Effect of Administration of an Opioid Antagonist (Naloxane) to Treat Ovarian Cysts in Dairy Cows

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Abstract

The role of stress in the pathogenesis of ovarian cysts 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. Therefore, the aim of this study was to determine the effect of epidural administration of Naloxone (NX) on cystic regression and follicle development leading to normal oestrus in dairy cattle. This study was conducted on 91 dairy cattle affected by follicular cysts, divided randomly into 3 groups. Group 1 (n=30) cows were treated epidurally (lumbo-sacral) with 0.8 mg Naloxan hydrochloride (NX). Group 2 (n=30) cows were treated epidurally with GnRH analogue (100 mcg gondorelin acetate). Group 3 (n=31) cows were considered as control and received 5 ml normal saline epidurally. Although the results of this study showed that Naloxane was better than the other two groups to treat follicular cysts, there was no significant difference in different criteria, such as CL formation, increasing plasma P4 concentrations and 1st service conception rates among 3 groups.

Key words: Cow, Follicular cyst, Naloxane

Introduction

The severe economic influence of ovarian cysts on the dairy industry is well – known, and losses caused by prolonged calving intervals and increased culling rates must be added to the direct costs of medical treatment (Ribadu et al. 2000). The etiopathogenesis of follicular cysts (FC) is still not completely understood (Pecket al. 1998; Pivaet al. 1986; Rizzo et al. 2009a; Rizzo et al. 2009b; Short et al. 1987). It is generally accepted that the etiology is multifactorial, in which genetic, phenotypic and environmental factors are involved (Wanget al. 1998). The most widely accepted hypothesis is based on neuro- endocrinological dysfunction of the hypothalamic- pituitary – gonadal axis (Lee et al. 2005; Lopez et al. 1997). The role of stress in the pathogenesis of COD is believed to be mediated by the discharge of endogenous cortisol, which inhibits LH release (Peck et al. 1988). Endogenous opioid peptides are involved in many responses to stress (Proboet al. 2011) as they regulate various endocrine systems including the hypothalamic-pituitary-adrenocortical axis. The latter has been particularly demonstrated in cattle (Palomar et al. 2008).

Endogenous opioid peptides produced in hypophysis and brain are believed to block the estrogen – induced LH surge and the release of hypothalamic GnRH (Mehmanesh et al. 1998). The opioid system exerts a physiological tone inhibitory effect on GnRH neurons as revealed by the enhancement of LH release after treatment with the opioid antagonist (naloxone) (Brownet al. 1994;
Przewlocki, 1993). This opioidergic block is related to the increase of free endorphins and receptors, which results in the formation of endorphins and receptors, and leads to formation of endorphin-receptor complexes that determine calcium channel blockade with deficit of intracellular calcium (\(\text{Ca}^{2+}\)). Conversely, administration of opioid agonists, just before pro-oestrus, inhibits the release of pre-ovulatory LH surge and hence ovulation (Grossman et al. 1989; Kavej et al. 2012). There is some evidence that opioids can affect GnRH release directly. Because beta-endorphins blocks release of LHRH in the hypophyseal portal vessel by stimulating muopioate receptor, there by inhibiting secretion of LH (Minoia et al. 2001). There is also evidence indicating that opioids act directly by influencing the brain monoaminergic systems (Diez–Guerraet al. 1987; Nishiharaet al. 1991; Palomaret al. 2008). In veterinary medicine, many pharmacological agents, above all local anesthetic and analgesic drugs, are administered into the epidural space (Tsuruokaet al. 1997). This route can also be used for administering hormone and analogues, achieving a selective local Pharmacological response. The main objective of this study was to assess the effect of naloxone on follicular cystic regression and follicle development leading to normal oestrus.

**Material and methods**

**Animals**

This study was carried out during the period of October 2013 to December 2015 on two commercial dairy herds in Isfahan in Iran on 5600 lactating dairy cows. The participating farmers and the local veterinarian were informed about all relevant characteristics of the study and agreed with the study design. The rolling-herd averages were approximately 12,000 kg of milk. Lactating dairy cows were housed in freestall facilities and milked three times daily. Within herds, cows were fed the same total mixed ration, formulated to meet or exceed the NRC (2001) nutrient requirements for lactation Holstein cows weighing 680 kg and producing 45 kg of 3.5 fat-corrected milk.

