Theriogenology 123 (2019) 100–107

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Evaluation of SexedULTRA 4M™ sex-sorted semen in timed artificial insemination programs for mature beef cows

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ARTICLE INFO

Article history:
Received 26 July 2018
Received in revised form
12 September 2018
Accepted 26 September 2018
Available online 27 September 2018

ABSTRACT

An experiment was designed to compare fertility of SexedULTRA 4M™ sex-sorted semen and conventional, non-sex-sorted semen following either fixed-time artificial insemination (FTAI) or split-time artificial insemination (STAI) of mature suckled beef cows. Units of sex-sorted and conventional semen were produced using contemporaneous ejaculates from three commercially available sires. Units of conventional semen were generated with 25.0×10^6 live cells per 0.25 ml straw prior to freezing, and units of sex-sorted semen were generated using the SexedULTRA$^{\mathbf{TM}}$ Genesis III sorting technology with 4.0×10^6 live cells per 0.25 ml straw prior to freezing. Sex-sorted units were sorted to contain X chromosome-bearing sperm cells at an accuracy level of >90%. Cows ($n = 1620$) across four herds were treated with the 7-d CO-Synch + CIDR protocol [administration of gonadotropin-releasing hormone (GnRH) and insertion of a progesterone insert (CIDR) on Day -10, followed by administration of prostaglandin $F_{2\alpha}$ (PG) and removal of CIDR inserts on Day -3]. Cows were preassigned based on age, body condition score, and days postpartum to one of the following four treatments: FTAI with SexedULTRA 4M™ sex-sorted semen, FTAI with conventional semen, STAI with SexedULTRA 4M™ sex-sorted semen, or STAI with conventional semen. On Day -3, estrus detection aids (Estrotect®) were applied. For cows in FTAI treatments, AI was performed on Day 0 at 66 h after PG administration and CIDR removal, and 100 μg GnRH was administered concurrent with AI. For cows in STAI treatments, AI was performed on either Day 0 or 1, at 66 or 90 h after PG administration and CIDR removal, based on timing of estrus expression. On Day 1 at 90 h after PG administration and CIDR removal, 100 μg GnRH was administered concurrent with AI to any STAI-treated cows that had failed to express estrus. Pregnancy rates to AI were affected ($P = 0.04$) by the interaction of bull and semen type. Greater pregnancy rates were obtained with conventional semen versus SexedULTRA 4M™ sex-sorted semen when using semen from Bull A (64% [176/277] versus 36% [100/278]; $P < 0.0001$) and Bull B (72% [200/277] versus 57% [156/276]; $P < 0.01$), whereas pregnancy rates to AI did not differ between conventional and SexedULTRA 4M™ sex-sorted semen when using semen from Bull C (58% [149/258] versus 52% [131/254]). Pregnancy rates did not differ significantly between cows inseminated using a STAI versus FTAI approach, regardless of whether insemination was performed with conventional semen (65% [265/409] versus 65% [260/403] or SexedULTRA 4M™ sex-sorted semen (50% [200/403] versus 48% [187/405]). However, due to the additional 24 h for potential estrus expression when performing STAI, total estrous response prior to AI was greater ($P < 0.001$) among cows receiving STAI (84%; 686/812) compared to FTAI (72%; 585/808), and greater pregnancy rates ($P < 0.0001$) were obtained among cows that expressed estrus prior to AI. In summary, the relative fertility of SexedULTRA 4M™ sex-sorted semen and conventional semen varied across bulls. Although overall pregnancy rates to timed AI did not differ between STAI and FTAI approaches, use of a STAI approach allowed for greater total estrous response prior to AI. Therefore, to achieve acceptable conception rates per unit and service the maximum number of cows with sex-sorted semen, one viable approach may be to use STAI

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https://doi.org/10.1016/j.theriogenology.2018.09.039

to maximize total estrous response and restrict use of SexedULTRA 4M™ sex-sorted to only those cows expressing estrus.

1. Introduction

Technologies that allow for predetermination of progeny sex have long been of interest to commercial animal agriculture due to inherent value differences between male and female progeny. Although which sex is more valuable differs across animal industries and within various segments of each industry, cost-effective preselection of progeny sex presents an opportunity to enhance the profitability and sustainability of animal agriculture systems. One such example is the dramatically increased rate of genetic progress facilitated through combined use of sex-predetermination technologies and genomic selection technologies [1–3].

