Evaluating impact of season and corpus luteum cavitation on serum progesterone concentration and pregnancy in a Holstein dairy herd

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Abstract

The corpus luteum (CL) is vital to bovine reproduction and can present in 2 forms on ultrasound examination: homogeneous or cavitary. Impact of CL cavitation on fertility in cattle has been debated. Many variables influence fertility including heat stress, which is highly dependent on management, climate and season. Little is known regarding the impact of season and risk of association with a cavitary CL. The objectives of this study were to evaluate: (1) effect of CL form on serum progesterone, and (2) effect of reproductive status and season on the likelihood of diagnosing a cavitary CL. Blood from Holstein cows (n = 787) on a commercial dairy farm was collected (n = 1,062) at 39-45 days post-insemination from September 2021 to October 2022. Some cows were sampled more than once. Corpora lutea were categorized as homogeneous or cavitary, and cows were diagnosed as either pregnant or non-pregnant via transrectal ultrasonography at time of blood collection. When adjusted for season and CL status, pregnant cows produced higher serum progesterone (6.880 ng/mL; 95% CI [6.606, 7.153]) than non-pregnant cows (4.664 ng/mL; 95% CI [4.398, 4.931], $P < 0.001$). When adjusted for progesterone levels and season, pregnant cows were 94.7% less likely (OR = 0.053, $P < 0.001$) to be diagnosed with a cavitary CL compared to non-pregnant cows. No difference was observed in serum progesterone by CL form ($P = 0.393$). Serum progesterone was significantly lower in summer than fall ($P < 0.001$), winter ($P < 0.001$) and spring ($P = 0.005$). Season did not impact likelihood of diagnosing a cavitary CL.

Key words: bovine, corpus luteum, progesterone, pregnancy, CL cavitation

Abbreviations

CL: corpus luteum/corpora lutea

$PGF_{2\alpha}$: prostaglandin

GnRH: gonadotropin releasing hormone

AI: artificial insemination

DIM: days in milk

Introduction

The importance of progesterone in establishment and maintenance of pregnancy in cattle cannot be overstated and has been studied extensively.[1-10] In pregnant cattle, serum progesterone levels averaged 4.6 ng/mL between 19- and 22-days post-insemination.[4] Serum progesterone concentrations exceeding 5 ng/mL at 35 days post-insemination up to 9 weeks in gestation are highly suggestive of pregnancy retention.[9] Use of intravaginal progesterone products in conjunction with estrous synchronization through Ovsynch increases pregnancies per artificial insemination (AI) over cows without progesterone supplementation.[10] Furthermore, supplemental progesterone after AI improves pregnancy retention in the first 2 months of gestation.[10] Progesterone levels in blood have a positive impact on follicular development and, subsequently, on efficacy of timed-AI protocols as well.[6,11] Additionally, a meta-analysis by Yan et al. illustrated that supplementary progesterone positively impacted pregnancy outcomes in cattle bred by estrus detection in the absence of timed AI.[12] Conversely, others have observed no effect of supplemental progesterone on pregnancy rates in early gestation.[13] In cattle, the corpus luteum (CL) is a significant source of progesterone which exerts a complex effect on fertility.[3] Until approximately 200 days in gestation, the CL is considered the primary source of progesterone serving to maintain pregnancy.[1-3] To optimize fertility in cattle, it is necessary to gain a heightened understanding of the role of the CL, its physiologic importance to reproduction, and the variables that influence its functionality.

