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The Effect of a Subcutaneous Implant of Naloxone on the Pulsatile Secretion of LH in the Prepuberal Goat During Anoestrus

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Abstract: From a commercial flock, 15 prepuberal does of the Alpine breed were selected, body weight and age averaged 35.8 kg and 8 months, respectively. The experiment was carried out during the months of May and June when anoestrus is more incipient in this latitude (19°13'N). Feeding and handling was conventional with water ad libitum. In groups of 5 chosen at random, they were treated as follows: Group A was used as control, group B received an implant of 15 mg naloxone. And group C received an i.m. injection of 0.5 mg naloxone i.m. at 12 h intervals for 15 days. To observe the effect of this experimental treatment on the pattern of secretion of LH, on day 0 a blood sample (2 mL) was obtained through a cannulae implanted in the jugular vein every 20 min for 7 h continuous. Blood samples were allowed to coagulate at room temperature and thereafter, centrifuged at 2.500 rpm for 15 min; plasma was decanted into small plastic vials and stored frozen until LH assay was carried out. Blood samples were obtained on day 0, 5, 10, 15 and 20. Plasma concentration of LH was determined by RIA as previously reported. And the statistical analysis of LH concentrations was studied using a descriptive method followed by an inferential screening and a variance analysis. It was observed that the plasmatic concentrations of LH changed through time in all groups, changes were more pronounced in the naloxone treated animals as compared with the control group. It was observed that there was no significance on the interaction between treatment and the sampling day. An influence of time upon the plasmatic levels of LH was observed, showing a high degree of significance ($f = 12.58$ (4.44) $p = 0.0000005$). There was tendency of an increase in LH secretion through time observed in all groups. A large difference was detected when comparing plasma LH from the first 2 samples with samples obtained in days 10, 15 and 20 of the experiment. It was observed that the effect of naloxone through time did not show significant differences until after the second week of treatment. This results give further support of the important role that endogenous opioids on the control of reproduction of sheep.

Key words: Naloxone, LH, prepuberal goats, anoestrus, pulsatile secretion

INTRODUCTION

The survival of species is determined through their capacity for reproduction and this event is controlled by the interaction of different biological systems characteristic of each species. Reproductive behaviour is under the control of external and internal factors. All of which permit birth to happen during the appropriate time of the year, in order to insure the survival of the new born (Fabre-Nys *et al*., 1993; Mori, 1989).

Among the many species that are under the influence of ambient factors, the ewe and goat are good examples. These small ruminants have a period of anestrus followed by a period of sexual activity (oestrus). The expression of sexual behaviour is stimulated during the beginning of the short days, while, the period of anoestrus is onset during the long days (Fuentes, 1988). This behaviour is more determinant in the north hemisphere, where the European breeds originated (Chemineau *et al*., 1988). In countries near the ecuator where photoperiod changes are not so marked, European goat breeds behave in much the same way (Silva *et al*., 1994; Mellado, 1997).

The period of anestrus is of interest because productivity in intensive production units is not optimal and many research teams are studying the mechanisms that determine the presence of periods of anoestrus and oestrus, using small ruminants as a model for such observations (Currie *et al*., 1991a; Chemineau *et al*., 1988; Malven, 1995). The capacity for connecting and disconnecting sexual behaviour in these species, means

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that there is an internal mechanism that through external and internal sensors are able to change the pattern of activity of the hypothalamic-hypophisial-gonadal axis. Modulating the secretion of the gonadotrophic hormones such as LH (Edqvist and Stabenfeldt, 1993a b; Fabre-Nys *et al*., 1993). During anoestrus the pulsatile secretion of LH is dimished (Schall *et al*., 1991) and during the breeding season pulsatile secretion of LH is increased (Scaramuzzi and Baird, 1977). The secretion of gonadotrophins is under the influence of releasing hormones that are originated in the median eminence of the hypothalamus. Furthermore, releasing hormones are secreted at rates determined by the influence of central nervous transmitters and neurohormones. Among the latter there is evidence that melatonin (Malpaux *et al*., 1998), dopamine and peptide opioids are important modulators of GnRH secretion (Rossier and Chapouthier, 1981; Currie, 1989; Currie and Rawlings, 1991b; Malven, 1995; Malpaux *et al*., 1998; Fuentes *et al*., 1998a).

