Central and peripheral oxytocin profiles during milking in ewes

Perfil de ocitocinea central e periférica em ovelhas lactantes

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Abstract

The present work investigated the possible relationship between central and peripheral oxytocin (OT) release during milking in experimental ewes. Ten multiparous ewes were divided into four groups according to milk ejection stimuli: exclusive machine milking (EM), mixed-management milking and suckling, lambs separated during the night and reunited with their mother after morning milking (MMS); mixed-management with manual milking (MMM), and exclusive suckling (ES) lambs also separated during the night. Simultaneous sampling of cerebrospinal fluid (CSF) and blood was performed during milking. The means, standard deviations, variation coefficients, and minimum and maximum CSF and plasma OT concentrations were the following, respectively: 257.88 ± 265.90 pg/ml, 103.11%, and 11.70 and 1000.00 pg/ml. No statistically significant correlations were found between OT concentrations in the CSF and plasma samples (EM: -0.26; ES: -0.19; MMM: 0.05; MMS: 0.04). The OT concentration in CSF was not influenced by milk ejection stimuli, although plasma OT was higher in the MMM (679.80 ± 25.63) and MMS (591.82 ± 30.56) groups compared with the EM and ES groups. Additionally, plasma OT concentrations were higher in the OME group (381.04 ± 22.09) compared with the AE group (218.82 ± 27.04). In conclusion, no positive correlations were found between central and peripheral OT concentrations during milking and suckling. Plasma OT concentrations differed as a function of milking management and had consequences for both milk ejection and production. Plasma but not CSF oxytocin concentrations were influenced by different milk ejection stimuli.

Keywords: Catheterization. Milk ejection. Cerebrospinal fluid. Sheep. Lactation.

Resumo

Foi investigada a possível relação entre as concentrações de ocitocina no líquido cefalorraquidiano e no soro em diferentes formas de ordenha em ovinos. Foram utilizadas dez ovelhas multíparas, divididas em quatro grupos de acordo com o estímulo para ejeção do leite: ordenha exclusivamente mecânica (EM), ordenha mista mecânica e mamada com os carneiros separados das mães durante a noite e reunidos a elas pela manhã para amamentação (MMS); ordenha mista com ordenha manual (MMS); apenas amamentação natural (ES). Foram coletadas amostras de fluido cerebroespinhal e de sangue, simultaneamente, durante as ordenhas. A média, o coeficiente de variação e os valores máximos e mínimos de ocitocina do plasma foram respectivamente 257,88 ± 265,90 pg/ml, 103,11%, e 11,70 e 1000,00 pg/ml. Não foram encontradas correlações entre as concentrações centrais e plasmáticas de ocitocina (EM: -0,26; ES: -0,19; MMM: 0,05; MMS: 0,04). Não foi evidenciada influência do tipo de estímulo para ejeção do leite nas concentrações centrais de ocitocina. Entretanto, as concentrações plasmáticas de ocitocina foram maiores nos grupos MMM (679,80 ± 25,63) e MMS (591,82 ± 30,56), quando comparadas às dos grupos EM e ES. Além disso, as concentrações plasmáticas de ocitocina foram maiores no grupo de OME (381,04 ± 22,09) em relação ao grupo AE (218,82 ± 27,04). Os resultados obtidos sugerem que as concentrações plasmáticas de ocitocina são mais sensíveis ao tipo de ordenha que as concentrações centrais deste hormônio.

Palavras-chave: Cateterização. Ejeção. Líquido cefalorraquidiano. Ovelha. Lactação.

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Introduction

In vivo neuroscience methods, such as microdialysis, and electrophysiology have provided valuable information about neurotransmitter release, neuronal activity, and a diverse range of brain functions (HUCKE et al., 1998; CRUZ-CASALLAS et al., 1999). Studies of different species should consider relevant differences. For example, the onset of maternal behavior in sheep is strictly dependent on peri-parturition activity, in contrast to rats. Once a mother-young bond is established, the selectivity for their own lamb is intense and persists until the end of lactation. In the rat, lactating mothers can easily accept alien pups (POINDRON; LÉVY, 1990; OLAZÁBAL et al., 2013; DWYER, 2014).

Oxytocin is a neurohypophysial hormone with a well-known relationship to milk release, delivery-inducing alveolar myoepithelial contractions, and uterine myometrium contractions (KIMURA et al., 1998). This hormone can also stimulate the immune system (MIRCIC; BELESLIN; JANCOVIC, 1998, MANN; WATHES; ROBINSON, 2013; LEE et al., 2013). This peptide is involved in the expression of reproductive behaviors and establishment of social bonds in mammals (WINSLOW et al., 2003; HOLLANDER, et al., 2007). In females, oxytocin facilitates maternal behavior (KENDRICK et al., 1988; PEDERSEN et al., 1982). The importance and complexity of such central actions has been the subject of scientific studies. Some authors were not able to establish relationships between the levels of oxytocin found in cerebrospinal fluid (CSF) and plasma (JONES; ROBINSON; HARRIS, 1983; MORRIS; BARNARD; SAIN, 1984). Other studies have suggested that these actions may be interrelated (KENDRICK, 2000; LIPSCHITZ; CROWLEY; BEALER, 2003; TYZIO et al., 2014).

