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Treatment of cystic ovarian disease with Naloxone in high production dairy cows

M. M. PALOMAR*
J. C. ACOSTA*
N. R. SALVETTI*
F. BARBERIS†
P. M. BELDOMENICO‡
O. GARNERO† &
H. H. ORTEGA*

**Department Morphological Sciences, Faculty of Veterinary Sciences, National University of Litoral, Santa Fe, Argentina; †Department of Animal Clinic, Faculty of Veterinary Sciences, National University of Litoral, Santa Fe, Argentina; ‡Leahurst Veterinary Field Station, University of Liverpool, Liverpool, UK*

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Dr Hugo H. Ortega, Department of Anatomy and Histology – Faculty of Veterinary Sciences, National University of Litoral. R.P. Kreder 2805, CP 3080, Esperanza, Santa Fe, Argentina. E-mail: hhortega@fcv.unl.edu.ar

Cystic ovarian disease (COD) is an important cause of abnormal oestrous behaviour and infertility in dairy cows. Ovarian cysts are defined as follicle-like structures of diameter >25 mm persisting for at least 10 days in the absence of a corpus luteum (Garverick, 1997). The prevalence of COD in dairy herds has been reported to vary from 5% to 30% (Vanholder *et al.*, 2006) and this condition may result in significant economic losses to the dairy industry because of increased calving to conception and inter-calving intervals (Peter, 2004).

The COD is mainly observed in high yielding dairy cows during the first months postpartum, as this is a period of high stress (Peter, 2004). The role of stress in the pathogenesis of COD is believed to be mediated by the discharge of endogenous cortisol, which inhibits LH release. Endogenous opioid peptides are involved in many responses to stress (Przewlocki, 1993), as they regulate various endocrine systems including the hypothalamic–pituitary–adrenocortical axis. The latter has particularly been demonstrated in cattle (Nanda *et al.*, 1992; Gazal & Anderson, 1995), which has direct implications on the modulation of anterior pituitary hormones (Barb *et al.*, 1991). Endogenous opioid peptides produced in hypophysis and brain are believed to block the oestrogen-induced LH surge and the release of hypothalamic GnRH (Malven, 1986). This opioidergic block is related to the increase of free endorphins and receptors, which results in the formation of endorphin–receptor complexes that determine calcium channels blockage with deficit of intracellular calcium (Ca^{2+}; Minoia & Sciorsci, 2001).

In cattle, the administration of an opioid antagonist [i.e. Naloxone (Nx)] results in increased serum LH release in a variety of physiological states (Byerley *et al.*, 1992). In fact, when Nx is administered to an individual with high endorphin levels, it also produces a fast intracellular calcium increase, which stimulates the production and release of LH (Minoia & Sciorsci, 2001).

To directly antagonize the effects of endogenous opioids on the ovary, Sciorsci *et al.* (2000a) proposed the epidural administration of Ca-Nx. In a preliminary field study, these authors determined the disappearance of follicular cysts and related clinical signs after this treatment. However, little is known about how is Nx involved in such clinical recovery. To address this question, the objectives of the present study were to (i) evaluate ovarian changes in cows with COD following treatment with Ca-Nx and (ii) determine the association between serum concentrations of reproductive hormones, before and after Ca-Nx treatment, and their relationship with the cyst disappearance.

This study was carried out during the period of January–November of 2005 at three commercial dairy farms in Colonia Aldao, Santa Fe, Argentina. Forty pluriparous (mean 3.3 ± 1.5 lactations, range: 2–7), high yielding (mean 29.68 ± 6.23 kg of milk per day at diagnosis) Argentinean Holstein cows affected by COD at least 45 days after calving were selected (95.9 ± 37.8 days in milk). For this study, a COD case was defined as a cow with one ovarian cyst >25 mm that persisted for at least 10 days in the absence of a corpus luteum. The ovarian cysts were diagnosed using a portable B-mode scanner with a 7.5 MHz transducer.

Immediately after diagnosis, the animals were injected, via epidural at lumbo-sacral level, with 0.6 mg of Nx hydrochloride (Diosynth B.V.; OSS, the Netherlands) dissolved in 3 mL of Ca^{2+} gluconate 20% together with 2 mL of GnRH analogue (Buserelin 4 μg/mL; Receptal, Hoescht, Buenos Aires, Argentina). The ovaries were re-examined by transrectal ultrasonography 2 weeks after treatment.

The cows were observed twice daily for 30 days following treatment for the detection of oestrus, and artificially inseminated if palpation per rectum confirmed the presence of a preovulatory follicle and the absence of genital disorders. Therapy was considered successful only if normal oestrus (followed by the appearance of a corpus luteum and no recurrence of cysts) was detected within 30 days of treatment.

