Enhancing prognostication of corpus luteum function in cows through B-mode and Doppler sonography

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SUMMARY

The utilization of B-mode ultrasound and Doppler techniques in ruminants has emerged as a promising avenue for investigating the intricacies of female reproductive physiology, with a specific focus on the dynamic processes occurring within the follicles and corpus luteum (CL). The primary objective of this investigation was to comprehensively characterize and evaluate the activity of the CL in cows using B-mode and Doppler sonography. To achieve this, 31 Algerian brown atlas cows were subjected to synchronization using the well-established Ovsynch protocol. Ovulation monitoring was conducted using an ultrasound scanner equipped with an endocavitary probe operating at a frequency of 7.5 MHz, precisely 24 and 48 hours after the final administration of GnRH. Subsequently, daily ultrasound assessments were carried out to closely monitor the development of the resulting CL, commencing from the day of ovulation (D0) and continuing up until D20. By meticulously observing morphological changes within the CL, detecting variations in blood flow through ultrasound-Doppler technology, and measuring serum progesterone (P4) levels, three distinct phases were discerned within the comprehensive summary curve, illustrating the relative evolution of these parameters: a phase characterized by luteal growth, a subsequent static phase, and finally, a regression phase. Notably, a robust correlation was observed between the area of the CL and P4 (0.67; $p < 0.001$), as well as between the CL blood flow area and P4 (0.93; $p < 0.001$). The application of colour Doppler imaging facilitated the estimation of blood flow quantity and patterns within the CL, which indirectly served as an indicator of its functional status. Consequently, assessing the vascularity of the CL holds great promise as a superior marker for evaluating its secretory activity, thereby providing an invaluable tool for making informed reproductive management decisions. These decisions encompass various aspects, including the objective selection of recipients within bovine embryo transfer programs, as well as the timely detection of pregnancies in both heifers and cows.

KEY WORDS

Blood flow; corpus luteum; cow; Doppler; ultrasound .

INTRODUCTION

Ultrasound technology has facilitated significant advancements in comprehending the physiological changes and characteristics of the CL during the oestrous cycle in cattle. As the CL plays a crucial role in establishing and maintaining pregnancy in all domestic animals, particularly in embryo transfer programs for recipient female selection, its precise assessment assumes paramount importance [1,2]. Ultrasound provides a means to evaluate various ovarian morphometric features, including follicles and the CL [3]. Utilizing ultrasound to assess the CL enables determining the reproductive status of cattle and monitoring its dynamics, formation, development, and regression over time [4]. The ultrasound image of the CL exhibits uniformity, circumscription, and lower echogenicity compared to the ovarian stroma [5]. The intensity of the return sound waves reflected from the grey scale generated in the image can be used to assess echogenicity and echotexture, making it possible to assess the luteal size by measuring both the horizontal and vertical diameters of the CL [6]. However, debates persist regarding whether the luteal size is the most appropriate parameter for assessing the functional state of a CL. Observations have indicated that large CL may already be inactive during the regression phase [7]. In human physiological and morphometric studies using Doppler ultrasound, a positive correlation has been observed between the blood perfusion index of the CL and P4 concentrations in the early stages of pregnancy [8]. Such correlation may also be applicable in cattle to predict P4 levels [9], presenting new avenues for research [10]. Nonetheless, factors such as breed and environmental conditions need to be considered, as they can lead to slight variations in the physiology of domestic animals compared to studies conducted on

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other breeds [11].
Considering these aspects, it is crucial to employ ultrasound to study the reproductive physiology in domestic animals of local breeds, such as the indigenous Algerian cattle breed «Atlas Brown», known as an ideal recipient for embryos. The main objective of this research is to evaluate the secretory activity of the CL using B-mode ultrasound and Doppler, aiming to gain deeper insights into their reproductive physiology.

