

Caracterización ultrasonográfica B-mode and Doppler of the corpus luteum in llamas

Caracterización ultrasonográfica modo-B y Doppler del cuerpo lúteo en llamas

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Abstract

B-mode and Doppler ultrasonography in ruminants, as a technique has allowed establishing new concepts on the reproductive physiology of females, through the study of follicular dynamics and morphometry of the corpus luteum, therefore, the objective was to characterize by means of B-mode and Doppler ultrasonography the corpus luteum in recipient llamas. Thirty-seven recipient llamas were used (27 for B-mode ultrasonography and 10 Doppler) that were synchronized by applying buserelin acetate 0.0096 mg, nine days later 0.048 mg of prostaglandin F2 α analog was applied two days later, the second dose of buserelin acetate was applied at the same dose to guarantee ovulation and subsequent formation of the corpus luteum, the evaluation in mode B of the morphometry consisted of observing the echotexture, area, diameter and volume of the corpus luteum with the use of a SonoStar SS-8[®] ultrasonograph at 6.5 MHz frequency and 6 cm depth equipped with a transrectal linear transducer; using the same technique with a Draminski 4Vet[®] Doppler equipment, the percentage of luteal area of vascularization (% AVL) was determined. The data were subjected to a descriptive analysis being 0.119 \pm 0.032 cm² of area, 12.7 \pm 1.7 mm of diameter and 0.84 \pm 0.32 cm³ of CL volume, to determine the relationship between metric measures the Pearson correlation was used observing a positive relationship between area/diameter of 0.7506, area/volume of 0.9289 and diameter/volume of 0.6602, observing a high positive correlation between area and volume, finally the % AVL characteristics was 34.97 for the CL of recipient llamas. In conclusion, the morphometric characteristics evaluated in mode B (area, diameter and volume) have a positive correlation and the % AVL could be applied as a tool in the efficient reproductive management for the selection of recipients in camelids, understanding that it is one of the first reports of these characteristics in llamas

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Resumen

La ultrasonografía modo B y Doppler en los rumiantes, como técnica ha permitido establecer nuevos conceptos sobre la fisiología reproductiva de las hembras, a través del estudio de la dinámica folicular y morfometría del cuerpo lúteo, por tanto, el objetivo fue caracterizar mediante ultrasonografía modo B y Doppler el cuerpo lúteo en llamas receptoras. Se utilizaron 37 llamas como receptoras (27 para ultrasonografía modo B y 10 Doppler) que fueron sincronizadas aplicando acetato de buserelina 0.0096 mg, nueve días posteriores se aplicó 0.048 mg de análogo de prostaglandina F2 α , a los dos días posteriores se aplicó la segunda dosis de acetato de buserelina en la misma dosis para garantizar la ovulación y posterior formación del cuerpo lúteo, la evaluación en modo B de la morfometría consistió en observar la ecotextura, área, diámetro y volumen del cuerpo lúteo con el uso de un equipo ultrasonógrafo SonoStar SS-8[®] a 6.5 MHz de frecuencia y 6 cm de profundidad equipado con un transductor lineal transrectal; con la misma técnica con un equipo Draminski 4Vet[®] Doppler se determinó el porcentaje de área luteal de vascularización (% AVL). Los datos fueron sometidos a un análisis descriptivo siendo de 0.119 \pm 0.032 cm² de área, 12.7 \pm 1.7 mm de diámetro y 0.84 \pm 0.32 cm³ de volumen de CL, para determinar la relación entre medidas



Palabras clave:

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ultrasonografía.

métricas se utilizó la correlación de Pearson observando una relación positiva entre área/diámetro de 0.7506, área/volumen de 0.9289 y diámetro/volumen de 0.6602, observando una correlación alta positiva entre área y volumen, finalmente las características de % AVL fue de 34.97 para los CL de llamas receptoras. En conclusión, las características morfométricas evaluadas en modo B (área, diámetro y volumen) tienen una correlación positiva y el % AVL podría aplicarse como herramienta en el manejo reproductivo eficiente para la selección de receptoras en camélidos, entendiendo que es uno de los primeros reportes de estas características en llamas

