Original Research

Correlations between ultrasonographic characteristics of corpora lutea and systemic concentrations of progesterone during the discrete stages of corpora lutea lifespan and secretory activity in cyclic ewes

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Abstract

Associations between physical characteristics and functionality of corpora lutea (CL) have previously been reported in monovulatory species, albeit several studies in cattle and humans have refuted the existence of temporal relationships between CL size, echotexture and serum progesterone (P_4) concentrations. The main objective of the present study was to examine whether or not there were correlations between ultrasonographic image attributes of CL and systemic concentrations of P_4 during the discrete stages of the luteal phase in two breeds of sheep differing in ovulation rates (non-prolific Western White Face [WWF] ewes and prolific Finn [F] sheep). Transrectal ovarian ultrasonography utilized a 7.5-MHz linear-array transducer connected to a portable scanner (Aloka SSD-500) and the images were analyzed using commercially available image analytical software (Image ProPlus®) validated for the present application in sheep. The correlations were assessed using the Pearson's Product Moment (PPM) analysis and also, to increase the accuracy of statistical tests, the analysis of covariance (ANCOVA), with the number of CL as a co-factor. In WWF ewes, serum concentrations of P_4 correlated significantly with the total luteal area (TLA) during the CL growth phase (days 3–6; day 0 = ovulation) and functional luteolysis (days 12–15), and with numerical pixel values (NPVs – pixel intensity) during luteolysis; the results obtained by using two different statistical methods were generally similar. In prolific F ewes, serum P_4 concentrations were directly correlated with TLA during CL growth (days 3–6; ANCOVA), functional luteolysis (days 13–14; PPM), and structural CL regression (days 11–14; PPM and ANCOVA), and with NPVs during functional luteolysis (PPM and ANCOVA). We concluded that systemic P_4 concentrations could only be accurately predicted from the changes in luteal area during CL growth and regression, and from NPVs during luteolysis, in both prolific and non-prolific ewes, but the changes in size and echotexture of the luteal glands at mid-cycle were not indicative of serum P_4 concentrations in sheep.

Keywords: ewe, corpus luteum, progesterone, ultrasonography, computerized image analysis

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Introduction

It is widely known that progesterone (P_4) is mainly secreted by the corpus luteum (CL) in non-pregnant females of mammalian species.[1] The pattern of circulating P_4 concentrations during the ewe's estrus cycle was one of the first to be described accurately[2] and has been confirmed several times since.[3–7] It has also been held, based largely on the observations of the ovaries postmortem, that serum concentrations of P_4 closely reflect the physical changes in ovine luteal glands.[8] The concentrations of P_4 are very low immediately after ovulation (day 0), followed by increasing amounts to days 5–6, a plateau to days 11–12, and a rapid decline to basal or undetectable levels preceding the next estrus.[3–7] This temporal pattern appears to be related to the growth, static and regression phases of CL lifespan,

and it also appears to be consistent between various breeds of sheep.[3] It was, however, reported that less prolific breeds had higher progesterone concentrations than prolific ewes as they produced fewer but larger CL, with a greater P_4 secretory ability.[3,4]

Non-invasive, realtime transrectal ovarian ultrasonography has become commonplace in large domestic livestock, especially cattle.[9–12] This technology was later adapted in sheep and goats in the early 1990s.[13–15] It allows for the monitoring of major anatomical changes in ovarian structures (e.g. antral follicles and luteal tissue) based on their ability to reflect or transmit high-frequency ultrasound beams[16,17] and regardless of their position or depth in the ovary. These morphological changes can be visualized on a screen of an ultrasound scanner, and can be accurately and objectively analyzed using computer-assisted image enhancement and/or analysis algorithms.[4,16,18] A grayscale digital image is an image in which the value of each picture element (pixel) carries only one intensity information, varying from black at the weakest (numerical value of 0) to white at the strongest intensity (numerical value of 255). Changes in numerical pixel values (NPVs) of ultrasonographic images depend on the cellular composition of the tissue.[16] It was expected that a combination of ultrasonographic imaging and hormone assays would confirm the temporal associations between CL morphology and P_4 production.