Currently, flow-cytometric cell sorting can be used effectively to generate sorted populations of X- or Y-chromosome-bearing bovine sperm cells for use in commercial artificial insemination (AI) programs [4–7]. However, fertility of frozen-thawed sex-sorted semen has generally been reduced relative to conventional, non-sex-sorted semen [8]. Reduced fertility of sex-sorted semen has been attributed to several factors, namely the reduced number of sperm cells placed in units of sex-sorted semen and the fact that sperm cells undergo cellular damage during the sorting, cryopreservation, and thawing processes. Timing of insemination relative to ovulation affects fertility with conventional semen, likely in a bull dependent manner [9–13]. As a result of damage occurring to sperm cells during the sorting process, the time window for optimal fertility is likely narrower and closer to ovulation when using sex-sorted semen [14,15]. Therefore, use of sex-sorted semen in fixed-time artificial insemination (FTAI) programs has generally been discouraged [16,17].

Recently, sex-sorted semen produced using an enhanced proprietary sex-sorting technology has become commercially available for both dairy and beef breeds under the trade name SexedULTRA**TM** (Sexing Technologies, Navasota, TX). Improvements made to the technologies and processes used to produce sex-sorted semen now allow units of SexedULTRA**TM** sex-sorted semen to be generated economically at 4.0×10^6 cells per unit, as compared to the 2.0×10^6 cells per unit that had previously become the industry standard dose for sex-sorted semen [6,18,19]. Extensive evaluations of SexedULTRA**TM** semen have indicated both improved fertility associated with the SexedULTRA**TM** process in comparison to sex-sorted semen produced using previous generation technology [20] as well as improved fertility when using the higher dose rate of 4.0×10^6 cells per unit of SexedULTRA 4M™ [21]. Research efforts also suggest improved comparability between pregnancy rates obtained using SexedULTRA 4M™ sex-sorted semen and those obtained using conventional semen [21]. Due in large part to these improvements in fertility, an increasing number of producers perceive sex-sorted semen as a viable technology. Dairy industry data compiled by the United States Department of Agriculture indicate increases both in use of sex-sorted semen and in the relative pregnancy rates of sex-sorted compared to conventional semen between 2007 and 2015 [22]. However, U.S. beef producers have been much slower to adopt use of sex-sorted semen, in large part because decreases in first service pregnancy rates present a high indirect cost due to the seasonality of beef production systems.

In addition to basic research efforts to improve the quality of sex-sorted semen, translational research efforts in estrous cycle control may also result in strategies that improve results when using sex-sorted semen for timed AI of beef cows and heifers. For example, strategies that maximize estrous response and enhance synchrony of estrus expression may offer improvements in timed AI pregnancy rates specifically with sex-sorted semen. In other publications, pregnancy rates following FTAI with sex-sorted semen have been particularly low among beef females that have not expressed estrus prior to FTAI [23–25]. A disparity between FTAI pregnancy rates of estrous and non-estrous females is also observed with conventional semen (see meta-analysis by Richardson et al. [26]), although we have previously proposed the degree to which pregnancy rates of estrous and non-estrous females differ may be bull dependent and may be exacerbated by the sex-sorting process [27].

Previous work from our lab led to the development of a timed AI approach known as split-time artificial insemination (STAI), in which AI is delayed by 20-24 h for females failing to express estrus prior to the standard time of FTAI. This approach was first found to improve pregnancy rates with sex-sorted semen among mature beef cows following the 7-d CO-Synch + CIDR protocol [25]. Subsequent work with conventional semen demonstrated similarly improved fertility among beef heifers following the 14-d CIDR-PG protocol [28] and among beef cows following the 7-d CO-Synch + CIDR protocol [29]. Research efforts were undertaken to evaluate the effectiveness and optimal timing of administration of gonadotropin-releasing hormone (GnRH) when performing STAI [30,31]. Together, these experiments and subsequent similarly designed experiments from other labs [32–34] largely support the conclusion that STAI is an effective opportunity for producers to optimize pregnancy rates to timed AI.

In a recent experiment, Thomas et al. [35] evaluated use of SexedULTRA™ sex-sorted semen in comparison to conventional semen when performing STAI among beef heifers following the 14-d CIDR-PG protocol. Although pregnancy rates were greater among heifers inseminated with conventional semen (60%), the authors noted the pregnancy rates observed among heifers inseminated with SexedULTRA 4M™ sex-sorted semen (52%) were well within the range of what many producers may consider acceptable in commercial production settings. To date, however, use of SexedULTRA 4M™ sex-sorted semen has not been evaluated for timed AI of mature beef cows, nor has the relative effectiveness of STAI versus FTAI when using SexedULTRA 4M™ sex-sorted semen. Therefore, the present study was designed as a two-by-two factorial to compare pregnancy rates following either FTAI or STAI with either conventional or SexedULTRA 4M™ sex-sorted semen.

2. Materials and methods

All experimental procedures were approved by the University of Missouri Animal Care and Use Committee.