A CL can take on 1 of 2 structural forms on examination by transrectal ultrasonography: cavitary or homogeneous (non-cavitary).[14-17] Cavitary CL are defined by the presence of a central, anechoic fluid compartment, whereas homogeneous CL consist entirely of echogenic luteal tissue.[18] Clinical importance of cavitary CL in cattle has been debated, and many have explored theories regarding the significance of cavitary CL to reproductive physiology and clinical practice.[9,14-23] In embryo transfer recipients, heifers with cavitary CL have been shown to have higher pregnancy rates and higher serum progesterone levels than those with homogeneous CL.[21,22] Pregnant cows with cavitary CL have also been shown to have higher serum progesterone concentrations than pregnant cows with homogeneous CL.[23] Others have found no correlation between serum progesterone concentrations and CL cavity dimensions.[24] Disparity of findings to date regarding the influence of cavitary CL on serum progesterone levels warrant further investigation to elucidate the clinical significance of identifying a cavitary CL at time of pregnancy diagnosis.

Although the impact of season on fertility in cattle has been investigated, questions remain regarding a possible seasonal pattern of cavitary CL development and implications for fertility. Studies have shown that heat stress is an environmental factor which has deleterious effects on feed efficiency, milk production, milk quality and fertility in dairy cattle.[25,26] Delayed luteolysis and reduced folliculogenesis have been observed under conditions of heat stress in cows.[25,27] However, to the best of the authors' knowledge, no current literature exists exploring odds of developing a cavitary CL in seasons during which heat stress may pose a threat to fertility. Investigating whether such an association exists between CL cavitation and season would add to the body of research investigating the clinical significance of cavitary CL.

Therefore, the objectives of this study were to evaluate: (1) whether an association exists between CL form and serum progesterone concentration, and (2) whether odds of diagnosing a cavitary CL are associated with reproductive status or season. We hypothesize that there is no association between CL form and serum progesterone concentrations. We furthermore hypothesize that there is no association between season or reproductive status and the odds of diagnosing a cavitary CL.

Materials and methods

Housing, feeding and animal health

Cows included in this study were housed in a free-stall, deep-bedded sand dairy system on a commercial Holstein dairy farm in southeastern Pennsylvania. This herd was selected due to convenience for sampling. Cows were fed a total mixed ration formulated for lactating dairy cows producing, on average, 92 lb. of milk per head per day with ad libitum access to fresh, clean water. Cows were milked 3 times daily. Seventy-five percent of the herd was housed in 2, 6-row barns with the remaining 25% housed in a 3-row barn. Manure was managed through a flush system in each barn. Barns were equipped with side-wall curtains, fans over free stalls and feed bunks, and sprinklers over feed bunks. Fans and sprinklers were activated at ambient temperatures of 60 °F (15.6 °C) and 75 °F (23.9 °C), respectively. Cows were routinely vaccinated approximately 50 days prior to calving with a combination viral respiratory and leptospiral vaccine[a] as well as clostridial[b] and coliform[c] vaccines. The coliform vaccine[c] was repeated at 21 days pre- and post-partum. When pregnancy was diagnosed at or near 39-45 days post-insemination, a leptospiral vaccine[d] was administered. Once every 6 months, a topical parasiticide[e] was administered to all lactating cows. All medications and treatment protocols on the farm were implemented with veterinary oversight through a valid veterinarian-client-patient relationship with the co-author clinicians of this study.

Ovulation synchronization and reproductive management

The herd consisted of approximately 1,100 lactating cows bred by timed AI following ovulation synchronization with a Pre-synch-Ovsynch protocol ($PGF_{2\alpha 1}$—14d—$PGF_{2\alpha 2}$—11d—$GnRH_1$—7d—$PGF_{2\alpha 3}$—56hr—$GnRH_2$—16hr—AI). The first prostaglandin ($PGF_{2\alpha}$) injection of the Pre-synch protocol was administered at 35 to 42 days in milk (DIM). Occasionally, cows were bred if they were observed to be in standing estrus following the second $PGF_{2\alpha}$ of the Pre-synch protocol, however, these incidents accounted for less than 1% (0.67%) of first breedings. For most first breedings (99.33%), Ovysnch was initiated at 60 to 67 DIM, and a 73 to 80 day voluntary waiting period was established. One week prior to first examination for pregnancy diagnosis at 39-45 days post-insemination (112 to 119 DIM), the first dose of GnRH in a second course of the Ovsynch protocol was given so that cows determined to be open (non-pregnant) at 39-45 days post-insemination who were eligible for repeat breeding were given $PGF_{2\alpha}$ at time of examination, then bred again by AI 3 days later.

