# Artificial Insemination of Beef Heifers with Multi-Sire Sexed Semen

Dempster M. Christenson
John G. Maddux
Jordan M. Thomas
Rick N. Funston


Figure 1. Spit-Time AI: Melengestrol acetate (MGA)—prostaglandin F2α (PG) protocol used for estrus synchronization in beef heifers. MGA is fed from Day 1 to 14 and PG is administered on Day 33 along with an estrus detection aid. Heifers exhibiting estrus by Day 36 or 37 are AI. All remaining heifers are administered gonadotropin releasing hormone and AI.


## Summary with Implications

*This study observed pregnancy outcomes resulting from artificial insemination of beef heifers with multi-sire sexed semen. It was hypothesized, pregnancy rates resulting from multi-sire sexed semen would be increased compared to pregnancy rates from prior studies using single-sire sexed semen. Producers looking to maximize the proportion of calves of one sex may consider this technique. Ultrasound determined pregnancy rate of heifers given multi-sire sexed semen was 65%, which is 12% greater than the average pregnancy rate reported in previous studies using single-sire sexed semen. After accounting for date of birth however, the adjusted AI conception rate was calculated to range between 55 and 62% with a calving rate between 51 and 57%. In summary, multiple sires may outperform single sires' pregnancy rate to artificial insemination with sexed semen.*


## Introduction

Multi-sire (aka. heterospermic or sperm pack) semen is rarely used for artificial insemination (AI) when assignment of calves' paternity is important, and the value of genotyping is too low. However, previous studies have shown pregnancy success increased 11—13% in heifers using multi-sire semen.

Sexed semen has been available for many years, but it has only recently become cost-effective for commercial producers. There are still challenges associated with utilizing sexed semen because it requires a more intensive protocol. Normal viability of conventional semen is >24 h following deposition, while the period of viability for sexed semen is generally considered to be much shorter. Due to differences among bulls, length of viability, reduced quantity of sperm per unit of sexed semen, and the timing of peak ovum viability, pregnancy rates are decreased among heifers not exhibiting estrus at the time of AI, particularly when sexed semen is used.

Bull semen resiliency can vary due to sorting and subsequent cryopreservation, and thus exhibit varied viability of sexed sperm cells post-deposition. A mixture of semen from multiple bulls in a semen straw may increase fertility and/or provide a longer period of optimal viability than that of an individual bull.

## Procedure

Twelve, unproven 5-way cross two year old bulls, born and raised on a ranch outside Imperial Nebraska were sent to ORIgen Inc. (Huntley, MT) for collection of semen. Bulls passed a breeding soundness exam and sperm passed post thaw evaluation. Sperm cells from three sires were pooled together in equal proportions and sexed (SexedULTRA4M) to favor female progeny with an expected 85–90% heifers and 15–10% bulls, forming four groups of multi-sire sexed semen straws (labeled: A, B, C, and D). Summer calving crossbred composite beef heifers (n=937) raised on the same ranch were transported to a feedlot on the ranch for estrus synchronization in June 2021. All heifers were assigned to the melengestrol acetate (MGA)—prostaglandin $F_{2a}$ (PG) protocol for split-time AI (Figure 1). In this protocol, MGA was mixed into a total ration from Day 1–14 and PG was administered on Day 33 along with an estrus detection aid. Heifers exhibiting estrus by Day 36 or 37 are AI. There were 841 heifers retained through the study: 706 AI with multi-sire sexed semen and 135 AI with conventional semen.

