Timing of A.I. is critical for fertility in lactating dairy cows

New information supports that timing of A.I. is imperative, but the optimal insemination window is wider than previously thought.

by Paul Fricke, Vanda Santos, and Paulo Carvalho

ONE of the most common questions we get about breeding dairy cows involves the importance of insemination timing for cows identified with increased activity using an automated activity monitoring system or submitted to timed A.I. after a synchronization protocol. Farms have widely adopted these technologies, and achieve optimal fertility to A.I., both require that the timing of A.I. occurs within a given time window, either relative to increased activity or induction of ovulation after the final GnRH treatment.

We recently published a paper in the *Journal of Dairy Science* in which we present three data sets; one that is a randomized controlled study, and two other large observational data sets on timing of A.I. relative to induction of ovulation after a double-ovsynch protocol or after an estrus alert using an automated activity monitoring system. Let's look at what we found.

Three experiments

Timing of timed artificial insemination (TAI): The first experiment revisited an old question that we are often asked: Can we inseminate cows at the same time as the last GnRH treatment of a TAI protocol (cosynch), or do we have to wait 16 hours after the last GnRH treatment to inseminate the cows? And if we choose to insert concurrently with the last GnRH treatment, how much fertility will we lose? This study was conducted from January 2016 to October 2016 in 1,924 multiparous lactating Holstein cows across six commercial dairy farms in Wisconsin that used conventional dairy semen for TAI. Cows were submitted to a double-ovsynch protocol for first insemination, and on the day of the last GnRH treatment, cows were randomly assigned to one of two groups: cosynch-56 (TAI at 0 hours, concurrent with the last GnRH treatment) or ovsynch-56 (TAI 16 hours after the last GnRH treatment).

Results from this experiment showed that cows in the ovsynch-56 treatment had more pregnancies per A.I. (P/A.I.; 46%) than cows in the cosynch-56 treatment (36%). Although delaying TAI until 16 hours improved P/A.I. by 10 percentage units, this represents a 28% bump in pregnant cows, which is a lot of pregnancies. This finding supports the hypothesis that delaying A.I. until 16 hours after the final GnRH treatment of a double-ovsynch protocol improves pregnancy outcomes compared to a cosynch protocol. These results also agree with an older study using ovsynch and TAI that I described in a *Hoard's Dairyman Intel* article titled, "Stop doing cosynch" in which cosynch protocols result in decreased fertility. So, the take-home message from Experiment 1 is simple: Stop doing cosynch!

Effect of timing of TAI (13 to 23 hours) after the final GnRH treatment: The longstanding recommendation for TAI relative to the final GnRH treatment of a synchronization protocol is 16 hours based on an experiment published in 1998, three years after the first publication of the ovsynch protocol. This experiment was conducted to determine how variability in the timing of TAI relative to the final GnRH treatment affects P/A.I. This is a common question for larger farms that must inseminate large numbers of cows across multiple pens which can dramatically increase the variability of TAI relative to the final GnRH treatment among cows submitted to a TAI protocol. How much variability in timing of TAI is allowable?

This experiment was an observational study conducted from May 2020 to August 2023 on two commercial dairy farms in Texas, and included a total of 13,318 lactating Holstein cows. Cows were submitted for their first TAI using a double-ovsynch protocol (n = 14,089) or subsequent TAIs (second or greater) using a GGPPG resynch protocol (n = 6,806). Because of the size of the farms, the timing of TAI varied from 13 to 23 hours after the last GnRH treatment of the double-ovsynch protocol. Cows were inseminated with either sexed dairy semen (4 million sperm cells per straw per freeze) or conventional beef semen (20 million sperm cells per straw per freeze). Electronic ID tags were used to precisely record the times of GnRH treatment and TAI for individual cows.

Results from this experiment are shown in Figure 1. The overall P/A.I. was 43%. For cows inseminated with sexed semen, there was no effect of timing of TAI after the last GnRH treatment on P/A.I. Similarly, for cows inseminated with conventional semen, no effect of timing of TAI on P/A.I. was observed. These results support that cows can be inseminated within a 13- to 23-hour window after the last GnRH treatment of a TAI protocol without affecting P/A.I. for either semen type. So, the take-home message from this experiment is that the optimal fertility window for TAI after the final GnRH treatment of a synchronization protocol is wider (13 to 23 hours) than the longstanding recommendation of TAI at 16 hours after the last GnRH treatment of a synchronization protocol. This is great news for breeding program management on dairy farms using synchronization protocols.

Effect of A.I. timing relative to onset of estrous alert using an automated activity monitoring system: A final question we are often asked involves the timing of A.I. relative to an activity alert for farms using an automated activity monitoring system to inseminate cows. This experiment was an observational study that included 10,927 lactating dairy cows (Holstein, Jersey, and crossbred) from two commercial farms in Texas and Arizona, with 20,461 A.I. records available for analysis.

Cows were fitted with neck-collar-mounted activity-monitoring tags, and the onset of an estrous alert was recorded when activity levels exceeded a predefined threshold. Timing of A.I. varied widely from 0 to 40 hours after the onset of an estrous alert on these farms. Similar to Experiment 2, cows were inseminated with either sex-sorted dairy or conventional beef semen, but randomization cows to semen

type was not performed.

