Iranian Journal of ruminants Health Research, 2016, 1 (2), 9-16
DOI: 10.22055/ijrhr.2017.12842

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

Derakhshesh, S. M[1]., Ghasemzadeh Nava, H[2*].,Tajik, P[2]., Akbari, Gh[1]

1-Department of Theriogenology, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University- Tehran-Iran

2 -Department of Theriogenology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

***Corresponding Author:** hghasem@ut.ac.ir*

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.Group1 (n=30) cows were treated epidurally (lumbo-sacral) with 0.8 mg Naloxan hydrochloride (NX). Group2 (n=30) cows were treated epidurally with GnRH analogue (100 mcg gondorelin acetate). Group3 (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 differnnt 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 (Peck*et al.* 1998; Piva*et 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 (Wang*et 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 (Probo*et 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) (Brown*et 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 formatio of endorphin-receptor complexes that determine calcium channel blockage with deficit of intracellular calcium (ca^{2+})(20). Conversely, administration of opioid agonists, just before pro-oestrus, inhibits the release of pre-ovulatory LH surge and henceovulation (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–Guerra*et al.* 1987; Nishihara*et al.* 1991; Palomar *et al.* 2008). In veterinary medicine, many pharmacological agents, above all local anesthetic and analgesic drugs, are administered into the epidural space (Tsuruoka*et 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 commerical 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 observeovarian structures.The diagnosis of ovarian follicular cysts was performed ultrasonographically, taking into account the criteria indicated by Silvia *et al* (2002).

Ultrasonographic 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 (p_4) concentration. The blood samples were collected by jugular venipuncture with refrigerated vacutainer serum tubes, maintained at 4^0C and then were taken to the laboratory at a minimumtime of 60 ± 20 minimum.Plasma concentration of p_4 was measured by the validated radioimmunoassay method (Bono *et*

*al.*1996) .The intra –and inter –assay cofficients 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 (α>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 relatedto 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).

Fig. 1: Plasma concentrations of p_4 in different groups

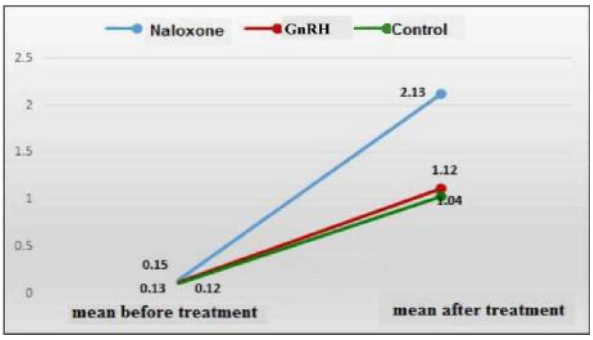


Fig.2: Distribution of CL formation in different groups after treatment

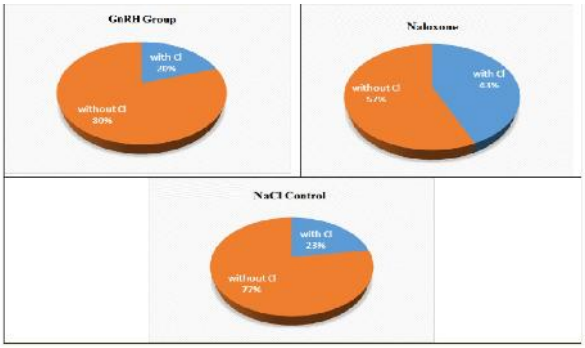
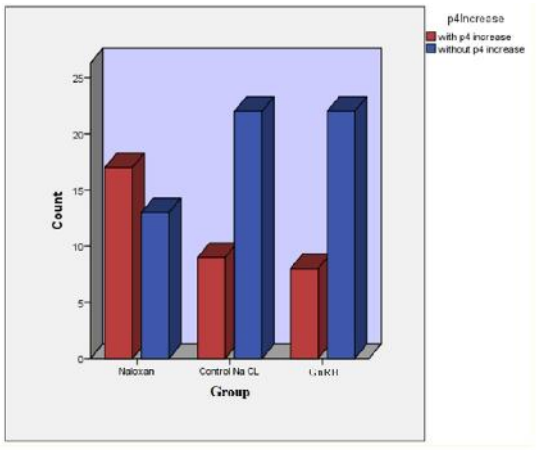
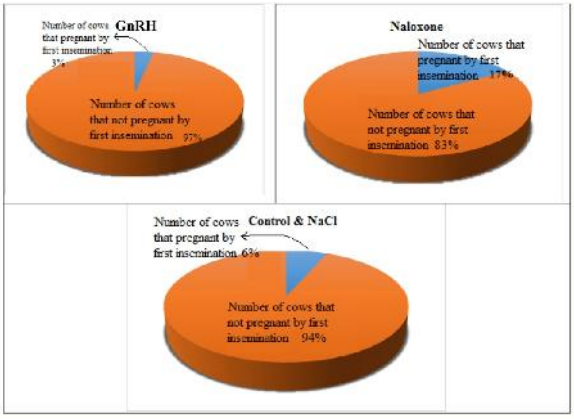


