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The authors thank all of the members of the KSU-DTRC and the KSU-ASI Repro Dairy Lab involved in the reproductive program of the dairy farm during record collections. We also thank Dr. Leticia Sanglard for her intellectual input and Parnell Living Science for the donation of the reproductive hormones used at the KSU-DTRC.

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M. Schultz, J. S. Stevenson, V.E. Gomez-Leon

Summary

The aim of this study was to evaluate the association between estrus expression and fertility outcomes in nulliparous Holstein heifers and high-producing lactating dairy cows submitted to GnRH-based timed artificial insemination (TAI) protocols. Retrospective data from the Kansas State University Dairy Teaching and Research Center from 2021 to 2024 were used in the study. Data included 1,154 first-service breeding records, 359 estrus events, pregnancy diagnoses, and calving incidence. Estrus's expression was recorded using automated activity monitors. Estrus incidence, intensity, duration, and timing were analyzed in relation to pregnancy per artificial insemination (P/AI) and calving risk with Generalized Linear Models in SAS. Parity (heifer, primiparous, and multiparous) was included in the statistical model. Heifers exhibited higher estrus incidence, intensity, and duration compared to primiparous and multiparous cows. While intensity and duration did not directly impact P/AI, the timing of estrus expression significantly influenced outcomes, with animals expressing estrus closer to insemination (within 18 hours) demonstrating greater odds of pregnancy. Moreover, estrus expression was associated with a 15% increase in P/AI and improved calving rates (93% vs. 87%) regardless of parity (heifer, primiparous, or multiparous). Although previous studies have shown that estrus expression in high-producing cows may be detrimental or unnecessary for fertility at first service, our findings suggest that promoting estrus expression during TAI protocols may be beneficial. Future research should corroborate our findings, explore strategies to promote estrus expression during TAI, and assess the physiologic mechanisms leading to higher fertility in animals expressing estrus.

Introduction

The effect of estrus expression on the fertility of high-producing dairy cows remains controversial. Current TAI protocols, such as Double-Ovsynch and PG3-G, which include pre-synchronization of the estrous cycle, result in an 18 to 70% increase in P/AI compared with estrus-bred cows. This could be partially attributed to the high metabolism resulting from intense milk production selection, which has been shown to alter the balance of hormones associated with estrus and ovulation and with poor fertility. The TAI protocols in dairy cows address this issue by inducing ovulation of mid-size follicles, which may reduce estrus expression.

The influence of estrus expression before the breeding time within the TAI protocol is less consistent and varies across studies (Table 1). Given the variability in these findings, it is essential to better understand how estrus expression, intensity, duration, and timing affect fertility in dairy cattle submitted to TAI during the first service. Thus, this study aimed to explore the association of estrus characteristics with P/AI and calving risk in Holstein heifers and lactating cows submitted to GnRH-based TAI protocols at first service. The primary hypothesis was that estrus expression is associated with increased P/AI and calving risk, regardless of parity. Additionally, estrus intensity and duration, as measured by automated activity monitors, were hypothesized to correlate positively with fertility outcomes, providing further insights into optimizing reproductive strategies in dairy cattle.

Experimental Procedures

Data source, animals, and estrus synchronization protocols

Retrospective data from the Kansas State University Dairy Teaching and Research Center (KSU-DTRC) were used in this study. Records included 1,154 breeding, 359 estrus expression events, pregnancy diagnoses, and calving data between January 2021 and March 2024. Heifers were housed in open dirt lots, while cows were kept in open free-stall pens with seasonal heat stress mitigation measures (shades and sprinklers). Diets consisted of a total mixed ration (TMR) adjusted to meet nutrient requirements for 50 kg of milk production.

