

ORIGINAL ARTICLE

Benefit of oxytocin released by cervix stimulation in Mexican Holstein cattle

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ABSTRACT

Objective: The aim of this research is to investigate the relationship level of oxytocin released and the tactile stimulation of the cervix in Holstein cattle from the northwestern arid regions in México.

Materials and Methods: The cervixes of 10 Holstein Friesian dairy cows in mid-lactation were manually stimulated for 60 sec. Oxytocin released in the blood was analyzed before and after stimulation. The enzyme-linked immunosorbent assay was used in the analysis to make oxytocin detectable by spectrometry techniques and by converting the luminescence absorbance of each sample into pg per mol.

Results: The study revealed that tactile stimulation of the cervix increased oxytocin levels. The highest increase in oxytocin level was 10,940.8222 pg/mol, while the lowest increase in oxytocin level was 1,830.94254 pg/mol. Besides, the milk production by tactile stimulation of the cervix had a higher milk yield and a p -value of 9.4×10^{-4} ($p < 0.05$) in comparison to the monthly average without tactile stimulation.

Conclusion: Tactile stimulation of the cervix activates the Ferguson reflex and increases the oxytocin released in the blood and increases the milk released from the alveolar glands.

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Introduction

The cervix is an organ located between the vagina and the uterus. The cervix has a cylindrical structure within the reproductive tract. It has a length of approximately 13 cm and a diameter of 5 cm. The primary function of the cervix is to isolate the uterus from the external environment. A significant amount of mucus is produced by the cervix. The mucus lubricates the vagina for copulation. Generally, the mucus can be observed as a clear fluid released by the vulva [1–3]. In mammal species, oxytocin is released during and after the process of labor. The realizing of oxytocin is due to pressure applied to the cervix or vaginal walls by the calf. Oxytocin is a hormone generated by the hypothalamus. Oxytocin is segregated by the pituitary gland. Oxytocin has a crucial role in the labor process and male reproduction. In females, oxytocin has a relationship with the contractions of the womb during labor. Oxytocin

stimulates the uterine muscles to contract, so labor begins. Oxytocin increases prostaglandin production, which moves labor along, and increases the contractions. Once the calf is born, oxytocin promotes lactation by moving the milk into the breast of the mother. When the calf sucks at the mother's breast, oxytocin secretion causes the milk to be released so that the calf can feed. Simultaneously, oxytocin is released by the hypothalamus to induce further oxytocin production [4–7].

The health and production of dairy cows are closely correlated with traits that demand new and efficient management techniques at arid and semi-arid zones [8]. Hot weather depresses feed intake, milk production, and reproductive performance in dairy cattle. Hot weather is a serious problem that causes seasonal economic losses in the dairy industries [9,10]. The weather is an incredibly common situation in the arid northwestern part of México,

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and the bordering southwestern USA, where daytime temperatures can reach at least 50°C. This hot weather causes dairy cows to experience heat stress for prolonged periods [11]. Besides, more than 80% of the milk produced by a cow is stored in the alveolar glands and the remaining 20% in the cisternal part of the udder [12]. So, only the remaining 20% in the cisternal part of the udder is available for machine milking before milk ejection.

A standard procedure for complete and fast milk removal is to induce oxytocin release for milk ejection by brushing stimulation or tactile stimulation of the udder before milking. The effects of tactile stimulation on the oxytocin release and the milk flow profile have been investigated at the whole udder level [13] and a quarter level [14,15]. The results have proved that tactile pre-stimulation of the udder improves oxytocin release and the distribution of milk constituents during milking, and can be precisely described at quarter levels [16,17]. Tactile pre-stimulation before the milking process induces the let-down reflex (milk ejection reflex) and influences the milk composition. Similarly, it was demonstrated that brushing the teats and udder for 60 sec, and attaching the teat cup without continuous pulsation to the multi-box automatic milking systems [18–20] induce oxytocin release and milk ejection.

