

The echotexture and morphometric assessment of the uterus and ovarian structures coinciding with hormonal parameters during early gestation of Arabian mares

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ARTICLE INFO

Received: 08 July 2026

Accepted: 21 September 2026

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Keywords:

Accessory corpora lutea, Arabian mares, Colored area, Early pregnancy, echotexture, Primary corpora lutea

ABSTRACT

Arabian horses are characterized by distinctive features, such as beauty, athletic performance, and heat tolerance, which are supported by functional and structural genes. Hormonal profiling and ultrasonography (US) are considered non-invasive tools for routine pregnancy diagnosis and evaluation. Therefore, this study aimed to evaluate the uterine and ovarian structures' haemodynamic, echotexture, and morphometric characteristics in relation to the hormonal profile during early pregnancy in Arabian mares. Therefore, 12 Arabian mares with a mean age of 7.6 years were subjected to natural breeding by three fertile Arabian stallions every other day throughout estrus till ovulation was detected by ultrasound (US). After embryonic vesicles were detected on Day (D) 14, blood samples and US examinations were then performed on days 35, 45, and 60 of the pregnancy. A larger ($P < 0.05$) dominant follicle (>30 mm) was observed at D 35 with a lower ($P < 0.05$) uterine echotexture. Whereas the primary and accessory corpora lutea (CLs) size, count, and echotexture showed no significant difference. Conversely, the largest CLs colored areas (mm^2) were observed on D 45. Nitric oxide (NO), total antioxidant capacity (TAC), and hormonal values showed no significant differences. In conclusion, these findings suggest that these values are consistent with the normal physiological state of early pregnancy and may serve as a reference range providing a baseline for future research and clinical assessments in this breed. Moreover, the observed larger primary CL colored area on D 45 supports the hypothesis of its resurgence, which reflects the second luteal response to pregnancy in equines.

Introduction

Equine pregnancy is typically diagnosed at day (D) 14 after ovulation, enabling early identification and management of twins using the transrectal ultrasonography (US) approach (Renaudin and Conley, 2023). Consequently, this management can lessen the risk of unfavorable outcomes before endometrial cup formation. Mares are rechecked at 33-35 days to detect abnormal pregnancy development or loss, allowing for termination and re-breeding before endometrial cup formation. Subsequently, a US exam is performed between days 45 and 60 to monitor supplementary corpora lutea (CLs) and fetal well-being (Burden, 2022). Pregnancy losses are still a concern, particularly early in gestation (Renaudin and Conley, 2023). According to a previous study, 10–15% of equine pregnancies result in pregnancy loss before day 40 (Allen, 2001). Also, there may be a higher chance of loss for embryos that “fix” somewhere other than the base of a uterine horn (i.e., uterine body) (Burden, 2022). Ultrasound (US) findings may indicate impending embryonic loss, such as an embryo that is small for its exact gestational age or that fails to progress in development and increase in size, or that is not aligned properly with the ventral surface, failure to develop a visible embryo inside the embryonic vesicle, failure to develop a heartbeat at the expected time, or loss of a previously observed heartbeat (Pycok, 2007; Vanderwall, 2011). As a result, regular monitoring is a crucial clinical practice in equine medicine.

Recently, Doppler technology could be helpful to assess the uterus's hemodynamics using spectral data from the middle uterine arteries (MUAs) and their ramifications (Ferreira and Morais, 2021). Pregnant Trotter mares had higher blood flow velocity and lower resistive index (RI) in their uterine horns compared to empty mares on days 11 and 15–29 of gestation, as reported in a previous study (Bollwein *et al.*, 2003). Subsequently, the blood flow pattern in both uterine arteries (ipsilateral and contralateral to the equine fetus) was investigated in Trotter mares by Bollwein *et al.* (2004) every four weeks, starting from the second week

until the moment of foaling. They observed that blood flow volume in the ipsilateral and contralateral uterine horns significantly increased according to the week from the mid-gestation period. Another study indicated that embryonic vesicle mobility and fixation are accompanied by temporary alterations in vascular perfusion (Silva *et al.*, 2005). Moreover, a spectral Doppler of the uterine artery insertion in the mesometrium was evaluated by Ferreira *et al.* (2015), who reported that it is an efficient approach for the objective evaluation of uterine blood flow during the first 20 days of equine gestation. Till now, no studies have investigated the utility of Doppler technology to assess uterine hemodynamics during early pregnancy in Arabian mares.

