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## Resynchronization of Lactating Dairy Cows at Open Pregnancy Diagnosis Based on the Presence or Absence of a Corpus Luteum: A Practical Approach

J. A. Sauls  
*Kansas State University, Manhattan, sauls@k-state.edu*

J. S. Stevenson  
*Kansas State University, jss@ksu.edu*

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# Resynchronization of Lactating Dairy Cows at Open Pregnancy Diagnosis Based on the Presence or Absence of a Corpus Luteum: A Practical Approach

## Abstract

Lactating Holstein cows in three herds were enrolled in a study at the time of nonpregnancy diagnosis. Cows were assigned to a resynchronization program based on ovarian structures determined by transrectal ultrasonography. Three resynchronization treatments were employed starting on the day of open diagnosis to test: (1) accuracy of ultrasound technician's ability to identify a functional corpus luteum (CL); (2) whether an initial GnRH injection is required to start resynchronization when a CL is present at nonpregnant diagnosis (Short Synch: PGF<sub>2α</sub> — 24 hours — PGF<sub>2α</sub> — 32 hours — GnRH — 16 hours — timed artificial insemination [AI]); and (3) whether applying progesterone to cows without a CL as part of a traditional Ovsynch program (CIDR + Ovsynch: GnRH + CIDR insert — 7 days — PGF<sub>2α</sub> + CIDR removal — 24 hours — PGF<sub>2α</sub> — 32 hours — GnRH — 16 hours — timed AI) would be equivalent to a standard Ovsynch program (same as CIDR-Ovsynch treatment but no CIDR was applied). Treatments produced similar proportions of pregnancies per AI, with a tendency for increased fertility when the first injection of GnRH was administered as part of a standard Ovsynch. The technician's ability to detect a functional CL was more accurate when the CL visualized was actually functional (progesterone ≥ 1 ng/mL) than when it was not functional (progesterone < 1 ng/mL). Although pregnancy outcomes tended to improve when cows were treated with Ovsynch compared with Short Synch, when a functional CL was accurately detected, pregnancy outcomes did not differ. Technician accuracy for detecting a functional CL is important for improving pregnancy outcomes when applying the Short Synch treatment.

## Keywords

Corpus luteum, Resynchronization, CIDR insert, Gonadotropin-releasing hormone, Short Synch, PGF<sub>2α</sub>

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*J. A. Sauls and J. S. Stevenson*

### Summary

Lactating Holstein cows in three herds were enrolled in a study at the time of nonpregnancy diagnosis. Cows were assigned to a resynchronization program based on ovarian structures determined by transrectal ultrasonography. Three resynchronization treatments were employed starting on the day of open diagnosis to test: (1) accuracy of ultrasound technician's ability to identify a functional corpus luteum (CL); (2) whether an initial GnRH injection is required to start resynchronization when a CL is present at nonpregnant diagnosis (Short Synch: PGF<sub>2α</sub> — 24 hours — PGF<sub>2α</sub> — 32 hours — GnRH — 16 hours — timed artificial insemination [AI]); and (3) whether applying progesterone to cows without a CL as part of a traditional Ovsynch program (CIDR + Ovsynch: GnRH + CIDR insert — 7 days — PGF<sub>2α</sub> + CIDR removal — 24 hours — PGF<sub>2α</sub> — 32 hours — GnRH — 16 hours — timed AI) would be equivalent to a standard Ovsynch program (same as CIDR-Ovsynch treatment but no CIDR was applied). Treatments produced similar proportions of pregnancies per AI, with a tendency for increased fertility when the first injection of GnRH was administered as part of a standard Ovsynch. The technician's ability to detect a functional CL was more accurate when the CL visualized was actually functional (progesterone  $\geq$  1 ng/mL) than when it was not functional (progesterone  $<$  1 ng/mL). Although pregnancy outcomes tended to improve when cows were treated with Ovsynch compared with Short Synch, when a functional CL was accurately detected, pregnancy outcomes did not differ. Technician accuracy for detecting a functional CL is important for improving pregnancy outcomes when applying the Short Synch treatment.

