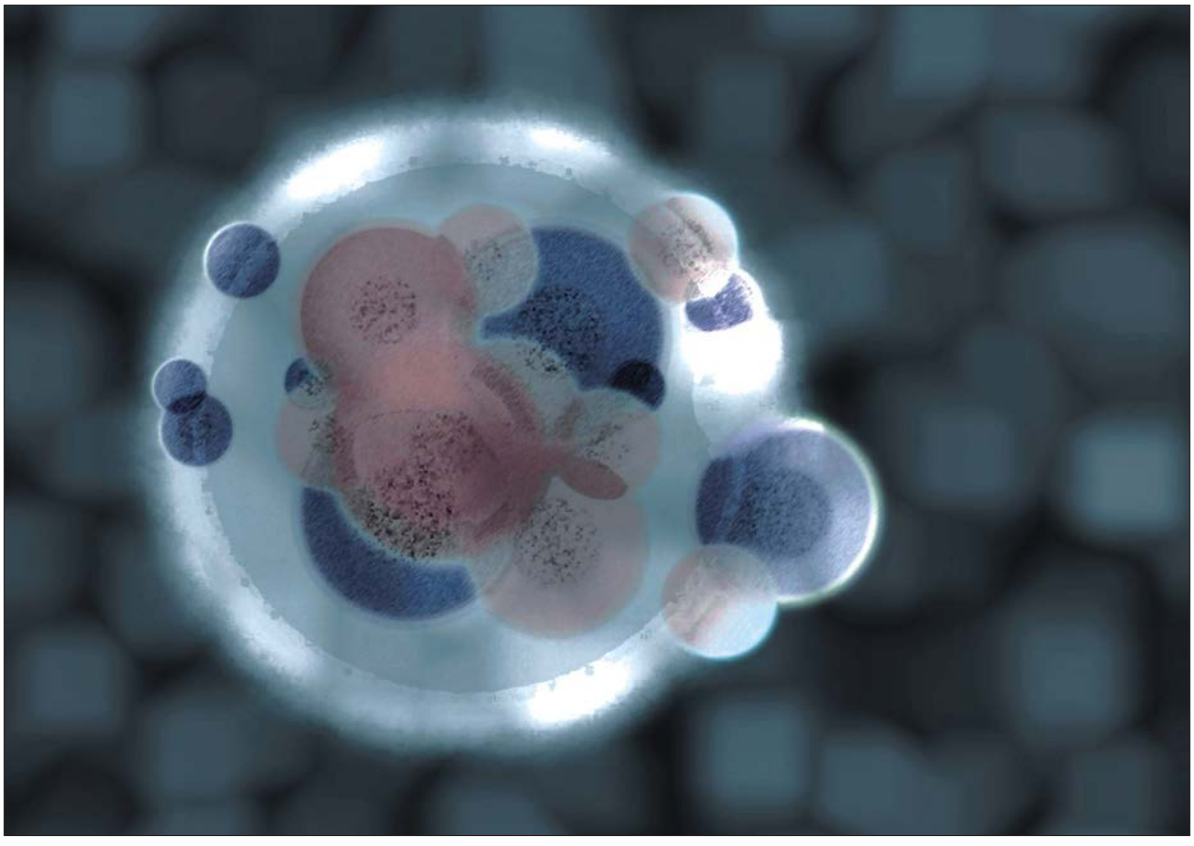

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 9 (8): 833-836, 2009
ISSN 1727-3048

A Comparative Study using an Opioid Antagonist and GnRH for the Treatment of Follicular Cyst in Dairy Holstein Cows

[1]V.O. Fuentes-Hernandez, [1]A. Bernal-Canseco, [2]P.I. Fuentes-Castro,
[1]H. Flores-Lopez and [1]R. Orozco-Hernandez
[1]Centro Universitario de los Altos, Universidad de Guadalajara,
Km 7.5 Carretera a Yahualica, 47600, Tepatitlan, Mexico
[2]Hospital de Alta Especialidad PEMEX Sur Mexico DF, Mexico

Abstract: With the objective of studying the effect of naloxone on the presence of Follicular cysts in dairy cattle, 60 high production cows with follicular cysts were selected. Divided at random in three equal Groups. Group 1 (n = 20) were treated with an im injection of 100 μg GnRH. Group 2 (n = 20) was treated with 5 mg naloxone im at 12 hour intervals for three consecutive days. Group 3 was considered as control and injected with saline solution. In group 1, 13/20 (65%) treated with GnRH showed follicular dehiscence and in group 2 treated with naloxone 18/20 (90%) showed follicular dehiscence. Group 3 (n = 20) saline treated cystic cows; 4/20 presented spontaneous estrous and pregnancy. After the statistic analysis χ^2 a significant difference ($p<0.0001$) was observed between control and GnRH and naloxone treated cows. The statistical outcome between GnRH and Naloxone was significant ($p<0.05$). It was concluded that naloxone in small doses would be considered as an alternative novel therapy for Follicular cysts in dairy cattle.

