# THE EFFECT OF NALOXONE ON REPRODUCTIVE BEHAVIOR AND PLASMA PROLACTIN LEVELS AFTER WEANING IN THIRD LACTATION SOWS

## El efecto de naloxona sobre el comportamiento reproductivo y los niveles plasmáticos de prolactina en cerdas de tercera lactación

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**ABSTRACT**

The present study was undertaken to study the effect of small doses of naloxone on behavior, prolactin plasma levels, interval of weaning to first estrus, and duration of estrus in third lactation sows. Thirty York x Landrace sows weaned at 25 to 27 days postpartum were selected and separated at random in two groups of 15. One group served as control and the other received every twelve hours two mg of naloxone im. Treatment with small doses of naloxone started three days before and continued for three days after weaning similarly the control group was injected with two mL of a saline solution. Naloxone treated sows showed estrus 88.8 ± 6.2 hours after weaning (P<0.1), control sows estrus was evident 102.37 ± 7.2 hours after weaning. Duration of estrus in treated and nontreated was 85.6 ± 3.8 and 42.6 ± 3.7 hours, respectively. Prolactin levels decreased rapidly after weaning in both groups, but Prolactin plasma levels in naloxone treated sows were below control levels (15 ± 2 and 7 ± 0.3 Ng, respectively; P<0.1). Behaviour scores showed that naloxone treated sows accepted mounting with a significant reduction in aggressive behaviour as compared with controls. It was concluded that opioids are important modulators of sow sexual behaviour.

**Key words:** Sow, weaning, naloxone, estrus, prolactin.

**RESUMEN**

El presente trabajo se realizó con la finalidad de estudiar el efecto de la administración de pequeñas dosis de naloxona sobre el comportamiento sexual, los niveles plasmáticos de prolactina, el intervalo entre el destete y el primer estro y la duración del mismo. Con este propósito se seleccionaron treinta cerdas York x Landrace destetadas a los 25 y 27 días posparto. Se dividieron al azar en dos grupos de 15. Uno de los dos grupos se utilizó como control y el otro recibió una inyección intramuscular de dos mg de naloxona cada 12 horas, el tratamiento con el antagonista opioide se inició desde tres días antes del destete y se continuó por tres días después. El grupo control recibió el mismo tratamiento con inyecciones de solución salina. Las cerdas tratadas con naloxona presentaron estro a las 88 ± 6,2 horas después del destete, mientras que en las cerdas controles el estro se hizo evidente 102,37 ± 7,2 horas después (P<0,01). La duración del estro en cerdas tratadas y no tratadas fue de 85,6 ± 3,8 y 42,6 ± 3,7 horas, respectivamente. Los niveles de prolactina disminuyeron rápidamente en ambos grupos después del destete, pero los niveles plasmáticos de prolactina en las cerdas tratadas se mantuvieron por debajo de los controles (15 ± 2 y 7 ± 0,3 ng, respectivamente; P<0,01). Los resultados del comportamiento mostraron que las cerdas tratadas con naloxona aceptaron la monta con un comportamiento significativamente menos agresivo que las cerdas controles. Se llegó a la conclusion que los opioide endógenos son moduladores importantes del comportamiento reproductivo en la hembra porcina.

**Palabras clave:** Marranas, destete, naloxona, estro, prolactina.

Recibido: 06 / 07 / 2010. Aceptado: 25 / 03 / 2011.

## INTRODUCTION

The weaning-to-estrus interval is a major contributor to sow (*Sus scrofa domesticus*) nonproductive days. Shortening

this interval may be achieved by zootechnical management (egg, feeding, flushing, and breeding practices) or biotechnical methods (egg, use of exogenous gonadotropins) [17].

Methods to induce estrus during lactation and to increase piglets per sow have been reported [7, 8, 18]. A major concern is the time taken by the sow to display estrus after weaning. It is generally observed that estrus is present 4 to 7 days after piglets are weaned, event followed by artificial insemination or direct mating, it is also reported that the interval between weaning and estrus would be increased to 10 days.