However, there have been inconsistent reports regarding the relationship between ultrasonographic image attributes of CL and circulating P_4 concentrations in several mammalian species. The relationship between estimates of luteal tissue area and peripheral blood P_4 concentrations has been demonstrated in cyclic and pregnant heifers, but those studies were restricted to single time-point observations.[10,19] During natural and prostaglandin $F_{2\alpha}$-induced luteolysis in sheep, goats and cattle, serum concentrations of P_4 are positively correlated to ultrasonographically-determined luteal area or volume.[20–22] A number of studies in humans and cattle have reported that there was no correlation between the size of CL and P_4 concentrations in peripheral blood plasma.[23–26] Bartlewski *et al.*[3] showed that correlations between luteal volumes and systemic concentrations of P_4 in cyclic ewes were only seen during relatively short periods of CL growth and regression. There exists a physiological divergence between functional and morphological luteolysis; a decline in progesterone synthesis (functional luteolysis) precedes the onset of structural luteolysis by 1–2 days.[1,3,21] Therefore, the analysis of ultrasonographic images of luteal structures for determining luteal volumes or area may have limited value in predicting serum P_4 concentrations.

It has recently been suggested that quantitative ultrasonographic characteristics of CL such as NPVs (i.e. pixel brightness or intensity) and pixel heterogeneity are related to their histomorphology and P_4 biosynthesis.[4,19,21] Pixel values of bovine CL *ex situ* were significantly correlated with luteal and plasma P_4 concentrations.[21] Duggavathi *et al.*[18] showed that an increment in the luteal tissue content and a concomitant decline in echogenicity of developing CL up to 72 h post-ovulation was reflected in an initial increase in serum P_4 concentrations in cyclic ewes. Davies *et al.*[4] have reported that mean pixel values were only moderately correlated with serum P_4 concentrations in cyclic ewes during the entire period when luteal structures could be detected with ultrasonography.

Considering this postulated association between echotextural characteristics and P_4 secretory ability of CL, the main objective of the present study was to determine if ultrasonographic image attributes of the ovine CL, at specific stages of their development and secretory function, were correlated with circulating P_4 concentrations. In this study, the luteal phase was partitioned into three discrete periods on the basis of changes in CL area or assessment of daily serum concentrations of P_4. In individual ewes, different numbers of CL can be detected after ovulation, but there has been no study of the effects that CL numbers have on the correlations among CL echotexture and systemic concentrations of P_4. Therefore, the analysis of covariance (ANCOVA), with the number of CL as a co-factor, was employed as an additional test of the relationships between ultrasonographic characteristics of the luteal glands and serum P_4 concentrations in ewes.

Materials and methods

Animals and experimental procedures

All experimental procedures were in compliance with the guidelines of the Canadian Council on Animal Care and had been approved by the local animal care committees. The present experiment was conducted in Saskatoon, Saskatchewan, Canada (latitude: 52°08′N; longitude: 106°39′W). The care and nutrition of experimental animals studied during the middle portion of the breeding season (October–December) (8 Western White Face [WWF] ewes, 5 years of age and an average body weight of 90 ± 7 kg; and 7 pure-bred Finn [F] ewes, 3–4 years of age and an average body weight of 57 ± 4 kg) have been described previously.[3,4] The mean ovulation rate of the WWF and F sheep employed in this study was 1.7 ± 0.2 and 2.7 ± 0.2, respectively ($P < 0.05$).

Ovarian ultrasonography

Ultrasonography utilized a 7.5-MHz linear-array transducer connected to a portable B-mode echo camera (Aloka SSD-500, Tokyo, Japan). The images obtained by Bartlewski *et al.*[3] were analyzed retrospectively. All ovarian images were displayed at 1.5× magnification, and the gain and focal points have been left unchanged. Ovarian images were initially recorded on high-grade videotapes (Fuji S-VHS, ST-120 N, Fuji Photo Film Co Ltd, Tokyo, China), using a composite video signal (VCR: Panasonic AG 1970 Proline; Matsushita Electric, Markham, ON, Canada). Ultrasound scanning was performed daily, from the first day of estrus to the onset of the next estrus to record the diameter and position of the CL.