Since the US provides structural information on embryonic and fetal growth, endocrine profiles provide additional functional information on fetal and placental competence and development (Renaudin and Conley, 2023). The most notable alteration in hormone levels during pregnancy is the prolongation of luteal activity and increased progesterone (P4) secretion (Swegen, 2021). Yet, P4 levels are commonly measured to discriminate the non-pregnant mares at D 14 after breeding. The thyroid gland plays a crucial role in various biological processes, including growth, metabolism, body heat production, etc (Yen, 2001). The potential positive association between the nitric oxide (NO) levels and uterine hemodynamics has been previously investigated in mares (Welter *et al.*, 2005). Estrogen (E2), another key hormone in female reproduction, may stimulate endothelial NO production, mediate its vascular effects, contributing to the regulation of blood flow and vascular tone within reproductive tissues (Mendelsohn and Karas, 1994). The oxidant system is involved in various reproductive processes, including pregnancy maintenance (Rizzo *et al.*, 2012). Since there is limited research on hormonal and Doppler indices of the MUA in Arabian mares during early gestation, the present study was planned for a profound understanding of the hormonal events taking place during early pregnancy, as well as NO and total antioxidant capacity (TAC) Levels in Arabian mare serum. Concomitantly, hemodynamic, echotexture, and

morphometric assessment of the uterus and ovarian structures.

Materials and methods

Animals

The present study was conducted at Al Khadijah Stud (Giza, Egypt), which housed (n=20) total Arabian mares and (n=3) breeding stallions at the time of the experiment, from June 2024 to October 2024, after being authorized by the Animal Care and Ethical Use Committee of Cairo University's Faculty of Veterinary Medicine (Approval ID: Vet CU13102024986). Currently, 12 regular cyclic Arabian mares, with a mean age of 7.6 years (range, 6–10 years), with at least two previous foalings and a mean body weight of 330 kg (range, 320–350 kg), with a body condition score of 3.5, were stabled individually at night, and during the day, they were released into sand paddocks. Their diet consisted of hay and pelleted rations, with ad libitum water. Mares were examined for two consecutive reproductive cycles to ensure their regular cyclicity and the absence of any reproductive affections. Mares were subjected to daily real-time US during estrus to determine ovulation. Concurrently, natural breeding by three proven fertile Arabian stallions was performed every second day until ovulation. Day -1 was defined as the last day a visible dominant follicle (DF) was present, while Day 0 was defined as the first day without a preovulatory follicle (Bollwein *et al.*, 2003). Mares were diagnosed pregnant by US with embryonic vesicles on D 14 post-ovulation; subsequent US pregnancy monitoring and blood sampling were conducted on D 35, 45, and 60, as shown in Fig.1.

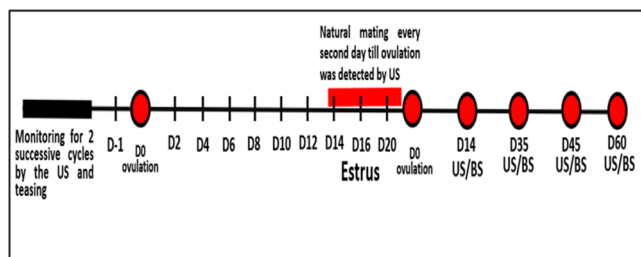


Fig. 1. Schematic diagram of the experimental design. Arabian mares were monitored for two successive cycles by ultrasound and teasing. In the next cycle, mares were monitored from the beginning of the estrus phase by ultrasound (US). Once a dominant follicle of 35 mm was observed, mares were mated every second day by proven fertile purebred stallions in the same stud till ovulation was detected by US. Transrectal US examination was performed 14-, 35-, 45-, and 60-days post-ovulation. Blood sampling was performed on the same days. US= Ultrasound BS= Blood sampling.