It is known that prolactin (PRL) modulates behavior during the final stages of gestation in the sow [26]. There is a rapid fall in prolactin secretion during estrus in gilts and during weaning. PRL has being related to different physiologic functions such as promotion of labour, milk ejection, lactogenesis and behavior [24, 29],and furthermore, PRL has also being related with inhibition of the release of $\beta$-endorphin from hypothalamic organ cultures while decreasing the secretion of gonadotrophin hormone-releasing hormone (GnRH) [5]. The latter effect of PRL is blocked by naloxone, indicating that hyperprolactinemia- induced depression of gonadotrophin secretion is mediated by endogenous opioids [5]. The connection between hyperprolactinemia and the suppression of GnRH secretion by persistent activation of an endogenous opioid system is supported by several other observations [6]. Thus, it is possible to postulate that high levels of PRL during lactation in the sow, modulate return to estrus, and the rapid fall in plasma PRL levels during weaning facilitates the prompt resumption of estrus cycles in the sow.

Previous work using naloxone in sows has being carried out using doses of 1 to 4 mg/kg [9, 10], but side effects of this medication were not considered. In ewes it was reported that sudden death was followed after the administration of 25 mg of an opioid antagonist [28]. In humans, the administration of 1 to 4 mg/kg produced significant dose-dependent behavioral, hormonal, and physiological effects. Including dysphoric effects, a deterioration of performance on memory testing, increasing systolic blood pressure and respiratory rate, and increasing plasma cortisol and growth hormone levels. Continuous infusion of naloxone produced nausea and vomiting hypotension bradicardia, seizures, and is some cases sudden death was also reported in humans [1, 4, 19, 20].

From the pharmacological point of view, the dose of naloxone used by different research teams is extremely elevated, non physiological: It is known that the use of high doses of Endogenous Opioid Peptides (EOP) antagonists such as naloxone can produce an interaction of the antagonist with other receptors, besides those related to the control of gonadotrophin secretion [21, 31]. On the other hand, when naloxone is administered in low dose (0.4 to 0.8 mg) there is a selective interaction with μ EOP receptors with duration of action of 1 to 4 hours [22].

In previous work, it was reported that the administration of low doses of the hypothalamic opioid antagonist naloxone, facilitates the expression of oestrus in the ewe (*Ovis aries*), goat (*Capra hircus*) and sow [11-15]. Also, in previous work, it was reported that naloxone decreased PRL levels in small ruminants [15]. Hence the objective of the present study was to study the effect of intramuscular injections of small doses of naloxone on behaviour, PRL plasma levels, interval of weaning to first oestrus, and duration of oestrus in the sow.

## MATERIAL AND METHODS

During the study, 30 York X Landrace sows (on their third lactation, 185 ± 6 kg body weight), 25 to 27 days in lactation were randomly separated in two groups. The animals were lodged in individual crates provided with two kg/day of commercial concentrated food and water ad libitum. The experimental group received intramuscular injections of naloxone every twelve hours, three days before and continued for three days after weaning, two mg of naloxone chlorhydrate diluted in two mL of saline solution. The 15 control sows were sham treated with saline im injections. The onset of oestrus was estimated as midway between the last refusal and first acceptance to stands. Three times (7:00, 12:00 and 19:00 h) daily, after weaning, the standing reflex was elicited and the male was introduced among the sows to detect heat, this procedure was carried out by the same operator.

### Behavior score

A note was made by a designated observer in each group; on behavior of treated and control sows during the onset of oestrus and mating, points were given to signs such as lordosis, redness of the vulva, vaginal discharge, mounting, nudging others, erect ears and loss of appetite as suggested by Seguin et al. [25]. Loss of appetite was considered as the % of concentrated food left over at the end of the day.