Hormone assays

Jugular blood samples (10 mL) were collected before each ultrasonographic examination and were allowed to clot at room temperature for 18–24 h. Subsequently, serum samples were stored at −20°C until assayed. The profile of serum concentrations of P_4 during the luteal phase, for both breeds of sheep, was determined by validated radioimmunoassay, as described earlier.[3]

Image acquisition and analysis

Selected ovarian images in the plane closest to the central axis of CL were digitized at standardized settings, with a resolution of 720 × 480 pixels and 256 shades of gray. Image digitization was performed using the commercially available image-acquisition software (Adobe Premiere®Pro 1.5; Adobe Systems Inc, San Jose, CA, USA). All images were saved as bitmap (*.bmp) graphic images. Spot analysis, as described by Davies *et al.*,[4] was performed to determine the ultrasonographic attributes to the CL using Image ProPlus® analytical software (Media Cybernetics Inc, San Diego, CA, USA). In order to measure the total luteal area (TLA), the area encompassing the CL was outlined. A fluid-filled central cavity was also outlined, if detected within a CL, and its area was subtracted from the outer CL area. We opted to use spot analysis to represent the echotextural data as this technique is fast and easy to use, and therefore it would be a more suitable method to employ in practical settings.[4] Four computer-generated spots were randomly placed on the cross-sectional area of the CL, avoiding central cavities and reflection artifacts. The size of the spots was large enough to cover approximately 70% of the entire CL area. NPVs and pixel heterogeneity (standard deviation [SD] of mean NPVs) were computed for each spot, and then the four spots were averaged. There were no differences in the results between the three individuals independently performing image analyses; therefore, data for each CL and animal were combined before the correlations were analyzed on a *per* animal basis. Daily ovarian and endocrine data from animals in which all luteal structures could not be clearly delineated were withdrawn from statistical analyses (Figure 1 and 2).

Statistical tests

In evaluating the relationships between CL echotextural attributes and serum P_4 concentrations, the luteal phase was divided into three periods (Figure 1). Period 2 was defined as a period encompassing all days on which daily TLA/serum P_4 concentrations did not differ ($P > 0.05$) from the maximum mean values for these parameters. The time from first ultrasonographic detection of CL and the beginning of Period 2 was Period 1, or the phase of luteal growth/development. Lastly, Period 3, or luteal regression, was the time from the end of Period 2 until complete regression of CL confirmed with ultrasonography. One-way repeated measures analysis of variance (RM-ANOVA; SimgaStat®3.0 for Windows®; 2003; Systat Software, Inc, Richmond, CA, USA) was used to assess