Key words: Follicular, cysts, dairy cattle, GnRH, naloxone

INTRODUCTION

Cystic ovaries in cattle represent not only and endocrine dysfunction but also an economic problem for dairy producers since cows are infertile as long as the condition persists (De Vries *et al*., 2006). There are follicular and luteal cysts, in this study the objective was to study the effect of an opioid antagonist on the persistence of Follicular Cysts (FC).

The presence of FC represents a universal problem wherever dairy cattle are produced (Hamilton *et al*., 1995). On the beginning of this century the treatment of FC was carried out by manual rupture (via rectal palpation), with this procedure return to normal estrus was around 45%.

During the 40's and 50's hormones were introduced for the treatment of FC, among this, pregnant mare's serum gonadotrophin (eCG) and human chronic gonadotrophin (hCG) were the battle horses for the treatment of FC. But success was diminished by immunologic reactions, never the less hCG, is still in use for the treatment of FC.

At present gonadotrophin releasing hormone (GnRH) and its analogs are the treatment of choice for FC. The objective of GnRH treatment is to induce LH release from the anterior pituitary in quantities such as to promote follicular dehiscence and resumption of a normal estrual cycle.

In the repeater cow due to many factors, pulsatile release of LH is diminished, but the concentration of serum LH is high and a preovulatory-like surge of LH is not present, when compared with normal cows (Hamilton *et al*., 1995). The preovulatory LH surge is essential for the physical rupture of the follicle and oocyte release (ovulation) (Monniaux *et al*., 2008). The preovulatory LH surge is also critical for the transformation of cells from the wall of the ovulatory follicle into progesterone producing luteal cells. Furthermore, if LH pulses are inadequate there is insufficient follicle growth and insufficient production of estradiol by the follicle. Thus, this cows never have a follicle that reaches sufficient size or/and estradiol production to induce the cow to come into estrus and have an LH surge and ovulate (Wiltbank *et al*., 2002). The latter observations gives way to postulate that FC presence is due to inadequate LH secretion.

It is well known that the release of GnRH at the hypothalamic level is under the control of endorphins and the administration of an endorphin like substance such as morphine inhibits the release of GnRH. It is also well documented that the administration of an opioid antagonist such as naloxone facilitates the release of LH and the expression of sexual behavior (Wójcik-Gładysz *et al*., 2006; Katz and Mazer, 2009).

Corresponding Author: Victor O. Fuentes, Centro Universitario de los Altos, Universidad de Guadalajara, Tepatitlan, Jalisco 47600, Mexico Tel: (52) 378 7828036

If we consider that the treatment of FC with GnRH aims at inducing an endogenous release of pituitary LH with the objective of inducing follicular dehiscence; on the other hand, if we consider that naloxone facilitates the release of GnRH in to portal hypothalamic veins, inducing the release of pituitary LH. Therefore, when naloxone is administered in low doses, a continuous release of LH will be facilitated after each small dose, the latter effect gives way to postulate that the administration of low doses of naloxone to dairy cattle with FC will facilitate an increase in the frequency of LH pulses in sufficient concentration to induce luteinization of the FC and the resumption of normal estrus cycles in cattle that are suffering of FC. Therefore it was considered of interest to study the effect of GnRH and naloxone on the prevalence of follicular Cysts in dairy cattle.

MATERIALS AND METHODS

In this study 60 multiparous dairy cows with a history (July 2007 January 2008) of repeat breeder and with a clinical diagnosis of cystic ovarian disease, were selected from a local intensive farm unit. With the objective of comparing the therapeutic effect of naloxone vs GnRH and Saline treatments for FC they were divided at random in three groups, group 1 (n = 20) was treated with a GnRH analog (100 mcg cistorelin im, CEVA Mexico). Group 2 was treated with 5 mg naloxone by im injection at 12 h intervals for 3 consecutive days (total 6 doses/5 mg = 30 mg naloxone). And group 3 (n = 20) were sham treated with saline solution im.

The presence of FC in all groups of cows was monitored through rectal examination at 48 h intervals. If the cyst suffered dehiscence and corpus luteum was present followed by estrus, the treatment was considered successful.

Furthermore, days from treatment to first signs of estrus and positive pregnancy after artificial insemination were also noted.