MATERIALS AND METHODS

Animals

For this research, a total of 31 cows were made available, all belonging to the local breed known as « Atlas Brown». The selected cows had previously undergone at least one calving, were more than 60 days post-partum, lack of abnormality and disease of the genital tract, and maintained a body condition score of 3 on a scale ranging from 1 to 5. Their diet primarily consisted of natural pasture; however, due to harsh winter conditions, they received supplementary feed consisting of coarse bran, flattened barley, and straw.

Experimental design

Oestrous cycle synchronization was achieved for all selected cows using the Ovsynch protocol, consisting of 100- g i.m. injections of GnRH (Cystoreline, CEVA Santé Animale, Libourne, France) 7 days before and 48 hours after a 25-mg i.m. injection of PGF2 (Enzaprost, CEVA Santé Animale, Libourne, France). Ovulation monitoring through ultrasound was conducted 24 and 48 hours after the final GnRH injection using an ultrasound scanner (MEDISON®, model SONOACE X6, South Korea) equipped with a 6 to 8 MHz endocavitary probe, set at 7.5 MHz. Daily ultrasound assessments of the resulting CL were performed, commencing from the day of ovulation (D0) and continuing until D20. The 2D mode was employed to monitor the morphological evolution of the CL (including surface area calculation). In contrast, the colour Doppler mode was activated to evaluate the surface area of detectable blood flow. Subsequent blood samples were collected after each examination to measure P4 levels. The CL's surface area was measured on a recorded image of the CL by selecting its section with the maximum diameter. This surface area was then calculated using Image J software version 1.54. In the case of a CL with a cavity, the surface area of the cavity was excluded from the total surface area. The Color-Doppler mode (Color-Flow-Mapping) was activated to visualise the detectable blood flow. Subsequently, a distinct, artefact-free image of the blood flow was acquired and duly recorded. Multiple images of the same CL were captured, and measurements were specifically performed on the images containing the highest number of coloured pixels. Utilizing the Image J software, the coloured pixels evident on the surface of the CL within the recorded images were meticulously selected. Subsequently, the area occupied by these coloured pixels was accurately calculated.

Statistical analysis

The statistical correlations between the P4 and CL area and between the P4 and CL blood flow area were calculated using the Bravais-Pearson correlation coefficient. Student-paired t-

