



Impact of delayed insemination on pregnancy rates to gender selected semen in a fixed-time AI system

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ABSTRACT

The objectives of the current experiment were to determine if delaying insemination by 8 h in a FTAI protocol would alter estrus expression and pregnancy rates in cows inseminated with sex-sorted semen, characterize bull variation in pregnancy rates to sex-sorted semen and examine the impact of repeated years of FTAI to sex-sorted semen on calving distribution. Over three breeding seasons, postpartum cows ($n = 839$) were estrous synchronized using the 5-day CO-Synch + CIDR system. Cows were given GnRH (100 µg i.m., Factrel) at time of insertion of a controlled internal drug releasing device (CIDR; Eazi-Breed CIDR). Five d later CIDR was removed and PGF_{2α} (25 mg i.m., Lutalyse) was given at removal and 8 h later. Estrus detection aids were applied at CIDR removal. Cows were inseminated with X-sorted or Y-sorted sex-sorted semen at 72 h (NORM) or 80 h (DELAY) after CIDR removal, and GnRH was administered at AI. At insemination, estrus status was categorized as positive (YES), partial (QUES), unknown (NR) or negative (NO). Bulls were introduced to cows at 14 d and removed at 60 d after FTAI. Pregnancy diagnosis was performed by ultrasound at d 60 after FTAI and via palpation at 60 d after bull removal. There was no difference ($P > 0.05$) in pregnancy rates to sex-sorted semen or final pregnancy rates between NORM and DELAY cows. Pregnancy to sex-sorted semen averaged 35.2% whereas final pregnancy rates were 90.6%. More cows ($P < 0.05$) in the DELAY group expressed estrus before FTAI, but this increase did not alter pregnancy rates to sex-sorted semen. Expression of estrus before FTAI increased ($P < 0.02$) pregnancy rates to sex-sorted semen across treatments with differences being YES > QUES or NR > NO. There was considerable variation in pregnancy rate by bull ($P < 0.05$) with pregnancy rates ranging from 55.6% to 19.3%. Whole herd calving distribution was altered ($P < 0.05$) after 3 y of use of sex-sorted semen compared to the previous 3 y when conventional semen was used. We conclude that delaying insemination by 8 h in an FTAI protocol did not improve pregnancy rates to sex-sorted semen despite more cows exhibiting estrus before FTAI. In addition, a high bull to bull variation in pregnancy rates to sex-sorted semen is a limitation in FTAI systems. Further research into FTAI strategies for use with sex-sorted semen is warranted.

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1. Introduction

Preselection of sex of offspring by separating sperm containing X- and Y-chromosomes is a powerful reproductive technology impacting animal industries. In the United States, commercially available sex-sorted bull semen is produced using high-speed flow

cytometry. The current method is a modification of the individual cell sorting process developed by US Department of Agriculture researchers in the 1980's and 1990's [1]. Sex-sorted semen is routinely processed in purities of 90% of the desired gender. The sorting process results in a high percentage of damaged and gender unidentified sperm; therefore, sperm yields per ejaculate can be reduced by up to 50%. The resulting sex-sorted product is packaged in straws containing 2.1 million to 4 million sperm.

Utilization of sex-sorted semen technology in the beef industry has lagged behind its use in the dairy industry despite increasing numbers of beef bulls with sex sorted semen available [2,3]. In the

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beef industry, applications of sex-sorted semen include using a small group of elite females to produce replacement heifers while mating the remaining cows to terminal sires, and/or increasing the percentage of steers produced [4]. However, producer adoption of this technology is hampered by the depressed pregnancy rates to sex-sorted semen compared to conventional semen.

When inseminated with sex-sorted semen, beef cows and heifers have a 10%–20% decrease in AI pregnancy rates compared to females inseminated with conventional semen [5–7]. The reduction in conception rates is a result of damage to the sperm during sorting which may include damage to DNA, premature capacitation, and acrosomal alterations [8]. This damage is primarily uncompensable as increasing sperm numbers in units of sex-sorted semen only produces minimal increases in pregnancy rates [9]. Bulls may be differentially affected by the sex-sorting process resulting in varying levels of sperm damage and changes in fertility. Variation in fertility in dairy bulls was greater when semen was sex-sorted compared to conventional packaged semen [10]. Limited information is available on variation in pregnancy rates observed in beef bulls after sex-sorting.

Pregnancy rates to sex-sorted semen are improved if females are inseminated 12–18 h after an expressed estrus or have been in estrus prior to fixed-time AI (FTAI) [7,11]. It has been suggested that delaying insemination after detected estrus until 18–24 h may improve pregnancy rates compared to the standard 12–18 h after detected estrus [5]. Therefore, timing of insemination in FTAI systems may be critical to success with sex-sorted semen.

Over the past 10 years, FTAI protocols for beef cows and heifers were developed that resulted in acceptable pregnancy rates (50%–65%) to conventional semen [12]. Use of FTAI may partially account for the 2-fold increase in beef semen sales in the US since 2005 [13]. Large scale adoption of sex-sorted semen in the beef industry will require development of FTAI procedures that result in acceptable (>50%) pregnancy rates.

