Ovarian Structures and Circulating Steroids in Heifers and Lactating Cows in Summer and Lactating and Dry Cows in Winter

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ABSTRACT

Two experiments compared follicular and luteal development and circulating steroid concentrations from induced luteolysis to ovulation in lactating Holstein cows ($n = 27$; 40.0 ± 1.5 kg milk/day) vs. nulliparous heifers ($n = 28$; 11 to 17 mo-old) during summer (Experiment 1), and in lactating ($n = 27$; 45.9 ± 1.4 kg milk/d) vs. dry cows ($n = 26$) during winter (experiment 2). All females received PGF_{2 α} 6 d after ovulation and were monitored until next ovulation by daily ultrasound and assay of serum progesterone (P_4) and estradiol (E_2). Every female was used two or three times. In Experiment 1, lactating cows had high incidence of multiple ovulation (63.5%) compared with heifers (1.3%). Among single ovulators, there was no difference in maximal size of ovulatory follicles between lactating cows and heifers (15.8 vs. 16.5 mm, respectively). However, lactating cows had lower peak serum E_2 (8.6 vs. 12.1 pg/ml), took longer to ovulate after luteolysis (4.6 vs. 3.8 d), developed more luteal tissue volume (7293.6 vs. 5515.2 mm³), and had lower serum P_4 on d 6 after ovulation (2.0 vs. 3.0 ng/ml) than heifers (data included multiple ovulators). In experiment 2, multiple ovulations were similar between lactating and dry cows (17.9 vs. 17.2%, respectively). Peak serum E_2 was also similar between lactating and dry cows (7.6 vs. 8.5 pg/ml) although lactating cows had larger ovulatory follicles (18.6 vs. 16.2 ± 0.4 mm). Lactating cows took longer to ovulate (4.8 vs. 4.2 d), developed more luteal tissue (7599 vs. 5139 ± 468 mm³), but had similar serum P_4 (2.2 vs. 1.9 ng/ml) compared with dry cows. Therefore, lactating cows had similar or lower circulating steroid concentrations than dry cows or heifers, respectively, despite having larger ovarian structures.

(**Key words:** ovary, estradiol, progesterone, dairy cattle)

Abbreviation key: CL = corpus luteum or corpora lutea, CR = conception rate, E_2 = estradiol, P_4 = progesterone.

INTRODUCTION

The increase in milk yield for lactating dairy cows is associated with a decrease in fertility (Faust et al., 1988; Nebel and McGilliard, 1993; Butler, 1998; Washburn et al., 2002), and is also associated with an increase in DMI (Harrison et al., 1990). In sheep (Parr et al., 1993a, 1993b), increased DMI increases the rate of blood flow to the liver and consequently increases the clearance rate for progesterone (P_4) because about 90% of P_4 that flows through the liver is metabolized. In pregnant lactating dairy cows, Vasconcelos et al. (1998) also showed that increased feed consumption decreases circulating P_4 concentrations.

Many studies in cows (Sirois and Fortune, 1990; Ahmad et al., 1996; Kinder et al., 1996) have identified that low-circulating progestin concentrations (as produced by many programs for synchronization of estrus) produce aberrant growth of the dominant follicle such that the follicle becomes very large due to prolonged stimulation with LH (persistent follicle). Vasconcelos et al. (1999) detected a direct correlation between milk production and the size of the ovulatory follicle, and an inverse correlation between milk production and the serum P_4 concentrations on d 16 of the estrous cycle. They also observed that cows beginning the Ovsynch protocol (GnRH-7d-PGF_{2 α} -2d-GnRH-1d-AI; Pursley et al., 1995) either early or late in the estrous cycle, when serum P_4 concentrations were low, ovulated larger follicles. Thus, it appears that “persistent” follicles may naturally occur in cows with high milk production, and this may result in a larger corpus luteum (CL) in lactating dairy cows because there is a direct positive relationship between the diameter of the ovulatory follicle

Received November 7, 2001.

Accepted May 3, 2002.

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and the diameter of the resulting CL (Vasconcelos et al., 2001). Nevertheless, it seems likely that the potential increase in circulating P_4 and estradiol (E_2) concentrations that would be expected by the increased follicular and luteal sizes could be blunted by the increase in P_4 and E_2 metabolism in lactating cows.