Blood samples were taken at the same time (from 07:00 to 08:00 hours) immediately before, and 2 weeks after treatment. All blood samples were centrifuged at 1500 ***g*** for 15 min, and serum samples were stored at −20 °C until assayed. Serum LH, FSH, oestradiol, progesterone and cortisol concentrations were determined by radioimmunoassay using previously described protocols (Ortega *et al.*, 2006; Baravalle *et al.*, 2007).

Results were expressed as mean ± standard error of the mean. A Wilcoxon signed rank test for paired samples was used to compare both hormonal levels and ultrasonography data at pretreatment and post-treatment (2 weeks after). Mann–Whitney *U*-tests were used to compare number of lactancies, yield and hormonal levels between recovering and nonrecovering animals 2 weeks after treatment.

The ultrasound examination identified thin-walled cystic structures in one or both ovaries of all cows. Mean cyst size before treatment was 3.36 ± 0.19 cm in diameter, which was larger than the mean diameter 2 weeks after treatment (1.83 ± 0.34 cm; $P = 0.008$); 32.5% of the cows achieved at least a 50% reduction in cysts sizes.

In this study, 31 of 40 (77.5%) cows with ovarian cysts began cystic regression or cyclic ovarian activity within 2 weeks post-treatment. Of these 31 respondents, nine (22.5% of the total) expressed oestrous with formation of CL within 2 weeks after treatment, while 22 (55%) showed oestrous later but within 4 weeks after treatment. The remaining nine cows (22.5%) displayed either turnover or persistence of cysts throughout the experimental period. In the 31 animals that responded to treatment, the average interval from treatment to oestrous was 23.93 ± 1.35 days and the interval from treatment to pregnancy was 60.85 ± 9.38 days. We have observed no adverse effects following treatment.

The descriptive statistics for serum oestradiol LH, FSH, oestradiol, progesterone and cortisol are summarized in Table 1. The progesterone level in all animals before treatment was <1 ng/mL with a significant increase 2 weeks after treatment ($P = 0.036$), however, this data could not be relevant due to the progesterone concentrations naturally vary during the oestrus cycle. No significant differences were observed in concentrations of LH, FSH, oestradiol and cortisol; and hormonal levels, lactancies and yield were not significantly different for responding and nonresponding individuals.

Table 1. Hormonal levels immediately before and 2 weeks after the treatment with Ca-Naloxone in cows with follicular ovarian cyst

	Pretreatment	Post-treatment
LH (ng/mL)	1.06 ± 0.27	1.45 ± 0.82
FSH (ng/mL)	2.98 ± 0.47	2.85 ± 0.44
Oestradiol (pg/mL)	14.41 ± 2.01	9.33 ± 1.95
Progesterone (ng/mL)	0.65 ± 0.31*	5.43 ± 1.62*
Cortisol (ng/mL)	22.94 ± 3.97	18.76 ± 2.51

Values are expressed as mean ± SEM.
*$P < 0.05$.

To our knowledge, this is the first comprehensive examination of the hormonal and follicular response to Ca-Nx plus GnRH in high yielding cows with COD. The results of the present study are strong evidence that this treatment was able to trigger cystic regression and follicle development leading to a normal oestrus with sexual behaviour and fertile ovulations, as it had been suggested in a preliminary field study (Sciorsci *et al.*, 2000a).

Also, the therapeutic efficacy of Ca-Nx indirectly demonstrates an involvement of endogenous opioids in the cystic pathogenia. This, in addition to the high levels of cortisol observed (substantially greater than the values of 3.62–11.69 ng/mL in nonpregnant heifers of the same farm; unpublished data), agrees with the demonstrated role of opioids on the effects of stress (factor associated with cattle that have COD).

It is known that stress induces the release of cortisol, which inhibits LH preovulatory discharge (Ribadu *et al.*, 2000). Furthermore, during any type of stress, β-endorphins increase and interplay with metabolic processes. In COD, it has been reported that β-endorphins are linked to the gonadotrophin receptors and block G-protein activity (Wang *et al.*, 1988). Sciorsci *et al.* (2000b) states that Ca-Nx directly competes with G-proteins for binding to opioid receptors and allows the transduction of gonadotrophin hormonal message while exogenous GnRH supply the deficit of hypothalamic GnRH. The ovulation and increased progesterone levels determined after treatment indirectly support this hypothesis of cease in the inhibition of LH secretion. Unfortunately, the frequency of blood sampling used in the present study (limited by sanitary regulations of the farms) did not allow an analysis of LH pulse frequency or amplitude, neither a proper evaluation of the changes in the other hormones.

Taking into account the above-mentioned findings, we may conclude that Ca-Nx is an effective treatment for COD in dairy cattle that it resulted in ovulation and formation of CL. To fully understand the mechanism of action of Nx in relieving COD, frequent monitoring of the ovaries using urtrasonography, coupled with circadian LH, FSH, oestradiol and progesterone profiles will be necessary in further experiments.

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