Animal Reproduction Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Ovulation and fertility response to commercially available GnRH products in lactating cows synchronized with the Double-Ovsynch protocol

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ARTICLE INFO

Keywords:
Ovulation
GnRH
Double-Ovsynch

ABSTRACT

This study was designed to evaluate whether commonly used gonadorelin products that are commercially available in the United States results in comparable ovulation and pregnancy per AI (P/AI) in synchronized lactating dairy cows. A total of 1411 Holstein cows receiving a Double-Ovsynch protocol (DOV) for conducting the first postpartum AI were randomized to receive one of the following GnRH products throughout the Double-Ovsynch: 1) Cystorelin® (CYS, gonadorelin diacetate tetrahydrate, $n = 484$); 2) Factrel® (FAC, gonadorelin hydrochloride, $n = 482$) or; 3) Fertagyl® (FER, gonadorelin diacetate tetrahydrate, $n = 515$). A subgroup of cows ($n = 487$) received ovarian ultrasound exams and collection of blood samples for progesterone (P4) analysis. Proportion of cows ovulating following the 3[rd] GnRH of DOV tended ($P = 0.07$) to differ between GnRH salts (hydrochloride = 61.5% vs. diacetate = 72.7%) but was similar for GnRH products (FER = 74.1% vs. FAC = 61.5% vs. CYS = 72.2%). Interestingly, a logistic regression analyses that considered the circulating P4 at the time of GnRH treatment indicated lower ovulation responses to FAC compared to FER and CYS; although greater circulating P4 decreased ovulation response to all GnRH products. Results for P/AI at 60 d post-insemination differed between GnRH salts ($P = 0.02$) as well as GnRH products (FER = 47.8% vs. FAC = 42.0% vs. CYS = 49.8%; $P = 0.04$). In conclusion, fertility following use of the Double-Ovsynch was less following a hydrochloride-based GnRH product likely due to lesser ovulatory responses throughout the synchronization protocol.

1. Introduction

Reproductive performance is a major factor affecting profitability of dairy farms. The use of GnRH and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in a coordinated fashion allows cows to be inseminated as a result of the use of a timed artificial insemination (TAI) protocol without the need for detection of estrus (Pursley et al., 1995, 1997). The use of Ovsynch protocol results in an increase in the percentage of cows inseminated earlier after the end of the voluntary waiting period, thus increasing the proportion of cows that become pregnant earlier in lactation (Pursley et al., 1997). The Double-Ovsynch (Souza et al., 2008) hormonal treatment regimen is a later

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https://doi.org/10.1016/j.anireprosci.2019.01.006
Received 13 September 2018; Received in revised form 22 December 2018; Accepted 24 January 2019

development of the original Ovsynch program, and is widely used in high producing dairy herds in USA.

In the United States, several GnRH products are commercially available and approved by the Food and Drug Administration to be used as a part of breeding protocols for dairy and beef herds. These products may have different gonadotropin salts and diluent composition that may affect whether there is an ovulation as a result of treatment with the compound (Martínez et al., 2003; Souza et al., 2009). As a result, it is possible that there are lesser ovulatory responses as a result of GnRH administration for induction of ovulation with hormonal treatment regimens which ultimately results in a lesser cow fertility. In a previous study (Martínez et al., 2003), both the surge release of LH and ovulatory responses were less for cows treated with gonadorelin hydrochloride compared with cows treated with gonadorelin diacetate-based products; however, the effect of type of GnRH on P/AI was not assessed in the previous study.

Results of another study indicated there were no differences in P/AI between cows where gonadorelin hydrochloride and gonadorelin diacetate were used in the hormonal treatment regimen for conducting the first postpartum AI (Poock et al., 2015). Poock et al. (2015) used a hormonal treatment regimen in cows for conducting the first postpartum AI that included two $PGF_{2\alpha}$ treatments 14 days apart or enrolled cows to a resynch protocol. Both factors (poor wave pre-synchronization with the two PGF treatments during presynch as well as random stage of the estrous cycle at start of resynch) will set cows to start Ovsynch at variable stages of follicle growth, which will lower the ovulatory responses to the first GnRH of Ovsynch (Souza et al., 2008; Ayres et al., 2013). In addition, when there was use of a single $PGF_{2\alpha}$ treatment, there was an increase in P/AI as a result of the relatively greater ovulatory response to the 1st GnRH injection during the Ovsynch protocol; however, the advantages from the greater ovulation rate may be negated when complete luteal regression is not accomplished (Poock et al., 2015).