2.1. Semen collection

Semen was collected from three Angus bulls (age 26–29 months at the time of collection) commercially available for AI, and units of SexedULTRA 4M™ sex-sorted and conventional semen were produced from contemporaneous ejaculates. Semen was collected over an 11 d period (May 16th through 26th, 2017) for Bull A, a 15 d period (June 2nd through June 16th, 2017) for Bull B, and an 11 d period (June 6th through 16th, 2017) for Bull C. For Bull A, semen from four ejaculates was used, with two ejaculates used for production of sex-sorted semen and two for conventional. For Bull B, semen from six ejaculates was used, with four ejaculates used for production of sex-sorted semen and two for conventional. For Bull C, semen from five ejaculates was used, with four ejaculates used for production of sex-sorted semen and one for conventional. All the semen passed the standard quality control criteria use for sex-sorted and conventional semen respectively. Units of conventional semen were generated with 25.0×10^6 live cells per 0.25 ml straw prior to freezing, based on sperm cell concentrations commonly used in industry. Units of sex-sorted semen were generated using the SexedULTRA™ Genesis III sorting technology (Sexing Technologies, Navasota, TX) with 4.0×10^6 live cells per 0.25 ml straw prior to freezing, based on sperm cell concentrations typically used in SexedULTRA 4M™ sex-sorted semen. Sex-sorted units were sorted to contain X chromosome-bearing sperm cells at an accuracy level of >90%, based on the accuracy level at which SexedULTRA™ sex-sorted semen is typically produced and marketed.

2.2. Animals and treatments

Estrus was synchronized for 1620 suckled beef cows of varying age and parity across four herds. Estrus synchronization was performed using the 7-d CO-Synch + CIDR protocol as follows: administration of a GnRH analogue (100 μg gonadorelin acetate [Fertagyl®, Merck Animal Health, Madison, NJ] in Locations 1 and 3, and 100 μg gonadorelin diacetate tetrahydrate [Cystorelin®, Merial, Athens, GA] in Locations 2 and 4,) and insertion of a 1.38 g progesterone insert (CIDR®; Zoetis, Madison, NJ) on Day -10, followed by administration of 500 μg cloprostenol sodium (PG; Estrumate®, Merck Animal Health, Madison, NJ) and removal of CIDR inserts on Day -3. On Day -3, estrus detection aids (Estrotect®, Rockway Inc, Spring Valley, WI) were applied to determine estrous status of animals at AI. Estrus expression was defined as removal of >50% of the rub-off coating on the estrus detection aid.

Within each location, cows were preassigned to balanced treatments based on age, days postpartum, and an assessment of body condition score (BCS) using a 1 to 9 scale (1 = emaciated and 9 = obese) on Day -3 [36]. Cows were assigned to one of the following four treatments: FTAI with SexedULTRA 4M™ sex-sorted semen (FTAI-SS), FTAI with conventional semen (FTAI-CON), STAI with SexedULTRA 4M™ sex-sorted semen (STAI-SS), or STAI with conventional semen (STAI-CON). For cows in FTAI treatments, AI was performed on Day 0 at 66 h after PG administration and CIDR removal, and 100 μg GnRH was administered concurrent with AI. For cows in STAI treatments, AI was performed on either Day 0 or 1, at 66 or 90 h after PG administration and CIDR removal, based on timing of estrus expression. Cows having expressed estrus by 66 h after PG administration and CIDR removal received timed AI at that time, whereas insemination was delayed by 24 h until 90 h for cows failing to express estrus by 66 h. On Day 1 at 90 h after PG administration and CIDR removal, 100 μg GnRH was administered concurrent with AI to any STAI-treated cows that had failed to express estrus. A diagram of the AI approaches used in the FTAI versus STAI treatments is presented in Fig. 1.