Outcomes of the activity monitoring experiment are shown in Figure 2. The overall P/A.I. was 48%. For both sexed dairy and conventional beef semen, there was a significant effect of timing of A.I. after the onset of an estrous alert on P/A.I. Cows inseminated early (between 0 and 2 hours after estrous alert onset) had fewer P/A.I. than cows inseminated at the recommended time of 15 to 16 hours after estrous alert. For conventional semen, P/A.I. was 38% for early A.I. versus 49% for recommended timing. For sexed semen, P/A.I. was 37% for early A.I. versus 54% for recommended timing. Cows inseminated late, more than 23 hours after an estrous alert, also had fewer P/A.I. than cows inseminated in a 15- to 16-hour window (conventional: 44% versus 49%; sexed: 42% versus 54%).

Figure 3 shows a second analysis from the activity monitoring system experiment that we conducted in which timing of A.I. was limited to the 13- to 23-hour window after an estrous alert. No effect of timing of A.I. on P/A.I. was observed for either semen type when the insemination window was restricted to 13 to 23 hours after an estrous alert. So, the take-home message from this is the optimal fertility window for timing of A.I. after an estrous alert is 13 to 23 hours. This means, however, that lists of cows identified with increased activity must be generated twice daily (a.m. and p.m.) to optimize fertility when inseminating cows based on estrous alerts.

Timing matters

Taken together, these three studies support that lactating dairy cows inseminated too early (equal to or less than three hours) or too late (equal to or more than 24 hours) relative to the onset of estrous alert, or too early relative to a synchronized ovulation, experience fewer P/A.I. Optimal fertility is achieved when insemination occurs between 13 and 23 hours after the last GnRH treatment of a synchronization protocol or after the onset of an estrous alert, and this optimal timing was consistent for both conventional and sexed semen. It is important to note that cows were not randomized to semen type in second and third experiments; therefore, direct comparisons of fertility between semen types could not be made, and only associations between A.I. timing and P/A.I. could be inferred. The large data sets in these studies and the ability to record precise timing achieved through electronic identification and the activity monitoring system enhance the accuracy of these conclusions.

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Figure 1. Effect of timing of TAI from 13 to 23 hours after the last GnRH treatment on P/A.I.

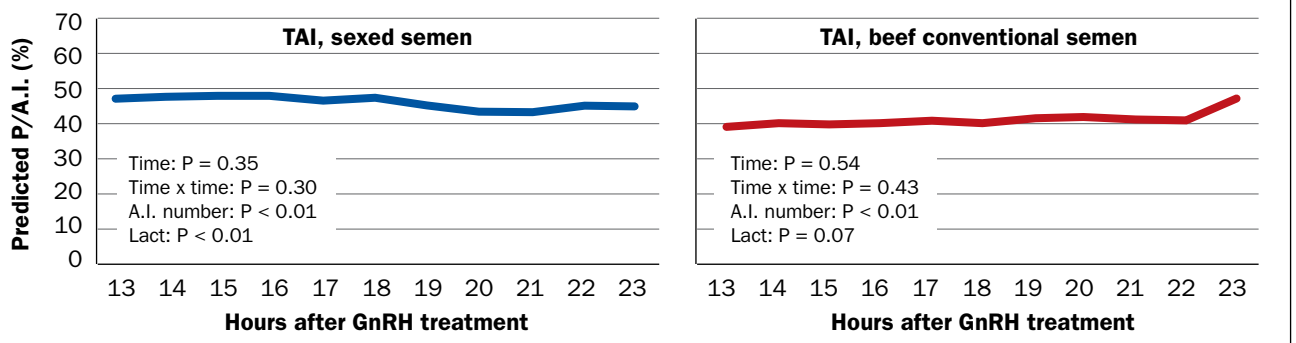

Figure 2. Effect of time of A.I. from 0 to 40 hours after an estrous alert on P/A.I.

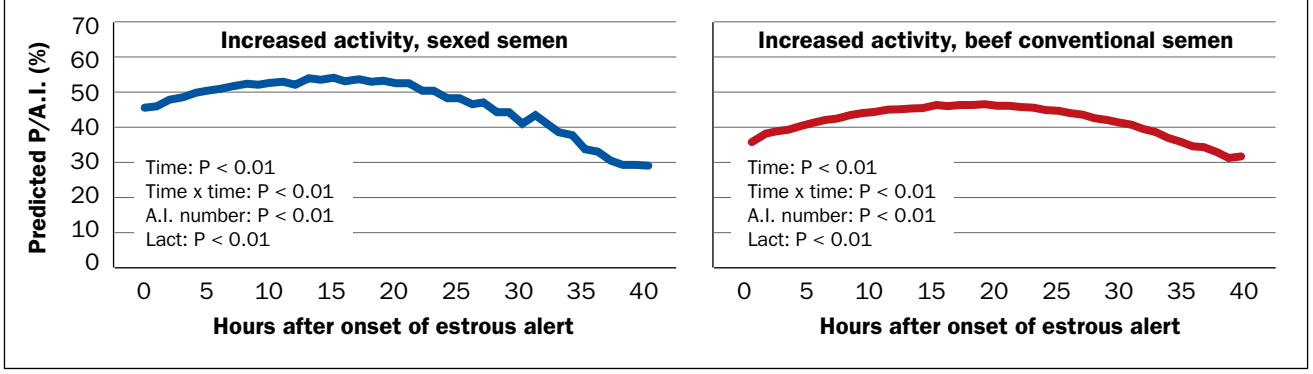

Figure 3. Effect of timing of A.I. from 13 to 23 hours after an estrous alert on P/A.I.