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Original article

Effect of repeated low doses of GnRH analogue (buserelin) on fertility performance of dairy cows with anovulation type I

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Abstract

The aim of this study was to evaluate the fertility response of dairy cows with anovulation type I on repeated low doses of GnRH agonist buserelin. The study was conducted on 83 anovulatory and 60 cyclic Polish Holstein Friesian cows. Anovulation type I was defined as small ovaries with follicles of ≤ 5 mm in diameter and without corpus luteum on two examinations in a 7-10 day interval between 50–60 days after parturition. Cows from the experimental group (n=58) received 0.4 µg of buserelin i.m. once a day for 5 consecutive days. Cows from the negative control group (n = 25) received saline. Sixty cyclic cows receiving no treatment served as positive controls. Intervals from calving to estrus and from calving to conception, pregnancy rate 30-35 days and 260 days after AI, and pregnancy loss were calculated. The anovulatory cows had a substantially prolonged calving to conception interval, decreased pregnancy rate and increased pregnancy loss and culling rate compared to cyclic herd mates. The average calving to conception interval was significantly ($p<0.05$) shorter in treated cows compared to non-treated anovulatory cows (153.7 days vs 209.3 days). In conclusion, repeated low doses of GnRH analogue buserelin led to a significant shortening of calving to conception interval. More clinical trials are needed to determine the practical usefulness of this method for the treatment of anovulation type I in dairy cows.

Keywords: anovulation type I, cows, fertility, repeated doses of GnRH

Introduction

Postpartum anovulation is considered to be one of the main causes of infertility in dairy cattle (Santos et al. 2009, Walsh et al. 2011). In lactating dairy cows, the interval from calving to first ovulation typically lasts 2 to 4 weeks (Darwash et al. 1997, McCoy et al. 2006, Crowe et al. 2008). The lack of ovarian cyclicity affects 25% of dairy cows within 60 days after calving, but in some herds the incidence of anovulation can reach up to 40% (Walsh et al. 2007, Santos et al. 2009). Recently, anovulation has been classified into three types (I, II and III) on the basis of functional states of follicular development. Type I is characterized by the

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growth of follicles to emergence without further deviation or establishment of a dominant follicle (Wiltbank et al. 2002, Peter et al. 2009). Previously, such cows were clinically classified as having inactive ovaries. Ovarian inactivity with poor follicular growth and low progesterone level until day 50-60 pp was also referred to as true anoestrus, acyclia or ovarian afunction (Opsomer et al. 1996, Mwaanga and Janowski 2000).

The pathophysiology of anovulation type I is not well understood. It is presumed that this condition may result from a suppression of GnRH and gonadotropins pulse frequency (Roche and Diskin 2001, Peter et al. 2009, Crowe et al. 2014). Risk factors for this condition are severe negative energy balance, BCS loss, parity, periparturient disorders, season of calving and stress factors (Walsh et al. 2007, Dubuc et al. 2012, Monteiro et al. 2021). Anovulation type I can be diagnosed by ultrasound or determination of progesterone concentration at an interval of 7-10 days (Gümen et al. 2003, Stevenson et al. 2006). Management of anovulatory dairy cows consists, in the first instance, of correcting the negative energy balance (Beam and Butler 1999, Peter et al. 2009, Crowe et al. 2014). Hormonal treatments were used in many studies with variable effectiveness to stimulate ovarian follicular growth and induce ovulation in acyclic cows. Intravaginal progesterone inserts (PRID – progesterone-releasing intravaginal device and CIDR – controlled internal drug releasing device) are recommended for the therapy of non-cyclic postpartum cows (McDougal et al. 2004, Yaniz et al. 2004). The increased circulatory concentration of progesterone supresses GnRH and gonadotropins release and causes their storage. Removal of the progesterone insert produces a surge of GnRH, followed by FSH and LH release with subsequent resumption of ovarian cyclicity (Zerbe et al. 1999). Several studies showed that treatment of anestrous cows using intravaginal progesterone inserts alone or in combination with equine chorionic gondotrophin (eCG) reduced the interval to first estrus and to conception compared with untreated controls (Mwaanga et al. 2004, Bryan et al. 2013, Shephard 2013, de Graff and Grimard 2018), but one study did not show this (Rhodes et al. 2003). Progesterone intravaginal devices may also be included in estrus synchronisation protocols for acyclic cows (Stevenson et al. 2006, Chebel et al. 2010, McDougall 2010, Colazo et al. 2013).

