**ABSTRACT**

The objective was to compare pregnancy per AI (P/AI) with conventional (CON) or sex-sorted (SS) semen from a single sire within a fixed-time AI (FTAI) program designed for dairy heifers. Holstein heifers (n = 240) were assigned to treatment (CON or SS) according to body weight and reproductive tract score. All heifers underwent FTAI by using the “Show-Me-Synch” protocol [controlled internal drug release (CIDR) insert from d 0 to 14 followed by PGF2α (25 mg i.m.) 16 d after insert removal (d 30) with GnRH (100 μg i.m.) and FTAI at 66 h after PGF2α]. A single professional technician performed the FTAI. Heifers were fitted with heat detection patches at PGF2α to characterize estrous response. Estrous response did not differ between CON (63/120; 53%) and SS (70/120; 58%) treatments. The CON heifers, however, achieved greater FTAI P/AI (82/120; 68%) compared with SS (45/120; 38%) heifers. The P/AI did not differ for CON heifers that exhibited or failed to exhibit estrus before FTAI [44/63 (70%) vs. 38/57 (67%), respectively]. For SS heifers, however, those that exhibited estrus had greater P/AI compared with those that failed to exhibit estrus [32/70 (46%) vs. 13/50 (26%)]. Pregnancy per AI resulting from FTAI was greater for heifers that were inseminated with CON semen compared with those that received SS semen. The expression of estrus before FTAI did not affect P/AI when CON semen was used, whereas the P/AI with SS semen was greater for heifers detected in estrus. Further studies are required to develop strategies for using sex-sorted semen when inseminating heifers at predetermined fixed times on the basis of the expression of estrus before FTAI.

**Key words:** controlled internal drug release insert, dairy heifer, fixed-time artificial insemination, sex-sorted semen

**INTRODUCTION**

Sex-sorted (SS) semen can increase the profitability of dairy farms by increasing the number of genetically superior heifers born after AI (Holzenbock, 1999). Fertility after AI is lower, however, with SS semen than with conventional (CON) semen (Seidel, 2011). The reduced fertility when SS semen is used may be caused by a lower sperm dose compared with CON (approximately 2 million vs. 20 million sperm/dose), in addition to noncompensable damage to sperm cells during the sorting procedure (Catt et al., 1997; Carvalho et al., 2010; DeJarnette et al., 2011). Based on these considerations, synchrony of estrus and ovulation become more critical when using SS semen in conjunction with an estrous synchronization protocol designed to facilitate AI at predetermined fixed times.

The timing between expected ovulation and AI requires careful consideration because the viability of SS sperm is reduced compared with that of CON sperm. Patterson et al. (2011) recently reported a 65% fixed-time AI (FTAI) pregnancy per AI (P/AI) from field trials involving 2,656 beef heifers that were treated with the “Show-Me-Synch” protocol for FTAI. When compared with alternative controlled internal drug release (CIDR)- or melengestrol acetate (MGA)-based protocols for beef heifers, the Show-Me-Synch protocol had greater synchrony of estrus (Leitman et al., 2009b; Mallory et al., 2010) accompanied by high P/AI resulting from FTAI (Mallory et al., 2011). Although use of SS semen in conjunction with FTAI has not been recommended to date, the repeatability in timing and synchrony of estrus following treatment administration with the Show-Me-Synch protocol (Leitman et al., 2009a,b; Mallory et al., 2010) may provide a sufficient degree of synchrony to facilitate use of SS semen in conjunction with FTAI. These considerations formed the basis of our hypothesis that dairy heifers treated with the Show-Me-Synch protocol would have equivalent P/AI after insemination with SS and CON semen.
MATERIALS AND METHODS

The experiment was conducted at 2 locations within the Doss Dairy Farm (West Plains, MO). Holstein heifers (n = 140 at location 1 and n = 100 at location 2) were randomly assigned to 1 of 2 treatments (insemination with SS or CON semen). The heifers were blocked and processed as CON (20 million sperm per straw) or SS (2.1 million sperm per straw). All inseminations were performed by a single professional technician. Semen from a single ejaculate was divided and processed as CON (20 million sperm per straw) or SS (2.1 million sperm per straw). All inseminations were performed by a single professional technician. Pregnancy per AI resulting from FTAI by using the Show-Me-Synch protocol (Figure 1). The treatment sequence for the protocol is an Eazi-Breed CIDR insert (1.38 g of progesterone; Pfizer Animal Health; New York, NY) from d 0 to 14 followed by an injection of PGF2α (25 mg, i.m.; Lutalyse, Pfizer Animal Health) 16 d after CIDR removal (d 30). An injection of GnRH (100 μg, i.m.; Cystorelin, Merial, Athens, GA) was administered concurrently with FTAI at 66 h after the PGF2α injection.