Ultrasonography and Image Analysis

Mares were examined using a 7.5 MHz transrectal linear array transducer (EXAGO, Echo Control Medical, Angoulême, France) at 14, 35, 45, and 60 days of pregnancy, with all US device settings adjusted and fixed. The cross-sectional diameters of the largest follicle, primary, and accessory CLs were recorded. The luteal and uterine echogenicity was measured as the mean grey value using a computer-assisted image analysis tool (ImageJ), as shown in Figs. 2 and 3. The CL vascular perfusion was initially assessed subjectively based on the percentage (0%-100%) of luteal tissue exhibiting color Doppler signals during at least a 1-minute scan (Romano *et al.*, 2015). The entire luteal tissue area was centered within the Doppler window. Subsequently, the selected luteal tissue area was enlarged by 1.5 mm using the freehand tool in ImageJ to evaluate the maximum extent of colored particles in mm² invading and surrounding it.

Doppler evaluation of the middle uterine artery

The middle uterine artery (MUA) was visualized for hemodynamic evaluation by transrectal US at 14, 35, 45, and 60 days as previously described (Bollwein *et al.*, 1998). In brief, after identification of the bladder, the external iliac artery was traced laterally, followed by the identification

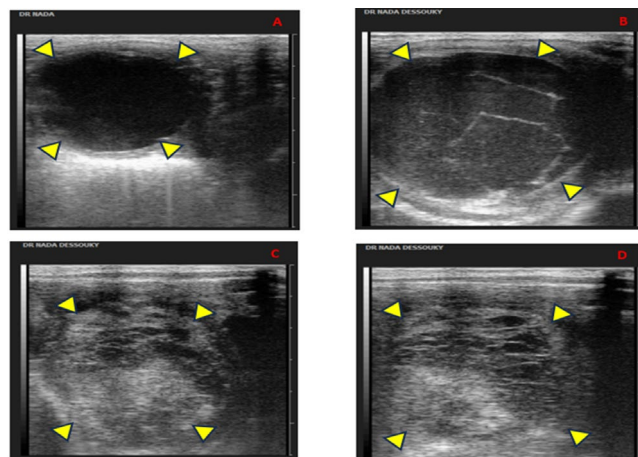


Fig. 2. Ovarian structure morphology assessment using B-mode ultrasound at different time points, including days 15, 35, 45, and 60 of early pregnant Arabian mares. (A) represented the Dominant follicle (DF) at 35 days of pregnancy. (B) represented accessory corpora lutea morphology at 45 days of pregnancy. (C) and (D) represented accessory corpora lutea organization morphology at 60 days of pregnancy in Arabian mares, all structures illustrated with yellow arrows.

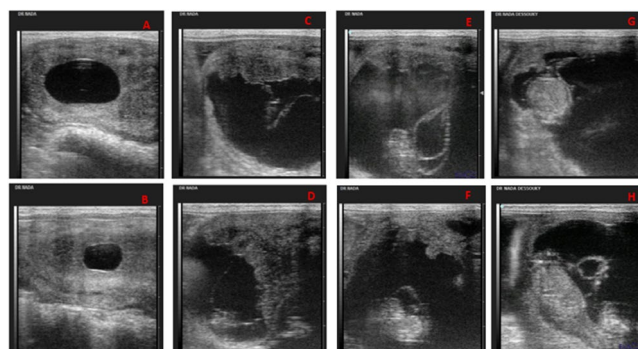


Fig. 3. Uterine echotexture corresponds to pregnant Arabian mares at 14 days (A, B), at 35 days (C, D), at 45 days (E, F), and at 60 days post-breeding (G, H).

