



# Conception risk of beef cattle after fixed-time artificial insemination using either SexedUltra™ 4M sex-sorted semen or conventional semen

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## ABSTRACT

The objective of this study was to compare conception rates of female beef cattle inseminated at a fixed-time with either conventional (**CON**) or SexedUltra™ sex-sorted (**SU**) semen. Treatments included CON or SU with two sires represented within each treatment. Cows ( $n = 316$ ) and heifers ( $n = 78$ ) from six locations were randomly assigned treatment. Ovulation was synchronized in all females using the industry-standard 7-d CO-Synch + controlled internal drug release (CIDR) protocol (100  $\mu$ g GnRH + CIDR [1.38 g progesterone] on d 0, 25 mg PGF2 $\alpha$  at CIDR removal on d 7, and 100  $\mu$ g GnRH on d 10, 54 h (heifers) or 60 h (cows) after CIDR removal). EstroTECT™ estrous detection aids were applied at CIDR removal and patch activation was recorded at insemination. Animals were assumed estrual if greater than 50% of the patch coating was removed. The results from this study indicated no main effects of treatment ( $P = 0.82$ ), sire ( $P = 0.64$ ), or age ( $P = 0.8$ ) on AI conception rates. Additionally, there were not significant interactions between sire and treatment ( $P = 0.19$ ) or age and treatment ( $P = 0.29$ ). There was however, a significant ( $P = 0.0005$ ) effect of estrous expression on conception rates. Conception rate for estrual females (62.8%) was greater ( $p = 0.0001$ ) than non-estrual females (38.7%) at FTAI regardless of treatment. Furthermore, the conception rates were similar ( $P = 0.61$ ) between conventional (61.9%) and sex-sorted semen (63.8%) when estrus was expressed prior to FTAI. Larger studies are warranted to determine appropriate timing of insemination with sex-sorted semen in FTAI protocols to maximize pregnancy potential.

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## 1. Introduction

Estrous synchronization and artificial insemination are reproductive technologies that beef cattle producers are progressively using to improve the reproductive performance of their herds. Benefits of estrous synchronization and artificial insemination include a more condensed calving season, increased pregnancy rates, increased calf uniformity, and increased weaning weights [1–3]. Fixed-time artificial insemination (**FTAI**) allows all females to be inseminated at the same time and reduces labor requirements. Protocols for FTAI are designed to maximize the

number of females that are in estrus before the time of breeding and eliminate the need for estrus detection. Condensing the calving season increases calf uniformity and allows producers to sell calves in larger lot sizes. Selling a group of five calves resulted in an increase of \$11/cwt over selling an individual animal [4]. Cows enrolled in a FTAI program had increased weaning percentages, weaning weight per cow exposed, and a net advantage of \$49.14 compared with natural service without synchronization [2].

Controlling the gender of the calf can also improve the opportunity for increased revenue and profit. In the dairy industry, increasing the number of females born is desirable, as male calves generally have lower economic value [5]. Commercial dairies have targeted use of sex-sorted semen in heifers rather than cows, as they have greater levels of fertility [6]. In commercial beef production, steer calves are more valuable than their heifer

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counterparts [7]. Producers are able to shift and/or control the gender ratio of their calf crop by incorporating sex-sorted semen into their AI programs. Previous research however, has observed decreased AI conception rates when sex-sorted semen was used in comparison with conventional semen of the same sires [8–10]. Recent research suggests that SexedUltra™ sex-sorted semen can be effectively used with good conception rates in a split-timed AI program in beef heifers [9,11]. In a split-timed AI program, FTAI occurs around 66 h for females that have expressed estrus and insemination is delayed by 20 h after GnRH is administered for females that fail to express estrus before 66 h. Delayed insemination for non-estrus females requires an additional handling of those females and therefore increases labor requirements. Currently, research is lacking comparing conception rates in cows and heifers after insemination with either conventional or sex-sorted semen using industry-standard protocols for FTAI. The objective of this study is to determine if conception rates will differ in females inseminated with conventional semen or sex-sorted semen when ovulation is synchronized using the 7-d CO-Synch + Controlled Internal Drug Release (CIDR) protocol for FTAI.

## 2. Materials and methods

The experimental procedures in this project were approved by the Institutional Animal Care and Use Committee at the University of Kentucky, protocol number 2016–2546.