The major cause of delayed resumption of ovarian function in post-partum cows is inadequate secretion of GnRH and gonadotropins. However, a single injection of GnRH or GnRH analogues failed to induce ovarian follicle development and ovulation in acyclic cows (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).

Intermittent (pulsatile) injections of GnRH at 1 to 4 h intervals to mimic endogenous patterns of gonadotropins secretion were reported to be successful in some studies (Edwards et al. 1983, Vorstermans and Walton 1985, Spicer et al. 1986, D'Occhio et al. 1989, Bishop and Wettemann 1993, Vizcarra et al. 1997); however, this technique is very difficult to use in practice.

Hussein et al. (1992) administered repeat injections of GnRH twice weekly for 6 weeks in anestrous dairy cow with low progesterone levels. However, there were no significant differences between treated and control cows in the number of days from calving to first observed estrus or the number of days open.

In a previous study we showed that repeated low doses of GnRH analogue buserelin (0.4 µg i.m.) once a day for 5 consecutive days stimulated the development of ovarian follicles in anovulatory dairy cows with follicle growth to emergence (Barański et al. 2022). The aim of the present study was to evaluate the effect of this treatment regime on fertility performance in dairy cows with anovulation type I.

Materials and Methods

The study was conducted on 83 anovulatory and 60 cyclic Polish Holstein Friesian cows from four dairy herds in North-East Poland. Herd sizes ranged from 60 to 100 milking cows. The cows were housed in loose housing barns and fed total mixed ration based on grass silage, maize silage and concentrate according to their requirements. The average milk yield was 8000 l per year. Cows were examined by ultrasound using Honda 1500 scanner with a 5 MHz linear transducer twice in a 7-10 day interval between 50-60 days after parturition. Anovulation type I was diagnosed if small ovaries with follicles of ≤ 5 mm in diameter and without corpus luteum were found on both examinations. The cows were divided into three groups. Cows from the experimental group (n=58) received 0.4 µg of buserelin (Receptal, MSD, Poland) i.m. once a day for 5 days. Cows from the negative control group (n=25) received saline. Sixty cyclic cows that showed estrus before 60 days postpartum served as positive controls. Cows included into the study were 4 to 6 year old and showed no clinical symptoms of endometritis, lameness and mastitis. After detection of estrus the cows were artificially inseminated (AI). Pregnancy was diagnosed using ultrasonography between days 30 and 35 after insemination. The cows diagnosed pregnant were re-examined on day 260 ± 3 d after AI.

The following reproductive performances were calculated for the treated and control cows: intervals from

Table 1. Reproductive performance and culling rates in anovulatory treated, non-treated and cyclic cows.

Variables	Anovulatory treated (n=58)	Anovulatory non-treated (n=25)	Cyclic (n = 60)
Interval, calving to estrus (mean ± SD)	100.1 ± 24.7[a]	133.5 ± 61.8[b]	50.4 ± 7.2[c]
Interval, calving to conception (mean ± SD)	153.7 ± 67.3[a]	209.25 ± 85.6[b]	109.0± 23.5[c]
Pregnancy rate 30-35 days after AI (%)	63.8 (37)	56.0 (14)[a]	75.0 (45)[b]
Services per conception	2.2	2.7	1.9
Pregnancy rate 260 days after AI (%)	51.7 (30)[a]	40.0 (10)[a]	68.3 (41)[b]
Pregnancy loss (%)	18.9 (7)	28.6 (4)	8.9 (4)
Culling rate (%)	17.2 (10)	28.0 (7)[a]	6.7 (4)[b]

Different superscript letters indicate statistical significance at p<0.05.

