Kansas Agricultural Experiment Station Research Reports

Volume 11
Issue 6 *Dairy Research*

Article 3

2025

Effect of Administering GnRH at the Time of Insemination on the Fertility of Dairy Heifers Bred at Estrus

V. E. Gomez-Leon
Kansas State University, vgomezleon@ksu.edu

S. A. Paez-Hurtado
Alta Genetics Inc., Watertown, WI

L. Smulders
Kansas State University

See next page for additional authors

Recommended Citation

Gomez-Leon, V. E.; Paez-Hurtado, S. A.; Smulders, L.; Ohlde, T.; Gamboa, E.; Cruz Mota, L. A.; Schrag, N.; and Lamberto, P. (2025) "Effect of Administering GnRH at the Time of Insemination on the Fertility of Dairy Heifers Bred at Estrus," *Kansas Agricultural Experiment Station Research Reports*: Vol. 11: Iss. 6. https://doi.org/10.4148/2378-5977.8733

Effect of Administering GnRH at the Time of Insemination on the Fertility of Dairy Heifers Bred at Estrus

Funding Source

The authors would like to thank the heifer yard management and the AI company involved in the study – especially the technicians conducting all the animal work. The authors also thank Parnell Living Science for the donation of the GONAbreed used to conduct the experiment. This work was supported by the USDA National Institute of Food and Agriculture, Research Capacity Fund/Hatch Multistate, project accession no. 1014097 to V.E. Gomez-Leon.

Authors

V. E. Gomez-Leon, S. A. Paez-Hurtado, L. Smulders, T. Ohlde, E. Gamboa, L. A. Cruz Mota, N. Schrag, and P. Lamberto

Effect of Administering GnRH at the Time of Insemination on the Fertility of Dairy Heifers Bred at Estrus

V.E. Gomez-Leon, S.A. Paez-Hurtado[1], L. Smulders, T. Ohlde[1], E. Gamboa[1], L.A. Cruz Mota[1], N. Schrag[2], P. Lamberto[2]

Summary

Heifer reproductive performance has gained attention from both the dairy industry and academia. Thus, strategies tested in cows have been proposed to be used in heifers at the field level. The current field trial tested the hypothesis that administering GnRH at the time of insemination would increase pregnancy per artificial insemination (P/AI) in dairy heifers bred at estrus. Using Holstein heifers (12 months and older) from a heifer yard in KS, a total of 5,809 breedings were randomized to a CONT (control, untreated) or a GNRH group (a single dose of 100 mcg of GnRH at estrus-AI). The heifers were subject to the regular reproductive program of the heifer yard, which prioritized breeding to estrus, and were randomized without any blocking factor. Pregnancy per AI (P/AI) at 45 ± 3 days post-AI was compared between CONT and GNRH groups using generalized linear models. The AI technician, AI sire, breed, and semen type (sexed and conventional) were considered in the statistical analysis. Data curation consisted of removing groups that lacked representation, and the final statistical analysis included 4,774 breedings (CONT n = 2,376 and GNRH n = 2,398). Our hypothesis was not supported by the results, as evidenced by a lack of significant difference between the P/AI of CONT (41.4 ± 3%) and GNRH (42.9 ± 3%). An interaction of group by service number was also not observed. Thus, the use of GnRH at the time of AI is not recommended in heifers bred at detected estrus under the current experimental conditions.

Introduction

Reproductive performance in lactating dairy cows has increased over the years due to strategies to increase service risk (SR), P/AI, and decrease pregnancy losses. Heifer reproductive performance is starting to gain attention, and some strategies tested in cows are being proposed in heifers at the field level. This creates the need for studies to test such strategies in heifers. Of particular interest for this study, the use of GnRH when breeding animals at estrus aims to ensure ovulation and timing of ovulation in relation to breeding. This approach has generated interesting but contrasting results in the literature. However, it remains to be tested in dairy heifers. In that sense, the state of KS provides an excellent opportunity to test these strategies due to the high number

[1] Alta Genetics Inc., Watertown, WI.
[2] Parnell Living Science, Overland Park, KS.

of heifers from dairy states (e.g., MI, WI, PA, etc.) that are raised and bred in KS. Thus, herein, we tested the hypothesis that administering GnRH at the time of insemination will increase P/AI in dairy heifers bred at estrus.