Mammary gland suckling, which occur during lactation, stimulates oxytocin release (MIRCIC; BELESLIN; JANCOVIC, 1998; WAGNER et al., 1997). Thus, the evaluation of the *in vivo* release of oxytocin may be relevant to studies that determine the patterns of release of this neurohormone during milk ejection, and other physiological situations. In ewes, the mother takes exclusive care of her own lamb (LÉVY et al., 1995). The release of oxytocin can be influenced by the type of stimulus (i.e., the suction exerted by the calf or milking, which can interfere with the milk ejection fraction and its production) (MARNET; NEGRÃO; LABUSSIERE, 1998; WEISS; DZIDIC; BRUCKMAIER, 2003).

Several factors can cause a disturbance in the milk ejection reflex, such as the completion of milking in unfamiliar surroundings, the presence of strangers, and weaning (BRUCKMAIER, 2005; HOPSTER et al., 2002). Even in a mixed management system, where females are allowed to feed their young part of the day, a loss of milk production occurs after weaning (MARNET; NEGRÃO; LABUSSIERE, 1998). Thus, suction and mother-young interactions always more potently increase plasma oxytocin secretion compared with the milking machine (MARNET; NEGRÃO, 2000).

The activity of groups of oxytocinergic neurons can be dissociated from the peripheral concentrations of this hormone. Low plasma oxytocin concentrations may not interfere with CSF concentrations (JONES; ROBINSON; HARRIS, 1983; MORRIS; BARNARD; SAIN, 1984). However, evidence indicates that the permeability of the blood-brain barrier can be modified by stress and therefore by the presence of circulating adrenocortical hormones, which may occur prenatally and during milking (LONG; HOLADAY, 1985; LIPSCHITZ; CROWLEY; BEALER, 2003).

These data indicate the importance of using an integrated approach to compare oxytocin

concentrations in CSF and plasma. Thus, the main objective of the present study was to quantify oxytocin release in the central nervous system and plasma, respectively, during milking. Developing an accurate surgical technique was necessary, and the puncture and catheterization of the subarachnoid space used for the collection and laboratory analysis of CSF (COWELL et al., 1999) was shown to be efficient with a low incidence of injury. A radiographic contrast technique known as myelography was also used to achieve our aims.

Material and Methods

The experiments were conducted on the farm of the Veterinary Hospital of the Faculty of Veterinary Medicine, UNIFEOB, using 23 Santa Ines breed sheep (*Ovis aries*) with similar weights and numbers of lactations that gave birth to a total of 33 lambs. The sheep had free access to water and a mineral and vitamin supplement. The sheep were given a mixture of grain (corn and soybean meal) and corn silage according to their productive potential. The animals were kept in semi-confinement, in which they were confined in a stall overnight, with free access to a paddock (between 10 and 16 h) with coast-cross grass with water and shade. At birth, the lambs were identified using earrings and weighed daily using a Kern CH 15K20 digital scale until weaning at 60 days of life. The experimental protocol was in accordance with the Standards of Ethical Animal Experimental Procedures, UNIFEOB, and Faculty of Veterinary Medicine, University of São Paulo.

Experimental procedures

Subarachnoid space catheterization: The surgical procedures of catheterization of the subarachnoid space were preceded by food fasting. Three days before the anesthetic procedure, the food was reduced by half. Two days before the anesthetic procedure, the dry food was removed completely. One day before the anesthetic procedure, both food and water were withdrawn. The anesthetic protocol was performed, including premedication with acepromazine (2% acepran, 0.2 mg/kg, i.m.) and meperidine (dolosal, 2 mg/kg, i.m.). Approximately 20 min after premedication, anesthesia was induced with ketamine (vetaset, 10 mg/kg, i.m.) and xylazine (rompum, 0.5 mg/kg, i.m.). Once the animal showed a loss of the laryngotracheal reflex, intubation was performed, and anesthesia was maintained with halothane (tanohalo) inhalation.

The animal was positioned in right lateral recumbency with subsequent introduction of an intravenous catheter (BD Angiocath; 14-gauge) between the C1 and C2 cervical vertebrae to reach the subarachnoid space. The output of the CSF was observed. Subsequently, an epidural catheter (Portex; 16-gauge) was introduced approximately 12 cm via an intravenous mandrel catheter to the subarachnoid space. Nonionic radiographic contrast (iodixanol, Visipaque) was injected through the catheter, and radiographic examination of the cervical region was performed to confirm the location of the epidural catheter. After this confirmation, the intravenous catheter was removed, and the epidural catheter remained in the subarachnoid space to maintain access and enable the periodic collection of CSF. The epidural catheter was filled with deionized water and gentamicin. The skin was then sutured, and the catheter was securely affixed to the skin. The epidural catheter, which was fixed to the neck of the animal, was protected and housed using a bandage and medical tape around the animal's neck. Antibiotic therapy was performed using benzyl penicillin benzathine (Benzetacil, 40,000 IU/kg/day for 3 days, i.m.).