### Introduction

Approximately 65 to 70% of lactating dairy cows fail to conceive after AI. Implementing an efficient strategy to identify and inseminate non-pregnant cows is crucial to achieving acceptable reproductive performance in dairy herds. Re-insemination strategies minimize interbreeding intervals and maximize pregnancy per AI (P/AI). In well-managed dairy farms, where cows are housed partly in pasture or dry lots, more than 60% of cows are inseminated after detection of estrus. In the absence of detected estrus, cows often are enrolled in a resynchronization ovulation control program resulting in

a timed AI. Initiating Ovsynch (GnRH — 7 days — PGF<sub>2α</sub> — 24 hours — PGF<sub>2α</sub> — 32 hours — GnRH — 16 to 20 hours — timed AI) to resynchronize ovulation in open cows is a widely adopted program in U.S. dairy herds. The resynchronization can be initiated 7 days before or at nonpregnancy diagnosis. When pregnancy diagnosis occurs weekly once cows are 30 to 36 days since the last AI, the interbreeding interval can range from 40 to 46 days for cows started on the program at a nonpregnancy diagnosis or even shorter (33 to 40 days) when the program is initiated 7 days before pregnancy diagnosis.

The first GnRH injection in an Ovsynch protocol is intended to induce ovulation and initiate a new follicular wave that will give rise to an ovulatory follicle, and the formation of a new CL after ovulation and AI. A disadvantage to this type of approach is the suppression of estrus expression that occurs after GnRH treatment partly because GnRH induces an ovulatory LH surge from the pituitary gland and suppresses estradiol production from the dominant follicle, thus precluding estrus expression. Previous research shows fewer cows were detected in estrus when GnRH was administered 17 to 32 days after AI. Cows bearing a CL at the initiation of Ovsynch may not require the first injection of GnRH because the CL would be responsive to PGF<sub>2α</sub> to initiate estrus, thus eliminating the need for GnRH to start a 7-day program, and reduce the interbreeding interval by 7 days compared with initiating a full Ovsynch program.

Previous reports have evaluated ovarian structures and subsequent fertility of resynchronized cows after timed AI. One limitation to the success of the Ovsynch resynchronization program is the absence of a functional CL at the time of the PGF<sub>2α</sub> injection. A CL is considered to be functional when concentrations of progesterone are  $\geq 1$  ng/mL. Cows starting Ovsynch without a functional CL conceive at rates approximately 50% less than cows with a functional CL. Improving fertility of cows with poor responses to a resynchronization program is important because cows diagnosed not pregnant incur the same expenses of completing the program as cows that become pregnant but have significantly longer delays to pregnancy establishment, thus reducing overall herd profitability. Because fertility is decreased when an Ovsynch protocol is initiated in cows without a functional CL, providing supplemental progesterone during Ovsynch may be a suitable alternative for cows starting the program without a CL.

Management strategies aimed at reducing interbreeding intervals usually involve early pregnancy diagnosis using transrectal ultrasonography between 30 and 36 days after insemination. This method of pregnancy testing allows the determination of ovarian structures of nonpregnant cows with minimal time and effort. Choosing resynchronization programs tailored to the ovarian status of cows could increase P/AI.

Therefore, we proposed to address three questions: (1) necessity of the initial injection of GnRH in an Ovsynch protocol to resynchronize ovulation in cows bearing a CL at non-pregnancy diagnosis; (2) necessity of applying supplemental progesterone to a standard Ovsynch protocol in the absence of a CL; and (3) accuracy of detecting a functional CL by one transrectal ultrasound examination.

