

Evaluation of corpus luteum vascularization in pregnant mares using colour Doppler ultrasonography

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Abstract

This study aimed to assess changes in corpus luteum (CL) vascularization using Colour Doppler Ultrasonography (CDU) based on pregnancy status and gestational age. A total of 26 Arabian mares were included, comprising 16 pregnant (Group P) and 10 non-pregnant (Group C) mares. The CL images were obtained on post-ovulation days 15, 25, 35, 45, 60, 75, 90, and 120. On day 15, the coloured pixel area (CPA) values of pregnant mares were significantly larger than those of non-pregnant mares ($P < 0.001$), while no significant differences were found in coloured pixel mean (CPM) and corpus luteum area (CLA) values. The lowest CPA and CLA values were observed on D120 which was significantly lower than at all time points except D90 (for CPA) and D60 (for CLA) ($P < 0.05$), but CPM values remained stable. A significant decrease in vascularization was detected between early and mid-pregnancy ($P < 0.05$). In Group P, a negative correlation between age and CPA ($\rho = -0.35$ to -0.52) was observed, indicating a significant decline in CL vascularization efficiency with advancing age. Time dependent differences in CPA and CLA were significantly related to mares' age, parity, and foetal sex ($P < 0.001$). Both CPA and CLA values on D35 were higher in younger (< 10 years) compared to older mares (> 10 years) ($P < 0.05$). The CLAs on D35 were higher in the 1–3 parities group compared to the 4–6 parities group ($P < 0.05$), while CPA did not exhibit significant changes related to the parity. These findings suggest that pregnancy status significantly affects CL vascularization patterns. Older mares exhibit reduced vascularization capacity, which may impact reproductive management strategies.

Equine reproduction, gestational haemodynamics, vascular imaging

Reproductive efficiency in mares is a critical factor in equine breeding programs, and early embryonic losses present a significant challenge. Bollwein et al. (2002) demonstrated that the viability and functionality of the corpus luteum (CL) depend on vascular support, and a reduction in blood flow leads to a rapid decrease in progesterone production, initiating luteolysis. Additionally, prostaglandin F2 α (PGF2 α) accelerates this process by inducing vasoconstriction, leading to CL regression (Bollwein et al. 2002). Maternal recognition of pregnancy modifies CL function to sustain progesterone secretion, which is essential for pregnancy maintenance, as the primary CL remains the sole source of progesterone during the first 40 days of gestation (Allen 2001a). Early embryonic losses in mares are significantly influenced by the functional capacity of the CL, which produces progesterone necessary for pregnancy maintenance. Without adequate CL support, pregnancy loss may occur within the first few weeks, emphasizing the need to assess luteal vascularization and function (Herzog et al. 2011).

The CL plays a crucial role in pregnancy maintenance by producing progesterone, which supports embryonic development and prevents luteolysis (Şenünver and Gültiken 2015). Understanding the vascularization dynamics of the CL throughout pregnancy is essential for evaluating reproductive efficiency and predicting pregnancy outcomes. Advances

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in ultrasonographic techniques, particularly Colour Doppler Ultrasonography (CDU), have enabled non-invasive assessment of luteal blood flow, which is strongly associated with progesterone secretion and luteal function (Ginther et al. 2006; Kurban and Günay Uçmak 2023). Several studies have demonstrated that CL blood flow serves as an indicator of corpus luteum functionality (Salim et al. 1994; Guerriero et al. 1999). Colour Doppler Ultrasonography is widely used to assess ovarian blood flow, follicular development and luteal perfusion (Bollwein et al. 2002; Uçmak et al. 2020a; Kurban and Günay Uçmak 2023). Previous studies suggested that luteal function decreases with increasing age, thereby reducing reproductive efficiency and pregnancy maintenance rates (Hori et al. 2019).

Despite the growing literature on CDU applications in equine reproduction, there is limited data on the temporal changes in CL vascularization during pregnancy and its correlation with reproductive parameters. This study aims to evaluate the temporal changes in CL vascularization in pregnant mares using CDU and to provide a comprehensive analysis of CL area (CLA), the coloured pixel area (CPA), and coloured pixel mean (CPM) values at different gestational stages. Additionally, by including a control group of non-pregnant mares, this study seeks to identify significant differences in CL vascularization patterns between the two groups and contribute to understanding luteal function in equine reproduction.

