

## Dominance of Right Ovary Structures in Lactating Dairy Cows

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### Summary

The objectives were to determine the absolute and relative ovary location of dominant and preovulatory follicles and corpora lutea (CL) present in ovaries before and during hormone synchronization and their associations with CL regression, ovulation, and pregnancy rates in lactating dairy cows. Cows were exposed to presynchronization treatments of PGF<sub>2α</sub>, GnRH, or both, 3 to 4 weeks before initiating a timed artificial insemination (AI) program (GnRH-1 – 7 days – PGF<sub>2α</sub> [1 dose or 2 doses 24 hours apart] – 56 hours after first or only dose of PGF<sub>2α</sub> – GnRH-2 – 16 hours – timed AI) in which cows were first inseminated at 72 ± 3 days in milk. Blood samples were collected to assess progesterone concentration before ovarian structures were mapped in 691 cows and before each hormone treatment (GnRH-1, PGF<sub>2α</sub> and GnRH-2).

Follicles that ovulated and CL were detected more often in right than left ovaries. Location of dominant follicles before GnRH-1 tended to be more right than left with co-dominant follicles in both ovaries. In response to GnRH, more left-ovary follicles ovulated contralateral to CL than right-ovary follicles, but fewer left-ovary follicles ovulated ipsilateral to CL (left to left and right to right). Dominance of right-ovary CL was less than 50% because of multiple CL in both ovaries before GnRH-1 (15.8%) and before PGF<sub>2α</sub> (35.6%), resulting from GnRH-1-induced ovulations in the latter case. Preovulatory follicles before PGF<sub>2α</sub> were detected more often ipsilateral than contralateral to CL induced by GnRH-1, but were of equal frequency ipsilateral or contralateral to older CL present before GnRH-1.

Death of the CL in response to PGF<sub>2α</sub> was greater for cows bearing older CL (95.3%) or older CL + younger CL (93.7%) compared with cows bearing only younger GnRH-1-induced CL (80.6%). Risk of CL death did not differ regardless of the ipsilateral or contralateral location of younger CL relative to older CL. Pregnancy rate was greater in cows having both older and younger CL (i.e., these cows had a CL at GnRH-1 and ovulated in response to GnRH-1) compared with cows having only a younger CL at PGF<sub>2α</sub>. Pregnancy rate also was greater for cows with younger CL when contralateral to older CL compared with cows bearing only younger or older CL before PGF<sub>2α</sub>.

Percentage of female calves born resulting from eggs produced by left or right ovaries did not differ. In contrast, a tendency ( $P = 0.14$ ) existed for more heifers to be born

resulting from eggs produced by right than left ovaries for cows that conceived at first service than for cows that conceived at repeat AI services.

## Introduction

The two ovaries in most domestic farm animals do not function equally during the estrous cycle; one of the ovaries is often more active than the other. Ovulation occurs more frequently from right than left ovaries varying from 54 to 60% in ewes and goats and from 60 to 65% in cows. In contrast, the left ovary in the sow is more functional, producing 55 to 60% of the oocytes, and the mare ovulates approximately 60% of the oocytes from the left ovary. Explanations for right-dominated ovulations in ruminants have included the proximity of the left ovary to the rumen and other extrinsic factors such as temperature or pressure fluctuations and mechanical contractions of the rumen. It is not clear if any intrinsic factors play a role.

Relative ovarian location of preovulatory follicles and corpora lutea (CL) resulted in shorter interovulatory intervals when follicle and CL were in the same (ipsilateral) than when in the contralateral ovary in spontaneously cycling heifers. In cattle, several studies have found positive CL-follicle intraovarian relationships during the estrous cycle, but other studies have not.

To our knowledge, no information is known about the spatial relationship (ipsilateral [same side] vs. contralateral [opposite side]) of ovarian follicles with CL and subsequent risks for ovulation, CL regression, or pregnancy during the estrous cycle when GnRH treatments are applied to accomplish hormonal synchronization before timed AI. For example, do younger GnRH-induced CL regress at different rates in presence or absence of an older CL or because of their different spatial relationship to the dominant or preovulatory follicle? Do follicles in the left or right ovary respond differently to GnRH-induced LH secretion to form CL when ipsilateral or contralateral to a younger or older CL?