Jugular vein catheterization: Blood collection was performed using a 19-gauge scalp Solidor that was implanted into the jugular vein and fixed at the time of collecting blood and CSF samples simultaneously. This was repeated during the 5 days of collection. Between one sample and the next, the scalp was heparinized (100 IU heparin [Parinex]/ml water for injection). The left and right jugular veins were

alternated between days for obtaining blood, thus minimizing the possibility of phlebitis.

Sample collection: For the collection of blood and CSF samples, the animals were yoked and habituated to the milking parlor where milking crop was performed simultaneous in vivo without anesthesia. Samples were taken before (-0.5 min), during (0.5, 1, and 4 min) and after (10 and 15 min) milking. We also compared the plasma concentrations before experimental sampling in the cage. This was done to exclude the possibility that the sheep were already releasing OT before being placed in the sampling cage. A disposable 10 ml syringe was attached to the scalp to withdrawal blood. The whole-blood collection system was previously heparinized, and the dead volume of the system was discarded. Six milliliters of blood were then placed in heparinized 10 ml Falcon tubes. The blood samples were centrifuged at 3000 x g for 15 min at 4ºC for 30 min at the end of the collection days. The plasma was stored in freezers (-20°C).

For CSF disposable collection syringes were connected to an epidural catheter adapter. An average of 0.5 ml of CSF was obtained per sample. The samples were stored (-20°C).

Procedures for determination of oxytocin by immunoenzymatic assay: The samples were analyzed at the Laboratory of Physiology and Pharmacology, School of Veterinary Medicine, UNIFEOB, São João da Boa Vista. The quantification was performed using an immunoenzymatic OT assay. The cross-reactivity of the antiserum used was 100% for OT, 131 % mesotocine for 42.2% for vasotocin, 15.4% for OT-SH, 0.6% for vasopressin and less than 0.04% for GH, TRH, and vasoactive intestinal peptide. The detection limit of the method was 11.7 pg/ml, and the intra- and interassay coefficients of variation were 14.5% and 9.1% for OT concentrations of 19.3-21.4 pg/ml and 12.4% and 5.2% for an OT concentration of 300 pg/ml.

Experiments: The experimental procedures related to breastfeeding and milking were adopted from previous studies (NEGRÃO; MARNET, 2002; 2003; NEGRÃO; MARNET; LABUSSIERE, 2001). The same routine was followed every feeding or milking. The animals were accustomed to the milking yoke and milking management 20 days before the start of the experimental procedures. Teat cups were attached by the beginning of milking, which were removed approximately 3 min afterward. In the exclusively breastfeeding group (AE group), the same procedure was performed in the milking yoke, but rather than teat cups, the lambs sucked their mother's udder. Milking and CSF and blood sampling were performed in all of the ewes in the groups the same way.

In the management of exclusive suckling milking (ES group), the lambs were separated from their mothers daily beginning on day 16 at 6:00 PM. The next day, the sheep were placed with their young, and the lambs were allowed to suckle for more than 4 min during the sampling of blood and CSF. After this procedure, the mothers and lambs remained together for the remainder of the day. This procedure was repeated until the lamb was weaned, which occurred at 60 days of life.

In the exclusive milking group (EM group), the lambs remained with their mothers for 3 days after birth for colostrum intake. On day 4, they were subjected to early weaning and isolated from their mothers. These lambs received thermal comfort in the stall and were fed a mixture of milk and substitute milk at 8:00 AM, 12:00 PM, 4:00 PM, and 7:00 PM. At night, buckets were made available with five nozzles that contained only the substitute milk for 12 h. Daily fresh water and corn silage mixed with concentrate based on corn and soybean meal were available.

In the mixed breastfeeding management and milking group (MMM group), the lambs remained with their mothers for 3 days under conditions that were suitable for the complete ingestion of colostrum. Beginning on day 4, they underwent combined management that lasted until the lamb was weaned on day 60 of life. Therefore, the lambs were separated

from their mothers for 57 days at approximately 6:00 PM and taken to a "day care" cubicle that was located far from the sheep's barn. The next day, the lambs were returned to their mothers at approximately 8:00 AM. They remained there until 6:00 PM. Ewes were mechanically milked at 7:00 AM using combined management with breastfeeding and a milking machine during those 57 days. In the "day care" barn, the lambs had free access to water, grass, minerals, and corn bran.