Fig. 3: Distribution of serum progesterone concentrations in different study groups after treatment



The highest and lowest increase in progestrone levels are in naloxone and GnRH treatment groups.

Fig. 4: First service conception rates in different study groups



Naloxone is a pure opioid antagonistand blocks each of the opiod receptortypes (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 progestrone concentration (p_4). 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 -endorphins are linked to the gonadotraing 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 concentions 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 – Hernandes*et al.*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, opioidsand 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 differnnt criteria, such as CL formation, increasing plasma P4 concentrations and ^{1}st 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

Annalisa, R., Debora, C.,Maddalena, M., Giuseppe, M., Massimo, S., and Raffaele Luigi S. (2011). Epidural vs intramuscular administration of lecirelin, a GnRH analogue, for the resolution of follicular cysts in dairy cows. Anim. Reprod. Sci., 126, 19-22.

Brown, C.H., Gilmore, D.P., Kalia, V., Leigh, A.J. and Wilson, C.A. (1994). Dose–dependent opioid of the pre–ovulato.ryluteinising hormone surge in the rat. Mediation by catecholamines. Biogenic. Amines., 10, 119–128,.

Byerley, J.D.,kiser, E.T., Bertrand, K.J. and Kereling, R.R. (1992). Release of luteinizing hormone after administration of Naloxone in pre-and peripuberal heifers. J. Anim. Sci. 70, 2794-2800.

Crowe, M.A. and Williams, E.J. (2012). Effect of stress on postpartum reproduetion in dairy cows. J. Anim. Sci. 90, 1722- 1727.

Diez–Guerra, F.J., Augood, S., Emson, P.C. and Dyer, R.G. (1987). Opioid peptides inhibit the release of noradrenaline from slices of rat medial preoptic area. Exp. Brain. Res. 66, 378–384.

Dolan, S., Evans, N.P., Richter, T.A. and Nolan, A.M. (2003). Expression of gonadotropin-releasing hormone and gonadotropin-releasing hormone receptor in sheep spinal cord. Neurosci. Lett. 346, 120–122.

Fuentes –Hernandes, V.O., Bernal- Canseco, A., Fuents – Castro, P.I., Floves – Lopes, H. and Orozoco-Hernandez, R. (2009). A comparative study using an opoiodantagonist and GnRH for the treatment of follicular cyst in dairy holsteincow . J. Bio. Sci. 9, 833-836.

Grossman, R.and Dyer, R.G. (1989). Opioid inhibition of brain stem projections to the medial preoptic area in female rats. Brain Opioid Systems in Reproduction (Dyer, RG. and Bicknell, RJ.). Oxford University Press, Oxford, New York, 1st ed., pp: 112–124.

Johnson, R.A., Lopez, M.J., Hendrickson, D.A. and Kruse-Elliot, KT.(1996). Cephalad distribution of three differing volumes of new methylene blue injected into the epidural space in adult goats. Vet. Surg. 25, 5, 448–451.

Kalra, S.P., Allen, L.G. andKalra, P.S. (1989). Opioids in the steroid–adrenergic circuit regulating LH secretion: dynamics and diversities. Brain Opioid Systems in Reproduction, (Dyer, RG. and Bicknell, RJ.). Oxford University Press, Oxford, New York, 1st ed. pp: 95–111.

kaveh, A.A ., Bahramini, A.F., Abdi, k., kabirian, A. and Kochakzadeh, H. (2012). Effect of naloxon on post-partum ovarian follicular size in Holstein cows. Europe. J. Exper. Bio. 2,4, 1214-1219.

Kesler, D.J. and Garverik, H.A. (1982). Ovarian cyst disease in dairy cattle. J. Anim. Sci. 55, 1147–1159.

Lee, I., Yamagishi, N., Oboshi, K., Ayukawa, Y., Sasaki, N. and Yamada, H. (2005). Distribution of new methylene blue injected into the caudal epidural space in cattle. Vet. J. 169, 257–261.