The herds were maintained on a weekly reproductive health program and the reproductive tract of each animal was examined by ultrasound scanning, beginning 28-34 days postpartum to verify normal uterine involution and to observe ovarian structures. The diagnosis of ovarian follicular cysts was performed ultrasonographically, taking into account the criteria indicated by Silvia et al (2002).

**Ultrasoundographic examination**

The animals were then subjected to a further three ultrasound examination at 7-day intervals. A single practitioner performed the examination by rectal palpation together with an ultrasound examination for better evaluation of the morphology and evolution of ovarian structures, using a portable B-mode ultrasound scanner (ECM, IMAGO user manual version 1.04). Each ovary was scanned in several planes by moving the transducer along the ovarian surface to identify the different structures. The size of the cysts was measured using the built in electronic caliper after freezing the image on the screen.

**Blood samples**

Blood samples were collected before and after treatment for the analysis of progesterone (\(\text{p}_4\)) concentration. The blood samples were collected by jugular venipuncture with refrigerated vacutainer serum tubes, maintained at 4°C and then were taken to the laboratory at a minimum time of 60 ± 20 minutes. Plasma concentration of \(\text{p}_4\) was measured by the validated radioimmunoassay method (Bono et
The intra– and inter– assay coefficients of variation were 6.9% and 6.98%, respectively; assay sensitivity was 13 pg/ml.

**Experimental design**

The experimental design was completely randomized with blocks. Weekly, a cohort of cows from 28 to 34 DIM (days in milk) were blocked by parity (primiparous or multiparous) and randomly assigned to one of three treatment groups.

Group 1 (Naloxon) (30 cows): Cows of this group were administered 0.8 Mg of Naloxan (Nx) hydrochloride (Hospira, Inc., Lake Forest, IL 60045 USA) in the epidural space at lumbo-sacral level.

Group 2 (GnRH) (30 Cows): Cows of this group were administered GnRH analogue (100 mcg gondorein acetate, Parnell Technologies Pty-Ltd Australia) in the epidural space at lumbo-sacral level.

Group 3 (Normal saline) or Control group (30 Cows): Cows of this group were injected 5 ml of physiologic solute onin the epidural space at lumbo-sacral level. Seven to 14 days after treatment, all animals underwent a transrectal ultrasonography to detect the eventual regression of follicular ovarian cyst.

**Results**

Plasma progesterone levels, before and after treatment, were compared in different treatment groups (fig. 1). It is not able that the most and the least mean increase is in naloxone and control group, respectively. Cows in 3 groups were divided into two possible categories with or without CL (corpus luteum). To evaluate their distribution and to find out whether there is any relationship between the treatment and CL development, chi-square test was used. P-value showed less than the significance level (a>P-value); therefore, the null hypothesis is rejected and it becomes obvious that there is a significant relationship between post-treatment CL development and the treatment group. Thus, naloxone treatment group and GnRH group had the most and the least effect, respectively, on CL occurrence (fig. 2).

Since the chi-square test and correlation coefficient were less than the significance level (pvalue=0.02), it can be concluded that the amount of progesterone increase is varied varies in each treatment group and is related to the specific group.

The optimum increase in progesterone level is more than 1 ng/ml Levels of progesterone were divided into two groups to determine the relevance between treatment groups and progesterone increase.

Group one (red column) covered cows with preferred increase in progesterone levels and group two (blue column) included cows with no or no higher than 1 ng/ml increase in progesterone levels (fig. 3).

First service conception rates were evaluated in different groups (Fig. 4). As can be seen, the naloxone treatment group has the highest number of pregnant cows and the GnRH group has the least. Chi-square test was used to evaluate a significant relevance between first service conception rates and the treatment group. Although, the number of pregnant cows was different in different groups, this difference was not considered statistically significant (P > 0.05).

**Discussion**

The stress axis (i.e., hypothalamic–pituitary–adrenal axis) shares endocrine glands with the reproductive axis (i.e., hypothalamic–pituitary–gonadal axis) (Crowe et al. 2012). It has also been suggested that high endorphin level in situations of stress, blocks the membrane channels by which calcium normally enters cells (Moyse et al. 1997).
Naloxone is a pure opioid antagonist and blocks each of the opioid receptor types (Moyse et al. 1997). The result of our study provides strong evidence that this treatment was able to trigger cystic regression and follicle development leading to a normal estrus, fertile ovulation and increased progesterone concentration (p₄). In agreement with this observation are the findings of two preliminary field study by Sciorsci et al (2000) and Palomar et al (2008). In cystic ovarian disease, it has been reported that β-endorphins are linked to the gonadotroing receptors and block G protein activity (Whisnant et al. 1986).

