# Strategies for Artificial Insemination of Cattle at Synchronized Ovulation or Synchronized Estrus

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#### Summary

Two synchronization protocols in lactating dairy and beef cows and in dairy heifers were tested for efficacy of breeding by artificial insemination (AI) with or without estrus detection. Controls received three prostaglandin  $F_{2}a$  (PGF<sub>2</sub>a) injections 14 days apart before AI at observed estrus. Pregnancy rates were compared with animals on the Ovsynch protocol that combined gonadotropin releasing hormone (GnRH) and PGF<sub>2</sub>a treatments with a timed AI 16 to 20 hours after the second GnRH injection. The pregnancy rates were similar at synchronized ovulation to fixed-time AI in lactating cows, but not effective in heifers because of the lack of synchronization.

#### Introduction

Net receipts from beef and dairy cattle exceeded \$20 billion for the farmers of our nine midwestern states during 1993, which is greater than agricultural receipts from any other animal or plant species (USDA, 1995). Reproductive inefficiency is one of the most costly and productionlimiting problems facing the cattle industry; \$2.7 billion could be saved annually by maximizing reproductive efficiency in cattle from the states in this north central region (Hoards, 1996). A 1995 survey identified herd health and reproduction as the most important (livestock producers) or second most important (agribusiness) issue affecting future profitability of farm operations in this region. During the past five years our basic knowledge has dramatically increased about mechanisms regulating bovine reproduction and particularly regulation of ovarian function. This report focuses on progress toward development of a practical protocol to precisely synchronize the time of ovulation to increase service rate (percentage of cows bred every 21 days) for lactating cows; it did not improve pregnancy rate per breeding (fertility). This protocol reduced days open and increased farm profitability.

Management of artificial insemination (AI) is more efficient when synchronization reduces the variation of first and subsequent AI. Prostaglandin  $F_2a$  (PGF<sub>2</sub>a) can be used in synchronization protocols because it regulates the life span of the corpus luteum on the ovary. Pregnancy rates per AI are similar in cows bred at detected estrus after synchronization of estrus with PGF<sub>2</sub>a or after spontaneous estrus. However, estrus cannot be synchronized precisely with  $PGF_{2}a$  because it does not synchronize growth of ovarian follicles. Thus, estrous detection is needed over a 7day period after doses of  $PGF_{2}a$  given 11 to 14 days apart. Consequently, when cows receive a fixed-time AI 72 to 80 hours after a second injection of  $PGF_{2}a$ , pregnancy rates per AI are considerably lower than those for cows AI at a detected estrus.

Gonadotropin releasing hormone (GnRH) stimulates ovarian follicle growth and maturation. Recently, a novel protocol, Ovsynch, was developed to synchronize ovulation in lactating cattle using GnRH and PGF<sub>2</sub>a (Pursley et al., 1995). Ovulation was synchronized within an 8-hour period from 24 to 32 hours after the second injection of GnRH. This precise synchrony may allow for successful AI without detection of estrus.

The current study evaluated pregnancy rates obtained by timed AI after synchronization of follicular growth and ovulation following the Ovsynch protocol compared with those after a traditional synchronization program using  $PGF_{2a}$  and detection of estrus (Pursley et al., 1997). A second objective was to compare pregnancy rates per AI for lactating cows and nulliparous heifers in response to these two synchronization strategies. This study was a collaborative project among stations of the NC-113 Project of the Cooperative States Research service of the USDA entitled "Methods for Improvement of Fertility in Cows Postpartum."

# Materials and Methods

#### Dairy cows

A total of 310 lactating dairy cows, 60 to 289 days postpartum, from three experiment stations, and 155 dairy heifers, 13 to 23 months old, were assigned randomly by location to two treatments described in Figure 1. Cows in the control group received up to three intramuscular injections of 25 mg of PGF<sub>2</sub>a 14 days apart.