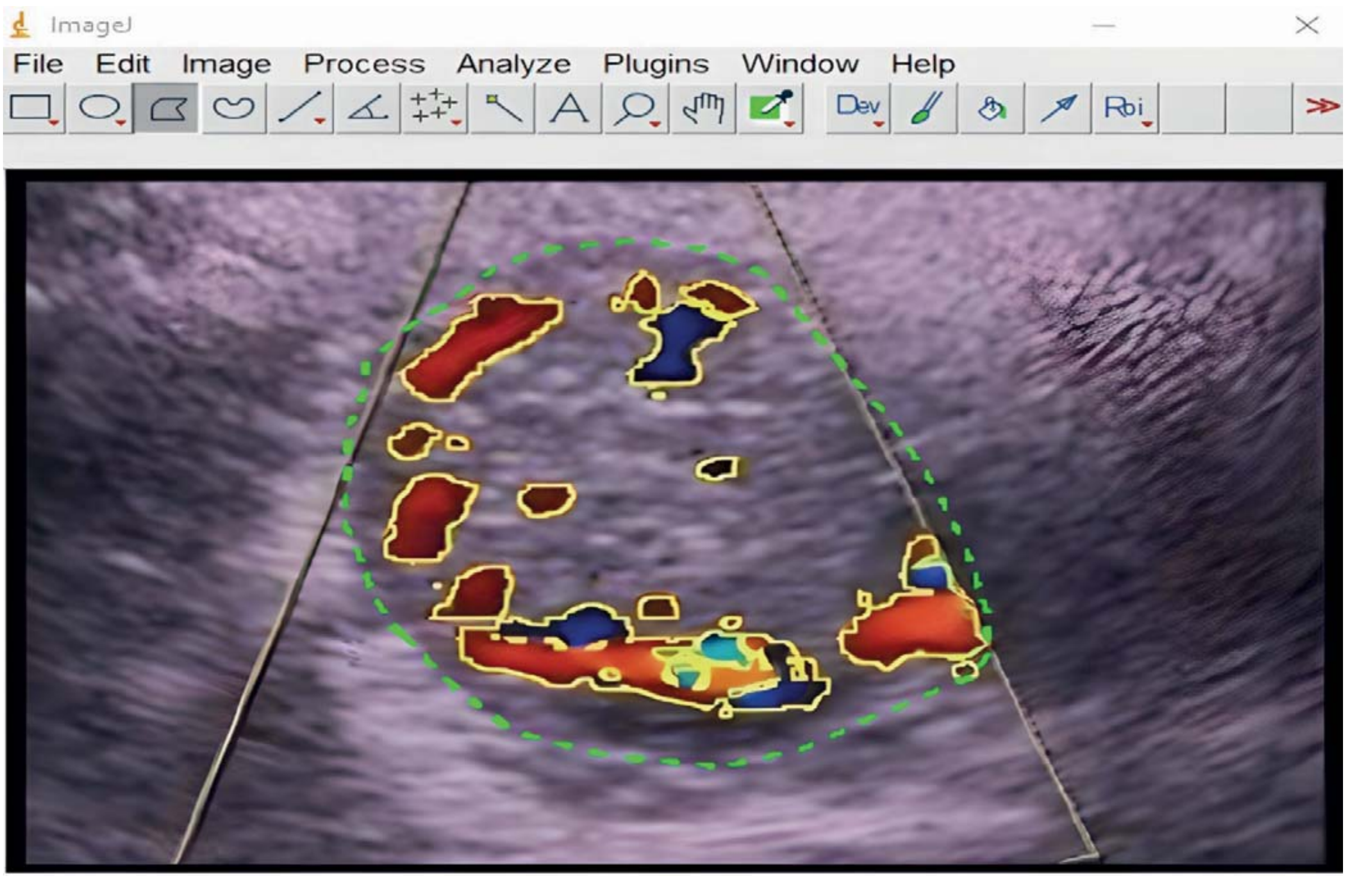

Figure 1 - Image processing program (Image J 1.54). for the calculation of the CL area (dotted green line). CL blood flow area was determined by calculating the combined area of colored pixels within the luteal parenchyma (yellow line).

tests were employed to determine significant changes in the means of the three parameters studied. The significance level was set at 5% ($p<0.05$). All statistical analyses were conducted using the SPSS software (version 26.0; SPSS Inc., Chicago, IL, USA).

