ALTERED INSEMINATION TIMING IMPROVES PREGNANCY RATES AFTER A CO-SYNCH + PROGESTERONE INSERT PROTOCOL

by

CASEY DOBBINS

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Department of Animal Sciences and Industry
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Approved by:

Major Professor
Dr. Jeffrey S. Stevenson

ABSTRACT

Our objective was to determine the optimal time to inseminate artificially cows following the standard CO-Synch protocol that also included a progesterone-releasing intravaginal controlled internal drug release (CIDR) insert. Lactating females from 3 Kansas locations were utilized. Crossbred Angus cows (n = 212) from the Agriculture Research Center in Hays (ARCH; location 1); Angus-Hereford crossbred cows (n = 249) from the Kansas State University Cow-Calf Unit (location 2); and purebred Angus, Hereford, and Simmental cows (n = 144) from the Kansas State University Purebred Beef Unit (location 3) were used in this study. Cows within each location were blocked by parity and assigned randomly within blocks to be artificially inseminated (AI) at 4 different times after the $PGF_{2\alpha}$ injection of the protocol: 48, 56, 64, or 72 h. Pregnancy diagnosis occurred at 32 and 63 d after insemination. Blood samples were collected 9 to 10 d and just before the first GnRH injection. Radioimmunoassays were performed on the blood sera samples to determine progesterone concentrations. Progesterone concentrations determined that approximately 60% of cows were cycling at the initiation of the study. A difference in cyclicity was observed with regards to age as well as body condition score. Pregnancies per AI (P/AI) at d 32 varied according to location and cycling status. Pregnancy loss between d 32 and 63 also was greatest for cows inseminated at 48 and 72 h. As pregnancy rates at d 63 increased with the 56- and 64-h treatments, pregnancy loss decreased. A significant difference in calving interval was detected among treatments, the shortest calving interval at 56 h. Results indicated that in

most situations, the 56- and 64-h treatments presented the most desirable outcomes. The 56-h treatment presented the greatest number of P/AI for younger cows (≤ 3 yr), but for older cows, inseminations anytime 56 h or later produced the most P/AI. Overall pregnancy rates at d 63 were greatest for the 56-h treatment, with the fewest pregnancy losses. Given the interactions that seem to exist among location, cycling status, and age, further work is necessary to better define these relationships with the applied protocol.

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With sincere appreciation, Casey.

Dedication

I dedicate this report to my friends and family: to my loving fiancé, Kyle, whose dedication and selfless service to our country is a constant source of inspiration; to my parents for their continued guidance and support; to my sister who has been a life-long friend; and to Kelly without whom I would have never survived graduate school.

With love, Casey.

CHAPTER 1 - Timed Artificial Insemination after a CO-Synch + Progesterone Insert (CIDR) protocol

ABSTRACT

Our objective was to determine the optimal time to inseminate artificially cows after using the standard CO-Synch protocol that also included a progesterone-releasing intravaginal controlled internal drug release (CIDR) insert. Lactating beef cows from 3 Kansas locations were utilized in this study. Crossbred Angus cows (n = 212) from the Agriculture Research Center in Hays (ARCH; location 1); Angus-Hereford crossbred cows (n = 249) from the Kansas State University Cow-Calf Unit (location 2); and purebred Angus, Hereford, and Simmental cows (n = 144) from the Kansas State University Purebred Beef Unit (location 3) were used in this study. Cows within each location were blocked by parity and assigned randomly within blocks to be artificially inseminated (AI) at 4 different times after the $PGF_{2\alpha}$ injection of the CO-Synch + progesterone insert protocol: 48, 56, 64, or 72 h. Pregnancy diagnosis occurred at 32 and 63 d after insemination to determine pregnancies per AI (P/AI) and pregnancy loss. Blood samples were collected 9 to 10 d and just before the first GnRH injection and placement of the progesterone insert. Radioimmunoassays were performed on the blood sera samples to determine concentrations of progesterone. Concentrations of progesterone determined that approximately 60% of cows were cycling at the initiation of the study. We also found that the greatest percentage of cycling cows were at the location 1 (63.7%), followed by the location 2 (62.6%) and the lowest percentage at location 3 (47.5%). A significant difference in cyclicity was observed with regards to age of the

