P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com
Research Article https://doi.org/10.47278/journal.ijvs/2022.156

Ex Vivo Study of Macroscopic Feature and Sonogram Imaging of Bovine Ovarian Corpus Luteum

Yosua Kristian Adi[1]*, Surya Agus Prihatno[1], Erif Maha Nugraha Setyawan[1] and Bina Rusmawati[2]

[1]Department of Reproduction and Obstetrics, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta-55281, Indonesia
[2]Department of Agriculture, Food, and Fisheries of Sleman Regency, Yogyakarta-55511, Indonesia
***Corresponding author:** yosua.kristian.a@ugm.ac.id

Article History: 22-588 Received: 04-Apr-22 Revised: 25-Apr-22 Accepted: 28-Apr-22

ABSTRACT

In the clinical procedure, corpus luteum identification in the ovary is important to determine the reproductive status of cows. There are two methods to determine corpus luteum in live cows, rectal palpation and ultrasound examination. However, real imaging cannot be obtained by these methods. Thus, the current study aimed to describe the macroscopic feature and sonogram imaging of the corpus luteum in the bovine ovary as an ex vivo study. Twenty-eight pairs of bovine ovaries from a slaughterhouse were used in this study. Ovaries were classified based on the diameter of the corpus luteum crown into five categories (≤0.5, 0.5-1, 1-1.5, >1.5cm, and none). We observed and measured the corpus luteum crown and corpus luteum tissue using a vernier calliper and ultrasound. Descriptive and statistical analyses were carried out using the SAS program. The corpus luteum crown was identified as a circular folding tissue on the ovary surface, during corpus luteum tissue as a yellowish lobed structure by macroscopic observation and hypoechoic structure by ultrasonography. Ovaries with corpus luteum crown diameter 0.5-1.0cm were dominant (41%). However, we found no correlation between the area of the corpus luteum crown with the area of corpus luteum tissue by macroscopic (P=0.121) and ultrasound observation (P=0.151). The Corpus luteum crown and corpus luteum tissue described in this study can be used as a reference by practitioners. Further correlation analysis is needed between corpus luteum crown and corpus luteum tissue in live cows.

Key words: Cow, Crown, Correlation, Luteal tissue, Ovarium, USG.

INTRODUCTION

Identification of the structure in the ovary can be used to determine the reproductive status of cows (López-Helguera et al. 2016; Skovorodin et al. 2020a). During the estrus cycle, there are some changes in the structure of the ovary due to follicular and corpus luteal development (Quezada-Casasola et al. 2014). Based on the presence of specific structure in the ovary, estrus cycle can be divided into two main phases, follicular phase and luteal phase. In the follicular phase there is dominant follicle from final wave of follicle growth that is ovulated, while the luteal phase is started when the corpus luteum (CL) is formed following the ovulation (Forde et al. 2011; Crowe and Mullen 2013). Combination of the observation of estrus signs and identification of dominant follicle prior to artificial insemination is helpful and recommended to determine the heat status in cattle (Hansar et al. 2014). While identification of CL can be used to know the occurrence of ovulation. CL will be formed when the ovulation occurred and will remain throughout the gestation if there is fertilization (Aréchiga-Flores et al. 2019). However, the size of CL tissue changes during the luteal phase (Kayacik et al. 2005; Felipez et al. 2019). In some reproductive disorders, such as ovary cyst, persistent corpus luteum, ovarian hypofunction, ovarian atrophy, and ovarian hypoplasia, abnormalities of the ovary can be detected (Jeengar et al. 2014; Mushonga et al. 2017; Amin and Mohammed 2018; Salman et al. 2021).