## Experimental Procedures

We enrolled 1,626 lactating dairy cows (mostly Holstein with a few crossbreeds) from three herds for 12 months (June 2016 through May 2017) at time of nonpregnancy diagnosis (NPD) in three resynchronization treatments based on the presence or absence of a CL (Figure 1). Pregnancy diagnosis occurred in all herds by employing transrectal ultrasonography to determine NPD and ovarian structures 30 to 36 days after previous insemination. Technicians performing pregnancy diagnosis were trained to detect pregnancies and ovarian structures. When a CL was present at NPD, cows were assigned randomly to two resynchronization treatments: (1) Short Synch (PGF<sub>2α</sub> — 24 hours — PGF<sub>2α</sub> — 32 hours — GnRH — 16 hours — timed AI); or (2) Ovsynch (GnRH<sup>3/4</sup> 7 days — PGF<sub>2α</sub> — 24 hours — PGF<sub>2α</sub> — 32 hours — GnRH — 16 hours — timed AI). In the absence of a CL at NPD, cows were enrolled in Ovsynch + CIDR (GnRH + CIDR insert — 7 days — PGF<sub>2α</sub> + CIDR removal — 24 hours — PGF<sub>2α</sub> — 32 hours — GnRH — 16 hours — timed AI).

Blood samples were collected at NPD for later determination of progesterone concentrations, which were used to determine accuracy of the CL diagnosis by individual technicians. An active functional CL was defined to have progesterone  $\geq 1$  ng/mL and was the gold standard to determine if the CL diagnosis was accurate.

Pregnancy diagnoses to assess treatment performance were made at 30 to 36 days and again at 60 to 66 days to reconfirm the pregnancy with subsequent pregnancy loss calculated from any losses that occurred between pregnancy diagnoses. Pregnancy per AI (pregnancy rates) was calculated by determining the proportion of cows diagnosed pregnant in each treatment divided by the number of cows receiving AI. Any cows not completing the treatment or culled before pregnancy diagnosis were deleted from the results.

## Results and Discussion

### *Technician Accuracy*

Using concentrations of progesterone as the gold standard, accuracy of technicians detecting a functional CL and accurately placing cows in either of the two CL resynchronization treatments ranged from 76 to 94.4% (Figure 2). Technician accuracy of detecting cows without a functional CL and utilizing a synchronization protocol with supplemental progesterone ranged from 43.3 to 89.1% (Figure 2).

### *Pregnancy Outcomes*

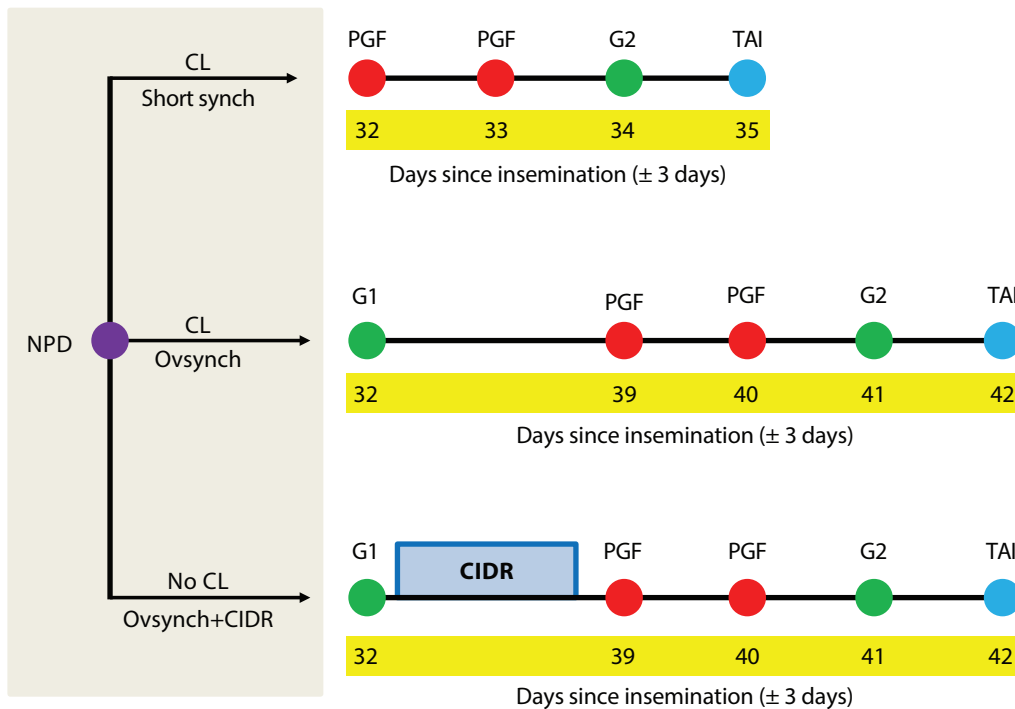
Pregnancy risk for all herds at 30 to 36 and 60 to 66 days after insemination tended ( $P = 0.06$ ) to be greater for cows enrolled in the Ovsynch treatment compared with Short Synch (Figure 3). No differences, however, occurred between Ovsynch and Ovsynch + CIDR treatments. Pregnancy loss ranged from 5.8 to 9.2% and did not differ among treatments.