female where cows 3 yr of age or greater had a higher cyclicity rate at 72.7%, whereas cows younger than 3 yr of age had a 43.2% cycling rate. Rates of cycling females also increased as body condition score increased. Pregnancies per AI (P/AI) at d 32 varied according to location and pretreatment cycling status. At the location 1, timed AI at 64 h was best for cycling cows, but 56 h seemed to be more optimal for anestrous cows. At location 2, 56 h was greatest for cycling cows and noncycling cows. At location 3, AI at 72 h was best for cycling cows and at 64 h for anestrous cows. Pregnancy loss between d 32 and 63 also was greatest for cows inseminated at 48 and 72 h. As pregnancy rates at d 63 increased with the 56- and 64-h insemination times, pregnancy loss decreased. Calving rates did not differ among insemination times. A significant difference in calving interval was detected among treatments, with the shortest calving intervals occurring in cows inseminated at 56 h. These results indicate that in most situations, the 56- and 64-h time of AI presented the most desirable fertility outcomes. The 56-h insemination time presented the greatest number of P/AI for younger cows (≤ 3 yr), but for older cows, inseminations anytime 56 h or later produced the most P/AI. Overall pregnancy rates at d 63 were greatest for the 56-h treatment, with the fewest pregnancy losses. Given the interactions that seem to exist among location, cycling status, and age, further work is necessary to better define these relationships in lactating beef cows to which the CO-Synch + progesterone insert program is applied before AI.

INTRODUCTION

Ability to synchronize estrus, ovulation, or both presents many options to cattle producers who desire to use artificial insemination (**AI**). Programs are available that use fixed-time insemination and produce acceptable pregnancy rates (Lamb et al., 2001;

Patterson et al., 2003; Larson et al, 2006). Applying estrus-or ovulation-synchronization programs to facilitate use of AI allows greater opportunity to increase genetic progress and selection of desirable, economic traits. Results from well-managed Kansas ranches indicate 40 to 60% of cows may be cycling at the beginning of the breeding season (Stevenson et al., 2003). When winter conditions are harsh, forage availability limited, and/or winter supplementation was insufficient, many cows may not be cycling at the onset of the breeding season (Stevenson et al., 2003). Thus, synchronization protocols that induce ovulation in both anestrous as well as cycling cows are essential.

When no detection of estrus is possible or desirable, the best protocol must include GnRH to stimulate noncycling and cycling cows to ovulate (manage follicle populations), a source of progestin to alter uterine function and tighten ovulation synchrony, and $PGF_{2\alpha}$ to control corpus luteum function (Patterson et al., 2003). The CO-Synch protocol provides the appropriate sequence, GnRH-$PGF_{2\alpha}$-GnRH, to stimulate ovulation associated with AI at an appointed time after $PGF_{2\alpha}$.

Addition of a progesterone-releasing intravaginally placed controlled internal drug release (**CIDR**) insert is also desired to prevent premature estrus during the protocol and thus tighten synchronization of ovulation as part of an optimal program for timed AI (Lamb et al., 2001; Larson et al., 2006). The CO-Synch protocol + progesterone insert seems to provide these desirable benefits that are needed in a timed insemination program (Larson et al., 2006). A similar study (Bremer et al., 2004) compared the CO-Synch protocol + progesterone insert to the 7-11 Synch estrus-synchronization program and reported pregnancy rates to be greater with the CO-Synch + progesterone insert protocol.

The CO-Synch + progesterone insert protocol also provides an advantage to many producers because it is a simple and easy protocol to administer.

Experiments to determine optimal timing of AI in the CO-Synch + progesterone insert protocol, however, have been lacking. The objective of the current study was to determine the optimal timing of insemination when applying a CO-Synch + progesterone insert protocol.

MATERIALS AND METHODS

Animals and Experimental Design

In the spring of 2005, lactating beef cows at 3 Kansas locations were utilized. Crossbred Angus cows (n = 212) from the Agriculture Research Center in Hays (ARCH; location 1); Angus-Hereford crossbred cows (n = 249) from the Kansas State University Cow-Calf Unit (location 2); and purebred Angus, Hereford, and Simmental cows (n = 144) from the Kansas State University Purebred Beef Unit (location 3) were used in this study. Cows within each location were blocked by parity and assigned randomly within blocks to be inseminated at 4 different times after the $PGF_{2\alpha}$ injection of the CO-Synch + progesterone insert protocol: 48, 56, 64, or 72 h (Figure 1.1). Numbers of cows differed slightly among treatment times due to a variation in pasture sizes. All cows were body-condition scored (1 = thin and 9 = obese; Whitman, 1975) and a blood sample was collected on d –17. On d –7, all cows were injected i.m. with 100 µg of GnRH (2 mL of OvaCyst, IVX Animal Health, St. Joseph, MO), a second blood sample was collected, and all cows received a CIDR insert (Eazi-Breed CIDR insert, Pfizer Animal Health, New York, NY) containing 1.38 g of progesterone. On d 0, the CIDR inserts were removed and each cow received i.m. a 25-mg injection of $PGF_{2\alpha}$ (5 mL of Lutalyse, Pfizer Animal Health). Each cow was then inseminated and injected i.m. with a second 100-µg injection of GnRH according to their designated treatment AI time (48, 56, 64, or 72 h).

