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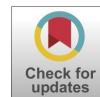
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# **B mode and Doppler ultrasonographic characteristics of the dominant follicle and corpus luteum in Criollo cows subjected to an oestrus synchronization protocol**

## **Característica ultrasonográficas B mode y Doppler del folículo dominante y cuerpo lúteo en vacas Criollas sometidas a un protocolo de sincronización de celo**

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### Abstract

The objective was to determine the ultrasonographic B mode and Doppler characteristics of the dominant follicle and corpus luteum in Criollo cows subjected to an oestrus synchronization protocol. The study was conducted at the Agricultural Experimental Station ILLPA during the months of May to July using 15 Criollo cows with regular body condition and fed with natural pasture, the protocol consisted of placing estradiol benzoate and intravaginal device on the first day, then on day 7 the device was removed and an analogue of Prostaglandin F<sub>2α</sub> was applied, On day 8 estradiol benzoate was applied again and on day 9 artificial insemination was performed with semen from a single Fleckvieh sire, ultrasonographic evaluation was performed on days 7 and 9 (synchronization started) to determine the diameter of the dominant follicle, growth rate and diameter of the uterine horn, the evaluation of the corpus luteum was performed on day 19 determining the diameter, volume and luteal vascular area (%), all the analyses were performed with the Jamovi 1 Programme. 6.23.0. The results of the dominant follicle on day 7 was 8.69 mm and day 9 was 10.7 mm with a growth rate of 1.14 mm/day, the diameter of the right uterine horn was 11.85 mm (day 7) which increased to 15.32 mm (day 9) while the left horn increased from 12.72 to 16.27 mm. 72 to 16.27 mm, the characteristics of the corpus luteum were 7.96 mm diameter, volume 0.23 mm<sup>3</sup> and luteal vascular area was 20.96 %, finally, the pregnancy rate obtained was 6 cows out of 15 inseminated which represents 40 %. In conclusion, the diameters and volume of the dominant follicle and corpus luteum are lower than those reported in other breeds in the same environmental conditions, the luteal vascular area was 20.96 %, the proportion of blood irrigation of the CL in Criollo cows and finally the pregnancy rate was 40 %. However, it is necessary to point out that these are the first reports of Doppler characteristics in Criollo cows

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### Resumen

El objetivo fue determinar las características ultrasonográfica B mode y Doppler del folículo dominante y cuerpo lúteo en vacas Criollas sometidas a un protocolo de sincronización de celo. El estudio fue realizado en la Estación Experimental Agraria ILLPA durante los meses de mayo a julio utilizando 15 vacas de raza Criolla con condición corporal regular y alimentadas con pasto natural, el protocolo consistió en colocar benzoato de estradiol y dispositivo intravaginal el primer día, posteriormente el día 7 se retiró el dispositivo y se aplicó un análogo de Prostaglandina F<sub>2α</sub>, el día 8 se aplicó nuevamente benzoato de estradiol y el día 9 se realizó la inseminación artificial con

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**Palabras clave:**

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semen de un solo reproductor de raza Fleckvieh, la evaluación ultrasonográfica fue realizada los días 7 y 9 (iniciado la sincronización) para determinar el diámetro del folículo dominante, tasa de crecimiento y diámetro de cuerno uterino, la evaluación del cuerpo lúteo fue realizada el día 19 determinando el diámetro, volumen y área vascular luteal (%), todos los análisis fueron realizados con el programa Jamovi 1.6.23.0. Los resultados del folículo dominante el día 7 fue de 8.69 mm y día 9 de 10.7 mm con una tasa de crecimiento de 1.14 mm/día, el diámetro del cuerno uterino derecho fue 11.85 mm (día 7) mismo que aumento a 15.32 mm (día 9) mientras el cuerno izquierdo aumento de 12.72 a 16.27 mm, las características del cuerpo lúteo fueron diámetro 7.96 mm, volumen 0.23 mm<sup>3</sup> y área vascular luteal fue de 20.96 %, finalmente, la tasa de preñez obtenida fue 6 vacas de 15 inseminadas que representa el 40 %. En conclusión, los diámetros y volumen del folículo dominante y cuerpo lúteo son inferiores a lo reportado en otras razas en las mismas condiciones ambientales, el área vascular luteal fue de 20.96 % proporción de irrigación sanguínea del CL en vacas Criollas finalmente la tasa de preñez fue del 40 %. Sin embargo, es necesario indicar que son los primeros reportes de características Doppler en vacas Criollas.