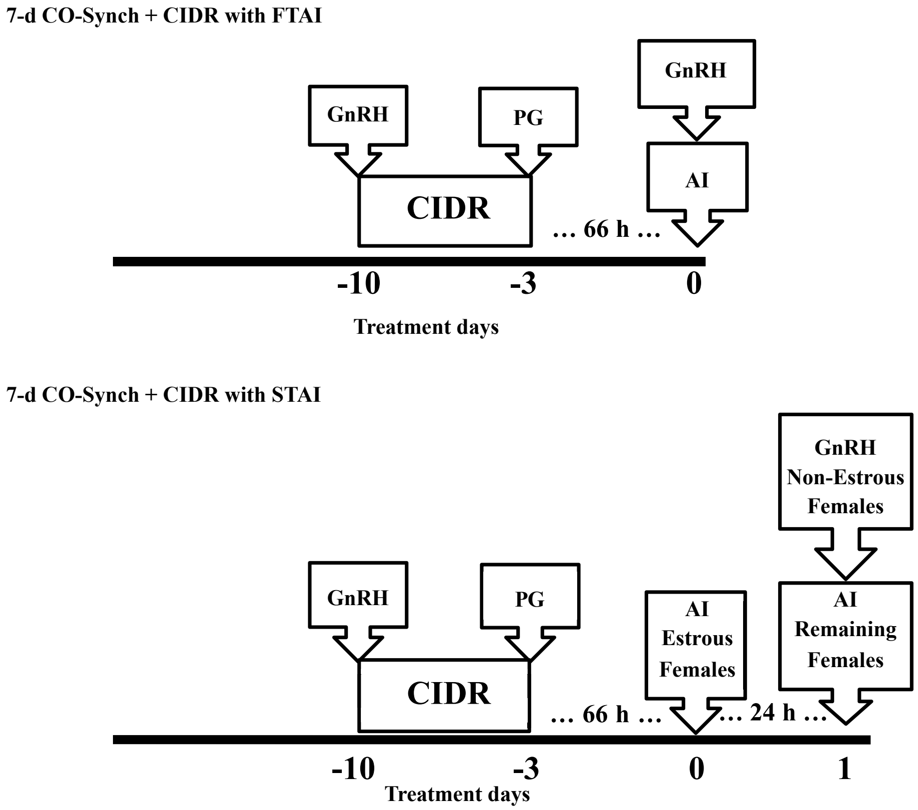

Fig. 1. Treatment schedule for the 7-d CO-Synch + CIDR protocol with fixed-time AI (FTAI) or split-time AI (STAI). Cows received a 1.38 g progesterone (CIDR) insert and GnRH administration (100 μg im) on Day -10. On Day -3, CIDR inserts were removed, PG (25 mg im) was administered, and estrus detection aids (Estrotect) were applied. On Day 0, 66 h after PG administration, cows in the FTAI treatments received GnRH administration and AI was performed. Cows in the STAI treatments received AI at this time if detection aids were activated. On Day 1, 90 h after PG administration, all remaining cows in the STAI treatment received AI, and GnRH was administered to cows with non-activated estrus detection aids.

2.3. *Artificial insemination*

Artificial insemination was performed with either conventional or SexedULTRA 4M™ sex-sorted semen based on treatment. A single technician, blinded to semen type, performed AI in each location. Sires were used proportionally across all treatments in all locations and were preassigned to cows based on age, DPP, and BCS. Fourteen d after AI, cows were exposed to fertile bulls for the remainder of a 60 d breeding season.

2.4. *Pregnancy diagnosis*

Pregnancy rate to AI was determined by transrectal ultrasonography (SonoSite EDGE equipped with a L52 10.0–5.0 MHz linear-array transducer; SonoSite Inc., Bothell, WA). Pregnancies resulting from AI were distinguished from those resulting from natural service based on fetal size [37,38], as cows were not exposed to natural service bulls until 14 d after AI. Ultrasonography was performed 84–95 d after AI in each location.

2.5. *Statistical analyses*

Potential treatment differences for age, DPP, and BCS were analyzed using the ANOVA procedure of SAS (SAS Inst. Inc., Cary, NC). Chi-square contingency tables (PROC FREQ; SAS Inst. Inc., Cary, NC) were used to analyze treatment differences in estrous response rates, pregnancy rates based on estrous response, and final pregnancy rates at the end of the breeding season. Pregnancy rates to AI were analyzed using a generalized linear model via the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) using the binomial distribution, link logit function. The model included AI approach, semen type, bull, AI approach x semen type, AI approach x bull, semen type x bull, and AI approach x semen type x bull. Location and bull were included as random effects, and the error term was specified as the interaction of location x bull x AI approach x semen type. Least squares means were generated using this model and compared using Fisher's least significant difference.

Table 1
Cow age, days postpartum (DPP), and body condition score (BCS) by treatment and location (mean ± SD).