Information on the large scale use of sex-sorted semen on calving patterns in beef herds is limited. Herd wide use of sex-sorted semen in beef operations may alter calving distribution [14]. Information on the effect of sex-sorted semen on calving distribution in commercial operations is needed. Commercial beef operations usually have a 60–90 d calving season which is crucial to match herd nutritional needs to forage availability. Alterations in calving distribution may also create barriers to adoption of sex-sorted semen.

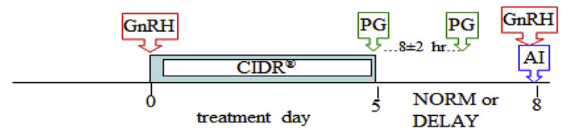
The objectives of the present experiment were to:

1. Determine if delaying insemination by 8 h in an FTAI protocol would alter estrus expression and pregnancy rates in cows inseminated with sex-sorted semen
2. Characterize bull variation in pregnancy rates to sex-sorted semen
3. Examine the impact of repeated years of FTAI to sex-sorted semen on calving distribution

2. Materials and methods

All procedures were approved by the University of Idaho Animal Care and Use Committee (Protocol number 2012-39). Over a 3 y period, postpartum crossbred (Angus X Hereford) beef cows ($n = 839$) from the Nancy M. Cumming Research, Extension and Education Center, Carmen, ID were stratified by age and days postpartum and randomly assigned to FTAI at 72 h (NORM; $n = 460$) or 80 h (DELAY; $n = 379$) after removal of a controlled internal drug release insert (CIDR; 1.38 g progesterone; Zoetis, Madison, NJ, USA) in the 5-day CO-Synch + CIDR protocol [15];

5-day CO-Synch + CIDR®



NORM = TAI at 72 ± 2 hr after 1st PG with GnRH at timed AI.

DELAY = TAI at 80 ± 2 hr after 1st PG with GnRH at timed AI.

GnRH = 100 µg i.m. Factrel; PG = 25 mg i.m. Lutalyse;

CIDR = Eazi-Breed CIDR; 1.38 g progesterone

Fig. 1. 5-day CIDR CO-Synch protocol.

Fig. 1. Analogs of GnRH (Gonadorelin) and $\text{PGF}_{2\alpha}$ (Dinoprost) were used in this experiment. As other commercially available analogs to these hormones can be used for estrous synchronization, the description of the estrous synchronization protocol will refer to these analogs by the hormones they mimic GnRH and $\text{PGF}_{2\alpha}$. Briefly, cows were administered GnRH (100 µg i.m., Factrel; Zoetis, Madison, NJ, USA or Cystorelin; Merial, Duluth, GA, USA) at time of CIDR insertion. Five d later CIDR was removed and $\text{PGF}_{2\alpha}$ (25 mg i.m., Lutalyse; Zoetis, Madison, NJ, USA) was given at removal and 8 h later. All cows were administered GnRH at FTAI. Due to logistical constraints more cows were assigned to NORM in year 1. In all other years, equal number of cows were assigned to NORM and DELAY. Cows were randomized to treatment each year, and primiparous cows were added as needed to compensate for cows removed from the experiment for reproductive or health reasons. Synchronization and timing of insemination treatments were applied to individual animals with cow as the experimental unit.

All cows were weighed and body condition scored (1, emaciated to 9, obese; [16]) at CIDR insertion and final pregnancy diagnosis. Body condition score classes were <5, 5 and 6, and >6. Cows were also characterized as Young (≤ 4 y), Mature (5–10 y), and Aged (>10 y). For analysis, cows were also characterized by four postpartum periods: ≤ 60 d, 61–80 d, 81–100 d, and >100 d.

At CIDR removal, heat detection patches (Estrotest, Denver, CO, USA; Years 1 and 2) or tail paint (Year 3) were applied to cows. Estrus status was determined at time of FTAI. Cows were considered to be in estrus (YES; patch fully activated or no tail paint), probably in estrus (QUES; patch partially activated or partial tail paint), unknown (NR; patch lost or failure to record), or not in estrus (NO).

Cows were inseminated by professional technicians with either Y-sorted ($n = 669$) or X-sorted ($n = 170$) semen. All sex-sorted semen was purchased from major AI companies and was sorted using flow cytometry by SexingTechnologies. All straws were $\frac{1}{4}$ cc and contained 2.1×10^6 cells. No semen used in this study was SexedUltra. Time from CIDR removal to AI was recorded. Six to eight AI sires were used per year. Within years, AI sires were used equally across the two timing treatments. Different AI sires were used during different years, so AI sire and year are confounded.

Fourteen days after FTAI, cows were divided into three groups and placed on similar irrigated pastures with natural service sires for clean-up breeding. All bulls had passed a breeding soundness exam 45 d prior to introduction to cows. The bull to cow ratio was 1:40 to 1:50. Between 55 and 60 d after FTAI, pregnancy status and fetal age were determined by transrectal ultrasonography. Bulls were removed 60 d after FTAI and final pregnancy status was determined via transrectal palpation between 60 and 70 d after bull removal.

Julian calving dates from all multiparous cows calving for three calving seasons before the current experiment, and the 3 calving

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