Sample collection

Clinicians co-authoring this study visited the farm once weekly for routine herd health monitoring including pregnancy diagnosis of the lactating herd. Cows enrolled in the study (n = 787) were examined by transrectal ultrasound for pregnancy diagnosis at or near 39-45 days post-insemination over a 13-month period from September 2021 to October 2022. Total number of individual cows sampled (n = 787) was less than the summation of samples (n = 1,062) as some cows were inseminated once (n = 550) whereas others were inseminated multiple times (n = 237). Some cows were sampled in more than one season over the course of the study period.

Pregnancy was defined by observation of an embryo within an amniotic vesicle by transrectal ultrasound[f] (B mode, 5.0MHz) with detection of a viable heartbeat. At the time of pregnancy diagnosis, cows with a single CL were enrolled in the study. Corpora lutea were categorized as homogeneous when they consisted entirely of well-demarcated, echogenic tissue as described by others (Figure 1 [a through f]).[17] Cavitary CL were defined as those with a total CL diameter < 25mm and presence of an echogenic rim of luteal tissue > 3mm in thickness surrounding a central, anechoic fluid compartment approximately < 19mm in diameter (Figure 2 [a through f]).[28] Neither luteal tissue volume, CL cross sectional area, nor luteal blood flow were recorded due to limitations in accurate measurement afforded by the instrumentation used in this study. The ultrasound machine[f] used did not have the capacity to perform color Doppler or to store images for retrospective measurement of luteal tissue volumes. Images depicted in Figure 1 (a through f) and Figure 2 (a through f) were recorded using a different ultrasound unit[g] which was not available for all practitioners involved in data collection throughout the study.

Cows diagnosed with more than one CL, follicular cysts, twins, pyometra, or other uterine or ovarian pathology were excluded. Cows with cavitary CL with a total CL diameter ≥ 25mm and > 3mm thick rim of echogenic tissue were excluded to exclude luteal cysts.[26] Blood samples were collected via coccygeal venipuncture with 20-gauge x 1.5" Vacutainer® needles[h] into 10 mL glass serum Vacutainer® tubes[h]. Blood samples were centrifuged at 36,000 rpm for 10 minutes, then frozen at -4 °F (-20 °C) within 6 hours of collection. Separated, frozen serum was then submitted for progesterone radioimmunoassay to the endocrinology section within the Animal Health Diagnostic Center at Cornell University (Ithaca, NY).

Progesterone radioimmunoassay

Serum progesterone concentrations were measured using a commercially available human progesterone radioimmunoassay[i] validated for use on bovine serum samples as part of this study. Serial dilutions of samples from 3 pregnant cows with assay buffer were parallel to the standard curve, and samples that were spiked with 3 different known quantities of progesterone tested with concentrations that averaged 80.9% of expected. Calculated sensitivity of the assay was 0.06 ng/mL. Mean intra- and inter-assay coefficients of variation were 12.1 and 9.2%, respectively.

Figure 1 (a through f): Ultrasound images of examples of homogeneous CL. Luteal tissue is outlined by white lines.

