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Reinsemination Intervals After Timed Artificial Insemination or Estrus-Detected Inseminations

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Summary

The objective was to quantify the reinsemination intervals of lactating dairy cows that were either inseminated at estrus or received a timed AI (**TAI**) at first service. Cows in Experiment 1 were enrolled in a TAI program before first AI after calving. Cows detected in estrus after 50 days in milk (**DIM**) were inseminated, whereas the remainder continued in the TAI program and were inseminated as scheduled. Cows in Experiment 2 also were enrolled in a TAI program and were inseminated accordingly at first service after calving. On day 7 after TAI, cows were assigned randomly to receive either saline (control) or 1,000 IU human chorionic gonadotropin (hCG) to induce accessory luteal structures (corpora lutea) in an attempt to improve pregnancy outcome. First-repeat insemination dates were recorded for all cows after the initial AI and grouped as <18 days, 18 to 25 days, or >25 days since first AI. More cows in Experiment 1 that were inseminated at estrus returned to estrus before 25 days than TAI cows and during summer months had shorter average return intervals by 1.7 days. More cows in Experiment 2 that received saline and had no accessory luteal structures also returned to estrus before 25 days than cows receiving hCG. Equal proportions of saline and hCG-treated cows (25%) in Experiment 2 had retained at least one of their original luteal structures until day 28 after TAI, but were not pregnant at day 32. Of those nonpregnant cows that retained luteal structures, average concentrations of pregnancy-specific protein B (BioPRYN test) concentrations were slightly elevated, but failed to retain the embryos to day 32 after AI. Furthermore, progesterone concentrations of these cows that lost their embryos were compromised compared with pregnant cows by day 21 after AI. Regardless of the number of luteal structures after first insemination, 25% were retained up to 28 days after AI, indicating pregnancy had occurred but embryo loss occurred between pregnancy recognition (day 15) and days 28 to 32 after insemination. Cows receiving TAI also had longer reinsemination intervals than cows inseminated at estrus, a phenomenon that is exaggerated during summer heat stress.

Key words: human chorionic gonadotropin, estrus, reinsemination, embryo loss

Introduction

Timed AI (**TAI**) programs facilitate control of estrous cycles in lactating dairy cattle and provide viable options to AI programs solely based on detection of estrus. Although pregnancy outcomes are often similar between cows inseminated at estrus or after TAI, reinsemination intervals have not been evaluated to determine if these intervals are in any way different in terms of timing or distribution among cows. Dairy producers often report that a proportion of these cows receiving TAI are observed in estrus 10 to 12 days after AI. Furthermore, we desired to determine whether multiple-ovulating cows that failed to conceive would show differences in the onset or distribution pattern for returns to estrus after insemination.

Our objective was to determine the pattern and timing of reinsemination intervals of lactating dairy cows previously inseminated after observed estrus or TAI (Experiment 1) or after accessory luteal structures were induced by human chorionic gonadotropin (**hCG**) after insemination (Experiment 2).

Experimental Procedures

Experiment 1

Lactating dairy cows from 4 herds in northeast Kansas were enrolled in the study. Three herds comprised cows calving from September 2010 through September 2011, with cows from the remaining herd calving from September 2009 through September 2011. All herds included cows that were milked thrice daily and fed diets consisting of alfalfa hay, corn silage, soybean meal, whole cottonseed, corn or milo grain, corn gluten feed, vitamins, and minerals.

At calving, 3,285 dairy cows (>95% were Holsteins with the residual representing crosses of Holstein with either Jersey, Brown Swiss, or Scandinavian Red) were clustered into breeding groups on a weekly (Herds 2, 3, and 4) or biweekly (Herd 1) basis. Enrollment in the study began at a median 42 days in milk (**DIM**) (41 ± 0.1 d; mean ± SE). All cows were enrolled in a TAI program. Cows were at a median of 75 DIM (74 ± 0.1 days) when inseminated at TAI.