The present study was designed to compare the size of follicles and CL (by ultrasound) and circulating P_4 and E_2 concentrations between lactating and nonlactating dairy cattle. We hypothesized that lactating cows would ovulate larger follicles but would have similar peak E_2 concentrations. Further, we hypothesized that lactating cows would have larger CL but would have similar circulating P_4 concentrations. The discrepancies between circulating steroids and follicular or luteal sizes were hypothesized to occur due to increased steroid metabolism in lactating as compared with nonlactating females. To investigate these hypotheses, two experiments examined follicular and luteal sizes and serum steroid concentrations from the time of induced luteolysis to ovulation in lactating cows and nulliparous heifers during summer (experiment 1) and in lactating cows and dry cows during winter (experiment 2).

MATERIALS AND METHODS

Experiment 1

Animals. A detailed description of the cows, feed, treatments of anovulatory cows, and reproductive management program for experiment 1 can be found in Sartori et al. (2002). Lactating Holstein cows ($n = 27$) and nulliparous Holstein heifers ($n = 28$) were compared during summer 1999. All cows and heifers had undergone one normal estrous cycle, and data collection began on the follicle leading up to the natural ovulation following this estrous cycle. Previously anovulatory cows were only included in the experiment after completing a normal estrous cycle. All cows and heifers were bred to this natural estrus/ovulation, and embryo collection occurred on d 6 (d 0 = day before detected ovulation). On d 7 and 8, all females received i.m. injections of a $PGF_{2\alpha}$ analog (Estrumate; Bayer Corporation, Shawnee Mission, KS; 500 μg on d 7 and 250 μg on d 8) and were observed for estrus and ovulation. Ovarian ultrasonography was performed, and blood samples were collected by coccygeal venipuncture on the day of $PGF_{2\alpha}$ treatment and daily thereafter until the next ovulation. Serum samples were stored at $-20^\circ C$ until assayed for P_4 and E_2 . Every cow or heifer was monitored from ovulation until subsequent $PGF_{2\alpha}$ -induced luteolysis either two or three times. Transrectal ultrasound (Aloka 500-V with a 7.5 MHz linear-array transducer; Corometrics Medical Systems Inc., Wallingford, CT) was used to determine size of the CL and ovulatory

follicle(s), and to confirm ovulation. Ultrasound measurements of follicles and CL (length [L] and width [W]) were recorded and used to calculate the average diameter and volume (V). Volume was calculated with the formula $V = 4/3 \times \pi \times R^3$ using a radius (R) calculated by the formula $R = (L/2 + W/2)/2$. For CL with a fluid-filled cavity, the volume of the cavity was calculated and subtracted from the total volume of the CL.

Experiment 2

Animals. A detailed description of the cows, feed, treatments of anovulatory cows, and reproductive management program for experiment 2 can be found in Sartori et al. (2002). Lactating Holstein cows ($n = 27$) and nonpregnant nonlactating (dry) Holstein cows ($n = 26$) were compared in experiment 2 during the winter of 1999–2000. Before collection of data, all cows received an i.m. GnRH injection (100 μg) followed 7 d later by i.m. $PGF_{2\alpha}$ (25 mg; ProstaMate; Phoenix Pharmaceutical Inc., St. Joseph, MO). Cows were observed for estrus twice daily (20 min each time) using an androgenized cow. Cows were also fitted with a pressure-activated heat mount detector (Kamar; Kamar Inc., Steamboat Springs, CO) to aid in detection of estrus. Previously anovulatory cows were included only after a natural ovulation. Ovarian ultrasonography was performed, and blood samples were collected on d 7 (d 0 = day before detected ovulation). Cows were treated on d 7 with $PGF_{2\alpha}$ (25 mg), and daily ovarian ultrasonography and blood collection were performed until next ovulation. As in experiment 1, collection of data following $PGF_{2\alpha}$ -induced luteolysis was performed two or three times in each cow.

Hormonal Assays

Serum was evaluated for P_4 concentration using double extraction of serum with petroleum ether and subsequent ELISA as previously reported (Rasmussen et al., 1996). For analysis of E_2 , samples were extracted twice with diethyl ether, and serum concentrations of E_2 were evaluated as previously reported (Kulick et al., 1999). The intraassay CV was 8.9% for P_4 and 3.3% for E_2 .

Statistical Analyses

Data were collected from the time of first treatment with $PGF_{2\alpha}$ until the time of last ovulation in both experiments. However, data were not utilized for luteal tissue volume or serum P_4 at the time of first $PGF_{2\alpha}$ treatment in experiment 2 because of the potential confounding effects from the GnRH treatment 7 d before $PGF_{2\alpha}$.