Few studies have been reported related to oxytocin release by tactile stimulation of the cervix [21,22]. Hence, this study aimed to investigate the effect on oxytocin release via the Ferguson reflex (FR) of tactile stimulation of the cervix [23] in Holstein-Friesian cows from northwestern arid regions of México. The cervixes of 10 dairy cows in mid-lactation were manually stimulated for 60 sec. Measurements of oxytocin release into the blood were taken before and after stimulation. The measurement results showed that all the treatments increased the percentage of oxytocin release by cervix stimulation. Besides, all treatments increased the milk release after stimulation compared to their monthly average milk delivery.

Materials and Methods

Ethics approval

The Animal Care and Use Committee from the Autonomous University of Baja California (UABC) considered the ethical approval for this study unnecessary. All blood samples for oxytocin extraction were collected under the direction of the Institute for Research in Veterinary Science of UABC (IICV-UABC), according to the laws of animal studies (NOM-062-ZOO-1999).

Population description

The animal study comprised 10 Holstein-Friesian dairy cows registered in the Holstein Association of México. The

herd was located 3.5 km from the San Felipe-Mexicali highway, Baja California at 32° 24' 27" N and 115° 23' 03" W in IICV-UABC, Mexicali, Baja California, México. The farm has an altitude of 8 m, with a maximum temperature in the summer of 50°C and a minimum temperature in the winter of -5°C. Cows were born by artificial insemination and were between 47 and 120 months old. They were between their first and fourth lactation period, non-pregnant, clinically healthy, and free of brucellosis and tuberculosis.

Stimulation

The cervixes of 10 Holstein cows in mid-lactation were tactile stimulated for 60 sec. A human arm performed tactile stimulation of the cervix via the rectum. The human arm manually shook the hot spot of the cow.

Blood sample

An enzyme-linked immunosorbent assay (ELISA) kit was used to analyze the plasma for measuring the oxytocin levels. Blood samples were extracted from each cow from the start section of the cow's tail by using a syringe. The blood extraction was realized 5 min before tactile stimulation commenced and 1 min after tactile stimulation was completed. The protocol that consisted of adding Na-EDTA, cooled on ice, and centrifuged at 4°C and 3,000 g for 15 min prevented the blood samples from coagulation. Plasma analysis was carried out 1 h later from the blood sample extraction.

The Bovine Oxytocin EasyTest™ competitive ELISA kit from BioAim Scientific Inc. by duplicates established the protocol to measure the oxytocin levels. The measurement of oxytocin levels used antibodies to make the hormone detectable by spectrometry technique. Oxytocin levels were obtained by converting the luminescence absorbance of each sample into pg per mol. After tactile cervix stimulation was applied, each cow was routinely milked in a 2 × 5 herringbone milking parlor by the BouMatic equipment. The milking process' performance consisted of a vacuum level of 42 kPa, pulsation rate of 60 cycles/min, and pulsation ratio of 60:40.

Statistical analysis

We made a comparison of oxytocin levels before and after tactile stimulation (Table 1). The first column, "Sample," identifies the number of cows. The second column, "Identification earring," indicates the number for internal controls of the animal at the stable. The third column, "Before," indicates the oxytocin levels obtained from the blood samples by the ELISA kit. The fourth column, "After," indicates the oxytocin levels of the animal after tactile stimulation. The fifth column, "Δ in oxytocin level (pg/ml)," indicates the difference values between before

stimulation (the third column) and after stimulation (the fourth column). Since every subject has two samples, one before and one after, a *t*-test for two dependent samples was employed to evaluate the difference between the samples of every cow. The null hypothesis was “the difference of the samples is zero (or negligible), $\Delta = 0$,” and the alternative hypothesis was “they are different $\Delta \neq 0$.” The test was evaluated with a *p*-value of 0.05. The sample means were calculated with 95% of confident interval (CI).