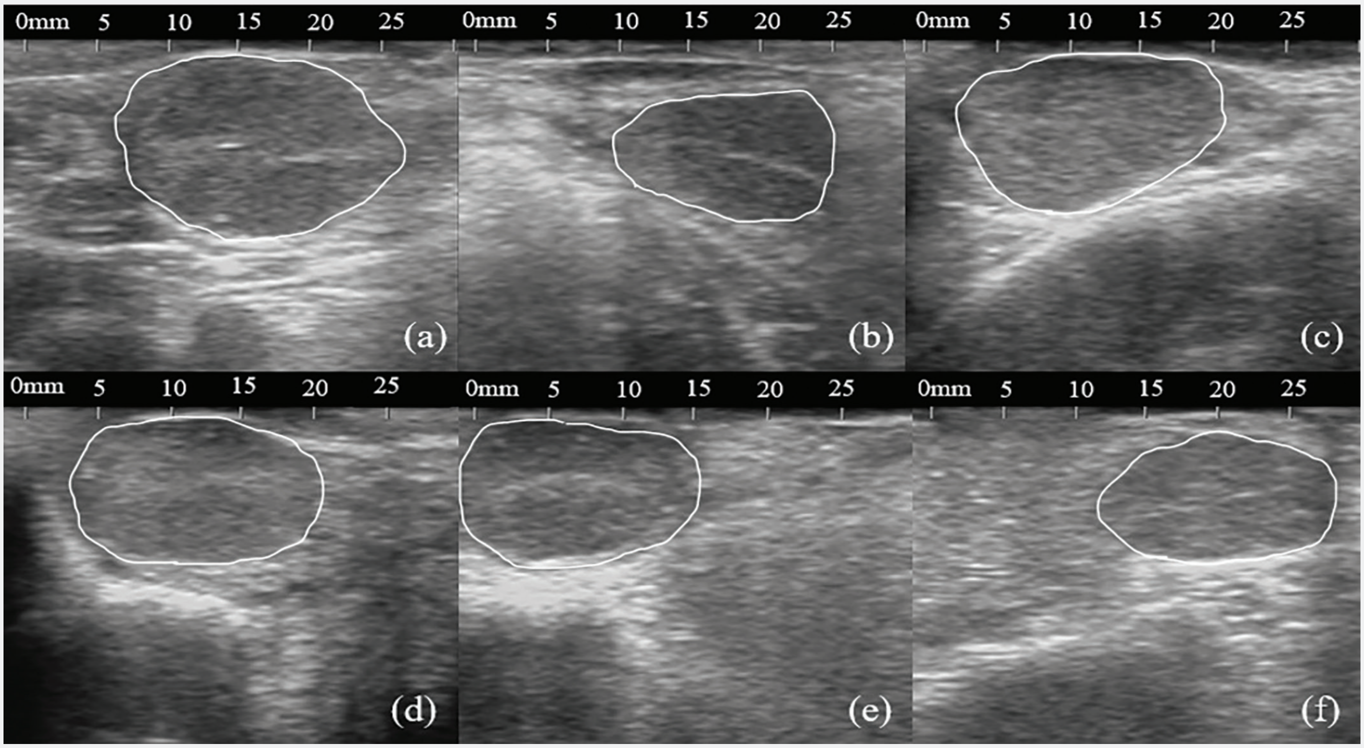
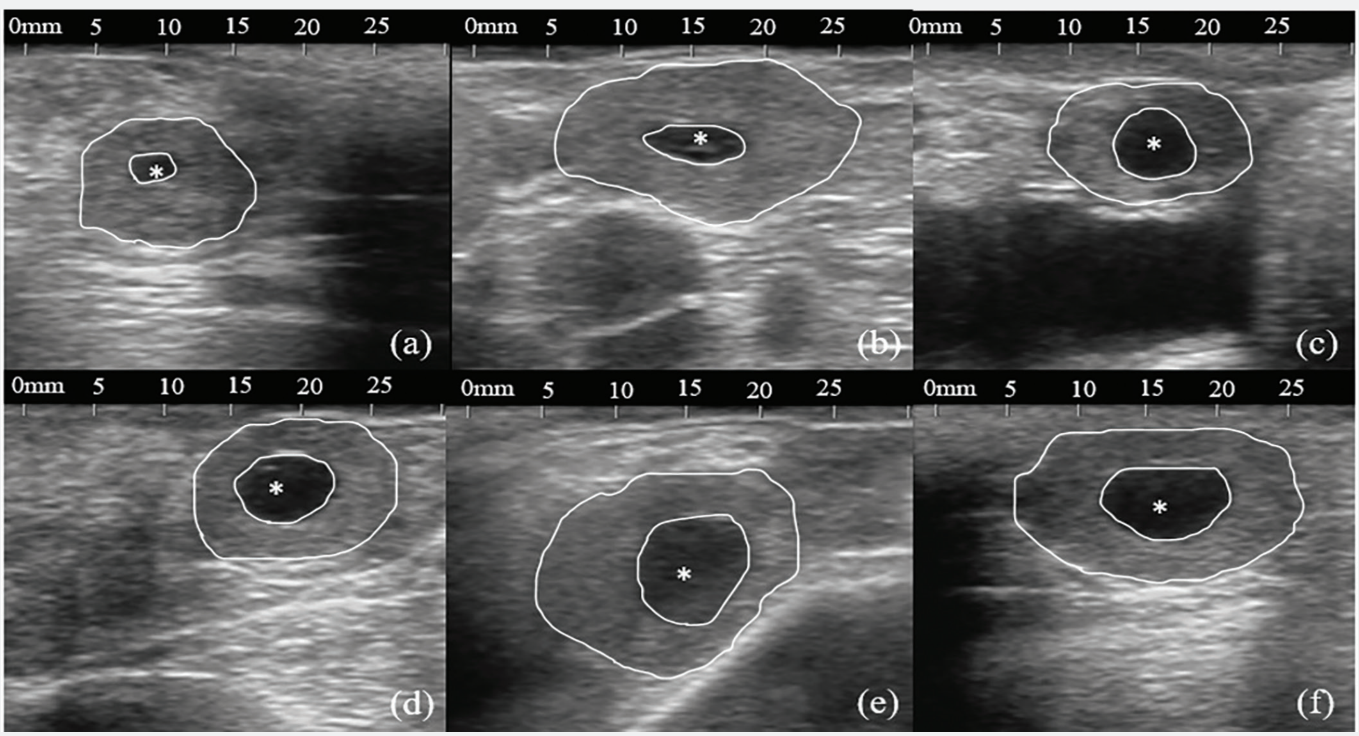