Each heifer was fitted with an Estrotect Heat Detector patch (Rockway Inc., Spring Valley, WI) at the time of PGF2α. A heifer was defined as having a positive estrous response when an Estrotect patch was completely activated (i.e., all of the scratchable material was worn off). Semen from a single sire (Genex Cooperative Inc., Shawano, WI) that was available as CON and SS was used for FTAI. Semen from a single ejaculate was divided and processed as CON (20 million sperm per straw) or SS (2.1 million sperm per straw). All inseminations were performed by a single professional technician. Pregnancy per AI resulting from FTAI was determined 62 d after FTAI by using transrectal ultrasonography (Aloka 500V ultrasound equipped with a 5.0-MHz linear-array transducer; Hitachi Aloka Medical Corp., Tokyo, Japan). Fetal size (crown–rump measurement ~ 61 mm) and presence of a heart beat were used as determinants of pregnancy via ultrasound on d 62. Body weight and RTS were analyzed by ANOVA using the PROC MIXED (SAS Institute Inc., Cary, NC) fitting a normal distribution. Models included the effects of treatment, location, and the interaction of treatment and location. Means are reported as least square means ± standard errors. Pregnancy per AI was analyzed by logistic regression using PROC GENMOD with a binomial distribution using the link function of logit. The model included the main effects of location, treatment, estrous response, and interactions.

RESULTS AND DISCUSSION

We observed an effect of location on heifer BW because heifers at location 2 were heavier (P < 0.01) than heifers at location 1 (413 ± 2.7 vs. 352 ± 2.3 kg). However, location had no effect on RTS (4.5 ± 0.1; P = 0.33). Body weight (P = 0.99) and RTS (P = 0.20) were similar for heifers assigned to CON and SS treatments. Estrous response did not differ (P = 0.20) between heifers assigned to CON (63/120, 53%) or SS (70/120, 58%) groups (Table 1). Heifers inseminated with CON semen had greater (P < 0.001) P/AI compared with heifers inseminated with SS semen (Table 1). Heifers that exhibited estrus before FTAI tended (P = 0.06) to have greater P/AI than heifers that failed to exhibit estrus. Pregnancy per AI was similar for heifers inseminated with CON semen that were either observed or not observed in estrus (Table 1). Conversely, heifers that were inseminated with SS semen that exhibited estrus had greater (P = 0.04) P/AI after FTAI compared with heifers that failed to exhibit estrus (Table 1). We acknowledge that by using a single ejaculate from a single sire, results are restricted to that particular individual. Semen from different bulls responds differently to the sorting process; therefore, it is possible that interpretation of these results may differ when using a different sire, supporting the need for future research.

Sex-sorted semen is currently recommended for use only after an observed estrus. Expanded use of sex-sorted semen in many cases, however, will rely on development of protocols that facilitate its use in conjunction with FTAI. Pregnancy per AI resulting from FTAI using conventional semen in heifers is expected to be greater following treatment with long-term CIDR-based protocols because of improvements in synchrony of estrus following treatment compared with short-term CIDR-based or melengestrol acetate-based protocols (Busch et al., 2007; Mallory et al., 2011). Although synchrony of estrus was not measured in this study, our results and those reported previously by Mallory et al. (2011) demonstrate that the Show-Me-Synch protocol is effective in facilitating FTAI in both beef and dairy heifers when conventionally processed semen is used. The high P/AI for CON semen observed in...
the current study did not depend on the expression of estrus. For SS semen, P/AI resulting from FTAI was greater among heifers that exhibited estrus before insemination. The fertility percentage (SS compared with CON), however, was markedly lower than that reported by DeJarnette et al. (2009), supporting the need to more closely match timing of insemination with estrus expression. This suggests that the timing of FTAI following administration of the Show-Me-Synch protocol should be evaluated more carefully for heifers inseminated with SS semen so that insemination and estrus expression are more closely matched. Re-evaluation of the timing of insemination in situations involving FTAI following administration of the Show-Me-Synch protocol may result in improvements in P/AI in breeding programs involving SS semen. Although further studies are needed, perhaps a sensible approach to follow when FTAI is preferred is to use SS semen for those heifers that exhibit estrus and to use less expensive conventional semen when FTAI is preferred is to use SS semen for those heifers that fail to exhibit estrus.

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REFERENCES


