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## Delayed Timing of Insemination Relative to Estrus Improves Pregnancy to Artificial Insemination With Sex-Sorted Semen in Beef Heifers

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## **Delayed Timing of Insemination Relative to Estrus Improves Pregnancy to Artificial Insemination With Sex-Sorted Semen in Beef Heifers**

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## Delayed Timing of Insemination Relative to Estrus Improves Pregnancy to Artificial Insemination With Sex-Sorted Semen in Beef Heifers

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### Abstract

The objective was to evaluate the effect of timing of insemination relative to the onset of estrus when using sex-sorted semen in beef heifers. Estrous cycles of beef heifers were synchronized using the melengestrol acetate with prostaglandin (MGA-PG) protocol and were visually observed for signs of estrus every four hours for five days following injection of PGF<sub>2α</sub>. Following visual detection of estrus, heifers were inseminated with semen sorted to contain X-chromosome bearing sperm cells from either an Angus or Simmental sire (packaged at  $4.0 \times 10^6$  live cells per 0.25 mL straw of SexedULTRA 4M). Heifers were retrospectively categorized into one of the three following intervals from time of estrus onset to insemination: 1) 12.5–15.9 hours; 2) 16.5–21.0 hours; and 3) 21.4–27.5 hours. Heifers with the shortest interval (12.5–15.9 hours) from estrus onset to insemination had a similar ( $P > 0.10$ ) AI pregnancy rate (51.4%) as compared with heifers with the estrus onset to insemination interval of 16.5 to 21 hours (56.3%). Heifers inseminated 21.4 to 27.5 hours following estrus onset achieved a greater ( $P \leq 0.05$ ) artificial insemination pregnancy rate (75.9%) than heifers inseminated 12.5 to 15.9 hours following estrus onset (51.4%).

### Introduction

The ability to determine gender at conception has been a widely desired technology that has been available commercially for less than 20 years (Garner and Seidel, 2008; Seidel and DeJarnette, 2021). A beef producer can potentially benefit from use of sex-sorted semen in decreasing incidence of dystocia or in targeting production of replacement females, seedstock bulls, or male calves to be fed for harvest. Sex-sorted semen use in fixed-time artificial insemination programs for beef females, however, has been shown to result in pregnancy rates that are 75–85% of those observed with conventional semen (Oosthuizen, et al., 2021; Perry et al., 2020; Thomas et al., 2019). The sorting process affects function and life span of sex-sorted sperm (Carvalho et al., 2013, Moce et al., 2006) and fewer sperm cells are packaged in a dose of sex-sorted semen, contributing to decreased pregnancy rates. It has been suggested that semen deposition of sex-sorted semen nearer time of expected ovulation in beef females may improve pregnancy outcomes (Thomas et al., 2014; Oosthuizen et al., 2021). Chebel and Cunha

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(2020) did not observe an improvement in pregnancy outcomes to artificial insemination (AI) in dairy heifers with delayed insemination. These studies were conducted using fixed-time AI programs and thus actual onset of estrus and timing from estrus onset to insemination were not necessarily known. The objective of this study was to evaluate the effect of timing of insemination relative to the onset of estrus on pregnancy outcome when using sex-sorted semen in beef heifers.

## Experimental Procedures

This trial was conducted on a commercial beef ranch in north-central South Dakota. Ninety-eight crossbred beef heifers housed in a drylot setting were subjected to the MGA-PG estrous synchronization protocol. Heifers were fed MGA for 14 days in an amount of 0.5 mg/head/day. Eighteen days after the last feeding of MGA, heifers were injected with 5 mL of PGF<sub>2α</sub> (Lutalyse; Zoetis, Madison, NJ). Estrous detection aids (Estroject, Rockway Inc., Spring Valley, WI) were applied at the time of PGF<sub>2α</sub> injection. Following injection of PGF<sub>2α</sub> heifers were visually observed for estrus every 4 hours for the next 5 days. Heifers were determined to have exhibited estrus when they were seen standing to be mounted, when >50% of the Estroject patch coating was removed, or the Estroject patch was missing. After the earliest observation of estrus, heifers were inseminated 12.5–27.5 hours later (retrospectively determined) with semen sorted to contain X-chromosome bearing sperm cells from either an Angus or Simmental sire (packaged at  $4.0 \times 10^6$  live cells per 0.25 mL straw of SexedULTRA 4M, STgenetics, Navasota, TX). Ninety-eight heifers were visually observed in estrus and retrospectively divided into one of the three following intervals from time of estrus onset to insemination: 1) 12.5–15.9 hours (n = 37); 2) 16.5–21.0 hours (n = 33); and 3) 21.4–27.5 hours (n = 28) for this analysis.