Pregnancy per AI for herds B and C did not differ at either time of pregnancy diagnosis when Ovsynch was compared with Short Synch (Figure 4). In contrast, herd A achieved more ( $P < 0.05$ ) pregnancy per AI at 30 to 36 days (Figure 4) and 60 to 66 days (Figure 5) for cows treated with Ovsynch compared with Short Synch. Pregnancy loss ranged from 0.4 to 13% and did not differ among treatments.

Comparisons of treatments were made when observations were sorted into two categories based on concentrations of progesterone at the time of NPD. Cows with progesterone  $\geq 1$  ng/mL at NPD were those correctly assessed as having a functional CL, whereas the remaining cows with concentrations  $< 1$  ng/mL were those with a nonfunctional CL (either regressing or newly formed). Pregnancy per AI for cows with concentrations of progesterone  $\geq 1$  ng/mL ranged from 26.7 to 31.5%. Cows with progesterone  $< 1$  ng/mL achieved P/AI between 20.2 and 28% (Figure 6). Pregnancy per AI of cows treated with Ovsynch or Short Synch, regardless of CL functional status, did not differ from those supplemented with progesterone or from each other (Figure 6).

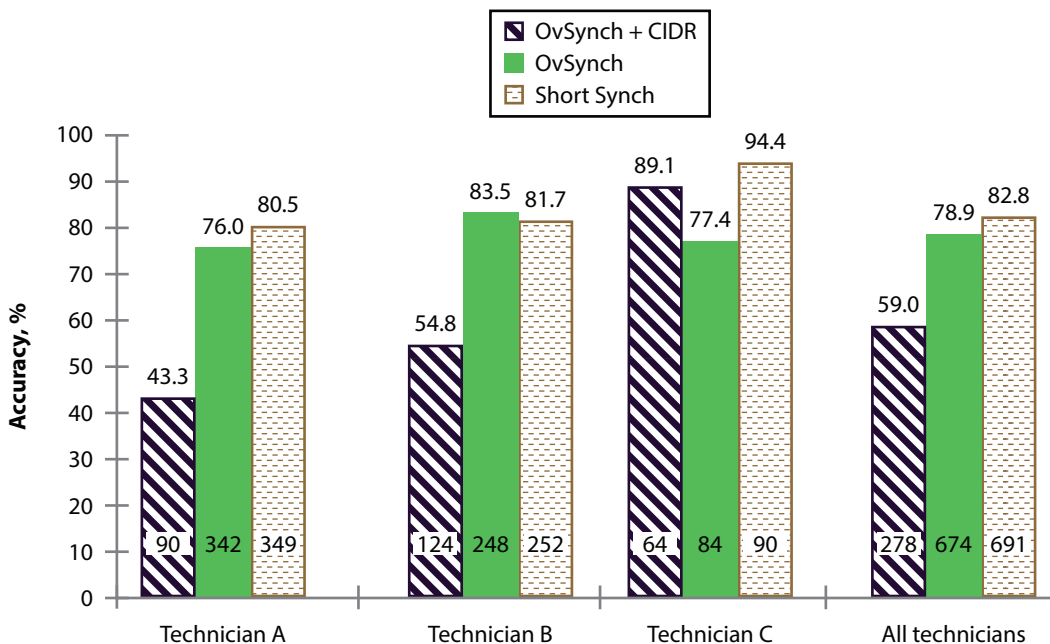
Results from these experiments demonstrate that when cows have a functional CL at NPD, applying the Short Synch treatment by eliminating the initial injection of GnRH in Ovsynch produced comparable fertility to the standard Ovsynch protocol. Accuracy of detecting a functional CL with transrectal ultrasound is largely technician-dependent, and is more accurate when declaring a CL is present and functional than when declaring a CL is absent and nonfunctional.