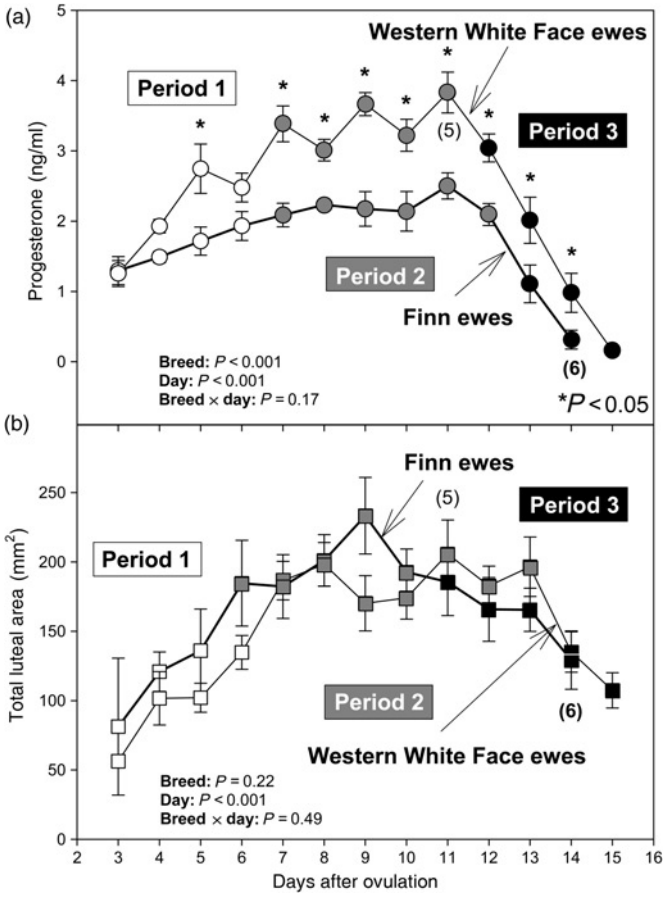

Figure 1 Serum progesterone (P_4) concentrations (a) and total luteal area (b) during Period 1 (○ □), Period 2 (● ■) and Period 3 (● ■) in eight cyclic Western White Face ewes (WWF) (thin lines) and seven Finn (F) ewes (thick connecting lines). Please see text for details of statistical analyses. Numbers in parentheses are the actual numbers of ewes used for data analyses on the indicated days (bold font – F ewes and regular font – WWF ewes)

differences in the luteal area and echotextural variables of the CL. Total luteal area, as well as mean spot pixel values and heterogeneity, and serum P_4 concentrations were analyzed by Pearson's Product Moment (PPM) in SimgaStat® program and by ANCOVA in SAS® program (SAS®9.2 software; SAS Institute Inc, Cary, NC, USA). ANCOVA considers additional variables (covariates) into the statistical model; the power of the statistical analysis can be increased when the covariates are associated with the treatment effect. Statistical significance was regarded as $P < 0.05$.

Results

General

Mean daily P_4 concentrations in cyclic WWF ewes increased ($P < 0.05$) from days 3 to 7 after ovulation, decreased ($P < 0.05$) from days 12 to 15, and did not change ($P > 0.05$) from days 7 to 11 after ovulation (Figure 1a). Progesterone concentrations in F ewes increased ($P < 0.05$) from days 3 to 8 after ovulation, decreased ($P < 0.05$) from days 11 to 14 after ovulation, and did not change ($P > 0.05$) from days 7 to 13 (Figure 1a). In WWF ewes, the TLA increased

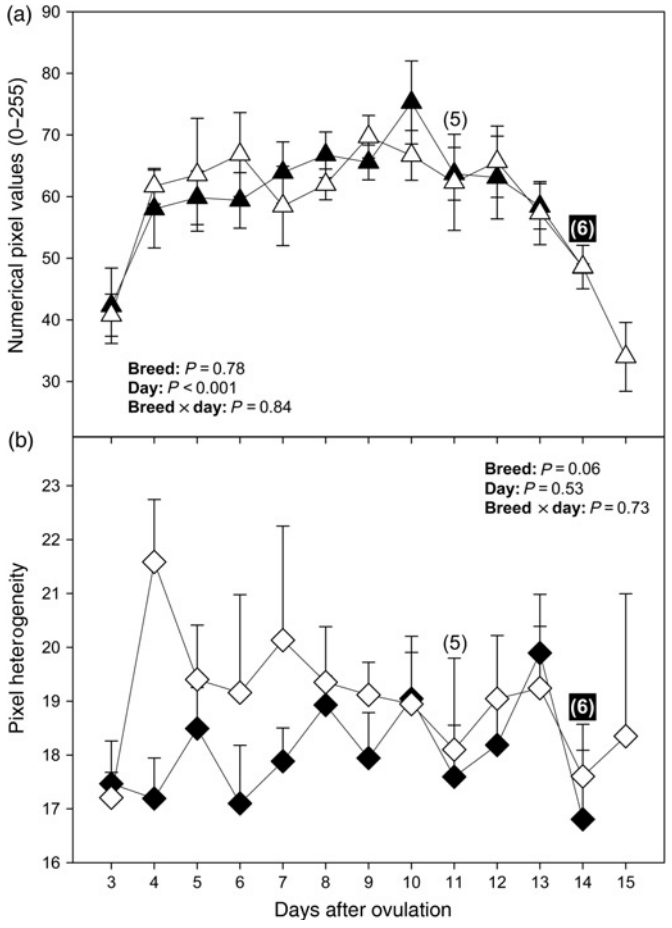

Figure 2 Summary of changes in daily values (mean ± SEM) for echotextural attributes of corpora lutea (numerical pixel values (a) and pixel heterogeneity (b)) recorded in eight cyclic Western White Face (WWF) ewes (◇△) and seven Finn (F) ewes (◆▲). Numbers in parentheses are the actual numbers of ewes used for data analyses on the indicated days (bold white font – F ewes and regular black font – WWF ewes)

($P < 0.05$) from days 3 to 9 after ovulation, decreased ($P < 0.05$) from days 13 to 14, and did not change from days 7 to 13 after ovulation (Figure 1b). In F ewes, the TLA increased from days 3 to 5 after ovulation ($P < 0.05$), declined from days 11 to 14 ($P < 0.05$), and did not change from days 6 to 10 after ovulation ($P > 0.05$; Figure 1b).