Rectal palpation and ultrasonography are the most common methods to identify the structure within the ovary in cows (Hanzen et al. 2000; Hansar et al. 2014). Although, these two methods have some limitations when performed alone. Manual palpation of the ovary can be used to detect the presence of follicle through the consistency of the ovary surface and CL through the existence of CL crown, but it is quite difficult to estimate the size of follicle and CL tissue within the ovary exactly. While ultrasonography is quite

Cite This Article as: Adi YK, Prihatno SA, Setyawan EMN and Rusmawati B, 2023. *Ex vivo* study of macroscopic feature and sonogram imaging of bovine ovarian corpus luteum. International Journal of Veterinary Science 12(1): 31-36. https://doi.org/10.47278/journal.ijvs/2022.156

accurate to estimate the size of follicle and CL tissue within the ovary but might be difficult to perform by unskilled operator. Interpret the feature of CL using ultrasonography is not easy either. Inexperienced operator might be wrong interpreting the CL tissue and other parenchyma tissue of the ovary from the ultrasound imaging. Whereas the use of ultrasound has become important for imaging of normal and abnormal ovarian structures in cattle today (Whitfield 2018). Thus, reference about macroscopic feature and ultrasonography imaging of CL is needed to help interpreting the sonogram examination results. The current study aimed to describe the macroscopic feature and sonogram imaging of the corpus luteum in the bovine ovary as an ex vivo study.

MATERIALS AND METHODS

The research design conducted in this study has been approved by Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta with ethical clearance number 00041/EC-FKH/Int./2021. Ovary samples were obtained from a slaughterhouse in Sleman, Yogyakarta from April – June 2021. Twenty-eight pairs of ovaries from unproductive crossbreed beef cows that slaughtered in abattoirs were included in this study. After the cow was slaughtered, the ovaries were removed and fixed immediately by putting the organ into a 10% formalin solution before analyzed furthermore. Left and right ovary from 28 female beef cattle were analyzed. Ovaries were observed for the presence of CL crown. After that, digital imaging of CL crown in the surface of ovary was captured using digital camera. Two diameters (D1 and D2) of the CL crown that were perpendicular to each other were measured in each ovary using vernier calliper (Tricle Brand®). Measurements were repeated three times and the results were averaged to obtain an accurate measure. Area of CL crown was calculated using ellipse formula. Ovaries were classified according to CL crown into five categories: ovary with CL crown diameter ≤0.5cm, ovary with CL crown diameter 0.5-1cm, ovary with CL crown diameter 1-1.5cm, ovary with CL crown diameter >1.5cm and ovary without CL crown. One sample of ovary from each category was used for ultrasonography test and macroscopic analysis of the CL tissue. Ultrasonography test was performed using ultrasonography machine HONDA HS-2000 with convex probe in the frequency of 5megahertz. Before performing ultrasonography, the sample was washed with sterile water and embedded in the gel. Convex probe was placed over the ovary in the site of the CL crown. Ultrasound imaging was captured and analyzed for its echogenicity. The diameter of CL tissue within the ovary was determined using the measurement menu in the ultrasonography machine. Area of CL tissue within the ovary from ultrasound imaging was calculated using ellipse formula. After that, the ovary was sliced into two parts in the middle of CL crown. The diameter of CL tissue within the ovary was determined using vernier calliper and the area of CL tissue was calculated using ellipse formula. Digital imaging of CL tissue was captured using digital camera. Data analysis was performed using SAS version 9.4 (SAS Inst. Cary, NC, USA). Diameter and area of CL crown, CL tissue from ultrasound imaging, and CL tissue from macroscopic observation were presented as descriptive data. Correlation analysis was performed for area of CL crown, area of CL tissue from ultrasound imaging, and area of CL tissue from macroscopic observation. CL crown feature and CL tissue feature within the ovary from ultrasound and digital imaging were described.