Three conclusions can be made based on the results of the present study. Regardless of treatment applied in this study, when cows had a CL to start the treatment, P/AI was superior to cows without a CL. Based on the correct retrospective diagnosis of a functional CL by progesterone, improving the technician's ability to detect accurately a functional CL should enhance pregnancy per AI when using the Short Synch treatment. Thus, with more accurate diagnosis of a functional CL and employing the Short Synch treatment, cows became pregnant 7 days earlier at the same level of fertility as cows treated with the traditional Ovsynch protocol. Furthermore, applying supplemental progesterone via the CIDR insert did not improve pregnancy outcomes in cows, regardless of their CL status.



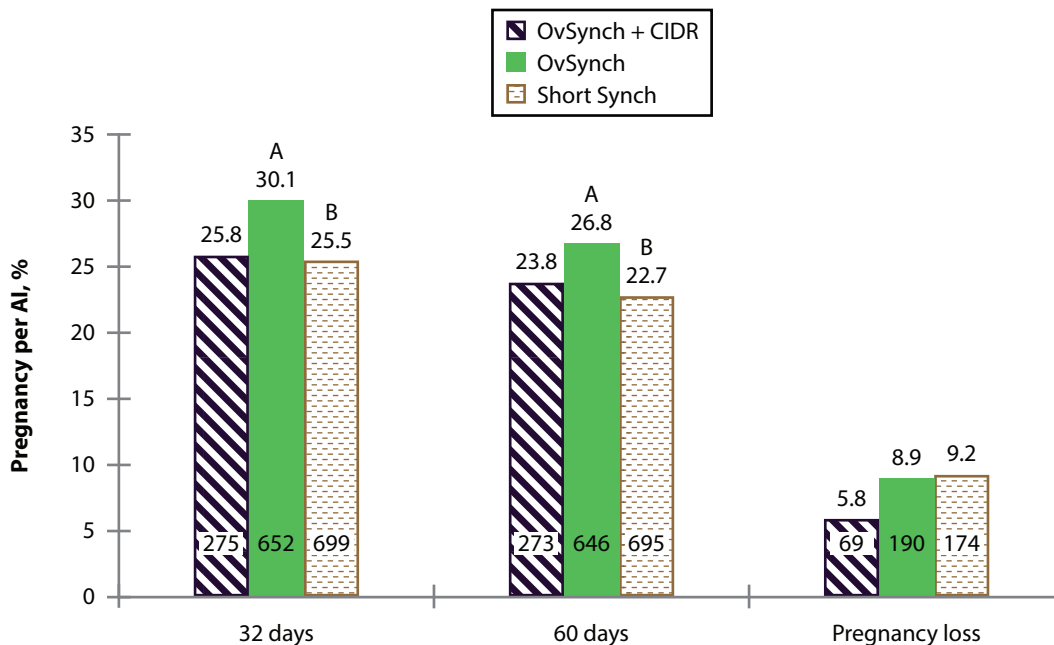
**Figure 1. Design of experimental procedures and resynchronization treatments.**

NPD: nonpregnancy diagnosis; CL: corpus luteum; CIDR: controlled internal drug release; PGF: PGF<sub>2α</sub>; G1/G2: GnRH; TAI: timed artificial insemination.