of the caudal aspect of the abdominal aorta. At this point, the MUA and the deep circumflex artery diverged. Thereafter, Doppler indices were measured as previously described (Bailey *et al.*, 2012; Esteller-Vico *et al.*, 2017). Briefly, Doppler color flow was used to describe the direction, velocity, and turbulence of blood flow within the MUA segment after the vessel was identified using B-mode US. Thereafter, the pulse repetition frequency (PRF) was adjusted to minimize background noise and maximize arterial boundaries. After a PWD window was placed in the artery's centre, a spectral wave was obtained at an angle of incidence of 50°. All Doppler parameters, including peak systolic velocity (PSV), end-diastolic velocity (EDV), mean velocities, pulsatility index (PI), and resistive index (RI), were automatically calculated and displayed by the US software as shown in Fig. 4. To avoid tachycardia, which might impair the precision of Doppler parameter measurements, stressful situations and phases of mare excitement were avoided. Transrectal pulsed-wave Doppler (PWD) US examinations lasted approximately 15 minutes per mare.

Hormonal, nitric oxide, and total antioxidant capacity Profiling

A 5 mL jugular blood sample was taken into plain blood tubes, allowed to clot for 20-40 min at room temperature, then kept at 4-5°C. Thereafter, sample tubes were spun at 2500 rpm for 10 min at 25°C. Harvested sera were kept in 2 mL Eppendorf tubes at -20 °C until analysis. Hormonal profiles were measured in sera using commercially available solid-phase ELISA Kits for E2 (Cat. No. EIA-2693, sensitivity: 9.714 pg/mL, intra- and inter-assay coefficients of variation (CV) were < 6.81% and < 7.25%, respectively), P4 (Cat. No. EIA-1561, sensitivity: 0.045 ng/mL, intra- and inter-assay coefficients of variation (CV) were = 6.86% and = 5.59%, respectively), T3 (Cat. No. E0149HO, sensitivity: 0.088nmol/L, intra- and inter-assay coefficients of variation (CV) were < 8% and < 10%,

respectively), T4 (Cat. No. E0148HO, sensitivity: 1.55nmol/L, intra- and inter-assay coefficients of variation (CV) were < 8% and < 10%, respectively), whereas NO and TAC were determined spectrophotometrically using commercial colorimetric kits (Bio-diagnostic, Egypt. CAT. No. NO 25 33 and TA 25 13), according to the manufacturer's recommended protocol at the national research centre lab (Animal Reproduction and Artificial Insemination Department-Veterinary Research division, Dokki, Giza, Egypt).

A probability of less than 5% was considered a significant difference ($P < 0.05$).

Results

The morphometric changes of the ovarian structures and echotexture of the uterus and luteal tissues, and color signals of primary and accessory CLs during early gestation days are presented in Table 1. There were no significant differences in follicular and luteal count between the studied time points. In contrast, the DF diameter (mm) on D 35 was significantly ($P < 0.05$) larger than on D 14, 45, and 60 (34.46 ± 2.06 vs 27.5 ± 2.03 , 26.48 ± 0.94 , and 22.78 ± 0.29 , respectively). The uterine echotexture on D 35 was significantly ($P < 0.05$) lower than that on other days (57.37 ± 2.05 vs 74.03 ± 2.34 , 68.33 ± 3.23 , and 77.59 ± 2.21 , respectively). Conversely, the primary and accessory CLs diameters (mm), as well as echotexture showed no significant difference. However, the primary CLs colored areas (mm²) were significantly ($P < 0.05$) higher on D 45 than on the other days (79.81 ± 5.89 vs 56.16 ± 5.85 , 43.76 ± 4.59 , and 22.25 ± 4.57 , respectively), as shown in Fig. 5. In contrast, the colored areas of the accessory CLs (in mm²) did not exhibit significant changes. All hemodynamic parameters of the blood flow within the MUA presented in Table 2 had no significant differences during the studied times. Similarly, all circulating concentrations of E2, P4, T3, T4, NO, and TAC during the studied days of gestation are presented in Table 3 with no significant differences ($P > 0.05$).