Figure 2 (a through f): Ultrasound images of examples of cavitary CL. Luteal tissue is outlined by white lines. Cavities are indicated by white asterisks (*).



Statistical analysis methods

Analyses were conducted using Stata 17MP[j] with 2-sided tests of hypotheses (outlined below) and a *P*-value < 0.05 as criterion for statistical significance. Frequency counts and percentages were used for summarizing categorical variables. Inference statistical analysis was based on multivariable linear regression model with blood progesterone concentration as the outcome and CL form and season as fixed effects. Both fixed effects (also known as independent variables) were categorical. For analysis, seasons were defined as outlined in Table 1. Animals sampled more than once in the study period were considered to be independent samples for each timepoint. Multivariable logistic regression was used to estimate the likelihood of cavitary CL, where progesterone levels, reproduction status and season were considered main fixed effects in the model.

Post-hoc analysis was used for estimation of the model adjusted (marginal) means and effects. Fisher protected least significant difference method (LSD), was used for adjusting for multiple comparisons. When using LSD, no adjustment is made to confidence intervals or P-values. However, it is protected in the sense that no pairwise comparisons are tested unless the joint test for the corresponding term in the model is significant.[29] All results were reported as marginal means or effects with their respective 95% confidence interval.

Results

A total of 1,062 CL and associated serum samples were assessed from 787 cows between September 2021 and October 2022. During this study, most CL identified were homogeneous (Table 2). Regardless of CL form, summer resulted in the fewest total CL observed (Table 2).