Experimental Procedures

Animals, location, and reproductive management

The current experiment comprised 5,809 breedings of Holstein heifers (n = 5,155 heifers; 12 months old and older) at a single heifer yard in KS. Heifers were housed in dry open pens and were offered *ad libitum* access to water and fed a total mixed ration (TMR) formulated to meet body weight standards according to NASEM (2021).

The reproductive management of the heifer yard is described in Figure 1. During the entire reproductive program, heifers were declared on estrus based on visual assessment. The AI was performed by trained technicians using bulls from a single AI company. All heifers were eligible to be bred from D 0 to D 141 of the reproductive program through a combination of intramuscular administration of PG (prostaglandin F 2 alpha analog), 5-day CIDR synch program, PG at pregnancy diagnoses, or estrus return after AI. Briefly, on D 0, heifers started the reproductive program by being moved to breeding pens and receiving a PG dose to induce estrus expression. On D 5, heifers that failed to be detected in estrus receive another PG. On D 19, the same was repeated. Heifers were submitted to TAI on D 26 if not detected in estrus.

Experimental groups, pregnancy diagnosis, and data retrieval

On a given day, heifers detected in estrus were randomized into two different groups: (1) control group (CONT, untreated) and (2) GNRH group (a single dose of 100 mcg of GnRH at the time of AI in estrus; Gonadorelin acetate, GONAbreed, Parnell, New South Wales, Australia). Since all the herd management procedures and the experimental procedures were performed by technicians from the heifer yard and from a single AI company, no blocking factor was used for the randomization in order to facilitate conducting this field trial. The randomization was performed as AI technicians walked the breeding pens. For this purpose, two groups of technicians worked with the animals. Within the first group, technician 1 walked behind the locked-up heifers, checking for the absence of tail chalk paint and other secondary signs of estrus (mucus, scratches, vulvar edema, etc.). Technician 1 identified animals in estrus and marked them for insemination. A second technician walked in front of the locked-up heifer (at the same speed as technician 1) with an RFID stick reader connected to a cellphone running the dairy management software to access all the heifer data. This technician scanned and entered all heifers to be bred within a day. The cellphone with the dairy management software assigned a consecutive number (1 to 100) to each breeding. When moving across pens, the consecutive number was continued rather than reset. The consecutive number was written on the rump of each heifer so that a third technician administered the GONAbreed to heifers with even numbers while skipping those with uneven numbers (CONT). The second group of technicians performed the AI on the previously identified in-estrus heifers.

Pregnancy diagnoses were performed with ultrasound every week at 45 ± 3 days after AI. All estrus detection, AI, and pregnancy diagnoses were conducted once daily in the morning. Pregnancy was confirmed by the presence of an embryo with a heartbeat, whereas heifers rebred before the scheduled pregnancy diagnosis were considered to be nonpregnant. Daily backups were retrieved from the dairy management software

(DC305 software, Valley Agricultural Software Inc.) to ensure that details on each breeding were used for the analysis. The retrieved backups contained the heifer ID and information related to the AI: service number, date, hour, sire, technician, and treatment group (CONT and GNRH).

Data handling and statistical analysis

Each breeding was considered the experimental unit. Moreover, based on an expected 53% P/AI in CONT and 56% P/AI in the GNRH group, a power analysis indicated that 2,300 breedings were required in each group to show statistical differences at $P = 0.05$.

Data curation consisted of removing breedings with annotation errors (n = 2), breedings that were service number 7 or greater (n = 57), breedings with Jersey, beef, or beef on dairy semen (n = 310 [7 sires]), and breedings of heifers that had been synchronized with CIDR synch (n = 47). Subsequently, breedings from sires that were used on < 100 AI during the study (n = 468 breedings [9 sires]) and breedings from technicians with < 100 AI (n = 151 [5 technicians]) were removed. Thus, from the initial 5,809 breedings, a total of 4,774 breedings (CONT n = 2,376 and GNRH n = 2,398) were used for the statistical analysis.

A logistic regression model was used to assess the fertility effect of using GNRH when breeding heifers detected in estrus. Pregnancy on day 45 ± 3 days post-AI was used as the response. The initial model included the fixed effects of service #, hour of the AI, and the group (CONT and GNRH). The interactions of group by service # and group by hour of the AI were also included as fixed effects. Sire and technician were used as random effects. Fixed effects with a $P > 0.20$ (group by service #) were eliminated from the model using a stepwise backward process. The main effect of the group was forced to stay in the model even if $P > 0.20$. A second logistic regression model was used to assess any potential interaction of GnRH with semen type (Holstein sexed or Holstein conventional). The model included the main effects of service #, semen type, group, and the interaction of group by service number. The AI technician was included as a random effect.