Table 1. Follicular and luteal sizes, serum steroid concentrations at the time (d 7; ovulation = d 1) and/or after PGF_{2α} injection, growth rate of the ovulatory follicle and days from PGF_{2α} injection to ovulation in lactating cows with single (n = 19) and multiple (n = 33) ovulations (experiment 1).¹

	Single-ovulating cows	Multiple-ovulating cows	P-value
Ovulatory follicles (no.)	1.0 ± 0.0	2.5 ± 0.1	<0.0001
Size of the largest ovulatory follicle at PGF _{2α} (mm)	12.2 ± 0.4 (n = 19)	10.5 ± 0.3 (n = 33)	0.0023
Maximal size of the largest ovulatory follicle (mm)	15.8 ± 0.4 (n = 19)	13.7 ± 0.3 (n = 33)	0.0002
Growth rate of the largest ovulatory follicle after PGF _{2α} (mm/d)	1.0 ± 0.1 (n = 19)	0.8 ± 0.1 (n = 33)	0.1061
Estradiol peak near estrus (pg/ml)	9.0 ± 1.0 (n = 15)	8.3 ± 0.7 (n = 28)	0.5836
Days from PGF _{2α} to ovulation	4.5 ± 0.2 (n = 19)	4.7 ± 0.2 (n = 33)	0.4908
Progesterone on d 7 (ng/ml)	1.8 ± 0.2 (n = 29)	2.2 ± 0.2 (n = 38)	0.1500
Luteal tissue volume on d 7 (mm ³)	6178.7 ± 516.4 (n = 29)	8542.3 ± 453.8 (n = 38)	0.0010

¹Results are expressed as mean ± SEM.

Data related to luteal tissue volume, serum P₄ and E₂ concentrations, and number of days from PGF_{2α} injection to ovulation were analyzed using a linear mixed-effects model, with the (fixed) effect of group (heifers [H], cows with single ovulation [CS], and cows with multiple ovulations [CM], for experiment 1; and dry [D] and lactating [L] cows for experiment 2), and two residual terms, within and between animals. For Experiment 1, F tests were used to study two orthogonal contrasts of interest: H vs. cows (CS and CM), and CS vs. CM; and for experiment 2, F tests were used to compare D vs. L. For experiments 1 and 2, data for size and growth rate of the largest ovulatory follicle were analyzed using a similar approach, but considering just females with single ovulation. The analyses were performed using the MIXED procedure of SAS (Littell et al., 1996). Ovulation rate was studied using the chi-square test. Pearson correlation coefficient tests were performed to study the relationships between maximal size of the ovulatory follicle, subsequent CL volume on d 7, and serum P₄ concentrations on d 7. The procedure CORR of SAS (SAS, 1996) was used to study relationships within groups; the MANOVA statement was considered for correlations using data from all three groups. Regression studies of volume of the ovulatory follicle and serum P₄ concentration vs. CL volume for single-ovulating heifers, lactating cows, and dry cows were carried out by using the procedures GLM and REG of SAS (SAS, 1996).

RESULTS

Experiment 1

Unexpectedly, during experiment 1, lactating cows had a very high incidence of multiple ovulation (two to five follicles being ovulated simultaneously from the same cow; Wiltbank et al., 2000; Tables 1 and 2). This

necessitated the division of lactating cow ovulations into two subgroups with either single (n = 19) or multiple (n = 33) ovulations and comparison of several reproductive values between these two subgroups. There was no difference between single- or multiple-ovulating cows for peak serum E₂ near estrus even though there were more ovulatory follicles per cow for the multiple-ovulating cows. The largest ovulatory follicle was greater in diameter at the time of PGF_{2α} and at maximal size in single- compared with multiple-ovulating cows. Similarly, the average size (using all ovulating follicles) of the ovulatory follicle(s) at the time of PGF_{2α} (12.2 ± 0.4 vs. 9.4 ± 0.3 mm) as well as close to ovulation (15.8 ± 0.4 vs. 12.7 ± 0.3 mm) was larger (*P* < 0.0001) for single- than for multiple-ovulating cows. The multiple-ovulating cows had greater (*P* = 0.03) follicular volume (2674.4 ± 126.8 vs. 2202.8 ± 168.5 mm³) and luteal volume (Table 1) than single-ovulating cows. There was no difference between single- and multiple-ovulating cows for the growth rate of the largest ovulatory follicle after luteolysis, days from PGF_{2α} injection to ovulation, or for serum P₄ concentrations on d 7 (Table 1).