Materials and Methods

All animal experiments were performed according to the following guidelines in compliance with the Istanbul University-Cerrahpaşa Veterinary Faculty Unit Ethics Committee (Approval number: 2020/04).

Animals and study design

This study was conducted between February and June in the Northern Hemisphere (41°N latitude) on 26 Arabian mares aged 4 to 16 years. The mares were housed in open shelters under natural lighting conditions, with *ad libitum* access to water and mineral salts. They were fed a diet of roughage supplemented with 2.5 kg of concentrated feed per meal. Their body condition scores (BCS) ranged from 5 to 6 (Kentucky Equine Research, USA). All mares were randomly selected from those that had delivered at least one healthy foal. To ensure reproductive health, endometritis screening was performed using three non-invasive techniques as described by Uçmak et al. (2020b); rectal palpation, transrectal ultrasonography and cytobrush technique. Only mares without signs of endometritis were included in the study. Selected mares were divided into two groups: pregnant mares (Group P, $n = 16$) and non-pregnant control mares (Group C, $n = 10$). Mares in Group P were naturally bred with different stallions during the breeding season. Ovulation was monitored, and pregnancy was confirmed via transrectal ultrasonography equipped with a 7.5 MHz linear-array transducer (Esaote MyLab One Vet, Esaote Pie Medical, Genoa, Italy) on day 15 post ovulation (Gain 28%, Pulsed Repetition Frequency 1 kHz). In contrast, mares in Group C were not mated, but their ovulation was tracked, and CL was evaluated using transrectal ultrasonography on day 15 post ovulation.

Ultrasound examinations were performed on days 15 (K1), 25 (K2), 35 (K3), 45, 60, 75, 90, and 120 post ovulation. As suggested by Sales et al. (2021), areas (cm^2) of the corpus luteum (CLA) were measured from the fixed image in B-mode ultrasonography. To assess temporal changes in CL vascularization, CDU was used on predetermined pregnancy days in Group P. However, for comparative analysis, only K1 data from Group P was used to compare with Group C. Doppler images were analysed using ImageJ software, allowing measurement of CPA (mm^2) and CPM with coloured pixel areas of the corpus luteum. The characteristics of CL in pregnant mares were also evaluated related to mares' age, parity, and foetal sex.

Statistical analyses

Statistical analyses were conducted using IBM SPSS Statistics (SPSS 31.0, IBM, USA). Shapiro-Wilk test was used to assess data distribution normality. Independent t -tests were used for normally distributed variables. Mann-Whitney U tests were applied for non-normally distributed variables. Time dependent differences about mare age, parity, and foetal sex in pregnant mares were evaluated with Repeated Measures ANOVA. The differences between the groups at the same time-points were assessed by independent samples t -test. Additionally, Spearman correlation analysis was conducted to examine relationships between mare age, parity, and CL indices over time. A P value ≤ 0.05 was considered significant.

Results

The mean age of the mares in Group P was 7.56 ± 0.81 years, whereas that of Group C was 9.00 ± 0.90 years. When previous births were evaluated, Group P had an average parity

of 4.00 ± 0.59 , while Group C had an average parity of 3.10 ± 0.45 . Additionally, of the mares included in Group P, five gave birth to female foals and eleven to male foals. The pregnant mares were divided into two age groups as < 10 years mares ($n = 7$) and > 10 years mares ($n = 9$), and the characteristics of CL were evaluated between the different age groups. Likewise, the pregnant mares were divided into two groups in terms of parity as mares with 1–3 parities ($n = 7$) and mares with 4–6 parities ($n = 9$), and they were evaluated in terms of CL characteristics.

Comparison between pregnant and non-pregnant mares on day 15 post ovulation

On day 15 post ovulation (K1), the CPA value of CL showed significant differences between pregnant and non-pregnant mares ($P < 0.05$), whereas CPM and CLA values did not show significant differences ($P > 0.05$). Additionally, the CPA value of CL in pregnant mares was significantly higher than that in non-pregnant mares ($P < 0.001$) (Plate VI, Fig.1). These findings indicate that pregnancy status influences luteal CPA, but does not have a statistically significant effect on CPM and CLA. The CL parameters recorded on day 15 post ovulation are presented in Table 1.

