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## The Effect of High and Low Doses of Naloxone on Estrous Display and Ovulation Rate in Creole Ewes During the Mating Season

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**Abstract:** The aim of this study was to determine the effect of administering high and low doses of the opioid antagonist on the ovulation rate of Creole ewes during the mating season. Sixty ewes were allocated at random in three groups of 20. Group A (n = 20) received an intravaginal sponge with 40 mg medroxy progesterone acetate for 14 days and since, one day before and for two more days after sponge withdrawal they were injected with 2 mL of saline solution at 12 h intervals. Group B (n = 20) received the same treatment as group A, but on the day before sponge withdrawal a fast iv infusion of 1 mg kg<sup>-1</sup> naloxone HCl in saline solution was administered at intervals of 12 h, infusion of naloxone continued until 2 days after sponge withdrawal. Group C (n = 20) was treated as group A but they received 0.5 mg of naloxone im at intervals of 12 h for three days. Treatment was initiated since 24 h before sponge withdrawal. Ovulation rate was 1.8±0.3, 1.9±0.5 and 2.9±0.6. in ewes of group A, B and C, respectively. It was observed that low doses of naloxone are effective in increasing duration of estrus and ovulation rate giving further support to endogenous opioids as modulators of reproduction in the ewe.

**Key words:** Ovulation rate, estrus, naloxone

### INTRODUCTION

The study of opioid control of reproduction in the ewe has been carried out using opioid antagonists as tools in order to block different types of Endogenous Opioid Receptors (EOR). In this study we are interested in hypothalamic opioid  $\mu$  receptors. The latter are identified as modulators of GnRH release. When this hypothalamic site is affected by agonists such as  $\beta$  endorphins a decrease in GnRH release is observed and the administration of antagonists such as naloxone evoke a release of GnRH.

Naloxone has been used extensively to study the endogenous opioid involvement in the control of sexual behavior and reproduction in laboratory and farm animals. In earlier study in this laboratories it was observed that the parenteral administration of low doses of the opioid antagonist naloxone (0.5 mg) induces a significant release of LH in the ewe. Furthermore, it was reported that low doses of naloxone influences directly sexual behavior in the ewe and buck (Fuentes, 1989; Fuentes *et al.*, 1997a, b). In the ewe the administration of low doses of 0.5 mg naloxone at 12 h intervals increases the duration

of estrus (Fuentes *et al.*, 2001). And in bucks naloxone (0.5 mg/12 h/15 days) induces an increase over time in the plasma level of testosterone (Fuentes *et al.*, 1998). It was also observed that in the ewe small doses of naloxone advances de preovulatory surge of LH (Fuentes *et al.*, 2001) and decreases the plasma level of prolactin in the dog with primary infertility the administration of 0.5 mg of naloxone at 12 h intervals for 15 days increased libido and fertility (Fuentes and Fuentes, 1998).

Other research groups using high doses (1 mg kg<sup>-1</sup> every 4 h for 7 days) of naloxone in soay rams resulted in no change in testosterone plasma levels (Lincoln, 1988). Singh *et al.* (2000) using 1 and 2 mg kg<sup>-1</sup> b.wt. reported a significant increase of LH, a decrease of prolactin and a significant increase in testosterone levels. It has also being reported that when naloxone is administered in doses of 1 mg kg<sup>-1</sup> the experimental animals showed different degrees of distress, while some collapsed and died after the bolus injection of opioid antagonists (Nanda *et al.*, 1989).

Others have used naloxone (0.75 or 1.5 mg of naloxone kg<sup>-1</sup> of b.wt) to predict sexual performance in rams (Stellflug, 2003; Stellflug *et al.*, 2004).

Goodman *et al.* (1995) used naloxone at a rate of 1 mg/kg/h for 16 continuous hours and they report an increase in the amplitude and shape of GnRH pulses in the ewe.

In view of the different doses of naloxone used by different research teams and the variations in their findings, it was considered of interest to compare the effect of low vs high doses of naloxone on the ovulation rate and estrous duration of Creole Mexican ewes during the mating season.

### MATERIALS AND METHODS

During the mating season (September 1, 2007 to November 30, 2007) 60 Creole ewes were chosen from an intensive production unit in the Highlands of the State of Jalisco, México. They were allocated at random in groups of 20. Age fluctuated between 2 and 3.5 years and  $40 \pm 3.5$  kg b.wt. They were housed in open paddocks with water ad libitum and fed with sheep concentrate (Purina Mexico) 250 g per ewe and 3 kg of dry chopped hay. Care was taken to separate groups as far as 400 m from each other to avoid biostimulation.

Group A Control (n = 20) received an intravaginal sponge with 40 mg medroxyprogesterona acetate (MAP) for a period of 14 days and since one day before and for two more days after sponge withdrawal they were injected with 2 mL of saline solution im at 12 h intervals. Group B High Naloxone (HN) (n = 20) received the same treatment as group A (received an intravaginal sponge with 40 mg medroxyprogesterona acetate (MAP) for a period of 14 days), on the day before sponge withdrawal a fast iv infusion of 1 mg kg<sup>-1</sup> naloxone HCl in saline solution was administered at intervals of 12 h. Infusions of naloxone continued until 2 days after sponge withdrawal, each ewe received a total of 160 mg in 4 doses. Group C Low Naloxone (LN) (n = 20) was treated as group A (received an intravaginal sponge with 40 mg medroxyprogesterona acetate (MAP) for a period of 14 days) and were injected with 0.5 mg of naloxone im at intervals of 12 h for three days, each ewe received a total of 3 mg. Treatment with naloxone in low dose was initiated since 24 h before and continued for 2 days after sponge withdrawal.

