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*Original article*

# Effect of repeated low doses of GnRH on follicular development and ovulation in anovulatory dairy cows with follicle growth to emergence size

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## Abstract

The aim of the study was to evaluate the effect of repeated low doses of GnRH agonist buserelin once a day for 5 days on follicle development and ovulation in anovulatory dairy cows with follicles growth only to emergence. The study was conducted on 71 anovulatory Polish Holstein Friesian cows. Anovulation with growth of follicles to emergence was defined as small ovaries with follicles of  $\leq 5$  mm in diameter and without corpus luteum on two examinations in a 7-10 day interval between 50-60 days after parturition. Cows were allocated to one of two groups. Cows from group 1 ( $n = 58$ ) received 0.4  $\mu\text{g}$  of buserelin (Receptal, MSD, Poland) i.m. once a day for 5 days. Control cows from group 2 ( $n = 13$ ) received saline. Ovarian structures were monitored weekly after the end of treatment by ultrasound for 4 weeks. The diameter of ovarian follicles on the ovaries was measured and recorded. Occurrence of ovulation was determined by the presence of corpus luteum. Overall, ovulation occurred in 46.6% (27/58) of cows treated with repeated doses of GnRH, while no corpus luteum was observed in the control group during the study period. There were significantly ( $p < 0.05$ ) more follicles 6-9 mm in diameter and 10-20 mm in diameter in cows treated with GnRH than in control cows. In conclusion, repeated low doses of GnRH analogue buserelin once a day for 5 days stimulate the development of ovarian follicles in anovulatory dairy cows with small ovarian follicles and led to ovulation in 46.6% of cows during 4 weeks after the end of the treatment.

**Key words:** cows, anovulation, repeated doses of GnRH, ovulation

## Introduction

An early resumption of normal ovarian cyclicity after calving is important for later fertility. Cows that have displayed estrus once or more before the start of breeding have higher fertility compared with cows inseminated at their first estrus (Thatcher and Wilcox

1973, Stevenson and Call 1983, Santos et al. 2009, Galvão et al. 2010). Higher fertility in cows that ovulated earlier can be explained by more estrous cycles, which provide progesterone priming and uterine cleansing during estrus (Thatcher and Wilcox 1973, Darwash et al. 1997, Gümen et al. 2003).

Follicular growth generally resumes within 7 to 10

days in the majority of cows. About 30% of the cows ovulate within 21 days postpartum (pp) and about 30% - 40% of the cows ovulate within 30-50 days pp. The prevalence of anovulation by day 60 after calving is about 20%-40% (Butler 2003, Santos et al. 2009, Galvão et al. 2010). Walsh et al. (2007) reported herd-specific range for anovulation from 5% to 45%. An extended interval from calving to first ovulation is associated with reduced conception and pregnancy rates and increased intervals from calving to conception (Stevenson and Call 1983, Shrestha et al. 2004, Galvão et al. 2010, Walsh et al. 2011). Risk factors for delayed resumption of cyclicity include BCS loss associated with severe negative energy balance, parity, periparturient disorders, season of calving, management, mastitis, lameness and heat stress (Walsh et al. 2007, Garnsworthy et al. 2008, Dubuc et al. 2012, Crowe et al. 2014).

Previously, anovulatory cows were clinically classified as having inactive ovaries or ovarian cysts. Ovarian inactivity with poor follicular growth and low progesterone level until day 50-60 pp was also referred to as true anoestrus or ovarian afuction. Ovarian cysts were defined as fluid-filled ovarian structures of at least 2,5 cm in diameter that persist at last 10 days in the absence of corpus luteum (Opsomer et al. 1996, Mwaanga and Janowski 2000, Peter et al. 2004). More recently, ovarian cysts has been defined as anovulatory follicles (> 2 cm) that are present on the ovary in the absence of corpus luteum and that clearly interfere with normal ovarian cyclicity (Vanholder et al. 2006). Wiltbank et al. (2002) based on evaluation of follicular growth patterns by ultrasound defined three categories of anovulation: 1) with growth of follicles only to emergence (< 4 mm), 2) with follicle growth to deviation size (< 9 mm), and 3) with follicle growth to ovulatory (10 to 20 mm) or larger size.