In the mixed breastfeeding management and milking group (MMS group), the lambs remained with their mothers for 3 days under conditions that were suitable for the complete ingestion of colostrum. Beginning on day 4, they underwent combined management that lasted until 60 days of life, at which time the lambs were weaned, and the mothers were separated from the lambs daily at approximately 6:00 PM. The next day, the lambs were collected immediately after milking. Ewes were manually milked at 7:00 AM. This allowed combined management with breastfeeding and hand milking. In the "day care" barn, the lambs had free access to water, grass, minerals, and corn bran.

The oxytocin concentrations and weight gain of the lambs were analyzed by analysis of variance (ANOVA) using PROC GLM. Values of $p < 0.05$ were considered statistically significant. Correlation coefficients among oxytocin concentrations in milk and CSF samples was also evaluated according to treatment. Because significant effects were observed for power variation double interactions with the type of treatment the sample type, interaction was evaluated according to the collecting sites within each type of treatment. The same procedure was used to evaluate the types of processing within each sample type. To assess the types of treatment within each sample type, we used a multiple-comparisons Student's t-test. These analyses were performed by considering the different animals as random effects and discrete repeated oxytocin measurements over time.

Results

A comparison between the concentrations of OT during "stall" time and -0.5 min before the start of milking is shown in Table 1. The concentrations of OT in CSF and plasma in lactating ewes were quantified in the ES, MMM, EM, and MMS groups. A total of five multiparous ewes were subjected to ES management. Three multiparous ewes were subjected to EM management. Seven multiparous ewes were subjected to MMM management. Two multiparous ewes were subjected to MMS management with 30 days of lactation (± 5 days). The samples were collected and analyzed, for a total of 60 CSF samples and 98 plasma samples. Most of these samples were precisely matched to allow further analysis of the correlation between central and peripheral OT.

Estimates of the mean, standard deviation, coefficient of variation, and minimum maximum correlation coefficients for the concentrations of OT in CSF and plasma are shown in Table 2.

The correlation coefficient was calculated only in plasma and CSF samples that were paired. To perform the statistical analysis, only animals with at least 2 days of collected both CSF and plasma samples were

Table 1 – Descriptive statistics of the quantification of OT concentrations (pg/ml) in plasma samples during "stall" time and -0.5 min in some animals, obtained by immunoenzymatic assay. No significant difference was found between "stall" time and -0.5 min. Thus, the need to collect the first plasma sample outside the "stall" was not required – São Paulo – 2013

Time	N	Mean	SD	CV (%)	Min	Max
Stall	27	478.28	197.54	41.30	92.74	822.56
-0.5 min	27	470.27	204.14	43.41	75.11	838.69

($p > 0.05$, Student's t-test for independent samples)

Table 2 – Results of the quantification of the concentration of OT (pg/ml) in CSF and plasma samples from sheep in the exclusive breastfeeding group (ES), exclusive milking group (EM), mixed breastfeeding management and milking group (MMM), and mixed breastfeeding management and milking group (MMS) obtained by enzyme immunoassay – São Paulo – 2013

Group	Sample	N	Mean	SD	CV (%)	Min	Max	Correlation*
ES	CSF	60	68.46	53.83	78.63	12.07	247.75	-0.19
	Plasma	98	237.77	215.97	90.83	13.06	880.25	
EM	CSF	80	45.00	27.41	60.91	14.65	138.51	-0.26
	Plasma	89	375.42	189.97	50.60	75.11	874.37	
MMM	CSF	39	44.40	21.84	49.19	12.59	132.90	0.05
	Plasma	183	326.33	282.88	86.69	21.15	982.98	
MMS	CSF	41	33.31	20.21	60.67	12.78	81.37	0.04
	Plasma	45	579.40	109.91	18.97	361.99	838.69	

*The correlation coefficient was calculated using only paired samples of CSF and plasma

used to perform the statistical analysis. Estimates of the mean, standard deviation, coefficient of variation, and minimum and maximum concentrations of OT were 257.88 ± 265.91 pg/ml, 103.12%, 11.70 pg/ml, and 1000.00 pg/ml, respectively. The summary of the ANOVAs of the OT concentrations is shown in Table 3.

Table 3 shows that the factors day and time were nonsignificant ($p > 0.05$). The Treatment x Moment, Sample Type x Moment, and Treatment x Sample Type x Moment interactions were not significant.

The Treatment x Sample Type interaction was significant ($p < 0.05$; Table 4).

ANOVA showed that there were significant differences when comparing OT plasma concentrations of the four treatments OT tested (EM, MMM, MMS and ES; * $p < 0.001$). No significant differences were found when comparing the OT concentrations in CSF in the four treatment groups (Table 5).

There were significant differences when comparing plasma and CSF OT concentrations within each treatment tested ($p < 0.001$; Table 5).