RESULTS

There were 56 ovaries identified in this study. The highest number of ovaries was in the category of CL crown diameter 0.5-1cm while the lowest number of ovaries was in the category of CL crown diameter >1.5cm. The percentage of ovary based on the CL crown diameter <0.5, 0.5-1.0, 1.0-1.5, >1.5cm and none were 14.3, 41.1, 14.3, 7.1 and 23.2%, respectively. The smallest CL crown diameter was 0.33cm, while the largest CL crown diameter was 1.66cm. The average of CL crown diameter observed in this study was 0.84±0.37cm. The data of CL crown diameter, CL tissue diameter from ultrasound imaging, CL tissue diameter from macroscopic observation, area of CL crown, area of CL tissue from ultrasound imaging, and area of CL tissue from macroscopic observation are presented in Table 1. Correlation analysis between area of CL crown, area of CL tissue from ultrasound imaging, and area of CL tissue from macroscopic observation is presented in Table 2.

Based on the macroscopic observation, CL crown was identified as circular folding tissue in the ovary surface. The CL crown was varying in diameter. Some of the ovaries have more than one CL crown and some other have no CL crown observed. The color of CL crown was yellowish for the big one and become white in the small one (Fig. 1. A-D). In some ovaries, there was large orifice in the middle of the CL crown but in some other the orifice was smaller and in some other there was no orifice observed (Fig. 3. A-C). In the sliced ovary, the CL tissue was identified as yellowish lobed structure. The color of the CL tissue within the ovary was same in the ovaries from categories of CL crown diameter 0.5-1, 1-1.5, and >1.5cm. However, in the ovaries from category of CL crown diameter ≤0.5cm, the color of the CL tissue was orange (Fig. 1. E-H). In line with the observation of CL crown with large orifice, there was hollow structure in the middle of CL tissue. However, this hollow structure was not observed in the CL tissue from the CL crown with smaller orifice and no orifice (Fig. 3. D-F). Ultrasound imaging of ovary tissue showed similar feature with other soft tissue. Parenchyma tissue of ovary resulted in hyperechoic imaging. While CL tissue resulted in hypoechoic imaging. The border between parenchyma tissue and CL tissue could be distinguished clearly (Fig. 2). Hollow structure filled with liquid, such as follicular antrum and CL cavity, resulted in anechoic imaging (Fig. 3. G-I).

DISCUSSION

In this study, most of the ovaries (41.1%) have CL crown with the diameter of 0.5-1cm. The sample of ovary in this category has diameter of CL tissue approximately 1.61cm from ultrasonography imaging and 1.53cm from macroscopic observation. Kayacik et al. (2005) reported that in Holstein cows with normal estrus cycle, CL with diameter

Table 1: Descriptive data of the corpus luteum crown and corpus luteum tissue on the ovary sample in each CL crown category

CL crown category		D of CL crown (cm)	D of CL tissue (USG/cm)	D of CL tissue (macros/cm)	Area of CL crown (cm^2)	Area of CL tissue (USG/cm^2)	Area of CL tissue (macros/cm^2)
<0.5	D1	0.40	0.33	0.35	0.148	0.132	0.165
	D2	0.47	0.51	0.60			
0.5-1	D1	0.81	1.49	1.43	0.541	1.885	1.719
	D2	0.85	1.61	1.53			
1-1.5	D1	1.32	1.53	1.74	1.452	2.693	3.103
	D2	1.40	2.24	2.27			
>1.5	D1	1.35	1.39	1.37	1.718	2.294	2.400
	D2	1.62	2.10	2.23			

D: diameter, D1: shortest diameter, D2: diameter perpendicular to D1, CL: corpus luteum

Table 2: Correlation analysis between area of corpus luteum crown, area of corpus luteum tissue from ultrasound imaging, and area of CL tissue from macroscopic observation

Pearson Correlation Coefficients, N=4 Prob > |r| under H0: Rho=0			
	Area of CL crown	Area of CL tissue (USG)	Area of CL tissue (macros)
Area of CL crown	1.00000	0.84930 0.1507	0.87905 0.1209
Area of CL tissue (USG)		1.00000	0.98553 0.0145
Area of CL tissue (macros)			1.00000

CL: corpus luteum.