Multiple ovulation rate was much greater in lactating cows (33 of 52) than in heifers (1 of 75). The values for size and growth rate of the ovulatory follicle were very different between single- and multiple-ovulating cows (Table 1), and it was unclear whether only the values for the largest follicle should be included or whether a mean value should be reported. Therefore, only data from single-ovulating cows were used for comparisons of follicular sizes and follicular growth rate to those in heifers. Data from all cows were utilized for comparison of the other measurements because comparison to only the subset (36.5%) of cows that had single ovulation did not seem appropriate (Table 2). Although heifers had larger preovulatory follicles at the time of PGF_{2α} injection than cows with single ovulation, there was no

Table 2. Multiple ovulation rate, follicular and luteal sizes, serum steroid concentrations at the time (d 7; ovulation = d 1) and/or after PGF_{2α} injection, growth rate of the ovulatory follicle and days from PGF_{2α} injection to ovulation in heifers (n = 28) and lactating cows (n = 27) (experiment 1).¹

	Heifers	Lactating cows	P-value
Multiple ovulation rate; % (no./no.)	1.3 (1/75)	63.5 (33/52)	<0.0001
Data from single-ovulating animals			
Size of ovulatory follicle at PGF _{2α} (mm)	13.3 ± 0.2 (n = 74)	12.2 ± 0.4 (n = 19)	0.0307
Maximal size of ovulatory follicle (mm)	16.5 ± 0.2 (n = 74)	15.8 ± 0.4 (n = 19)	0.1579
Growth rate of ovulatory follicle after PGF _{2α} (mm/d)	1.1 ± 0.0 (n = 74)	1.0 ± 0.1 (n = 19)	0.2905
Data from all animals			
Estradiol peak near estrus (pg/ml)	12.1 ± 0.5 (n = 56)	8.6 ± 0.6 (n = 43)	0.0074
Days from PGF _{2α} to ovulation	3.8 ± 0.1 (n = 75)	4.6 ± 0.1 (n = 52)	<0.0001
Progesterone on d 7 (ng/ml)	3.0 ± 0.1 (n = 79)	2.0 ± 0.1 (n = 67)	<0.0001
Luteal tissue volume on d 7 (mm ³)	5515.2 ± 273.4 (n = 79)	7293.6 ± 304.8 (n = 67)	0.0003

¹All results are expressed as mean ± SEM, except for multiple ovulation rate.

difference between groups for the maximal size of the ovulatory follicle (Table 2). Nevertheless, lactating cows had lower peak serum concentrations of E₂ near estrus (Table 2). There was no difference between groups in the growth rate of the ovulatory follicle after luteolysis; however, lactating cows took almost 1 d longer to ovulate after CL regression (Table 2 and Figure 1). On d 7, lactating cows had greater ($P < 0.01$) luteal tissue volume, but heifers had greater ($P < 0.01$) serum P₄ concentrations (Table 2).

Figure 1 shows the distribution for time from PGF_{2α} treatment until ovulation for lactating cows (n = 67 treatments) and heifers (n = 79 treatments). Most of the heifers (92.4%) ovulated on d 3 and 4 after PGF_{2α} treatment, whereas most of the cows (65.7%) ovulated on d 4 and 5. Four heifers (5.1%) and 15 cows (22.4%) did not ovulate after PGF_{2α} (Figure 1). Of these anovulatory

cows, two heifers and two lactating cows did not demonstrate complete luteolysis as determined by ultrasound evaluation of the CL and serum P₄ concentrations. The reason(s) for anovulation in the other cows was not clear.

Experiment 2

In contrast with experiment 1, lactating cows had a lower incidence of multiple ovulation during winter (17.9%), which was similar to the multiple ovulation rate for dry cows (17.2%) (Table 3). Lactating cows tended to have a greater ($P = 0.1$) size of the ovulatory follicle at time of PGF_{2α} treatment and greater ($P < 0.06$) growth rate of the future ovulatory follicle than dry cows (Table 3). In addition, lactating cows required a longer ($P < 0.01$) time from PGF_{2α} treatment until ovulation, and this resulted in ovulation of a larger ($P < 0.01$) follicle in lactating than dry cows (Table 3). In spite of the larger ovulatory follicle size, there was no difference in maximal serum E₂ concentrations near the time of estrus between the groups. On d 7, lactating cows had greater ($P < 0.01$) luteal tissue volume than dry cows, but serum P₄ concentrations were similar in the two groups (Table 3).