Trough the manipulation of opioidergic receptors with opioid antagonists it is possible to induce changes in the pattern of secretion of gonadotrophic hormones in several species (Brooks *et al*., 1986; Cosgrove *et al*., 1993; Fuentes, 1988; Aurich *et al*., 1995; Grossman, 1983). Some have considered the pattern and magnitude of naloxone-induced changes in endocrine function to facilitate identification of sexually active and inactive rams during the breeding season. Prediction accuracy of the naloxone-based test was 69-85% (Stellflug *et al*., 2004). In the latter experiment, Rams were treated with 1.5 mg of naloxone kg^{-1} BW. In experiments with different animal species, de dose used was in average 1 mg $body^{-1}$ weight. Some report undesirable side effects and death of experimental animals after the acute administration of such high doses (Nanda *et al*., 1989; Yang *et al*., 1988). In bucks naloxone was used to study opioid interaction with LH and testosterone secretion, with doses of 1 mg kg^{-1} BW (Baljeet *et al*., 2000). In previous research, we have observed that small doses produce significant changes in LH, Testosterone and prolactin plasma levels in different species (Fuentes *et al*., 1997, 2007).

It is well known that hypothalamic opioid receptors are sensitive to opioid antagonists, the higher the dose the higher effect on different opioid receptors. Naloxone administered in high doses affect various opioid receptors and it is possible to postulate that small doses show a tendency to affect selectively μ-receptors (Fuentes *et al*., 2007).

In previous researches, it was observed that small doses of opioid antagonists with selective affinity for μ-endorphinergic receptors, such as naloxone, induced changes in the sexual behaviour and blood hormones levels of sheep and goats (Fuentes, 1988). The administration of naloxone is limited by the pharmacological characteristic of its absorption and distribution through the experimental animal body. It is administered solely by the parenteral route, therefore all previous experiments with this opioid antagonist have being carried out using the intramuscular and intravenous routes. It was considered of interest to study the effect of a subcutaneous implant containing 15 mg naloxone on the plasmatic concentrations of LH of the goat during the anoestrus period.

MATERIALS AND METHODS

From a commercial flock, 15 does of the Alpine breed were selected, body weight and age averaged 35.8 kg and 8 months, respectively. The experiment was carried out during the months of May and June when anoestrus is more incipient in this latitude (19°13'N). Feeding and handling was conventional with water *ad libitum*. In groups of 5 chosen at random, they were treated as follows: Group A was used as control, group B received an implant of 15 mg naloxone. And group C received an i.m. injection of 0.5 mg naloxone i.m. at 12 h intervals for 15 days.

To observe the effect of treatment on the pattern of LH secretion, on day 0 a blood sample (2 mL) was obtained through a cannulae implanted in the jugular vein every 20 min for 7 continuous h. Blood samples were allowed to coagulate at room temperature and thereafter centrifuged at 2.500 rpm for 15 min, plasma was stored frozen until LH assay was carried out.

Blood samples were obtained on day 0, 5, 10, 15 and 20. Plasma concentration of LH was determined by RIA as previously reported (Fuentes *et al*., 1998b). And the statistical analysis of LH concentrations were studied using a descriptive method followed by an inferential screening and a variance analysis.

RESULTS

It was observed that the plasmatic concentrations of LH changed through time in all groups, changes were more pronounced in the naloxone treated animals as compared with the control group. This changes can be appreciated observing Fig. 1-5. Where plasma concentrations of all groups can be compared during the different times of the experiment.

After performing the variance analysis, it was observed that there was no significance on the interaction between treatment and the sampling day. But there is a treatment influence on the plasmatic levels of LH. An

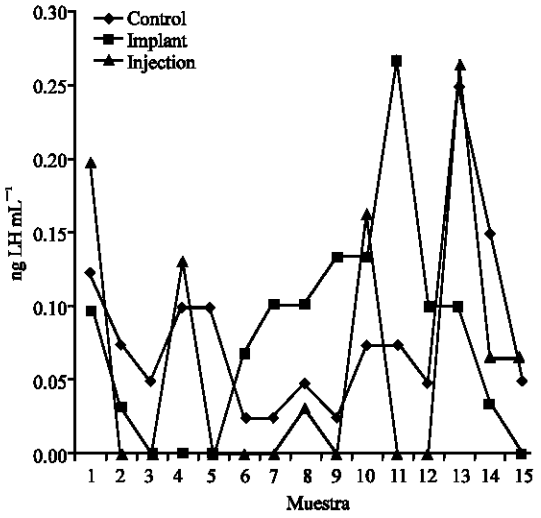

Fig. 1: The effect of nalexone on plasmatic levels of LH on the 1st day of the experiment