When adjusted for reproductive status and progesterone levels, season did not significantly impact risk of a cow exhibiting a cavitary CL (Table 3). However, when adjusted for season and progesterone levels, pregnant cows had a significantly lower risk of being diagnosed as having a cavitary CL than non-pregnant cows (Table 3).

When adjusted for CL form and season, serum progesterone concentrations were significantly lower for non-pregnant compared to pregnant cows (*P* < 0.001, Table 4). Model adjusted mean serum progesterone concentration was not different between CL forms (*P* = 0.393; Table 4). Summer resulted in a statistically significantly lower concentration of serum progesterone than winter (*P* < 0.001), fall (*P* < 0.001), and spring (*P* = 0.005). Marginal mean serum progesterone concentrations for cavitary and homogeneous CL are outlined in Table 5. Highest model-adjusted mean serum progesterone concentration was observed in winter (6.120 ng/mL; 95% CI [5.775, 6.464]), and the lowest in summer (4.921 ng/mL; 95% CI [4.518, 5.323]).

Discussion

This study adds to the body of evidence investigating the relationship between CL cavitation and serum progesterone levels. Of all CL observed in this study, 23.5% were cavitary CL, which is less than what has been described in previous studies enumerating cavitary CL in commercial dairy herds where 33.7 to 79% incidence of cavitary CL was observed.[14,17,23] With such a wide range of observed rates of cavitary CL, many variables may contribute to this difference such as timing of ovarian examination. For example, cavities have been shown to persist for 9.4 to 17.4 days in gestation depending on their size.[16] Corpora lutea in the present study were observed much later following insemination, which may have resulted in a different rate of cavitation than what has previously been reported.

No association was observed between CL form and serum progesterone concentration. Although mean serum progesterone concentration was numerically lower for cavitary (5.590 ng/mL; 95% CI [5.185, 6.993]) compared to homogeneous CL (5.795 ng/mL; 95% CI [5.585, 6.006]), there was no statistically significant difference between the 2 groups. Other studies have also noted that there is no difference in serum progesterone concentrations between cows with cavitary and homogeneous CL within the first 32 days of gestation.[14,20] While still others have shown higher levels of serum progesterone in heifers and pregnant cows with cavitary CL compared to those with homogeneous CL.[15,21-23]

Mean serum progesterone concentrations of homogeneous and cavitary CL in the present study exceed concentrations which have been described by others as evidence of the presence of a functional CL.[4,18] Furthermore, these results are above 5 ng/mL, the concentration of serum progesterone which has been found to be sufficient to maintain 90% of pregnancies to 9 weeks in gestation.[9] When controlling for reproductive status and season, presence of a cavitary CL did not affect serum progesterone levels, suggesting that secretory function was not compromised when a cavity was present at 39-45 days post-insemination.

A striking finding in these results was a markedly reduced likelihood of identifying a cavitary CL in pregnant cows relative to non-pregnant cows. When compared to non-pregnant cows, pregnant cows were 94.7% less likely to be diagnosed with cavitary CL at 39-45 days post-insemination (OR = 0.053, *P* < 0.001). Others have described no difference in proportion of cavitary and homogeneous CL identified in pregnant and non-pregnant heifers.[16] However, in the present study, only lactating cows were included, which may complicate this comparison to heifers. Furthermore, others have noted no difference in pregnancy rates for cattle with cavitary compared to homogeneous CL, suggesting that CL cavitation does not negatively influence fertility.[30]

Although serum progesterone concentration did not differ by CL form, progesterone was significantly lower in non-pregnant (4.664 ng/mL; 95% CI [4.398, 4.931]) compared to pregnant cows (6.880 ng/mL; 95% CI [6.606, 7.153]). These findings, combined with the observation that pregnant cattle were less likely to exhibit a cavitary CL complicate the conclusions that can be

Table 1: Definitions of seasons for sampling period of Holstein cows (n = 1,062) over a 13-month period (September 2021 to October 2022).