Discussion

To the best of our knowledge, this is the first study to comprehensively evaluate the hemodynamic parameters, echotexture, and morphometrical characteristics of the uterus and ovarian structures during the early stages of pregnancy in the Arabian mare. In addition to structural assessments, the study investigates the hormonal, NO, and TAC levels associated with early pregnancy by analyzing serum samples from Arabian mares. This dual approach offers a holistic view of this critical period of equine pregnancy to better understand the complex mechanisms in-

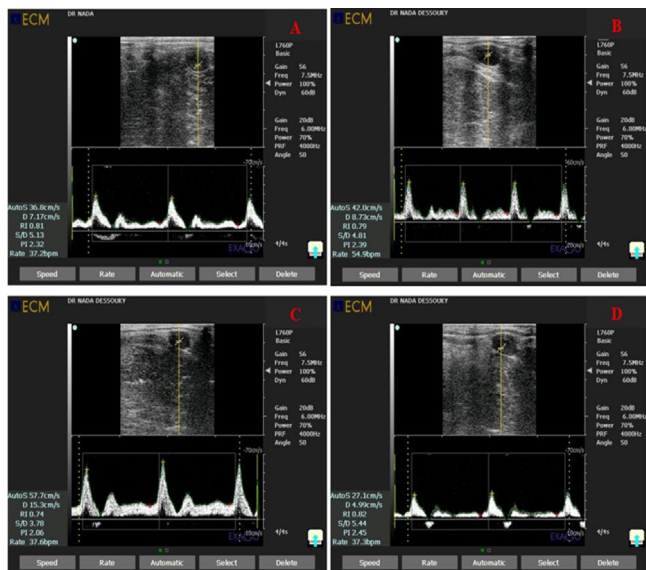


Fig. 4. Doppler indices of blood flow within the middle uterine artery (MUA) waveforms of pregnant Arabian mares on Day 14 (A), Day 35 (B), Day 45 (C), and Day 60 (D).

Statistical analysis

All data were normally distributed by the Shapiro-Wilk test and presented as means $\pm$ standard error of the mean (SEM). Differences in the examined parameters between different time points were assessed using repeated measures ANOVA followed by a Bonferroni post hoc test. Statistical analyses were performed using the GraphPad Prism 5 program.

Table 1. Morphometric changes in the ovarian structures as assessed by transrectal B-mode ultrasonography and Changes in the echotexture and color signals of the uterus and ovarian structures as assessed by color Doppler ultrasonography and computer image analysis software during early pregnancy at 14, 35, 45, and 60 days of Arabian mares (n=12).

	Day 14 of pregnancy	Day 35 of pregnancy	Day 45 of pregnancy	Day 60 of pregnancy	P-value
Follicular count	4.70 $\pm$ 0.67	5.30 $\pm$ 1.30	4.00 $\pm$ 0.00	5.00 $\pm$ 1.00	0.76
DF size (mm)	27.50 $\pm$ 2.03 ^a	34.46 $\pm$ 2.06 ^b	26.48 $\pm$ 0.94 ^a	22.78 $\pm$ 0.29 ^a	0.02
CL count	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.25 $\pm$ 0.25	1.25 $\pm$ 0.25	0.71
CL size (mm)	31.64 $\pm$ 1.57	32.83 $\pm$ 1.37	33.83 $\pm$ 1.23	29.45 $\pm$ 2.07	0.28
Acs CL size (mm)	-	-	42.91 $\pm$ 0.96	43.71 $\pm$ 0.54	0.94
Ut echotexture	74.03 $\pm$ 2.34 ^a	57.37 $\pm$ 2.05 ^b	68.33 $\pm$ 3.23 ^a	77.59 $\pm$ 2.21 ^a	0.00
CL echotexture	95.5 $\pm$ 5.86	81.16 $\pm$ 4.66	90.8 $\pm$ 5.14	82.44 $\pm$ 5.74	0.39
ACs CL echotexture	-	-	76.55 $\pm$ 9.23	90.41 $\pm$ 6.95	0.25
CL colored area (mm ²)	56.16 $\pm$ 5.85 ^a	43.76 $\pm$ 4.59 ^a	79.81 $\pm$ 5.89 ^b	22.25 $\pm$ 4.57 ^a	0.01
ACs CL colored area (mm ²)	-	-	38.24 $\pm$ 4.31	48.73 $\pm$ 4.65	0.12