Treatment[a] Location	N	Age	DPP	BCS[b]
FTAI Conventional	403	4.6 ± 2.2	80.6 ± 19.4	5.7 ± 0.6
Location 1	39	4.2 ± 1.8	67.5 ± 18.8	5.7 ± 0.4
Location 2	107	4.8 ± 2.6	91.1 ± 21.4	5.8 ± 0.6
Location 3	134	4.6 ± 2.0	72.9 ± 20.2	5.7 ± 0.6
Location 4	123	4.5 ± 2.3	82.8 ± 9.4	5.5 ± 0.5
STAI Conventional	409	4.6 ± 2.2	81.2 ± 17.8	5.7 ± 0.6
Location 1	38	4.3 ± 2.0	67.2 ± 16.3	5.9 ± 0.5
Location 2	108	4.9 ± 2.5	91.3 ± 19.4	5.8 ± 0.6
Location 3	137	4.7 ± 2.2	74.4 ± 17.8	5.8 ± 0.7
Location 4	126	4.4 ± 2.0	82.6 ± 10.2	5.6 ± 0.5
FTAI SexedULTRA 4M™	405	4.7 ± 2.2	80.8 ± 18.9	5.7 ± 0.6
Location 1	38	4.4 ± 1.9	69.9 ± 11.5	5.7 ± 0.6
Location 2	110	4.8 ± 2.5	92.1 ± 20.5	5.8 ± 0.6
Location 3	136	4.7 ± 2.1	71.5 ± 19.7	5.9 ± 0.7
Location 4	121	4.5 ± 2.2	82.2 ± 11.0	5.6 ± 0.5
STAI SexedULTRA 4M™	403	4.6 ± 2.2	80.1 ± 19.8	5.7 ± 0.6
Location 1	38	4.3 ± 2.0	66.3 ± 15.0	5.8 ± 0.5
Location 2	104	4.6 ± 2.3	92.1 ± 20.0	5.8 ± 0.6
Location 3	140	4.8 ± 2.1	71.8 ± 21.8	5.8 ± 0.6
Location 4	121	4.5 ± 2.2	82.2 ± 10.4	5.6 ± 0.5

[a] Cows received either conventional or SexedULTRA 4M™ sex-sorted semen in either a split-time artificial insemination (STAI) or fixed-time artificial insemination (FTAI) approach. See Fig. 1 for a depiction of timing of AI in STAI and FTAI treatments.

[b] Body condition score (mean ± SE) of cows at treatment assignment on Day 0 (1–9 scale, where 1 = emaciated and 9 = obese).

3. Results

Cow age, DPP, and BCS are presented for each treatment and location in Table 1. Treatments did not differ with respect to mean cow age, DPP, and BCS. Cows receiving FTAI were inseminated 66.9 ± 1.0 h (mean ± SD) after PG administration and CIDR removal. Cows receiving STAI and expressing estrus prior to this time point were inseminated 66.8 ± 0.9 h after PG administration and CIDR removal, and STAI-treated cows failing to express estrus by this time point were inseminated 89.5 ± 1.2 h after PG administration and CIDR removal. Rates of estrous response are presented for each treatment and location in Table 2. Treatments did not differ with respect to the proportion of cows expressing estrus prior to 66 h after PG administration and CIDR removal. However, due to the additional 24 h for potential estrus expression prior to AI when STAI is performed, total estrous response prior to AI was increased ($P < 0.0001$) among cows in STAI treatments (84%; 686/812) compared to FTAI treatments (72%; 585/808).

Across treatments, pregnancy rates to AI (Table 3) were affected by estrous response at 66 h after PG administration and CIDR removal. Cows expressing estrus prior to this time achieved greater ($P < 0.0001$) pregnancy rates to AI (61%; 701/1157) than cows failing to express estrus prior to this time (46%; 211/463). Likewise, in STAI treatments, greater ($P < 0.0001$) pregnancy rates to AI were obtained among cows expressing estrus during the 24 h delay period (60%; 68/114) than among cows failing to express estrus by 90 h (33%; 42/126). However, overall pregnancy rates to AI did not differ

Table 2
Estrous response based on treatment[1] and location.

Treatment Location	Estrous Response Prior to FTAI Time[2]		Estrous Response During 24 h Delay[3]		Total Estrous Response[4]	
	Proportion	%	Proportion	%	Proportion	%
FTAI Conventional	295/403	73			295/403	73[a]
Location 1	27/39	69			27/39	69
Location 2	88/107	82			88/107	82
Location 3	87/134	65			87/134	65
Location 4	93/123	76			93/123	76
STAI Conventional	292/409	71	53/409	13	345/409	84[b]
Location 1	27/38	71	5/38	13	32/38	84
Location 2	81/108	75	14/108	13	95/108	88
Location 3	87/137	64	20/137	15	107/137	78
Location 4	97/126	77	14/126	11	111/126	88
FTAI SexedULTRA 4M™	290/405	72			290/405	72[a]
Location 1	19/38	50			19/38	50
Location 2	91/110	83			91/110	83
Location 3	85/136	63			85/136	63
Location 4	95/121	79			95/121	79
STAI SexedULTRA 4M™	280/403	69	61/403	15	341/403	85[b]
Location 1	27/38	71	7/38	18	34/38	89
Location 2	89/104	86	9/104	9	98/104	94
Location 3	74/140	53	26/140	19	100/140	71
Location 4	90/121	74	19/121	16	109/121	90

[1] See Table 1 for a description of treatments.

[2] Estrous response prior to 66 h after CIDR removal and PG administration.