Figure 2 shows the distribution for time from PGF_{2α} treatment until ovulation for lactating (n = 66 treatments) and dry (n = 81 treatments) cows. Most of the dry cows (61.7%) had ovulated by d 4 after PGF_{2α} treatment in contrast with only 39.4% of lactating cows ($P < 0.05$; Figure 2). A number of cows in both groups (17 dry and 10 lactating cows) did not ovulate after PGF_{2α} treatment (Figure 2). Of these anovulatory cows, five of the dry cows and five of the lactating cows did not demonstrate complete luteolysis as determined by ultrasound evaluation of the CL and serum P₄ concentrations. The reasons for anovulation in the other cows were not clear.

Figure 1. Percentage distribution by days from the time of PGF_{2α} treatment until ovulation for nulliparous heifers (n = 79 treatments) and lactating cows (n = 67 treatments). Data within parentheses represent number of observations for each group at each day.

Table 3. Multiple ovulation rate, follicular and luteal sizes, serum steroid concentrations at the time (d 7; ovulation = d 1) and/or after PGF_{2α} injection, growth rate of the ovulatory follicle and days from PGF_{2α} injection to ovulation in dry (n = 26) and lactating cows (n = 27) (experiment 2).¹

	Dry cows	Lactating cows	P-value
Multiple ovulation rate [% (no./no.)]	17.2 (11/64)	17.9 (10/56)	0.9200
Data from single-ovulating animals			
Size of ovulatory follicle at PGF _{2α} (mm)	12.9 ± 0.5 (n = 53)	14.0 ± 0.5 (n = 46)	0.1000
Maximal size of ovulatory follicle (mm)	16.2 ± 0.4 (n = 53)	18.6 ± 0.4 (n = 46)	<0.0001
Growth rate of ovulatory follicle after PGF _{2α} (mm/d)	1.0 ± 0.1 (n = 53)	1.2 ± 0.1 (n = 46)	0.0580
Data from all animals			
Estradiol peak near estrus (pg/ml)	8.5 ± 0.6 (n = 64)	7.6 ± 0.5 (n = 56)	0.2660
Days from PGF _{2α} to ovulation	4.2 ± 0.1 (n = 64)	4.8 ± 0.1 (n = 56)	0.0060
Progesterone on d 7 (ng/ml)	1.9 ± 0.1 (n = 40)	2.2 ± 0.2 (n = 36)	0.1900
Luteal tissue volume on d 7 (mm ³)	5138.9 ± 459.8 (n = 40)	7598.9 ± 475.3 (n = 36)	0.0005

¹All results are expressed as mean ± SEM, except for multiple ovulation rate.

Table 4 summarizes the results from the correlation analyses between size of ovulatory follicle (for single ovulating cows), CL volume, and serum P₄ concentrations on d 7 in heifers (experiment 1), lactating cows (experiments 1 and 2 combined), and dry cows (experiment 2). There was a positive ($P < 0.05$) relationship between: 1) size of ovulatory follicle and CL volume; 2) size of ovulatory follicle and serum P₄ concentration; and 3) CL volume and serum P₄ concentration in both lactating cows and heifers. For dry cows, a significant ($P < 0.05$) positive correlation was only detected between size of the ovulatory follicle and CL volume (Table 4). Using data from single-ovulating females from all three groups, we found positive correlation coefficients between follicle size and CL volume ($r = 0.51$; $P < 0.0001$), follicle size and serum P₄ ($r = 0.24$; $P = 0.002$), and CL volume and serum P₄ ($r = 0.31$; $P < 0.0001$).

Figure 2. Percentage distribution by days from the time of PGF_{2α} treatment until ovulation for dry cows (n = 81 treatments) and lactating cows (n = 66 treatments). Data within parentheses represent number of observations for each group at each day.

Figure 3 shows scatter plots of volume of the ovulatory follicle and CL volume (Figure 3A), and of serum P₄ concentration and CL volume (Figure 3B). Regression lines shown in Figure 3 were estimated by using the regression-through-the-origin technique (Neter et al. 1996), i.e., forcing the regression to go through the origin at (0, 0). When the intercept was not forced to be zero, the estimated linear regressions were:

