase of basal levels during the day could be a result of increasing stress of manipulation, however it is more likely due to a nycthemeral rhythm already described in ewes [6, 21].

We conclude that the quality of udder stimulation (either from suckling or the machine) does not appear to influence CORT or PRL release in Lacaune ewes, and therefore, these two hormones are not likely to significantly influence OT concentrations at the time of suckling or machine milking.

Although our results imply a clear dissociation between PRL or CORT release and OT release, this phenomenon is not in agreement with at least two other reports that demonstrated that PRL release may be stimulated by OT during suckling [3, 32]. Under identical stimulation (suckling) of ascending neural pathways, our results would suggest that OT release from the posterior pituitary is not a prerequisite for PRL and ACTH release from the anterior pituitary at time of udder stimulation. Furthermore, Silveira et al. [35] demonstrated that in beef cattle, cows suckling their own calves had higher OT release over a 24-h period than cows suckling foreign calves; PRL release, however, was not different. These results in conjunction with those of the present study indicate that there is a selective inhibition of OT release during udder stimulation at the central level, quite possibly due to a modification of the oxytocinergic system. According to Theodosis et al. [37], the neuronal plasticity of the oxytocinergic system is profound and rapid, especially under the effects of olfactory stimulation in an adequate endocrine (estrogen) milieu in rats. Even if lactation is not defined in terms of specific steroidal requirements, the endocrine milieu is consistent in terms of PRL and ACTH releases for example. Additionally, maternal behavior is present including frequent contacts, licking and sniffing of the young. Thus, it could be hypothesized that a combination between this endocrine milieu and signal(s) coming from the young could act via the connections between hypothalamic magnocellular neurons and might play a role in either activating or inhibiting OT release very rapidly. That might provide another level of regulation**,** in addition to the system induced by opiate receptor neurons at the supraoptic and paraventricular nuclei levels. This is consistent with the observations of Bruckmaier and Blum [4] whose could not disinhibit OT release after weaning by the injection of opiate antagonists. From our results, we can conclude that there exists a selective inhibition of OT release in accord with maternal behavior.

Our results confirm that during early lactation, milk yield in dairy ewes remains much higher than what is required by the lambs. This justifies the use of a mixed-management weaning system, which permits commercial use of the excess milk and sustains milk secretory processes [8, 11, 16]. Furthermore, mean total daily milk yield obtained from the experimental ewes with the mixed weaning system was higher than that obtained from the rest of the flock that was machine milked twice per day (2 591 ± 57 vs. 1 830 ± 65 mL, respectively), during the same stage of lactation. Additionally, it appears that the frequency of milk removal from the udder limited the effect that the number of lambs had on milk yield.

The present study was unable to elicit any direct relationship between hormone release and milk yield obtained during either suckling or milking. This might be due to the fact that there was no significant difference in galactopoietic hormone (PRL and CORT) release at suckling and milking, and that only small amounts of OT (3 to 5 pg above baseline levels at milking in the present experiment) are sufficient to induce effective udder drainage [34]. More likely, however, is the fact that frequent milk removal from the udder proportionally reduces the volume of alveolar milk. Therefore, even if alveolar milk was neither properly ejected nor retrieved during milking, the accumulation of its small volume had little impact on subsequent milk secretion. The relatively low OT release during machine milking suggests that ewes are never totally adapted to milking during a mixed-management weaning system, which might explain why mixed management only partially reduces losses in milk production at the time of weaning.

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