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## Introduction

The cattle population in Peru is approximately 5 million, 78 % is in the Sierra, more than half (70 %) is in the hands of small and medium producers, the predominant breed, the Criolla, with crosses with the Brown Swiss breed<sup>1</sup>. This breed has an important economic role, as it is a source of labour, animal protein food and family savings in altitudinal zones, where other breeds do not produce efficiently<sup>2-4</sup>.

Criollo cattle, even though they are used for a triple purpose, meat, milk and work, are at a disadvantage when compared to other breeds such as Holstein and Brown Swiss. However, their strength lies in their adaptation to the environment, lower feed requirements, longevity, resistance to adverse conditions and higher fertility<sup>1,5</sup>. This high fertility can be exploited more efficiently through the use of reproductive biotechnologies, generating greater genetic progress by increasing the intensity of selection from the male through artificial insemination (AI) and from the female through embryo transfer (ET), in both cases, the use of oestrus synchronization (OS) protocols with different synthetic hormones is necessary<sup>6</sup>. The purpose of CS is to generate a new follicular wave at the beginning, in order to synchronize ovulation, in most of the protocols used in beef and dairy

cattle<sup>7</sup>, these technologies could be used in Criollo cattle, to schedule calving at times of the year with better environmental conditions<sup>4</sup>, currently being complemented with ultrasonography, in order to determine the morphometry of ovarian structures during the CS process<sup>8</sup>, these findings are complemented with Doppler ultrasonography to obtain characteristics related to the blood irrigation of different organs<sup>9,10</sup>. The need to generate information on reproductive physiology in Criollo cattle, hence the objective of the study was to determine the ultrasonographic B mode and Doppler characteristics of the dominant follicle (DF) and corpus luteum (CL) in Criollo cows subjected to a CS protocol.

## Materials and methods

*Study site and animals.* The study was carried out at the Agricultural Experimental Station ILLPA belonging to the National Institute of Agrarian Innovation (INIA) located in the district of Paucarcolla in the province and region of Puno. Fifteen cows were evaluated cows Criollo breed The study was carried out during the dry season, which is characteristic of

the Peruvian Altiplano from May to July. The animals had a regular body condition between 2.5 and 2.8 degrees with a feeding management based on natural pastures. *Oestrus synchronization and artificial insemination*. This process was preceded by an obstetric diagnosis by means of ultrasonography, selecting those apparently normal females (free of apparent reproductive problems, evaluated by means of a previous gynecological evaluation), those selected, on the first day the CIDR® device was inserted (Progesterone in intravaginal device, active Progesterone 10 %: 1.9 g, Zoetis, Argentina) in addition to the application of 2 mg intramuscularly (IM) of Estrovet® (Estradiol benzoate, Montana, Peru). After 7 days, the device was removed and 2 mg of Prostal® (D-Chlorprostenol, Over, Argentina) was applied IM. Finally, on day 8 Estrovet® 2 mg IM was applied again. Fixed-time artificial insemination (FTAI) was performed on day 9 with semen straws from a Fleckvieh bull named INTI from the Ministry of Agriculture and Rural Affairs, the semen had a total motility at unfreezing of approximately 50% and a concentration of  $25 \times 10^6$ . When the straws were thawed at 37° C for 30 to 45 seconds, all females were inseminated with straws from the same bull.

