Artificial Insemination of Beef Heifers with Multi-Sire Semen

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Summary with Implications

This study compared pregnancy rates of beef heifers artificially inseminated with multi-sire semen to single-sire semen at ranch 1 and demonstrated pregnancy to multi-sire sexed semen at ranch 2. It was hypothesized pregnancy rates resulting from multi-sire semen would be increased compared to single-sire semen. Ranch 1 heifers were inseminated with either single-sire or multi-sire semen and all heifers expressing estrus at ranch 2 were inseminated with multi-sire sexed semen. Heifers inseminated with multi-sire semen averaged numerically greater pregnancy rate than the average single-sire pregnancy rate and pregnancy outcomes from multi-sire sexed semen exceed previous literature but cannot be directly compared. Despite similar pregnancy results between each single-sire treatment, paternity results suggest sires produce unequal proportions of offspring when their semen is mixed. In summary, producers looking to maximize pregnancy rate to artificial insemination may consider multi-sire insemination but more data is required.

Introduction

Multi-sire (aka. heterospermic or sperm pack) semen is rarely used for artificial insemination (**AI**) when assignment of paternity is important, and the value of genotyping is low. However, previous studies reported pregnancy success increased 11—13% in heifers inseminated with multi-sire semen compared to single-sire AI. This increase is believed to be the result of interactions between semen from different sires and differences in optimal viability between sires, which may optimize matching of the peak sperm and ovum viability.

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. Due to reduced semen quality after the sex sorting process, pregnancy rates are decreased among heifers not exhibiting estrus at the time of AI. Bull semen differ in resiliency to sex sorting and subsequent cryopreservation, and thus exhibit varied viability of sexed sperm cells post-deposition, but a mixture of semen may provide a longer period of optimal viability than an individual bull. The objective of this study was to compare pregnancy rates of beef heifers artificially inseminated with multi-sire semen to single-sire semen at ranch 1 and demonstrated pregnancy to multi-sire sexed semen at ranch 2.

Procedure

Ranch 1 utilized 441 Angus crossbred spring calving beef heifers (762 ± 64 lb) from Sutherland, NE in 2022. Estrus was synchronized with the melengestrol acetate—prostaglandin $F_{2\alpha}$ (**MGA-PG**) timed-AI protocol (Figure 1). Estrotect™ patches were applied to identify behavioral estrus before AI and the response was compared by treatment.

Three black Angus bulls were chosen for AI from the ABS Global (DeForest, WI) AI directory based on non-relation to each other and the heifers, availability for simultaneous collection, ranch management choice, and consistent prior AI success rate. One collection was made from each bull (1, 2, 3) and allotted to either the single sire treatments (**SS1, SS2, or SS3**; n = 75 each) or the multi-sire (**MS**; n = 216) treatment, which contained a one third sample from each bull. A breeding soundness exam was performed on all three bulls and the mixture of sperm, which determined sperm morphology, motility, and survivability exceeded industry standards.

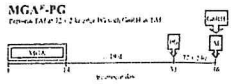

Fig. 1. Melengestrol acetate—prostaglandin $F_{2\alpha}$ (PG) timed artificial insemination protocol used for synchronization of estrus in ranch 1 beef heifers. Melengestrol acetate is fed from Day 1 to 14 and PG is administered on Day 33 along with an aid to detect estrus. Heifers are inseminated on Day 36 and heifers not exhibiting estrus are administered gonadrotropin releasing hormone. Beef Reproduction Task Force; BeefRepro.org.

Treatment assignment alternated between the three SS and MS treatments utilizing 10 semen straws for each SS and 30 straws for MS as heifers entered the chute before repeating. Unrelated bulls were introduced 7 days after AI and remained with the heifers for 29 days. Pregnancy rate to AI was determined by fetal aging using ultrasound 82 days post AI. Due to drought conditions, half the pregnant heifers were sold before calving, but 57 calves born to the remaining MS heifers were genetically tested (Quantum Genetix, Saskatoon, SK, Canada) to determine paternity within the MS treatment. Paternity of 49 SS calves were also confirmed.