Lactating cows:

$$\text{Follicle} = 14.031 + 0.00048 \text{ CL } (R^2 = 0.44, P < 0.0001)$$

$$P_4 = 1.177 + 0.00011 \text{ CL } (R^2 = 0.20, P < 0.0010)$$

Dry cows:

$$\text{Follicle} = 13.400 + 0.00053 \text{ CL } (R^2 = 0.22, P = 0.006)$$

$$P_4 = 1.2784 + 0.000077 \text{ CL } (R^2 = 0.08, P = 0.11)$$

Heifers:

$$\text{Follicle} = 13.683 + 0.00040 \text{ CL } (R^2 = 0.20, P < 0.0001)$$

$$P_4 = 2.2454 + 0.00014 \text{ CL } (R^2 = 0.08, P = 0.01)$$

DISCUSSION

These studies were designed to determine physiological differences in follicular and luteal sizes and circulating P₄ and E₂ between lactating cows and virgin heifers and between lactating and dry cows. They were done in conjunction with studies of embryonic quality in lactating and nonlactating females (Sartori et al., 2002) in order to provide potential physiological explanations for differences in embryonic quality that were observed in those studies. The high multiple ovulation rate (63.5%) in lactating cows in experiment 1 was an unexpected and particularly puzzling aspect of this study. It is not possible to determine the exact cause of this increased ovulation rate; however, lactating cows in experiment 1 were treated with rbST every 12 d to match requirements for a simultaneous milk production trial in our herd. Previous studies have reported

Table 4. Correlation (r) tests among size of ovulatory follicle (in single-ovulating cows), corpus luteum (CL) volume on d 7, and serum progesterone (P_4) concentration on d 7 (d 1 = ovulation) in nulliparous heifers during summer, lactating cows during summer and winter, and dry cows during winter. Number of observations for each test is expressed within parentheses.

	Heifers		Lactating cows		Dry cows	
	CL	P_4	CL	P_4	CL	P_4
Follicle	$r = 0.44$ $P < 0.0001$ ($n = 78$)	$r = 0.32$ $P = 0.0044$ ($n = 78$)	$r = 0.67$ $P < 0.0001$ ($n = 51$)	$r = 0.33$ $P = 0.0196$ ($n = 51$)	$r = 0.47$ $P = 0.0063$ ($n = 33$)	$r = 0.10$ $P = 0.5698$ ($n = 34$)
CL	—	$r = 0.29$ $P = 0.0114$ ($n = 78$)	—	$r = 0.45$ $P = 0.0010$ ($n = 51$)	—	$r = 0.28$ $P = 0.1148$ ($n = 33$)

rbST treatment did not change (reviewed by Lucy, 2000), increased (Jimenez-Krassel et al., 1999), or decreased (Santos et al., 2000) incidence of double ovulation. Alternatively, heat stress decreased follicular growth (Wilson et al., 1998a, 1998b) and may have been responsible for the decreased size of the future ovulatory follicle at the time of PGF_{2 α} treatment. Cows with multiple ovulations were treated with PGF_{2 α} just after follicular diameter deviation (Ginther et al., 1996), and the subsequent reduction in circulating P_4 could have altered the follicular deviation mechanisms similar to what was observed after LH treatment (Sartori et al., 2001). The high double ovulation rate allowed some interesting comparisons of single- and multiple-ovulating lactating dairy cows although it may have con-

founded some of the comparisons of heifers and lactating cows. In addition, the differing location, diets, and parity of animals may have confounded results in experiment 1. Regardless, the physiological conditions that produced this high ovulation rate appeared to be specific for experiment 1 because a much lower frequency of multiple ovulations (17.9%) was found in experiment 2.

Results from experiment 1 showed greater peak E_2 concentrations in heifers compared with lactating cows; however, no difference in size of the ovulatory follicle was detected. In our study, evaluating normal estrous cycles (Sartori et al., 2000), we found that heifers had both a greater peak E_2 concentration (9.4 vs. 7.1 pg/ml) and a significantly smaller ovulatory follicle size (14.8 vs. 17.4 mm) compared with lactating cows. A recent

Figure 3. Scatter plot showing the relationship between: A) volume of the ovulatory follicle (Fol) and corpus luteum (CL) volume on d 7 of the estrous cycle; and B) serum progesterone (P_4) concentration and CL volume on d 7 for single-ovulating females (heifers, $n = 78$ observations; lactating cows, $n = 51$ observations; dry cows, $n = 33$ observations). Estimated regression-through-the-origin models: Fol = 0.3833 CL and $P_4 = 0.00025$ CL for lactating cows (solid lines); Fol = 0.4506 CL and $P_4 = 0.00032$ CL for dry cows (dotted lines); and Fol = 0.380 CL and $P_4 = 0.00050$ CL for heifers (dashed lines).

study by Inbar et al. (2001) and the Cooperative Regional Project NE-161 (Ahmad et al., 1996) also reported similar differences between heifers and lactating cows. Moreover, studies comparing lactating with dry cows observed that lactating cows developed larger dominant follicles (De La Sota et al., 1993; Beam, 1995) but had lower (De La Sota et al., 1993) or similar (Beam, 1995) circulating concentrations of E_2 . In the present study, the average size of the ovulatory follicle of lactating cows was especially large in experiment 2 (18.6 mm), but this was similar to the average diameter of ovulatory follicles in our study of lactating cows during the normal estrous cycle (17.4 mm; Sartori et al., 2000).