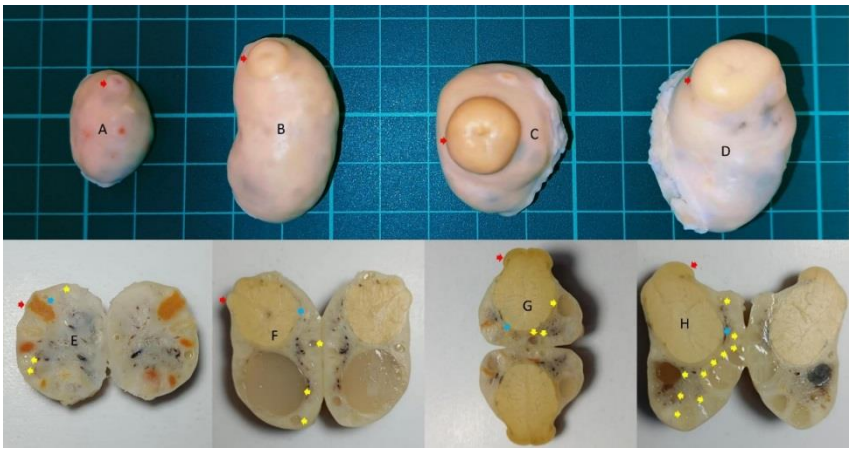

Fig. 1: Macroscopic feature of CL crown and CL tissue on the ovary sample in each CL crown category (A,E=<0.5cm; B,F=0.5-1cm; C,G=1-1.5cm; D,H=> 1.5cm). Red arrow: CL crown, blue arrow: CL tissue, yellow arrow: follicle.

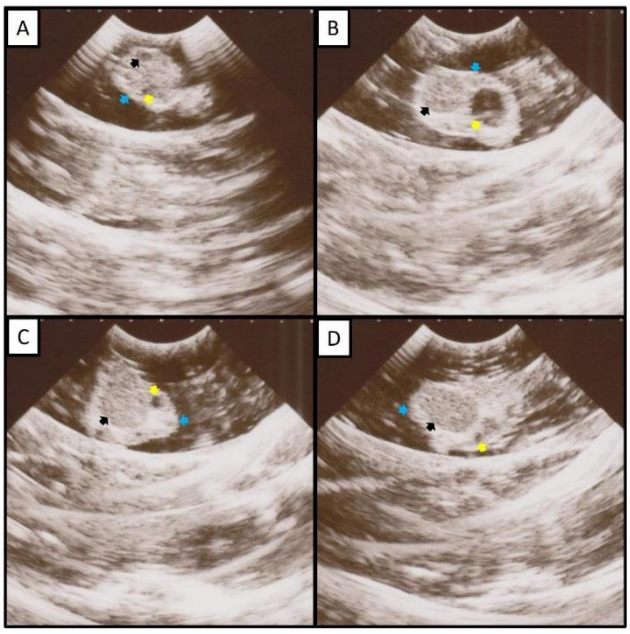

Fig. 2: Ultrasound imaging of ovary sample in each CL crown category (A=<0.5cm; B=0.5-1cm; C=1-1.5cm; D=>1.5cm). Blue arrow: ovary tissue, black arrow: CL tissue, yellow arrow: follicle.

1-1.5 cm was observed in four days, 1.5-2cm in 6 days and 2-2.5cm in 11 days during luteal phase. In Aceh cattle, CL with diameter 1-1.5cm was observed in major proportion (11 days) during estrus cycle (Siregar et al. 2016). However, in this study the stage of estrus cycle could not be defined. In Criollo cattle, the maximum of CL diameter is ranging from 1.1 to 1.6cm (Quezada-Casasola et al. 2014) while the maximum of CL diameter of beef cattle that maintained in Nebraska, USA is 2.88±0.12cm (Quintal-Franco et al. 1999). This indicates that CL size is influenced by breed. In addition, Rocha et al. (2019) reported that area of CL strongly correlates with progesterone concentrations during CL development. In this study, there is a possibility that CL with a diameter of 0.5-1cm was the dominant phase of CL development in the estrus cycle of beef cattle in Sleman Regency. However, this is not supported by data of estrus cycle day, thus it cannot be concluded. Further studies on the CL development of beef cattle in Sleman regency need to be carried out with the association of estrus cycle day and progesterone concentration.