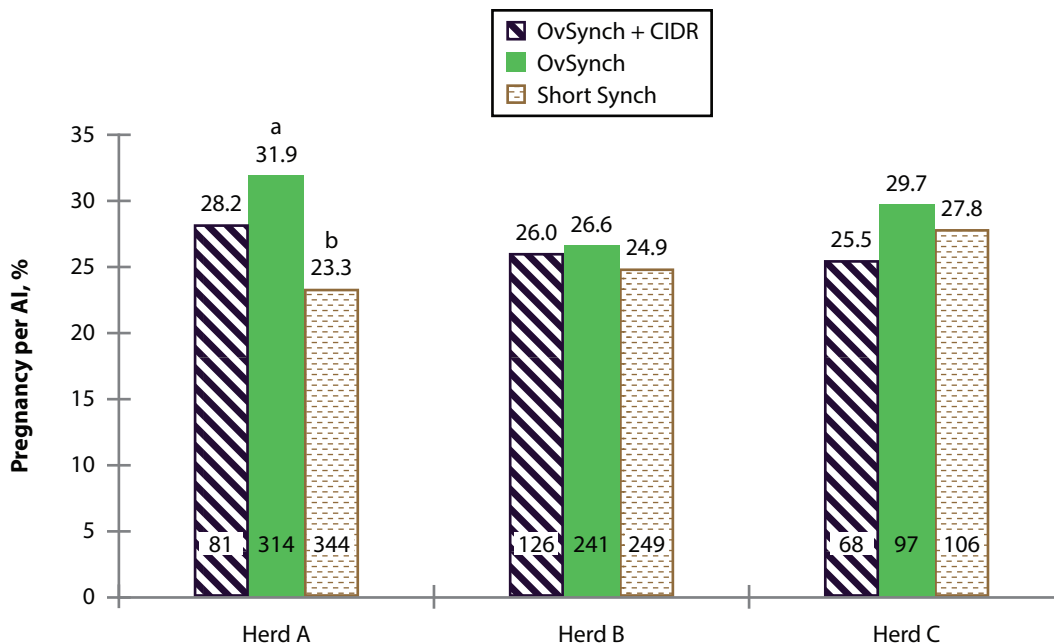


**Figure 2. Accuracy of technicians' ability to identify a functional or nonfunctional corpus luteum (CL) and placement into appropriate resynchronization treatments. A functional CL was defined as the visual presence of a CL when concentrations of progesterone  $\geq 1$  ng/mL.**