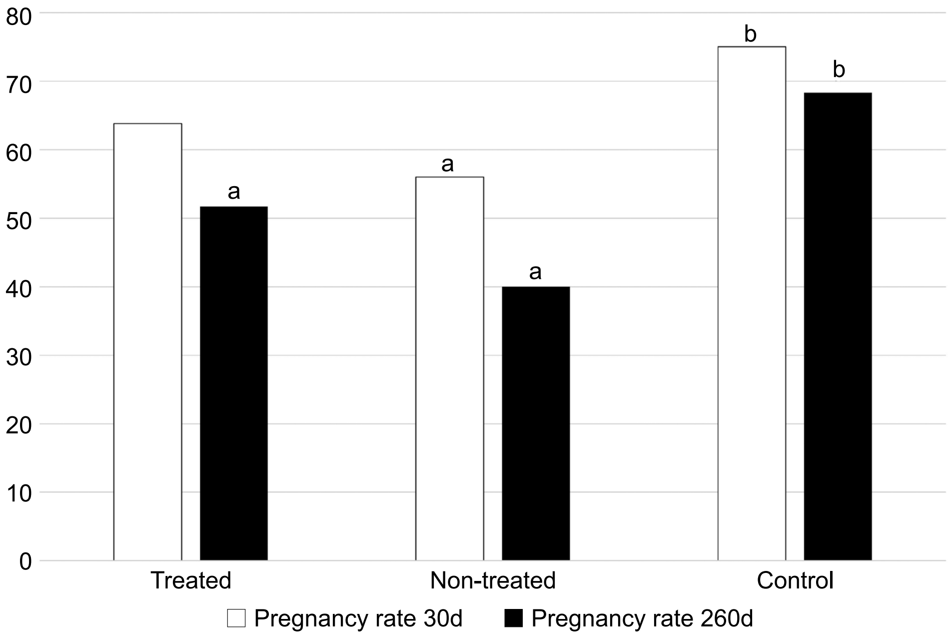

Fig. 1. Pregnancy rates 30-35 days and 260 days after AI (%) in anovulatory treated, non-treated and cyclic cows. Different superscript letters indicate statistical significance at p<0.05.

calving to estrus and from calving to conception, pregnancy rate 30-35 days after AI, pregnancy rate 260 days after AI and pregnancy loss. Pregnancy loss was defined as the percentage of non-pregnant cows 260 days after AI diagnosed 30-35 days after AI as pregnant.

Statistical analysis

The data were analysed using the Mann-Whitney test and GraphPad Prism version 9.00 (GraphPad Software, San Diego, CA, USA). The level of significance was considered as p<0.05.

Results

Fertility performance of anovulatory and cyclic cows is presented in Table 1. The average length of calving to conception interval was significantly (p<0.05) greater in non-treated cows with anovulation type I than in cyclic cows (209.3 days vs 109.0 days). The pregnancy rates 30-35 days after AI and 260 days after AI were significantly (p<0.05) lower in anovulatory non-treated cows compared to cyclic cows, 56.0% vs 75.0% and 44.0% vs 68.3%, respectively (Fig. 1). The average number of services per conception was 2.7 in anovulatory non-treated cows and 1.9 in cyclic cows. There was a tendency towards higher pregnancy loss in anovulatory non-treated cows than in cyclic cows (28.6% vs 18.3%). The culling rate was significantly (p<0.05) higher in non-treated anovulatory cows compared to cyclic cows (28.0% vs 6.7%).