*Ultrasonographic evaluation of the dominant follicle and uterine horns (B mode)*. It was preceded by cleaning the perianal area of the females for the examination, using Draminski 4Vet® equipment (Draminski ul. Owocowa 17, Poland) with a 6 MHz linear transducer was used to evaluate the morphometric characteristics of the FD and uterine horns on days 7 and 9 of CS to determine the following characteristics: i) FD diameter on days 7 and 9, measured in mm, ii) Growth rate, measured in mm/day, iii) Uterine horn diameter on days 7 and 9, measured in mm.

All the images were stored in a USB which were later analyzed, finally, the diagnosis of gestation was made on day 30 post IATF, to determine the presence or not of the gestational sac and the embryo using the same ultrasonographic equipment.

*Ultrasonographic evaluation of the corpus luteum (Colour Doppler)*. It was performed on day 19 post IATF using Draminski 4Vet® equipment, first, identifying the CL only with B mode to determine the diameter (mm) and volume (mm<sup>3</sup>), then, the Colour Doppler mode was activated to determine the luteal vascular area (AVL %) which was determined in percentage, once the cinestore (short video of the evaluation of the CL) was stored it was analyzed with the Image J® programme on a computer, the methodology was performed as recommended by Acosta et al.<sup>11</sup>.

*Statistical analysis*. Statistical analysis was performed using Jamovi 1.6.23.0<sup>12</sup> statistical software. The data were analyzed with descriptive statistics, determining the mean and standard deviation for the characteristics of FD diameter, uterine horns and FD growth rate, in addition to a Student's test to compare the diameter of the FD and uterine horns during days 7 and 9 of the SC protocol, while for the characteristics of the CL the mean, standard deviation and confidence intervals at 95 % were evaluated.

## Results

Table 1 presents the B-mode morphometric characteristics of the FDs of synchronized Criollo cows, as well as the uterine diameter of both horns on days 7 and 9 after the start of the protocol.

**Table 1** Ultrasonographic morphometric characteristics (Mode B) of the uterine follicle and uterine horns (n=15)

	Dominant follicle (mm)	Uterine diameter (mm)		Growth rate (mm/day)
		Right	Left	
<b>Day 7</b>	8.69±1.57 <sup>a</sup>	11.85±2.66 <sup>a</sup>	12.72±1.43 <sup>a</sup>	1.14±0.58
<b>Day 9</b>	10.7±1.35 <sup>b</sup>	15.32±2.76 <sup>a</sup>	16.27±1.75 <sup>a</sup>	

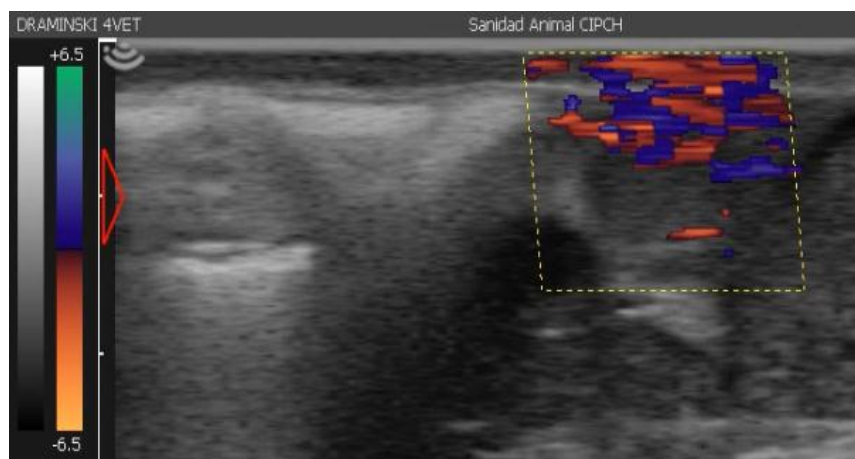
(a, b) different letters in columns show statistical significance ( $p>0.05$ ).