Transrectal palpation or ultrasonography are common methods to identify ovarian structures in the cow. However, ultrasonography can give more precise results

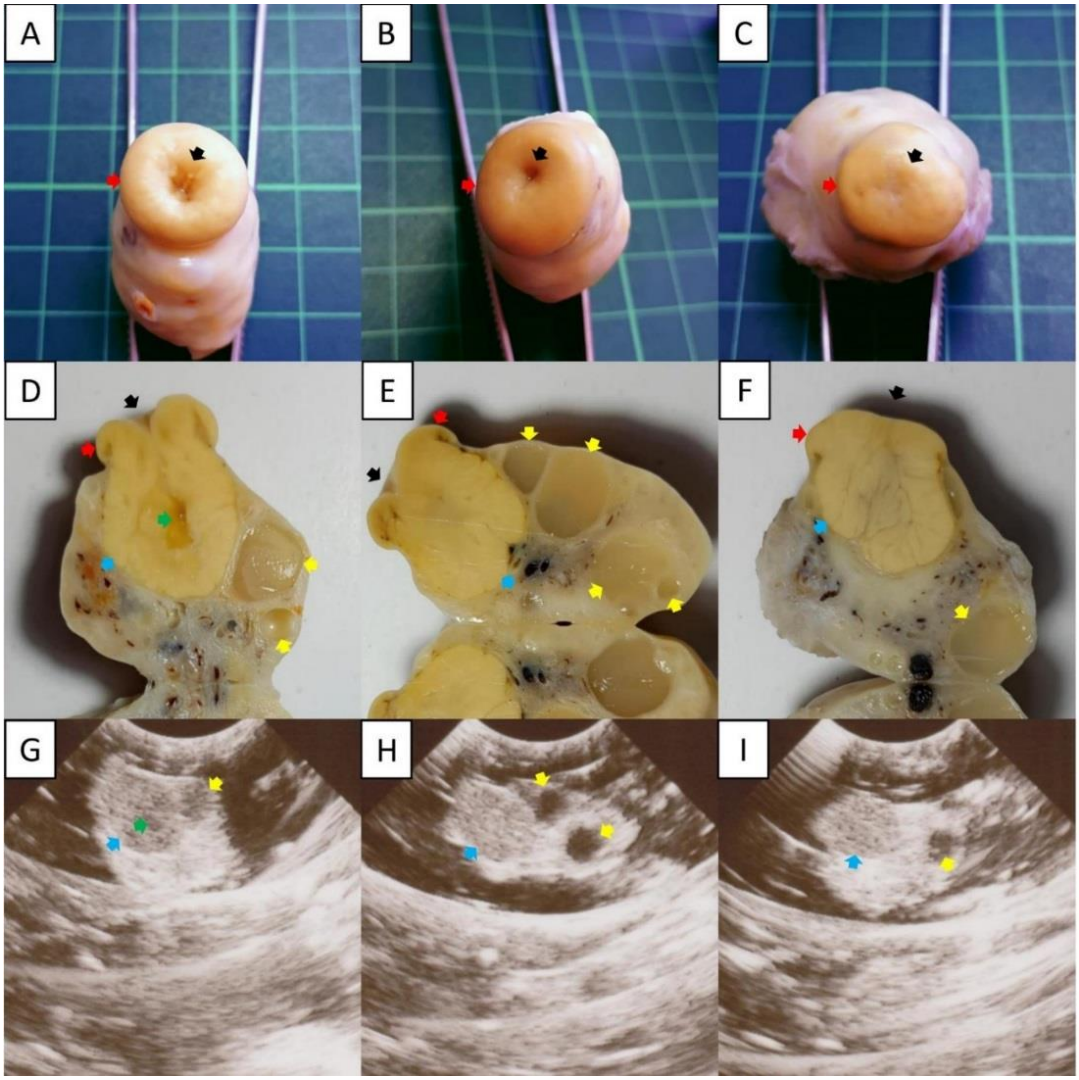

Fig. 3: Macroscopic feature and ultrasound imaging of ovary with large CL crown orifice (A), small CL crown orifice (B), and no CL crown orifice (C). Red arrow: CL crown, blue arrow: CL tissue, yellow arrow: follicle, black arrow: CL crown orifice, green arrow: CL cavity.

when determining the number and the size of the mature corpus luteum compared to manual palpation (McDougall and Rhodes 1999; Hanzen et al. 2000). This is in line with our findings that revealed strong positive and significant correlation between area of CL tissue from ultrasound imaging and area of CL tissue from macroscopic observation. Although, ultrasonography also has limitation to identify young and old-CL or CL with small size (Pieterse et al. 1990; Aslan et al. 2000). In this study, non-significant correlation was revealed between area of CL crown with both area of CL tissue from ultrasound imaging and area of CL tissue from macroscopic observation. This indicate that the CL crown size cannot be used to estimate the CL size within the ovary. Although it is easy to determine the presence of CL by identifying the CL crown during transrectal palpation, it depends on the experience of the operator.

The feature of CL tissue from macroscopic observation in this study was same with the description of CL by Skovorodin et al. (2020b). In a few days after ovulation, the CL is large, light red or yellow, slightly protruding above the surface of the ovary, and the lobulation of the incision is not visible (Skovorodin et al. 2020b). This description similar with our finding of the CL feature with large orifice in the middle of the CL crown (Fig. 3D). After that, CL become dense, and the luteal tissue is bright yellow with clear lobulation in the incision (Skovorodin et al. 2020b). This description similar with our findings of the CL feature with no orifice in the middle of the CL crown (Fig. 3F). Ultrasound imaging has been used to identify the structure in the ovary for decades and still develop until now (Pieterse 1989; Boyd and Omran 1991; Viana et al. 2013; Jaśkowski et al. 2021). In general, CL