Table 1. Values of corpus luteum (CL) parameters in the study mares on day 15 post ovulation (K1).

CL indices	Group P ($n = 16$)	Group C ($n = 10$)	<i>P</i> value
Coloured pixel area (mm^2)	16.48 ± 1.61^a	4.21 ± 1.16^b	< 0.001
Coloured pixel mean	126.45 ± 3.38	112.65 ± 9.42	0.19
CL area (cm^2)	5.42 ± 0.39	4.52 ± 0.40	0.14

^{a,b} Different superscripts in the same row indicate significance at $P < 0.05$

Temporal changes in CL vascularization in pregnant mares

The changes in CL vascularization over time were evaluated in pregnant mares (Group P) to analyse the progression of vascularization patterns. As pregnancy progressed, CLA ($P < 0.001$) and CPA ($P < 0.001$) showed a significant decrease (Fig. 2) in Group P regardless of the mare's age, parity, and foetal sex. However, time dependent changes were not significant in the CPM value ($P > 0.05$). The lowest CPA value was recorded in D120 which was significantly lower than in all time points except D90 (Fig. 2A) ($P < 0.05$). A significant decrease in vascularization was detected from between early and mid-pregnancy (K1, K2 vs. D75 to D120, $P < 0.05$). Similarly, the lowest CLA value was observed in D120 which was significantly lower than in all time points except D60 (Fig. 2B) ($P < 0.05$). The highest CLA was observed in D45 which was significantly higher than in all time points except D60 ($P < 0.05$). Although the time dependent differences were significant in CPA and CLA, CPM remained stable throughout pregnancy ($P > 0.05$).

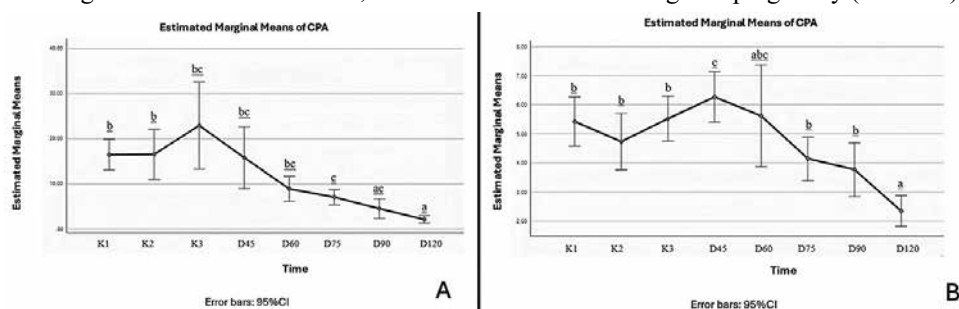


Fig. 2. Time-dependent changes in corpus luteum (CL) characteristics in Group P. A: Time-dependent differences in coloured pixel area (CPA); B: Time-dependent differences in corpus luteum area (CLA).

^{a,b,c} Values with different superscripts are significantly different ($P < 0.05$)

Table 2. Time dependent differences in coloured pixel area (CPA) and corpus luteum area (CLA) related to mares' age, parity, and foetal sex in pregnant (values are presented as means $\pm$ SEM).