Data are expressed as Mean $\pm$ standard error of the mean (SEM). DF: Dominant follicle, CL: Corpus luteum, Acs: Accessory, mm: Millimetre, Ut: Uterine. Means with different superscripts are statistically significant, i.e., ^a^b difference between the same rows at different time points.

Table 2. Hemodynamic parameters of blood flow of the middle uterine artery, including resistive index (RI), systolic/ diastolic ratio (S/D), pulsatility index (PI), and mean velocities during early pregnancy at 14, 35, 45, and 60 days of Arabian mares (n=12) as assessed by pulsed wave Doppler ultrasonography.

	Day 14 of pregnancy	Day 35 of pregnancy	Day 45 of pregnancy	Day 60 of pregnancy	P-value
RI	0.78 $\pm$ 0.022	0.76 $\pm$ 0.018	0.72 $\pm$ 0.046	0.78 $\pm$ 0.02	0.31
S/D	4.98 $\pm$ 0.54	4.26 $\pm$ 0.32	3.85 $\pm$ 0.65	4.91 $\pm$ 0.57	0.47
PI	2.13 $\pm$ 0.16	2.11 $\pm$ 0.14	1.84 $\pm$ 0.18	2.26 $\pm$ 0.17	0.47
Rate (bpm)	39.3 $\pm$ 2.31	40.43 $\pm$ 2.61	37.1 $\pm$ 3.61	36.45 $\pm$ 0.96	0.56

Data are expressed as Mean $\pm$ standard error of the mean (SEM). bpm: beats per minute

Table 3. Hormonal profile, nitric oxide, and total antioxidant capacity concentrations during early pregnancy at 14, 35, 45, and 60 days in Arabian mares (n=12).

	Day 14 of pregnancy	Day 35 of pregnancy	Day 45 of pregnancy	Day 60 of pregnancy	P-value
Progesterone (ng/mL)	9.98±0.41	8.46±0.35	9.68±0.63	9.57±0.49	0.4714
Estrogen (pg/mL)	6.69±0.23	6.88±0.28	7.01±0.27	7.14±0.52	0.9828
Triiodothyronine (nmol/mL)	1.19±0.07	1.08±0.09	1.02±0.12	1.03±0.13	0.6075
Thyroxine (nmol/mL)	2.78±0.24	3.08±0.29	3.47±0.29	3.46±0.21	0.2987
nitric oxide (umol/L)	3.78±0.32	4.67±0.81	4.64±0.26	3.39±0.62	0.5658
Total antioxidant capacity (mM/L)	0.71±0.07	0.66±0.11	0.74±0.13	0.63±0.03	0.8342

Data are expressed as Mean ± standard error of the mean (SEM). ng: Nanogram, pg: Picogram, nmol/mL: Nanomole/millilitre, µg: Microgram, umol: Micromole, mM: Millimole, mL: Millilitre, L: Litre

involved. The findings of this research are significant for veterinary practitioners and reproductive specialists, as they establish reference values of ultrasound and hormonal parameters for early pregnancy monitoring in this valuable breed. These benchmarks can improve early diagnosis, management, and intervention strategies, ultimately enhancing reproductive success rates.