Treatment options for anovulatory cows include hormonal, nutritional and management strategies. Hormonal treatments aim to stimulate maturation of ovarian follicles and induce ovulation. They may include the use of progesterone-releasing intravaginal devices (PRID – progesterone-releasing intravaginal device and CIDR – controlled internal drug releasing device), gonadotrophin releasing hormone (GnRH), gonadotrophins (human chorionic gonadotrophin – hCG or equine chorionic gonadotropin – eCG) and protocols to synchronize ovulation for timed breeding (Ovsynch and others) (Gümen et al. 2003, Rhodes et al. 2003, Mwaanga et al. 2004, Bryan et al. 2013). Progesterone devices may be used alone, followed by hCG or eCG or in combination with Ovsynch (CIDR or PRID-Ovsynch) (Stevenson et al. 2006, Chebel et al. 2010, McDougall 2010, Colazo et al. 2013).

Single injection of GnRH or its analogues can successfully induce ovulation in anovulatory cows provided a functional ovarian follicle is present. However, this method has been proven ineffective in treating anovulation with growth of follicles only to emergence (ovarian afuction) (Dailey et al. 1983, Zdunczyk et al. 1992, Mwaanga et al. 2004, Hussein and Yaurb 2021). Follicular growth is stimulated by pulsatile secretion of GnRH and gonadotrophins (Crowe et al. 2014). Several attempts have been made, injecting GnRH with intermittent (pulsatile) regimes at 1- to 4-h intervals to mimic endogenous patterns of gonadotrophin secretion, but the results were inconsistent (Walters et al. 1982, Edwards et al. 1983, Vorstermans and Walton 1985, Spicer et al. 1986, Jagger et al. 1987, Bishop and Wettemann 1993). The requirement of intermittent injections of GnRH makes this technique very impractical. Hussein et al. (1992) proposed repeated injections of GnRH twice weekly for 6 weeks for the treatment of anoestrus with low progesterone levels in dairy cow.

The aim of the study was to evaluate the effect of repeated low doses of GnRH agonist buserelin once a day for 5 days on follicle development and ovulation in anovulatory dairy cows with follicles growth to emergence.

## Materials and Methods

The study was conducted on 71 anovulatory Polish Holstein Friesian cows from three dairy herds under a herd health program located in North-East Poland. Herd size ranged from 60 to 100 milking cows. All herds were housed in a loose housing barn and fed total mixed ration based on grass silage, maize silage and concentrate. The average milk yield was about 8000 l per year. Cows were examined by ultrasound using a Honda 1500 scanner with a 5 MHz linear transducer twice in a 7-10 day interval between 50-60 days after parturition. Anovulation with growth of follicles to emergence was defined as small ovaries with follicles of  $\leq 5$  mm in diameter and without corpus luteum on both examinations. Cows included into the study were 4 to 6 year old. Cows were allocated to one of two group. Cows in group 1 ( $n = 58$ ) received 0.4  $\mu\text{g}$  of buserelin (Receptal, MSD, Poland) i.m. once a day for 5 days. Control cows from group 2 ( $n = 13$ ) received saline. Ovarian structures were monitored weekly for 4 weeks after the end of treatment by ultrasound. The diameter (average of 2 dimensions) of ovarian follicles on the ovaries were measured and recorded. The follicles were divided into small ( $\leq 5$  mm in diameter), medium (6-9 mm in diameter), large (10-20 mm in diameter) and cystic ( $> 20$  mm in diame-

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Table 1. Occurrence of ovulation (presence of corpus luteum) in anovulatory dairy cows after treatment with repeated doses of GnRH (group 1) and in control cows (group 2).