can be identified using ultrasound 3 days after ovulation. Ultrasound image of a developing CL is poorly defined, irregular, greyish-black structure with echogenic spots all within the ovary. While CL in a mid-cycle can be defined well as granular greyish echogenic structure. Demarcation line is clear visible between CL tissue and the ovarian stroma. In a regressing CL, the demarcation line become faint owing to the slight difference in echogenicity between the tissues (Pieterse et al. 1990). Our findings were same with the description of ultrasound imaging in mid-cycle CL. In addition, CL with cavities also could be identified in this study (Fig. 3G). Small central cavity was identified in the CL with large orifice in the middle of CL crown. CL

cavity is identified as non-echogenic area surrounded by greyish echogenic luteal structure. This cavity is varying for its size and shape according to the cyclic stages of the CL (Kito et al. 1986; Kastelic et al. 1990).

Conclusion

Corpus luteum crown was identified as circular folding tissue in the ovary surface while corpus luteum tissue as yellowish lobed structure by macroscopic observation and hypoechoic structure by ultrasonography. We found that there was no correlation between the area of corpus luteum crown and area of corpus luteum tissue. Thus, area of corpus luteum crown could not be used to predict the area of corpus luteum tissue. This study of macroscopic feature and ultrasonography imaging of CL in the bovine ovary can be used to understand better about interpretation in sonogram examination by practitioners. However, further studies about CL crown and CL tissue development in beef cattle need to be carried out with special emphasis on association of estrus cycle day and progesterone concentration.

Acknowledgement

This study was supported by Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta through Research Development Department Grant 2021 number: 934/UN.1/FKH/HK4/2021.

Author's contribution

YKA designed the research project and obtained the fund. YKA and BR collected the sample and data in the field. YKA, SAP, and EMNS prepared the sample and collected the data in the laboratory. YKA analyzed the data and prepared the manuscript. All authors read and contributed to evaluate the manuscript.