The main objective of this study was to utilize and propose naloxone to antagonize the endogenous opiate peptides (Eop). It has been shown that the administration of naloxone increases LH concentrations in the postpartum anestrus beef cows. The effect of naloxone on LH release is well documented in laboratory and farm animal. When naloxone is administered by parental injection pulsatile LH release is increased in frequency and amplitude (Fuentes – Hernandeset 2009).

In cattle, the administration of an opioid antagonist result increased serum LH release in a variety of physiological states (Byerley et al. 1992):
puberal heifers (Silvia et al. 2002) postpartum and anestrous cows (Kesler et al. 1982; Xu et al. 2008), steers (Peter, 2004), and young bulls (Malven, 1986). In veterinary medicine, analgesic drugs, such as local anesthetics, opioids and ketamine are usually administered directly to the epidural space (Tsuruoka et al. 1997). Moreover, the pharmacokinetics of drugs administered in the epidural space depends on many factors, such as the volume injected, the speed of inoculation, lipophilicity and molecular weight (Johnson et al. 1996; Kalra et al. 1989; Liptrap and McNally, 1976; MacDonald et al. 1990; Tsuruoka et al. 1997). Furthermore, when administered epidurally, drugs could reach the central nervous system, and thus hypothalamus and hypophysis are vehicled by cerebrospinal fluid (Dolan et al. 2003; Skinner et al. 2009; Zulu et al. 1998). It was also concluded that a low dose parental regimen with naloxone can relieve pain by a central pathway mechanism (Vanholder and Opsomer, 2006). In our study, group 2 was the same as group 3 (control group), the result of which is not in agreement with those of Annalisa et al’s (2011) who demonstrated that the epidural administration of GnRH analogue can improve reproductive parameters and remission of follicular cysts compared to intramuscular administration.

**Conclusion**

Although the results of this study showed that naloxane was better than the other two groups to treat follicular cysts in dairy cows, there was no significant difference in different criteria, such as CL formation, increasing plasma P4 concentrations and 1st service conception rates. It seems that considering the effect of parity, history of previous treatments, on response to treatment, another study with more cows in each group is needed.

**References**


Effect of administration of an opioid antagonist


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تأثیر تجویز یک آنتاگونیست اپیوئیدی (نالوکسان) در درمان کیست تخمدانی گاو شیری

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چکیده:

این بیانیه از نظر ایزوئیدیکی در نقاطی که در بستر منطقه موارد بیماری نالوکس سنتی با تغییرات در نظارت از پاتوژن می‌گردند. نتایج آزمایشات از نگاهی ناشی از ایزوئیدیکی که به خوبی باعث ایجاد شود. در پی این بررسی، اثر تزریق آنتاگونیست اپیوئیدی (نالوکسان) در تحلیل كیست‌های تخمدانی در گزارش‌های طبیعی در گزارش‌های شیری باشد. این مطالعه پروپت 91 راس گاو مبتلا به کیست فولیکولی انجام شد که بطور تصادفی به 3 گروه تقسیم شدند. گروه دوم (تعداد 30 راس) با تزریق GnRH به مقدار 100 میکروگرم بوسترین استات، از راه اپیدورال تحت کمری-خاجی تحت درمان قرار گرفتند. گروه دوم (تعداد 20 راس) با تزریق GnRH به مقدار 100 میکروگرم بوسترین استات، از راه اپیدورال تحت درمان قرار گرفتند. گروه سوم (تعداد 31 راس) به علت این مطالعه نشان داد که تولید درمان کیست فولیکولی از دو گروه دیگر بهتر بوده است اما اندازه‌داده از نظر رنگ‌های مختلف منجمد: تکسکس جسم زرد، افزایش غلظت پلاسمایی پروپتسترون، و میزان آبستنی در اولین تلفیق است. درمان است. این گروه دیگری نشد.

واژه‌کلیدی: تولید، کاسیم، GnRH، کیست فولیکولی، گاو/شیری