The diameter of the follicle on day 7 after the beginning of the synchronization protocol was 8.69 mm while on day 9 a diameter of 10.7 mm was observed, allowing to observe that there was a development of the follicle, verified with the growth rate of 1.14 mm/day. As for the diameter of the right and left uterine horns, an increase in size was also observed between day 7 and 9 respectively, ranging from

11.85 to 15.32 mm for the right horn and from 12.72 to 16.27 mm for the left horn. The diameter of uterine horns increased in size from day 7 to day 9 with the latter day for right horn being 15.32 mm and left horn 16.27 mm. The growth rate was 1.14 mm/day. Table 2 shows the B-mode and Doppler morphometric characteristics of the CL of Criollo cows synchronized with a SC protocol.

**Table 2** Ultrasonographic morphometric characteristics (B-mode and Doppler) of the corpus luteum (n=15)

	Diameter (mm)	Volumen (mm <sup>3</sup> )	Vascular Luteal Area (AVL %)
<b>Mean±SD</b>	7.96±2.07	0.23±0.13	20.96
<b>Confidence Interval (95 %)</b>	6.82 - 9.10	0.16 - 0.31	13.16 - 28.76

**Figure 1** Colour Doppler ultrasound evaluation of a corpus luteum of Criollo cows (day 19 post AI)

The characteristics of the CL were 7.96 mm in diameter with sizes ranging from 6.82 to 9.10 mm, the volume of the CL was 0.23 mm<sup>3</sup> with intervals ranging from 0.16 to 0.31 mm<sup>3</sup>, the characteristic taken with Doppler ultrasonography was the AVL evaluated in

percentages being on average 20.96 % with intervals ranging from 13.16 to 28.76 %, all these characteristics were determined with 95 % reliability. The colour Doppler assessment, Figure 1.

## Discussion

The maximum diameter obtained on day 9 is similar to that reported in crossbred animals with Criollo animals in the same conditions synchronised with Progesterone protocol reaching a size of 10.2 mm<sup>13</sup>, likewise, in Criollo cows evaluated during the entire estrous cycle in high altitude conditions, diameters in the rainy season of 11.25 mm and in the dry season of 11.67 mm were reported<sup>14</sup>, the follicular development from day 7 to 9 reflects the positive feedback after the extraction of the progesterone device that allows the free development of the FD until the respective insemination, at this moment it is known that the FD will increase the number of receptors for FSH and later for LH to continue with the development and maturation of the FD<sup>15,16</sup>. Higher reports are obtained in altitude conditions of 1500 masl in Black and White Creole (BON) cows with a FD<sup>17</sup> size of 14.3 mm. The difference could be due to the effect of the higher altitude where the present study was carried out since it generates the highest production of reactive oxygen species (ROS) which are involved in reproductive functions such as follicular maturation, ovulation, steroidogenesis, CL functions and luteolysis<sup>18</sup>, therefore, smaller follicular diameters could be obtained compared to studies carried out at lower altitudes. The diameter of the CL in Criollo cows is related to the diameters of the FD (evaluated on day 9), these diameters are lower than those reported in Brown Swiss cows which report 17.92 mm in cows in production and 11.32 mm in heifers. 32 mm evaluated on day 15 post AI, while in the present study it was evaluated on day 19 post AI, this difference may be due to the fact that these structures, according to the physiology of the estrous cycle, are structures in regression process, as well as to racial effects of the

Brown Swiss breed due to its larger body size than Criollo cows<sup>17,19,20</sup>.

The uterine diameter obtained was lower than those reported in Holstein cows of approximately 460 kg live weight with diameters greater than 20 mm<sup>21</sup>, however, the increase in uterine diameter is related to the increase in estrogens also related to the follicular phase, in this phase estrogens increase blood irrigation which generates oedema at the level of the uterine horns which are observed with an increase in diameter on ultrasonography<sup>22,23</sup>. The growth rate was similar to that reported in cows crossed with Criollas in the same environmental conditions with a similar synchronization protocol reporting a rate of 1.2 mm/day, although the diameter of the FD was higher<sup>17</sup>, the growth rate reported was similar being 1.05 mm/day in Criollo cows called BON, also reported rates similar to those reported in Criollo cows in dry and rainy season which were 1.01 mm/day and 1.0 mm/day respectively<sup>14</sup>. The growth rate obtained suggests the positive development of the FD from the day the DIB (bovine intrauterine device) was removed until the day before IATF, which indicates the action of the gonadotropin hormones (FSH and LH) that produce the development of the FD<sup>24</sup>.