Hypotheses tested included: (1) a greater proportion of ovarian structures (dominant or preovulatory follicles and older or GnRH-induced younger CL) are found in the right compared with the left ovary; (2) relative ratio of right to left ovary-bearing CL is altered after exposure to GnRH; (3) younger, GnRH-induced CL found ipsilateral to older CL have greater CL regression than those contralateral to older CL; and (4) spatial and relative location of preovulatory follicles before AI do not affect ovarian characteristics and subsequent risks of ovulation and pregnancy.

The objectives were to determine various outcomes (changes in size of dominant follicles, progesterone concentration, risks for CL regression, ovulation, pregnancy, and calf gender at birth) in lactating dairy cows exposed to hormonal synchronization that were associated with the:

- absolute and relative spatial location of ovarian structures before and after exposure to GnRH, and their spatial relation to new GnRH-induced CL or older CL;
- ovary location of preovulatory follicles and their relative location to GnRH-induced younger and older CL present before GnRH; and
- luteal environment (younger CL, older CL, or both) at the time of PGF<sub>2a</sub>.

## Experimental Procedures

### *Source of Data*

Maps of ovarian structures obtained by transrectal ultrasonography constructed previously in four studies were re-examined to determine ovarian spatial locations of dominant and preovulatory follicles relative to older and younger GnRH-induced CL identified before and AI. Lactating Holstein cows were exposed to a standard 7-day timed AI program (GnRH-1 – 7 days – PGF<sub>2α</sub> – [1 dose or 2 doses 24 hours apart] 56 hours – GnRH-2 – 16 hours – timed AI) before first postpartum AI. In one study, some cows received either one 50-mg dose of PGF<sub>2α</sub> (dinoprost tromethamine, Zoetis Inc., Kalamazoo, MI) or two 25-mg doses of PGF<sub>2α</sub> administered 24 hours apart; otherwise, cows received 1 standard 25-mg dose of PGF<sub>2α</sub>. In that same study, cows exposed to a 5-day Ovsynch timed AI program were not included in the present study except to examine side of ovulation and calf gender. In each of the 4 studies, estrous cycles were presynchronized with either 2 doses of PGF<sub>2α</sub> administered 14 days apart (the last of which was administered 10 days before initiating Ovsynch) or presynchronization combinations of GnRH and PGF<sub>2α</sub> (PGF<sub>2α</sub> and GnRH administered 10 and 7 days before Ovsynch or GnRH and PGF<sub>2α</sub> administered 17 and 10 days before Ovsynch).

### *Data Collection*

Before GnRH-1, and at 0 and 48 hours after PGF<sub>2α</sub> (first or only treatment of PGF<sub>2α</sub>), ovaries were scanned by transrectal ultrasonography (7.5-MHz linear-array transducer, Aloka 500V; Corometrics Medical Systems Inc., Wallingford, CT) and all ovarian follicles > 5 mm were sized by electronic calipers and mapped relative to location of CL. Ovulation (single or multiple) was determined by disappearance of follicles previously mapped and recorded either 6 or 7 days after GnRH treatments. Frozen images of follicles and CL were assessed using electronic calipers and measuring diameters in 2 directions perpendicular to one another. Total volume of luteal tissue was calculated ( $\frac{4}{3} \times r^3 \times p$ , where  $r$  = radius  $[(W/2 + H/2)/2]$ ;  $W$  = largest width and  $H$  = largest height of the structure; and  $p$  = 3.14159). When a luteal structure contained a fluid-filled cavity, volume of the cavity was subtracted from the total luteal volume. Blood samples were collected by caudal vessel puncture at GnRH-1, and 0 and 48 hours after PGF<sub>2α</sub>.

Ovary location of each dominant and preovulatory follicle was determined (right, left, or both, only when co-dominant follicles ovulated) and their spatial location (ipsilateral, contralateral, or both) relative to CL identified and present before GnRH-1 (defined as older CL that was at least 7 days old but most likely 10 to 14 days old at the time of PGF<sub>2α</sub> treatment) or relative to new younger CL formed after treatment with GnRH-1 (approximately 5 days old at the time of PGF<sub>2α</sub> treatment). Co-dominant follicles were verified by post-ovulatory examinations of a newly revealed CL located on the ovarian map previously occupied by dominant follicle(s). For cows that became pregnant, the side of ovulation was determined as described previously and gender of calf at birth was recorded.