Correlation analyses

According to the discrete phases of CL development (i.e. changes in TLA), a PPM analysis in SimgaStat® (Table 1) indicated that there was a positive correlation between luteal area and daily P_4 concentrations in Period 1 (CL growth phase; $r = 0.60$, $P < 0.01$) and Period 3 (luteal regression phase; $r = 0.75$; $P < 0.001$) in WWF ewes. Only one significant correlation between the TLA and P_4 concentrations in F ewes ($r = 0.75$, $P < 0.05$) was recorded for Period 3. ANCOVA analysis (Table 2) revealed correlations between luteal area and serum P_4 concentrations in Period 1 ($r = 0.62$, $P < 0.05$) and Period 3 ($r = 0.80$, $P < 0.001$), and a correlation between mean pixel values and progesterone concentrations in Period 3 ($r = 0.75$, $P < 0.05$) of the WWF ewes. The only significant correlation between pixel values and P_4 concentrations in cyclic F ewes was found in Period 3 ($r = 0.46$, $P < 0.05$).

According to the discrete phases of P_4 secretory activity, PPM analysis indicated that there was a positive correlation between luteal area and P_4 concentrations in Period 1 ($r = 0.60$, $P < 0.01$) and Period 3 ($r = 0.75$, $P < 0.001$), and a correlation between mean pixel values and P_4 concentrations in Period 3 ($r = 0.57$, $P < 0.01$) in WWF ewes. There was a positive correlation between TLA and P_4 concentrations ($r = 0.75$, $P < 0.05$) and between mean NPVs and P_4 concentrations ($r = 0.71$, $P < 0.05$) during Period 3 in F ewes. ANCOVA analysis revealed a positive correlation between luteal area and P_4 concentrations in Period 1 ($r = 0.62$, $P < 0.05$) and Period 3 ($r = 0.80$, $P < 0.001$), and a correlation between mean pixel values and P_4 concentrations in Period 3 ($r = 0.77$, $P < 0.001$) in WWF ewes. TLA correlated with serum P_4 concentrations in Period 1 ($r = 0.72$, $P < 0.05$) and mean pixel values correlated with P_4 concentrations in Period 3 ($r = 0.87$, $P < 0.05$) in F ewes.

Discussion

The specific objective of the present study was to examine the utility of transercatal ovarian ultrasonography combined with computer-assisted image analyses as a tool to evaluate luteal function in cyclic ewes, by correlating morphological and echotextural characteristics with a functional trait (serum P_4 concentrations) during the discrete stages of the luteal phase. This method could potentially be used during on-farm examinations of embryo transfer recipients[21] and early pregnant ewes.

The pattern of changes in the physical characteristics of luteal glands varied substantially between non-prolific WWF ewes and prolific F sheep. A progressive increase in the size and number of luteal cells from metestrus to mid-diestrus, as evidenced by an increase in the total luteal tissue area (Figure 1a), was more gradual and took longer in WWF ewes as compared with F sheep. However, a plateau phase during diestrus was longer in WWF than F ewes by two days. The onset of structural luteolysis in WWF was abrupt and CL regression occurred over approximately two days, whereas luteal tissue in cyclic F ewes underwent a slower degradation spanning approximately four days. Clearly, prolific F ewes produced more but smaller CL than less prolific WWF sheep. Both small and large luteal cells contribute to an increase in the total luteal mass during the early and mid-luteal phase of the ewe's estrus cycle. Large luteal cells increase in size between days four and 12 of the cycle (day 0 = detection of behavioral estrus), but their number remains relatively constant until the onset of luteolysis.[27] In contrast, the size of small luteal cells does not change but, due to mitotic divisions, there is an increase in the number of these cells from days 4–8 of the estrus cycle.[27] The present observations may imply that there are differences in cellular content, composition and/or proliferating ability of the CL between prolific and non-prolific genotypes of sheep.