The objective of the present study, therefore, was to compare the ovulatory responses and P/AI after administration of three commercially available GnRH products for conducting the first postpartum AI in cows estrous synchronized using a modified Double-Ovsynch protocol with two PGF2α treatments towards the end of the program to assure complete luteal regression.

Based on previous results, the hypothesis for the present study was that ovulatory response and P/AI following use of a Double-Ovsynch is less for cows treated with gonadorelin hydrochloride (Factrel®) than cows treated with gonadorelin diacetate tetrahydrate-based products (Cystorelin® or Fertagyl®).

2. Materials and methods

2.1. Animal handling, housing, and feeding

Cows were housed in free stall facilities on two commercial dairy herds (*n* total = 1411; herd A = 614; herd B = 797) located in Wisconsin, USA from February 2016 through April 2017. Lactating Holstein cows (primiparous = 591 and multiparous = 910) were milked three times daily and fed twice daily a total mixed ration (TMR) that consisted of corn and alfalfa silage as forage with a corn and soybean meal-based concentrate. The TMR was balanced to meet or exceed minimum nutritional requirements for dairy cattle (Council, 2001) and cows were supplied water *ad libitum*. All procedures, including injections, blood sampling, artificial insemination, and ultrasonography, were conducted while cows were locked up at the feedline in Herd B. The free stall facilities from in herd A had no headlocks and cows, therefore, were utilized for conducting a fertility trial only. Experimental treatments and cow management of both herds was conducted in accordance to the Canadian Council on Animal Care (CCAC) guidelines for farm animals (http://www.ccac.ca/en_/standards/guidelines).

2.2. Randomization and hormonal protocol

Weekly cohorts of postpartum cows at 48 ± 3 days in milk (DIM) and producing 42.1 ± 3.6 kg/day were assigned to receive the Double-Ovsynch protocol for conducing the first postpartum AI as previously described (Souza et al., 2008), with the addition of administration of a second PGF2α treatment 24 h after the last PGF2α during the Double-Ovsynch protocol to improve luteolysis (Brusveen et al., 2009). Thus, all cows received the modified Double-Ovsynch protocol, as follows: D0: GnRH; D7: PGF2α; D10: GnRH; D17: GnRH; D24: PGF2α; D25: PGF2α; D26-pm: GnRH; D27: AI 16 h after the last GnRH administration. All hormonal treatments for timed AI were administered intramuscularly. The PGF2α used was Estrumate® (500 mcg of cloprostenol sodium, Merck & Co., Inc., Kenilworth, NJ, USA). Within each herd, weekly cohorts of cows were assigned to receive one of the three types of GnRH compounds throughout the entire hormonal program, as follows: 1) Cystorelin® (CYS, *n* = 484, 100 mcg of gonadorelin diacetate tetrahydrate, Merial Inc., Duluth, GA, USA); 2) Factrel® (FAC, *n* = 482, 100 mcg of gonadorelin hydrochloride, Zoetis LLC., Parsippany NJ, USA); 3) Fertagyl® (FER, *n* = 515, 100 mcg of gonadorelin diacetate tetrahydrate, Merck & Co., Inc., Kenilworth, NJ, USA). All GnRH products where kept refrigerated until treatment. Breeding records from both herds were collected and stored utilizing the Bovisync® (www.bovisync.com) herd management software.

2.3. Ovarian ultrasonography, ovulatory responses, and pregnancy diagnosis

Ultrasonographic evaluations of the ovaries were performed using an 8.0-MHz linear transducer (Ibex® Pro - portable ultrasound, E.I. Medical Imaging, Loveland, CO, USA) in a subset of 487 cows from Herd B (CYS, *n* = 163; FAC, *n* = 163; FER, *n* = 161) on D17 and D24 of the Double-Ovsynch hormonal treatment regimen to evaluate ovulation response as a result of administration of the three differing GnRH products. Data from ultrasound exams were used to determine presence of a mature CL on D17 and ovulation was assumed to have occurred for cows with the appearance of a new CL structure on D24. Pregnancy was diagnosed by a trans-rectal ultrasound examination 32 days after timed AI and reconfirmed 60 days after AI. Pregnancy loss was assumed for cows diagnosed pregnant on the first ultrasound exam on day 32, but were detected to be non-pregnant on day 60 post AI.

