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Effect of naloxone on post-partum ovaries' follicular size in holstein cows

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ABSTRACT

Pulsatile release of luteinizing hormone (LH) in postpartum cows is relatively rare immediately after parturition and its occurrence increases as time of first ovulation approaches by naloxone. The main objective of this study was to assessment of naloxone on post-partum ovaries' follicular size in Holstein cows. In this study, 60 dairy cows were selected and divided into 3 identical groups by chance. Control group received no treatment, but two others, received 20 and 30 mg naloxone respectively. Results showed that there is significant difference among control and treatments ($P<0.001$) which is not observed between treatments ($P>0.05$). Finally can declare that opioids are close relationship with GnRH secretion and can be used in the animal breeding for obtaining better results in post-partum period.

Keywords: Opioids, Naloxone, Dairy Cow, Follicle, GnRH**.**

INTRODUCTION

Reproductive efficiency in dairy and beef cows is dependent on achieving high submission rates and high conception rates per service. However, to achieve good submission and conception rates, cows must resume ovarian cyclicity, have normal uterine involution, be detected in oestrus and inseminated at an optimum time.

The pattern of resumption of ovarian function in both dairy and beef cows was reviewed 16 years ago by Roche [31]. Resumption of ovarian cyclicity is largely dependent on LH pulse frequency. Both dairy and beef cows have early resumption of follicular growth within 7–10 days post-partum. The fate of the dominant follicle within the first follicular wave is dependent on LH pulsatility.

At the time of parturition, progesterone and oestradiol concentrations cascade to basal concentrations. This allows for the almost immediate resumption of recurrent transient increases in FSH concentrations (within 3–5 days of parturition) that occur at 7- to 10-day intervals [12]. The first of these increases stimulates the first post-partum wave of follicle growth that generally produces a dominant follicle by 7–10 days post-partum [11,27,33]. The fate of this

first follicular wave dominant follicle is dependent on its ability to secrete sufficient oestradiol to induce a gonadotrophin surge. The capacity for oestradiol secretion is in turn dependent on the prevailing LH pulse frequency during the dominance phase of the follicle wave, the size of the dominant follicle and IGF-I bioavailability [2,9]. So the major driver for ovulation of a dominant follicle during the post-partum period is the LH pulse frequency. This has been tested and validated by the LH pulsatile infusion studies of Duffy in early post-partum anoestrous beef cows. The LH pulse frequency required to stimulate a dominant follicle towards ovulation is one LH pulse per hour [15].

Naloxone is an opioid inverse agonist [37] drug developed by Sankyo in the 1960s [4]. Naloxone is a drug used to counter the effects of opiate overdose, for example heroin or morphine overdoses. Naloxone is specifically used to counteract life-threatening depression of the central nervous system and respiratory system. Naloxone is also experimentally used in the treatment for congenital insensitivity to pain with anhidrosis (CIPA), an extremely rare disorder (1 in 125 million) that renders one unable to feel pain. It is marketed under various trademarks including Narcan, Nalone, and Narcanti, and has sometimes been mistakenly called "naltrexate." It is not to be confused with naltrexone, an opioid receptor antagonist with qualitatively different effects, used for dependence treatment rather than emergency overdose treatment [35]. Naloxone has an extremely high affinity for μ-opioid receptors in the central nervous system. Naloxone is a μ-opioid receptor competitive antagonist, and its rapid blockade of those receptors often produces rapid onset of withdrawal symptoms. Naloxone also has an antagonist action, though with a lower affinity, at κ- and δ-opioid receptors. Naloxone is synthesized from thebaine. The chemical structure of naloxone resembles that of oxymorphone, the only difference being the substitution of the N-methyl group with an allyl (prop-2-enyl) group. The name naloxone has been derived from N-allyl and oxymorphone. Also, naloxone has other pharmacologic effects in high doses, those, can be mention to dopaminergic mechanisms and GABA [8]. The main objective of this study was to assessment of naloxone on post-partum ovaries' follicular size in Holstein cows.

MATERIALS AND METHODS

In present study which carried out during February 2012 in the station No. 2 of Moghan dairy cow breeding industry, 60 healthy dairy cows which 15-20 days had elapsed from their parturition, were selected. Then, were divided into the 3 groups include treatments (2 groups) and control (1 groups), 20 in each. All animals were in same management and breeding condition and milk production rate was 25-30 kg per day in average. On day's 15-20 post-partum, naloxone hydrochloride at the dose of 20 mg and 30 mg was administrated intramuscularly to treatment groups 1 and 2, respectively. Normal control groups were received saline normal for 10 ml intramuscularly. All cow's ovaries were tested ultrasonographically one day prior study and during the study by trans-rectal, 8 MHz linear array rectal transducer. Follicular sizes were recorded as one of the ovarian activity indexes.

At the end of the study, data were analysed by SPSS software and compared each other by Mann-Whitney and Kruskal-Wallis statistical ways.

