



J. Dairy Sci. 100:1–5  
<https://doi.org/10.3168/jds.2016-12318>  
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## Short communication: Optimization of a timed artificial insemination program for reproductive management of heifers in Canadian dairy herds

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### ABSTRACT

Pregnancy per AI (P/AI) following the use of 1 of 2 timed AI (TAI) protocols and 2 different intervals between TAI and resynchronization were compared in heifers that were inseminated with either conventional or sex-sorted semen. Holstein heifers ( $n = 317$ ; 527 inseminations) were submitted to a 5-d Cosynch protocol with (+) or without (-) GnRH at the time of controlled internal drug release (CIDR) insertion on d 0, CIDR removal and a single PGF<sub>2α</sub> treatment on d 5, and TAI plus GnRH on d 8 (72 h later). Visual estrus detection (ED) was conducted on d 6 in the afternoon and d 7 in the morning and heifers observed in estrus were artificially inseminated on d 7 in the afternoon. Heifers were alternately assigned conventional or sex-sorted semen. Pregnancy was diagnosed by ultrasound 27 and 42 d after AI, and heifers diagnosed as nonpregnant were resynchronized, up to 3 times, starting on d 27 or 34 to provide an interbreeding interval of 35 or 42 d. Overall, TAI protocol had no effect on P/AI at 27 or 42 d after artificial insemination or on pregnancy loss, but P/AI following the first service tended to be higher in the -GnRH TAI group (66.3 vs. 56.8%). Pregnancy per AI at 27 d (61.9 vs. 55.5%) tended to differ between conventional and sex-sorted semen. Heifers artificially inseminated based on ED tended to have a greater P/AI (67.6 vs. 58.2%) and had decreased pregnancy loss (0.0 vs. 4.1%) than those submitted to TAI. A greater number of heifers in the -GnRH TAI protocol were artificially inseminated on ED than the +GnRH TAI protocol (21.5 vs. 13.7%). No difference in P/AI was observed between the 35- and 42-d interbreeding intervals; however, more heifers in the 42-d group were artificially inseminated based on ED than in the 35-d group (22.7 vs. 7.8%). A 5-d Cosynch+CIDR TAI protocol without the initial GnRH and with a single PGF<sub>2α</sub>

at CIDR removal is an acceptable alternative to achieve high P/AI when either conventional or sex-sorted semen is used in Holstein heifers. Breeding heifers based on detected estrus increases labor, but has the potential to increase fertility.

**Key words:** dairy heifer, timed artificial insemination, resynchronization, sex-sorted semen

### Short Communication

As the raising of replacement heifers represents a large cost (Tozer and Heinrichs, 2001), reducing age at first calving by increasing reproductive efficiency is advantageous. Management of heifer breeding involves many factors, including the use of different synchronization protocols, intervals between AI (resynchronization), and the use of sex-sorted semen, all of which represent important decisions in a breeding program.

Timed AI (TAI) protocols are beneficial, as they reduce the interval to first AI, the need for estrus detection, and allow for tight synchronization of breeding groups (Ribeiro et al., 2012). The 5-d Cosynch and controlled internal drug release (CIDR) TAI protocol achieved pregnancy per AI (P/AI) in heifers that were comparable to estrus detection and AI (Silva et al., 2015; Colazo and Mapletoft, 2016). Although the 5-d Cosynch+CIDR protocol includes an initial injection of GnRH to synchronize the growth of the ovulatory follicle, an earlier study revealed that only 25% of heifers actually ovulated and no difference existed in P/AI when the initial GnRH was not used (Colazo and Ambrose, 2011). Additionally, approximately 25% of heifers ovulated before TAI in the Colazo and Ambrose (2011) study, so a systematic estrus detection plan was necessary to identify early ovulators and potentially improve the overall P/AI.

Seidel et al. (1999) described a decrease in fertility with the use of sex-sorted semen when compared with conventional semen, which may be due to reduced sperm concentrations, damage suffered during sorting, or reduced duration of optimal fertility in the female

Received November 16, 2016.

Accepted January 11, 2017.

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reproductive tract (Seidel, 2014). Improved fertility is expected when sex-sorted semen is deposited in the uterus close to the time of ovulation. In this regard, Colazo and Ambrose (2011) reported that more than 75% of heifers ovulated within 24 h of TAI when the initial GnRH was not used in the 5-d Cosynch+CIDR protocol, which has implications for the use of sex-sorted semen.

Timely reinsemination after heifers are diagnosed nonpregnant is important to reduce the interval to pregnancy and increase pregnancy rate in a fixed breeding season (Ribeiro et al., 2012). However, a longer interval between TAI and resynchronization increased the number of cows AI on detected estrus, which also improved P/AI (Sinedino et al., 2014).