Group	Week after the treatment				Overall
	1	2	3	4	
	% (n/n)	% (n/n)	% (n/n)	% (n/n)	
Group 1	10.3 (6/58)	17.3 (9/52)	16.3 (7/43)	13.8 (5/36)	46.6 (27/58)
Group 2	0	0	0	0	0

Table 2. Distribution of follicles in in anovulatory dairy cows after treatment with repeated doses of GnRH (group 1) and in control cows (group 2).

Follicles	Week after treatment							
	1		2		3		4	
	group 1	group 2	group 1	group 2	group 1	group 2	group 1	group 2
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
≤ 5 mm	234 (74.3)	88 (86.3)	259 (73.1) <sup>a</sup>	118 (86.1) <sup>b</sup>	180 (65.2)	61 (78.2)	203 (70.2) <sup>a</sup>	81 (79.4) <sup>b</sup>
6-9 mm	50 (15.9)	9 (8.8)	51 (14.4)	14 (10.2)	53 (19.2)	9 (11.3)	38 (13.1)	13 (12.7)
10-20 mm	30 (9.5)	5 (4.9)	42 (11.9) <sup>a</sup>	5 (3.7) <sup>b</sup>	40 (14.5)	8 (10.3)	46 (16.0) <sup>a</sup>	8 (7.8) <sup>b</sup>
> 20 mm	1 (0.3)	0	2 (0.6)	0	3 (1.1)	0	2 (0.7)	0

a, b – difference between groups in a respective week statistically significant (p<0.05)

ter). Occurrence of ovulation was determined by the presence of corpus luteum. Cows that ovulated were excluded from the analysis of follicle development.

**Statistical analysis**

The distribution of follicles was presented as the percentage of follicles of different sizes in the total number of follicles at each examinations. The data were analysed by the Mann-Whitney test using GraphPad Prism version 6.00 (GraphPad Software, San Diego, CA, USA). The level of significance was considered as p<0.05.

**Results**

In cows treated with GnRH ovulation was detected by the presence of corpus luteum in 10.3% (6/58) of cows in the first week after treatment, in 17.3% (9/52) of cows in the second week, in 16.3% (7/43) of cows in the third week and in 13.8% (5/36) of cows in the fourth week. Overall, ovulation occurred in 46.6% (27/58) of cows treated with repeated doses of GnRH (Table 1). No corpus luteum was observed in the control group during the study period.

Before treatment, the diameter of the ovarian follicles in all cows was small (≤ 5 mm). In the first week after the end of the treatment the distribution of ovarian follicles in cows that not ovulated was similar in both

group (p>0.05). In both groups most follicles were small (≤ 5 mm in diameter) (74.3% vs. 86.3% of all follicles), followed by medium (6 to 9 mm) (15.9% vs. 8.8%) and large (10-20 mm) (9.5% vs. 4.9%). In group 1 one (0.3%) ovarian follicle > 20 mm in diameter was found. In the second week after the end of the treatment the percentage of follicles of 10-20 mm in diameter was statistically significantly higher (p<0.05) in the group 1 compared to the control group (11.9% vs. 3.7%). There were 2 cases (0.6%) of cystic follicles in the group 1. In the third week after the end of the treatment the cows treated with GnRH showed statistically significantly (p<0.05) less follicles with diameter ≤ 5 mm (65.2% vs. 78.2%) than control cows. In the fourth week after treatment there were statistically significantly (p<0.05) more follicles with a diameter of 10-20 mm in the cows treated with GnRH compared to control cows (16.0% vs. 7.8%) (Table 2).

**Discussion**

Anovulation with follicle growth to emergence is common during the early postpartum period in lactating dairy cows and suckled beef cows (Williams 1990, Beam and Butler 1997, Wiltbank et al. 2002). It can also be an important clinical form of anestrus in dairy cows over 60 days postpartum (Opsomer et al. 1996, Zduńczyk et al. 2002, Mwaanga et al. 2004).