Pregnancy status was evaluated via transrectal ultrsonography on d 32 (range of 27 to 35 d) after AI and pregnancy status was reconfirmed on d 63 (range of 60 to 68 d).

Blood Collection and Radioimmunioassays

Blood samples were collected 10 d before and at the time of the first GnRH injection and CIDR insertion were analyzed for progesterone content by radioimmunoassay (Skaggs et al., 1986). When either sample contained concentrations of progesterone ≥1 ng/mL, the cow was considered to be cycling. When concentrations of progesterone in both samples were <1 ng/mL, the cow was considered to be noncycling or anestrous.

Statistical Analyses

Binomial variables were analyzed using logistic regression (procedure GENMOD, SAS Institute Inc., Cary, NC). The model for testing the effects of pregnancies per AI (**P/AI**) at d 32 and 63, and pregnancy loss between those times, included the following independent variables: treatment (48, 56, 64, of 72 h timing of AI), location (n = 3), sire and AI technician, each nested within location, cycling status (0 vs. 1), interactions of treatment × cycling status, treatment × age, treatment × location × cycling status, body condition score (≤ 4, 4 to 5, and > 5), and days postpartum at the onset of the breeding season (<60, 60 to 75, and >75).

Calving rate and calving interval were analyzed similarly including treatment, location, interaction of treatment × location, cycling status, interaction of treatment × cycling status, body condition score, and days postpartum.

When F-tests were significant ($P < 0.05$), differences among least-square means were detected by the method of least-significant difference.

RESULTS AND DISCUSSION

Before the CO-Synch + progesterone insert protocol was initiated in our study, 60% of the 605 cows across all 3 locations had initiated estrous cycles (Table 1.1). Rate of cyclicity in the statistical model was 63.7% for cows at location 1, 62.6% at location 2, and 47.5% at location 3. As expected, cyclicity rates were less ($P < 0.001$) in 2- and 3-year old cows (43.29%) compared with 4-yr or older cows (72.7%). In the current study, fewer 2-yr-old cows were cycling (30%), despite calving on average, 23 and 20 days earlier ($P < 0.001$) than the 3-yr or older cows (Table 1.1). As BCS in cows increased from ≤4 to >5, the percentage of cows cycling increased ($P \leq 0.05$). Fewer ($P \leq 0.05$) cows < 60 d postpartum were cycling before treatment than those ≥ 60 d (Table 1.1).

The time between the $PGF_{2\alpha}$ injection and timed insemination varied slightly within treatments. The means for the 48, 56, 64 and 72 h treatments were 48.4 (range of 46.9 to 49.3 h), 56.2 (range of 55.0 to 57.2 h), 64.5 (range 63.0 to 66.8 h) and 72.6 (range of 71.4 to 73.3 h), respectively.

Pregnancies per AI of cows by treatment, cycling status, location, age, and days postpartum are summarized in Table 1.1. Insemination at 56 h improved ($P < 0.01$) P/AI compared with inseminations made at 48 h, but only tended ($P = 0.08$) to be better than 64 and 72 h. The P/AI response fit ($P < 0.05$) a quadratic and cubic curve, indicating the pregnancy rates probably peaked between 56 and 64 h.

The reported P/AI were somewhat different than in an earlier study (Bremer et al., 2004) in which 3 fixed-times after $PGF_{2\alpha}$ (48, 54, and 66 h) were tested after a CO-Synch

+ progesterone insert. They reported P/AI increased with increased time between $PGF_{2\alpha}$ and AI (56%, 67%, and 71%), respectively, whereas ours seemed to peak after 56 h.