Group P characteristics		K1	K2	K3	D45	D60	D75	D90	D120	P value
CPA	Age	<10 years	19.04 $\pm$ 2.81	17.71 $\pm$ 4.66	33.17 $\pm$ 8.73 ^a	17.48 $\pm$ 5.47	8.61 $\pm$ 2.14	6.44 $\pm$ 1.35	4.39 $\pm$ 1.94	2.50 $\pm$ 0.74
		>10 years	14.49 $\pm$ 1.66	15.67 $\pm$ 3.12	15.01 $\pm$ 2.41 ^b	14.54 $\pm$ 4.04	9.22 $\pm$ 1.71	7.62 $\pm$ 0.99	4.69 $\pm$ 1.06	1.87 $\pm$ 0.40
	P value	ns	ns	<0.05	ns	ns	ns	ns	ns	<0.05
	Parity	1-3 parities	19.53 $\pm$ 2.80	15.22 $\pm$ 4.67	30.51 $\pm$ 8.41	16.53 $\pm$ 5.57	6.64 $\pm$ 1.0	6.69 $\pm$ 1.19	4.77 $\pm$ 1.85	2.83 $\pm$ 0.66
CLA		4-6 parities	14.12 $\pm$ 1.57	17.61 $\pm$ 3.10	17.08 $\pm$ 4.15	15.28 $\pm$ 4.01	10.75 $\pm$ 2.03	7.43 $\pm$ 1.12	4.40 $\pm$ 1.16	1.61 $\pm$ 0.40
	P value	ns	ns	ns	ns	ns	ns	ns	ns	>0.05
	Foetal sex	Male	17.02 $\pm$ 1.96	19.75 $\pm$ 3.33	27.48 $\pm$ 5.83	15.81 $\pm$ 3.53	8.39 $\pm$ 1.53	7.42 $\pm$ 1.07	5.06 $\pm$ 1.44	2.59 $\pm$ 0.47
		Female	15.30 $\pm$ 3.08	9.55 $\pm$ 1.69	13.00 $\pm$ 4.81	15.87 $\pm$ 7.41	10.20 $\pm$ 2.61	6.42 $\pm$ 1.08	3.45 $\pm$ 0.40	1.17 $\pm$ 0.46
CPA	P value	ns	=0.05	=0.09	ns	ns	ns	ns	ns	=0.07
	Group P characteristics	K1	K2	K3	D45	D60	D75	D90	D120	P value
	Age	<10 years	6.02 $\pm$ 0.74	5.55 $\pm$ 0.76	6.38 $\pm$ 0.43 ^a	6.73 $\pm$ 0.59	5.97 $\pm$ 1.70	3.48 $\pm$ 0.55	2.95 $\pm$ 0.57	1.89 $\pm$ 0.19
		>10 years	4.96 $\pm$ 0.37	4.10 $\pm$ 0.48	4.85 $\pm$ 0.44 ^b	5.91 $\pm$ 0.56	5.34 $\pm$ 0.73	4.67 $\pm$ 0.39	4.19 $\pm$ 0.61	2.72 $\pm$ 0.38
CLA	P value	ns	ns	<0.05	ns	ns	=0.09	ns	ns	=0.09
	Parity	1-3 parities	6.11 $\pm$ 0.72	5.57 $\pm$ 0.76	6.47 $\pm$ 0.42 ^a	6.48 $\pm$ 0.60	4.43 $\pm$ 0.87	4.06 $\pm$ 0.62	3.43 $\pm$ 0.55	1.97 $\pm$ 0.17
		4-6 parities	4.89 $\pm$ 0.36	4.08 $\pm$ 0.47	4.79 $\pm$ 0.42 ^b	6.11 $\pm$ 0.58	6.53 $\pm$ 1.25	4.21 $\pm$ 0.42	4.04 $\pm$ 0.65	2.65 $\pm$ 0.40
	P value	ns	ns	<0.05	ns	ns	ns	ns	ns	<0.05
CPA	Foetal sex	Male	5.28 $\pm$ 0.53	4.47 $\pm$ 0.63	5.56 $\pm$ 0.44	6.08 $\pm$ 0.56	5.94 $\pm$ 1.06	4.04 $\pm$ 0.41	3.95 $\pm$ 0.56	2.24 $\pm$ 0.32
		Female	5.75 $\pm$ 0.53	5.32 $\pm$ 0.40	5.44 $\pm$ 0.69	6.69 $\pm$ 0.45	4.90 $\pm$ 1.32	4.38 $\pm$ 0.72	3.38 $\pm$ 0.67	2.61 $\pm$ 0.39
	P value	ns	ns	ns	ns	ns	ns	ns	ns	>0.05

^{a,b} Different superscripts in the same column indicate significance ($P < 0.05$).

CPA: Colour pixel area; CLA: corpus luteum area; ns: not significant; SEM: standard error of the mean; K1: Day 15, K2: Day 25, K3: Day 35.

