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Effects of Routinely Oxytocin Injection to Induce Milk Ejection on Some Reproductive Parameters of Crossbred Cows in the Tropics of Veracruz

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MAL and BDM designed the study, wrote the protocol and interpreted the data. Authors MAA, AC and AID, anchored the field study, gathered the initial data and performed preliminary data analysis. Authors MAL and BDM, managed the literature searches and produced the initial draft. All authors read and approved the final manuscript

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ABSTRACT

The objective was to assess the effects of routine intramuscular injection ofoxytocin in crossbred milked cows in days to first service (DPS), number of services per conception (SC), percentage of abortions (PA), cumulative percentage of pregnant cows(PAVG) length of the embryo (LE), serum cortisol concentrations and milk production (PL). Cows were milked twice daily and randomly assigned to: (i) Received a routine intramuscular injection of oxytocin (10 IU / milking Injected Group, n=31) or (ii) not receive injection (Control group, n=29). Pregnancy diagnosis and measurement of the embryo was performed by ultrasonography 33(±4) days after artificial

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insemination. At 152.2±10 days in milk, several blood samples were taken before and after injection of the two groups of cows (Control, n=8 and Injected, n=7). The serum was processed and cortisol concentrations were determined by radioimmunoassay. Statistical analysis was performed using ANOVA and chi-square. The Control cowshad lower (P=0.05) percentage of abortions (3.8%) than Injected cows (PA=23%). The PAVG was higher (P=0.01) in Control group. Embryos from Control cow (19.0±2.0 mm) had greater (P=0.0001) length than those of Injected cows (14.22±1.4 mm). There were no differences (P=0.10) in DPS, SC and PL. Cortisol concentrations were higher (P=0.0003) in Injected cows (33.4±2.8 ng / ml) than Control cows (11.6±2.4 ng/ml) cows. In conclusion, routine intramuscular injection of oxytocin increased serum cortisol concentrations and affected some reproductive profiles; we suggest not using intramuscular injection of oxytocin routinely.

Keywords: Cortisol; oxytocin; embryo viability; milk ejection.

1. INTRODUCTION

Due to the need for more efficient livestock production in the tropics, farmers have adopted modern technologies to improve milk production, such as mechanical milking. Mechanical milking is becoming so popular that it is replacing traditional hand milking very quickly. However, in the tropics, most cows are genetically influenced with Zebu breeds to tolerate tropical conditions such as humidity, heat, mud, insects and extensive grazing. Machine milking of these crossbred cows has not been easy, mainly due to their nervous temperament and stressful milking conditions (inefficient parlor infrastructure, noise environment, screamer and impatient staff [1]. It is noteworthy that the cow milking process starts since the cows are herded from the paddock until the milking unit is removed and if at any stage of this process cows goes under stress, adrenaline is released and the oxytocin effect on milk ejection might be blocked up to 30 minutes; therefore a good milking process should be stress free and have positive stimuli for the cow [1].

Farmers have found a way to partially solve this problem by injecting oxytocin intramuscularly and improve milk ejection. It has been reported that injection of the hormone oxytocin could improve ejection of milk up to 12%, especially in cows milked in poor conditions [2]. However, the side effects of routinely intramuscular injection of oxytocin to induce milk ejection in crossbred cows under tropical conditions have not been well documented. Villa et al. [3] suggested that routine intramuscular injection of oxytocin could affect reproductive performance.

Another aspect that has not yet been considered is the welfare of the cow in relation to the implementation of routine intramuscular injection. The aim of this study was to assess the effects of routine intramuscular injection of oxytocin to induce milk ejection in crossbred (*bos indicus x bostaurus*) cows milked twice a day on days to first service, number of services per conception, abortion rate, and percentage of pregnant cows accumulated over time, length of the embryo, serum cortisol concentrations and milk production under tropical conditions.