REFERENCES

Amin FAM and Mohammed MO, 2018. A Study of Congenital abnormalities of the non-pregnant genitalia in cows: 1. Ovaries and uterine tubes. Al-Anbar Journal of Veterinary Sciences 11: 37-45.

Aréchiga-Flores C, Cortés-Vidauri Z, Hernández-Briano P, Flores-Flores G, Rochín-Berumen F and Ruiz-Fernández E, 2019. Review: Function and regression of the corpus luteum during the estrous cycle. Abanico Veterinario 9: 1-21. http://dx.doi.org/10.21929/abavet2019.924

Aslan S, Findik M, Erünal-Maral N, Kalender H, Celebi M and Saban E, 2000. Comparison of various examination methods used in ovarian diagnostics in cattle. Deutsche tierarztliche Wochenschrift 107: 227-30.

Boyd JS and Omran SN, 1991. Diagnostic ultrasonography of bovine female reproductive tract. In Practice 13: 109-118. https://doi.org/10.1136/inpract.13.3.109

Crowe MA and Mullen MP, 2013. Regulation and Function of Gonadotropins Throughout the Bovine Oestrous Cycle. In: Gonadotropin. IntechOpen, London, Vizcarra J (ed), https://doi.org/10.5772/53870

Felipez MV, Acosta T and Ungerfeld R, 2019. Sexual stimulation as a luteolytic inductor in beef heifers. Theriogenology 132: 83-87. https://doi.org/10.1016/j.theriogenology.2019.04.003

Forde N, Beltman ME, Lonergan P, Diskin M, Roche JF and Crowe MA, 2011. Oestrous cycles in *Bos taurus* cattle. Animal Reproduction Science 124: 163-169. https://doi.org/10.1016/j.anireprosci.2010.08.025

Hansar E, Lemma A and Yilma T, 2014. Pre-service ultrasonic and manual evaluation of the reproductive organs of dairy cows presumed to be in estrus. Springer Plus 3: 529. https://doi.org/10.1186/2193-1801-3-529

Hanzen C, Pieterse M, Scenczi O and Drost M, 2000. Relative accuracy of the identification of ovarian structures in the cow by ultrasonography and palpation per rectum. Veterinary Journal 159: 161-170. https://doi.org/10.1053/tvjl.1999.0398

Jaśkowski BM, Bostedt H, Gehrke M and Jaśkowski JM, 2021. Ultrasound characteristics of the cavitary corpus luteum after oestrus synchronization in Heifers in relation to the results of embryo transfer. Animals 11: 1706. https://doi.org/10.3390/ani11061706

Jeengar K, Chaudhary V, Kumar A, Raiya S, Gaur M and Purohit GN, 2014. Ovarian cysts in dairy cows: old and new concepts for definition, diagnosis and therapy. Animal Reproduction 11: 63-73.

Kastelic JP, Pierson RA and Ginther OJ, 1990. Ultrasonic morphology of corpora lutea and central luteal cavities during the estrous cycle and early pregnancy in heifers. Theriogenology 34: 487-498. https://doi.org/10.1016/0093-691x(90)90006-f

Kayacik V, Salmanoğlu MR, Polat B and Özlüer A, 2005. Evaluation of the corpus luteum size throughout the cycle by ultrasonography and progesterone assay in cows. Turkish Journal of Veterinary and Animal Sciences 29: 1311-1316.