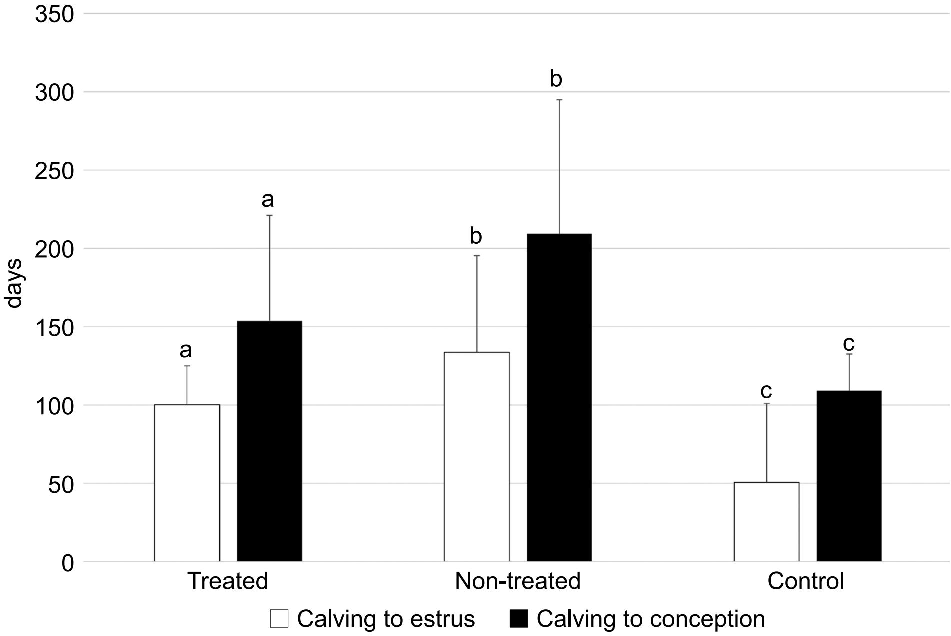

Fig. 2. Intervals calving to estrus and calving to conception (mean ± SD) in anovulatory treated, non-treated and cyclic cows. Different superscript letters indicate statistical significance at p<0.05.

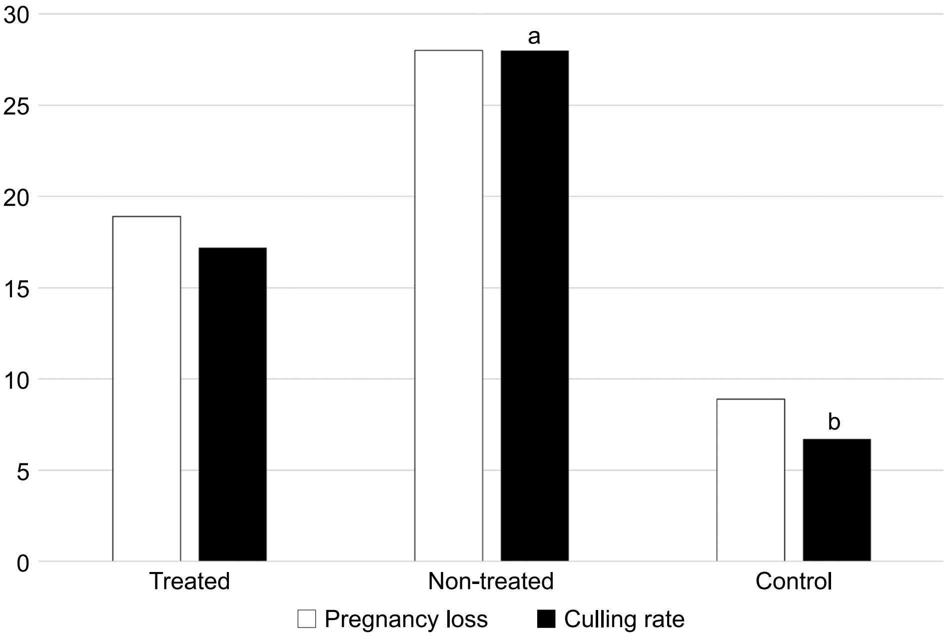

Fig. 3. Pregnancy loss (%) and culling rate (%) in anovulatory treated, non-treated and cyclic cows. Different superscript letters indicate statistical significance at p<0.05.

The average intervals of calving to estrus and calving to conception were significantly (p<0.05) shorter in anovulatory cows treated with repeated low doses of GnRH analogue buserelin compared to non-treated anovulatory cows (100.1 days vs 133.5 days and 153.7 days vs 209.3 days, respectively) (Fig. 2). There was no significant difference (p>0.05) in the pregnancy rates, pregnancy loss and culling rate between treated and untreated anovulatory cows (Fig. 3).

