Ultrasound image attributes of the bovine corpus luteum: structural and functional correlates

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Nulliparous heifers (n = 58) were studied to determine whether computer-assisted quantitative echotexture analysis of ultrasound images reflects the functional and histomorphological characteristics of the corpus luteum. The ovaries of heifers were examined daily by transrectal ultrasonography from day -2 (day 0 = ovulation) until the day of ovariectomy during metoestrus (day 3; n = 8), early dioestrus (day 6; n = 9), mid-dioestrus (mean, day 10; n = 7), or pro-oestrus (mean, day 18; n = 8; Expt 1). High resolution ultrasound images of corpora lutea were obtained in vitro, and were digitized and analysed using custom-developed computer algorithms optimized for ultrasonography. Cryostat sections of corpora lutea were examined for lipid distribution, and corpora lutea were homogenized to determine the content of progesterone, total protein, cholesterol and triglyceride. In Expt 2, heifers (n = 26) were ovariectomized as in Expt 1, and ovaries were prepared for histomorphometric evaluation. Pixel values (brightness of picture elements) of ultrasound images of corpora lutea were characterized as high during metoestrus, low during early and mid-dioestrus, and increasing again during pro-oestrus (P < 0.05). Changes (P < 0.001) in volume density of luteal cells were characterized as increasing from metoestrus (40.7 ± 0.4%) to mid-dioestrus (55.8 ± 2.8%) and decreasing again at pro-oestrus (41.5 ± 0.9%). The proportion of blood vascular components decreased (P < 0.001) progressively from 31.0 ± 1.0% in metoestrus to 15.6 ± 1.1% in pro-oestrus. Pixel values of ultrasound images of corpora lutea were correlated with luteal (r = -0.72, P < 0.05) and plasma (r = -0.71, P < 0.03) progesterone concentration, and to the volume densities of luteal cells (r = -0.75, P < 0.02) and connective tissue (r = 0.69, P < 0.03). Estimates of triglyceride, protein and cholesterol content of corpora lutea were not correlated with pixel values of ultrasound images. Protein and cholesterol content did not change while triglyceride concentration increased during pro-oestrus (P < 0.05). Results support the hypothesis that ultrasound images reflect luteal and plasma progesterone content, and histomorphological characteristics of the corpus luteum.

Introduction

The ovarian follicle wall, consisting of granulosa cells and theca cells, vascularizes and luteinizes after ovulation to form a corpus luteum. This temporary steroid-producing gland undergoes marked structural and functional changes in a short time-span during its development, functional life and regression, and has been the subject of extensive morphological studies (reviewed by O’Shea, 1987). Recent investigations have focused on cell ultrastructure, cell types and origins, and luteal changes taking place during regression (Peukert-Adam et al., 1987; Fields et al., 1989; O’Shea et al., 1990; Shah et al., 1991; Fields et al., 1992; Garcia-Iglesias et al., 1992; Yamada et al., 1994). There is a high correlation between plasma progesterone concentration and corpus luteum mass, volume (Marcel et al., 1992) and histomorphology (Gasse et al., 1984). The advent of ultrasonography has made it possible to monitor luteal gland development sequentially (Pierson and Ginther, 1987; Kastelic and Ginther, 1989; Kastelic et al., 1990a,b). A high correlation has been found in the ultrasonographic detection of bovine corpora lutea (Lean et al., 1992), and between luteal tissue area measured ultrasonographically and plasma progesterone concentration (Bergfelt et al., 1989; Kastelic et al., 1990a). It has been suggested that changes in luteal echotexture, as determined by measurement of pixel values (picture element brightness) composing ultrasound images of equine corpora lutea during early stages of the oestrous cycle (Townsend and Ginther, 1989), reflect structural, functional or haemodynamic alterations in the tissue. In another study (Carnes and Dunn, 1988), measurement of the ultrasonic absorption coefficients of excised ovaries of a number of

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species including cattle indicated that differences in physiological stage were associated with significant variations in acoustic impedance due to changes in macromolecular content and structure.

Grey-scale densitometry has been used for quantitative analyses in a number of different fields such as gel electrophoresis, microspectrophotometry and immunohistochemistry (Sternberger and Sternberger, 1986; Fritz et al., 1989; Remucci et al., 1991; Fritz et al., 1992; Ferrandi et al., 1993). However, to develop image analysis as a diagnostic and prognostic tool for evaluating ultrasound images, an objective measurement procedure for grey-scale evaluation is needed.