We compared the milk yield levels between the monthly average and after tactile stimulation (Table 2). The first column, “Sample,” indicates the number of cows. The second column, “Identification earring,” indicates the number for internal controls of the animal at the stable. The third column, “Monthly average,” indicates the average milk liters that the cow produces in one event over 1 month. The fourth column, “After stimulation,” indicates the number of milk liters extracted from the cow after the cow had

Table 1. Individual oxytocin levels obtained before and after tactile stimulation.

Sample	Identification earring	Oxytocin level (pg/ml)		
		Before	After	Δ in Oxytocin
1	112	5,560.88582	13,211.31630	7,650.43048
2	151	11,321.20560	22,262.02780	10,940.82220
3	190	4,090.37994	9,588.29586	5,497.91592
4	145	9,004.65818	12,376.13780	3,371.47962
5	228	7,061.88236	8,892.82490	1,830.94254
6	179	12,153.01510	14,574.91010	2,421.89500
7	104	18,649.20490	23,226.80340	4,755.59850
8	216	8,099.86420	11,032.07780	2,932.21360
9	207	6,192.07674	10,400.12530	4,208.04856
10	142	21,083.56930	31,118.59960	10,035.03030

Table 2. Individual milk production.

Sample	Identification earring	Milk yield (l)		
		Monthly average	After stimulation	Δ in milk (l)
1	112	6.00	9.00	3.00
2	151	8.00	18.00	10.00
3	190	4.00	7.50	3.50
4	145	24.90	27.00	2.10
5	228	6.00	7.50	1.50
6	179	7.50	9.00	1.50
7	104	24.90	27.00	2.10
8	216	6.20	7.80	1.60
9	207	5.50	7.60	2.10
10	142	15.10	21.00	5.90

been tactile stimulated. The fifth column, “ Δ in milk (L),” indicates the difference values between the monthly average of milk (the third column) and the milk extracted after stimulation (the fourth column).

The average absorbance values for each set of duplicate standards, samples, and controls were calculated. The average blank well optical density was subtracted from the average absorbance values. The MATLAB software carried out four parameters of logistic regression. It also created the standard curve plot, with standard concentration on the *x*-axis and percentage of absorbance on the *y*-axis:

$$\text{Percentage absorbance} = \frac{(B - \text{blank OD})}{(B_0 - \text{blank OD})} * 100$$

Where B = OD of sample or standard and B₀ = OD of zero standards (total binding).

The minimum detectable dose of oxytocin was 80 pg/ml. The minimum detectable dose of oxytocin was defined as two standard deviations above the mean optical density of 20 replicates of zero standards. A fitting curve converts the luminescence absorbance of each sample into oxytocin levels from 10¹ to 10⁵ pg/mol (Fig. 1). The fitting curve plot was obtained using the absorbance levels of six control samples. Samples from before and after tactile stimulation were fitted to the tight curve.

Results and Discussion

The udder manipulation creates sensory impulses that reach the hypothalamus. The hypothalamus controls the release of oxytocin from the neurohypophysis and the oxytocin contributes to the milk production [24].

Oxytocin can be released by the stimulation of sensory nerves at labor, lactation, and sexual activity. Oxytocin can be released by the low-intensity stimulation of skin [25,26].

In this work, we used a Bovine Oxytocin EasyTest™ competitive ELISA kit to study the effect on oxytocin release [5,6,27] via FR of tactile stimulation of the cervix in Holstein cattle [21,22,28] from the northwestern arid regions of México. Ten cervixes of Holstein-Friesian dairy cows in mid-lactation were tactilely stimulated for 60 sec. The measurements of oxytocin release from the blood were determined before and after stimulation.