Of the original cows assigned to the TAI program, 472 cows identified in estrus were inseminated early (early bred; **EB**) before completing the entire experimental protocol and did not receive further scheduled injections. These 472 early inseminations occurred a median of 58 DIM (61 ± 0.5 days). The EB cows included those identified by rubbed tail chalk or tail paint or by vaginal mucus.

Date of first repeat insemination was recorded for all cows after the initial AI. Cows reinseminated were considered to be not pregnant unless a subsequent pregnancy diagnosis confirmed the pregnancy to be established earlier at first service based on size of the fetus. Return intervals to a second AI were categorized as early (<18 d), normal (18 to 25 d), or late (>25 d) for purposes of analysis.

Experiment 2

Lactating dairy cows at the Kansas State University Teaching and Research Center milked thrice daily (n = 328) and previously inseminated at first service were assigned randomly to a completely randomized design consisting of two treatments when at least 1 corpus luteum (**CL**) was detected on day 7 post-AI. Treatment consisted of 1,000 IU hCG (1 mL Chorulon, Intervet Schering Plough Animal Health, Millsboro, DE) or 1 mL of saline (control) administered i.m. Blood was collected and luteal structures were mapped and sized by transrectal ultrasonography on days 7, 14, 21, 28, and 32 after AI. Blood also was collected on d 60 from all pregnant cows.

Repeat insemination dates were recorded after the initial insemination, and return intervals to a second AI were categorized as in Experiment 1. Blood was assayed for progesterone (days 7, 14, 21, 28, and 32 after AI) and pregnancy-specific protein B (BioPRYN test; days 21, 28, and 32) concentrations to determine luteal function and evidence of a viable embryo, respectively.

Results and Discussion

Experiment 1

Proportions of EB cows that returned to estrus and were reinseminated <18 days after AI were greater ($P < 0.05$) than those cows receiving TAI (Table 1). Fewer ($P < 0.05$) TAI cows returned to estrus in the normal 18- to 25-day interval compared with EB cows. More ($P < 0.05$) TAI cows were reinseminated after day 25.

Patterns of percentage distribution of cows returning to estrus during 30 days after first AI were similar between EB cows and those receiving the TAI (Figure 1). With the exception of day 21 after AI, the proportions of reinseminations between days 20 and 25 were similar between EB and TAI cows. Mean duration of the reinsemination interval for cows returning to estrus by day 30 during the cool months of the year did not differ between EB and TAI cows, but during the summer months, TAI cows had 1.7-day longer reinsemination intervals than EB cows (Figure 1).

Experiment 2

Multiple luteal structures were induced in 70% of the cows treated with hCG regardless of pregnancy status. Of those cows responding to hCG, 75% formed one accessory luteal structure and 25% formed two or more structures. Treatment with hCG reduced ($P < 0.05$) by half the proportion of cows reinseminated between 18 and 25 days after first AI (Table 2). As a result, more ($P < 0.05$) hCG cows were reinseminated after day 25. For cows with >25 days to reinsemination, concentrations of progesterone were greater ($P < 0.001$) in hCG- than saline-treated cows on day 14 (7.1 ± 0.3 vs. 5.2 ± 0.3 ng/mL) and day 21 (5.2 ± 0.3 vs. 3.9 ± 0.3 ng/mL), respectively, but concentrations were similar (2.5 ± 0.4 ng/mL) between treatments by day 28. Mean duration of the reinsemination interval for cows returning to estrus by day 30 was nearly 4 days longer for cows treated with hCG than with saline (Figure 2).