The P/AI response in our study differed among cows depending on treatment, cycling status, and location (treatment × location × cycling status interaction, $P < 0.01$; Figure 1.2). Noncycling cows seemed to have more P/AI than cycling cows (57.7 vs. 50.1%, respectively). At the location 1, timed AI at 64 h was best for cycling cows, but 56 h seemed to be more optimal for anestrous cows. At location 2, 56 h was greatest for cycling cows and noncycling cows. At location 3, AI at 72 h was best for cycling cows and at 64 h for anestrous cows.

A treatment × age group interaction ($P = 0.02$) also was detected. This was interpreted to mean that the optimal insemination timing may differ according to age of the cow. Figure 1.3 illustrates that in 2- and 3-yr-old cows, the 56-h timing was clearly greatest. In contrast, in 4-yr and older cows, timing of 56 h or later was superior to 48 h.

Pregnancy rate at d 63 of the breeding season, which included both AI and exposure of cows to natural-service bulls beginning 10 d after the timed AI, are summarized in Table 1.2. The advantage of the 56-h AI time continued compared with 48- and 72-h inseminated cows. Actual calving rates of cows were similar in the 48-, 56-, 64-, and 72-h treatments were 71.3, 77.7, 81.1, and 77.3%, respectively. Conception rates of cows failing to conceive to the timed AI were normal in response to natural mating (clean-up bulls). Actual calving intervals were 370, 360, 364, and 365 days for the 48-, 56-, 64-, and 72-h treatments.

Pregnancy loss for females that conceived from timed AI was determined on d 63 from the time of insemination in cows previously diagnosed pregnant at d 32 (Table 1.2).

Pregnancy loss was greatest in the 48- and 72-h treatments. The least pregnancy loss was detected in cows inseminated at 56 and 64 h.

The scientific literature indicates (Odde, 1990), that attempts to synchronize estrus in the bovine have been made for more than 50 yr, with the first efforts using a single dose of progesterone. When determining the optimal time for AI, an optimal protocol must first be determined. Greater conception rates with timed AI were observed after the Ovsynch protocol compared with Syncro-Mate-B (Geary et al., 2001). One study focused on the improved conception rates seen with calf removal in both Ovsynch and CO-Synch protocols (Geary et al., 2001). Geary et al. (2001) found that the CO-Synch protocol resulted in the greatest number of conceptions in addition to being the easiest protocol to administer because cows and calves were handled less than when applying the Ovsynch protocol.

The CO-Synch protocol seems to provide the desirable benefits that are needed in a timed AI program. The literature indicates (Lamb et al., 2001), however, that the use of additional progesterone may be needed to ensure tight synchronization for a timed AI to be more efficacious.

In addition, when a progesterone insert is added to synchronization protocols, it has potential to increase P/AI (Larson et al., 2006). Comparison of the CO-Synch with the CO-Synch + progesterone insert protocol demonstrated that the addition of supplemental progesterone increased P/AI after a timed AI. The P/AI averaged 43 and 54% when comparing the CO-Synch with the CO-Synch + progesterone insert protocol, respectively (Larson et al., 2006). Comparison of these 2 protocols in this study also led to the conclusion that addition of progesterone in the CO-Synch + progesterone insert treatment improved P/AI when a fixed timed AI occurred at 60 h (Larson et al., 2006).

In general, a limiting factor of the success of estrus- or ovuation synchronization is likely to be the proportion of noncycling, cows exposed to treatment (Stevenson et al., 2003; Short et al., 1990). This fact is reiterated in many studies conducted for the continued development of synchronization. A major barrier in estrus synchronization and achieving desirable pregnancy rates in suckled beef cows is conquering postpartum anestrus (Larson et al., 2006). In contrast, our results indicate that using the CO-Synch + progesterone insert actually produced more P/AI in noncyling than in cycling cows.

It is important to remember that both cycling cows as well as those who are not cycling will be present within each herd. Synchronization protocols must induce ovulation in both anestrous as well as in cycling females to maximize P/AI.

Age is an additional factor to consider. Limitations increase as calving increases throughout a cow's life. Primiparous cows generally have a more prolonged anestrus because of their additional growth requirements (Stevenson et al., 2003).

A surprising result in our study was observed in the interaction of age and AI timing. The optimal insemination timing is likely to vary with the age of the cow. We found that increasing the interval to insemination from 56-, 64-, and 72 h paralleled increased age of cows (Figure 1.3). A second study would be warranted to examine further this interaction of age and AI timing.