2. MATERIALS AND METHODS

The study was conducted in a commercial farm in Tuxpan, Veracruz (Mexico). The climate in Tuxpan is tropical with an average temperature of 24.9ºC, with abundant rains in the summer and early fall and less intense from November to May, the average annual rain fallis 1,241 millimeters.

Animal procedures were approved by the University Animal Care and Use Committee. The study was conducted from July to November (405 days). Crossbred (Holstein x Cebu and Brown Swiss x Cebu) pregnant cows (n=60) were used; the cows were multiparous (2-5 calf) with a body condition score of 5±0.5 (scale 1-9, [4]). Three weeks before the expected calving date, cows were placed in a pasture (0.5 hectare / cow) with fresh water and commercial minerals at libitum (Magnaphoscal® Bayer; mixed with sodium chloride 1:3). Cows were dewormed (ivermectin 0.2 mg/kg; 1 ml / 50kg subcutaneous), intramuscularly injected with vitamin E (60 mg), selenium (600 mg),vitamin ADE (A, 250,000 IU; D3, 375,000 IU) and E1 alfa-69 tocoferol acetato (250 mg)and started to receive 2 kg / head / day of a commercial concentrate (18% crude protein). After, parturition cows (n=60) were incorporated with the milking herd; five days after the parturition the cows were randomly assigned to: (i) Receive a hormone intramuscular injection (10 IU of oxytocin; Oxitocina U.S.P.20 UI / 1ml Virvac® México, [dose commonly used by farmers 0.5 ml]) to induce milk ejection, every time cows were milked (Injected; n=31) or (ii) Not to receive a hormone injection (Control; n=29). Due to the study had a strong practical and applied research tendency and a limited number of cows and working staff, a third group that would had been injected with only saline could not be included. Lactating crossbred cows were managed in an intensive rotational grazing system in which 61.5 hectares of tropical grasses were divided with electric fence in 30 pastures and each pasture (1.98 hectares/pasture) was grazed 1.5 days every 45 days. Cows grazed 15±3 hours daily and had fresh water and commercial mineral at libitum. Cows were milked in a stanchion barn milking system (capacity for 8 cows) at 6:00 am and 16:00 pm and they were fed with 1 kg of commercial concentrate (18% crude protein) for every 3 liters of milk produced on a daily basis. Milk yield was weighed every 14 days using Waikato milk meters (Alfa-Laval Agri; 30 kg capacity) from the third day, post-calving, until the end of lactation. Milk yield per cow was adjusted to 305 days.

Estrus detection was observed twice a day (am-pm), during the study (405 days) for 2 hour periods and cows were artificially inseminated approximately 10-12 hours after estrus detection. Days from parturition to first service (FS), number of services per conception (SC), number of cows not served (NS) and accumulated percentage of bred cows over-time were collected.

Pregnancy was diagnosed at 33±4 days using ultrasonography (Honda 1201; real time with rectal 5.0 MHZ transducer). Also, embryos were measured by taking crown-rump length (mm). After the first pregnancy diagnosis, pregnant cows were confirmed at 60±10, 90±10 and 150±10 days. Therefore, cows that had estrus behavior after pregnancy diagnosis were confirmed again, and aborted cows were recorded.

After 152.2(±10) days in production and treatment, 25% of the cows from each group (control; n=8, and hormone injected; n=7) were randomly selected at the pm milking and were bled via tail venipuncture as follow: Ten and five minutes before cows entering the milking parlor (-10 and -5), before connecting the unit (minute 0), and at 5, 15 and 45 minutes after connecting the unit. Blood was allowed to clog and then centrifuged at 2500 rpm for 20 minutes. Serum was stored at -5ºC until cortisol concentrations were determined using radioimmunoassay (Cortisol RIA Kit; Ref RK-240 CT; ISOTOP, INSTITUTE OF ISOTOPES Ltd.1535 Budapest).