RESULTS

All the cows examined (n=31) had a single ovulation, which led to the formation of 31 CL, 24 on the left ovary and 7 on the right ovary. Of these, 15 cavitary CLs were detected.
Echotexture of CL in cows, as shown in Figure 1, shows a uniform, well-defined, hypoechoic and homogeneous structure. Through the monitoring of morphological changes in the CL, variations in blood flow detectable by ultrasound-doppler and serum P4 levels, it became feasible to discern and demarcate three distinct phases within the summary curve illustrating the relative evolution of these parameters: a luteal growth phase, a static phase and a regression phase as illustrated in Figure 2.
P4 increases significantly during the luteal growth phase, from 0.57 (ng/ml) at D0 to 5.72 (ng/ml) at D6 ($p<0.01$). Another significant increase was observed during the static phase, from 8.02 ng/ml at D7 to 10.83 ng/ml at D16 ($p<0.001$). This was followed by a significant regression phase between D17 and D20, with P4 falling from 4.37 ng/ml to 0.69 ng/ml, respectively ($p<0.0001$). The CL area increased significantly during the luteal growth phase, rising from 2.46 cm² at D0 to 3.82 cm² at D6 ($p<0.05$). During the static phase, the CL continued to increase in size, rising from 4.29 cm² at D7 to 6.76 cm² at D16 ($p<0.01$). During the regression phase, there was a significant decrease in CL area, with the CL falling from 5.47 cm² at D17 to less than 3.37 cm² at D20 ($p<0.0001$). Detectable blood flow area also increased significantly during the luteal growth phase, rising from 0.12 cm² at D0 to 0.27 at D6 ($p<0.05$), and from 0.39 cm² at D7 to 0.74 at D16 ($p<0.01$), with a peak of 1.05 cm² at D12 during the static phase. Finally, a significant drop was observed between D17 and D20, from 0.30 cm² to 0.14 cm² ($p<0.0001$).
A strong correlation was observed between the CL area and P4 (0.67; $p<0.001$), as well as between the CL blood flow area and P4 (0.93; $p<0.001$), as shown in Figure 3. The correlations between the CL area and P4 during the luteal growth phase, the static phase and the regression phase were 0.69 ($p<0.001$), 0.56 ($p<0.001$) and 0.77 ($p<0.001$), respectively. The correlations between CL blood flow area and P4 during the growth phase, the static phase and the regression phase were 0.76 ($p<0.001$), 0.58 ($p<0.001$) and 0.97 ($p<0.001$) respectively.

DISCUSSION

In cattle breeding, luteal insufficiency in recipients stands out as a primary cause of abortion and failure in embryo transfer protocols [12-13]. This deficiency results in significant economic losses, especially when dealing with embryos of considerable genetic value, such as clones or transgenics. Diagnosing these anomalies typically involves measuring serum P4 levels, which requires vascular puncture, potentially causing stress to the animal and necessitates access to a well-equipped laboratory, which