Cows and heifers included in this study were subjected to normal reproductive management of the KSU-DTRC (Figure 1). Briefly, cows between 50 and 56 days postpartum were enrolled in the PG3-G protocol and received their first service between 70 and 76 days postpartum. Nulliparous Holstein heifers of 13 months of age and 800 pounds of body weight were submitted to a CIDR6 protocol with scheduled TAI at 48 h after CIDR removal. Pregnancy was diagnosed via transrectal ultrasonography at 35 ± 3 days post-TAI. Animals bred before the scheduled pregnancy diagnosis were considered to be nonpregnant.

Data collection and curation

Breeding records were extracted weekly from PCDART (Dairy Records Management Systems, Raleigh, NC) and included information on insemination date, parity, AI sire, technician, and breeding type (TAI or estrus). Estrus events, recorded by CowManager ear tags (CowManager B.V., Utrecht, The Netherlands), were retrieved biannually and included details on estrus date, intensity, duration, and timing. Calving data for pregnancies from January 2021 to February 2023 were retrieved from PCDART.

Data were curated to include only first-service TAI breedings. In addition, animals with incomplete records or estrus outside of -48 to 0 hours before AI were removed. Final records included 1,154 breeding animals (288 heifers, 866 cows), 359 estrus events, and 323 calving records.

Statistical analysis

Logistic regression models were used to assess the effects of parity (heifer, primiparous, multiparous), estrus expression (yes or no), intensity, duration, and timing of estrus on P/AI and calving risk. Random effects included AI sire, technician, and season/year of AI. Statistical significance was set at $P \leq 0.05$, with a tendency noted at $0.05 < P \leq 0.10$.

All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC). Data are shown as raw means and odds ratios.

Results and Discussion

Incidence of estrus expression and fertility

As expected, nulliparous heifers had a significantly higher estrus incidence (57%) compared to primiparous (26%) and multiparous cows (20%; $P < 0.0001$). Overall, 31% of animals in TAI protocols expressed estrus (Figure 2).

Estrus expression improved P/AI (Figure 3), with a 10% increase in nulliparous heifers and primiparous cows and a 16% increase in multiparous cows ($P = 0.002$). Overall, animals expressing estrus had 63% greater odds of pregnancy than those not expressing estrus. Estrus expression also tended to improve calving risk (93% vs. 87%, $P = 0.06$), while parity had no significant effect. The mechanisms behind these fertility improvements may be linked to hormonal dynamics. Our findings suggest that optimizing TAI protocols to enhance estrus expression could improve fertility outcomes, but this is not without challenges. Current protocols, such as those using GnRH, rely on the cow's natural hormone production, which may be limited in high-producing dairy cows. While some protocols based on estradiol have shown promise in boosting estrus expression, their use is restricted in certain regions, and the results on pregnancy rates have been mixed. A limitation of our study is that animals that did not respond to the reproductive program (out of synchrony) will fall within the no-estrus group. Nonetheless, previous studies have shown synchronization rates around 80 to 90%. Even with these challenges, animals that express estrus consistently outperform those that do not. This raises questions about how to better support estrus expression through management and protocol adjustments. Future research should focus on understanding why some cows fail to express estrus and how to improve synchronization without compromising fertility.

Our primary hypothesis was supported, which is that estrus expression is associated with increased P/AI and calving risk, regardless of parity. In fact, estrus expression was associated with a 15% increase in pregnancy rates and a greater likelihood of successful calving. The fertility improvement was consistent across heifers and cows, regardless of parity. This aligns with previous research showing that estrus expression before FTAI is linked to better pregnancy outcomes, likely because it reflects better synchronization and optimal hormonal balance.

Estrus duration, intensity, and timing

Timing of estrus was associated with fertility; animals expressing estrus between –18 hours and AI had greater odds of P/AI (2.11, $P = 0.05$) than those expressing estrus earlier (–48 to –18 hours). The highest incidence of estrus occurred between –12 and –6 hours before TAI for both heifers and cows, as per the TAI protocol design.