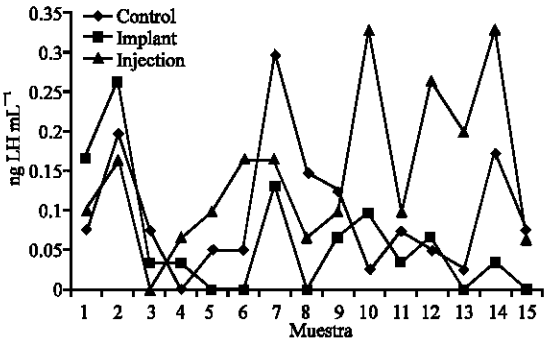

Fig. 2: Plasma levels of LH in nalexone injected, implanted and control goats on

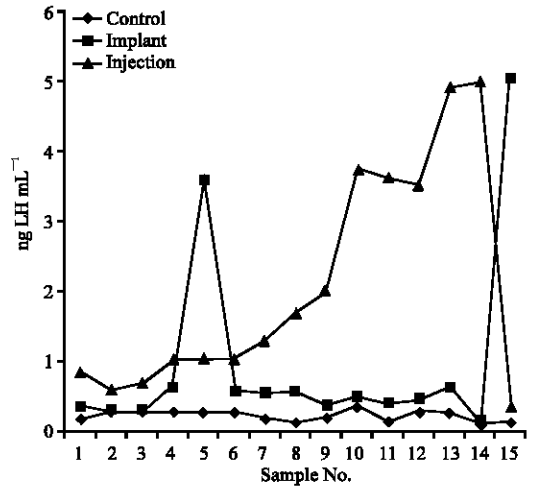

Fig. 3: Plasma levels of LH on the 14 day of the treatment in nalexone injected

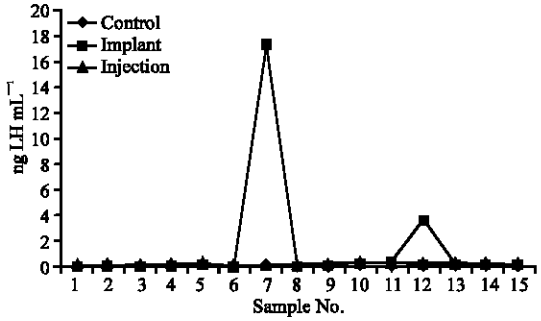

Fig. 4: Comparative level of plasma LH on day 21, in between groups

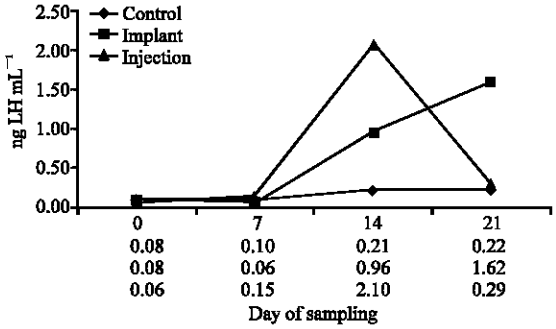

Fig. 5: Accumulated plasma levels of LH according to the day of sampling and group

influence of time upon the plasmatic levels of LH was observed, showing a high degree of significance ($f = 12.58$ (4.44), $p = 0.0000005$). This interaction is clearly illustrated in Fig. 5, where a contrast of the obtained averages is observed in the different groups and also is observed a tendency of an increase in LH secretion through time observed in all groups.

In order to study, the influence of treatment upon time on the plasmatic levels of LH a large difference was detected when comparing the first 2 samples with the samples obtained in days 10, 15 and 20 of the experiment. It was observed that the effect of naloxone through time did not show significant differences until after the second week of treatment.

DISCUSSION

The observed results in this study show that the plasmatic concentrations of LH are in agreement with the reports of similar observations in the ewe (Currie *et al*., 1991a, b; Fuentes *et al*., 1998a, b; Malven, 1995; Schall *et al*., 1991); goats (Fuentes *et al*., 1997; Cheminenau, 1988).