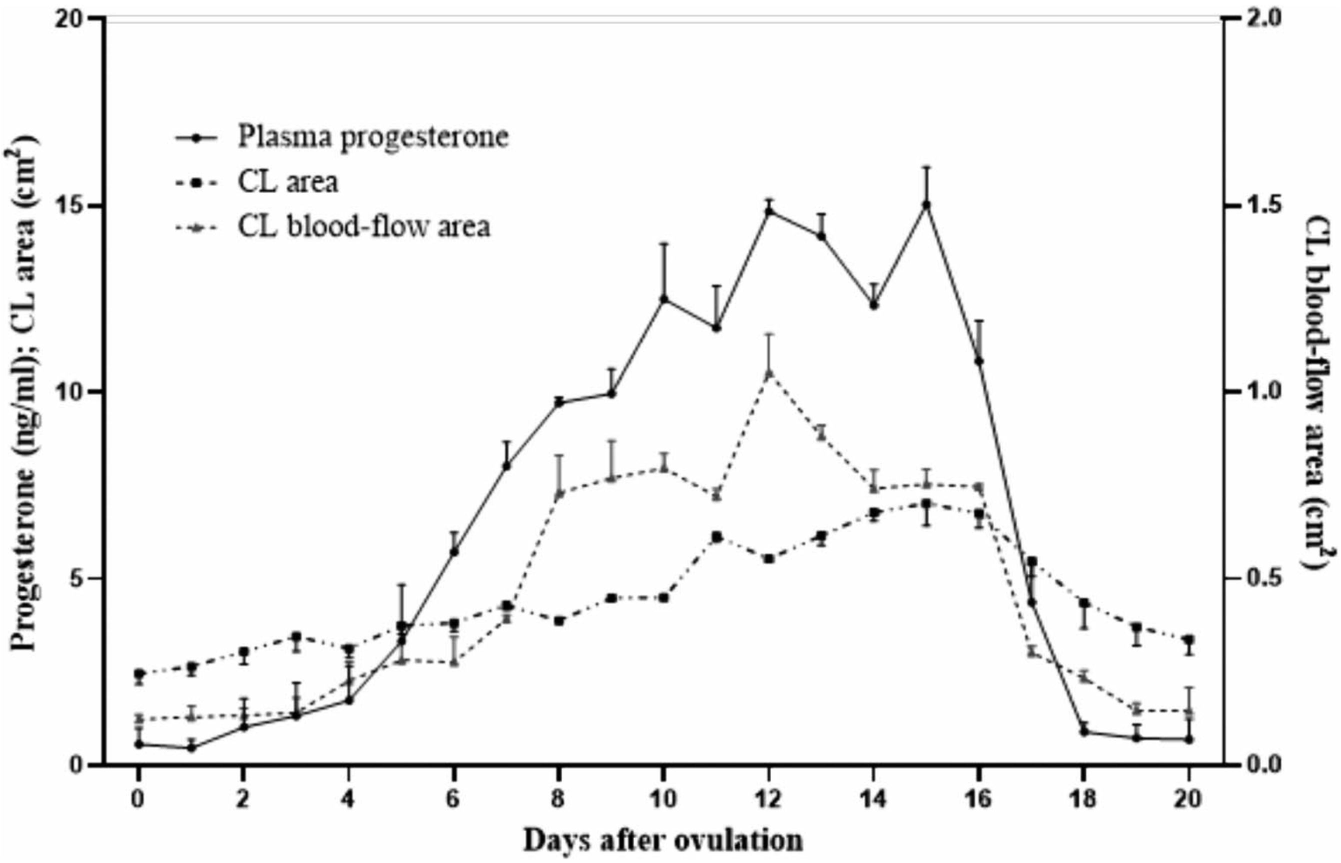

Figure 2 - Relative changes of plasma progesterone concentration, CL area, and CL blood-flow area. of 31 cows during the oestrous cycle; Mean (±S.E.M.).