The present study was conducted to determine whether quantitative changes in the echotextural components of bovine corpora lutea at different stages of development are reflective of structural and functional characteristics of the gland. Specific hypotheses under test were that pixel values of ultrasound images of corpora lutea are correlated with histomorphological components of the corpus luteum, luteal and plasma progesterone content, and luteal gland lipid content and distribution.

Materials and Methods

Two experiments were conducted in succession during May to August using 58 cross-bred nulliparous beef heifers (primarily Hereford) that were 16–18 months of age. Experiments involved ultrasonographic monitoring of follicular and luteal changes, ovariectomy at prescribed times, and correlation of ultrasound image attributes with histological and biochemical characteristics. As part of a related study on follicular characteristics, the days of ovariectomy were chosen on the basis of follicular wave status. The days chosen for ovariectomies satisfied the objectives of the present study by allowing the acquisition of corpora lutea during metoestrus (day 3; day 0 = day of ovulation), early diestrus (day 6), mid-diestrus (day 8 to day 13), and pro-oestrus (day 17 to day 21).

Experiment 1

Experiment 1 was designed to obtain direct correlation between ultrasound image attributes and luteal and plasma biochemical measurements. Thirty-two heifers were ovariectomized during metoestrus (n = 8 on day 3), early diestrus (n = 9 on day 6), mid-diestrus (n = 1 on day 8, 2 on day 9, 1 on day 10, 2 on day 11 and 1 on day 12), or pro-oestrus (n = 1 on day 17, 3 on day 18, 3 on day 19, 1 on day 20).

Heifers were ovariectomized by colpotomy (Hudson, 1986) under caudal epidural anaesthesia using 2% (w/v) lidocaine HCl with 0.001% (w/v) adrenaline. Clinbuterol was administered intravenously at a dose rate of 6 µg (10 kg)−1 body mass (Ventipulmin, Boehringer Ingelheim Ltd, Ontario) to induce relaxation of broad ligament (Hassett and Sloss, 1984). The ovaries were placed immediately in ice-cold phosphate buffered saline (PBS; 0.1 mol phosphate buffer 1−1, 0.9% (w/v) sodium chloride, pH 7.2–7.4) and transported to the laboratory within 45 min of ovariectomy. The ovaries were placed in a degassed PBS bath and images were obtained by using a broad-band (5–9 Mhz), convex-array, ultrasound transducer (ATI, Mark 9 HDI ultrasound machine; Advanced Technology Laboratories, Bothell, WA). Serial ultrasound images of both ovaries were digitally acquired at 0.5 mm increments directly from the ultrasound machine to a computer at a resolution of 640 × 480 pixels and 256 shades of grey (0 = absolute black; 255 = absolute white). Images from all corpora lutea were digitized at standardized settings. After image capture, corpora lutea were dissected from the ovaries, weighed, bisected, and frozen at −20°C until further processing.

In other tissue models, pixel values of ultrasound images were influenced by total lipid content and histomorphological fat deposit distribution (Layer et al., 1990). Steroid hormone precursors (cholesterol, triglycerides and total lipid) were biochemically measured and localized in corpora lutea to allow correlation with changes in echotexture. One half of each corpus luteum was sectioned at a thickness of 5 µm using a cryostat microtome at −15°C. Sections were stained with Sudan black B and Oil-red-O (Luna, 1968). Sudan black B was used to stain all lipids including phospholipids, and Oil-red-O was used to demonstrate neutral fats (Pearse, 1968). Slides from all animals were stained in a single staining dish with identical time treatments (that is, as a single batch). Images from the stained cryostat sections were digitized from the video signal using a video camera (Tamron Fotovix TF-56 WV, Tamron Co., Ltd, Ohmiya) fitted with a uniform transillumination light box to minimize the variations in pixel values due to uneven intensity of light. After image digitization, the Oil-red-O stained slide set was counterstained with haematoxylin, and the Sudan black B stained set was counterstained with Nuclear red. Counterstaining was done to study the lipid distribution in the luteal cells, and for the purposes of photography.