Data were confirmed to have normal distribution using the Shapiro-Wilk-test and the Bartlett test to confirm the homogeneity of groups (Statistical V.10.1; Stat Soft). Data were analyzed as a 2 x 2 factorial Using ANOVA from module GLM. Serum cortisol concentrations were analyzed for treatment, time and treatment x time using analysis of variance specific for repeated measures. The main effects of treatment on days to first servicing days open number of services per conception, milk production and embryo length were tested using analysis of variance. The effect of treatment on percentage of abortions and cumulative percentages of pregnant cows was tested Fisher-exact test. Milk yield was adjusted to 305 days.

3. RESULTS AND DISCUSSION

Cows in the intramuscular hormone injection group had a higher ($P<0.05$) abortion rate (23%) compared to cows in the control group (3.8%). Also, there were more ($P<0.05$) cows open at the end of the 405 day study in the hormone intramuscular injection group (34.3%) than cows in the control group (14.8%). The cumulative percentage of bred cows over time was greater ($P<0.01$) in the group that was not injected (Fig. 1).

The abortions rate was higher in cows that received intramuscular injection of oxytocin and embryos were smaller in these cows compared to cows that were not injected. These effects could be related to an exaggerated increase of oxytocin in blood due to the injected dose to induce milk ejection. Sagi et al. [5], reported that the physiological dose of oxytocin to induce milk letdown successfully only 0.1 UI and an exogenous application of 10 to 20 IU of oxytocin increased 120 to 240 times the physiological concentrations.

Additionally, these concentrations causing exogenous oxytocin may have a maximum of 120 minutes compared with 3-15 minutes of endogenous oxytocin [6]. This increase could have an effect on endogenous profiles of oxytocin and prostaglandin F2α concentrations affecting growth and viability of embryos, especially poor quality embryos [7,8]. There is also the possibility that a disorder in the serum concentrations of progesterone may have affected the rate of abortions, more open cows and embryonic growth. Increased blood estrogen concentrations prompts both synthesis and release of oxytocin and increased oxytocin receptors, stimulating uterine secretion of PGF, this effect would produce irreversible damage to the CL [9]. Oxytocin also contributes to the initiation of luteolysis [10]. In addition, it was reported that application of exogenous oxytocin on days 2 to 6 of the estrous cycle affected the growth of the corpus luteum and serum progesterone concentrations, affecting embryonic growth [11]. Lonergan [12], reported that a decrease in serum concentrations of progesterone in the early embryonic development affected this growth and increased the chances of embryonic loss. There is also possible that serum progesterone concentrations were lower even before injected cows ovulated and it has been reported that oocytes that developed under low progesterone concentrations formed embryos which pregnancies were at higher risk of been lost [13].

More open cows were present in the group injected intramuscularly with oxytocin compared with cows in the group not injected. These differences could be explained by results published by Lemaster et al. [14] and Yildiz and Erisir [15], who concluded in their studies that injections of exogenous oxytocin during certain days of the estrous cycle after artificial insemination drastically reduce the embryonic survival. In this study, cows were injected twice a day every day of the estrous cycle in cows inseminated, which might have contributed to higher abortion rate. Furthermore, exogenous oxytocin increases especially uterine contractions in pregnant sheep oviduct reducing embryonic survival [16].

The number of days to first service and number of services per conception were similar in both groups. These results are similar to those reported by Alejo and Basurto [17] and Nostrand et al. [2]; In addition, embryos from control cows had increased (P=0.0001) in length (19.0±2.0 mm) compared to embryos from cows in the intramuscular hormone injection group (14.22±1.4 mm). Cows intramuscularly injected with the hormone to induce milk ejection had similar (P=0.10) days to first service (172.5±20.7) and number of services per conception (2.28±0.28) than cows that were not injected (167.3±20.2; 1.90±0.27respectively).