Naloxone is an opioid antagonist that has being used as a tool to study the interaction of endogenous opioids

with sexual function, but the dose used is the first reports is extremely high, in the order of 0.5 mg kg^{-1} (Currie and Rawlings, 1989; Schall *et al*., 1991) and in this research, the dose used is 1.0 mg total dosis day^{-1}. Furthermore, in previous research Fuentes (1988) and Fuentes *et al*. (1998a, b) using the same dose (1 mg day^{-1}) has reported changes in sexual behaviour and changes in the pulsatile release of LH. In this research, the use of a subcutaneous implant with 15 mg naloxone produces much the same changes as those observed with injections of 0.5 mg at 12 h intervals and it was observed that the concentration of LH increased with time of treatment. When Tuckey's test is used to study this changes it was observed a significant difference between the plasmatic levels of control goats with those of the naloxone treated animals. The latter observation shows that the effect of naloxone is not inmediate, it takes time for naloxone to express the antagonist effect on μ receptors. When naloxone was administered to adult bucks during the anoestrus season, testosterone levels were not affected until after 7 days of treatment (Fuentes *et al*., 1997). Furthermore, in this research, significant changes are not present until after 7 days of treatment. It is interesting that when naloxona is injected epidrurally for the treatment of chronic pain, the effect of one single injection persists for several weeks (Blaise, 2003). The latter suggests that there is a time effect on the endocrine response to naloxona treatment (Fuentes *et al*., 2003). There are reports, in which the administration of high doses in acute or continuous intravenous infusions of naloxona does not consistently affect blood hormone concentrations (Ebling and Lincoln, 1985; Barb *et al*., 1991). The latter might be due to effect of high doses of naloxone on different opioid receptors (Barb *et al*., 1991).

CONCLUSION

This observation further supports that naloxone administered in low doses produces changes of significance both in sexual behaviour and hormone concentrations (LH). The effect of an implant of naloxone is similar to the effect observed when the opioid antagonist is administered in small doses at 12 h intervals for 15 days.

This findings show the need of further research using low doses of opioid antagonists in order to permit continuous interaction with opioid receptors with the aim of inducing physiologic changes as those needed to express sexual behaviour.

REFERENCES

Aurich, C.H., H. Hoppe and J.E. Aurich, 1995. Role of endogenous opioids for regulation of the oestrous cycle horse. Reprod. Domest. Anim., 30: 188-192.

Baljeet, S., V.D. Dixit, P. Singh, G.C. Georgie 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.

Barb, C.R., R.R. Kraeling and G.B. Rampacek, 1991. Opioid modulation of gonadotropin and prolactin secretion in domestic farm animals. Domest. Anim. Endocrinol., 8: 15-27.

Blaise, G., 2003. Should we use naloxone epidurally? Can. J. Anesth., 50: 875-878.

Brooks, A.N., G.E. Lamming and N.B. Haynes, 1986. Endogenous opioid peptides and the control of gonadotropin secretion. Res. Vet. Sci., 41: 285-299.

Chemineau, P., G. Martin, J. Suamande and E. Normant, 1988. Seasonal and hormonal control of pulsatile LH secretion in the dairy goat (*Capra hircus*). J. Reprod. Fert., 83: 91-97.

Cosgrove, J.R., F. Rensis and G.R. Foxcroft, 1993. Opiodergic pathways in animal reproduction: Their role and effects of their pharmacological control. Anim. Reprod. Sci., 33: 373-392.

Currie, W.D., S.J. Cook and N.C. Rawlings, 1991a. LH secretion in ovariectomized ewes: Effects of morphine and ovarian steroid interactions with naloxone during the breeding season and anestrum. Can. J. Anim. Sci., 71: 333-342.

Currie, W.D., I.B.J.K. Joseph and N.C. Rawlings, 1991b. Morphine, naloxone and the gonadotropin surge in ewes. J. Reprod. Fert., 92: 407-414.

Currie, D. and N.C. Rawlings, 1989. Fluctuation in responsiveness of LH and lack of responsiveness of FSH to prolonged infusion of morphine and naloxone in the ewe. J. Reprod. Fert., 86: 359-366.

Ebling, F.J.P. and G.A. Lincoln, 1985. Endogenous opioids and the control of seasonal LH secretion in Soay rams. J. Endocrinol., 107: 341-353.

Edqvist, L.E. and G.H. Stabenfeldt, 1993a. The Endogenous Control of Reproduction Processes. In: King, G. (Ed.). Reproduction in domesticated animals. Amsterdam: Elsevier Science Publishers, pp: 75-82. ISBN: 13-978-0-444-89530-1.