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Introduction

Efficient reproductive management and the use of reproductive biotechnologies such as artificial insemination (AI) and embryo transfer (ET) require an understanding of the follicular dynamics that govern ovarian activity¹. Several studies exist on follicular dynamics in South American camelids (SAC) characterizing a continuous and overlapping wave with induced ovulation²⁻⁴. The detection of corpus luteum (CL) by rectal palpation by experts has a positive predictive value of only 64 %, even confusing between follicles and CL, while ultrasonography (US) allows the identification of CL with an efficiency of 85 %^{5,6}. The wide availability of the US has facilitated the continuous advancement of knowledge of the physiological changes and characteristics of the CL during the estrous cycle in cattle⁷. The CL, through the production of progesterone (P4), plays a key role in the establishment and maintenance of pregnancy in all domestic animals⁸, including camelids, especially in ET programs for the selection of recipient females⁹⁻¹¹. US allows the evaluation of ovarian morphometric characteristics such as follicles, CL, among others. Studies with US evaluating the CL make it possible to determine the reproductive status in cattle, monitor its dynamics, formation, development, and regression of this structure¹². The ultrasound image of the LC is uniform, circumscribed and less echogenic than the ovarian stroma, the sound wave intensity. The return of the echogenicity and echotexture,

characteristics that are used as a potential measure for predicting LC function and steroidogenic capacity^{13,14} can be used for the evaluation of echogenicity and echotexture. Physiological and morphometric studies in reproduction evaluated with US Doppler of the LC in humans report a positive correlation between the LC blood perfusion index and progesterone concentrations in early pregnancies, so in cattle and camelids it could also predict early pregnancy, opening new knowledge even more if factors such as altitude are included on the physiology of domestic animals in these environmental conditions, assuming slight variations compared with studies carried out at other altitudes¹⁵⁻¹⁷, for this reason it is necessary to use US in the study of reproductive physiology in domestic animals that inhabit the Peruvian highlands, for this reason the objective of the present study was to characterize the corpus luteum in recipient llamas by means of B-mode ultrasonography and Doppler.

Materials and methods

Animals. A total of 37 recipient llamas were selected from an embryo transfer program for B-mode ultrasonographic evaluation and 10 of them for Doppler evaluation (due to the complexity and time-consuming nature of the Doppler study), the study was carried out at the Research and Production Center (RPC) "La Raya" of the National University of the Altiplano

Puno (UNAP), located at 4230 meters above sea level, where the llamas had at least one calving, more than 15 days post parturition (empty llamas), with a corporal condition of 3.44 on a scale of 1 to 5¹⁸, fed with natural pasture.

Wave synchronization and ovulation induction in recipients. For synchronization, GnRH (buserelin acetate: 0.0096 mg: Gestar[®] - Over - Argentina) was applied on day "0" and a prostaglandin F2 α analogue (PGF: 0.048 mg: Cloprostenol: Prostal[®] Over - Argentina), two days later (day 11) a second dose of GnRH analog (buserelin acetate: 0.0096 mg: Gestar[®] - Over - Argentina) was applied to ensure ovulation and formation of the CL, finally the ultrasonographic evaluation was performed on the 18th day of treatment¹⁹.

Ultrasonographic evaluation of CL morphometry. The evaluation of the morphometry in *mode B* consisted of taking characteristics such as: area, volume, and diameter with a SonoStar SS-8[®] (SonoStar Technologies, China) ultrasonographic equipment with 6.5 MHz frequency and 6 cm depth equipped with a linear transrectal transducer, the CL was located by means of the US equipment (SonoStar SS-8[®]) in mode 2B to capture two images through its *freeze* option and evaluate its characteristics as described below: i) *Volume of the LC.* Three diameters were taken over the CL by placing the calipers (electronic calipers of the US software) in the limit between the luteal tissue and the ovarian stroma to be valued and immediately the veterinary *software* of the US equipment (SonoStar SS-8[®]) allowed us to visualize the volume of the CL in cm³ (Figure 1). ii) *Area of the CL.* The option of *circular area* was chosen due to the characteristics of the CL, the calipers were placed in the limit between the luteal tissue and the ovarian stroma. This circular area was then opened, which allowed us to obtain the area of the CL in cm². iii) *CL*

diameter. calipers were placed between the luteal tissue and the visualized ovarian stroma, which allowed us to obtain the diameter of the CL in mm.