RESULTS

Frequency distribution of follicular size in normal controls:
As given in table 1, of 20 cases, 4 cases (20%) showed no follicle enlargement, 5 cases (25%) showed 1 enlarged follicle, 10 cases (50%) showed 2 and finally, 1 case showed 3 enlarged follicle.

Table 1: Frequency distribution of follicular size in normal controls

Enlargement rate	Frequency	%
Without increasing the follicle size	4	20
1	5	25
2	10	50
3	1	5
Total	20	100

Frequency distribution of follicular size in treatment 1 (20 mg):
As shown in table 2, of 20 cases, 1 case (5%) showed 2 enlarged follicles, 6 cases (30%) showed 4, 4 cases (20%) showed 5, 3 cases (15%) showed 6 and 6 cases (30%) showed 7 enlarged follicles.

Table 2: Frequency distribution of follicular size in treatment 1

Enlargement rate	Frequency	%
2	1	5
4	6	30
5	4	20
6	3	15
7	6	30
Total	20	100

Frequency distribution of follicular size in treatment 2 (30 mg):
Based on table 3, of 20 cases, 8 cases (40%) showed 4 enlarged follicles, 9 cases (45%) showed 5, 2 cases (10%) showed 6 and 1 case (5%) showed 7 enlarged follicles.

Table 3: Frequency distribution of follicular size in treatment 2

Enlargement rate	Frequency	%
4	8	40
5	9	45
6	2	10
7	1	5
Total	20	100

Comparison of data obtained from 3 groups:
Based on table 4 and Kruskal-Wallis test results it revealed that median follicular size in control, treatment 1 (20 mg) and treatment 2 (30 mg) was 2, 5 and 5 respectively that based on X^2=40.02 and confidential level 99% and P=0.000, so, there is a significant differences among groups, so that this value in control group was lesser than two others (P<0.001).

Table 4: Comparison of data obtained from 3 groups in median

Group	No	Median	Mod	X^2	p
Control	20	2	3	40.20	0.000
T_1	20	5	5		
T_2	20	5	3		

Comparison of data obtained from 2 groups:
Based on table 5 and Mann-Whitney test results it revealed that median follicular size in control and treatment 1 (20 mg) was 2 and 5 respectively that based on U=6 and confidential level 99% and P=0.000, so, there is a significant differences among groups, so that this value in treatment group was more than other (P<0.001).

Table 5: Comparison of data obtained from 2 groups in median

Group	No	Median	Mod	U	p
Control	20	2	3	6	0.000
T_1	20	5	5		

Comparison of data obtained from 2 groups:
Based on table 6 and Mann-Whitney test results it revealed that median follicular size in control and treatment 2 (30 mg) was 2 and 5 respectively that based on U=0.000 and confidential level 99% and P=0.000, so, there is a significant differences among groups, so that this value in treatment group was more than other (P<0.001).

Table 6: Comparison of data obtained from 2 groups in median

Group	No	Median	Mod	U	p
Control	20	2	3	0.000	0.000
T_2	20	5	3		

Comparison of data obtained from 2 groups:
Based on table 7 and Mann-Whitney test results it revealed that median follicular size in treatment 1 and treatment 2 was 5 and 5 respectively that based on U=155 and confidential level 95% and P=0.203, so, there is no significant differences among groups.

Table 7: Comparison of data obtained from 2 groups in median

Group	No	Median	Mod	U	p
T_1	20	5	5	155	0.203
T_2	20	5	3		

DISCUSSION AND CONCLUSION

The limited information available on ovarian antral follicular growth in cattle during postpartum anestrus suggests that follicular growth increases markedly after the first week postpartum [21,32,39], and that large antral follicles (> 10 mm diameter) may be present within 5 wk prior to the first postpartum estrus [40]. Thus, large antral follicles are present during postpartum anestrus, but they do not ovulate soon after they appear. Although challenge doses of gonadotropin-releasing hormone (GnRH) or estradiol can induce normal gonadotropin surges by this time [21,34], perhaps physiologic increases in estradiol (10 to 15 pg/ml blood) produced by large follicles may be incapable of stimulating preovulatory gonadotropin surges.

Alternatively, these large follicles may be unable to produce sufficient estradiol for appearance of normal estrual rise in estradiol. Results of these studies are based on observations of only the large follicles (>5 ram) present in ovaries; thus, quantitative dynamics of ovarian follicular growth in cattle during postpartum anestrus remain unknown. Recently, Dufour and Roy (1985) found that after microscopic evaluation of ovaries collected from dairy cows, the percentage of the total follicular population per ovary that was nonatretic, small antral follicles (0.16 to 0.28 ram) decreased significantly from d 15 to 25 postpartum, with no change in percentage of atretic follicles in this size category; whereas the percentage of slightly larger, nonatretic follicles (0.29 to 1.57 mm) increased during the same interval [16]. Although not significant, there was also a trend for nonatretic antral follicles >1.57 mm to increase 32% from d 15 to 25 postpartum [16]. These results suggest that there is growth of small antral follicles into larger follicles during this postpartum period in dairy cows. Additional preliminary data indicate that large follicles (1>8 ram) are present on the ovarian surface as early as d 7 postpartum, and that numbers of follicles 4 to 7.9 mm diameter increase with time after parturition in acyclic, suckled beef cows [38]. Rate of turnover of large antral follicles during postpartum anestrus is unknown.