Tactile stimulation increases oxytocin levels

The oxytocin levels (pg/mol) from the blood samples before and after tactile stimulation were between 10³ and 10⁵ pg/mol. The oxytocin levels from the blood samples before stimulation presented a mean of 10,321.7 ± 10,322.2, while the oxytocin levels from the blood samples after stimulation showed a mean of 15,668.3 ± 1,566.8

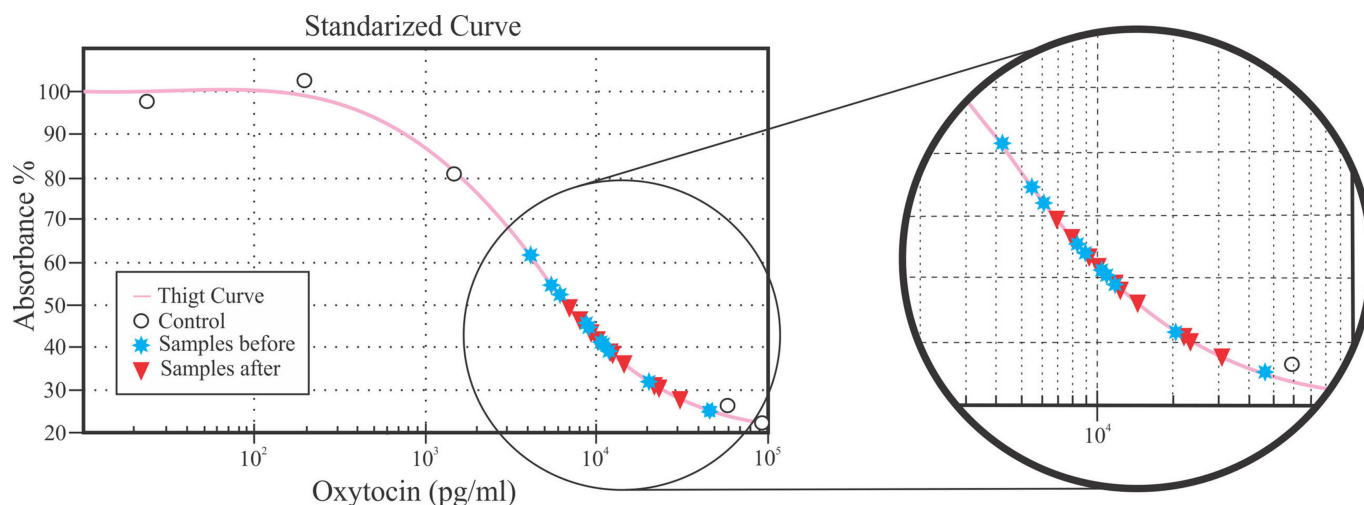


Figure 1. Oxytocin fitting curve with four parameters of logistic regression.

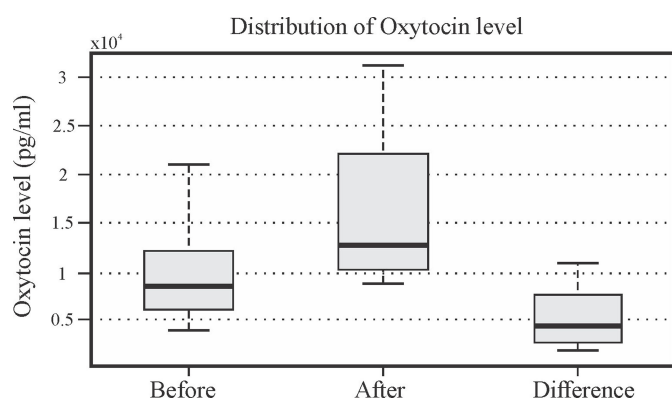


Figure 2. Box diagram of oxytocin levels obtained before and after tactile stimulation.

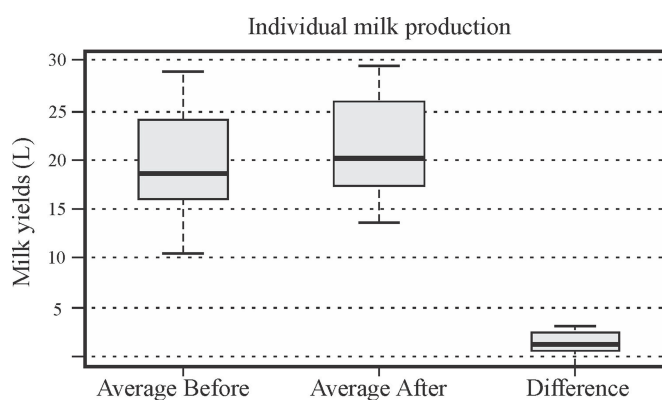


Figure 3. Box diagram of milk production. Monthly average before and after milk production stimulation delivery.