The greater size of the ovulatory follicle in lactating cows appears to be primarily related to an increased time for follicular growth as demonstrated by the greater time from $PGF_{2\alpha}$ to ovulation observed for lactating cows in both experiments 1 and 2. The time from $PGF_{2\alpha}$ to estrus or ovulation is influenced by day of the estrous cycle and follicle developmental stage in the follicular wave at the time of $PGF_{2\alpha}$ injection (Momont and Seguin, 1984; Pursley and Wiltbank, unpublished results; Stevenson et al., 1998). In our experiments, follicular development at $PGF_{2\alpha}$ was expected to be similar for all groups because $PGF_{2\alpha}$ was given at a known stage of the first follicular wave (6 d after ovulation). In experiment 2 there was a tendency for a greater follicular size at time of $PGF_{2\alpha}$ in lactating cows; however, lactating cows still required a greater time from $PGF_{2\alpha}$ to ovulation. Because peak E_2 concentrations were similar in lactating and dry cows (experiment 2), it seems clear that lactating cows require a larger follicular size and greater E_2 production to achieve the circulating E_2 concentrations necessary to produce an LH surge and ovulation. We have previously reported a very high rate of E_2 metabolism in lactating cows related to elevated DMI (Sangsrivong et al., 2002). The higher peak E_2 concentration in heifers as compared with lactating cows (experiment 1; Ahmad et al., 1996; Sartori et al., 2000; Inbar et al., 2001) is also consistent with high E_2 metabolism in lactating cows.

The potential physiological consequences of reduced peak E_2 and increased size of the ovulatory follicle are numerous. A reduction in peak circulating E_2 could be a cause of any reduced length or intensity of behavioral estrus in lactating cows (Nebel et al., 1997) or a low estrous detection rate observed in lactating cows (Senger, 1994). However, our studies as well as other studies comparing behavioral estrus in heifers and lactating cows have any parity effects confounded with type of housing (usually dirt for heifers vs. concrete for lactating cows) and location. Lower concentrations of E_2 before ovulation may also contribute to poor fertilization and poor early embryonic development (King et al.,

1994; DeSouza and Murray, 1995). In addition, reduced peak E_2 may also alter aspects of the LH surge that could account for some types of anovulation in lactating cows (Wiltbank et al., 2002). The increased size of the ovulatory follicle and a delayed time to ovulation after luteolysis in lactating dairy cows may also have important effects on fertility. Vasconcelos et al. (1999) observed that cows ovulating larger follicles had lower conception rate (**CR**) and a tendency for higher pregnancy loss than cows ovulating smaller follicles. Moreover, studies that induced persistent follicles in cattle (Sanchez et al., 1993; Savio et al., 1993; Wehrman et al., 1993; Ahmad et al., 1996; Kinder et al., 1996) observed lower fertility after ovulation of these persistent follicles compared with ovulation of smaller growing follicles. One study (Ahmad et al., 1996), for example, demonstrated that production of a persistent follicle decreased CR from 54 to 15% in lactating dairy cows.

There were also important differences in the CL between lactating and nonlactating females. Lactating cows had greater luteal tissue volume; however, they had either lower (experiment 1) or similar (experiment 2) circulating P_4 concentrations than heifers and dry cows, respectively. Greater luteal volume is likely related to greater size of the ovulatory follicle. Vasconcelos et al. (2001) reported a reduced luteal volume in cows that were induced to ovulate smaller follicles. In the present study, there was a significant positive correlation between size of the ovulatory follicle and size of the CL in all groups. The regression line explaining this relationship did not differ between the three groups of animals, suggesting that this relationship may be relatively constant for various physiological states. It seems likely that increased follicular size would lead to increased numbers of granulosa cells. Following the LH surge, granulosa cells differentiate into large luteal cells (Smith et al., 1994). Large luteal cells account for less than 4% of the luteal cell number but ~40% of the luteal volume (O'Shea et al., 1989). According to Niswender et al. (1985), the majority (~80%) of P_4 secreted by the mature ovine CL is derived from large luteal cells. Thus, an increased number of granulosa cells would likely result in an increased number of large luteal cells and subsequent increased size of the CL and increased P_4 secretion. Indeed, Milvae et al. (1991) reduced the number of granulosa cells in the ovulatory follicle of heifers and found a corresponding reduction in plasma P_4 concentrations. Murdoch and Kirk (1998) observed lower serum P_4 concentrations, lower luteal P_4 , and lower percentage of large luteal cells from ewes in which follicles were induced to ovulate earlier as compared with follicles that ovulated 24 h later. In that study, follicles induced to ovulate earlier had fewer granulosa cells but similar num-