From the remaining half of each corpus luteum, a known mass of luteal tissue proper (approximately 200 mg) was homogenized in PBS using a mechanical homogenizer (Polytron homogenizer, Brinkman Instruments, Inc., Westbury, NY) at medium speed for 20 s, then ground manually in a glass homogenizer. The homogenization process was carried out on ice. Homogenates were filtered through three layers of cheesecloth previously soaked in PBS. Finally, the homogenates were diluted with PBS to 10 mg wet mass of corpus luteum ml−1 for estimation of cholesterol, total protein and triglycerides, and to 0.625 mg wet mass of corpus luteum: ml−1 for progesterone measurement. Daily plasma samples were obtained from the jugular vein from day −2 until 3 days after ovariectomy from all the heifers; all samples were analysed for plasma progesterone in the pro-oestrus group but only the samples obtained on the day of ovariectomy were analysed in other groups.

Quantitative echotexture analysis of ultrasound images. Analysis of ultrasound images was performed using a series of custom-developed computer algorithms optimized for ultrasonography (SYNERGYNE T°, Saskatoon, Saskatchewan) on a Sun Sparc Station 10 (Sun Microsystems, Mt View, CA) computer (Pierson and Adams, 1995). From each corpus luteum, three images were selected from a sequence of images obtained during the inactive stage of the corpus luteum was measured. Quantitative echotexture analysis was performed based upon measurement of pixel values (that is, grey-scale value of individual pixel elements ranging from 0 to 255). Each image was divided into four quadrants (Fig. 1) and pixel value from each
region was measured by a computer-generated spot meter encompassing approximately 20% of each quadrant (Pierson and Adams, 1995). The pixel value was the mean of gray-scale values of all pixels falling under the measuring spot. Maximum and minimum pixel values were also measured from the grey-scale bar of each image to permit normalization of measurements among images. The mean pixel value for each corpus luteum was obtained as an average of 12 measurements (three images per corpus luteum and four regions per image). Pixel value measurements were performed by a single person who was not aware of the experimental design and to whom individual image identities were not disclosed.

**Densitometric analysis of cryostat sections.** Images from cryostat sections of corpora lutea, stained with Oil-red-O and Sudan black B, were analysed for pixel values in a fashion similar to the ultrasound images, using the same computer algorithms. The analysis was conducted at approximately the same magnification as for ultrasound images (× 3–4). Grey-scale values for background (without tissue), reactive area (luteal tissue proper), and nonreactive area (connective tissue capsule of corpus luteum) were recorded, and an absorption index was calculated for each corpus luteum (Fritz et al., 1992). Briefly, the transmittance values for reactive and nonreactive areas were calculated from grey-scale values as a percentage of background pixel value. These values were transformed to absorbance (log of transmittance value) and the absorption index was calculated by obtaining the ratio of absorbance of reactive versus nonreactive areas. This method of reporting the results provides considerable advantage over other methods because the absorption index value is independent of section thickness (Heitz, 1982).

**Biochemical estimations.** For all assays, serial dilutions of samples were parallel to their respective standard curves. Progesterone in plasma and corpus luteum homogenates was measured by radioimmunoassay (DPC coat-a-count, Diagnostic Products Corporation, Los Angeles, CA) using the non-extraction procedure validated with progesterone standards prepared in plasma from ovarioctomized heifers (for plasma progesterone measurements) and in PBS (for corpus luteum homogenates); the minimum detection limit of the assay was 0.03 ng ml⁻¹. All plasma samples were analysed in a single assay with an intra-assay coefficient of variation of 6.5%. Similarly, all corpus luteum homogenates were run in single assay with an intra-assay coefficient of variation of 6.1%. In the corpus luteum homogenates, cholesterol content was measured in a single assay (intra-assay coefficient of variation = 3.8%) by the method described by Allain et al. (1974) using an Abbott Spectrum kit (Diagnostic Division, Abbott Laboratories, Abbott Park, IL) and total protein by the method of Watanabe et al. (1986) with a total protein microassay kit (Diagnostic Chemical Ltd, Charlottetown, PEI, Canada) as a single assay with an intra-assay coefficient of variation of 4.9%. Triglycerides were measured on a Vet Test 8008 machine (Industrial Innovation Management Co., New Jersey) using Kodak Ektachem D1 slide dry chemistry kits (Eastman Kodak Co., Rochester, NY) as a single batch with an intra-assay coefficient of variation of 2.9%. For all biochemical estimations, if the detected amount was below the sensitivity of the procedure, then the lowest sensitivity value was substituted for the value.