(Table 1). It means that the oxytocin levels increased as a consequence of tactile stimulation of the cervix.

The highest increment of the oxytocin level was 10,940.8222 pg/mol, while the lowest increment of the oxytocin level was 1,830.94254 pg/mol. The mean value of the oxytocin levels was $5,364.4 \pm 2,273.3$ pg/mol. All of these ranges were calculated with a confidence interval of 95%.

The box diagram helps identify the increased value of the oxytocin levels before and after tactile stimulation (Fig. 2).

Tactile stimulation increases milk production

The milk production with cervix tactile stimulation has a higher milk yield and has a significantly high p -value of 9.4×10^{-4} ($p < 0.05$) than the monthly average without pre-stimulation milking (Table 2). The highest increment of milk production was 10 l and the lowest increment of

milk production was 1.5 l. The mean value of the samples was 3.33 ± 1.92 , and the confidence interval was 95%. The box diagram helps to identify the increased levels of monthly milk production before and after tactile stimulation (Fig. 3).

The results obtained show that all treatments lead to an increase in oxytocin release after tactile stimulation. This increase in oxytocin levels is conclusive proof that tactile stimulation of the cervix activates the FR [12,18,19], and can be a direct method to increase oxytocin releases in dairy cows in mid-lactation.

A t -test for two dependent samples was realized on the oxytocin data obtained before and after stimulation. The null hypothesis is that the difference in the samples is zero (or negligible), and the alternative hypothesis is that they are different. This test shows a p -value of 54.5×10^{-4} , less than 0.05, which leads to rejecting the null hypothesis and accepting the hypothesis of high significance

of the experiment. The obtained values show that the data acquired before and after stimulation are statistically entirely different. The rise in oxytocin levels is not a coincidence but confirms the difference between the two samples.

On the other hand, milking with tactile stimulation of the cervix had a numerically higher milk yield with a significant p -value of 9.4×10^{-4} compared to the monthly average milk delivered without pre-stimulation. Hence, this increase in milk production is attributed to tactile stimulation of the cervix, which activates the FR and in turn increases the oxytocin release and increases the release of milk stored in the alveolar glands.

This research analyzes the blood samples of 10 Holstein-Friesian dairy cows in mid-lactation. If the experiment would have hundreds of cows, it could give more significant statistical values, but it would require more financial investment to hire people to be tactile, stimulate the cervix of the cow, and buy hundreds of ELISA kits to analyze the blood samples before and after the stimulation.

With the state-of-the-art techniques, we can find that some experiments stimulate the cow by brushing or injections, but there are not reports about tactile stimulation of the cervix activating the FR to increase the oxytocin released in the blood and measured by the ELISA kits. One of the limitations of this experiment is that the 10 cows were not the same age, but at least they had a similar time since the last time they gave birth.

Conclusion

This study confirms that milking with tactile stimulation of the cervix increases the oxytocin levels and increases the release of milk stored in the alveolar glands. This can be a direct method to increase oxytocin releases in dairy cows in mid-lactation. Besides, this method helps the cow to achieve healthy calving.

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Conflict of interests

The authors declare that they have no conflict of interest for the research, authorship, and/or publication of this article.

Authors' contribution

MCR, CAMA, GMME, and VAC designed, performed the study, and drafted the manuscript. MBG realized the measurements of oxytocin levels according to the protocol of the Bovine Oxytocin EasyTest™ competitive ELISA kit from BioAim Scientific Inc. MNOM and GVVM selected the cow's treatments and coordinated the sample collection.

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