The analysis of the result was carried out using contingency tables and χ^2 through a computerized SAS program.

RESULTS

In cystic cows treated with GnRH (Table 1) it was observed that 13/20 showed follicular dehiscence and a corpus luteum was present.

When cows with FC were treated with low doses of naloxone it was observed that 18/20 showed follicular dehiscence and the presence of a corpus luteum was corroborated.

Table 1: The effect of GnRH and Naloxone on the prevalence of follicular cysts in Holstein dairy cow

Groups (n = 20)	Follicular corpus luteum	Dehiscence present pregnant	No. cows
Cows treated with GnRH	13/20a	yesa	11a
Treated with naloxone	18/20b	yesb	17a
Control saline	4/20	yes	4

ab: $p<0.001$

Table 2: The means between cistyc cows treated with naloxone and GnRH as compared with controls

Factors	Control	GnRH	Naloxone
Follicular dehiscence	1.80a	1.35b	1.10b*
Corpus luteum	1.80a	1.35b	1.10b*
Days to estrus	1.80a	13.80b	18.65c*
Pregnancy	1.85a	1.45b	1.25b*
Days pospartum	147.25	137.75	152.80NS

ab: $p<0.001$, *$p<0.05$, NS: Not significant

In control saline treated cystic cows; 4/20 presented spontaneous estrous and pregnancy. The presence of estrus was significant in between groups. After artificial insemination 17 cows treated with naloxone were positively pregnant.

Cows treated with GnRH only 11 resulted with positive pregnancy. When means were considered in between groups, as can be shown in Table 2, it was observed that there was a significant difference in between cows treated with GnRH and naloxone, when considering the days postpartum to treatment no difference was observed. It was noted that cystic cows treated with Naloxone presented the highest conception rate as compared with the GnRH treated group.

DISCUSSION

The results of treatment of FC with GnRH in this study; are similar to reports by Amer and Mhady (2008). The aim of the treatment with GnRH is to directly stimulate the anterior pituitary to release LH and FSH in sufficient amounts as needed for the luteinization of the follicular cysts (De Vries *et al*., 2006). The effect of GnRH is short lived and it is probable that repeater cows treated with GnRH might develop short luteal phases. The latter might be the cause of failed pregnancy in GnRH treated repeater cows. It should be considered that anovular cows show a low LH pulse frequency associated with postpartum anestrous (Duffy *et al*., 2000), while in normal cow's ovulation coincides with increases in LH pulse frequency from two to three pulses per 6-h period to five to seven pulses per 6 h (Savio *et al*., 1990; Stagg *et al*., 1998). Therefore, it is possible to assume that the administration of GnRH only stimulates short lived release of pituitary LH, but the needed frequency of LH pulses are not restored or/and not sufficient to achieve effective regression of the FC, explaining the failure to conceive of some cows treated with GnRH.

The effect of naloxone on LH release is well documented in laboratory and farm animals, when naloxone is administered by parenteral injection pulsatile LH release is increased in frequency and amplitude. In early experiments using naloxone, the dose of this opioid antagonist is extremely high (non physiologic) and at times it was lethal to the experimental animals (Ebling and Lincoln, 1985; Yang *et al*., 1988), or the response was variable or non significant (Horton and Clarke, 1988; Currie and Rawlings, 1989). Yang *et al*. (1988) report behavioral changes in treated ewes, such as excitement, circular movements round the pen and intense chewing at their food bowls, each other's skins and the person taking blood samples. They also reported that one sheep collapsed and died 15 min after the second administration of the opioid antagonist. Behavioral symptoms in the treated animals disappeared some 3 h later. A question comes forward, how many of the experiments were carried out without reporting undesirable effects after the administration of high doses of intravenous and continuous infusion of naloxone? And another question arises, why we use small doses of the opioid antagonist? The use of small doses of naloxone in this work was derived from the use of naloxone in humans. In humans the recommended dose is 0.4 mg for a 70 kg man. This dose is not free of secondary effects such as dizziness and discomfort. Therefore a simple approach was carried out using small doses such as those used therapeutically in humans. In previous study, it was observed that when naloxone was administered in small doses the pulsatile release of LH is increased, facilitated the expression of estrus behavior, decreased Prolactin serum concentrations and increased libido and testosterone levels in bucks and rams (Fuentes *et al*., 1997a,b; 1998a,b, 2001, 2003, 2007). This previous experiments using naloxone in small doses show that the effect of naloxone on LH, testosterone and behavior are facilitated after the continuous intermittent administration of low doses of naloxone and make way to postulate that each time that naloxone is administered in a very small dose, there is a selective effect on μ receptors inhibiting the effect of β endorphin and facilitating the release of LH. Furthermore, Endogenous opioids are among the factors involved in the inhibition of the hypothalamic pulse generator that directs gonadotrophin releasing hormone (GnRH) secretion, similar findings in women with polycystic ovarían syndrome LH concentrations were normalized after naltrexone treatment (Roozenburg *et al*., 1997) giving further support to endogenous opioids as important components of the endocrine events that regulate ovulation (Wójcik-Gładysz *et al*., 2006; Katz and Mazer, 2009). And with the results of this study, it is possible to postulate that ovulation might be under the control of hypothalamic endorphins.