## Results and Discussion

### *Dominant Follicle Location*

Frequency of finding the dominant follicle in the right ovary was greater ( $P \leq 0.05$ ) than in the left ovary before both GnRH treatments (Table 1). A numerical shift of

dominant follicle locations was observed after GnRH-1 and before GnRH-2. The proportion of dominant follicles in right ovaries did not change (57.3 vs. 55.9%), but proportions of dominant follicles in left ovaries numerically decreased after GnRH-1 (38.0 to 35.3%), whereas more multiple preovulatory follicles were found in both ovaries after GnRH-1 (4.7 to 8.8%; Table 1). This shift was not associated with any difference in ovulation risk between ovaries after GnRH-1 (Table 1). When the dominant follicle was in the right ovary before GnRH-2, frequency of ovulation after GnRH-2 tended ( $P = 0.06$ ) to be greater (95.1 vs. 91.3%) than when the dominant follicle was in the left ovary. Neither diameter of the dominant follicles, number of CL per cow, nor progesterone concentration differed between ovaries bearing the dominant follicle before either GnRH treatment (data not shown).

Switching ovary location of the CL dominance from before to after GnRH-1 or GnRH-2 treatments differed between ovaries. Switching of CL-ovary contralaterally from left (before GnRH) to right (after GnRH) was more ( $P < 0.001$ ) common than right to left (Figure 1). Furthermore, when the CL-ovary dominance did not change after GnRH, it remained ipsilateral right (before GnRH) to right (after GnRH) more ( $P < 0.001$ ) often than left to left (Figure 1). This effect did not differ in response to either GnRH-1 or GnRH-2 treatment and reinforces the greater activity of the right compared with the left ovary.

### *Corpus Luteum Location*

Frequency of finding CL in the right ovary was greater ( $P \leq 0.05$ ) than in the left ovary before and after GnRH-1 treatment when assessed before PGF<sub>2 $\alpha$</sub>  treatment (Table 2). The proportion of CL in the left or right ovary decreased by 13.2 or 6.5 percentage points from before to after GnRH-1, whereas the additive increase in CL detected in both ovaries doubled ( $P < 0.001$ ) from 15.8% before GnRH-1 to 35.6% at the time of PGF<sub>2 $\alpha$</sub>  such that the frequency of CL was not different between right ovaries and both ovaries (Table 2). Ovulation risk in response to GnRH-1 tended ( $P = 0.06$ ) to be less when CL were found in both ovaries (54.6%) compared with cows having 1 CL in the left ovary (67.3%) and none in the right ovary (60.7%). Diameter of dominant follicles did not differ between ovaries (data not shown). As expected, number of CL and progesterone concentration were greater ( $P \leq 0.05$ ) when a CL was detected in both ovaries compared with a CL in either the right or left ovary. Subsequent ovulation risk of preovulatory follicles in response to GnRH-2 did not differ between ovaries (Table 2).

### *Location and Death of the Corpus Luteum*

Progesterone concentrations were related to age and number of CL before PGF<sub>2 $\alpha$</sub>  treatment for cows having both younger CL + older CL at the time of PGF<sub>2 $\alpha$</sub>  treatment compared with cows having only younger or older CL (Table 3). Regression of CL was greater ( $P \leq 0.05$ ) in cows having older CL or younger + older CL compared with only younger CL (based on progesterone cut points of  $< 1.0$  or  $< 0.5$  ng/mL at 48 hours after PGF<sub>2 $\alpha$</sub>  treatment and 24 hours before timed AI; Table 3). Cows having only older CL also had greater ( $P < 0.05$ ) proportion of cows showing CL regression than that for cows bearing younger + older CL (cut point  $< 0.5$  ng/mL; Table 3).