Retention of at least one post-AI luteal structure in cows treated with hCG or saline through day 28 in nonpregnant cows was observed. In fact, by day 28, this proportion was 25% and did not differ between treatments. We further studied the retention of luteal structures by examining concentrations of progesterone and pregnancy-specific protein B (**PSPB**; BioPRYN test, BioTracking, Moscow, ID) in nonpregnant (nonpregnant–retained CL) and pregnant (pregnant–retained CL) cows that retained at least 1 luteal structure to day 28 post-AI as well as in cows in which all structures regressed (nonpregnant–no CL) before day 28. Pretreatment concentrations of progesterone on day 7 did not differ among groups before treatment with hCG or saline (Figure 3). On day 14, however, only saline-treated nonpregnant–no CL cows had lesser ($P < 0.05$) concentrations of progesterone, indicating that either hCG, pregnancy status, or both increased progesterone secretion. By d 21, cows that retained their luteal structures and were pregnant or retained their luteal structures and were not pregnant on day 28 or 32 had greater ($P < 0.05$) concentrations of progesterone than cows whose luteal structures regressed before day 28. In addition, on day 21, hCG further increased ($P < 0.05$) progesterone in pregnant cows compared with all other groups. By day 28, the 3 groups of cows differed ($P < 0.05$) from one another within treatment (Figure 3) and pregnant, hCG-treated cows had the largest concentrations compared with all other groups.

This potential for pregnancy in the nonpregnant–CL retained group that resulted in subsequent early embryonic loss is supported by elevated concentrations of PSPB in cows in the 3 groups. Although concentrations did not differ on day 21, 28, or 32 between the 2 groups with retained CL for which a full complement of samples were available for all 3 sampling days (days 21, 28, and 32), when all samples were included in the analysis, the nonpregnant–no CL group tended ($P = 0.08$) to differ from the nonpregnant–retained CL group (55.4 vs. 134.0 pg/mL) on d 32 (Figure 4). Only 6 of 31 cows in the nonpregnant–retained CL group (for which samples for PSPB were available on d 21, 28, and 32) had elevated PSPB (>100 pg/mL) on day 32. Furthermore, only 2 of these 6 cows had elevated PSPB on both days 28 and 32. A lack of any significant PSPB elevation on day 28 in nonpregnant–retained CL cows may be a dilution effect of the remaining cows having concentrations of PSPB at or near the sensitivity of the assay.

Why differences occurred in return to insemination intervals (Table 1) for EB cows and cows that completed both TAI programs is unclear. Fewer shorter returns (<18 d) in TAI cows are consistent with our observations that less than 5% of cows returned to estrus during this interval in Experiment 2 after a TAI at first service. In Experiment 2, approximately one-third returned to estrus in the normal 18- to 25-day period, which is consistent with Experiment 1. Furthermore, 25% of cows that retained their original luteal structures to d 28 after TAI at first service were diagnosed as not pregnant at d 32 in Experiment 2. These differences in return intervals could exist partly because EB cows are nearly "100% synchronized" compared with a lesser percentage of TAI cows and are a result of pregnancy failure in any cow after pregnancy recognition but before first pregnancy diagnosis.

The fact that 25% of cows in Experiment 2 retained at least 1 luteal structure to day 28 after TAI indicates that some of the previously observed long estrous cycles were associated with early embryo loss after pregnancy recognition. Only 6 of 31 had elevated PSPB on day 32 and only 2 of 6 had elevated PSPB on days 28 and 32, which may indicate that these embryos were late-developing. Although these embryos may have been sufficiently viable to prevent luteolysis by secreting adequate amounts of interferon-tau and survived variously beyond day 21, they lacked the ability to produce adequate concentrations of PSPB by the trophectoderm binucleate cells. Suggestion of early embryo loss also is supported by our observation that these nonpregnant cows with retained CL had progesterone concentrations intermediate between those in nonpregnant cows that returned to estrus by day 21 and those in cows diagnosed as pregnant on day 28 or 32 (Figure 3). Evidence that some of these nonpregnant retained-CL cows were pregnant also was supported by elevated concentrations of PSPB on day 32 (data not shown).

Many pregnancy losses occur before pregnancy recognition, but in high-producing lactating dairy cattle, substantial losses continue to occur up to 42 to 56 days after insemination. Several factors affect pregnancy losses in cattle, such as compromised oocytes, resulting in poorly developed embryos incapable of cross-talking with the endometrial epithelial cells, to inadequate uterine environment and infectious agents resulting in death of the embryo from undernourishment. Other studies indicated anovulation or anestrus, metabolic status of the cow, some dietary ingredients, as well as occurrence of diseases, predispose the cow to experience embryonic and fetal death. Although some insemination protocols might affect embryo survival, when TAI has been implemented properly, it has not influenced embryonic or fetal death in cattle.