### Plasma prolactin analysis

Since five days before weaning, *vena cava w*as catheterized via de *vena jugularis e*xterna, which is punctured while the sow is restrained by nose snaring according to the method described by Damm et al. [8]. Blood samples were collected at 12 hrs intervals for three days before and six days after naloxone treatment, for the determination of plasma prolactin. Prolactin concentrations were measured by an homologous double antibody radioimmunoassay using a commercial kit (Medidores Industriales, Mexico). Intra- and interassay CV's were 9.1% and 12.3%, respectively. Sensitivity of the assay, defined as 86% of total binding, was 1 ng/mL.

### Statistical

Data analysis was carried out using ANOVA through a repeated measures analysis of variance to examine the effect of treatment on PRL plasma concentrations; the level of significance chosen was < 0.01, and the Chi square test, establishing a 0,05 alpha to declare differences among the treatments [24].

## RESULTS AND DISCUSSION

### Oestrus detection and duration

Oestrus in the naloxone treated group was initially detected 88.8 ± 6.2 h after weaning, in the control group estrus was detected 102.3 ± 7.2 h (P<0.01) after weaning. In the naloxone group estrus duration was 85.6 ± 3.8 h, while in the control was 42.2 ± 3.7 h (P<0.01).

### Behaviour

The behaviour of naloxone treated sows at onset of oestrus was different from saline treated group, the lordosis standing reflex was significantly prolonged. Vaginal discharge was more evident, mounting others and food left over was less apparent in naloxone treated sows (TABLE I). It is worth to mention that naloxone treated sows when mated; they were very compliant and accepted mounting without running and grunting. Behaviour in the saline treated control group was characteristic with running and avoiding initially the boar's trusts and mounting, and finally after some time they finally accepted mounting.

### Prolactin plasma levels

Prolactin levels in the preweaning stage fluctuated between 160 and 180 ng in both groups, and naloxone did not affect PRL levels during the first three days of treatment before weaning, after weaning its level decreased to 70 ± 12 and 50 ± 9 ng in control and naloxone treated sows, thereafter levels remained lower in the naloxone treated group as compared with control sows (FIG. 1; P<0.05). It is interesting to observe that PRL levels in naloxone treated sows remained lower than control sows several days after the end of treatment.

At the end of suckling high concentrations of plasma oxytocin together with a decrease in plasma PRL levels has been reported [2], suggesting that lactation *per se* produces a certain degree of stress in the sow. During lactation, high levels of

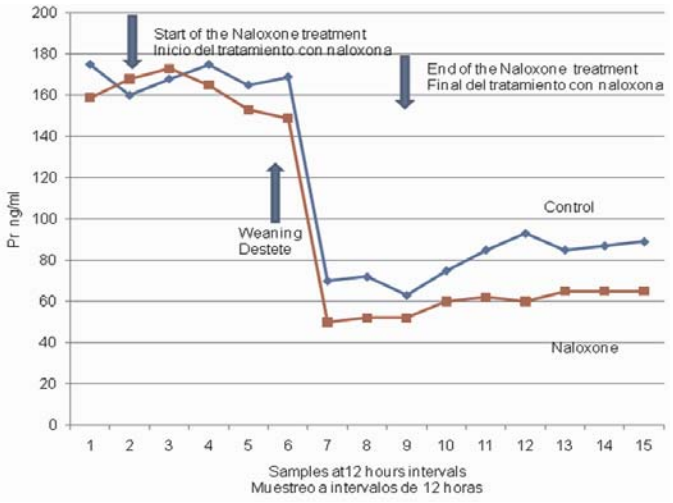


**FIGURE 1. EFFECT OF LOW DOSES OF NALOXONE ON PLASMA PROLACTIN LEVELS BEFORE AND AFTER WEANING.**

PRL facilitates the release of hypothalamic endorphins inhibiting the release of GnRH and subsequently the pituitary release of LH is diminished [3, 19, 30]. In this work, PRL level before and after weaning in control sows were similar to other report [30]. After weaning, there is a fall in plasma PRL levels, decreasing to 40 – 60 ng/mL, but in naloxone treated sows, prolactin levels appeared lower when compared with control. It is possible to postulate that high PRL levels suppress LH release, both by antagonizing the hypothalamic release of GnRH and by decreasing gonadotroph sensitivity to the latter.