Heifers exhibited greater estrus intensity (7.7 ± 0.2 units) and duration (18 ± 0.4 hours) than cows (5.8 ± 0.2 units; 14 ± 0.3 hours, $P < 0.001$). Although estrus intensity and duration were correlated ($r = 0.58$), neither influenced P/AI ($P \geq 0.77$). Interestingly, the intensity and duration of estrus, although greater in heifers than in cows, did not directly influence fertility outcomes. This suggests that while estrus behavior provides an indication of reproductive willingness, other factors, such as the timing of ovulation and follicular quality, could play a critical role in this issue.

Conclusions

Our findings indicate that estrus expression is associated with greater pregnancy P/AI and with greater calving risk in both nulliparous heifers and high-producing lactating dairy cows subjected to GnRH-based TAI protocols for first service. Further research should aim to uncover the mechanisms behind the fertility enhancement associated with estrus expression in dairy cattle and explore whether synchronization protocols designed to promote estrus expression in high-producing cows can improve reproductive success. Additionally, investigations are needed to understand the factors contributing to the absence of estrus in dairy cattle undergoing TAI, particularly in relation to milk production levels and hormonal dynamics.

Acknowledgments

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Table 1. Studies assessing the effect of estrus expression in dairy cattle

Manuscript	n	Incidence of es-trus (%)	P/AI (%) NoE ¹	P/AI (%) E ²	Fold change
A) Dairy cows (Estradiol + CIDR based protocols)					
Pereira et al. (2016)	5,430	84%	25.5	38.9	52.5%
B) Dairy heifers (GnRH + CIDR based protocols)					
Macmillan et al. (2021)	195	68%	33.0	62.1	88.2%
Current study	288	57	53.0	63.0	18.9%
Total or average	483	62%	46%	63%	36.6%
C) Dairy cows (GnRH based protocols)					
Santos et al. (2010)	819	27%	31.9	46.2	44.8%
Stevenson et al. (2010)	1,021	16%	33.5	51.8	54.6%
Lauber and Fricke (2023)	1,046	29%	53.8	59.5	10.6%
Laplacette et al. (2024)	2,135	26%	44.9	57.7	28.6%
Current study	866	22%	44.0	57.6	30.9%
Total or average	5,887	24%	42%	56%	33.14%

CIDR: Controlled internal drug release with progesterone; ¹No estrus animals; ²Estrus animals.

Estrus synchronization protocols

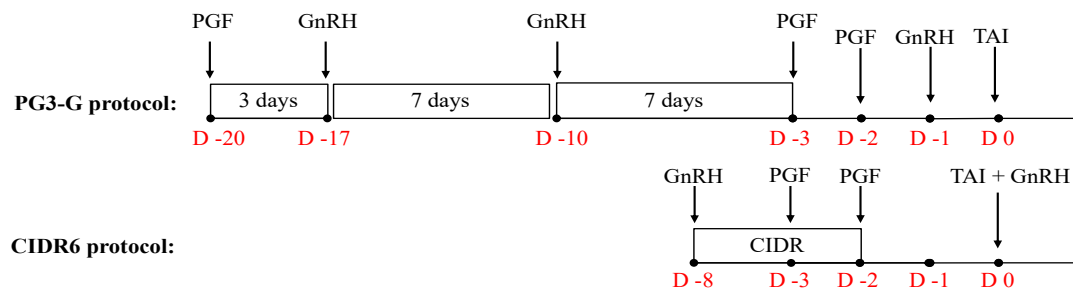


Figure 1. Schematic diagram of TAI protocols for the study. Holstein lactating cows between 50 and 56 days postpartum were enrolled in the PG3-G protocol to receive their first service between 70 and 76 days postpartum. Nulliparous Holstein heifers of 13 months of age were enrolled in a CIDR6 protocol with scheduled TAI fixed at 72 or 48 hours after CIDR removal.