**Experiment 2**

Experiment 2 was designed to study the histomorphology of perfusion-fixed corpora lutea at similar stages of development as in Expt. 1. The necessity of immediate perfusion fixation (Dharmarajan et al., 1983; Meyer, 1991) of each ovary precluded the use of the same set of corpora lutea for high-resolution ultrasonographic imaging and luteal biochemical estimations. Hence, histomorphological results of Expt 2 were, by design, intended to be correlated with ultrasonographic imaging and luteal biochemical estimates of Expt 1. Experiment 2 was conducted on 26 heifers examined and ovarioctomized as in Expt 1 during metoestrus (n = 7), early dioestrus (n = 6), mid-dioestrus (n = 1 on day 9, 2 on day 10, 2 on day 11, 1 on day 13), or pro-oestrus (n = 2 on day 17, 3 on day 18, 1 on day 19, 1 on day 21).

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**Fig. 1.** Image analysis technique for a bovine corpus luteum with a central cavity (a) or without a cavity (b). The corpus luteum is demarcated from the stroma by arrowheads. The image of each corpus luteum was divided into four equal quadrants. Pixel value was measured by placing a measuring spot (circles) covering approximately 20% of each quadrant. Pixel value was the mean of grey-scale values of all pixels falling under the measuring spot. The scale on the ultrasound images is in cm.
Immediately upon removal, the ovarian artery or its branches were cannulated, flushed with 15–20 ml PBS to remove blood, and perfused with 30 ml of Karnovsky’s fixative (Karnovsky, 1965) containing 4% (w/v) paraformaldehyde and 0.1% (w/v) glutaraldehyde in 0.1 mol phosphate buffer 1⁻¹ (pH 7.4) at a flow rate of 2 ml min⁻¹. Ovaries were immersed in the same fixative for a total of 3 h, after which a 3–4 mm slice was taken from the corpus luteum at its maximum diameter and placed in aqueous Bouin’s fixative (Luna, 1968) for another 3 h. Double fixation permitted the use of the same tissues for light microscopy, immunohistochemistry and electron microscopy for this and further studies. Tissues for the present study were processed for paraffin wax sectioning as described by Luna (1968); sections 5 µm thick, were placed on slides coated with poly-L-lysine and stained with haematoxylin and eosin, Masson’s trichrome, and Periodic acid–Schiff reaction (Sheehan and Hrapchak, 1973).

**Fig. 2.** Mean ± SEM of various endpoints from Expt 1 at metostrus (n = 8), early dioestrous (n = 9), mid-dioestrous (n = 7) and pro-oestrous (n = 8) in cows. Biochemical estimations of progesterone (b), cholesterol and triglyceride (c) of corpus luteum homogenates are based on per mg wet mass of corpora lutea. Values within parentheses indicate the probability value for a group effect. Within each endpoint, values with different letters were significantly different (P < 0.05).

Morphometric analysis of paraffin sections of corpora lutea. Measurements were made on paraffin sections stained with haematoxylin and eosin by projecting the image of each section on a point-counting grid at a final magnification of × 250 with the camera lucida (Weibel, 1979). Luteal cells were identified as polyhedral cells with round nuclei (Priedkaufs, 1993). No attempt was made to differentiate between small and large luteal cells. The point-counting grid consisted of 100 equidistant points with an interpoint distance of 10 mm. Relative volume densities (proportion of volume occupied) of luteal cells, blood vascular components (including capillaries and all larger vessels), and connective tissue were determined by calculating the percentage of points falling on each structure of interest in relation to the total number of points falling on all structures. At least four areas of each corpus luteum were counted, using systematic random sampling (Gundersen and Jensen, 1987).