Only cows and heifers that were not detected in estrus were given subsequent hormone injections. All controls detected in estrus after  $PGF_{2a}$  treatment were AI according to the a.m.-p.m. rule. Control animals that did not show estrus after the third  $PGF_{2a}$  injection received a fixed-time AI 72 to 80 hours later. Cows and heifers on the Ovsynch protocol were given an intramuscular injection of 100 mg GnRH. Seven days later, they received a  $PGF_{2a}$  injection followed by a second injection of GnRH 30 to 36 hours later, and a timed AI 16 to 20 hours later. The Ovsynch animals were not observed for estrus. In a subset of animals, blood was collected to evaluate progesterone status at the time of hormone injection to determine the effect of  $PGF_{2a}$  on corpus luteum regression. Effect of treatment on pregnancy rate per AI was evaluated by chi-square analysis. The interactions of treatment and stage of lactation, treatment and parity, and treatment and location were analyzed by logistic regression using the SAS, CATMOD procedure.

## Beef cows

Two-year old lactating beef cows averaging 87 days postpartum were assigned to two treatments according to calving data: group 1 received a GnRH analogue (cystorelin) to synchronize ovulation, and group 2 received PGF<sub>2</sub>a to synchronize estrus. In group 1, calves were separated from their dams for a 48-hour period before beginning the experiment to enhance response to GnRH (cystorelin) treatment. Seven days after the first GnRH analogue administration, cows were given PGF<sub>2</sub>a to regress the corpus luteum. To stimulate a luteinizing hormone surge release, a second dose of cystorelin (100 mg) was given 48 hours later; this was followed by AI of all cows 20 hours after the second cystorelin injection. Cows in group 2 were given three intramuscular injections of PGF<sub>2</sub>a (25 mg) 14 days apart. After each hormone treatment, estrus was detected and cows were AI according to the a.m.-p.m. rule. Cows that did not show signs of estrus throughout the treatment period were bred 80 hours after the third hormone treatment.

## **Results and Discussion**

## Dairy cows

The interval from the start of hormone treatment to AI averaged  $13 \pm 11$  days (mean  $\pm$  standard deviation) for cows treated with PGF<sub>2</sub>a, and  $9 \pm 0$  days for Ovsynch-treated cows. Lactating cows had a similar pregnancy rate per AI following the control PGF<sub>2</sub>a protocol or the Ovsynch protocol (Table 1). In contrast, control heifers had a greater (p < p0.01) pregnancy rate per AI than did heifers treated with Ovsynch. Pregnancy rate was greater for control (PGF<sub>2</sub>a) heifers than cows. Cows in the Ovsynch group that were greater than 76 days postpartum had a greater (p < 0.05) pregnancy rate per AI than cows from 60 to 75 days postpartum (Table 2). Most cows and heifers were AI after the first or second PGF<sub>2</sub>a injection; only 18% of lactating cows and 23% of heifers received a third PGF<sub>2</sub>a treatment. Of those given the third PGF<sub>2</sub>a injection, 92% were bred by timed AI, and pregnancy rate from this breeding was low (4.3% and 50.0%). Control cows that were inseminated at an observed estrus had a greater (p < 0.01) pregnancy rate per AI. However, pregnancy rate per AI was similar (p > 0.10) for control cows inseminated only at estrus and for cows in the Ovsynch group. Pregnancy rate per AI was similar for cows, regardless of whether concentrations of progesterone were high or low at the time of PGF<sub>2</sub>a, but heifers with low progesterone concentrations at the time of PGF<sub>2</sub>a injection had a lower pregnancy rate per AI than did heifers with high progesterone concentrations at the time of PGF<sub>2</sub>a treatment.

Although both protocols allowed all dairy cows to be bred, the Ovsynch protocol provided a predictable time of AI after start of treatment. The Ovsynch method could benefit dairy operations because it allows for timed AI of lactating cows without detection of estrus. For lactating dairy cows, synchronization of ovulation with GnRH and PGF<sub>2</sub>a resulted in a pregnancy rate per AI that was similar to those receiving only PGF<sub>2</sub>a every 14 days. For those cows, 83% were AI at detected estrus, and 17% had timed AI. Pregnancy rates per AI at observed estrus were almost twice as great in heifers as in lactating cows. Timed AI after PGF<sub>2</sub>a is ineffective in lactating dairy cows because of anestrous cows, as well as a large variation in time from luteal regression to observed estrus and differences in the developmental stage of the preovulatory follicle at the time of PGF<sub>2</sub>a treatment. The developmental stage of the preovulatory follicle can be controlled by GnRH treatment, the basis of the Ovsynch protocol. Most (85%) lactating dairy cows ovulate after GnRH treatment, whereas heifers are poorly synchronized by Ovsynch.