Start date	End date	Season
Sep 14, 2021	Sep 22, 2021	Summer
Sep 23, 2021	Dec 20, 2021	Fall
Dec 21, 2021	Mar 19, 2022	Winter
Mar 20, 2022	Jun 20, 2022	Spring
June 21, 2022	Sep 21, 2022	Summer
Sep 22, 2022	Dec 20, 2022	Fall

Table 2: Distribution of cavitary and homogeneous corpora lutea (CL, n = 1,062) observed via transrectal ultrasonography in a herd of Holstein cows at 39-45 days post-insemination by season over a 13-month sampling period between September 2021 and October 2022.

	Number of corpora lutea observed (% of that season*)				
CL form	Fall (n = 239)	Winter (n = 252)	Spring (n = 288)	Summer (n = 191)	Total (n = 787)
Homogeneous CL	187 (71.1)	223 (78.5)	254 (83.0)	148 (70.8)	812 (76.5)
Cavitary CL	76 (28.9)	61 (21.5)	52 (17.0)	61 (29.2)	250 (23.5)
Total	263	284	306	209	1,062

* Dates included in each season are defined in Table 1.

Table 3: Logistic regression model of odds of diagnosing a cavitary corpus luteum relative to reproductive status, serum progesterone concentration, and season for Holstein cows (n = 1,062) sampled over a 13-month period (September 2021 to October 2022).

	OR*	95% CI
Reproductive status		
Pregnant	0.053	0.032, 0.089
Non-pregnant	Reference category	
Serum progesterone concentration (ng/mL)	0.972	0.918, 1.030
Season†		
Fall	Reference category	
Winter	0.824	0.532, 1.276
Spring	0.714	0.454, 1.121
Summer	0.978	0.623, 1.535
Constant	0.965	0.638, 1.461

* Odds ratio

† Seasons are defined as listed in Table 1

Table 4: Marginal effects (model adjusted effects) on serum progesterone concentration adjusted for reproductive status, corpus luteum (CL) form, and season in a herd of Holstein cows over a 13-month period (September 2021 to October 2022, n = 1,062).

	Effect estimate	*P*-value	95% CI
Reproductive status			
Pregnant vs non-pregnant	2.215	< 0.001	1.810, 2.620
CL form			
Cavitary vs. homogeneous CL	-0.206	0.393	-0.680, 0.268
Season*			
Spring vs Fall	-0.408	0.105	-0.902, 0.085
Summer vs Fall	-1.163	< 0.001	-1.701, -0.625
Winter vs Fall	0.036	0.888	-0.463, 0.534
Summer vs Spring	-0.755	0.005	-1.281, -0.229
Winter vs Spring	0.444	0.069	-0.035, 0.923
Winter vs Summer	1.120	<0.001	0.668, 1.730

* Seasons are defined as listed in Table 1

Table 5: Marginal means (model adjusted means) on serum progesterone concentration adjusted for reproductive status, corpus luteum (CL) form, and season in a herd of Holstein cows over a 13-month period (September 2021 to October 2022, n = 1,062).

	Marginal mean serum progesterone concentration (ng/mL)	95% CI
Reproductive status		
Non-pregnant	4.664	4.398, 4.931
Pregnant	6.880	6.606, 7.153
CL form		
Homogeneous CL	5.795	5.585, 6.006
Cavitary CL	5.590	5.185, 6.993
Season*		
Fall	6.084	5.725, 6.443
Winter	6.120	5.775, 6.464
Spring	5.676	5.341, 6.010
Summer	4.921	4.518, 5.323

* Seasons are defined as listed in Table 1

drawn relative to the physiologic significance of a cavitary CL. It is possible that variables related to CL form influenced serum progesterone concentrations to produce these observations.