[3] Estrous response occurring during the 24 h delay period from 66 to 90 h after CIDR removal and PG administration.

[4] Total cumulative estrous response.

[ab] Rates of total estrous response with different superscripts differ ($P < 0.0001$).

between cows inseminated using a FTAI versus STAI approach, regardless of whether insemination was performed with conventional semen (FTAI: 65%; STAI: 65%) or SexedULTRA 4M™ sex-sorted semen (FTAI: 48%; STAI: 50%).

Pregnancy rates to AI (Table 4) were also affected by semen type ($P < 0.0001$) and the interaction of semen type and bull ($P < 0.01$). Across bulls, greater pregnancy rates ($P < 0.0001$) were obtained among cows inseminated with conventional semen (65%; 525/812) compared to cows inseminated with SexedULTRA 4M™ sex-sorted semen (48%; 387/808). Greater pregnancy rates were obtained with conventional semen versus SexedULTRA 4M™ sex-sorted semen when using semen from Bull A (63% [176/277] versus 36% [100/278]; $P < 0.0001$) and Bull B (72% [200/277] versus 57% [156/276]; $P < 0.001$), whereas pregnancy rates to AI did not differ between conventional and SexedULTRA 4M™ sex-sorted when using semen from Bull C (58% [149/258] versus 52% [131/254]). At locations in which fetal sex was determined via ultrasound among cows conceiving to AI, the proportion of cows carrying a heifer calf was greater ($P < 0.0001$) among cows serviced with SexedULTRA 4M™ sex-sorted semen (95%; 109/115) compared to conventional semen (53%; 85/161). Final pregnancy rate at the end of a 60 d breeding season did not differ between cows that had received AI with conventional semen (94%; 767/812) and those that had received SexedULTRA 4M™ sex-sorted semen (94%; 761/808).

4. Discussion

In many publications involving use of sex-sorted semen, pregnancy rates following AI with sex-sorted semen have been found to be reduced in comparison to pregnancy rates following AI with conventional semen. Specifically, publications reporting FTAI results using sex-sorted semen highlight the unique challenges associated with use of this product in timed AI programs [23,25,39–41]. Expanded use of sex-sorted semen in the beef industry will likely only occur if sufficiently high pregnancy rates can be obtained when using sex-sorted semen in timed AI systems, as the labor and time commitments associated with estrus detection-based programs present a significant challenge for many beef producers [42].

In the present study, pregnancy rates to timed AI were reduced among beef cows inseminated with SexedULTRA 4M™ sex-sorted semen. Reduced pregnancy rates to timed AI are effectively an indirect cost to producers when using sex-sorted semen [41,43]. Depending on the degree to which pregnancy rates are reduced, this reduction in pregnancy rates may in fact be the largest cost associated with use of sex-sorted semen. However, the magnitude of the pregnancy rates obtained with SexedULTRA 4M™ sex-sorted semen in this trial should be noted (48%; 387/808). In a recent publication evaluating use of SexedULTRA 4M™ sex-sorted semen for timed AI of beef heifers following the 14-d CIDR-PG protocol [35], pregnancy rates observed following STAI with SexedULTRA 4M™ sex-sorted semen (52%; 218/422) were similar to those observed in the present study among cows receiving SexedULTRA 4M™ sex-sorted semen. Although lower than the pregnancy rates that may be obtained when using conventional semen, pregnancy rates in this range when using sex-sorted semen may still be commercially acceptable in certain production settings when the value difference between male and female calves is sufficiently large. Further work is needed to evaluate the economic implications of use of SexedULTRA 4M™ sex-sorted semen for timed AI across different segments of the beef industry.

In contrast to previous work comparing STAI and FTAI approaches, no difference in pregnancy rates was observed between cows in STAI treatments and cows in FTAI treatments. However, this lack of effect may be due in part to exceptionally high rates of estrous response observed by 66 h after PG administration and CIDR removal in some herds (Locations 2 and 4). In addition to limiting statistical power to detect treatment differences among

Table 3

Pregnancy rates[1] to timed artificial insemination within location based on treatment[2] and estrous response[3].