Reinsemination intervals clearly differ between cows inseminated at estrus and those inseminated at TAI. Some of these differences may stem from pregnancies that have occurred after TAI, thus accounting for longer average reinsemination intervals. It is also clear that when cows have multiple luteal structures, such as induced by hCG in Experiment 2, reinsemination intervals are longer, but despite that, the proportion of cows with retained luteal structures are similar at 25% at day 28, even though all of these cows are nonpregnant at day 32.

Table 1. Distribution of cows according to reinsemination intervals after artificial insemination (AI) at first service (Experiment 1)

Days from timed AI	Early bred	Timed AI
	% (n)	
<18	13.2[a] (5/40)	6.8[b] (5/125)
18 to 25	38.3[a] (44/116)	27.9[b] (78/432)
>25	48.5[a] (71/147)	64.0[b] (634/990)

[a,b] Proportions within row having different superscript letters differ ($P < 0.05$).

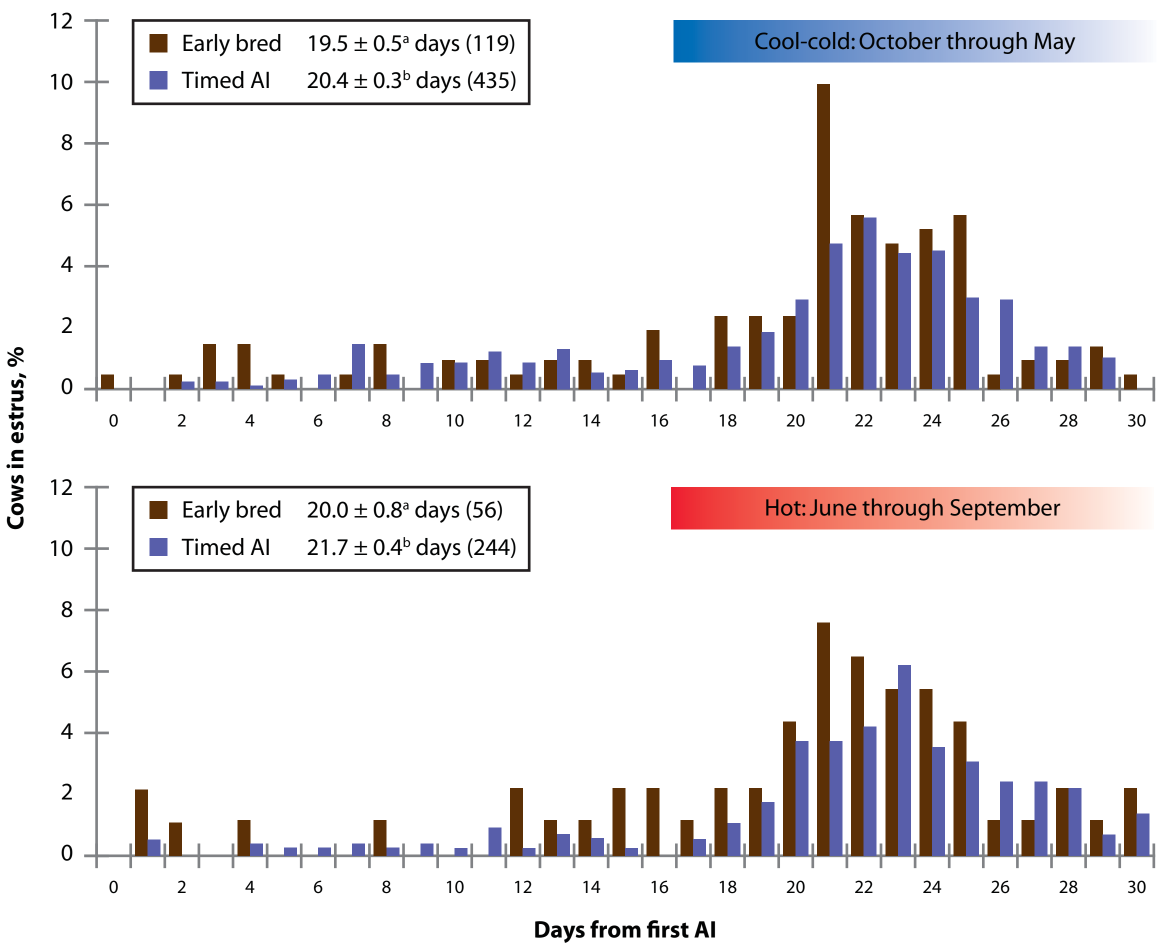