Table 1 Results of correlation analyses (Pearson's Product Moment) between the total luteal area and echotextural attributes of the ovine CL (input variables), and serum P_4 concentrations (output variable) in eight cyclic Western White Face and seven Finn ewes at different stages of the CL lifespan and secretory activity

Based on circulating P_4 concentrations						
Input variables	**Western White Face ewes (n = 8)**			**Finn ewes (n = 7)**		
	Period 1 (days 3–6)	**Period 2 (days 7–11)**	**Period 3 (days 12–15)**	**Period 1 (days 3–6)**	**Period 2 (days 7–12)**	**Period 3 (days 13–14)**
Luteal area (mm^2)	$r = 0.60$	$r = -0.01$	$r = 0.75$	$r = 0.41$	$r = 0.03$	$r = 0.75$
	$P < 0.01$	$P = 0.33$	$P < 0.001$	$P < 0.09$	$P = 0.86$	$P < 0.05$
Mean NPVs	$r = 0.22$	$r = 0.21$	$r = 0.57$	$r = 0.38$	$r = -0.03$	$r = 0.71$
	$P = 0.36$	$P = 0.31$	$P < 0.01$	$P = 0.11$	$P = 0.85$	$P < 0.05$
Pixel heterogeneity (SD)	$r = 0.02$	$r = 0.005$	$r = 0.14$	$r = 0.31$	$r = -0.18$	$r = 0.07$
	$P = 0.92$	$P = 0.98$	$P = 0.51$	$P = 0.19$	$P = 0.26$	$P = 0.87$
Based on changes in luteal area						
	Period 1 (days 3–6)	**Period 2 (days 7–13)**	**Period 3 (days 14–15)**	**Period 1 (days 3–5)**	**Period 2 (days 6–10)**	**Period 3 (days 11–14)**
Luteal area (mm^2)	$r = 0.60$	$r = 0.05$	$r = 0.73$	$r = 0.09$	$r = 0.03$	$r = 0.45$
	$P < 0.01$	$P = 0.78$	$P < 0.05$	$P = 0.77$	$P = 0.87$	$P < 0.05$
Mean NPVs	$r = 0.22$	$r = -0.04$	$r = 0.39$	$r = 0.48$	$r = 0.008$	$r = 0.40$
	$P = 0.36$	$P = 0.82$	$P = 0.24$	$P < 0.10$	$P = 0.97$	$P < 0.07$
Pixel heterogeneity (SD)	$r = 0.02$	$r = 0.009$	$r = -0.12$	$r = 0.52$	$r = -0.06$	$r = -0.17$
	$P = 0.92$	$P = 0.96$	$P = 0.73$	$P < 0.08$	$P = 0.76$	$P = 0.45$

CL, corpus luteum; P_4, progesterone; NPVs, numerical pixel values

Table 2 Results of the analysis of covariance (ANCOVA) for the total luteal area and echotextural attributes of the ovine CL (input variables), and serum P_4 concentrations (output variable) in eight cyclic Western White Face and seven Finn ewes at different stages of the CL lifespan and secretory activity