Heifers were estimated to weigh approximately 625 lb at feedlot entry and 725 lb at AI. At PG administration (2cc estroPLAN®), estrus detection patches (Estrotect™) were placed on the tail head. Patches were scored at AI (1 = < 25% removed, 2 = 25%—50% removed, 3 = > 50% removed, 4 = patch missing) with patch scores of 3 and 4 considered to be in estrus. Heifers exhibiting estrus after PG administration were removed from the herd at 72 h and 96 h for AI using the multi-sire sexed semen. Forty straws of semen from each sire group were administered in series to improve randomization of AI among heifers. Immediately following multi-sire sexed semen AI, the remaining heifers not previously removed from the herd by 96 h were administered 2cc Fertagyl (gonadotropin-releasing hormone) and artificially inseminated using conventional semen from proven sires unrelated to the original sires. Heifers sorted for exhibiting estrus could have been exhibiting estrus for 3–24 h before AI. Each day after AI, inseminated heifers were transported to pasture and placed with unrelated unproven bulls for a 60 d breeding season at heifer to bull ratio of 25:1.

At 101 d after timed-AI, pregnancy status was determined via transrectal ultrasonography, and fetal size determined age of the fetus. Based on fetal age, pregnant multi-sire sexed semen AI heifers were sorted into one of three groups: 1) 'EARLY' (80–101 d post AI; n = 457), 2) 'MIDDLE' (70–79 d post AI; n = 56), and 3) 'LATE' (<70 d post AI; n = 128). At birth, progeny sex and date of birth (DOB) were recorded. Heifers from the 'MIDDLE' and 'LATE' groups were not considered at parturition.

At parturition, calving rate was calculated based on the number of calves born from the EARLY group divided by the number of heifers administered multi-sire sexed semen. An adjusted conception rate was calculated based on the percentage of calves born in the EARLY group on each DOB multiplied by the number of heifers observed pregnant through ultrasound divided by the total number of heifers who received multi-sire sexed semen in order to account for heifers who lost their calf after pregnancy check. Heifer to bull rate was calculated by dividing the number of heifer calves born by the total number of calves born and was expected to approach 85–90% among heifers successfully bred by multi-sire sexed semen. Adjusted conception rate, calving rate, and heifer to bull rate were calculated for each DOB. The earliest DOB where heifer to bull rate equaled 85–90% was considered the last day heifers bred by multi-sire sexed semen would give birth, which ranged from 295–300 d post AI. Based on a DOB between 295 d and 300 d, a range for adjusted pregnancy rate and calving rate were assumed. In these calculations, twins were counted as one calf but were not considered as part of the heifer to bull rate. A control treatment was not used in this study and observations were compared to prior studies using single sire sexed semen.

**Table 1. Pregnancy results of heifers inseminated with conventional and sexed semen or bull bred by multi-sire groups and expected time of pregnancy.**

| | n | OPEN[1], % | EARLY[2], % | Adj[3], % | MIDDLE[4], % | LATE[5], % |
|---|---|---|---|---|---|---|
| Sexed | 706 | 9 | 65 | 55–62 | 8 | 18 |
| Sire group A | 197 | 9 | 62 | 50–57 | 10 | 19 |
| Sire group B | 176 | 11 | 69 | 56–66 | 6 | 14 |
| Sire group C | 187 | 5 | 69 | 63–68 | 8 | 18 |
| Sire group D | 146 | 12 | 58 | 50–54 | 8 | 23 |
| Conventional | 135 | 20 | 49 | | 6 | 25 |

[1]Open: Pregnancy was not observed through ultrasound after AI and a 60-day breeding period.

[2]EARLY: Fetal age was observed through ultrasound to be between 80 and 101 d post AI.

[3]Adjusted pregnancy rate was calculated based on the percentage of 'EARLY' calves born Day 295–300 post AI multiplied by the number of heifers observed pregnant through ultrasound to multi-sire sexed semen divided by the total number of heifers who received multi-sire sexed semen.

[4]MIDDLE: Fetal age was observed through ultrasound to be between 70 and 79 d post AI.

[5]LATE: Fetal age was observed through ultrasound to be less than 70 d post AI.