Plasma OT concentrations were significantly different between treatments. The animals in the MMM and MMS groups had the highest estimated

Table 3 – Summary of analysis of variance of OT concentrations – São Paulo – 2013

Source of Variation	df	GLRES	Fcalc	P	
Day	4	30	0.76	0.5615	ns
Treatment	3	6	20.63	0.0015	*
Sample Type	1	6	566.50	< 0.0001	**
Time	5	30	2.30	0.0693	ns
Treatment x Sample Type	3	6	39.01	0.0002	*
Treatment x Time	15	30	0.60	0.8554	ns
Sample Type x Time	5	30	1.87	0.1298	ns
Treatment x Sample Type x Time	15	30	0.88	0.5898	ns

ns, nonsignificant. *p < 0.05, **p < 0.01

Table 4 – Interaction Treatment x Sample Type – São Paulo – 2013

Source of variation	df	DFRES	Fcalc	P	
TREAT/CSF	3	6	0.63	0.63	ns
TREAT/PLASMA	3	6	61.77	< 0.0001	**

ns, non significant; **p < 0.01

Table 5 – Summary deployment of Interaction Treatment x Sample Type – São Paulo – 2013

Source of variation	df	DFRES	Fcalc	P	
Sample Type/ES	1	6	18.34	< 0.0052	**
Sample Type/EM	1	6	127.73	< 0.0001	**
Sample Type/MMM	1	6	331.06	< 0.0001	**
Sample Type/MMS	1	6	194.05	< 0.0001	**

**p < 0.01

plasma OT concentrations, which were different from the EM group, which had an intermediate estimated plasma OT concentration. The ES group had the lowest estimated plasma OT concentration. However, OT concentrations in CSF did not differ between treatments. With regard to the sample type, statistically significant differences were found between the plasma and CSF concentrations of OT in all of the treatments.

The factors Sex and Treatment were not significant ($p > 0.05$). The Treatment x Sex, Sex x Day, and Sex xTreatment x Day interactions were not significant (1, 7, 15, 30, 45, and 60.

Discussion

The present results showed that quantifying oxytocin in CSF is possible, similar to plasma (NEGRÃO; MARNET, 2002; 2003; NEGRÃO; MARNET; LABUSSIERE, 2001). We compared the patterns of central and peripheral hormone secretion during milking and found that central oxytocin does not behave like peripheral oxytocin, with no variations in hormone release caused by milking management.

A possible relationship between central and peripheral oxytocin concentrations was previously proposed under various experimental conditions (INSEL, 1990; JONES; ROBINSON; HARRIS, 1983; KENDRICK et al., 1986). Moreover, the co-localization of oxytocinergic neurons in the paraventricular nucleus of the hypothalamus and its projections is related to central and peripheral oxytocin concentrations, suggesting a functional relationship (INSEL, 1990; JONES; ROBINSON; HARRIS, 1983; KENDRICK et al., 1986).

The quantification of oxytocin concentrations using immunoenzymatic methods revealed that the concentrations found in plasma were always higher than those present in CSF. This occurred regardless of treatment. This result is consistent with the literature. The neurohypophysis secrets oxytocin into the bloodstream in response to specific stimuli. This implies that peripheral oxytocin concentrations are higher than those present in the central nervous system. In addition to the neurohypophysis, other CNS areas can secrete oxytocin (GOULD; ZINGG, 2003; INSEL, 1990). Oxytocin acts as a neurotransmitter, interacting with central oxytocin receptors (GOULD; ZINGG, 2003) and facilitating maternal behavior (FLEMING; O'DAY; KRAEMER, 1999; KENDRICK, 2000) and social bonds (HOLLANDER et al., 2007). Therefore, it can be present at lower concentrations in the CNS than in plasma. On the other hand, the target organs of oxytocin released by the neurohypophysis are not only the uterus and mammary gland. Oxytocin is also involved in the development of ovarian follicles (JO; FORTUNE, 2003). Thus, to be functionally relevant in these systems, peripheral oxytocin concentrations may have to be higher than those found in the central nervous system.

With regard to the collection days, no significant difference was found between the oxytocin concentrations found on different days (i.e., day 1 to day 5), suggesting that the pattern of oxytocin release under the present experimental conditions was always the same. This result is consistent with the literature. The sheep were catheterized at approximately 30 days postpartum in the present study when plasma oxytocin concentrations were already significant (NEGRÃO; MARNET, 2002; NEGRÃO; MARNET; LABUSSIERE, 2001). The oxytocin concentrations must remain at high levels until the end of lactation (NEGRÃO; MARNET, 2003).

Regarding the time of sample collection for both plasma and CSF, no significant difference was found between oxytocin concentrations at different times before (-0.5 min), during (0.5, 1, and 4 min), and after (10, 15 min) nursing or milking. This was possibly attributable to the inhibition of peak oxytocin caused by experimental stress. In fact, stress-induced peaks of catecholamines, cortisol, and opioids can jeopardize both the ejection of milk and the production and release of oxytocin (GOREWIT et al., 1992; NEGRÃO; MARNET, 2002; 2003; NEGRÃO; MARNET; LABUSSIERE, 2001).