One avenue which might explain differences observed in secretory capacity and form of CL in pregnant compared to non-pregnant cows in the present study may be a difference in overall luteal tissue mass. Variable conclusions have been drawn regarding the influence of total luteal tissue mass on secretory function of cavitary and homogeneous CL. One study has shown increased luteal tissue volume and secretory function of cavitary CL relative to homogeneous CL.[22] However, others have shown that cavitary CL have a higher secretory capacity in spite of exhibiting the same luteal tissue volume.[23] In contrast, other studies suggest that no difference exists in the secretory function of cavitary and homogeneous CL.[16,20] Blood flow within the CL as assessed by color Doppler has also been suggested as a useful diagnostic for assessing luteal function in cattle.[31,32] Neither luteal blood flow nor total luteal mass could be assessed in the current study due to limitations of available ultrasound machinery. Therefore, neither luteal tissue volume nor CL blood flow were explored as independent variables. Quantifying amount of luteal tissue and comparing vascularity in cavitary and homogeneous CL would be a beneficial next step in this research to further clarify effects of CL form on functionality in this population of cows.

While no association was observed between CL form and season, mean serum progesterone concentration was lower in summer than any other season. Furthermore, compared to fall, spring, and winter, summer resulted in the fewest total CL observed. Many environmental factors influence reproductive performance, including photoperiod and temperature humidity index (THI), the latter of which is calculated based on environmental temperature and relative humidity.[25,34-36] Thresholds for THI that have been established illustrate that high ambient temperatures and high relative humidity negatively impact fertility in cattle.[35,37] Under conditions of elevated ambient temperature, such as might be expected in summer months in the northeastern United States, lactating dairy cows have been shown to experience delayed luteolysis, reduced follicular development and reduced conception rates.[27,38] Although cows in the present study were all managed under conditions which reduce the risk of heat stress, neither THI nor core body temperatures were monitored, and therefore it cannot be concluded whether cows were indeed experiencing heat stress at any point during the 13-month study period. With these considerations in mind, seasonal effect observed in the present study may be explained by more than merely the impact of heat stress, and further investigation is warranted to understand more precisely the nuances of the association between season and serum progesterone levels observed. Because odds of identifying a cavitary CL were similar in all seasons, and there was no difference in progesterone levels for cavitary and homogeneous CL, diagnosis of a cavitary CL at 39-45 days post-insemination appears to bear questionable clinical significance for this population of cows. However, the results of this study are difficult to extrapolate to other herds under different environmental and management conditions as only one herd was included in the study.

Conclusion

This study illustrates no difference in serum progesterone concentrations in cows with cavitary CL compared to homogeneous CL. These results support the theory that the mere presence of a cavity within a CL does not necessarily reduce its secretory capacity at 39-45 days post-insemination as serum progesterone concentrations did not differ by CL form. Pregnant cows produced higher levels of circulating progesterone and were at lower risk of having a cavitary CL at 39-45 days post-insemination than non-pregnant cows. Serum progesterone concentrations were on average lowest in summer, and the odds of diagnosing a cavitary CL were similar across seasons. From these findings, it can be concluded that, on this farm, season did not impact likelihood of identifying a cavitary CL at 39-45 days post-insemination.

Endnotes

[a] Triangle® 10 HB, Boehringer Ingelheim, St. Joseph, MO

[b] Ultrabac® 8, Zoetis, Inc., Kalamazoo, MI

[c] Enviracor® J5, Zoetis, Inc., Kalamazoo, MI

[d] Leptoferm® 5, Zoetis, Inc., Kalamazoo, MI

[e] Cydectin®, Elanco, Shawnee, KS

[f] Honda HS-102V, B mode ultrasound, 5.0MHz rectal probe, Honda Electronics, Toyohashi Aichi, Japan

[g] Ibex® Pro ultrasound, EI Medical Imaging, Loveland, CO

[h] BD Vacutainer® Serum tubes and needles, BD, Franklin Lakes, NJ

[i] Progesterone double antibody RIA kit, MP Biomedicals, Costa Mesa, CA

[j] Stata 17MP, StataCorp, College Station, TX

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