Liptrap, R.M. and McNally, P.J.(1976). Steroid concentrations in cows with corticotropin-induced cystic ovarian follicles and the effect of prostaglandin F_2alpha and indomethacin given by intrauterine injection. Am. J. Vet. Res. 37, 369–375.

Lopez, M.J., Johnson, R, Hendrickson, D.A. and Kruse-Elliot, K.T.(1997). Cranial migration of differing doses of new methylene blue injected in the epidural space after death of calves and juvenile pigs. Am. J. Vet. Res. 58, 7, 786–790.

MacDonald, R.D., Peters, J.L. andDeaver, D.R. (1990). Effect of naloxone on the secretion of luteinizing hormone in infantile and prepubertal holstein bull calves. J. Reprod. Fertil. 89, 51-57

Malven, P.V. (1986) Inhibition of pituitary LH release resulting from endogenous opioid peptides. Dom. Anim. Endocrin. 3, 135–144.

Mehmanesh, H., Almedia, O.F.X., Nikolarakis, K.E. andHerz, A. (1988). Hypothalamic LH–RH release after acute and chronic treatment with morphine studied in a combined in vivo/in vitro model. Brain. Res. 451, 69–76.

Minoia, P. andSciorsci, R.L. (2001).Metabolic control through L-calcium channal, PKC and opioid receptor smodulation by an Association of

naloxone and calcium salts. Curr. Drug Targets Immune Endocrin. Metabal. Disord. 1, 2,131-137.

Moyse, E., Marcel, D., Leonard, K., and Beaudet, A. (1997). Electron microscopic distribution of opioid receptors on noradrenergic neurons of the locus coeruleus. Eur. J. Neurosci. 9, 128–139.

Nishihara,. M, Hiruma, H., and Kimura, F. (1991). Interactions between the noradrenergic and opioid peptidergic systems in controlling the electrical activity of luteinizing hormone–releasing hormone pulse generator in ovariectomized rats. Neuroendocrin. l54, 321–326.

Palomar, M.M., Acosta, J.C., Salvetti, N.R., Barberis, F., Beldomenico, P.M., Garnero, O. and Ortega, H.H. (2008). Treatment of cystic ovarian disease with naloxone in high production dairy cows. J. Vet. Pharmacol. Ther. 31, 184–186.

Peck, D.D., Thompson,F.N., Stuedeman, J.A**.,** Leshin, L.S. andKiser T.E. (1988). Evidence for endogenous opioid modulation of serum luteinizing hormone and prolactin in the steer. J. Anim. Sci. 66, 3197-3202

Peter, A.T.(2004). An update on cystic ovarian degeneration in cattle. Reprod. Domest. Anim. 39, 1, 1-7.

Piva, F., Maggi, R., Limonta, P., Motta, M. and Martini, L. (1985). Effects of naloxone on luteinizing hormone, follicle stimulating hormone and prolactin in the different phases of estrous cycle. Endocrin.11**7,**766–772.

Przewlocki, R. (1993). Opioid Systems and Stress. Opioids II. Reprod. Domest. Anim. 39, 1–7.

Probo, M., Comin, A., Mollo, A., Cairoli, F., Stradaioli, G. and Veronesi, M.C. (2011). Reproductive performance of dairy cows with luteal or follicular ovarian cysts after treatment with buserlin. Anim Repro Sci. 127, 135-139.

Ribadu, A.Y., Nakada, K., Moriyoshi, M., Zhang, W.C., Tanaka, Y. and Nakao, T. (2000). The role of LH pulse frequency in ACTH-induced ovarian follicular cysts in heifers. Anim. Reprod. Sci. 64, 21–31.

Rizzo, A., Minoia, G., Trisolini, C., Mutinati, M., Spedicato, M., Manca, R. and Sciorsci, R.L.(2009a). Renin and ovarian vascularization in cows with follicular cysts after epidural administration of a GnRH analogue. Anim. Reprod. Sci. 116, 226–232.

Rizzo, A., Minoia, G., Trisolini, C., Mutinati, M., Spedicato, M., Jirillo, F. and Sciorsci, R.L. (2009b). Reactive oxygen species (ROS): involvement in bovine follicular cysts etiopathogenesis Immunopharmacol. Immunotoxicol. 31, 4, 631–635.

Short, R.E., Brooks, A.N., Peters, A.R. and Lamming G.E. (1987). Opioid modulation of LH secretion during the oestrous cycle of heifers. J. Reprod. Fertil. 80,213-219.

Silvia, W.J.,Hatler, T.B., Nugent, A.M. and Laranja da Fonseca, L.F. (2002). Ovarian follicular cysts

in dairy cow: An abnormality in folliculogenesis. Domestic. Anim. Endocr. 23,167-177.