Kito S, Okuda K, Miyazawa K and Sato K, 1986. Study on the appearance of the cavity in the corpus luteum of cows by using ultrasonic scanning. Theriogenology 25: 325-333. https://doi.org/10.1016/0093-691x(86)90068-3

López-Helguera I, Colazo MG, Garcia-Ispierto I and López-Gatius F, 2016. Factors associated with ovarian structures and intrauterine fluid in the postpartum period in dairy cows. Journal of Dairy Science 99: 3925-3933. https://doi.org/10.3168/jds.2015-10615

McDougall S and Rhodes FM, 1999. Detection of a corpus luteum in apparently anoestrous cows by manual palpation, transrectal ultrasonography and plasma progesterone concentration. New Zealand Veterinary Journal 47: 47-52. https://doi.org/10.1080/00480169.1999.36110

Mushonga B, Kaurivi B, Chiwome B, Kandiwa E and Habarugira G, 2017. Persistent corpus luteum in a 9year-old Afrikaner cow: A case report. Global Veterinaria 18: 146-150.

Pieterse MC, 1989. Ultrasonic characteristics of physiological structures on bovine ovaries. In: Taverne MAM and Willemse AH (ed), Diagnostic Ultrasound and Animal Reproduction. Kluwer Academic Publishers, The Netherlands, pp:37-51.

Pieterse MC, Taverne MA, Kruip TA and Willemse AH, 1990. Detection of corpora lutea and follicles in cows: a comparison of transvaginal ultrasonography and rectal palpation. The Veterinary Record 126: 552-4.

Quezada-Casasola A, Avendaño-Reyes L, Macías-Cruz U, Ramírez-Godínez JA and Correa-Calderón A, 2014. Estrus behavior, ovarian dynamics, and progesterone secretion in Criollo cattle during estrous cycles with two and three follicular waves. Tropical Animal Health and Production 46: 675-684. https://doi.org/10.1007/s11250-014-0562-0

Quintal-Franco JA, Kojima FN, Melvin EJ, Lindsey BR, Zanella E, Fike KE, Wehrman ME, Clopton DT and Kinder JE, 1999. Corpus luteum development and function in cattle with episodic release of luteinizing hormone pulses inhibited in the follicular and early luteal phases of the estrous cycle. Biology of Reproduction 61: 921-926. https://doi.org/10.1095/biolreprod61.4.921

Rocha CC, Martins T, Cardoso BO, Silva LA, Binelli M and Pugliesi G, 2019. Ultrasonography-accessed luteal size endpoint that most closely associates with circulating progesterone during the estrous cycle and early pregnancy in

beef cows. Animal Reproduction Science 201: 12-21. https://doi.org/10.1016/j.anireprosci.2018.12.003

Salman A, Prihatno SA and Sumiarto B, 2021. Reproductive performance of beef cattle with ovarian hypofunction and repeat breeding in Jepara Regency, Central Java, Indonesia. Veterinary world 14: 784-787. https://doi.org/10.14202/vetworld.2021.784-787

Siregar TN, Hafizuddin H, Akmal M, Sayuti A, Aliza D, Melia J, Armansyah T, Syafruddin S, Panjaitan B and Adani DL, 2016. Follicle dynamics of aceh cattle during estrous cycle. Global Veterinaria 17: 424-429.

Skovorodin E, Mustafin R, Bogoliuk S, Bazekin G and Gimranov V, 2020a. Clinical and structural changes in reproductive organs and endocrine glands of sterile cows. Veterinary World 13: 774-781. http://doi.org/10.14202/ vetworld.2020.774-781

Skovorodin E, Bogolyuk S, Bazekin G, Sharipov A and Khokhlov R, 2020b. Morphology and histochemistry of the corpus luteum (CL) of ovaries of pregnant and infertile cows. American Journal of Animal and Veterinary Sciences 15: 257-265. https://doi.org/10.3844/ajavsp.2020.257.265

Viana JHM, Arashiro EKN, Siqueira LGB, Ghetti AM, Areas VS, Guimarães CRB, Palhao MP, Camargo LSA and Fernandes CAC, 2013. Doppler ultrasonography as a tool for ovarian management. Animal Reproduction 10: 215-222.

Whitfield L, 2018. Ovarian ultrasound for fertility management in dairy cattle. Livestock 23: 154-160. https://doi.org/10.12968/live.2018.23.4.154