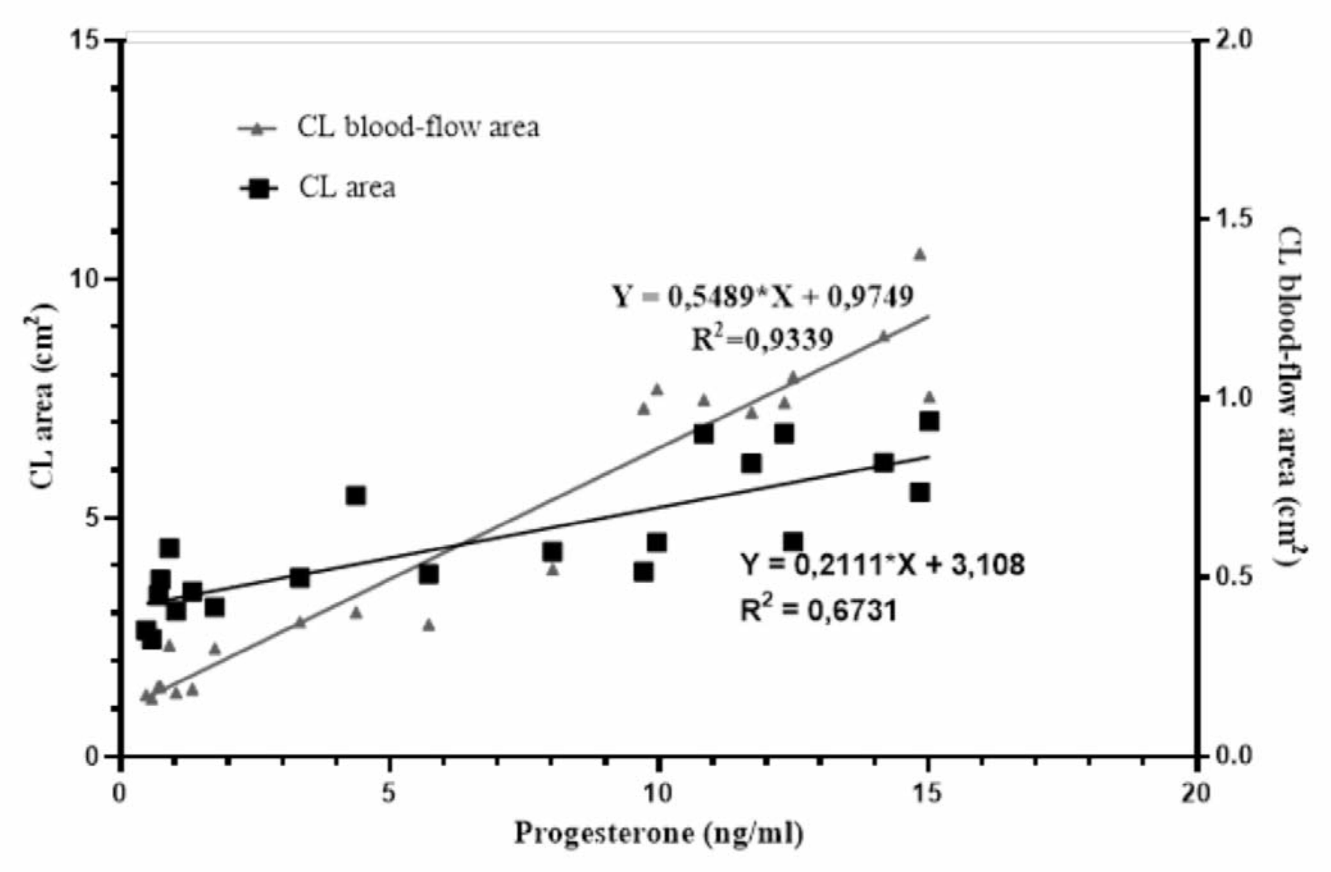

Figure 3 - Correlation between plasma progesterone concentration and CL area (■), as well as the correlation between plasma progesterone concentration and CL blood-flow area (▲).

is not always readily available.

When selecting embryo recipients, a common practice involves utilizing a rectal examination to detect the presence of the CL in the ovaries. This examination allows for the identification of females with prominent CL. However, relying solely on palpation is insufficient to assess the quality of CL [14], as some recipients may be excluded from transfer programs due to their CL not being prominently visible despite being of good quality. Conversely, relying solely on prominence may lead to selecting recipients whose P4 levels do not guarantee successful gestation [15]. Furthermore, ovulation from a larger pre-ovulatory follicle in cattle and dairy cows leads to a well-sized CL associated with fertility. In addition, genetics and the nature of production significantly impact the amount of P4 secreted and, therefore, fertility. The Atlas Brown breed, known for its exceptional genetic potential, demonstrates high fertility rates, hardiness, adaptability to limited food resources, and resistance to disease and climatic variations [16]. Although its production might not match that of improved breeds, it still possesses significant potential, with a fertility rate of 90% and an average carcass yield of 48.77%, comprising 61% muscle and 11% fat. On average, it produces approximately 700 kg of milk per lactation, primarily intended for calf suckling and potentially for family consumption [17]. Moreover, this local breed, originating from the Atlas Mountains of North Africa (Algeria, Tunisia, and Morocco), is distinguished by elevated progesterone levels, despite the relatively small size of the ovaries and the CL formed on them [18]. Additionally, it rarely experiences dystocia [19], making it an ideal recipient for embryo transfer.

For this study, the Ovsynch (G-P-G) protocol was chosen to synchronize the follicular waves of the females studied. This protocol allows precise control over physiological phenomena [20]. Additionally, the application of 2D medical imaging proves to be an intriguing solution for better highlighting intra-ovarian CL and cavitary CL [21]. The maximum follicular diameter is linked to the future diameter of the CL since these follicles exhibit active angiogenesis after the formation of the theca, favouring blood vessel permeability, ovulation, and subsequent CL formation [22].