Changes in Group P were evaluated in terms of the mares' age, parity, and foetal sex. According to these subgroups, time dependent differences in CPM were not significantly related to mares' age, parity, and foetal sex ($P > 0.05$). However, time dependent differences in CPA and CLA were significantly related to mares' age, parity, and foetal sex ($P < 0.001$) (Table 2). Both CPA and CLA values in K3 were higher in younger mares (< 10 years) compared to older mares (> 10 years) ($P < 0.05$). Also, the CLA in K3 were higher in the 1–3 parities group compared to the 4–6 parities group ($P < 0.05$), while CPA did not exhibit significant changes related to the parity ($P > 0.05$). In the evaluation of the difference in vascularization between foetal sexes in Group P, it was observed that CPA values were higher in mares carrying male foetuses compared to those carrying female foetuses only in K2 at a significance level of $P = 0.05$. Correlation analysis showed that CPA significantly decreased with increasing mares' ages ($\rho = -0.35$ to -0.52). Similarly, a negative correlation was observed between age and vascularization efficiency ($\rho = -0.36$ to -0.50), suggesting that as mares age, the vascular structure of CL deteriorates. Although the correlation between parity and CLA was weaker ($\rho = -0.15$ to -0.24), a decreasing trend was observed. These findings statistically support the hypothesis that aging reduces both the morphological characteristics and vascularization capacity of CL.

Discussion

To ensure fertility, mares must have a BCS of at least 5 (Henneke et al. 1984). In line with previous research, all mares included in this study had BCS values between 5 and 6. Reproductive performance in mares is generally optimal until 12 years of age, after which fertility rates gradually and time dependently decrease (Madill 2002). Similarly, the mean age of the mares in this study (Group P: 7.56 ± 0.81 years; Group C: 9.00 ± 0.90 years) falls within the most fertile years of their reproductive lifespan.

Several studies have highlighted that Doppler ultrasonography is a crucial tool for evaluating luteal function and CL vascularization (Bollwein et al. 2002; Ginther and Utt 2004). Consistent with these studies, CDU was used to assess luteal vascularization. However, comparative studies on CL vascularization between pregnant and non-pregnant mares remain limited. Ginther and Utt (2004) reported that CL vascularization in non-pregnant mares undergoes rapid regression, with luteal vascularization decreasing over time due to luteolysis. Similarly, the present study determined that CPA values in Group C were lower than those in Group P, likely because Group C mares lacked the physiological mechanisms required to sustain pregnancy.

Studies have shown that CL remains functionally active until days 140–180 of pregnancy but starts to regress around day 100 (Knowles et al. 1993). Limited studies exist on the changes in CL size and vascularization throughout equine pregnancy. Durfee and Frates (1999) conducted a detailed analysis of the CL size, ultrasonographic variations, and vascularization in early pregnancy, stating that low-resistance blood flow indicates continued CL function. In our study, it was observed that luteal vascularization was preserved and exhibited a more stable structure in Group P. In particular, CPM values remained constant throughout pregnancy, indicating that a certain level of blood flow is maintained for the continuation of pregnancy. However, similar to Durfee and Frates (1999), CLA and CPA values in Group P decreased in the later months of pregnancy. Brogan et al. (2016) reported that luteal blood flow was the highest in early pregnancy, decreasing as pregnancy progressed. A study conducted by Sales et al. (2021) in mares reported that luteal blood flow increased in the first three weeks of pregnancy but reached a balance in the following periods. Similarly, in Group P, the lowest CPA and CLA were observed in D120. These results reflect the regression of CL around day 100 and start to

decline in its function until days 140–180 of pregnancy as previously reported (Knowles et al. 1993).

In mares, maternal identification of pregnancy is achieved by embryonic migration (days 7–16) and prevention of luteolysis (Allen 2001b). When migration ends on day 17 of gestation, the fixation process begins and the contact of trophoblast cells to the uterus increases (Allen 2001b). Trophoblast cells exhibit MHC-I antigens of paternal origin on their surface and generate a response in the mare immune system (Antczak et al. 1982; Kydd et al. 1982). During early gestation and placental development, proinflammatory cytokines activate maternal immunity and promote the release of factors that increase placental invasion and blood flow (Cha et al. 2012). Enninga et al. (2015) reported that proinflammatory cytokines and proangiogenic growth factors were increased in pregnancies with male foetuses, whereas regulatory cytokines were increased in female foetuses. In our study, the K2 CPA values of mares giving birth to male foals were significantly higher than those of mares giving birth to female foals ($P = 0.05$). It is considered that this difference observed only in the K2 period may be due to embryonic fixation and sex-specific proinflammatory response to trophoblast cells.