Hormonal treatments may help to stimulate anovulatory cows to cycle (Wiltbank et al. 2002, Rhodes et al. 2003, Peter et al. 2009). GnRH treatment was used in numerous studies early postpartum to improve the fertility performance of dairy cows by induction of ovulation. However, the effects of single injection of GnRH on ovulation early postpartum were very variable (Becket and Lean 1997, Bittar et al. 2014). The effect of GnRH on ovulation depends on the stage of follicle development at treatment. When given at dominance of a follicular wave GnRH treatment resulted in ovulation. However, there was no effect of GnRH on follicular dynamics when given at emergence of a follicular wave (Ryan et al. 1998).

The use of GnRH to treat inactive ovaries has also been studied, but single injections of GnRH failed to stimulate follicle growth and induce ovulation (Dailey et al. 1983, Zduńczyk et al. 1992, Mwaanga et al. 2004, Hussein and Yaurb 2021). The major cause of anovulation in postpartum cows is low GnRH and gonadotrophins pulse frequency (Wiltbank et al. 2002, Crowe et al. 2014). Thus, attempts have been made to induce follicular development and ovulation in acyclic cows by pulsatile administration of GnRH. Intermittent administration of GnRH at 1- to 4-h intervals mimicked the pulsatile secretion of endogenous GnRH and induced pulses of FSH and LH (Walters et al. 1982). Infusion of GnRH (2 µg) with infusion pump at frequency of one pulse every hour induced luteal activity in nutritionally anestrus cows (Bishop and Wettemann 1993, Vizcarra et al. 1997). The intermittent injection of a small dose of GnRH (5 µg) every 4 hours advanced significantly the time of first ovulation (Vorstermans and Walton 1985). However, this method is not suitable for use in practice. Repeated injections of GnRH may be an alternative.

Our study showed that the repeated injections of GnRH analogue buserelin once a day for 5 days significantly increase the number and size of ovarian follicles and caused ovulation in about half of the anovulatory cows during 4 weeks after treatment. In 3 cows cystic follicles > 20 mm in diameter were found. The occurrence of ovarian cysts has not been noted in earlier studies on repeated or intermittent administration of GnRH in cows. However, Etherington et al. (1985) reported that treating normal cows with GnRH in the early postpartum period may increase the risk of ovarian cysts. Control cows remained anovulatory until the end of the study, although in some cows follicle development up to the diameter of 10-20 mm was observed. Gümen et al. (2003) found that 22% of cows that did not ovulate by day 60 postpartum had follicles of 9 to 14 mm in diameter.

There are only very few studies on repeated injections

of GnRH to stimulate ovarian function in cows. Vorstermans and Walton (1985) showed that the injection of a small dose of GnRH twice a day from day 5 to day 10 after calving advanced the time of first ovulation in dairy cows by 10 days. Hussein et al. (1992) injected GnRH analogue cystorelin (50 µg) twice weekly for 6 weeks in cows with low progesterone level until 32 to 36 days pp. This treatment resulted in significantly fewer days to first plasma progesterone above 1.0 ng/ml than in the controls (44.3 vs 62.3 days pp, respectively).

Prevention of anovulation is preferable to treatment (Wiltbank et al. 2002, Rhodes et al. 2003, Peter et al. 2009). It may be achieved in part by providing appropriate feeding and housing conditions, early recognition and treatment of postpartum disorders and minimizing stress factors. A combination of management changes and hormonal treatment may result in higher conception rates.

In conclusion, repeated low doses of GnRH analogue buserelin once a day for 5 days stimulate the development of ovarian follicles in dairy cows with small ovarian follicles and lead to ovulation in about half of anovulatory cows during 4 weeks after the end of the treatment. Thus, it seems that this method may be considered for the treatment of anovulation with growth of follicles only to emergence.

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