The objective of our study was to evaluate the use of a 5-d Cosynch+CIDR TAI protocol with (+) or without (-) initial GnRH and 2 different interbreeding intervals on P/AI of heifers inseminated with conventional or sex-sorted semen. We hypothesized that the 5-d protocol without the initial GnRH would result in comparable P/AI in heifers inseminated with either conventional or sex-sorted semen and that an interval of 42 d between AI would increase P/AI compared with a 35-d interval.

This study was conducted in a commercial dairy herd in Wetaskiwin, Alberta, Canada, from April 2014 to June 2015. All procedures were conducted in accordance with the guidelines of the Canadian Council on Animals Care (CCAC, 2009). Holstein heifers between 13 and 16 mo of age were housed in freestalls, had unrestricted access to water, and were fed a TMR once daily that was designed to meet the requirements of Holstein heifers weighing 350 kg and gaining 1.0 kg/d (NRC, 2001). The diet was based on a mixture of barley silage, alfalfa silage, haylage grass, hay, brewer's grain, barley grain, and a vitamin and mineral supplement.

Heifers were examined by transrectal ultrasonography (MicroMAXX, Sonosite, Bothell, WA) to determine cyclicity (presence of corpus luteum) and normalcy of the reproductive tract. All normal heifers ( $n = 370$ ; 5 noncycling) received a CIDR (Zoetis, Kirkland, Québec, Canada) on d 0 in the morning, either with (+GnRH) or without (-GnRH) an i.m. injection of 100  $\mu$ g of GnRH (Fertiline, Vetoquinol N.-A Inc., Lavaltrie, Québec, Canada). On the morning of d 5, CIDR were removed and an i.m. injection of 500  $\mu$ g of cloprostenol (PGF<sub>2 $\alpha$</sub> ; Estroplan, Vetoquinol N.-A Inc.) was given to both groups. Heifers were observed (~30 min each) for estrus detection (ED) on d 6 in the afternoon and d 7 in the morning, and those detected in standing estrus were AI on d 7 in the afternoon. Heifers not observed in estrus received a GnRH treatment concurrent with TAI on the morning of d 8, 72 h after CIDR removal. All

heifers were inseminated by the same technician with either sex-sorted or conventional semen from 8 sires with semen available commercially.

Pregnancy was diagnosed by transrectal ultrasonography 27 d after AI and heifers diagnosed as pregnant were rechecked on d 42. Nonpregnant heifers were resynchronized up to 3 times to the other TAI protocol, depending on which TAI protocol they received previously. The resynchronization was initiated 27 or 34 d after the previous insemination, resulting in interbreeding intervals of 35 or 42 d, respectively. Heifers observed in estrus before initiation of the resynchronization protocol were AI based on detected natural estrus. All the resynchronized heifers were inseminated with conventional semen.

All data were analyzed using SAS (version 9.3, SAS Institute, Cary, NC). Pregnancy per AI (at 27 and 42 d after AI), pregnancy loss (between 27 and 42 d after AI), and incidence of estrus were analyzed using general estimating equations in the GENMOD procedure, with model specifications including a binomial distribution, logit link function, repeated statement with subject equal to heifer, and an exchangeable correlation structure. The final model included treatment (TAI protocol), type of semen (sex-sorted vs. conventional), number of AI (1 vs.  $\geq 2$ ), type of AI (TAI vs. ED), interbreeding interval (35 vs. 42 d), and their interactions. A  $P$ -value of  $<0.05$  was considered statistically significant and  $0.05 \leq P < 0.10$  was considered a tendency.

A total of 527 inseminations were recorded, and after 4 AI the cumulative pregnancy rate was 96.8%. A total of 144 (27.3%) and 383 (72.7%) of inseminations completed were with sex-sorted and conventional semen, respectively. Overall, P/AI at 27 d did not differ between TAI protocols; however, P/AI after first AI tended to be greater ( $P = 0.08$ ) following the use of the -GnRH TAI protocol (Table 1). Conversely, TAI protocols had no effect on P/AI for subsequent AI, nor on pregnancy loss between 27 and 42 d after AI (Table 1). A greater number of heifers were inseminated based on detected estrus in the -GnRH compared with the +GnRH TAI protocol (21.5 vs. 13.7%;  $P = 0.03$ ). Pregnancy per AI at 27 d tended ( $P = 0.08$ ) to be greater for heifers AI following ED (Table 2). Heifers inseminated on ED had greater ( $P = 0.03$ ) P/AI at 42 d, as pregnancy loss was lower compared with heifers inseminated by TAI (Table 2).

Pregnancy per AI at 27 d tended to be higher with conventional than sex-sorted semen (61.9 vs. 55.5%;  $P = 0.10$ ). Despite a difference of 5.6%, we did not find an effect of type of semen on P/AI at 42 d (59.8 vs. 54.2%;  $P = 0.30$ ) or pregnancy loss between 27 and 42 d (3.4 vs. 2.5%;  $P = 0.90$ ). No interaction was observed

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