2.4. Blood sampling and hormonal assay

To quantify circulating progesterone (P4) concentrations at the time of GnRH administration on D17, blood samples were collected by coccygeal venipuncture immediately prior to the administration of GnRH on D17 in a subset of cows in Herd B (n = 487). Refrigerated samples were centrifuged (3000 x g for 20 min) within 1 h after collection and stored at – 20 °C until assayed for P4 concentrations. Circulating P4 was evaluated from unextracted sera using an antibody-coated-tube RIA kit (Diagnostic Products Corporation, Los Angeles, CA). The average intra-assay coefficient of variation was 8.0%.

2.5. Statistical analyses

Binomially-distributed data (ovulation to GnRH on D17 and P/AI) were analyzed using logistic regression with the GLIMMIX procedure of SAS (version 9.4). Explanatory variables considered for inclusion in the models were treatment, farm, month of AI, parity (primiparous compared with multiparous), circulating P4 concentrations at time of GnRH administration, ovulatory response as a result of administration of GnRH on D17, and meaningful two-way interactions. For the final logistic regression model, there was removal of variables by a backward elimination based on the Wald statistics criterion when $P > 0.20$. The variables that were included in the final model for analysis of fertility were: treatment, parity, and interaction between treatment and parity. The final model for estimating ovulation response also included circulating P4 concentrations at the time of GnRH administration and its interaction with GnRH treatment. In addition, the variable “cow” was included in all models as a random variable. The Logistic procedure of SAS (9.4) was utilized to produce continuous logistic regression curves for predicted ovulatory responses as a result of administration of the GnRH products when there were differing concentrations of circulating P4 on D17 of the Double-Ovsynch hormonal treatment regimen. Tendency was assumed for P-values between 0.05 and 0.10, and significance when the P-value was less than 0.05. All values are presented as LSmeans unless stated otherwise.

3. Results

Ovulatory response on D17 after treatment with the Double-Ovsynch hormonal regimen tended to differ as a result of type of GnRH salt compound administered. With use of the regression model, there was detection of a trend ($P = 0.07$) for a lesser ovulation rate when there was administration of hydrochloride-based GnRH compared with the Diacetate salt. Ovulation response as a result of administration of GnRH salts on D17 did not differ with administration of the three commercial GnRH products (Fig.1).

Circulating concentrations of P4 at D17 of Double-Ovsynch did not differ as a result of treatment with the different GnRH salts and averaged 3.7 ± 0.3, 3.5 ± 0.3, and 3.3 ± 0.3 in the CYS, FAC, and FER treatment groups, respectively. Similarly, proportion of cows with a CL on D17 of Double-Ovsynch was similar among the treatment groups (CYS = 98.4%; FAC = 95.3%; FER = 95.0%). As a result of a more precise analysis for predicted ovulatory responses as indicated by differing circulating P4 concentrations on D17, there were indications of lesser ovulation rates for cows treated with gonadorelin hydrochloride than cows treated with gonadorelin diacetate, as well as for cows treated with FAC compared with FER and CYS (Fig. 2). In addition, when there were greater P4 concentrations at the time of GnRH administration on D17, ovulatory response to the GnRH salt treatments was less ($P < 0.01$) for all treatment groups (Fig. 2). There was also no interaction in terms of ovulation response between P4 concentration on D17 and type of salt or GnRH product administered (Fig. 2).

Pregnancy per AI results at 32 days post TAI tended to differ among GnRH products and was greater for FER and CYS than FAC according to the Tukey-grouping analysis statistical approach (Table 1). In addition, P/AI was greater for cows treated with gonadorelin diacetate products than for cows treated with gonadorelin hydrochloride (Table 1). Similarly, at 60 d after TAI, P/AI differed among groups and was greater for cows treated with FER and CYS than FAC, and was also greater for cows treated with gonadorelin diacetate than cows treated with gonadorelin hydrochloride (Table 1). Pregnancy loss between 32 and 60 d after TAI did not differ as a result of treatment with different GnRH products or GnRH salts (Table 1).