Serum cortisol concentrations were affected by treatment (P=0.0003); cows receiving-intramuscular hormone injections (33.4±2.8 ng/ml) had higher concentrations than cows that did not received the injection (11.6±2.4 ng/ml). Serum cortisol concentrations were also affected by time (P=0.01), and by treatment X time interaction (P=0.01). Serum cortisol concentrations increased after intramuscular hormone injection, while cows that did not receive intramuscular hormone injection serum cortisol concentrations remained low over time (Fig. 2). Before intramuscular hormone injections, serum cortisol concentrations were similar in cows from both groups; however, after intramuscular hormone injections, serum cortisol concentrations increased in cows receiving the hormone injection. This may indicate a stress response to the hormone injection, and cows never habituated to the daily hormone injection routine. Bruckmaier et al. [18-20], showed that cows milked in unfamiliar places showed higher stress and serum cortisol and β-endorphin concentrations, and low concentrations of oxytocin during milking. This happened until the cows became accustomed to the new surroundings, once the cows became accustomed to the new place, serum concentrations of β-endorphin and cortisol decreased and serum concentrations of oxytocin increased. In our study, cows that were intramuscularly injected with the hormone, after several weeks under the same conditions, continued to respond with increased cortisol concentrations; this could indicate that crossbred milked cows did not became habituated to the routine intramuscular injection and it could be a daily stressful stimuli.

It is noteworthy that the chronic and long term stress can have a biological cost to the animals as would be negative effects on health, reproductive profiles and growth rates [1]. In this study the stress caused by the intramuscular injection of oxytocin routinely triggered release of cortisol and chronically stress and may have influenced some reproductive parameters. Cows from both groups had an average milk yield of 12.15±0.55 kg during 305 days. Milk yield adjusted to 305 days was similar (P=0.10) between cows that received intramuscular hormone injections (3,928.10±265.2 kg) and cows that did not receive intramuscular hormone injections (3,520.04±185.8 kg).

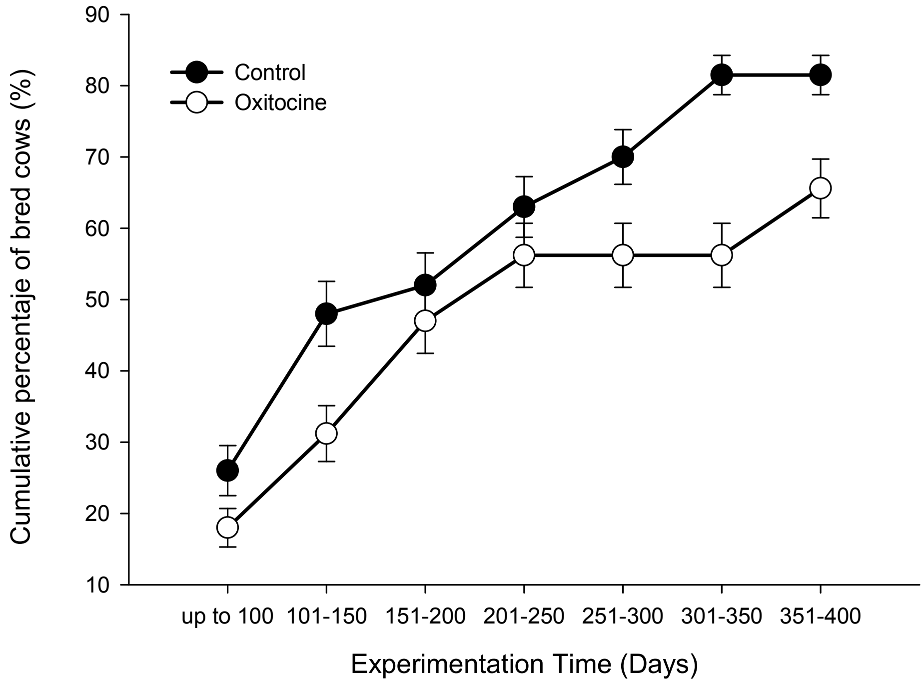

Fig. 1. Accumulated percentage of bred cows over time affected (P=0.01) by treatment