All statistical analyses were performed with PROC GLIMMIX in SAS v. 9.4 (SAS Institute Inc., Cary, NC, USA). Statistical significance was declared when $P \leq 0.05$. Data are presented as Least Square Means (i.e., adjusted means).

Results and Discussion

Our hypothesis was not supported, which was that administering GnRH at the time of insemination would increase P/AI in dairy heifers bred at estrus. That is, there was no significant difference ($P = 0.36$) for P/AI at 45 ± 3 days after AI between the CONT (41.4 ± 3%) and the GNRH groups (42.9 ± 3%). The rationale for using GnRH on estrus-bred heifers came from commercial farms interested in testing strategies previously used in cows. Potential benefits of GnRH could be associated with ensuring ovulation and aiding in the length from insemination to ovulation, especially when estrus is detected once a day or when using sexed semen. However, the interaction of semen type (sexed or conventional) and group (CON or GNRH) was not significant ($P = 0.9$) in our study. Moreover, to our knowledge, there have not been reports showing a lack of ovulation in heifers expressing estrus. Physiologically, an animal expressing estrus already had a preovulatory luteinizing hormone surge, which should trigger ovulation ~28 hours later. Reports from the literature would indicate that GnRH

could be beneficial when used in estrus-bred animals during challenging situations. For example, the use of GnRH when breeding animals in estrus has been shown to be beneficial in cows that are in their third lactation or greater and in cows with low estrus intensity measured by automated activity monitors. Although one could speculate that the heifers in the current study had challenges related to high environmental temperatures, as the experiment was collected during the summer season and heifers were housed in open dry lots, the GnRH did not present any advantages when used at the time of AI in estrus-bred heifers. Another speculation in our study was that heifers with multiple services could benefit from using GnRH at the time of estrus, perhaps due to physiologic hormonal challenges in those heifers. However, the interaction of service # and group (CONT or GNRH) was not significant ($P > 0.2$). Likewise, the interaction between the hour of insemination and the group was not significant ($P = 0.12$). Thus, based on the results of the current study, using GnRH at the time of insemination in estrus-bred heifers is not recommended.

Conclusions

In summary, the CONT and GNRH groups were not different, as demonstrated by the difference between the groups not exceeding the pre-specified non-inferiority margin of 3%. It is worth mentioning that although our study had an adequate sample size of 4,774 breedings to test the hypothesis, it was conducted on a single heifer yard that may not represent other locations. Nevertheless, the use of GnRH at the time of insemination is not recommended in heifers bred at detected estrus under the current experimental conditions.

Acknowledgments

The authors would like to thank the heifer yard management and the AI company involved in the study – especially the technicians conducting all the animal work. The authors also thank Parnell Living Science for the donation of the GONAbreed used to conduct the experiment.

This work was supported by the USDA National Institute of Food and Agriculture, Research Capacity Fund/Hatch Multistate, project accession no. 1014097 to V.E. Gomez-Leon

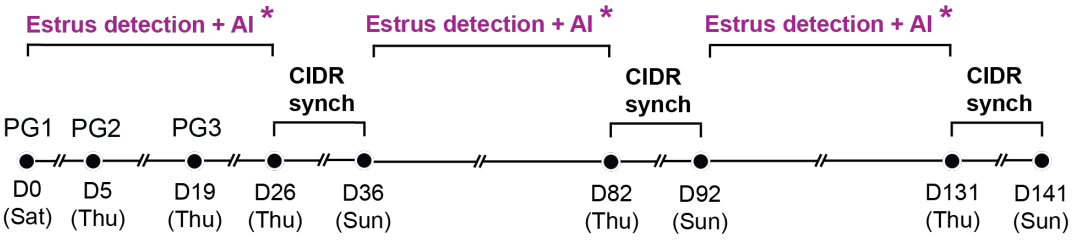

Figure 1. Reproductive management to which the dairy heifers are submitted at the heifer yard, where the experiment was conducted. PG = Prostaglandin F2 alpha analog; CIDR synch = 5-day CIDR synch protocol; * Heifers were randomized to Control (no treatment) or GnRH (100 ug of gonadorelin acetate) at the time of artificial insemination.