4. Discussion

A GnRH treatment induces a surge release of LH and FSH from the anterior pituitary and ovulation from a dominant follicle (Vasconcelos et al., 1999; Giordano et al., 2012, 2013). Interestingly, there were indications from results in a previous study that there was a lesser amplitude of the induced LH surge and ovulatory response in cows treated with gonadorelin hydrochloride compared to cows treated with gonadorelin diacetate (Martínez et al., 2003). These previous findings may indicate the lesser ovulatory responses as well as lesser P/AI in the current trial occurred because of a lesser amplitude of the induced preovulatory LH release when there was administration of gonadorelin hydrochloride as compared with what occurred as a result of the other GnRH salt treatments. The exact mechanism by which gonadorelin diacetate induces a greater GnRH-induced surge release of LH and subsequent ovulatory response and possibly fertility is unknown, but results of the present study indicate the lesser ovulation response to gonadorelin hydrochloride is independent of circulating P4 concentrations at the time of GnRH administration. Perhaps the hydrochloride salt has a lesser capacity to be transported to the GnRH receptors of the anterior pituitary. Alternatively, the two salts may differ in half life in circulation which could lead to lesser efficacy of the GnRH hydrochloride salt compound. Furthermore, these

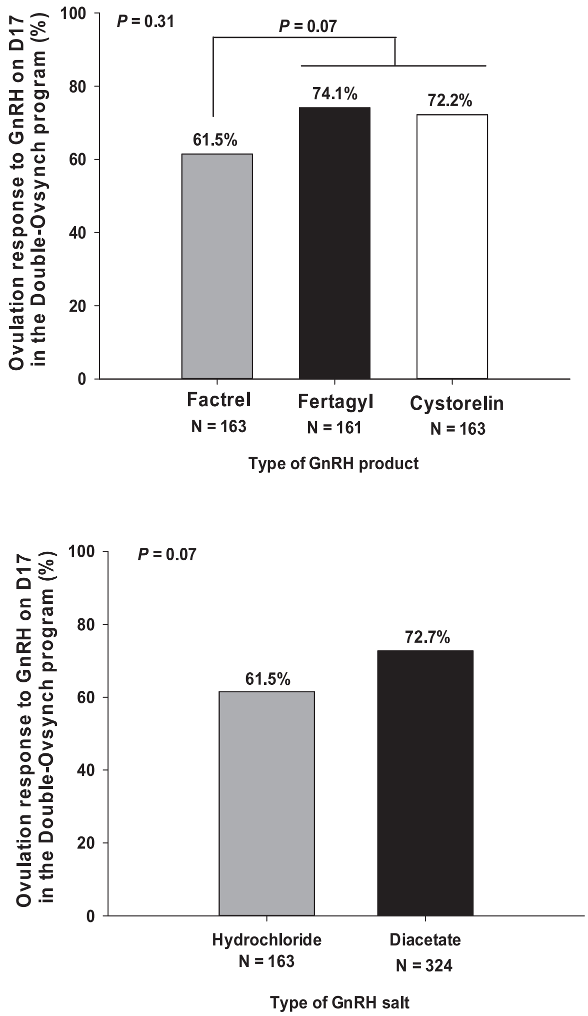

Fig. 1. Ovulation response with use of different GnRH products (Cystorelin vs Factrel vs Fertagyl – upper panel) or GnRH Salt (Hydrochloride vs Diacetate – lower panel) on ovulation response as a result of GnRH administration on D17 during the Double-Ovsynch protocol in lactating Holstein cows; Tendency was assumed for *P*-values between 0.05 and 0.10, and significance when *P*-value was less than 0.05; All values are presented as LSmeans.

differing GnRH salt compounds may have different tissue perfusion patterns that contribute to variation in capacity to induce LH release from the anterior pituitary. Basic research exploring pharmacological features of these differing GnRH salts is lacking and if conducted would lead to an enhanced understanding of the biological effects of these GnRH salts.

The findings from conducting the logistic regression analysis indicate that the greater the circulating P4 concentrations the lesser the ovulation induction with use of all types of GnRH products and salts in the current study, and these findings are consistent with those from previous reports. For example, Giordano et al. (2012) also evaluated the ovulation-induction effect of circulating P4 concentrations at the time of the GnRH treatment. Cows with greater (3.5 ng/mL) P4 concentrations had a lesser amplitude of the GnRH-induced LH release than cows with lesser (0.2 ng/mL) P4 concentrations (3.3 compared with 15.7 ng/mL). When there is consideration of previous and present research findings, it is obvious when P4 concentrations exceed 1 ng/mL the amount of LH secreted as a result of GnRH administration is less (Giordano et al., 2012). In a subsequent study, Giordano et al. (2013) evaluated the effect of relatively larger GnRH doses on ovulatory response. Interestingly, doubling the GnRH dose resulted in an increase in ovulatory response only in cows with P4 concentrations exceeding 1 ng/mL at the time of GnRH administration, and there was no effect of GnRH dose in cows with P4 concentrations of < 1 ng/mL. Thus, depending on the reproductive management schedule used on a farm and the stage of the estrous cycle at beginning of the reproductive programs, increasing the GnRH dose might be something to be explored by the herd veterinarian to maximize synchronization in the breeding protocol.