The largest preovulatory follicle tended ( $P = 0.08$ ) to be greater in cows bearing only a new CL compared with cows bearing only older CL or younger CL + older CL. The second largest preovulatory follicle was greater ( $P \leq 0.05$ ) in diameter for cows bearing only younger CL compared with cows having older CL and tended ( $P = 0.08$ ) to be greater than that in cows with both CL types (Table 3). Single ovulation risk after GnRH-2 did not differ among CL age classes, but multiple ovulation risk was 2.0 and 2.9 times greater ( $P \leq 0.05$ ) in cows bearing only younger CL after PGF<sub>2 $\alpha$</sub>  treatment compared with cows bearing both CL types and those bearing only older CL, respectively. Subsequent pregnancy rates at 32 days after AI tended ( $P = 0.08$ ) to be and was greater ( $P \leq 0.05$ ) for cows with both CL types compared with cows having older CL or younger CL, respectively (Table 3). Pregnancy rates at 60 days after AI were greater ( $P \leq 0.05$ ) for cows that had both CL types before PGF<sub>2 $\alpha$</sub>  treatment compared with cows having only older or younger CL, respectively. Cows bearing older CL before CL regression had greater ( $P \leq 0.05$ ) pregnancy rates at 60 days after AI than cows that had only new CL. Pregnancy loss tended ( $P = 0.08$ ) to be less for cows with both CL types compared with cows that had only older CL before timed AI (Table 3).

Trans-uterine migration of the embryo in cattle is extremely rare so the ovary from which the egg is derived determines the uterine horn in which the pregnancy will be carried (i.e., right ovulation, right-horn carried calf or left to left). In the case of twins, when ovulations occur from both ovaries, each uterine horn will bear one embryo. If double ovulation occurs from one ovary, one of the twins will eventually be found in the opposite uterine horn.

Based on single pregnancies in this study, the percentage of female calves born resulting from eggs produced by left or right ovaries did not differ for left or right uterine horns or for cows that conceived at first vs. repeat AI services (Figure 2). In contrast, a tendency ( $P = 0.14$ ) was detected for more heifers to be born resulting from eggs produced by right than left ovaries (i.e., more females carried in right than left horns) for cows that conceived at first AI services and the opposite was observed for cows that conceived at repeat AI services (i.e., fewer females carried in the right than left horn; Figure 3).

## Conclusions

We accept the first hypothesis that frequency of right ovarian structures was more prevalent in the present study, both before and after GnRH treatments. When exposed to GnRH more follicles in left ovaries ovulated contralaterally than right-ovary follicles, and the reverse was true for right ovaries; fewer left-ovary follicles ovulated ipsilaterally than right-ovary follicles. Preovulatory follicles before PGF<sub>2 $\alpha$</sub>  were detected more often ipsilateral than contralateral to CL induced by GnRH, but were of equal frequency ipsilateral or contralateral to older CL present before GnRH-1.

The second hypothesis is supported by more CL identified in right than left ovaries before and after GnRH. After GnRH-induction of ovulation, frequency of right-ovary CL was twice that found in left ovaries, mostly resulting from a doubling of multiple ovulation found in both ovaries.

The third hypothesis was rejected because, although CL regression was greater for cows bearing older CL or older CL + younger CL compared with cows bearing only younger CL, CL regression was unaltered regardless of ipsilateral or contralateral location of younger CL relative to older CL. Regression of CL was compromised in cows bearing younger CL, which was associated with greater-size ovulatory follicles that developed in a reduced progesterone environment leading to 2 to 3 times more multiple ovulation, and lesser subsequent pregnancy rates than in cows with at least 1 older CL before PGF<sub>2 $\alpha$</sub>  treatment.

Not only is the right ovary more active in producing follicles and CL in dairy cows based on present evidence, but more fertilized oocytes produced from right than left ovaries cleave and form blastocysts based on another study in the scientific literature. A tendency for more right-ovary derived heifer calves occurred when conceptions occurred at first AI service and the opposite tended to occur when conceptions occurred at repeat AI services.

**Table 1. Ovary location of the dominant follicle (DF) and subsequent ovulation risk to GnRH treatments during the Ovsynch timed AI program**

Ovary	Cows, no.	DF location, %	Ovulation risk, %
Before GnRH-1 (onset of Ovsynch)			
Left	253	38.0 <sup>a</sup>	67.2 <sup>a</sup>
Right	381	57.3 <sup>b</sup>	66.7 <sup>a</sup>
Both	31	4.7 <sup>c</sup>	100 <sup>b</sup>
Before GnRH-2 (end of Ovsynch)			
Left	244	35.3 <sup>a</sup>	91.3 <sup>A</sup>
Right	386	55.9 <sup>b</sup>	95.1 <sup>abB</sup>
Both	61	8.8 <sup>c</sup>	98.4 <sup>b</sup>

<sup>a,b</sup>Means within column and GnRH treatment differ ( $P \leq 0.05$ ).