Figure 1. Proportion of cows in estrus after first service after having been first inseminated at estrus or timed inseminated. Duration of cycles is the average reinsemination interval for cows reinseminated up to 30 days after first service.

Table 2. Proportion of cows reinseminated at various intervals after first insemination

Days to reinsemination	Treatment[1]	
	Saline	hCG
	------------------------------ % (n/n) ------------------------------	
<18	3.6 (3/83)	1.1 (1/95)
18 to 25	25.3[a] (21/83)	12.6[b] (11/95)
>25	71.1[a] (59/83)	86.3[b] (60/95)

[a,b] Means differ ($P < 0.05$) between treatments.
[1] Cows were treated with either hCG or saline on day 7 post-artificial insemination.

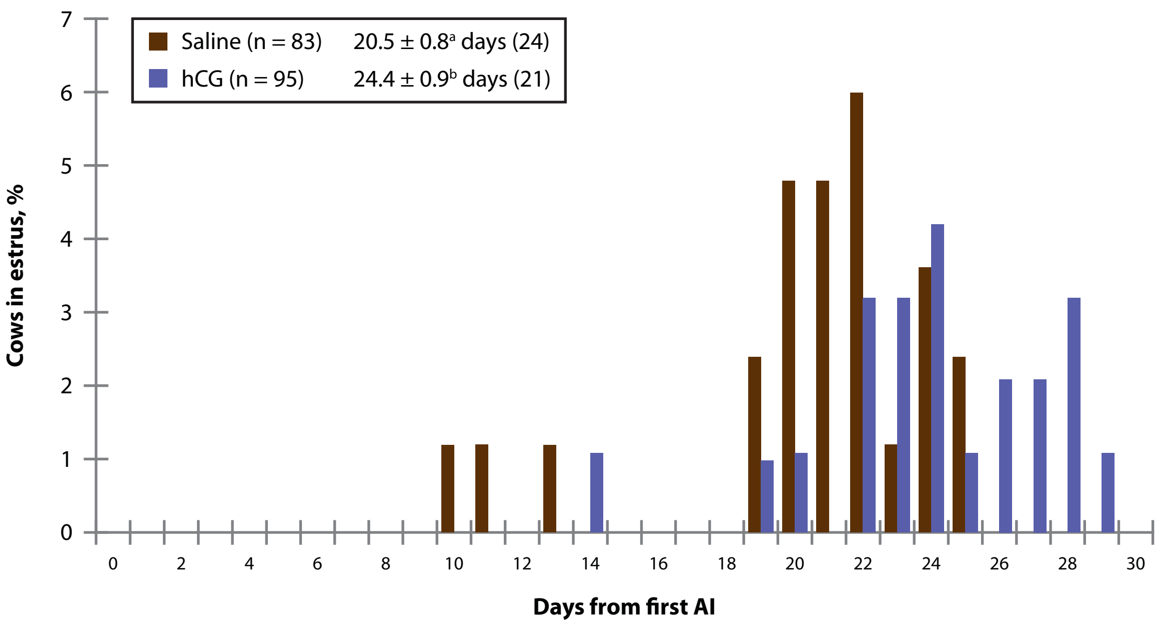

Figure 2. Proportion of cows in estrus after first service after having been timed artificially inseminated (TAI) at first service and received with saline or human chorionic gonadotropin (hCG) on day 7 after the TAI. Duration of cycles is the average reinsemination interval for cows reinseminated up to 30 days after first service.