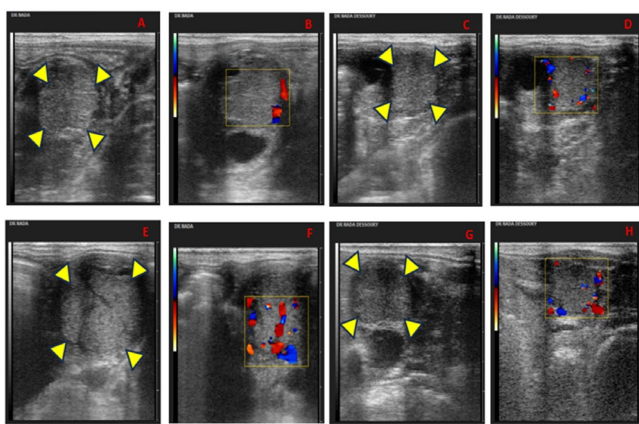


Fig. 5. Luteal morphology defined by B-mode and colored particles using color Doppler mode. (A), (C), (E), and (G) represent primary corpora lutea in B-mode at 14, 35, 45, and 60 days of pregnancy in Arabian mares, illustrated with yellow arrows, respectively. Whereas (B), (D), (F), and (H) represent primary corpora lutea colored particles, as measured by color Doppler US, on 14, 35, 45, and 60 days of pregnancy, respectively.

The current study observed an increase in DF diameter and reduced uterine echotexture on D 35, closely linked to the formation of endometrial cups, which occur around this time (Burden, 2022). Endometrial cups are notable for their prolonged activity, lasting from D 35 to beyond D 120 (Pycock, 2007). Conversely, circulating E2 levels did not show significant fluctuations. The authors suggest that this may be due to the P4 dominance during pregnancy (Samper *et al.*, 2006). This follicular phenomenon is stimulated by equine chorionic gonadotropin (eCG) (Burden, 2022). As a result, the supplemental CLs, including accessory and secondary types, were developed. Accessory CLs originate from the luteinization of anovulatory follicles, typically feature an intraluteal cavity, and exhibit a developmental trajectory analogous to that of haemorrhagic anovulatory follicles (Pierson and Adams, 1995; Samper *et al.*, 2006). Currently, this type was observed on D 45. This explains the larger diameter of supplemental CLs recorded in the present study and the lack of significant differences in the echotexture and colored area (mm²). Conversely, secondary corpora lutea, which result from the ovulation of a secondary follicular wave during pregnancy or diestrus when P4 levels are dominant (Samper *et al.*, 2006), were not detected in this study. This suggests that the development of secondary corpora lutea may be less prominent or occur under different physiological conditions during early pregnancy in mares.

The study revealed no significant difference in primary luteal echogenicity among the studied time points. Similar findings were reported by Samper *et al.* (2006). This consistency suggests that echogenicity alone may not be a reliable indicator of luteal activity or pregnancy status in mares. Moreover, the present study observed no significant variations in the size of the primary CLs or circulating P4 levels across different exam-

ination days compared with previously reported findings (Ginther, 1992; Samper *et al.*, 2006). Persistence of the primary CL beyond the anticipated period of luteolysis, which is 14-16 days post-ovulation in non-pregnant mares, constitutes the first luteal response to pregnancy (Ginther, 1992; Pierson and Adams, 1995). While a secondary luteal response was identified between D 30 and 35 of gestation, represented by the primary CL resurgence, which was characterized by increased systemic P4 levels around D 35 (Ginther, 1992), with a pronounced rise 2 days later. The decline in P4 levels, followed by a resurgence and significant increase, was not influenced by accessory CLs, as their contribution was not detected until after D 40 (Samper *et al.*, 2006). However, the limited number of sampling points in the present study may have restricted the ability to observe more nuanced hormonal fluctuations, compared to previous studies that monitored daily changes from D 0 to 40 post-ovulation. Interestingly, the present study identified a significant increase in the colored area of the primary CL (mm²) on D 45 of pregnancy, which could support the hypothesis of a secondary luteal resurgence morphologically. Consequently, this finding provides clinical evidence that could help veterinarians and practitioners to evaluate the second luteal response to pregnancy in this valuable breed. A positive correlation between luteal tissue vascular perfusion and plasma P4 levels in mares has been demonstrated by Romano *et al.* (2015). SO, further research with more frequent ultrasound monitoring is needed to better understand luteal vascular dynamics during early pregnancy in Arabian mares.