When the influence of milking management on plasma oxytocin concentrations was tested, we found that the MMM and MMS groups had the highest concentrations of oxytocin. The EM group had intermediate plasma oxytocin concentrations, whereas the ES group had the lowest plasma oxytocin concentrations. Thus, oxytocin release may be influenced by the type of stimulus (e.g., the suction exerted by the calf or milking) (MARNET; NEGRÃO, 2000; VAN REENEN et al., 2002). This stimulus interferes with the ejection of alveolar milk and milk production (MARNET; NEGRÃO, 2000; WEISS; DZIDIC; BRUCKMAIER, 2003).

With regard to mixed management, in which lactating females nursed the calf for part of the day, the amount of milk produced increased. This was attributable to the increase in milk ejection that occurred under these conditions. The lamb suction stimulus and mother-infant interaction are always more potent than mechanical milking at increasing plasma oxytocin secretion, thereby interfering with milk production (MARNET; NEGRÃO, 2000). This explains why plasma oxytocin concentrations in the MMM and MMS groups were higher compared with the EM group, which had intermediate concentrations of plasma oxytocin. However, this does not apply to the AE group, in which females were expected to have the highest plasma oxytocin concentrations because they exclusively breastfed their lambs. These animals had lower plasma oxytocin concentrations. This may be explained by the fact that the lambs were not provided with effective mammary gland stimuli during the suckling trial. Although the lambs were trained under experimental conditions, these procedures appeared to have interfered with physiological hormone release, leading to hormone inhibition caused by stress.

Oxytocin concentrations in CSF did not differ between treatments. Regardless of the type of management used, oxytocin concentrations were not statistically different in any of the groups. This indicates that the management might not modify the central release oxytocin. Alternatively, these data suggest that the central circuits that determine the release of central oxytocin may be influenced by the type of milking management adopted, in a far minor scale as compared to other stimuli such as olfactory clues (FLANAGAN et al., 1993). The present results found a low correlation between plasma and central oxytocin. This finding is consistent with other studies that did not establish relationships between oxytocin concentrations detected in CSF and plasma (JONES; ROBINSON; HARRIS, 1983; MORRIS; BARNARD JÚNIOR; SAIN, 1984).

Conclusion

The present study found the following:

- The immunoassay can be used to quantify oxytocin in both plasma and CSF.
- The method of catheterization of the subarachnoid space appeared to be a feasible and effective method for obtaining continuous CSF over prolonged periods of time.
- The experimental procedure may have both influenced and hidden plasma and CSF OT peaks as a result of the stress associated with the procedure.
- The average oxytocin concentrations in plasma were higher than those in CSF, regardless of treatment.
- The release of plasma oxytocin can be influenced by the type of stimulus (e.g., pup suckling and milking).
- A significant correlation was found between oxytocin in CSF and plasma.
- The concentration of oxytocin in CSF did not vary at different times, on different days of collection, or with the different treatments studied. This suggests that milking management did not modify the release of central oxytocin.

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References

BRUCKMAIER, R. M. Normal and disturbed milk ejection in dairy cows. **Domestic Animal Endocrinology**, v. 29, n. 2, p. 268-273, 2005.

COWELL, R. L.; TYLER, R. D.; MEINKOTH, J. H; DENICOLA, D. B. **Diagnostic cytology and hematology of the dog and cat**. 2. ed. St Louis: Mosby, 1999. 338 p.

CRUZ-CASALLAS, P. E.; NASELLO, A. G.; HUCKE, E. E.; FELICIO, L. F. Dual modulation of male sexual behavior in rats by central prolactin: relationship with in vivo striatal dopaminergic activity. **Psychoneuroendocrinology**, v. 24, p. 681-693, 1999.

DWYER, C. M. Maternal behavior and lamb survival: from neuroendocrinology to practical application. **Animal**, v. 8, n. 1, p. 102-112, 2014.

FLANAGAN, L. M.; PFAUS, J. G.; PFAFF, D. W.; McEWEN, B. S. Induction of FOS immunoreactivity in oxytocin neurons after sexual activity in female rats. **Neuroendocrinology**, v. 58, n. 3, p. 352-358, 1993.

FLEMING, A. S.; O'DAY, D. H.; KRAEMER, G. W. Neurobiology of mother-infant interactions: experience and central nervous system plasticity across development and generations. **Neuroscience and Biobehavioral Reviews**, v. 23, n. 5, p. 673-685, 1999.

GOREWIT, R. C.; SVENNERSTEN, K.; BUTLER, W. R.; UVNAS-MOBERG, K. Endocrine responses in cows milked by hand and machine. **Journal of Dairy Science**, v. 75, n. 2, p. 443-448, 1992.