bers of theca interna cells. Studies comparing heifers and lactating cows throughout a normal estrous cycle (Sartori et al., 2000; Inbar et al., 2001) and between dry and lactating cows after synchronization of estrus with norgestomet implant and PGF_{2α} (De La Sota et al., 1993) have reported lower circulating P₄ concentrations in lactating compared with nonlactating females. Thus, clearly, lactating cows ovulate larger follicles producing larger CL; however, circulating P₄ concentrations are reduced despite greater luteal volume, possibly due to an increased rate of P₄ metabolism in lactating cows. Similar to E₂ infusion, we also found that continuous infusion of P₄ at a constant rate produces much greater circulating P₄ concentrations in dry cows than lactating cows of similar size (Sangsritavong et al., 2002). It seems likely that high feed consumption in lactating cows may be responsible for the increased steroid metabolism (Wiltbank et al., 2000). One other observation that was consistent with differences in P₄ metabolism under different physiological conditions was the finding that the correlation between P₄ concentration and CL volume was explained by a different regression line in heifers than in cows.

Reduced peri- and postovulatory circulating P₄ concentrations could be responsible for some of the reduction in fertility in lactating dairy cows. Lower serum P₄ concentrations before AI were associated with reduced fertility (Folman et al., 1973; Fonseca et al., 1983) and supplementation of P₄ before AI increased PR (Folman et al., 1990; Wehrman et al., 1993; Xu et al., 1997). Low serum P₄ concentration allows increased pulse frequency of LH (Roberson et al., 1989; Bergfelt et al., 1991; Adams et al., 1992), causing premature maturation of the oocyte (Revah and Butler, 1996) with a resulting decrease in oocyte quality at the time of ovulation, and consequently, low embryo quality after fertilization (Ahmad et al., 1995). Reduced P₄ concentrations after AI also are associated with reduced fertility (Henricks et al., 1971; Lukaszewska and Hansen, 1980; Mann et al., 1995; Ahmad et al., 1996; Larson et al., 1997). Vasconcelos et al. (2001) showed that ovulation of very small follicles in lactating cows (11.5 ± 0.2 mm) resulted in smaller CL, lower serum P₄ concentrations, and lower CR compared with ovulation of larger follicles (14.5 ± 0.2 mm). This reduction in CR may be due to lower circulating P₄ concentration that may hamper embryo development (Mann et al., 1998), and/or allow earlier induction of luteolytic mechanisms (Mann and Lamming, 1995; Mann et al., 1995). Lynch et al. (1999) improved CR in cows using intravaginal P₄ releasing devices for 10 d starting on d 2 or 3 after estrus, combined with a GnRH injection on d 12 or 13. Studies in sheep and cattle also showed that P₄ administration during the first 4 to 6 d after insemination resulted in

increased embryonic or fetal growth (Garrett et al., 1988; Kleemann et al., 1994). Moreover, interferon τ production on d 16 is closely related to the circulating P₄ pattern (Kerbler et al., 1997; Mann et al., 1998). Thus, decreased embryonic development in lactating dairy cows (Sartori et al., 2002) could be due to multiple differences that were found between lactating cows vs. heifers or dry cows including increased size of the ovulatory follicle and/or decreased serum P₄ concentrations either before or after AI. Manipulative studies will be required to determine the importance of each of these reproductive changes in reducing embryonic development in lactating dairy cows.

CONCLUSIONS

In general, the results from the experiments supported our original hypotheses that lactating cows would ovulate larger follicles but have similar circulating E₂, and would have larger CL but similar circulating P₄ compared with heifers or dry cows. These discrepancies are expected to be due to greater steroid metabolism in lactating cows. Lower serum steroid concentrations have numerous potential physiological consequences that may compromise fertility in lactating cows.

ACKNOWLEDGMENTS

The authors thank Jerry N. Guenther and Siegrid A. Mertens for their technical assistance at the Dairy Cattle Center and Josie Lewandowski for her help with the steroid assays. We also thank Merial Ltd. for providing GnRH (Cystorelin). This research was supported by Wisconsin State experiment Station, USDA grant 2000-2276, and the fellowship BEX 1811/97-5 from CAPES of Brazil to Roberto Sartori.

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