Pregnancy diagnosis was conducted 65–70 days post-insemination via transrectal ultrasonography (ReproScan XTC equipped with a 4.0 MHz 60mm convex rectal probe; ReproScan, Winterset, IA). Fetal size was used to differentiate AI pregnancies from natural service pregnancies.

## Results and Discussion

The results of this study support the suggestion that delayed insemination following estrus may be advantageous for increasing AI pregnancy rates when using sex-sorted semen in beef heifers. A 24.5 percentage point improvement in AI pregnancy rate was observed among heifers inseminated 21.4 to 27.5 hours after observed estrus (75.9%) as compared with insemination 12.5 to 15.9 hours after observed estrus (51.4%; Table 1).

The timing from the onset of estrus to ovulation in cattle has been found to range from 24 to 32 hours (Senger, 2012). Research by White et al. (2002) showed that the average time of ovulation relative to the onset of estrus was 31.1 hours. Oosthuizen et al. (2021) have recently demonstrated improved pregnancy outcomes with delayed insemination and use of strategies that synchronize ovulation timing when using sexed semen in fixed-time AI protocols in beef heifers. Extent of synchrony of estrus among heifers and whether estrus is expressed at all before insemination in a fixed-time AI system could be affecting pregnancy outcomes which were relatively unknown. In the present study, with frequent observation of estrus, we were able to definitively categorize heifers into three time intervals to more precisely evaluate effect of interval from estrus to insemination alone.

Based on previous research, we speculate that the functional changes imposed on sperm from the sorting process may explain the benefit of delayed insemination on pregnancy outcomes. Sex-sorted sperm display altered motility and reduced capacity to fertilize oocytes (Steele et al., 2020). Embryos resulting from fertilization with sex-sorted sperm have impaired development as well. Deposition of sperm into the female reproductive tract at a later time following observation of estrus (and nearer time of ovulation) likely improves opportunity for these altered sex-sorted sperm to successfully reach and fertilize the oocytes. Additional research investigating more multiple estrus to insemination intervals, with greater numbers of females and across multiple herds, is warranted to further our understanding.

## Implications

We interpret the results of this study to indicate an advantage to delaying insemination of beef heifers from the traditional 12 hours following observation of estrus to 21 to 27 hours after onset of estrus when using sex-sorted semen.

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**Table 1. Effect of interval from observed estrus to insemination on AI<sup>1</sup> pregnancy rate using sex-sorted semen in crossbred beef heifers**

<b>Estrus onset to insemination interval range<sup>2</sup></b>	<b>Number</b>	<b>AI pregnancy rate</b>
Overall	98	60.2%
12.5–15.9 hours <sup>3</sup>	37	51.4% <sup>a</sup>
16.5–21.0 hours <sup>4</sup>	33	56.3% <sup>ab</sup>
21.4–27.5 hours <sup>5</sup>	28	75.9% <sup>b</sup>

<sup>ab</sup> Values within a column without a common superscript are different ( $P \leq 0.05$ ).

<sup>1</sup>Artificial insemination.

<sup>2</sup>Interval of time from when heifers were detected in estrus (with observation occurring every 3 to 4 hours) to the time of insemination. Average interval from estrus to insemination was 18.5 hours (range: 12.5–27.5 hours).

<sup>3</sup>Average interval from estrus to insemination was 14.7 hours.

<sup>4</sup>Average interval from estrus to insemination was 17.9 hours.

<sup>5</sup>Average interval from estrus to insemination was 24.3 hours.