GOULD, B. R.; ZINGG, H. H. Mapping oxytocin receptor gene expression in the mouse brain and mammary gland using an oxytocin receptor – LacZ reporter mouse. **Neuroscience**, v. 122, n. 1, p. 155-167. 2003.

HOLLANDER, E.; BARTZ, J.; CHAPLIN, W.; PHILLIPS, A.; SUMNER, J.; SOORYA, L.; ANAGNOSTOU, E.; WASSERMAN, S. Oxytocin increases retention of social cognition in autism. **Biological Psychiatry**, v. 61, n. 4, p. 498-503, 2007.

HOPSTER, H.; BRUCKMAIER, R. M.; VAN DER WERF, J. T. N.; KORTE, S. M.; MACUHOVA, J.; KORTE-BOUWS, G.; VAN REENEN, C. G. Stress responses during milking; comparing conventional and automatic milking in primiparous dairy cows. **Journal of Dairy Science**, v. 85, n. 12, p. 3206-3216, 2002.

HUCKE, E. E.; CRUZ-CASALLAS, P. E.; FLORIO, J. C.; FELICIO, L. F. Reproductive experience reduces striatal dopaminergic responses in freely moving female rats. **Neuroreport**, v. 9, n. 16, p. 3589-3593, 1998.

INSEL, T. R. Oxytocin and maternal behavior. In: KRASNEGOR, N. A.; BRIDGES, R. S. **Mammalian parenting**: biochemical, neurobiological and behavioral determinants. New York: Oxford University Press, 1990. p. 260-280.

JO, M.; FORTUNE, J. E. Changes in oxytocin receptor in bovine preovulatory follicles between the gonadotropin surge and ovulation. **Molecular and Cellular Endocrinology**, v. 200, n. 1-2, p. 31-43, 2003.

JONES, P. M.; ROBINSON, I. C. A. F.; HARRIS, M. C. Release of oxytocin into blood and cerebrospinal-fluid by electrical-stimulation of the hypothalamus or neural lobe in the rat. **Neuroendocrinology**, v. 37, n. 6, p. 454-458, 1983.

KENDRICK, K. M. Oxytocin, motherhood and bonding. **Experimental Physiology**, v. 85, p. 111S-124S, 2000.

KENDRICK, K. M.; KEVERNE, E. B.; BALDWIN, B. A.; SHARMAN, D. F. Cerebrospinal fluid levels of acetylcholinesterase, monoamines and oxytocin during labour, parturition, vaginocervical stimulation, lamb separation and suckling in sheep. **Neuroendocrinology**, v. 44, n. 2, p. 149-156, 1986.

KENDRICK, K. M.; KEVERNE, E. B.; CHAPMAN, C.; BALDWIN, B. A. Intracranial dialysis measurement of oxytocin, monoamines and uric acid release from the olfactory bulb and substantia nigra of sheep during parturition, suckling, separation from lambs and eating. **Brain Research**, v. 439, n. 1-2, p. 1-10, 1988.

KIMURA, T.; ITO, Y.; EINSPANIER, A.; TOHYA, K.; NOBUNAGA, T.; TOKUGAWA, Y.; TAKEMURA, M.; KUBOTA, Y.; IVELL, R.; MATSUURA, N.; SAJI, F.; MURATA, Y. Expression and immunolocalization of the oxytocin receptor in human lactating and non-lactating mammary glands. **Human Reproduction**, v. 13, n. 9, p. 2645-2653, 1998.

LEE, J.; McCRACKEN, J. A.; BANU, S. K.; AROSH, J. A. Intrauterine inhibition of prostaglandin transporter protein blocks release of luteolytic PGF2alpha pulses without suppressing endometrial expression of estradiol or oxytocin receptor in ruminants. **Biology of Reproduction**, v. 89, n. 2, p. 27, 2013.

LÉVY, F.; KENDRICK, K. M.; GOODE, J. A.; GUEVARA-GUZMAN, R.; KEVERNE, E. B. Oxytocin and vasopressin release in the olfactory bulb of parturient ewes: changes with maternal experience and effects on acetylcholine, g-aminobutirc acid, glutamate and noradrenaline release. **Brain Research**, v. 669, n. 2, p. 197-206, 1995.

LIPSCHITZ, D. L.; CROWLEY, W. R.; BEALER, S. L. Central blockade of oxytocin receptors during late gestation disrupts systemic release of oxytocin during suckling in rats. **Journal of Neuroendocrinology**, v. 15, n. 8, p. 743-748, 2003.

LONG, J. B.; HOLADAY, J. W. Blood-brain barrier: endogenous modulation by adrenal-cortical function. **Science**, v. 227, n. 4694, p. 1580-1583, 1985.