One of the most significant physiological functions of neural opioid peptides is their association with feeding behavior [3,13]. Activation of opioid pathways stimulates feeding, and blocking opioid receptors with the antagonist, naloxone, inhibits feed intake. A direct relationship with negative energy balance was suggested when Dyer et al. [17] reported impairment of LH secretion in fasting ovariectomized rats and that this could be prevented with naloxone pretreatment. The effect of fasting was a reduction in LH pulse amplitude. In the same study, fasting also impaired LH release during neural excitation of the ventral noradrenergic tract in the brainstem. Here again naloxone prevented the inhibitory effects of fasting. These important results and others describing the opioid mediation of LH secretion [5,14,22,25] support and further describe the neural pathways for opioid inhibition of GnRH release. As reviewed by Bicknell [6], excitatory noradrenergic neurons originating in the brainstem synapse on GnRH producing neurons located in the anterior hypothalamus and preoptic area. Presynaptic opioid inhibition of these noradrenergic neurons arises both from outside the hypothalamus (enkephalin pathway) or from within the basal hypothalamus near the arcuate nucleus (3-endorphin pathway). There is additional evidence suggesting that the /3-endorphin pathway may also directly impinge on the GnRH neurons as they terminate near the pituitary portal vessels in the median eminence. Although the evidence suggesting a neural site of action for inhibitory effects of opioids on GnRH mediated LH secretion is welldocumented, actions within the anterior pituitary gland must also be considered. Opiate peptides, acting through specific opiate-binding sites, exert direct inhibitory effects on anterior pituitary LH release in vitro [7]. The most prevalent endogenous opioid, 3-endorphin, is released from the hypothalamus into the pituitary portal system [23] and could thereby reach pituitary sites of LH production. Perhaps, just as importantly, j3-endorphin is produced within anterior pituitary gland corticotrophs from pro-opiocortin, the same precursor as for adrenocorticotropin [18].

Endogenous opioid peptides (EOP) have been strongly implicated as endogenous inhibitors of LH and stimulators of prolactin (PRL) release [20,29]. Significant increases in serum concentrations of LH following administration of the EOP receptor antagonist, naloxone (NAL), strongly implicate one or more EOP as inhibitors of LH release, but a failure of NAL to affect serum LH cannot be taken as definitive evidence against EOP involvement because some EOP are not readily antagonized by NAL [19].

Several lines of evidence point to an EOP involvement in suckling-induced suppression of LH and stimulation of PRL. Suckling stimuli appeared to increase EOP modulation of the release of several pituitary hormones [30,36]. Nursed rats had lower concentrations of B-endorphin in hypothalamic tissue than weaned controls [28]. Abrupt weaning of postpartum beef cows produced transient changes in hypothalamic concentrations of dynorphin-A, methionine-enkephalin and GnRH [26].

In one study by Ahmadzadeh et al., 1998 it revealed that Naloxone caused a transient increase ($P < 0.05$) in serum LH concentrations in both primi- and multiparous cows within 45 min after administration. In contrast, serum LH concentrations remained unchanged in saline-treated cows. GnRH increased ($P < 0.05$) LH and there was no effect of treatment. These results suggest that modulation of LH secretion, at least in part, may be mediated via endogenous opioids in dairy cows before first postpartum ovulation [1].

Leshin et al., 1991 stated that injections of naloxone alone did not affect LHRH secretion. Although there was no significant dose (10(-9) to 10(-7) M) effect, NAL increased (P less than .05) LHRH efflux from the median eminence (ME) and preoptic area (POA) when administered at 110 min from the initiation of perfusion and again at 200 min for ME but not for POA. All tissues responded to KCl (30 mM) administered at 290 min of perfusion with increased (P less than 0.001) LHRH efflux. They suggest that endogenous opioids suppress LHRH secretion by actions on specific opioid receptors located within the POA and ME of the brain [24].

Chao et al., 1986 demonstrated that the stimulatory effect of naloxone on the basal release of LH suggests that endogenous opioid peptides (EOP) may directly regulate pituitary cell function; the inhibitory effect of physiological concentrations of Met-enk on the basal in vitro release of LH suggests that EOP may directly affect the release of LH in vivo; the antagonism between the stimulatory effect of naloxone and the inhibitory effect of Met-enk is consistent with effects exerted through opioid receptors; and the stimulatory effect of GnRH may be partially reduced by Met-enk. These results are consistent with the hypothesis that opioids may directly modulate the release of LH at the pituitary level [10].

Finally can declare that opioids are close relationship with GnRH secretion and can be used in the animal breeding for obtaining better results in post-partum period.

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