<sup>A,B</sup>Means within column and GnRH treatment tended to differ ( $P = 0.06$ ).

**Table 2. Location of corpora lutea (CL) at the time of GnRH-1 and PGF<sub>2a</sub> treatments during the Ovsynch with number of CL per cow ( $\pm$  standard error), progesterone concentration before treatments, and subsequent ovulation risk after GnRH and PGF<sub>2a</sub> treatments**

Ovary	Cows, no.	CL location, %	CL per cow, no.	Progesterone, ng/mL	Ovulation risk, %
Before GnRH-1 (onset of Ovsynch)					
Left	214	34.8 <sup>a</sup>	1.2 $\pm$ 0.03 <sup>a</sup>	3.7 $\pm$ 0.2 <sup>a</sup>	67.3 <sup>aA</sup>
Right	303	49.3 <sup>b</sup>	1.2 $\pm$ 0.02 <sup>a</sup>	3.7 $\pm$ 0.1 <sup>a</sup>	60.7 <sup>ab</sup>
Both	97	15.8 <sup>c</sup>	2.2 $\pm$ 0.05 <sup>b</sup>	5.0 $\pm$ 0.2 <sup>b</sup>	54.6 <sup>bB</sup>
After GnRH-1 treatment (time of PGF <sub>2a</sub> )					
Left	150	21.6 <sup>a</sup>	1.5 $\pm$ 0.06 <sup>a</sup>	5.8 $\pm$ 0.3 <sup>a</sup>	94.7 <sup>a</sup>
Right	297	42.8 <sup>b</sup>	1.5 $\pm$ 0.04 <sup>a</sup>	5.3 $\pm$ 0.2 <sup>a</sup>	92.6 <sup>a</sup>
Both	247	35.6 <sup>b</sup>	2.4 $\pm$ 0.05 <sup>b</sup>	6.8 $\pm$ 0.2 <sup>b</sup>	95.5 <sup>a</sup>

<sup>ab</sup>Means within column and GnRH treatment differ ( $P < 0.05$ ).

<sup>A,B</sup>Means within column and GnRH treatment tended ( $P = 0.06$ ) to differ.

**Table 3. Characteristics of corpora lutea (CL) at the time of PGF<sub>2a</sub> treatment and concentrations of progesterone at 0 and 48 hours thereafter including preovulatory follicle diameters ( $\pm$  standard error), luteolytic risk, and subsequent ovulation and pregnancy risks**