Based on circulating P_4 concentrations						
	Western White Face ewes (n = 8)			**Finn ewes (n = 7)**		
	Period 1 (days 3–6)	**Period 2 (days 7–11)**	**Period 3 (days 12–15)**	**Period 1 (days 3–6)**	**Period 2 (days 7–12)**	**Period 3 (days 13–14)**
Luteal area (mm^2)	$r = 0.62$	$r = 0.41$	$r = 0.80$	$r = 0.72$	$r = 0.36$	$r = 0.85$
	$P < 0.05$	$P = 0.18$	$P < 0.001$	$P < 0.05$	$P = 0.71$	$P < 0.07$
Mean NPVs	$r = 0.40$	$r = -0.33$	$r = 0.77$	$r = 0.70$	$r = -0.36$	$r = 0.87$
	$P = 0.26$	$P = 0.56$	$P < 0.001$	$P = 0.81$	$P = 0.76$	$P = 0.05$
Pixel heterogeneity (SD)	$r = 0.30$	$r = 0.32$	$r = -0.30$	$r = 0.70$	$r = -0.36$	$r = 0.78$
	$P = 0.79$	$P = 0.94$	$P = 0.30$	$P = 0.97$	$P = 0.53$	$P = 0.21$
Based on changes in luteal area						
	Period 1 (days 3–6)	**Period 2 (days 7–13)**	**Period 3 (days 14–15)**	**Period 1 (days 3–5)**	**Period 2 (days 6–10)**	**Period 3 (days 11–14)**
Luteal area (mm^2)	$r = 0.62$	$r = -0.35$	$r = 0.82$	$r = 0.82$	$r = 0.44$	$r = 0.46$
	$P < 0.05$	$P = 0.29$	$P < 0.05$	$P = 0.68$	$P = 0.25$	$P < 0.05$
Mean NPVs	$r = 0.40$	$r = 0.35$	$r = 0.75$	$r = 0.85$	$r = 0.40$	$r = 0.42$
	$P = 0.26$	$P = 0.23$	$P < 0.05$	$P = 0.20$	$P = 0.82$	$P < 0.07$
Pixel heterogeneity (SD)	$r = 0.30$	$r = 0.30$	$r = 0.41$	$r = 0.88$	$r = -0.40$	$r = -0.20$
	$P = 0.79$	$P = 0.61$	$P = 0.90$	$P < 0.08$	$P = 0.73$	$P = 0.57$

CL, corpus luteum; P_4, progesterone; NPVs, numerical pixel values

Circulating P_4 concentrations were increasing until day seven post-ovulation and maximum P_4 concentrations were seen on day 11 in both breeds under study (Figure 1a). However, maximum values for the TLA were recorded on days nine and 11 after ovulation in F and WWF ewes, respectively (Figure 1b). The functional luteolysis appeared to begin one day earlier, but it took one day longer in cycling WWF than F ewes. Moreover, the gradual decline in serum P_4 began earlier than rapid structural degradation of luteal glands in WWF ewes, but the demise of CL in cyclic F ewes started two days before the significant decline in serum P_4 concentrations. The reasons for this apparent lack of synchrony between the shifts in morphological (luteal tissue area) and functional characteristics of the CL in both breeds of sheep remain to be elucidated.

Luteal tissue area and serum P_4 concentration were correlated in both genotypes only during CL growth and luteolysis. This would suggest that during the middle portion of the ewe's estrus cycle, factors other than luteal tissue content govern the amount of luteal P_4 in the systemic circulation. In WWF ewes, the primary decline in systemic P_4 concentrations was not caused by regression of luteal cells, but it could have occurred as a result of decreased blood flow to

the CL that affected steroidogenic capacity of luteal glands, the concept previously suggested by Niswender *et al.*[1] Physical, or structural, regression of the CL is a complex process of cellular death and tissue remodeling,[13,28] and it likely requires more time to complete than the onset of the decrease in blood flow, which may result in an earlier onset of functional luteolysis. However, the different mechanism appears to be in effect in prolific F ewes, where a more gradual decline in luteal tissue content preceded a rapid cessation in serum P_4 concentrations. The role of luteal vasculature in controlling luteal steroidogenesis and peripheral P_4 concentrations in prolific and non-prolific breeds of sheep remains poorly defined.

Our present results revealed the existence of a correlation between mean NPVs and serum P_4 concentrations during functional luteolysis in both breeds of sheep. The existence of a similar relationship during the structural CL regression was only evident in WWF ewes (ANCOVA). Hence, the suggestion that pixel intensity determined in ultrasonographic images could be an accurate indicator of CL endocrine function during their entire lifespan[4,19] was not supported in the present study. Our results are in agreement with the observations in cattle that the mean pixel values were not useful indicators of functional changes in the CL.[21] It has been suggested that NPVs correlate directly with the numbers of large and small luteal cells in bovine CL,[21] but in a more recent study, there has been no correlation between luteal echogenicity and cell density in cows.[29]