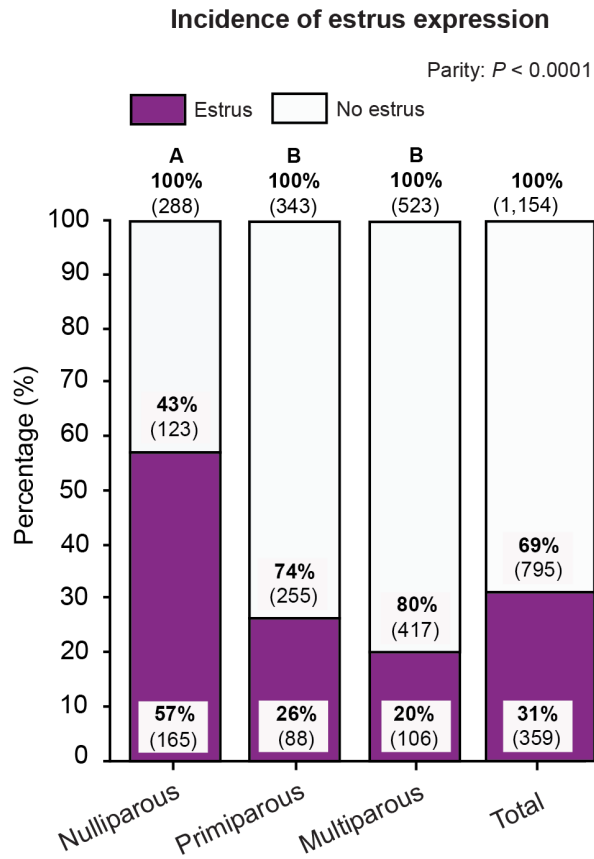


Figure 2. Incidence in percentage (and n) of estrus in nulliparous heifers and lactating cows that expressed estrus or not during a 48-hour window before timed artificial insemination at first service. Different capital letters on top of each bar indicate a significant difference among parity.

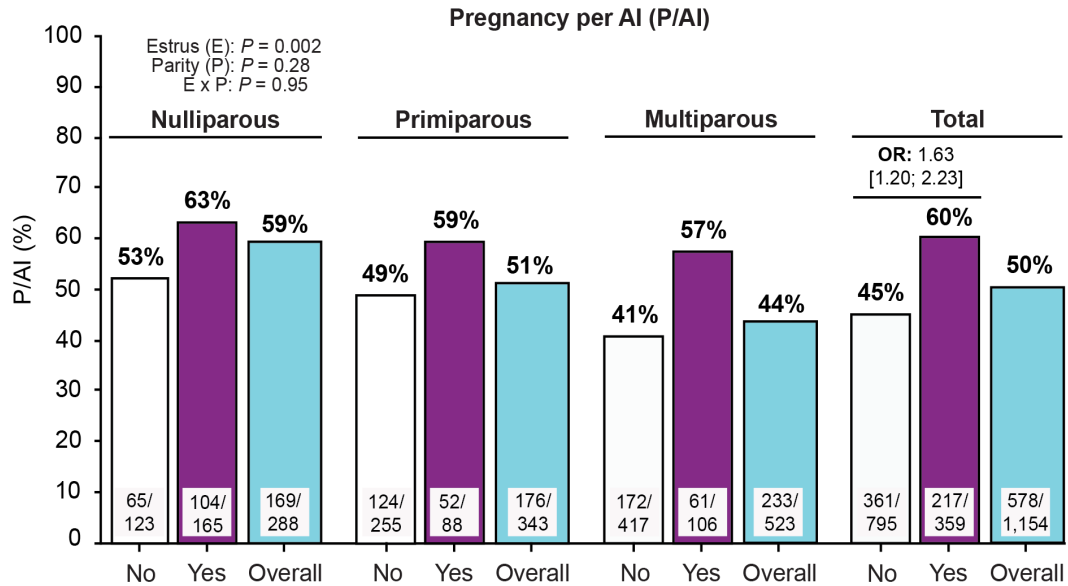


Figure 3. Pregnancy per artificial insemination (P/AI) in nulliparous heifers and lactating dairy cows that expressed estrus (Yes: purple bars) or not (No: white bars) during a 48-hour window before the timed artificial insemination day at first service. Overall P/AI is depicted for each parity (aqua bars).