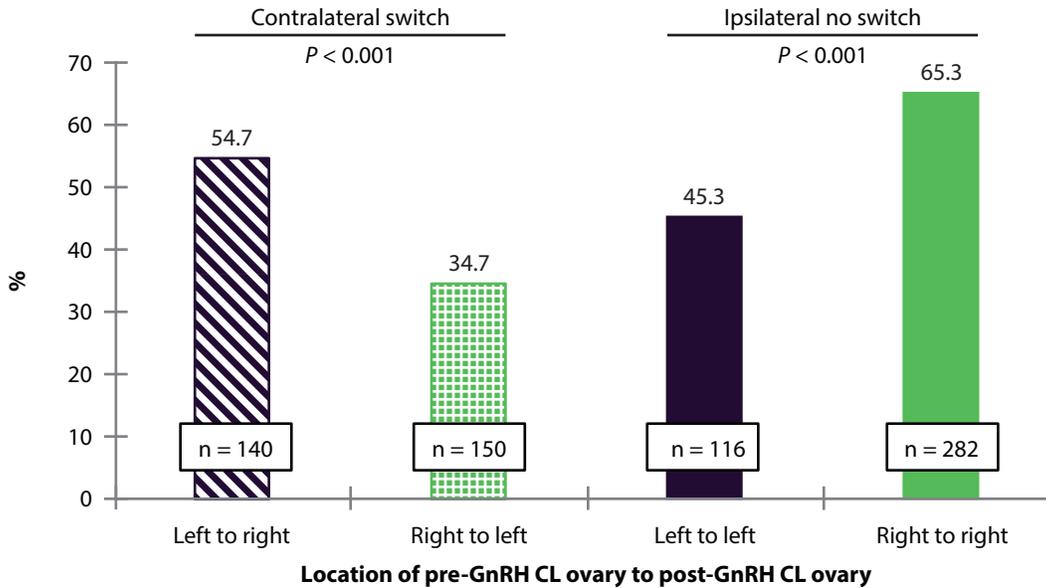
Item	CL age class		
	Younger CL	Older CL	Younger CL+ older CL
Cows, no.	72	215	368
CL/cow, no.	1.2 $\pm$ 0.08 <sup>a</sup>	1.4 $\pm$ 0.05 <sup>b</sup>	2.3 $\pm$ 0.03 <sup>c</sup>
Progesterone, ng/mL			
0 hours	3.4 $\pm$ 0.3 <sup>a</sup>	6.2 $\pm$ 0.2 <sup>b</sup>	7.0 $\pm$ 0.1 <sup>c</sup>
48 hours	0.8 $\pm$ 0.11 <sup>a</sup>	0.3 $\pm$ 0.06 <sup>b</sup>	0.4 $\pm$ 0.05 <sup>b</sup>
Proportion of cows, <sup>1</sup> %			
Progesterone < 1.0 ng/mL at 48 hours	80.6 <sup>a</sup>	95.3 <sup>b</sup>	93.7 <sup>b</sup>
Progesterone < 0.5 ng/mL at 48 hours	65.3 <sup>a</sup>	86.5 <sup>b</sup>	79.6 <sup>c</sup>
Largest preovulatory follicle (mm)	14.7 $\pm$ 0.3 <sup>A</sup>	13.8 $\pm$ 0.2 <sup>B</sup>	13.8 $\pm$ 0.2 <sup>B</sup>
Second preovulatory follicle (mm)	12.7 $\pm$ 0.4 <sup>aA</sup>	11.3 $\pm$ 0.4 <sup>b</sup>	11.9 $\pm$ 0.2 <sup>bb</sup>
Single ovulation risk after GnRH, %	91.7 <sup>a</sup>	93.4 <sup>a</sup>	94.3 <sup>a</sup>
Multiple ovulation risk after GnRH, %	39.4 <sup>a</sup>	13.4 <sup>b</sup>	19.6 <sup>b</sup>
Pregnancy rate at 32 days after AI, <sup>2</sup> %	26.4 <sup>a</sup> (72)	39.4 <sup>bA</sup> (213)	46.8 <sup>bb</sup> (363)
Pregnancy rate at 60 days after AI, <sup>2</sup> %	23.6 <sup>a</sup> (72)	33.3 <sup>a</sup> (213)	43.5 <sup>b</sup> (363)
Pregnancy loss 32-60 days after AI, %	10.5 <sup>AB</sup> (19)	15.5 <sup>A</sup> (84)	7.1 <sup>B</sup> (170)

<sup>abc</sup>Means within row differ ( $P < 0.05$ ).

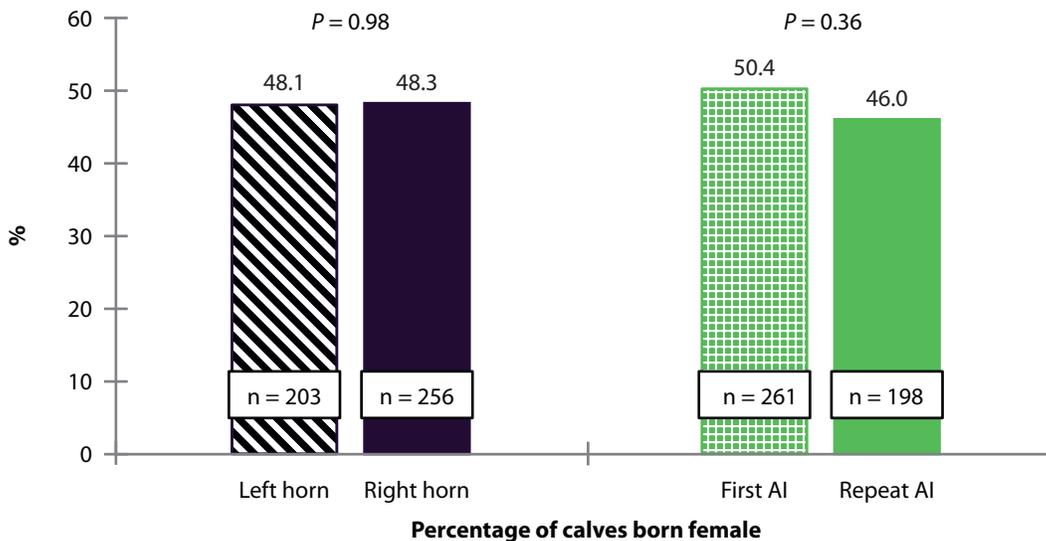
<sup>A,B</sup>Means within row tended to differ ( $P = 0.08$ ).