Data were analyzed using PROC GLIMMIX of SAS 9.4 (Cary, NC USA). Heifer was the experimental unit. Pregnancy status and estrus response were analyzed as a response to each SS treatment, each SS treatment and the MS treatment, or the combined SS treatments and the MS treatment.

Ranch 2 is a field demonstration evaluating AI to multi-sire sexed semen that utilized 937 and 914 crossbred composite summer calving beef heifers, in year 1 and 2 respectively (2021 and 2022), from Imperial, NE. Estrus was synchronized with the MGA-PG split-time AI protocol, (Figure 2). Please see (*2023 Nebraska Beef Report*, pp 19–21) for a more thorough description of the procedure.

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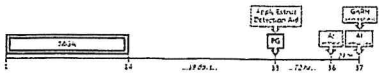

Fig. 2. Split-Time AI: Melengestrol acetate—prostaglandin F2α (PG) protocol used for synchronization of estrus in ranch 2 beef heifers. Melengestrol acetate is fed from Day 1 to 14 and PG is administered on Day 33 along with an aid to detect estrus. Heifers exhibiting estrus by Day 36 or 37 are inseminated with multi-sire sexed semen. All remaining heifers are administered gonadotropin releasing hormone and inseminated with conventional semen. Estrus Synchronization Recommendations for Artificial Insemination of Beef Heifers; extension.missouri.edu

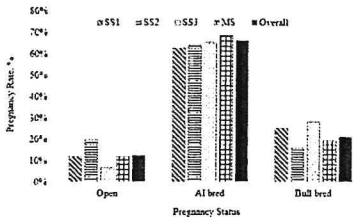

Fig. 3. Pregnancy rate to artificial insemination of semen from three bulls, a combined semen sample, and their average by pregnancy status after natural service at ranch 1.

Semen was collected from twelve 5-way cross bulls from year 1 and eight bulls from year 2, born and raised on ranch 2. Unlike ranch 1, semen volume and concentration were not equivalent between sires. Semen was sex sorted to favor female progeny (SexedULTRA4M) with progeny expected to be 85–90% heifers and 15–10% bulls. Semen from three random sires were mixed forming sire groups A, B, C, and D in year 1 and sire groups E, F, and G in year 2 (sire group G contained 2 bulls). Heifers exhibiting estrus were inseminated with sexed multi-sire semen on day 36 and 37 of the protocol. Different bulls were introduced immediately after AI for 60 days at a heifer to bull ratio of 25:1. Success of AI was determined by fetal aging using ultrasound 100 days post AI. Heifers determined pregnant within 20 days of AI were considered pregnant due to AI and all others were considered bull bred, but heifers considered to be bred to AI had a narrow opportunity to be bred by a different bull. At birth, progeny sex and date of birth (**DOB**) were recorded.

Due to the inability to differentiate between AI bred and bull bred heifers around the time of AI, pregnancy rate was recalculated based on DOB and the percentage of heifer and bull calves born, which was expected to approach 85–90% heifer calves among dams successfully bred by multi-sire sexed semen. The earliest DOB where heifer calves made up 85–90% of total progeny ranged from 295–300 days post AI in year 1 and ranged from 290–297 days post AI in year 2. Pregnancy rate and heifer calf percentage were calculated for each DOB where all calves born after the gestation date were considered bull bred. Based on a DOB between these time periods, a range for adjusted pregnancy rate was estimated. Genetic testing for paternity will be performed for year 2 to confirm pregnancy rate to AI and discover individual sire success within each multi-sire group but will not be completed until publishing. A control treatment was not used on Ranch-2 and observations cannot be directly compared to prior studies using single-sire sexed semen.