The evaluation of the *Doppler ultrasonographic morphometry* consisted of determining the area of luteal vascularization (AVL %) with a US Draminski 4Vet[®] (Draminski ul. Owocowa 17, Poland) with 6.5 MHz frequency and 6 cm depth equipped with a linear transrectal transducer, the CL was placed in B mode, then the Color Doppler mode was activated to determine the vascular activity of the CL, the images were stored and analyzed in the Image J[®] program, determining the AVL in percentage using the following formula²⁰:

$$\text{Area luteal vascularization (ALV: \%)} = \frac{\text{Area luteal vascularization}}{\text{Area of CL}} \times 100$$

Statistical analysis. The B-mode and Doppler morphometric characteristics, being a characterization study and one of the first reports, were determined by descriptive statistics (average and standard deviation) of volume, area, diameter and AVL (%), specifically the B-mode characteristics were subjected to Pear's correlation to determine their degree of association; all data were processed using the statistical program R version 3.5.1.²¹

Results

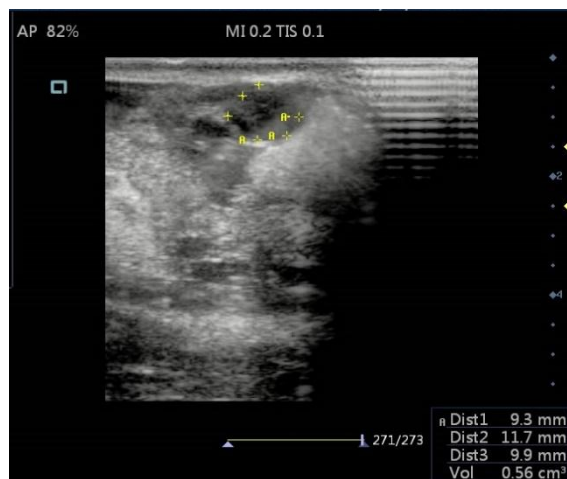
Table 1 Morphometric characteristics of CL (area, diameter and volume: average and standard deviation, respectively) of B-mode llamas

	N	CL B-Mode
Área (cm ²)	37	0.119 ± 0.032
Dimeter (mm)	37	12.7 ± 1.7
Volume (cm ³)	37	0.84 ± 0.32

The *echotexture of CL in llamas* is hypoechogenic (darker) compared to the ovarian stroma due to its

greater vascularization. The most important characteristic of the CL in llamas is that it is not embedded in the ovary but appears to be an appendage of the ovary. Table 1 shows the metric characteristics (area, diameter and volume) of the CLs evaluated by US mode B in llamas.

Figure 1 Ultrasonographic evaluation of the morphometric characteristics and echotexture of the CL of recipient llamas in B-mode

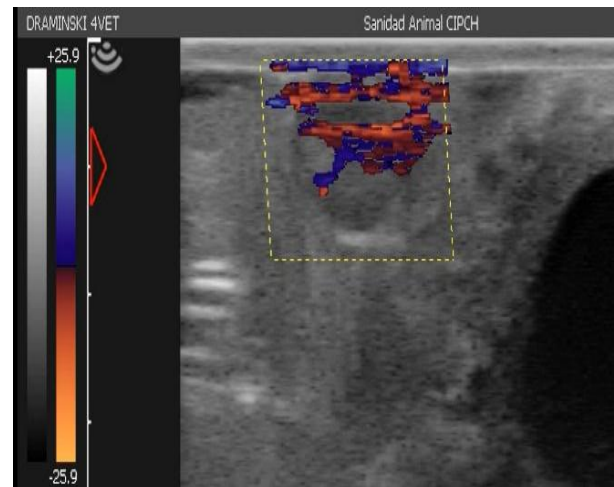


In all the characteristics evaluated, a positive correlation was observed between area/diameter of 0.7506, area/volume of 0.9289, and diameter of 0.9289. The significant association between area and volume of the CL would allow taking only one of these characteristics in subsequent ultrasonographic evaluations of CL in llamas, the coefficients of variation of the evaluated characteristics were for area of 26.9 %, for diameter 13.4 % and volume of 38.1 %, observing less variability in the determination of the diameter of CL.9 %, for diameter 13.4 % and volume of 38.1 % observing a lower variability in the determination of the diameter of CL, the morphometric characteristics by US are as shown in figure 1.

Figure 2 shows the AVL determined as a percentage of the proportion of blood flow that irrigates the CL

at the time of the evaluation (7 days after the application of GnRH), with an average of 34.97% of the total area of the LC evaluated, it should be noted that the red color of the image corresponds to the arterial vascularization while the blue color corresponds to the venous vascularization, which could be specified in subsequent studies.