<sup>1</sup>Hours after PGF<sub>2a</sub> treatment.

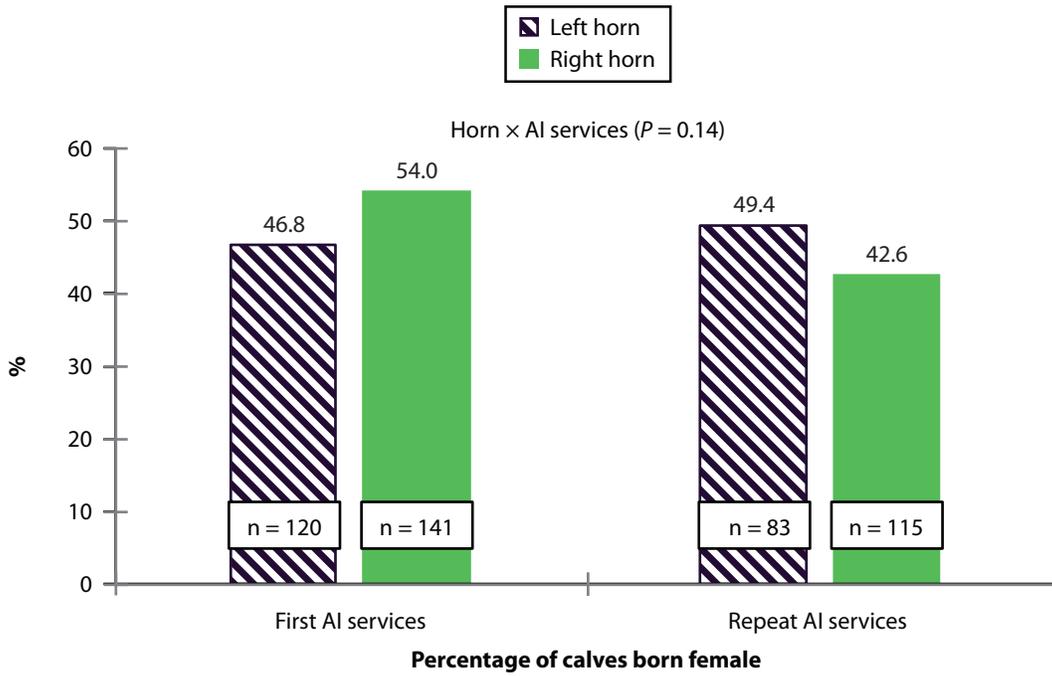
<sup>2</sup>Number of cows differed from total because of culling before pregnancy diagnosis occurred.



**Figure 1.** Ovary location of the GnRH-induced corpus luteum (CL) relative to CL present before GnRH treatment in lactating dairy cows. Results include cows exposed to GnRH-1 at the onset of Ovsynch and those exposed to GnRH-2 administered 56 hours after PGF<sub>2a</sub>. No differences were detected for relative locations after either GnRH treatment so data were combined. Switching of successive CL contralaterally from left (before GnRH) to right (after GnRH) ovaries was more ( $P < 0.001$ ) common than right to left. The new CL on right ovaries were detected more ( $P < 0.001$ ) frequently ipsilaterally right (before GnRH) to right (after GnRH) than for left to left.



**Figure 2.** Percentage of female calves born that were carried in either the right or left uterine horn and percentage of female calves born resulting from first or repeat AI services.



**Figure 3. A tendency for more female calves born resulting from right-ovary derived eggs at first services (carried in the right than left horns) compared with fewer right than left pregnancies for cows conceiving at repeat AI services.**