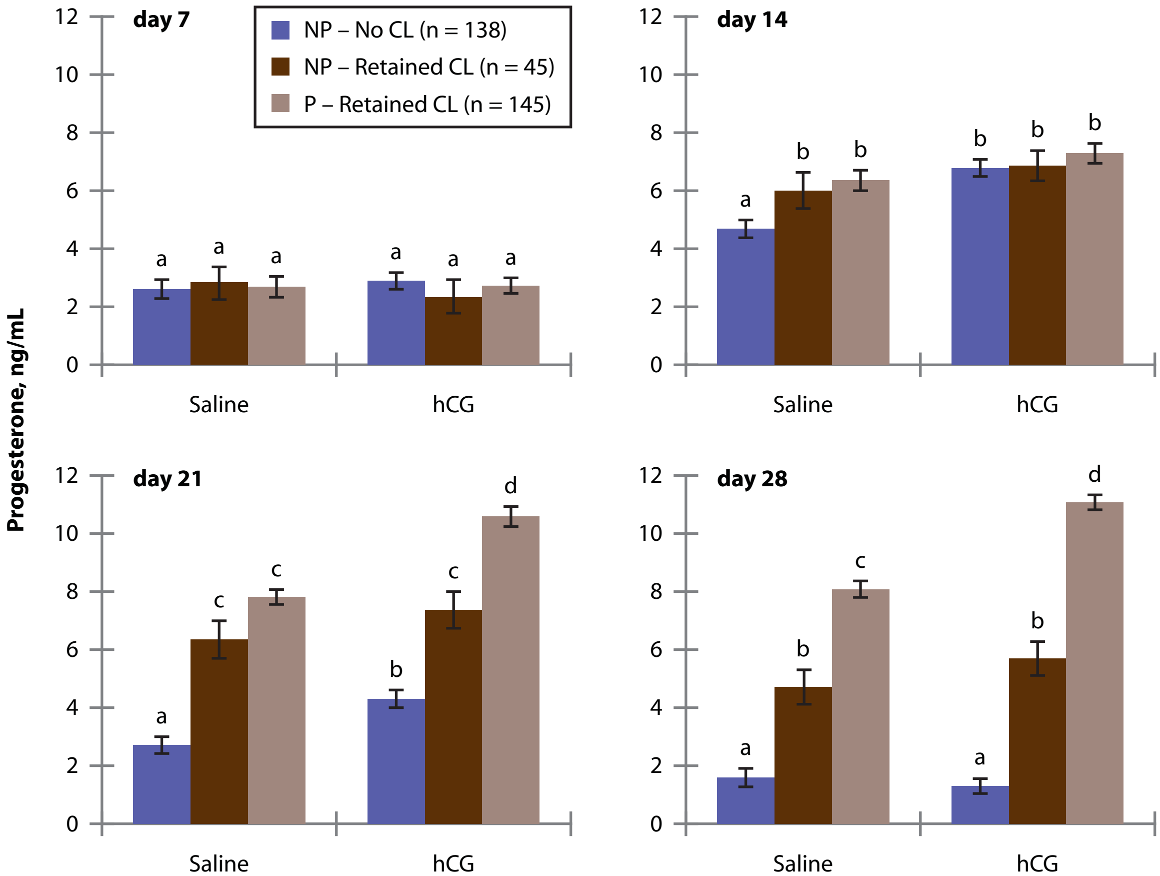

Figure 3. Concentrations of progesterone on days 7, 14, 21, and 28 post-GnRH-AI in cows whose: (1) original corpora lutea (CL) regressed before day 28 after first artificial insemination and were not pregnant (NP) on day 32 (NP – no CL), (2) original CL were retained until day 28 but were not pregnant on day 32 (NP – retained CL), and (3) original CL were retained until day 28 and were pregnant (P) on day 32. Bars with different superscript letters within day differ ($P \leq 0.05$).