Results

Ranch 1 estrus detection results did not significantly differ among sire groups (SS1 = 64%, SS2 = 73%, SS3 = 75%, MS = 70%; P = 0.43). The average pregnancy rate was 66% with no significant differences between treatments (SS1 = 63%, SS2 = 64%, SS3 = 65%, MS = 69%; P = 0.80, Figure 3). Pregnancy rate averaged 64% in SS (SS1=63%, SS2=64%, SS3=65%) and 69% in MS (P=0.32). Pregnancy rate was not significantly different between MS and SS1 (P=0.36). When heifers did not express estrus before timed-AI, pregnancy rate was 51% in SS and 60% in MS (P=0.31). Paternity was determined in 57 MS calves. Within this random sample of the MS treatment population, bull 1 sired 7%, bull 2 sired 56%, and bull 3 sired 37%. Although bull 1 was much less successful than bull 2 or 3, the values are not significantly different due to the small sample size (P = 0.11 and P = 0.24, respectively). It is quite surprising to see such a disparity between the bulls given the pregnancy rate of each sire in the SS treatments were similar. There are several theories about the interaction of semen from different sires in the female tract, but the influence of bull 1 on MS pregnancy rate isn't clearly positive or negative without more data comparing MS and SS pregnancy rate.

Ranch 2 percentage of heifers expressing estrus (87% and 88% in year 1 and 2 respectively) and overall pregnancy rate after AI and 60 days of bull breeding (89% and 92% in year 1 and 2 respectively) were consistent with other studies using MGA-PG split-time AI. Pregnancy rate to multi-sire sexed semen was 65% in year 1 and 75% in year 2. Although they should not be compared directly, prior studies have averaged 53% pregnancy success to single-sire sexed semen. Pregnancy rate differed by sire group ranging from 57 to 69% in year 1 and 74 to 76% in year 2 (Table 1).

Date of birth was used to informally attribute parentage and recalculate pregnancy rate to an adjusted pregnancy rate but does not directly negate the aforementioned pregnancy results. Adjusted pregnancy rate was 55–62% in year 1 and 58–67% in year 2 (Table 1). For reference, the expected value of 53% for pregnancy rate to single-sire sexed semen is below these ranges, but these values cannot be directly compared. Adjusted pregnancy rate among each sire group (Table 1) was decreased. One sire group from year 1 adjusted pregnancy rate ranged from 63–68% and another sire group from year 2 adjusted pregnancy rate ranged from 62–72%, but it is unknown if this high pregnancy success rate 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. Greater detail on the results of year 1 can be found in (*2023 Nebraska Beef Report*, pp 19–21).

Conclusions

Methods that increase pregnancy rate to AI in heifers increase the productivity of the herd by increasing the lifetime productivity of those heifers and their progeny and decreasing the costs associated with development of heifers who take more time and feed to produce a calf. Ongoing research may indicate artificial insemination with multi-sire semen increases pregnancy rate to AI and increase consistency of results by improving pregnancy rate in heifers that do not exhibit estrus during a timed AI protocol. However, more research is required to solidify these conclusions and understand what unexplored interactions are influencing these benefits and causing the unequal

Table 1. Pregnancy results of heifers at ranch 2 inseminated with sexed semen by multi-sire groups

	n	OPEN[1], %	AI[2], %	Adj[3], %
Year 1 total	706	9	65	55–62
Sire group A	197	9	62	50–57
Sire group B	176	11	69	56–66
Sire group C	187	5	69	63–68
Sire group D	146	12	58	50–54
Year 2 total	763	7	75	58–67
Sire group E	232	7	74	50–62
Sire group F	241	7	76	62–72
Sire group G	290	7	76	59–66

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

[2]AI: 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 calves born day 295–300 (year 1) or 290–297 (year 2) 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. Gestation length was chosen based on the DOB when the percentage of heifer to bull calves equaled 85–90% heifers.

sire representation in progeny. Adoption of sexed semen AI is reduced due to economically relevant considerations by producers that may be improved if pregnancy rate to AI can be increased. Multi-sire sexed semen continues to show promise as a potentially improved method for AI with sexed semen, but no conclusions can be made without further study.

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