Edqvist, L.E. and G.H. Stabenfeldt, 1993b. The Hormones of Reproduction. In: King, G. (Ed.). Reproduction in domesticated animals. Amsterdam: Elsevier Sci. Publishers, pp: 55-74. ISBN-13: 978-0-444-89530-1.

Fabre-Nys, C., P. Poindron and J.P. Signoret, 1993. Reproductive Behaviour. In: King, G. (Ed.). Reproduction in domesticated animals. Amsterdam: Elsevier Sci. Publishers, pp: 147-182. ISBN-13: 978-0-444-89530-1.

Fuentes, H.V.O., P.I. Fuentes and A. García, 1997. Chronic treatment with naloxone enhaces libido in the male goat during anoestrus. Vet. Rec., 141: 52.

Fuentes, V.O., V. Sanchez, R. Rosiles and P.I. Fuentes, 1998a. The effect of low doses of naloxone on the preovulatory surge of LH and on the onset and duration of oestrus in the ewe with induced oestrus during the non-breeding season. Small Rum. Res., 29: 89-92.

Fuentes, V.O., H. Gonzalez, V. Sanchez, A. García and P. Fuentes, 1998b. The effect of naloxone on the duration of oestrus ovulation rate and oestradiol 17 β in crossbred ewes with induced oestrus during seasonal anoestrus. Small Rum. Res., 29: 89-92.

Fuentes, V.O., 1988. 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., J.J. Alvarez, A. Hernández, P.I. Fuentes and R. Sanchez, 2003. The effect of small doses of naloxone on the initiation and duration of the first estrus after weaning in sows. Anim. Reprod. Sci., 70: 121-125.

Fuentes, V.O., H. Gonzalez, V.M. Sanchez and P.I. Fuentes, 2007. Effect of small doses of naloxone on the pusatile secretion of prolactin in the crossbreed ewe during the non breeding season. Anim. Reprod. Sci., 100: 44-50.

Grossman, A., 1983. Brain opiates and neuroendocrine function. Clin. Endocrinol. Metab., 12: 725-746.

Malpaux, B., A. Daveau, F. Maurice-Mandon, G. Duarte and P. Chemineau, 1998. Evidence that melatonin acts in the premammillary hypothalamic area to control reproduction in the ewe: Presence of binding sites and stimulation of luteinizing hormone secretion by *in situ* microimplant delivery. Endocrinology, 139: 1508-1516. PMID: 9528928.

Malven, P.V., 1995. Role of endogenous opioids for regulation of the oestrous cycle in sheep an cattle. Reprod. Domest. Anim., 30: 183-187.

Mellado, M., 1997. The creole goat in Latin America (*La cabra criolla* en América Latina). Vet. Mex., 28: 333-337.

Mori, Y., 1989. Central Integration of Photoperiodicity for Gonadotropin Release in Ruminants. In: Dyer, E.G. and R.J. Bicknell (Eds.). Brain Opioid Systems in Reproduction. Oxford: Oxford Science Publication, pp: 93-118. ISBN: 9780198576945.

Nanda, D.A., W.R. Ward and H. Dobson, 1989. Opiois modulation of tonic luteinizing hormone release on ovariectomized dairy cows. J. Vet. Pharmacol. Ther., 12: 397-403.

Rossier, J. and G. Chapouthier, 1981. Encefalinas and endorfinas. La Recherche, pp: 504-513.

Scaramuzzi, R.J. and D.T. Baird, 1977. Pulsatile release of LH and the secretion of ovarian steroids in sheep during anestrus. Endocrinol., 101: 1801-1806.

Schall, R.E., F.J.P. Ebling, F.J. Karsch and D.L. Foster, 1991. Postpubertal maturation of endogenous opioid regulation of luteinizing hormone secretion in the female sheep. Biol. Reprod., 44: 760-768.

Silva, E., M. Galina and B. López, 1994. Reproductive behaviour of the dairy goat during estrous sychronization on the anestrous season. Av. Inv. Agropecuaria, 3: 39-47.

Stellflug, J.N., A. Perkins and V.A. LaVoie, 2004. Testosterone and luteinizing hormone responses to naloxone help predict sexual performance in rams1. J. Anim. Sci., 82: 3380-3387.

Yang, K., N. Haynes, G.E. Lamming and A.N. Brooks, 1988. Ovarian and steroid involment in endogenous opioid modulation of LH secretion in mature ewes during the breeding and non-breeding season. J. Reprod. Fertil., 83: 129-139.