Estrus was detected since, 24 h after sponge withdrawal by the introduction of a ram to each group three times a day at 7:00, 12:00 and 21 h and detection continued until 4 days after sponge withdrawal.

Six to ten days after estrus was displayed ovulation rate was observed through laparoscopy under ketalar xylazine anesthesia as previously described (Fuentes *et al.*, 1998a, b). Statistical analysis of the results was carried out using a variance analysis (ANOVA) through a computer assisted SAS program.

### RESULTS AND DISCUSSION

It was observed that ovulation rate in ewes of group A Control was  $1.8 \pm 0.3$ ; ovulation rate of ewes of group B HN was  $1.9 \pm 0.5$ . And ovulation rate of ewes of group C LN was  $2.9 \pm 0.6$ .

Statistical analysis showed a significant effect ( $p < 0.01$ ) when group C-LN was compared with groups A and B.HN (Table 1).

Estrous duration in ewes of group C-LN treated with low doses of naloxone was  $41 \pm 4$  h and duration of estrous in groups A and B-HN was  $26 \pm 5$  h and  $25 \pm 7$  h (Table 1).

Significance between duration of estrus between ewes treated with low doses of naloxone as compared with ewes treated with saline high doses of naloxone.

Some ewes treated with high doses of naloxone showed respiratory distress chewing doors and clothing.

The selectivity of EOP receptors to different antagonists is well established and it is also well known that the antagonist effect of naloxone is specifically directed to  $\mu$  receptors, but if the dose of naloxone is increased there is a probability that naloxone may interact with other EOP receptors (Panerai *et al.*, 1985).

In some reports naloxone did not affect ovulation rate when it was administered in doses of 1 mg/kg/h (Ebling and Lincoln, 1985) It is possible that the dose used by Ebling and Lincoln (1985) was too high, consequently opioid receptors can become acutely tolerant (Tachyphylaxia) to the opioid antagonist (Owens and Cicero, 1981; Fuentes *et al.*, 2001). On the other hand, Currie *et al.* (1991) observed that naloxone in high doses (1 mg kg<sup>-1</sup>) facilitated the release of LH in the ewe. This early findings using naloxone to study sexual behavior in the ewe shows that the antagonist can exert its effect in GnRH neurons, showing that there are very complex opioid influences on sexual behavior and that the sites of naloxone action on  $\mu$ - and  $\kappa$ -receptors determine different effects on sexual behavior when used in different doses. In present study it was observed that the administration of low doses of naloxone is capable of influencing the hypothalamic pituitary axis facilitating sexual behavior (Fuentes *et al.*, 1998a, 2001). But it must be mentioned that when naloxone affects libido in the buck (Fuentes *et al.*, 1998b); the effect of naloxone is not immediate, it takes up to 7 days of treatment with naloxone

Table 1: The effect of high and low doses of naloxone in the ovulation rate and duration of oestrus in the creole Mexican ewe

Groups	Ovulation rate	Duration of estrus h
A control	$1.8 \pm 0.3a$	$26 \pm 5a$
B HN	$1.9 \pm 0.5a$	$25 \pm 7a$
C LN	$2.9 \pm 0.6b$	$41 \pm 4b$

High degree of significance between a and b  $p < 0.01$  ANOVA. Group B HN doses of 1 mg/kg/iv at 12 h intervals for 3 days. Group C LN single doses of 0.5 mg ewe<sup>-1</sup> at 12 h intervals for 3 days. Values with different letter(s) are significant different

in small doses to reach maximum concentrations of testosterone. But in the study of Stellflug (2003) and Stellflug *et al.* (2004) using one dose of naloxone (0.75 or 1.5 mg of naloxone kg<sup>-1</sup> of BW) to predict sexual performance in rams, they observed that this high dose of naloxone was capable of eliciting an LH response within 15 min after administering the antagonist and the LH release was correlated with testosterone concentrations. The latter experiment shows that a single dose of naloxone is capable of facilitating LH release. On the other hand Ebling and Lincoln (1985) using continuous high doses of naloxone is unable to observe an LH surge.

The duration of estrus is increased in naloxone low dose treated ewes and this observation is similar to previous findings in this laboratory (Fuentes *et al.*, 1998a). In the earlier study it was observed that the preovulatory surge of LH was significantly advanced and probably explains why estrous duration was increased. It might be possible to postulate that after the administration of Naloxone there is an increase in the pulsatile release of LH before ovulation, therefore estradiol was secreted in sufficient quantities to stimulate and facilitate a more intense display of estrus. LH is an important modulator of sexual behavior, therefore if LH is secreted in higher concentrations before the onset of estrus (Perkins *et al.*, 1992; Perkins *et al.*, 1995), this preovulatory increase of LH stimulates the ovary during the preovulatory stage to secrete Estradiol, explaining the increase in the duration of estrus in present and earlier study (Fuentes *et al.*, 1998b).