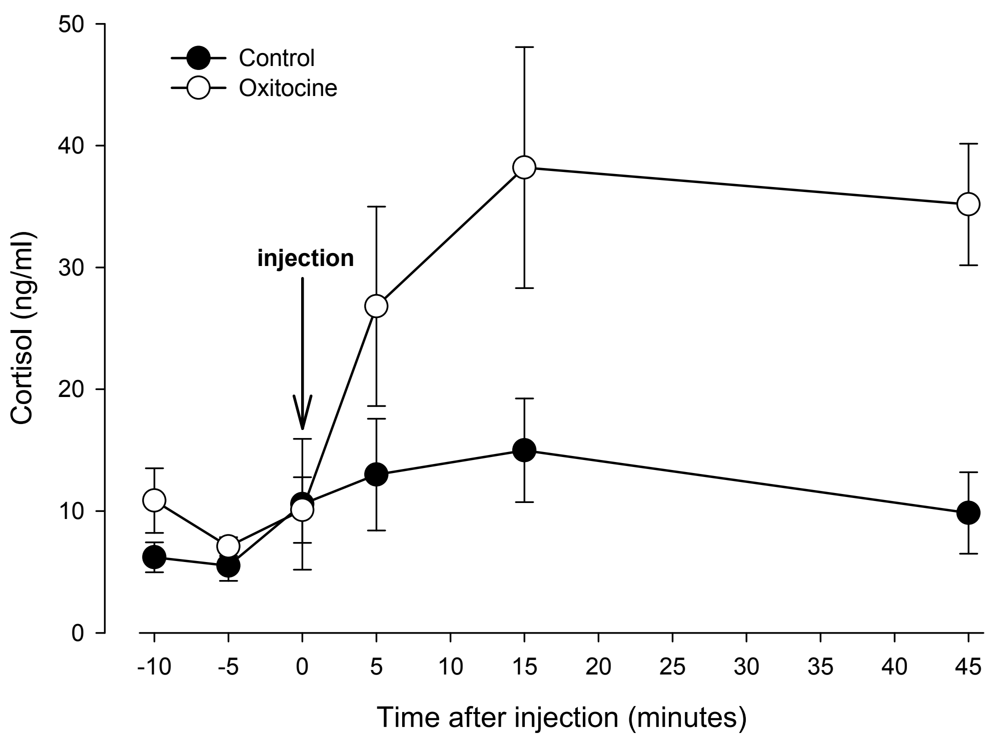

Fig. 2. Serum cortisol concentrations affected by treatment (P=0.0003), time (P=0.01), and treatment by time interaction (P=0.01) in crossbred milked cows

In this study, milk production was not affected by the intramuscular injection of oxytocin. This is corroborated by Knight et al. [21], who concluded that the application of exogenous oxytocin does not increase milk production. However, Macuhová et al. [22], reported an increase in milk production in cows that were injected with oxytocin. Similarly, Nostrand et al. [2], found a 12% increase in milk from cows injected with oxytocin than control cows, even a higher dose (50 IU) further increased the crop milk [23]. The differences between studies may be due to the fact that in this study, the average daily milk production was 12.5 liters; while other studies in tropical milk production is only 3 liters per day [24]. It is possible that management in the parlor, as feeding the cows during milking balanced food, stimulates further release of endogenous oxytocin and milk ejection, and application of exogenous oxytocin not greatly improved milk ejection injected in cows. Uvnas-Moberg et al. [25], and Lindstrom and Redbo [26], reported that cows fed during milking had elevated blood levels of oxytocin compared with those who were not fed. It is also possible that cows in this study had a free or low-stress environment in the parlor and generated adequate endogenous oxytocin release and milk ejection.

4. CONCLUSION

Routine intramuscular injection of oxytocin to induce milk ejection in crossbred cows under tropical conditions of Veracruz reduced the cumulative percentage of pregnant cows and embryo size. It also increased serum cortisol concentrations, abortion rate and the number of open cows, but did not improve milk production. It is suggested that cows do not habituate to routine intramuscular injection and it would be advisable not to use it in this way or at least minimize the number of cows with this management.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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