Compared to cyclic cows, the length of calving to conception interval was significantly (p<0.05) greater and the pregnancy rate 260 days after AI significantly (p<0.05) lower in treated anovulatory cows (153.7 days vs.109.0 days and 51.7% vs 68.3%, respectively).

Discussion

Fertility of the cows was negatively influenced by the incidence of anovulation type I. The anovulatory cows had a substantially prolonged calving to conception interval, decreased pregnancy rate and increased pregnancy loss and culling rate compared to cyclic cows. Thus, anovulation type I leads to a considerable economic loss. Previous studies also showed lower fertility performance in anovulatory cows both inseminated in a detected oestrus and after a synchronisation programme (Gümen et al. 2003, Santos et al. 2004, Walsh et al. 2007, Bisinotto et al. 2010). Of interest is the high pregnancy loss in anovulatory cows. In our study it was 16.7% in treated and 21.4% in non-treated cows with anovulation type I. Similarly, in other studies, pregnancy loss in anovulatory cows ranged between 15% and 30% (Gümen et al. 2003, Galvão et al. 2004, Santos et al. 2004, Bisinotto et al. 2010). Low pregnancy rates and high pregnancy loss may be due to the lack of progesterone priming from earlier estrous cycles, resulting in low oocyte quality and inadequate preparation of the uterus for pregnancy (Santos et al. 2016).

The hormonal treatment of anovulation type I is difficult due to the many factors affecting its occurrence. In numerous studies progesterone intravaginal insert devices alone or in combination with eCG were used with good results in induction of estrus and ovulation in anovulatory cows, but the effects on fertility were inconsistent (Rhodes et al. 2003, Mwaanga et al. 2004, McDougall 2010, Bryan et al. 2013).

Single injections of GnRH failed to stimulate follicle growth and induce ovulation in acyclic cows (Dailey et al. 1983, Zduńczyk et al. 1992, Mwaanga et al. 2004, Hussein and Yaurb 2021). In post-partum cows resumption of ovarian cycles is stimulated by an increase in pulse frequency of GnRH and gonadotrophins (Shrestha et al. 2004, Crowe et al. 2014). Some studies showed that frequent administration of small doses of GnRH at 1-4 hour intervals with an infusion pump stimulated ovarian follicle development in acyclic cows (D'Occhio et al. 1989, Bishop and Wettemann 1993, Vizcarra et al. 1997); however, this method is not suitable for use in practice. Repeated injections of GnRH at longer intervals may be an alternative. There is only one study on the effect of repeated injections of GnRH on fertility in acyclic cows. Hussein et al. (1992) injected GnRH analogue cystorelin (50 µg) twice weekly for 6 weeks in cows with alow progesterone level. However, there were no significant differences among treatment groups in the number of days from calving to first observed estrus or the number of days open. In our study the treatment of anovulatory cows with repeated low doses of GnRH analogue buserelin (0.4 µg i.m.) once a day for 5 days led to a significant shortening of the intervals between calving to estrus and calving to conception and an increase in the pregnancy rate 260 days after AI. This was apparently related to a higher frequency of GnRH administration than in the study of Hussein et al. (1992). It seems that repeated low doses of GnRH analogue buserelin (0.4 µg i.m.) once a day for 5 days may be useful for the treatment of anovulation type I in dairy cows.

However, despite treatment, fertility in cows with anovulation type I was lower than in cyclic cows. This underlines the importance of preventive strategies for anovulation type I. The strategy to counteract the negative effects of anovulation on fertility is based on appropriate nutrition in the dry period and early lactation, early recognition and treatment of postpartum diseases and minimizing stress factors (Wiltbank et al. 2002, Rhodes et al. 2003, Peter et al. 2009).

In conclusion, anovulation type I had detrimental effects on reproductive performance in dairy cows. Repeated low doses of GnRH analogue buserelin once a day for 5 days led to a significant shortening of the calving to estrus and calving to conception intervals and numerically, but not significantly, increased the pregnancy rate. More clinical trials are needed to determine the practical usefulness of this method for the treatment of anovulation type I in dairy cows.

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