If we take in to consideration previous experiences, we can also postulate that the use of naloxone in small doses in dairy cattle with FC produces a sustained release of LH with a direct effect on the FC, inducing luteinization and resumption of a normal estrus and fertility.

CONCLUSION

We finally conclude that naloxone can be used as an alternative therapy for FC in anovulatory repeater cows suffering with FC.

REFERENCES

Amer, H. and A.B. Mhady, 2008. Hormonal profiles associated with treatment of cystic ovaries with GnRH and PGF2α with and without CIDR in dairy cows. Livest. Res. Rural Dev., 20: 52-52.

Currie, W.S. 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. Fertil., 86: 359-366.

De Vries, A., M.B. Crane, J.A. Bartolome, P. Melendez, C.A. Risco and L.F. Archibald, 2006. Economic comparison of timed artificial insemination and exogenous progesterone as treatments for ovarian cysts. J. Dairy Sci., 89: 3028-3037.

Duffy, P., M.A. Crowe, M.P. Boland and J.F. Roche, 2000. Effect of exogenous LH pulses on the fate of the first dominant follicle in postpartum beef cows nursing calves. J. Reprod. Fertil., 118: 9-17.

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, V.O., V. Sanchez, H. Gonzalez, P.I. Fuentes, A. Garcia and R. Rosiles, 1997a. La función endocrina del testículo en el carnero criollo mexicano durante la diferentes épocas del año y su control opioidergico durante el anestro. J. Vet. Med. A., 44: 259-263.

Fuentes, V.O., P.I. Fuentes and A. Garcia, 1997b. The chronic treatment with naloxone enhances libido in the male goat during anoestrus. Vet. Rec., 141: 52-52.

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 anestrus. 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.

Fuentes, V.O., J.J. Alvarez, A. Hernandez, 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 5 in sows. Anim. Reprod. Sci., 79: 121-125.

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

Hamilton, S.A., H.A. Garverick, D.H. Keisler, Z.Z. Xu, K. Loos, R.S. Youngquist and B.E. Salfen, 1995. Characterization of ovarian follicular cysts and associated endocrine profiles in dairy cows. Biol. Reprod., 53: 83-90.

Horton, R.J.E. and I.J. Clarke, 1988. Lack of effect of morphine or naloxone on the oestrogen-induced LH surge in anoestrous ewes. J. Endocrinol., 119: 89-93.

Katz, N. and N.A. Mazer, 2009. The impact of opioids on the endocrine system. Clin. J. Pain, 25: 170-175.

Monniaux. D., N. Di Clemente, J.L. Touzé, C. Belville and C. Rico *et al*., 2008. Intrafollicular steroids and anti-müllerian hormone during normal and cystic ovarian follicular development in the cow. Biol. Reprod., 79: 387-396.

Roozenburg, B.J., H.J.H.M. van Dessel, J.L.H. Evers and R.S.G.M. Bots, 1997. Successful induction of ovulation in normogonadotrophic clomiphene resistant anovulatory women by combined naltrexone and clomiphene citrate treatment. Hum. Reprod., 12: 1720-1722.

Savio, J.D., M.P. Boland, N. Hynes and J.F. Roche, 1990. Resumption of in the early post-partum period of dairy cows. J. Reprod. Fertil., 88: 569-579.

Stagg, K., L.J. Spicer, J.M. Sreenan, J.F. Roche and M.G. Diskin, 1998. Effect of calf isolation on follicular wave dynamics, gonadotropin and metabolic hormone changes and interval to first ovulation in beef cows fed either of two energy levels postpartum. Biol. Reprod., 59: 777-783.

Wiltbank, M.C., A. Gumen and R. Sartori, 2002. Physiological classification of anovulatory conditions in cattle. Theriogenology, 57: 21-52.

Wójcik-Gładysz, A., K.W. Nowak, K. Pierzchała-Koziec, M. Wańkowska and T. Misztal, 2006. Aspects of central and peripheral regulation of reproduction in mammals. Reprod. Biol., 1: 89-103.

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