For the evaluation of the CL, its volume was evaluated, observing differences that were referenced by various authors, who reported smaller CL diameters in Criollo cows compared to other studies carried out in other breeds such as Holstein, with the same reasons found after comparing the diameter of CL<sup>13,14,17,25</sup>. In addition, Doppler Colour Doppler was evaluated in altitude conditions and an average of 20.96 % AVL was reported, which is interpreted as the percentage of blood irrigation in relation to the total area of the CL, with no marked difference between those cows that were pregnant and those that

were empty respectively therefore, this percentage is descriptive, indicating that in Creole cows at 15 days post AI or post estrus reported a CL with an irrigation of approximately 20.96 %, this method measures the speed and intensity of blood flow and the number of red blood cells moving through the blood vessels per min<sup>19,26</sup>, it is also necessary to indicate that the colouring of the colour Doppler is based on the principle that the red colouring are those blood vessels that are approaching the transducer, while the blue colouring are the blood vessels that are moving away, all this at the time of the ultrasound examination<sup>25,27</sup>, compared to the results, no differences between pregnant and non-pregnant cows could be reported, because the day of evaluation (19 days post AI) coincides with the CL regression of those cows that could not get pregnant and in this process it has been studied that before the secretion of Prostaglandin F2 $\alpha$  occurs there is a sudden increase in blood supply<sup>11,25</sup>, which could be taken as a false positive (diagnosing a pregnant cow as non-pregnant), as well as mentioning that the assessment of blood flow by Doppler ultrasound alone was insufficient for diagnosis and would have to be accompanied by assessment of progesterone concentrations<sup>28</sup>. Finally, the pregnancy rate obtained after ultrasonographic evaluation on day 30 post AI was 6 cows out of 15 that were synchronised which would represent 40%.

Colour Doppler ultrasonography in Criollo cows. The ultrasonographic characteristics B mode before AI for FD diameter were lower, compared to other breeds, similarly the diameter and volume of the CL was lower while AVL of 20.96 % evaluated on day 19 post AI. However, the study aims to determine the morphometry of ovarian structures and uterine horns in Criollo cows. Finally, the cows subjected to SC achieved a pregnancy rate of 40 %.

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## Conflicts of interest

The undersigned authors declare that they have no potential personal or financial conflicts of interest with other persons or organizations that could unduly influence this manuscript.

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## Ethical considerations

The authors declare that the study presented has been carried out in accordance with the Code of Ethics conducted in accordance with the Code of Ethics for Animal Experiments for animal experiments, as reflected in the regulations as reflected in the regulations described in the web page: [http://ec.europa.eu/environment/chemicals/lab\\_animals/legislation\\_en.htm](http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm)

## Authors' contribution to the article

*Pérez Durand Manuel Guido, Perez Guerra Uri Harold, Luque Mamani Natalio, Condori Apaza Rafael, Escobedo Arizaca Ángel, preparation and execution. Perez Guerra Uri Harold, Manrique Quispe Yan Pierr, Condori Apaza Rafael, Urviola Sánchez Jesús Martin, methodology development. Pérez Durand Manuel Guido, Urviola Sánchez Jesús Martin,*

*Escobedo Arizaca Ángel*, conception and design. *Pérez Durand Manuel Guido*, *Perez Guerra Uri Harold*, *Mamani Cato Ruben*, *Manrique Quispe Yan Pierr*, article editing. *Pérez Durand Manuel Guido*, *Luque Mamani Natalio*, *Mamani Cato Ruben*, study supervision.

## Research limitations

There were limitations with respect to the information on the research, since it was a new topic.

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