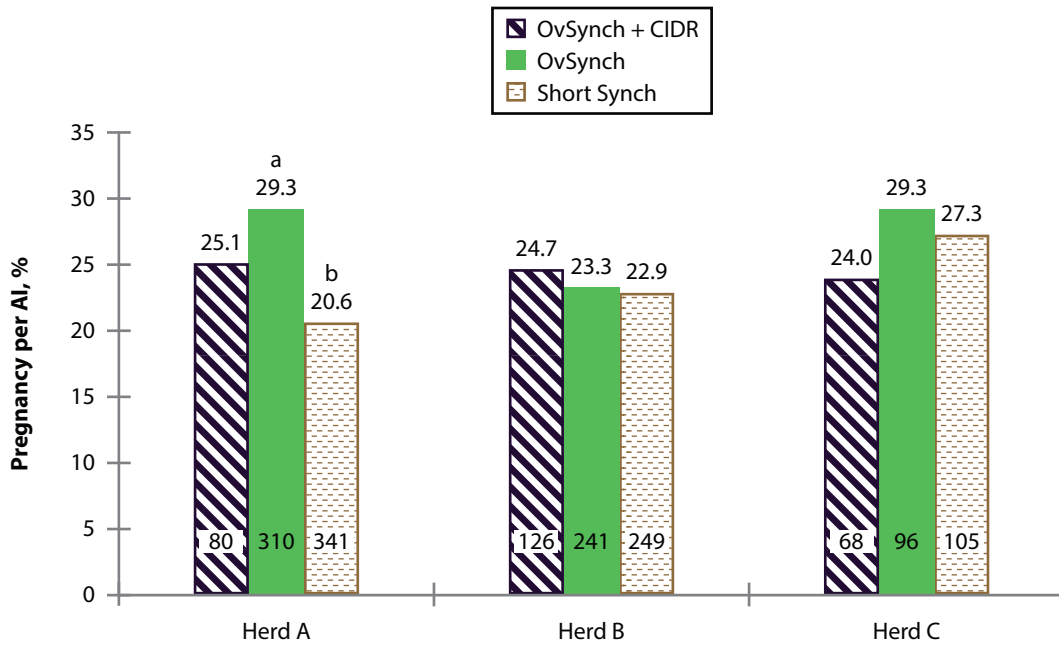


**Figure 3. Pregnancy per AI at 30 to 36 days and 60 to 66 days after insemination in all three herds by resynchronization treatment. Pregnancy losses illustrated occurred between 30 to 36 days and 60 to 66 days after insemination for all herds.** Upper case letters indicate a tendency ( $P = 0.06$ ) for P/AI to differ between cows treated with OvSynch and Short Synch.

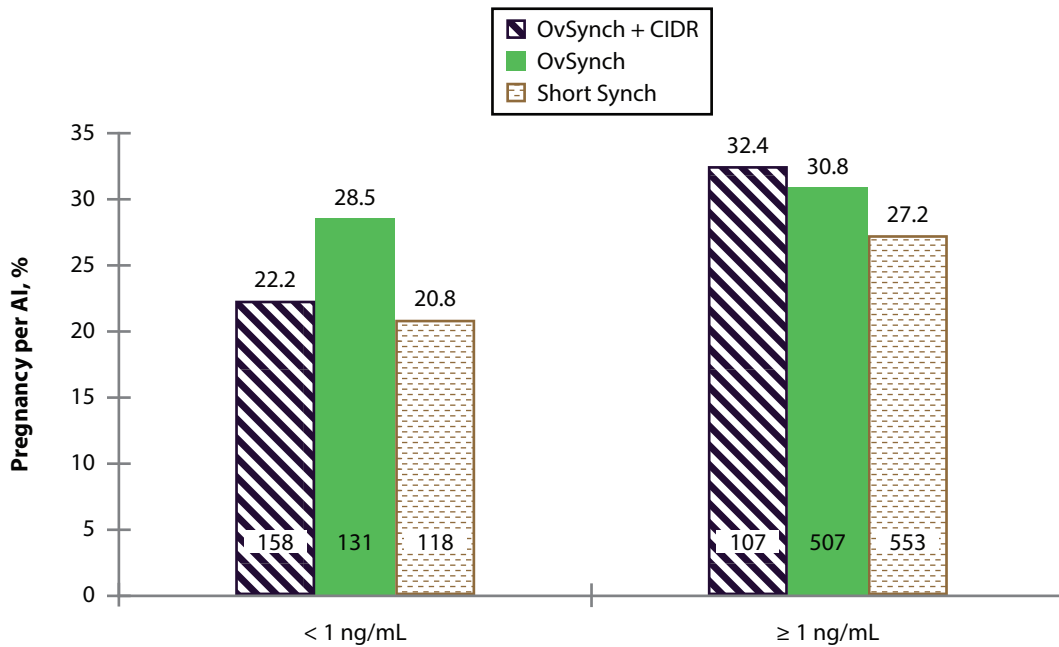


**Figure 4. Pregnancy per AI 30 to 36 days after insemination by herd.** Lower case letters indicate treatment differences ( $P < 0.05$ ) in herd A.





**Figure 5. Pregnancy per AI 60 to 66 days after insemination by herd.** Lower case letters indicate treatment differences ( $P < 0.05$ ) in herd A.



**Figure 6. Pregnancy per AI based on the concentrations of progesterone at the nonpregnancy diagnosis and resynchronization treatment used.**