Analysis of 2D ultrasound images reveals that the size of the CL increases during the luteal growth phase, from 2.46 cm² at D0 to 3.82 cm² at D6. During the static phase, the CL continues to grow, increasing from 4.29 cm² at D7 to 6.76 cm² at D16. A reduction in surface area is observed during the regression phase, where the CL decreases to less than 3.37 cm² at D20. However, it's important to note that this technique is not entirely free from error, as some CL that appears satisfactory on 2D imaging may produce very little P4, which could indicate inactivity, particularly during the regression phase [5,23].

Notably, CL is characterized by intense angiogenesis, with CL blood flow increasing 8-9-fold in a few days after ovulation in cattle [9,24]. In this study, we also observed a detectable increase in blood flow during the luteal growth phase, with significant values ranging from 0.12 cm² at D0 to 0.27 cm² at D6 and from 0.39 cm² at D7 to 0.74 cm² at D16, peaking at 1.05 cm² at D12 during the static phase This observation aligns with previous findings reported by Shirasuna et al. [25]. However, a decline in blood flow was observed between D17 and D20, from 0.30 cm² to 0.14 cm². When conception fails, luteolysis occurs, characterized by reduced blood flow and suppression of P4 production. Luteal cells then undergo apoptosis, followed by necrosis and tissue remodeling. Luteolysis is divided into two main phases: functional luteolysis, which involves the loss of function, and structural luteolysis, which refers to the physical regression of luteal tissue. This reduction in blood flow and P4 is crucial to initiate a new estrous cycle. Thus, the vascularization of the CL is essential not only for P4 production but also for its rapid regression in case of non-pregnancy.

Consistent with this finding in the present study, Pugliesi et al. (2019) [1] reported similar values for luteal blood flow across various breeds, albeit with some variations. These discrepancies may be attributable to differences in the histological structure of the CL, particularly in breeds where the Conjunctive Stroma occupies a substantial portion of the luteal tissue, resulting in reduced vascularization and P4 production. Such structural differences can also be observed among individuals of the same breed and age or between different cycles.

There was previously reported that luteal vascularization correlates with the rate of P4 secretion in humans [26], as well as in several other mammals, such as llamas [27] and buffalo [28]. However, this process occurs at a relatively slower rate and supports longer menstrual or estrous cycles. Differences in vascularisation dynamics are linked to variations in progesterone levels and the duration of the luteal phase between species.

Moreover, the vascularization of the CL is crucial for its progesterone production capability. A sufficient blood supply guarantees the delivery of vital nutrients, oxygen, and hormonal precursors required for P4 synthesis. Gaining insight into and controlling this relationship can significantly impact reproductive health and the treatment of luteal phase deficiencies. Assessing its vascularity may, therefore, prove to be a superior indicator of its secretory activity and a valuable tool for reproductive management decisions, such as the objective selection of recipients in bovine embryo transfer programs [29-30], along with the prompt detection of pregnancy in heifers and cows [3]. In cases like these, the presence of non-functional CL with negligible or absent blood flow serves as an indicator for animals that are not pregnant [12]. This inference is further supported by the strong correlation observed between vascularization and P4 levels during the three luteal phases [19].

In conclusion, enhancing reproductive outcomes in livestock farming necessitates adopting more precise and early methods to optimize the profitability of each animal in the herd. Successful reproductive management relies on a profound understanding of the physiological processes governing the various follicular and luteal phases. In this context, researchers have explored using B-mode ultrasonography to assess morphometric characteristics of the CL. Positive correlations have been observed, suggesting that this technique could assist in selecting recipients among cattle. Doppler ultrasonography has emerged as a non-invasive tool in animal gynaecology, enabling visualization and analysis of blood flow in organs, particularly ovarian structures. Through this evolving approach, researchers have developed models to track organ blood flow during key stages of sexual activity in cows. With the help of reference values derived from healthy subjects, the prognosis for the likelihood of pregnancy can now be established, especially in bovine embryo transfer programs.

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Conflict of interest

The named authors have no conflict of interest, financial or otherwise.

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