Treatment Location	Overall		Estrous by 66 h		Non-Estrous by 66 h		Estrous 66–90 h		Non-Estrous by 90 h	
	Proportion	%	Proportion	%	Proportion	%	Proportion	%	Proportion	%
FTAI Conventional	260/403	65^{x}	200/295	68^{a}	60/108	56^{b}				
Location 1	25/39	64	18/27	67	7/12	58				
Location 2	80/107	75	67/88	76	13/19	68				
Location 3	78/134	58	54/87	62	24/47	51				
Location 4	77/123	63	61/93	66	16/30	53				
STAI Conventional	265/409	65^{x}	203/292	70^{a}	62/117	53^{b}	36/53	68^{a}	26/64	41^{c}
Location 1	25/38	66	19/27	70	6/11	55	5/5	100	1/6	17
Location 2	77/108	71	58/81	72	19/27	70	11/14	79	8/13	62
Location 3	83/137	61	59/87	68	24/50	48	11/20	55	13/30	43
Location 4	80/126	63	67/97	69	13/29	45	9/14	64	4/15	27
FTAI SexedULTRA 4M™	187/405	48^{y}	146/290	50^{b}	41/115	36^{c}				
Location 1	15/38	39	9/19	47	6/19	32				
Location 2	60/110	55	49/91	54	11/19	58				
Location 3	56/136	41	40/85	47	16/51	31				
Location 4	56/121	46	48/95	51	8/26	31				
STAI SexedULTRA 4M™	200/403	50^{y}	152/280	54^{b}	48/123	39^{c}	32/61	52^{b}	16/62	26^{c}
Location 1	22/38	58	16/27	59	6/11	55	4/7	57	2/4	50
Location 2	56/104	54	51/89	57	5/15	33	4/9	44	1/6	17
Location 3	59/140	42	39/74	53	20/66	30	10/26	38	10/40	25
Location 4	63/121	52	46/90	51	17/31	55	14/19	74	3/12	25

[1] Pregnancy rate to AI determined by transrectal ultrasonography 84–92 d after STAI.

[2] See Table 1 for a description of treatments.

[3] Estrous response as determined by activation of an estrus detection aid (Estrotect).

[xy] Overall pregnancy rates with different superscripts differ ($P < 0.0001$).

[ab] Pregnancy rates within row or column with different superscripts differ ($P < 0.05$).

Table 4
Pregnancy rates[1] to artificial insemination based on treatment[2], bull, and estrous response[3] during STAI.

Treatment[2]	Overall		Estrous by 66 h		Non-Estrous by 66 h		Estrous66 – 90 h		Non-Estrous by 90 h	
Bull	Proportion	%	Proportion	%	Proportion	%	Proportion	%	Proportion	%
FTAI Conventional	260/403	65^{x}	200/295	68	60/108	56				
Bull A	87/134	65^{a}	73/100	73	14/34	41				
Bull B	98/139	71^{a}	76/102	75	22/37	59				
Bull C	75/130	58^{b}	51/93	55	24/37	65				
STAI Conventional	265/409	65^{x}	203/292	70	62/117	53	36/53	68	26/64	41
Bull A	89/143	62^{a}	65/94	69	24/49	49	18/27	67	6/22	27
Bull B	102/138	74^{a}	81/103	79	21/35	60	9/12	75	12/23	52
Bull C	74/128	58^{b}	57/95	60	17/33	52	9/14	52	8/19	42
FTAI SexedULTRA 4M™	187/405	48^{y}	146/290	50	41/115	36				
Bull A	42/139	30^{c}	33/103	32	9/36	25				
Bull B	75/138	54^{b}	61/99	62	14/39	36				
Bull C	70/128	55^{b}	52/88	59	18/40	45				
STAI SexedULTRA 4M™	200/403	50^{y}	152/280	54	48/123	39	32/61	52	16/62	26
Bull A	58/139	42^{c}	46/97	47	12/42	29	9/23	39	3/19	16
Bull B	81/138	59^{b}	58/96	60	23/42	55	16/22	73	7/20	35
Bull C	61/126	48^{b}	48/87	55	13/39	33	7/16	44	6/23	26

[1] Pregnancy rate to AI determined by transrectal ultrasonography 84–92 d after STAI.
[2] See Table 1 for a description of treatments.
[3] Estrous response as determined by activation of an estrus detection aid (Estrotect).
[xy] Overall pregnancy rates with different superscripts differ ($P < 0.0001$).
[ab] Pregnancy rates with different superscripts differ ($P < 0.05$).

cows failing to express estrus prior to 66 h, high rates of estrous response by this time likely limit any potential improvement in pregnancy rate associated with use of STAI. In previously published data [28–30], we noted that improved pregnancy rates associated with STAI were primarily associated with increased total rates of estrous response, as cows that fail to express estrus by 66 h after PG administration are afforded an additional 20–24 h to potentially express estrus prior to AI. Although the total rate of estrous response prior to AI was enhanced in the present data through use of STAI rather than FTAI (84% versus 72%), the magnitude of this improvement is rather moderate compared to that observed in previous work noting improved fertility associated with STAI. For example, Bishop et al. [29] found that total estrous response prior to AI was enhanced from 60% among FTAI treated cows to 86% among STAI treated cows. Pregnancy rates for cows inseminated at 66 h that exhibited estrus did not differ between treatments (FTAI = 58%; STAI = 58%; $P = 0.93$); however, pregnancy rates among cows that failed to express estrus by 66 h were improved in STAI treatments when insemination and GnRH administration were postponed by 24 h (FTAI = 35%; STAI = 51%; $P = 0.01$). Consequently, total AI pregnancy rate tended to be higher for cows that received STAI (FTAI = 49%; STAI = 56%; $P = 0.06$). In contrast, the potential for any improvement from STAI is much more limited in the present data set due to the high rates of estrous response observed by 66 h after PG administration and CIDR removal.