Sales et al. (2021) identified a positive correlation ($P < 0.05$) between the CLA and CL vascular perfusion. Also, they observed a positive correlation ($P < 0.05$) between the vascular perfusion in CL and the progesterone concentration in mares (Sales et al. 2021). Hori et al. (2019) reported that CL vascularization capacity decreased in aged cows, which had a negative effect on progesterone production. Similarly, Miyazaki et al. (1998) demonstrated that luteal vascularization decreases with increasing age in humans and this has effects on fertility. Although the species in our study was different from other studies, CPA and CLA values in K3 were significantly higher in younger mares (< 10 years) than in older mares (> 10 years) ($P < 0.05$). Significant results obtained in both CPA and CLA should be explained by Sales et al. (2021) who reported positive correlation ($P < 0.05$) between the CLA and CL vascular perfusion. The decrease in CPA and CLA values in older mares suggests that reproductive management strategies should be optimized in older mares. As highlighted in humans (Miyazaki et al. 1998), decreased luteal vascularization with age can affect the fertility of mares.

The present study is largely in agreement with previous studies in the literature and provides a detailed description of how luteal vascularization changes during pregnancy in mares. At 17 to 30 days after ovulation, increased follicular activity in the ovaries occurs and secondary ovulation or follicular luteinization takes place from about 40 days (Allen 1970). Formation of secondary CL coincides with the presence of pregnant mare serum gonadotropin (PMSG). Also, increase in progesterone between days 35 and 45 was coincidental with the initial increase in PMSG (Squires et al. 1979). The significant results obtained in K3 in our study can be explained by the stimulatory effect of increased progesterone and PMSG on luteinization and luteal vascularization, consistent with the previous reports (Allen 1970; Squires et al. 1979).

Rosa Filho et al. (2025) investigated oxidative changes in mares during the last third of gestation and the postpartum period, considering age and parity. They indicated that both parity and age were considered crucial factors for the oxidative status in healthy mares (Rosa Filho et al. 2025). In this study CLA in K3 was significantly higher in mares with 1–3 parities compared to the mares with 4–6 parities. As Rosa Filho et al. (2025) highlighted similar effects of age and parity on fertility, it is thought that the same endocrinological mechanism (PMSG and progesterone) plays a significant role in luteal activity in mares with 1–3 births at K3 as in younger mares.

This study provides new evidence that pregnancy status significantly influences early CL vascularization, and maternal factors such as age and parity contribute to structural regression of the CL. These findings emphasize that monitoring CL dynamics through CDU could serve

as a predictive marker for pregnancy success and has important clinical implications for equine breeding programs.

Given the negative impact of mare age on luteal function, CDU can serve as a non-invasive tool for identifying mares at risk of early embryonic loss and guiding reproductive management strategies for improved pregnancy outcomes. This study contributes to the understanding of pregnancy-associated changes in CL vascularization and highlights the importance of maternal factors in CL regression.

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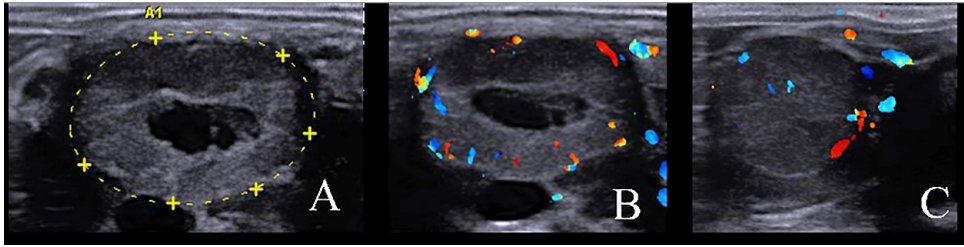


Fig. 1. Images of corpus luteum (CL). A: CL area measurement in a 15-day pregnant mare; B: vascularization of the CL (high vascularization) in a 15-day pregnant mare; C: vascularization of the CL (moderate vascularization) in a non-pregnant mare 15 days after ovulation.