In this study the effect of high doses (1 mg kg<sup>-1</sup>) versus low doses (0.5 mg) of naloxone were compared and the result favored the use of small doses of naloxone to produce changes in sexual behavior an ovulation rate. Therefore, it was concluded that the opioid antagonist when administered in low doses produces significant changes in the reproductive axis of the ewe.

This study gives further support to endogenous opioids as modulators of sexual behavior in the ewe.

## REFERENCES

- Currie, W.D., I.B. Joseph and N.C. Rawlings, 1991. Morphine, naloxone and the gonadotrophin surge in ewes. *J. Reprod. Fertil.*, 92: 407-414.
- Ebling, F.J.P. and G. Lincoln, 1985. Endogenous opioids and the control of seasonal LH secretion in Soay Rams. *J. Endocrinol.*, 107: 341-353.
- Fuentes, H.V.O., 1989. Effect of Naloxone, nalbuphine, progesterone and pregnant mare's serum gonadotrophin on the sexual behaviour of ewes. *Vet. Rec.*, 124: 274-276.
- Fuentes, V.O., P.I. Fuentes and A. Garcia, 1997a. The chronic treatment with naloxone enhances libido in the male goat during anoestrus. *Vet. Rec.*, 141: 52-52.
- Fuentes, V.O., V. Sanchez, H. Gonzalez, P.I. Fuentes, A. Garcia and R. Rosiles, 1997b. La función endocrina del testículo en el carnero criollo mexicano durante la diferentes épocas del año y su control opioidérgico durante el anestro. *J. Vet. Med. A.*, 44: 259-263.
- Fuentes, V.O. and P.I. Fuentes, 1998. A new approach to the therapeutic treatment of infertility in the male dog: Report of two clinical cases. *Short commun. Vet. Rec.*, 143: 507-508.
- Fuentes, V.O., H. Gonzalez, V. Sanchez, A. Garcia and P.I. Fuentes, 1998a. The effect of naloxone on the duration of oestrus, ovulation rate and oestradiol 17-beta in crossbreed ewes with induced oestrus during seasonal anoestrus. *Small Rum. Res.*, 29: 89-92.
- Fuentes, V.O., P.I. Fuentes and A. Garcia, 1998b. The effect of naloxone on plasma concentrations of testosterone in male goats. *Small Rum. Res.*, 27: 173-176.
- Fuentes, V.O., V. Sanchez, R. Rosiles and P.I. Fuentes, 2001. The effect of naloxone on the preovulatory surge of LH and on the duration of oestrus in the ewe with induced oestrus during the non breeding season. *Anim. Reprod. Sci.*, 65: 225-230.
- Goodman, R.L., D.B. Parfitt, N.P. Evans, G.E. Dahl and F.J. Karsh, 1995. Endogenous opioid peptides control the amplitude and shape of gonadotropin-releasing hormone pulses in the ewe. *Endocrinology*, 136: 2412-2420.
- Lincoln, G.A., 1988. Endogenous opioids and the control of LH secretion during the reproductive cycle in the ram induced by treatment with melatonin. *Reprod. Nutr. Dev.*, 28: 527-539.
- Nanda, D.A., W.R. Ward and H. Dobson, 1989. Opioids modulation of tonic of tonic luteinizing hormone release on ovariectomized dairy cows. *J. Vet. Pharmacol. Ther.*, 12: 397-403.
- Owens, D.P. and T.J. Cicero, 1981. Development of acute tolerance to the effects of naloxone on the hypothalamic-pituitary-luteinizing hormone axis in the male rat. *J. Pharmacol. Exp. Ther.*, 216: 135-141.
- Panerai, A., F. Petraglia, P. Sacerdote and A.R. Genazzani, 1985. Mainly mu opiate receptors are involved in LH and prolactin secretion. *Endocrinology*, 117: 1096-1099.
- Perkins, A., J.A. Fitzgerald and E.O. Price, 1992. Luteinizing hormone and testosterone response of sexually active and inactive rams. *J. Anim. Sci.*, 70: 2086-2093.

- Perkins, A., J.A. Fitzgerald and G.E. Moss, 1995. A comparison of LH secretion and brain estradiol receptors in heterosexual and homosexual rams and female sheep. *Horm. Behav.*, 29: 31-41.
- Singh, V.D.B., P.S. Dixit and V.P. Dixit, 2000. Effect of naloxone on the plasma levels of LH, FSH, prolactin and testosterone in Beetal bucks. *Small Rum. Res.*, 37: 51-55.
- Stellflug, J.N., 2003. Use of naloxone challenge to predict sexual performance of young rams. *J. Anim. Sci.*, 81: 2111-2117.
- Stellflug, J.N., A. Perkins and V.A. LaVoie, 2004. Testosterone and luteinizing hormone responses to naloxone help predict sexual performance in rams. *J. Anim. Sci.*, 82: 3380-3387.