Sciorsci, R.L., Robbe, D., DI-matteo, A., LA-calandra, G.M. andMinoia, P. (2000). The treatment of ovarian follicular cystis in dairy cow with epidural calciumnaloxone and systemic gnarl administration: A preliminary field study. 14th. International Congress on Animal Reproduction, Stockholm. 12, 7-13.

Skinner, D.C., Albertson, A.J., Navratil, A., Smith, A., Mignot, M., Talbott, H. andScanlan-Blake, N., (2009). GnRH effects outside the hypothalamopituitary- reproductive axis. J. Neuroendocrinol. 21, 4, 282–292.

Thurmon, J.C., Tranquilli, W.J. and Benson, G.J. (1996). Lumb and Jones' Veterinary Anesthesia, 3rd ed. Lea &Febiger, Baltimore, MD. pp: 115–147.

Tsuruoka, M., Hiruma, Y. andWillis, W.D. (1997). The subnucleusreticulais dorsalis is involved in antinociception produced by a low dose of naloxone during carrageenan-induced inflammation. Brain. Res. 762, 264-268.

Vanholder, T. andOpsomer, G. (2006). Aetiology and pathogenesis ef cystic ovarian follieles in dairy cattle: A review. Repord. Nutri. Dey. 46, 105-119.

Wang, D., Sadee, W.,Quillan, J.M. (1988). Calmodulin binding to G protein-coupling domain of opioid receptors. J. Biol. Chem. 274, 22081–22088.

Whisnant, C.S., Thompson, F.N., Kiser, T.E. and Barb, CR.(1986). Effect of naloxone on serum luteinizing hormone, cortisol and prolactin concentrations in anestrous beef cows. J. Anim. Sci. 62, 1340–1345.

Xu, C., Roepke, T.A., Zhang, C., Ronnekleiv, O.K. and Kelly, M.J. (2008). Gonadotropin-releasing hormone (GnRH) activates the M-current in GnRH neurons: An auto regulatory negative feedback mechanism. Endocrin. 149, 5, 2459–2466.

Zulu, V.C. and Penny, C.(1998). Risk factors of cystic ovarian disease in dairy cattle. J. Reprod. Physiol. 44, 191–195.

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

سید محمد درخشش[1]، حمید قاسم زاده نوا[2]، پرویز تاجیک[2]، قاسم اکبری[1]*

۱- گروه علوم بالینی، دانشکده دامپزشکی، شاخه علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

۲- گروه مامایی و بیماری های تولید مثل دام، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران

*نویسنده مسئول: **hghasem@ut.ac.ir***

چکیده:

این باور وجود دارد که نقش استرس در پاتوژنز کیست تخمدانی بواسطه مهار آزادسازی LH ناشی از آزادسازی کورتیزول اندوژن میانجیگری می‌گردد. نقش پپتیدهای اپیوئیدی اندوژن در پاسخ‌های متعدد به استرس مورد بحث می‌باشد. لذاهدف از انجام این مطالعه، بررسی اثر تزریق اپیدورال نالوکسان در تحلیل کیست‌های تخمدانی و رشد فولیکول منجر به فحلی طبیعی در گاوهای شیری بوده است. این مطالعه برروی۹۱ راس گاو مبتلا به کیست فولیکولی انجام شد که بطور تصادفی به ۳ گروه تقسیم شدند.گاوهای گروه اول (تعداد ۳۰ راس) با تزریق ۰/۸ میلی گرم از داروی نالوکسان هیدروکلراید از راه اپیدورال (ناحیه کمری-خاجی) تحت درمان قرار گرفتند.گاوهای گروه دوم (تعداد ۳۰ راس) با تزریق GnRH به مقدار۱۰۰ میکروگرم بوسرلین استات، از راه اپیدورال تحت درمان قرار گرفتند. گاوهای گروه سوم (تعداد ۳۱ راس) بعنوان گروه شاهد درنظر گرفته شدند و تزریق اپیدورال سرم فیزیولوژی به مقدار۵ سی‌سی در آنها انجام شد. با وجودی‌که نتایج این مطالعه نشان داد که نالوکسان در درمان کیست فولیکولی از دو گروه دیگر بهتر بوده است اما تفاوت معنی‌داری از نظر اندیس‌های مختلف منجمله: تشکیل جسم زرد، افزایش غلظت پلاسمایی پروژسترون، و میزان آبستنی در اولین تلقیح بعد درمان بین ۳ گروه دیده نشد.

واژه‌گان کلیدی: نالوکسان- کلسیم، GnRH، کیست فولیکولار، گاوشیری