Figure 2 Ultrasonographic evaluation of the Doppler characteristics of the CL of recipient llamas



Discussion

The *echotexture* of the LC of recipient llamas effectively shows them to be uniform, circumscribed, hypoechoic and homogeneous structures (Figure 1), due to the fact that their ovarian stroma is related to the active angiogenic process that occurs during the initial stages of luteal development in mammals, mediated by angiogenic factors such as vascular endothelial growth factor (VEGF), whose increase leads to rapid mitosis of the endothelial cells of the capillaries of the LC^{14,22,23}, in addition to the fact that most steroidogenic cells are adjacent to one or more capillaries and the perfusion of the luteal blood flow is so intense that it affects the acoustic impedance of the organ, resulting in the characteristic echotexture of the LC^{12,24}, as occurs in other species with the same

hypoechoic characteristics in relation to the ovarian stroma^{25,26}.

In *B-mode ultrasonography of the CL in recipient llamas* show morphometric characteristics such as area, volume and diameter highly related to studies on follicular dynamics in llamas specifically with sizes of donor follicles reaching diameters of 10.0 ± 2.0 mm and 11.8 ± 1.6 mm respectively^{3,27}, the maximum follicular diameter is related to the future diameter of the CL since these follicles have active angiogenesis after theca formation, which promotes the permeability of blood vessels, ovulation and subsequent formation of the CL²⁸, in cows for meat and milk production, ovulation of a preovulatory follicle of greater diameter leads to a CL of good size; this characteristic seems to be associated with fertility^{29,30}. The diameter of the CL of the llamas was 12.7 ± 1.7 mm, compared to other studies, are similar to those reported at 7 days post hormone application reaching diameters of 12.3 ± 0.6 mm, 10.4 ± 0.4 mm, 11 ± 1.9 , 11.19 ± 0.32 mm³¹⁻³³. While the area of the CL was 0.119 ± 0.03 cm² and the volume of the CL evaluated was 0.84 ± 0.34 cm³, these being the first results reported with various morphometric measurements of the CL and their correlation between them in llamas by US. In *ultrasonographic Doppler morphometry of the CL in recipient llamas* and specifically Color Doppler allows to determine the blood flow denoted as AVL in percentage, because the CL is highly vascularized and shows higher blood flow per unit of body tissue, the CL is characterized by intense angiogenesis and the CL blood flow increases 3 to 4 times in the first 96 h after ovulation in cattle²⁰, in the present study it was determined that the blood flow represented by the AVL was 34. In contrast, in llamas, the AVL between day 6 and 8 post application of GnRH was 20 and 25 %³⁴, while other authors report between 30 and 40 % of AVL, similar results to the present study³⁵, which is why similar percentages of AVL are

determined in high altitude conditions, This evaluation performed with color Doppler allows the evaluation of the quantity and pattern of blood flow in the LC, which indirectly indicates its functionality, a useful tool for reproductive management decisions such as the objective selection of recipients in embryo transfer programs in camelids, as well as mainly in cattle^{36,37}. In conclusion, the morphometric characteristics evaluated in B-mode (area, diameter and volume) have a positive correlation, therefore, any of the metric characteristics of the CL could be used for the selection of recipients in camelids, and the AVL percentage could be applied as a predictive tool for efficient reproductive management, diagnosis of early pregnancy and for the selection of recipients in camelids, as occurs in cattle.

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Conflicts of interest

The authors declare that the present research was carried out at the National University of the Altiplano Puno and there is no conflict of interest between the authors of this article.

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Ethical considerations

The study was approved by the Ethics Committee of the Faculty of Veterinary Medicine and Zootechnics of the National University of the Altiplano Puno and the guidelines established by this Committee were followed.

Authors' contribution to the article

Perez Guerra Uri Harold performed the experimental design and drafting of the manuscript, *Bustamante Quispe Carlos Washington* developed the experimental part of the ultrasonographic B-mode, *Luque Mamani Natalio* reviewed and wrote the manuscript, *Huayta Arizaca Rito* developed the experimental part of the ultrasonographic Doppler, *Condori Chuchi Eloy Amador* design of the experiment and statistical analysis, *Catacora Flores Nubia Lilia* performed the statistical analysis and revision of the manuscript, *Pérez Durand Manuel Guido* performed the writing and final revision of the manuscript.

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