**Statistical analyses**

Data for all endpoints were analysed for a day effect during days 8–13 (mid-dioestrous) and days 17–21 (pro-oestrous); however, as no differences were detected, the data were pooled in the respective groups. Individual endpoints were analysed for a group effect using one-way analysis of variance. Percentage data were transformed (arcsin) before statistical analyses. If the main effect was significant, individual comparisons were made using Duncan’s multiple range test. Data were categorized by presence or absence of a central cavity of corpus luteum and compared using Student’s t test; however, as the two

**Fig. 3.** Ultrasound morphology (a, c, e, g) and histology (b, d, f, h) of bovine corpora lutea during metostrus (a, b), early dioestrous (c, d), mid-dioestrous (e, f) and pro-oestrous (g, h). Corpora lutea are delineated from surrounding stromal tissue by arrowheads. Arrows indicate luteal cells. Higher angiogenesis during metoestrous and hyalinization of blood vessels (double arrow) during pro-oestrous (regression) was evident. Scale on the ultrasound images (a, c, e, f) is in cm. Paraffin wax sections of corpora lutea were 5 µm thick and stained with haematoxylin and eosin. Scale bar on each micrograph represents 10 µm.
categories did not differ from each other, this categorization was disregarded. Pixel values of the ultrasound images of corpora lutea before and after normalization were also compared using Student's t test. Pearson correlation coefficients between pixel values of ultrasound images and other endpoints from Expts 1 and 2 were calculated on the mean values for a particular day of the cycle (that is, day 3, 6, 9, 10, 11, 12, 17, 18, 19 and 20). Results are presented as means ± SEM unless otherwise indicated.

Results

Experiment 1

Development and regression of the corpus luteum. Luteal gland diameter (measured ultrasonographically) increased from metoestrus to mid-dioestrus and decreased with regression during pro-oestrus (Fig. 2a). Similarly luteal gland mass increased (P < 0.001) from 1.7 ± 0.2 g in metoestrus to 3.9 ± 0.3 g in early dioestrus and 5.1 ± 0.5 g in mid-dioestrus with a subsequent decrease to 3.1 ± 0.5 g in pro-oestrus. Luteal diameter was highly correlated with luteal mass (r = 0.74, P < 0.05).

In 42 of 58 corpora lutea studied (72.4%), a cavity of at least 3 mm diameter was observed by ultrasonography as a central anechoic area. In all but one instance, the cavity present on the day of ovariectomy was filled with a clear serous transudate. In one corpus luteum (metoestrus) the cavity contained coagulated blood. Pixel value measurements of the ultrasound images of luteal tissue, tissue progesterone content, and biochemical and morphometric endpoints were not different among the corpora lutea with or without a central cavity (P > 0.15). Therefore, data were combined and analysed without categorization of a central cavity.

Quantitative echotexture analysis of ultrasound images. Data were analysed both before and after the normalization procedure and results were not different (Fig. 2a); however, the values after normalization were used for statistical interpretation and reporting since they accounted for uncontrolled image drift and better represented relative differences among images. Pixel values were not significantly different among days 8–12 for the mid-dioestrus group, and among days 17–20 for the pro-oestrus group. There was a group effect (P < 0.05) for the pixel value data; values were high during metoestrus, low during early and mid-dioestrus, and started to increase in the regressing corpus luteum of pro-oestrus. Darker and more homogeneous echotexture of ultrasound images during dioestrus (Fig. 3c, e) than metoestrus or pro-oestrus (Fig. 3a, g) was associated with higher volume density and larger luteal cells during dioestrus (Fig. 3d, f) and more stromal components during metoestrus and pro-oestrus.

Progesterone content of plasma and corpus luteum homogenates. Plasma progesterone concentration (Fig. 2b) measured on the day of ovariectomy increased (P < 0.001) progressively from metoestrus to mid-dioestrus and decreased (P < 0.001) to a nadir at pro-oestrus. Similarly, the daily plasma progesterone profile measured in the pro-oestrus group was characterized by undetectable concentrations until day 1, rising to a maximum on day 13, and a decrease again to undetectable concentrations on Day 18 (P < 0.001); values remained below the minimum detectable limits of the assay for the 3 days following ovariectomy. Progesterone concentrations of corpus luteum homogenates (Fig. 2b) were higher during mid-dioestrus than metoestrus and pro-oestrus (P < 0.001). Plasma and luteal progesterone concentrations were highly positively correlated (r = 0.72, P < 0.02).