Results from the current study are not consistent with those of a previous study (Poock et al., 2015) where there was no differences in ovulation induction response when there was administration of gonadorelin hydrochloride and gonadorelin diacetate salts. These differences in results are likely due to the type of synchronization protocol utilized in the two studies. Poock et al. (2015) used a Pre-synch hormonal treatment regimen with two PGF2α treatments followed by Ovsynch 2 weeks later for cows at first

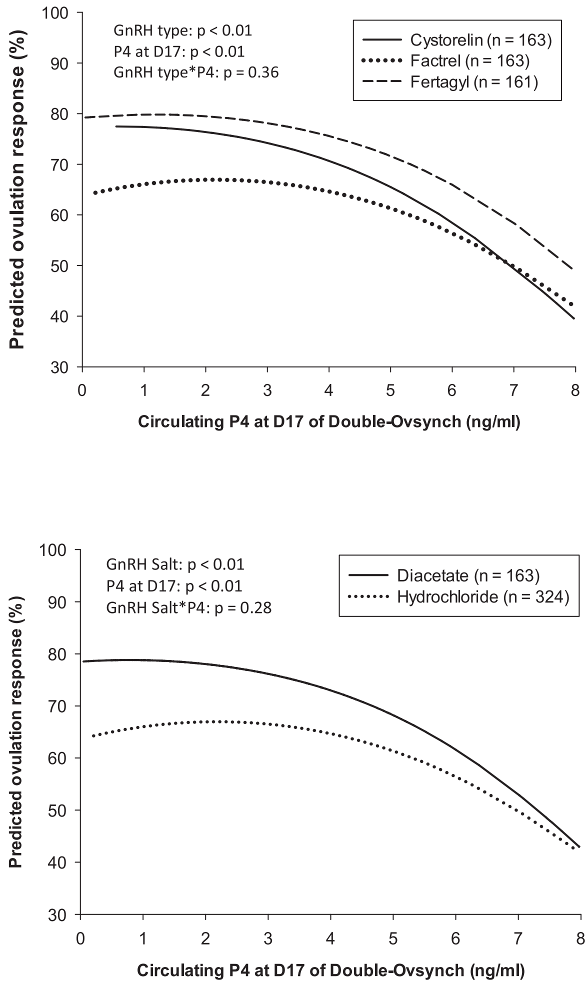

Fig. 2. Estimated effect of GnRH product (Cystorelin vs Factrel vs Fertagyl – upper panel) or GnRH Salt (Hydrochloride vs Diacetate Tetrahydrate – lower panel) on ovulation response to GnRH administration on D17 of the hormonal treatment regimen as affected by circulating P4 concentrations during the Double-Ovsynch protocol in lactating Holstein cows.

Table 1
Effect of GnRH product on pregnancies per AI (P/AI) and pregnancy loss in lactating Holstein cows treated with the Double-Ovsynch protocol for conducting the first postpartum AI.

	Factrel $n = 482$	Fertagyl $n = 515$	Cystorelin $n = 484$	GnRH product *P*-value	GnRH Salt[1] *P*-value
P/AI 32d (%)	44.1	49.3	51.3	0.07	0.03
P/AI 60d (%)	42.0[a]	47.8[b]	49.8[b]	0.04	0.02
Preg loss (%)	4.7	3.1	2.8	0.42	0.26

Means with different letters (a,b) within each line are different, $P < 0.05$.
[1] Contrast: Factrel (gonadorelin hydrochloride) compared with Fertagyl + Cystorelin (gonadorelin diacetate tetrahydrate).