The degree to which the sex-sorting process negatively impacts fertility of sperm cells appears to vary based on bull [44], and bull differences observed in this study highlight this variability. For example, pregnancy rates with sex-sorted semen were 56% of those obtained with conventional semen for Bull A, 79% for Bull B, and 90% for Bull C. While routine semen analysis can easily identify collections that are obviously unsuitable for sex-sorting, such as those with high rates of non-motile or morphologically abnormal cells, development of further screening methods is needed in order to identify bulls or collections with moderately impaired fertility following sex-sorting. Several promising opportunities include genomic-based predictions of sire fertility, flow-cytometric evaluation of sperm, and large-scale collection of bull fertility data (see review by Amann et al. [18]).

We have previously proposed [27] that bull or ejaculate variability may also contribute to variability in pregnancy rates specifically in the context of timed AI, as these systems inherently involve more variability in estrous status at AI and in timing of insemination relative to ovulation. One may speculate, for example, that sires or ejaculates of particularly high semen quality may perform equally well in FTAI and STAI systems, whereas others may greatly benefit from the higher rates of estrous response and more forgiving timing of AI when using STAI. This could offer an explanation as to why a previous evaluation of STAI with sex-sorted semen [25] demonstrated more marked improvements in pregnancy rates in comparison to those observed here. Alternatively, the recent improvements made in the SexedULTRA™ sex-sorting process may have mitigated some damage negatively impacting the fertile lifespan of sorted sperm produced with previous technologies, and there may likewise be improvements associated with the higher dose of 4 million compared to 2 million sperm cells per unit.

Despite the lack of net improvement in pregnancy rates associated with use of STAI in this study, producers using sex-sorted semen should consider opportunities associated with use of STAI rather than FTAI. For example, in addition to potentially mitigating the risks previously discussed regarding bull variability, use of STAI also results in an increased proportion of cows having expressed estrus prior to AI. To minimize risk when performing timed AI with sex-sorted semen, producers could be advised to evaluate total rates of estrous response prior to the recommended time point for FTAI and consider use of STAI when low rates of estrous response are observed. Another alternative management approach for cost-conscious producers may be to restrict use of sex-sorted semen to only those cows with activated estrus detection aids, while using less expensive conventional semen among cows failing to express estrus. In doing so, producers could achieve strong conception rates per unit of sex-sorted semen among estrous cows, yet still provide cows failing to express estrus with the maximum opportunity to become pregnant early in the breeding season. If such an approach is used, use of STAI rather than FTAI is highly advisable, as STAI would result in a larger proportion of cows expressing estrus and receiving sex-sorted semen at AI.

In summary, pregnancy rates to timed AI among mature cows following the 7-d CO-Synch + CIDR were reduced when using SexedULTRA 4M™ sex-sorted semen compared to conventional semen. Given the differences observed among bulls in this experiment, basic research efforts are warranted to better characterize sire fertility and develop predictive assessments of sperm fertility following sex-sorting. Nevertheless, as in a previous evaluation of SexedULTRA 4M™ sex-sorted semen for timed AI of beef heifers following the 14-d CIDR-PG protocol [35], pregnancy rates to timed AI with SexedULTRA 4M™ sex-sorted semen are near 50% and, as such, may be commercially acceptable in some beef production settings. Future work should evaluate economic implications of use of SexedULTRA 4M™ sex-sorted semen for timed AI of beef females, and translational research efforts are needed to develop timed AI approaches specifically tailored for use of sex-sorted semen.

Acknowledgements

The Missouri Agricultural Experiment Station (Columbia, MO) contributed. The authors gratefully acknowledge Sexing Technologies (Navasota, TX) for providing semen and funding for this project; Zoetis (Madison, NJ) for providing the EAZI-Breed CIDR® cattle inserts; Merck Animal Health (Madison, NJ) for providing the Estrumate® and Fertagyl®; Merial (Athens, GA) for providing the Cystorelin®; Estrotect® Inc. (Spring Valley, WI) for providing estrus detection aids; and the Mason-Knox Ranch (Frankfort, SD), Mershon Cattle Company (Buckner, MO), D&R Ogren Farm (Langford, SD), and Philip Brooks (Cassville, MO) for use of the animals in this project.

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