The effect of naloxone in plasma PRL has being studied in the 80´s and 90´s. Armstrong et al. [2] used multiparous crossbreed sows, infused naloxone at a dose of 200 mg/h iv for 8 h, with this high dose they observed that PRL levels decreased after weaning and in naloxone treated sows prolactin levels were similar to those in control treatment, however the

*TABLE I*
**EFFECT OF NALOXONE ON SEXUAL BEHAVIOUR AND THE WEANING TO ESTRUS INTERVALS AND DURATION OF ESTRUS IN THIRD LACTATION SOWS**

| | Control | Naloxone |
|---|---|---|
| Lordosis Reflex (Standing) | 60 ± 3a | 88 ± 7b |
| Redness of Vulva | 56 ± 9 | 75 ± 7 |
| Vaginal discharge | 60 ± 3 | 70 ± 3 |
| Mounting | 45 ± 5a | 30 ± 4b |
| Nudging others | 75 ± 9a | 50 ± 7b |
| Erect ears | 55 ± 7 | 60 ± 6 |
| Appetite loss, % | 30 ± 2 | 25 ± 3 |
| Weaning to estrus interval,hours | 88.8 ± 6.2a | 102 ± 7.2b |
| Duration of estrus, hours | 42.2 ± 3.7 | 85.6 ± 3.8 |

Different literals means statistical difference (P<0.01).

hormone levels continued low until 4 - 5 hs after the naloxone infusion, thereafter, prolactin levels were similar in control and naloxone treated weaned sows.

In similar work with late gestation sows that received an iv bolus of 2 mg/kg of naloxone and 6 h after, treatment with naloxone was followed by two further one mg/kg bolus injections of the opioid antagonist at hourly intervals [30]. The latter work showed that the opioid antagonist decreased plasma prolactin levels and also blocked the afternoon increase in PRL secretion. It is interesting to observe that in this experiment, a significant difference is observed in the naloxone dose used (two mg im at 12 h intervals vs 200 mg/h iv for eight continuous h). In this work, PRL levels behaved in a similar pattern as reported by Armstrong et al. [2], there is a decrease in concentration immediately after weaning, followed days after by a continuous increase. However, in both groups immediately after weaning prolactin levels decreased synchronously, but the group receiving the small dose of naloxone maintained prolactin levels below control saline treated sows.

Behavior signs of estrus in saline treated sows were similar to those observed during daily management, sows treated with naloxone were significantly different in behavior corroborating previous findings [14]. There was a significant number of positive back pressure tests, mounting was reduced with slight increase in daily vaginal discharge. This behavior might be related to the interaction of naloxone with opioid micro receptors facilitating positive physiological or psychological changes associated with the weaning related stress [32].

The effect of naloxone on estrus duration was previously reported in ewes [13], and an increase in the expression of sexual behavior was also related to the administration of small doses of naloxone in bucks [12], sows [14] and male rabbits (*Oryctolagus cuniculus)* [15].

Opioid receptors for PRL and GnRH are of the same type and localized in the hypothalamus [23, 27], suggesting that naloxone might be affecting both the secretion of PRL and GnRH at the hypothalamic level. Therefore, when naloxone is administered to the third lactation sow during the weaning phase it might be facilitating the release of GnRH and inhibiting the release of PRL, events both necessary for the initiation and facilitation of estrus in the sow. The latter is based on previous work, when using small doses of naloxone in the ewe and sow it was observed that the presence and duration of estrus is facilitated [13, 14] and furthermore, naloxone in small doses decreased the plasma levels of Prolactin in anoestrous ewes [16]. Findings that give way to postulate that endogenous opioids are important modulators of GnRH and PRL release in the sow.

## CONCLUSION

Small doses of naloxone interact with endogenous opioids at the level of the Central Nervous system postulating that EOPs are important modulators of sexual behavior in the sow and previous work carried out with high doses of naloxone should be reconsidered.

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