## Results

This observational study observed 87% of heifers expressed estrus with 83% inseminated with sexed semen and 4% inseminated with conventional semen. Sixty-four percent of heifers exhibited estrus by 72 h and 22% exhibited estrus between 72 h and 96 h. According to the protocol, 74% of heifers should exhibit estrus by 72 h and an additional 14% by 96 h totaling 88%, which suggests the estrus response was normal in the current study.

Overall pregnancy rate was 89% with 61% 'EARLY', 8% 'MIDDLE, and 19% 'LATE'. Within the 'EARLY' group, multi-sire sexed semen heifers had 65% pregnancy success and conventional semen heifers had 49% during the same time period (Table 1). Pregnancy rates of 53% have been reported in other studies utilizing single-sire sexed semen administered to heifers exhibiting estrus. The expected success rate of conventional semen on heifers not exhibiting estrus could be as low as 22%, but this does not account for the 4% of heifers that were exhibiting estrus and given conventional semen or the 20 day window in which some may have exhibited estrus later and been bred naturally by bulls in the pasture.

The four multi-sire groups had differing rates of success ranging from 58% to 69% (Table 1). Conventional sire success was combined due to much wider ranges of success between individual sires with low numbers of individual inseminations on heifers in various stages of their estrous cycle (Table 1). It is hypothesized the variability between sire groups may be a result of the viability of some individuals that make up that sire group or the random assignment of heifers. Given the 20–21 day window for the 'EARLY' group, some heifers may have been bull bred immediately or completed another estrous cycle before being bull bred 18–24 d later, resulting in a much later DOB. It is equally likely that heifers completing another estrous cycle after the end of the synchronization protocol would be successfully bull bred at the 'MIDDLE' time period. Any heifers that failed to retain a fertilized egg would have had the highest chance of re-fertilization by the 'LATE' time period. Despite the potential for sire misassignment, multi-sire sexed semen performed better than is normally expected from sexed semen, but scientific research is required to make these conclusions.

Separation of AI and bull bred heifers within the EARLY group was calculated using DOB and the accepted heifer to bull rate of 85–90% to informally attribute parentage, a concept that does not directly negate the aforementioned pregnancy results. Based on these rates it was assumed the heifers bred by multi-sire sexed semen gave birth by Day 295–300 post AI and all other calves born after this period were considered bull bred. During this period the calving rate ranged from 51–57%. Due to calf loss at or before calving, an adjusted pregnancy rate to AI was calculated based on the number of calves born and the number of heifers observed pregnant through ultrasound. Between Days 295 and 300 post AI, the adjusted pregnancy rate to AI ranged from 55–62%. The expected value of 53% for pregnancy rate to single-sire sexed semen is below this range, which suggests multi-sire sexed semen may have outperformed single-sire sexed semen in other studies. Adjusted pregnancy rate among each sire group (Table 1) was decreased. One sire group adjusted pregnancy rate ranged from 63–68%, but it is unknown if this is due to random chance or the sires that make up this group. In either scenario, the relationship between pregnancy rate and parentage to AI with multi-sire sexed semen requires more research.

## Conclusion

When the sex of the calf produced from a mating is an economically relevant consideration (e.g.: replacement heifers or seedstock), there may be value associated with the use of sexed semen. However, adoption of these technologies may be reduced due to variable pregnancy outcomes with sexed semen. Pregnancy rate determined by ultrasound suggests a 12% improvement compared to prior studies and pregnancy rate based on DOB suggests an improvement of as much as 9%. Results from the current study suggest sexed semen from 3 sires pooled together may have outperformed single sire sexed semen when heifers exhibiting estrus are inseminated during an MGA-PG split-time AI protocol. However, further observations will be needed to validate this technique and increase adoption.

Dempster M. Christenson, research technician and graduate student

John Maddux, producer, Imperial NE

Jordan M. Thomas, assistant professor, Animal Science (University of Missouri)

Rick N. Funston, full professor, animal science, West Central Research and Extension Center, North Platte NE