postpartum AI and cows found non-pregnant after a previous AI and as a result the Ovsynch protocol initiated at random stages of the estrous cycle. In the current study, however, there was only cows included to conduct the first postpartum AI with the Double-Ovsynch protocol to ensure most of the cows were in the same stage of the estrous cycle at the time of initiation of the Ovsynch hormonal treatment regimen. This treatment approach ensures there was a LH-responsive follicle at the time of initiation of the Ovsynch treatment regimen in most of the cows (D17 of Double-Ovsynch). Double-Ovsynch increases proportion of cows ovulating to the first GnRH administration of the Ovsynch compared with use of the standard pre-synch regimen with two PGF2α treatments (Souza et al., 2008). Cows having ovulations as a result of administration of the first GnRH treatment of Ovsynch hormonal regimen generally have greater P/AI than cows in which ovulations do not occur (Chebel et al., 2006; Galvão et al., 2007; Giordano et al., 2013; Carvalho et al., 2015b). When complete luteal regression at the end of the Double-Ovsynch protocol does not occur, the expected increase in P/AI in cows having ovulations as a result of GnRH administration on D17 might be negated (Wiltbank et al., 2011a; Ribeiro et al., 2012; Carvalho et al., 2015a; Stevenson, 2016). This is the primary reason why two PGF2α treatments were used in the current experimental design. Thus, it is possible that in the study of Poock et al. (2015) there was not detection of differences when there was administration of different gonadorelin salts in terms of fertility because in the design of the previous study there was use of a single $PGF_{2\alpha}$ treatment to induce luteolysis during Ovsynch, which may cause incomplete CL regression possibly diluting the effects of better ovulatory capacity from differing GnRH products and making it difficult to interpret the effects of differing GnRH products on P/AI.

Incomplete luteal regression has a profound negative impact on P/AI (Carvalho et al., 2015a; Fricke et al., 2015; Wiltbank et al., 2015). The bovine corpus luteum begins to acquire luteolytic capacity approximately 7 days after ovulation (Momont and Sequin, 1984); thus, cows having ovulations as a result of the 1st GnRH of the Ovsynch treatment regimen are likely to have a greater incidence of incomplete luteal regression when a single $PGF_{2\alpha}$ treatment is used (Lopes et al., 2013). This possibly explains, at least partially, why in the study of Poock et al. (2015) there was failure to detect differences in fertility when comparing different GnRH products.

The improved fertility following greater ovulation responses as a result of GnRH administration at the beginning of the Ovsynch (D17 in Double-Ovsynch) associated with complete luteolysis and are likely related to an enhanced embryo quality. There has been evaluation of the effect of ovulatory response to the first GnRH administration of Ovsynch treatment regimen on subsequent embryo development on day 7 after AI (Cerri et al., 2009). Although fertilization rate did not differ, based on ovulatory responses of cows to the first GnRH administration of the Ovsynch treatment regimen (87.5% compared with 83.3%), the percentage of embryos classified as excellent and good quality was substantially less (38.9% compared with 77.5%) and the percentage of degenerate embryos was greater (22.2% compared with 5.0%) for cows that did not have ovulations. The lesser quality of embryos from cows failing to have ovulations as a result of the first GnRH administration of the Ovsynch treatment regimen was attributed to ovulation from persistent/aged follicles. Ovulation as a result of GnRH administration at beginning of Ovsynch-like treatment regimens appears to be a very important response for fertility to occur due to a more consistent stage of dominant follicle development at the end of the treatment regimen, which eventuates in development of embryos of greater quality on day 7 after AI (Vasconcelos et al., 1999; Cerri et al., 2009; Wiltbank et al., 2011b).

5. Conclusion

In conclusion, the use of the gonadorelin hydrochloride-based GnRH salt (Factrel) tended to result in lesser ovulations on D17 of the Double-Ovsynch compared to use of the diacetate tetrahydrate salts (Fertagyl and Cystorelin). Interestingly, these differences in ovulatory response with different GnRH products were unrelated to P4 concentrations at the beginning of the Ovsynch. As a result, P/AI 60 days post AI was less for cows treated with Factrel during Double-Ovsynch compared to cows treated with either Fertagyl or Cystorelin, presumably, due to lesser ovulation responses as a result of these treatments at critical times during the Double-Ovsynch program.

Author statement

The authors verify that they have no competing interests with the work presented in this manuscript. This study was supported by Merck Animal Health, E.I. Medical Imaging, and Bovisync® herd management software.

Acknowledgments

The authors thank participating herds, their vet practitioners, and all their staff for their collaboration during the study.

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