Thyroid hormones play a vital role in maintaining normal ovarian function and supporting follicular growth and development. Beyond reproductive health, these hormones are crucial regulators of systemic metabolic processes, influencing energy expenditure, thermoregulation, and overall metabolic balance. In the current study, T3 and T4 concentrations did not change significantly across the time points examined. This finding aligns with previous research conducted on different horse breeds, such as Italian Saddle, Sanfratellano, Thoroughbred (Fazio *et al.*, 2016), and broodmares (Meredith and Dobrinski, 2004). Such consistency suggests that thyroid hormone levels remain relatively stable during early pregnancy across different equine species. Notably, Fazio *et al.* (2016) found elevated T3 levels in pregnant mares compared to non-pregnant ones only during the 7th and 12th weeks of gestation. Thyroid-stimulating hormone (TSH) was not quantified in the present study due to notable methodological and physiological constraints. Primarily, commercially available assay kits lack the requisite structural cross-reactivity for the reliable quantification of endogenous equine TSH, rendering peripheral evaluation of T3 and T4 the established and practical methodology for monitoring systemic metabolic status during early gestation. Additionally, our primary objective in the current study focused on evaluating peripheral thyroid status and metabolic adjustments during early equine gestation, where circulating pools of active T3 and T4 directly dictate maternal metabolic and systemic support for early embryonic development. However, TSH measurements could be an important avenue for future, specialized equine neuroendocrine investigations. Indeed, accurately assessing thyroid function during pregnancy necessitates the use of specific reference ranges tailored to the test methods and species involved. Establishing these ranges is essential for distinguishing physiological

variations from pathological conditions. The present study contributes valuable data during the early gestation stages in the Arabian breed, providing a baseline for future research and clinical assessments. While fluctuations are minimal in the initial stages, breed, environmental factors, and precise ranges should be considered to interpret hormonal data accurately and ensure optimal reproductive outcomes.

TAC level is significantly modulated by the level of oxidative stress present (Chrysohoou *et al.*, 2007). Previous studies have established that breed is a significant determinant of both total oxidative stress (TOS) and TAC (Bażanów *et al.*, 2020). The latter study reported that the Arabian mares exhibited higher levels of oxidative stress, as indicated by a greater mobilization of cellular antioxidant agents, measured by TAC, compared to Hucul mares. This suggests that Arabian mares may have a more pronounced physiological response to external stressors due to environmental and constitutional differences. In the present study, non-significant changes in the serum TAC levels could be attributed to constant oxidative stress levels during the normal early days of equine pregnancy. Also, the present study findings could provide valuable reference data during the early gestation stages in the Arabian breed, providing a baseline for future research and clinical assessments.

All non-significant Doppler indices of the MUA could be attributed to fewer changes in embryonic development in the first 60 days of equine pregnancy. These findings coincide with those reported in an earlier study in trotter mares by Bollwein *et al.* (2003), who observed higher blood flow velocity and lower RI on days 11 and 15 to 29 of pregnancy. Also, the present study results are in agreement with previous study findings reported by Bollwein *et al.* (2004), who observed that blood flow volume in the ipsilateral and contralateral uterine horns started to significantly increase during the mid-gestation weeks. The potential positive association between the NO levels and uterine hemodynamics has been previously investigated in mares (Welter *et al.*, 2005). Consequently, the non-significant MUA Doppler indices during the present study could be explained by the non-significant changes in NO levels.

Conclusion

Doppler US showed that the primary CL had increased colored particle areas at D 45 of gestation, providing morphological evidence of its resurgence. Combining ultrasonographic and hormonal data offers a holistic view of early pregnancy in Arabian mares. The NO, TAC, and hormonal values in this study require further research on a large scale in Arabian horse studs to establish reference ranges, aiding veterinary practitioners and reproductive specialists in assessing this crucial early pregnancy period.

Acknowledgments

The authors are grateful to acknowledge Al Khadijah Stud for purebred Arabian horses in the Giza governorate, Egypt, for allowing the use of animals.

Conflict of interest

The authors have no conflict of interest to declare.

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