### 2.1. Animals

Crossbred lactating beef cows and yearling beef heifers ( $n = 394$ ) across six locations were subjected to ovulation synchronization using the 7-d CO-Synch + CIDR protocol (Supplemental Fig. 1). Animals were administered GnRH (100 µg, i.m.; Factrel®, Zoetis, Parsippany, NJ) and an Eazi-Breed™ CIDR insert (1.38 g progesterone, Zoetis, Parsippany, NJ) on day 0. The CIDR insert was removed, animals were injected with dinoprost tromethamine (25 mg, Lutalyse®, Zoetis, Parsippany, NJ) and estrous detection patches were applied on day 7 (EstroTECT™, Rockway Inc., Spring Valley, WI). The condition of the patches was observed at insemination and they were recorded as: estrual (patch > 50% activated; orange), proestrual (patch < 50% activated; orange), non-estrual (not activated; gray), or unknown (missing). Fixed-time AI occurred at 54 h for yearling heifers ( $n = 78$ ) and 60 h for mature cows ( $n = 316$ ) following CIDR removal. All females were administered GnRH (100 µg, im) at FTAI. Within age group (yearling heifer, two-year-old, mature cow) animals were randomly assigned to treatment. Treatments consisted of conventional semen (CON) or SexedUltra™ 4M sex-sorted semen (SU). Two sires (A and B) were used in each treatment.

### 2.2. Semen treatments

Semen from two bulls from contemporaneous ejaculates were processed as either sex-sorted semen using the SexedUltra™ procedure or as conventional semen. The conventional semen was packaged at  $25\text{--}40 \times 10^6$  cells per 0.5 ml straw. SexedUltra™ 4M sex-sorted semen (Sexing Technologies, Navasota, TX) was processed using the technology described by Gonzalez-Marin et al. (2017) and using the flow cytometry process previously described by Sharpe and Evans (2009) [12,13]. Sex-sorted semen was packaged at  $4 \times 10^6$  cells per 0.25 ml straw. All inseminations were performed by the same technician at all locations.

### 2.3. Pregnancy diagnosis

Conception rate to AI was determined via transrectal ultrasonography (Ibex Pro equipped with an 8-5 MHz, E.I. Medical Imaging, Loveland, CO) at 47–58 days post FTAI.

### 2.4. Statistical analysis

All statistical analyses were conducted using the SAS (version 9.4; SAS Inst. Inc.) statistical program. Conception rates to AI were analyzed using the GLIMMIX procedure using the binomial distribution, link logit function. The model consisted of location, age, age by treatment, sire, treatment, and the sire by treatment interaction. All variables were analyzed as fixed factors except for location. In order to analyze the impact of estrous expression, an additional model for conception rates to AI was created using the GLIMMIX procedure with the binomial distribution, link logit function. Variables in this model included location, age, age by treatment, sire, treatment, sire by treatment, estrous expression, and the estrus expression by treatment interaction. Again, all factors except for location were analyzed as fixed factors. Location was analyzed as a random variable in the model in order to increase the inference of the study and location means are not of interest. Animal age was analyzed as a fixed variable since it was classified as one of three categories, instead of using actual days of age. Least squares means were generated using these models and were assessed using pairwise comparisons. For all statistical analyses, differences were considered significant at  $P < 0.05$ .

## 3. Results

The number of females represented in each age group and treatment within location are shown in Table 1. Sizes of these operations ranged from 11 to 184 head; with an average herd size of 66 head. No main effects of treatment ( $P = 0.82$ ), sire ( $P = 0.64$ ) or age ( $P = 0.8$ ) on AI conception rates were observed. Additionally, there was not a significant interaction between sire and treatment ( $P = 0.19$ ), age by treatment ( $P = 0.29$ ), or patch by treatment ( $P = 0.13$ ). The conception rates by sire and treatment are displayed in Table 2.

The EstroTECT™ patch status seemed to influence AI conception rates ( $P = 0.0005$ ). The four EstroTECT™ patch categories were

**Table 1**  
Number of females based on location, age<sup>a</sup>, and treatment<sup>b</sup>.

Location	Age <sup>a</sup>	Treatment <sup>b</sup>	
		Conventional	SexedUltra™ 4M
1	1	1	1
	2	1	1
	3	4	4
2	2	1	0
	3	5	5
	3	18	16
3	2	4	3
	3	72	71
	4	4	4
4	1	4	4
	2	4	5
	3	8	8
5	1	10	8
	6	8	8
6	2	11	10
	3	50	49

<sup>a</sup> Female age was classified as 1 = yearling heifer, 2 = two-year old, and 3 = mature cow. <sup>b</sup> Females in the conventional treatment received conventional, non-sex-sorted semen and females in the SexedUltra treatment received SexedUltra 4M sex-sorted semen.

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