## Beef cows

Plasma progesterone concentrations decreased within 48 hours after each PGF<sub>2</sub>a injection in these postpartum beef cows. After the first PGF<sub>2</sub>a injection, average peripheral blood progesterone decreased from 5.36 to 1.49 ng/ml. This trend was consistent throughout the three PGF<sub>2</sub>a treatments (after the second hormone injection, progesterone decreased from  $7 \pm 0.63$  to  $1 \pm 0.06$  ng/ml [mean  $\pm$  standard error]; after the third treatment it decreased from  $8 \pm 0.18$  to  $1.14 \pm 0.10$  ng/ml). Conception rates of beef cows AI after PGF<sub>2</sub>a control or Ovsynch treatments were similar (Table 3). From these results there is no evidence that treatment with the GnRH analogue (cystorelin) increases conception rates in two-year-old postpartum suckled beef cows. The shift in plasma progesterone concentration was a reliable indicator for estrous cycle status in these lactating beef cows.

The success of the Ovsynch protocol depends on synchrony of both the ovulatory follicle and the corpus luteum on the ovary. Greater synchrony of corpus luteum function in lactating cows likely results from high blood progesterone concentrations detected at the time of PGF<sub>2</sub>a injection in a greater proportion of cows (86%) than heifers (59%). Follicle growth seems to be more rapid and the follicular wave lasts longer in heifers than in lactating cows. Thus, some heifers would be at day 9 or 10 after initiation of a follicle wave then this older follicle loses dominance and likely becomes unresponsive to the second GnRH treatment at 30 to 36 hours after PGF<sub>2</sub>a injection.

# Implications Breeding to a synchronized ovulation in lactating cows would allow more control in AI programs and would remove dependence on estrus detection

for AI. The Ovsynch protocol is a strategy to program-breed lactating cows and achieve pregnancy rates equivalent to cows synchronized with PGF<sub>2</sub>a treatment only but requiring estrus detection. Thus, the Ovsynch method may benefit a reproductive management scheme by eliminating the need for estrus detection at AI. The Ovsynch protocol synchronizes follicular and luteal development in lactating cows but not in heifers.

#### References

- Pursley, J.R., Mee, M.O. and Wiltbank, M.C. 1995. Synchronization of ovulation in dairy cows using  $PGF_{2}a$  and GnRH. Theriogenology 44:915.
- Pursley, J.R., Wiltbank, M.C., Stevenson, J.S., Ottobre, J.S., Garverick, H.A. and Anderson, L.L. 1997. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. J. Dairy Sci. 80:295.

Figure 1. Description of the timing of injections for the two synchronization methods used in this experiment. Control cows received AI in relation to estrus unless they were not detected in estrus after any of the three  $PGF_{2}a$  injections. Ovsynch cows had timed AI.

Control Group



Ovsynch Group



Table 1. Pregnancy rates in dairy cows and heifers after treatment with  $PGF_2a$  (control) or synchronization of ovulation (Ovsynch).

	Pregnancy rate		
	Control	Ovsynch	P
Cows	38.9 (n=154)	37.8 (n=156) >0	).10
Heifers	74.4 (n=78)	35.1 (n=77) <0	).01
Р	< 0.01	>0.10	

Table 2. Influence of stage of lactation on pregnancy rates in lactating dairy cows after treatment with  $PGF_{2}a$  (control) or synchronization of ovulation (Ovsynch)<sup>1</sup>

Stage of	Pregnancy rate				
lactation	Control	Ovsynch	Total		
	(%)				
60 to 75 d	39.4 (n=33)	26.0 (n=50)	31.3 (n=83)		
<u>≥</u> 76 d	38.8 (n=121)	43.4 (n=106)	41.0 (n=227)		
Р	0.88	0.04	0.12		

<sup>1</sup>No differences between treatments.

Table 3. Pregnancy rates in beef cows after treatment with  $PGF_{2}a$  (control) or synchronization of ovulation (Ovsynch).

Pregnancy					
Treatment	No. bred	No. pre	gnant %		
PGF <sub>2</sub> a cont Ovsynch P	rol 29 30	21 22	72 73 >0.10		