Protein, cholesterol and triglyceride content of corpus luteum homogenates. The protein and cholesterol contents of corpus luteum homogenates were not different among groups (overall mean ± SEM, 47.2 ± 1.4 and 2.6 ± 0.4 µg mg⁻¹ of wet mass of the corpus luteum, respectively). Triglyceride content of corpus luteum homogenates was higher (P < 0.05) in pro-oestrus than in any other group (Fig. 2c). Results of analyses of progesterone, cholesterol and triglyceride concentration of corpus luteum homogenates, calculated per mg of protein, were similar to results of analyses based on per mg wet mass of the corpus luteum.

Densitometric analysis of cryostat sections. The absorption index of Sudan black B was higher than that of Oil-red-O (Fig. 2d); however, the pattern of change for both types of stain was similar. Their absorption indices increased (P < 0.05) with luteal maturity (metoestrus to mid-dioestrus) and again with luteal regression (dioestrus to pro-oestrus). Histologically, a minimum amount of Sudan black B and Oil-red-O reactive components was detected in metoestrus (Fig. 4a), an intermediate amount during early dioestrus and mid-dioestrus (Fig. 4b, c), and the highest amount was detected in pro-oestrus (Fig. 4d). Lipid droplets were fine and uniformly distributed in luteal cells during mid-dioestrus (Fig. 4e) compared with coarser droplets during pro-oestrus (Fig. 4f).

Experiment 2

Histomorphometric analysis of corpora lutea. The volume density of luteal cells (Fig. 5) increased (P < 0.001) from metoestrus to mid-dioestrus (40.7 ± 0.4% to 55.8 ± 2.8%) with a decrease during pro-oestrus (41.5 ± 0.9%). The proportional volume occupied by blood vascular components progressively decreased (P < 0.001) from metoestrus (31.0 ± 1.0) to pro-oestrus (15.6 ± 1.1%). Connective tissue volume density varied (P < 0.001) with highest values in pro-oestrus during luteal regression (42.9 ± 1.3%) and lowest during mid-dioestrus (24.9 ± 2.8%). Stromal components (blood vascular components and connective tissue combined) decreased (P < 0.001) from metoestrus and early dioestrus to mid-dioestrus, and increased again during pro-oestrus. Histologically, corpora lutea at metoestrus exhibited high vascularity with smaller individual luteal cells (Fig. 3b) and these cells underwent hypertrophy during dioestrus (Fig. 3d, f) resulting in higher volume density. During luteal regression in pro-oestrus, connective tissue components predominated (Fig. 3h) with hyalinization of larger blood vessels and smaller individual luteal cells.
Fig. 4. Cryostat sections of bovine corpora lutea at metoestrus (a), early dioestrus (b), mid-dioestrus (c, e) and pro-oestrus (d, f) showed a sequential increase in lipid (arrowheads) from metoestrus to pro-oestrus. (a–d) were stained with Oil-red-O and counterstained with haematoxylin; (e–f) were stained with Sudan black and counterstained with Nuclear red. Scale bars represent 10 µm. Note the decrease in luteal cell size during pro-oestrus (f versus e).

**Correlation between ultrasound images and other characteristics**

A strong correlation was observed between mean pixel values of ultrasound images and volume densities of luteal cells ($r = -0.75, P < 0.02$), connective tissue ($r = 0.69, P < 0.03$) and stromal components ($r = 0.75, P < 0.02$). Pixel values were not correlated with volume density of blood vascular components; however, there was a high correlation with plasma ($r = -0.71, P < 0.03$) and luteal tissue ($r = -0.72, P < 0.05$) progesterone concentration. No correlation was observed with cholesterol or triglyceride content, or the absorption index of Sudan black B or Oil-red-O.

**Discussion**

The results of the present study support the hypothesis that pixel values of ultrasound images are correlated with histomorphological characteristics, and luteal and plasma progesterone concentration of the corpus luteum of cattle; however, pixel values were not correlated with luteal gland lipid content and distribution. Higher pixel values, obtained by computer-assisted quantitative echotexture analysis during luteal gland formation (metoestrus) and regression (pro-oestrus group) were similar to the pattern observed in horse and pony mares by subjective scoring of echotexture (Pierson and Ginther, 1985).
and pixel analysis (Townson and Ginther, 1989) of corpora lutea recorded in vivo by transrectal ultrasonography.