Using optimal timed AI in conjunction with the CO-Synch + progesterone insert protocol provides the potential to attain outcomes demanded by producers and desired within today's beef production systems.

IMPLICATIONS

At the onset of this study we hypothesized that the optimal range of times to conduct timed AI of suckled beef cows as part of the standard CO-Synch + progesterone insert protocol was approximately 56 to 64 h following the injection of $PGF_{2\alpha}$. Our goal in utilizing the CO-Synch + progesterone insert protocol was not only to synchronize ovulation in cycling cows, but more importantly, to induce a fertile ovulation in noncycling females. The P/AI peaked when AI was performed between 56 and 64 h, with a greater percentage of P/AI detected in anestrous females.

The window for timed AI for younger cows may be smaller than for mature cows. Although overall conception rates were greatest between the 56 and 64 h, some variation occurred among age groups. Younger females may benefit more from an earlier insemination time, closer to 56 h, whereas older females obtained more P/AI from a later insemination time such as the 64 to 72 h. More information is needed before specific age recommendations might be made for beef cows. Our results indicate that those wishing to use fixed-time insemination with a CO-Synch + progesterone insert protocol may have a broader window of insemination times from 56 to 64 h after $PGF_{2\alpha}$. This larger window may facilitate application of the CO-Synch + progesterone insert protocol in a wider range of production settings.

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Table 1.1. Cycling status and overall pregnancies per AI at d 32 after timed AI according to time of insemination (treatment), cycling status, location, age, days postpartum, and body condition score at the beginning of the breeding season

Item	No. of cows	Cycling, %		Pregnancies per AI, %	
		Unadjusted[1]	Adjusted[2]	Unadjusted[1]	Adjusted[2]
Treatment, h after $PGF_{2\alpha}$					
48	136			42.6	45.4[a]
56	157			62.4	63.2[b]
64	170			54.1	53.6[ab]
72	142			51.4	53.4[ab]
Cycling status before treatment					
Not cycling	242			55.8	57.7
Cycling	363			51.2	50.1
Location					
Agriculture Research	212	67.9	63.7[a]	51.9	49.0
Center-Hays	249	65.1	62.6[a]	56.2	54.4
Cow-Calf Unit	144	39.6	47.5[b]	49.3	58.2
Purebred Beef Unit					
Age, yr					
≤ 3	276	44.6	43.2[a]	50.4	50.4
> 3	329	72.9	72.7[b]	55.3	57.4
Days postpartum at $PGF_{2\alpha}$					
< 60	292	56.7	52.2[a]	50.9	48.3[a]
60 to 75	155	70.3	64.5[b]	49.7	52.3[ab]
> 75	159	56.0	57.2[ab]	60.4	61.0[b]
Body condition score					
≤ 4	102	41.1	47.3[a]	41.2	40.4[a]
4.0 to 5.0	315	55.9	56.2[a]	51.4	53.8[b]
> 5.0	188	77.1	70.4[b]	62.2	67.5[c]

[a,b,c] Means having different superscript letters differ ($P \leq 0.05$).

[1]Actual percentages.

[2]Adjusted mean percentages resulting from statistical analyses.

Table 1.2. Overall pregnancy rate at d 63 and embryo loss for cows that conceived to timed AI[1]

	Time of insemination after $PGF_{2\alpha}$, h			
Item	48	56	64	72
Pregnancy rate at d 63, %	53.60^{a} (136)[2]	73.9^{b} (157)	68.6^{bc}(169)	66.0^{ac} (141)
Pregnancy loss[3], %	10.3^{a} (58)	2.0^{b} (98)	4.3^{b} (92)	8.2^{a} (73)
Calving rate, %	71.3 (136)	77.7 (157)	81.1 (170)	77.3 (142)
Calving interval[4], d	370^{a} ± 2 (97)	360^{b} ± 2 (122)	363^{ab}± 2 (137)	365^{ab} ± 2 (109)

^{a,b} Means having different superscript letters differ ($P \leq 0.05$).

[1]Includes exposure to clean-up or continuous AI to d 63 of the breeding season.

[2]Number of cows.

[3]Loss between d 32 and 63 of pregnancy.

[4]Least squares means ± SE.

Figure 1.1. Experimental protocol. Blood samples were collected at d −17 and −7 for later determination of concentrations of progesterone.