MANN, G. E.; WATHES, D. C.; ROBINSON, R. S. The progesterone receptor antagonist, onapristone has differential effects on the timing and control of the luteolytic mechanism depending on timing of administration in sheep. **Molecular and Cellular Endocrinology**, v. 376, n. 1-2, p. 1-11, 2013.

MARNET, P. G.; NEGRÃO, J. A. The effect of a mixed-management system on the release of oxytocin, prolactin, and cortisol in ewes during suckling and machine milking. **Reproduction, Nutrition, Development**, v. 40, n. 3, p. 271-281, 2000.

MARNET, P. G.; NEGRÃO, J. A.; LABUSSIÈRE, J. Oxytocin release and milk ejection parameters during milking of dairy ewes in and out of natural season of lactation. **Small Ruminant Research**, v. 28, n. 2, p. 183-191, 1998.

MIRCIC, G. M.; BELESLIN, D. B.; JANCOVIC, S. M. Hormoni zadnjeg reznja zlezde hipofize. **Srpski Arhiv za Celokupno Lekarstvo**, v. 126, p. 111-118, 1998.

MORRIS, M.; BARNARD JUNIOR, R. R.; SAIN, L. E. Osmotic mechanisms regulating cerebrospinal fluid vasopressin and oxytocin in the conscious rat. **Neuroendocrinology**, v. 39, n. 5, p. 377-383, 1984.

NEGRÃO, J. A.; MARNET, P. G. Cortisol, adrenalin, noradrenalin and oxytocin release and milk yield during first milkings in primiparous ewes. **Small Ruminant Research**, v. 47, n. 1, p. 69-75, 2003.

NEGRÃO, J. A.; MARNET, P. G. Effect of calf suckling on oxytocin, prolactin, growth hormone and milk yield in crossbred Gir x Holstein cows during milking. **Reproduction, Nutrition, Development**, v. 42, n. 4, p. 373-380, 2002.

NEGRÃO, J. A.; MARNET, P. G.; LABUSSIÈRE, J. Effect of milking frequency on oxytocin release and milk production in dairy ewes. **Small Ruminant Research**, v. 39, n. 2, p. 181-187, 2001.

OLAZÁBAL, D. E.; PEREIRA, M.; AGRATI, D.; FERREIRA, A.; FLEMING, A. S.; GONZÁLEZ-MARISCAL, G.; LÉVY, F.;

LUCION, A. B.; MORRELL, J. I.; NUMAN, M.; URIARTE, N. Flexibility and adaptation of the neural substrate that supports maternal behavior in mammals. **Neuroscience Biobehavioral Reviews**, v. 37, n. 8, p. 1875-1892, 2013.

PEDERSEN, C. A.; ASCHER, J. A.; MONROE, Y. L.; PRANGE JR., A. J. Oxytocin induces maternal behavior in virgin female rats. **Science**, v. 216, n. 4546, p. 648-650, 1982.

POINDRON, P.; LÉVY, F. Physiological, sensory, and experiential determinants of maternal behavior in sheeps. In: KRASNEGOR, N. A.; BRIDGES, R. S. **Mammalian parenting**: biochemical, neurobiological and behavioral determinants. New York: Oxford University Press, 1990. p. 133-156.

TYZIO, R.; NARDOU, R.; FERRARI, D. C.; TSINTSADZE, T.; SHAHROKHI, A.; EFTEKHARI, S.; KHALILOV, I.; TSINTSADZE, V.; BROUCHOUD, C.; CHAZAL, G.; LEMONNIER, E.; LOZOVAYA, N.; BURNASHEV, N.; BEN-ARI, Y. Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring. **Science**, v. 343, n. 6171, p. 675-679, 2014.

VAN REENEN, C. G.; VAN DER WERF, J. T.; BRUCKMAIER, R. M.; HOPSTER, H.; ENGEL, B.; NOORDHUIZEN, J. P.; BLOKHUIS, H. J. Individual differences in behavioral and physiological responsiveness of primiparous dairy cows to machine milking. **Journal of Dairy Science**, v. 85, n. 10, p. 2551-2561, 2002.

WAGNER, K. U.; YOUNG, W. S.; LIU, X.; GINNS, E. I.; LI, M.; FURTH, P. A.; HENNIGHAUSEN, L. Oxytocin and milk removal are required for post-partum mammary-gland development. **Genes and Function**, v. 1, n. 4, p. 233-244, 1997.

WEISS, D.; DZIDIC, A.; BRUCKMAIER, R. M. Effect of stimulation intensity on oxytocin release before, during and after machine milking. **Journal of Dairy Research**, v. 70, n. 3, p. 349-354, 2003.

WINSLOW, J. T.; NOBLE, P. L.; LYONS, C. K.; STERK, S. M.; INSEL, T. R. Rearing effects on cerebrospinal fluid oxytocin concentration and social buffering in rhesus monkeys. **Neuropsychopharmacology**, v. 28, n. 5, p. 910-918, 2003.