A central cavity was commonly observed in corpora lutea in the present study (72.4%), similar to observations made by others (37%, Kito et al., 1986; 47–53%, Kahn, 1989; 79%, Kastelic et al., 1990b). The cavity of the corpus luteum was filled with a serous transudate, and remained anechoic during its entire existence as opposed to the organized clot and appearance of a network of echogenic lines within the central cavity of the equine corpus luteum (Ginther, 1992). No differences in pixel values or tissue progesterone production were detected between corpora lutea with and without a central cavity, which lends credence to earlier observations (Kito et al., 1986; Kahn, 1989; Kastelic et al., 1990a).

The pattern of development and regression of the corpus luteum observed during the present study is consistent with reported observations (Kito et al., 1986; Pierson and Ginther, 1987; Kastelic et al., 1990b; Juengel et al., 1993). During metoestrus (period of luteal growth), the percentage volume occupied by blood vascular components was highest. The luteal cells underwent hypertrophy at mid-dioestrus, during peak progesterone production, in association with their highest volume density. Increase in bovine luteal cell size during the formation phase and decrease during the regression phase have been shown to parallel progesterone secretion (Peukert-Adam et al., 1987). During pro-oestrus, the regressing corpus luteum decreased in mass, in volume densities of luteal cells and blood vascular components, and in progesterone production. There was a concurrent increase in connective tissue and hyalinization of blood vessels. Individual luteal cells underwent degenerative changes reflected by decreased cell size and accumulation of coarse lipid droplets in the cytoplasm.

High pixel values observed during the formation phase of the corpus luteum were attributed to a smaller volume occupied by the luteal cells and greater volume occupied by newly forming angiogenic tissue (blood vessels and connective tissue combined). High pixel values during the regression were also attributed to a smaller volume density of luteal cells and greater volume occupied by connective tissue. Minimum pixel values observed during mid-dioestrus were attributed to luteal cell hypertrophy and greater volume occupied by these cells. Lower plasma and luteal tissue progesterone values at the beginning and at the end of dioestrus coincided with higher pixel values of the gland, as observed in mares (Pierson and Ginther, 1985). Changes in the blood vascular components per se did not appear to be reflected in the ultrasound images; however, combined with connective tissue components (as stroma) the correlation was significant. It appears that the structural components of blood vessels have echogenic characteristics similar to those of other connective tissue. The ultrasound images in the present study were recorded in vitro to minimize confounding due to intervening tissues, changes in position of the ovary in relation to the transducer and to allow direct digitization of the images from high-resolution ultrasound equipment without video recording. However, images acquired in vitro may differ from images acquired in vivo in terms of blood flow through vessels of the luteal tissue.

Computer-assisted quantitative echotexture analysis offers the potential of a highly sensitive and quantitative method of assessing the attributes of ultrasound images. In horses (Pierson and Ginther, 1985) and ponies (Townson and Ginther, 1989), changes in luteal echotexture were pronounced enough to be recognized by an eight-zone grey-scale scoring system, but scoring was subjective and contingent upon operator consistency and expertise. Furthermore, differences among species and tissues other than the corpus luteum may not be recognized by a subjective scoring system and may not be apparent on an individual-observation basis for diagnostic purposes. Analysis
of pixel values ranging from 0 to 255 shades of grey enables a quantitative approach that minimizes inconsistencies among operators and has the potential to be included in the hardware and software of ultrasound equipment.

In summary, pixel values, obtained by quantitative echotexture analysis, of ultrasound images decreased from metoestrus to mid-dioestrus, and increased during pro-oestrus. Pixel values of ultrasound images were highly correlated to plasma and luteal tissue progesterone concentrations and volume densities of luteal cells and stroma, but not to cellular lipid content or distribution. It was concluded that quantitative changes in the pixel values of ultrasound images occur concurrent with changes in structural and hormonal characteristics of the bovine corpora lutea and that computer-assisted echotexture analysis may be developed into a powerful diagnostic and prognostic tool to assess the physiological or pathological status of the bovine corpus luteum. The diagnostic accuracy of computer-assisted analysis of a single ultrasound image, however, remains to be tested. Further refinements or evaluation of other characteristics may be required to improve the structure–function correlations, particularly with respect to distinguishing between the growing and the regressing phases of luteal gland development.

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