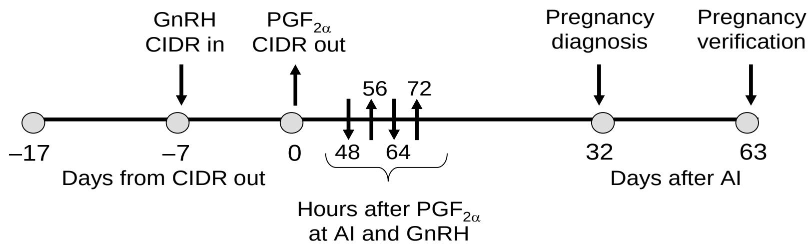

Figure 1.2. **Pregnancies per AI of lactating beef cows at d 32 after timed AI according to time of insemination (48, 56, 64, or 72 h), location, and whether they had initiated estrous cycles (cycling vs. not cycling) before the onset of the CO-Synch + progesterone insert protocol (treatment × location × cycling status interaction [*P* < 0.003]). Numbers of cows represented per bar are listed across the bottom of each bar.**

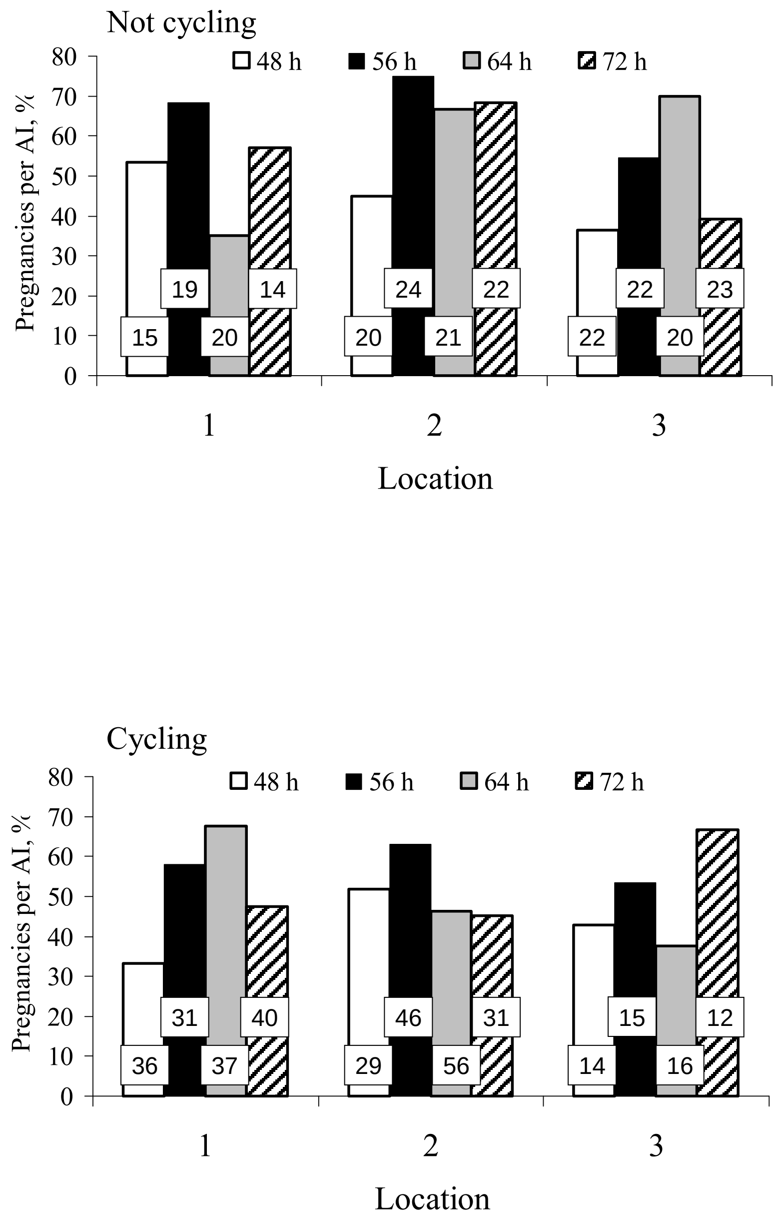

Figure 1.3. **Pregnancy rates of lactating beef cows at 32 d of the breeding season according to time of insemination (48, 56, 64, or 72 h) and age (treatment × age interaction [*P* = 0.02]). Numbers of cows represented per bar are listed across the bottom of each bar.**