In the present study, there was no correlation between CL pixel heterogeneity and serum concentrations of P_4 (Tables 1 and 2). Previous studies investigating the associations between CL pixel heterogeneity and their functional status in ruminants have provided inconsistent results.[4,19,22] In cattle[21] and goats,[22] the greatest values for pixel heterogeneity were observed at the beginning (metestrus) and the end (proestrus) of the luteal phase, whereas the least pixel heterogeneity values were recorded during diestrus. In both species, there was a negative correlation between CL heterogeneity and plasma P_4 concentrations. It was suggested that the decrease in pixel heterogeneity from metestrus to middiestrus in cattle and goats resulted from the cellular differentiation process.[21] During metestrus, the volume and number of luteal cells are small, systemic P_4 concentrations are low, and the ultrasonographic images of CL are heterogeneous. As the estrus cycle progresses and the luteal cells increase in volume and number (diestrus), the luteal glands producing large amounts of P_4 become more homogenous. However, Liu *et al.*[29] did not observe any correlations between ultrasonographic attributes, cell density and proliferating index in bovine CL *ex situ*. After the onset of luteolysis, the luteal cells undergo cell death and their remnants are removed from the ovaries. During this stage (proestrus), ultrasonographic images of the bovine and caprine CL have the greatest values of pixel heterogeneity, and circulating P_4 concentrations are declining. In cycling sheep, however, pixel heterogeneity of the luteal glands was a poor indicator of CL secretory ability.[4] Apart from a transient increase in pixel heterogeneity observed in WWF ewes on day 4 and a rapid decline in pixel SD values on day 14 in F sheep, there were no apparent changes in CL pixel heterogeneity from day 3 until the end of luteolysis (Figure 2b).

Previous studies have shown an increase in serum concentrations of P_4 in response to feed restriction;[30] however, by feeding congruent diets to each breed in this study, the confounding variable was controlled for. It can also be hypothesized that a greater P_4 synthesizing ability of CL in WWF versus F ewes is mainly caused by the fact that the small multiple CL of F ewes contain fewer and/or smaller luteal cells. O'Shea *et al.*[31] demonstrated that small luteal cells in sheep were two to three times as numerous as large luteal cells; however, no studies have been performed to compare the ratio of small to large luteal cells in prolific and non-prolific strains of sheep.

The metabolic clearance rate of P_4 affects plasma P_4 concentrations, and therefore the inherent differences in P_4 intermediate metabolism may have also been responsible for skewing the results of the present study. Progesterone catabolism occurs largely in the liver, and is accomplished by cytochrome P450 enzymes, specifically the cytochrome P450 3A and 2C subfamilies.[32–35] It has also been found that the level of expression of these enzymes differs significantly between different breeds of cattle.[34,35] It is, therefore, possible that a higher level of expression of cytochrome P450 enzymes in F compared with WWF ewes resulted in a higher metabolic clearance rate of P_4.

A countercurrent exchange mechanism has been implemented to explain the theory of hormone localization in the utero-ovarian vasculature.[36,37] The model proposes a localized distribution of specific serum components that are exchanged between adjacent blood vessels and lymphatics. This allows for the presence of more concentrated biological components in the gonadal region, as compared with the systemic circulation. The reproductive system countercurrent transfer is a highly dynamic process; it can even be affected temporarily by the locally secreted ovarian products.[37] A blood sample from the jugular vein is representative of systemic P_4 concentrations, which could be altered by the efficacy of the countercurrent hormonal exchange. Therefore, it would be noteworthy to investigate both systemic and localized concentrations of P_4 in relation to changes in CL morphology and echotexture in ewes.

In summary, the results of the present study show that size and echotextural characteristics of the ovine CL are not consistently correlated with serum P_4 concentration through all of the three stages of the luteal lifespan. These characteristics do not seem to be good predictors of serum P_4 concentrations and, therefore, luteal functionality during diestrus in ewes. Systemic P_4 concentrations only correlated with luteal area during CL growth and regression, and with pixel intensity (NPVs) during luteolysis, in both prolific and non-prolific ewes, but the changes in size and echotexture of the luteal glands at mid-cycle were not indicative of serum P_4 concentrations in ewes. Further research into possible confounding factors such as breed-dependent differences in CL cellular composition, intermediate P_4 metabolism (i.e. hepatic enzyme expression), and subovarian countercurrent transfer of steroid hormones may prove useful; if these variables are

accounted for, there is a possibility that the reasons for a lack of correlations between CL physical/echotextural characteristics and circulating P_4 concentrations during diestrus in ewes will be elucidated.

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