

Discrimination of Early Pregnancy and Endometrial Cyst by Ultrasonographic Assessment of Uterine Echotexture in Mares

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Abstract

The present study was performed to demonstrate the tissue differentiation of uterine ultrasonographic image by computer assisted analysis programs in mares with endometrial cyst and early pregnancy. A total of 124 thoroughbred Arabian mares were used in the study, with early pregnancy and endometrial cyst. Mares were divided into six groups according to stage of early pregnancy (Group I, n=12 (13th day pregnancy); Group II, n=15 (14th day pregnancy); Group III, n=29 (15th day pregnancy); Group IV, n=17 (16th day pregnancy); Group V, n=12 (17th day pregnancy) and as endometrial cysts (Group VI, n=39). The mean gray value (MGV), heterogeneity (HET) and contrast (CON) of the uterine images that were recorded during ultrasound scan were measured through computer assisted program. The MGV values of the echotexture parameters were significantly higher in the pregnant mare (55.20 ± 4.95 , 58.31 ± 3.88 , 56.23 ± 1.88 , 54.15 ± 3.47 and 56.08 ± 2.94 , respectively) than the endometrial cyst mares (42.71 ± 0.71) ($P < 0.001$). The HET values were significantly higher in the pregnant mare (20.75 ± 1.53 , 19.23 ± 1.39 , 21.42 ± 0.88 , 22.37 ± 1.32 and 22.20 ± 1.09 , respectively) than the endometrial cyst mares (15.06 ± 0.44) ($P < 0.001$). CON values were lower in pregnant mare (152.83 ± 7.02 , 144.02 ± 8.18 , 141.03 ± 4.83 , 145.93 ± 4.70 and 154.65 ± 4.86 respectively) than endometrial cyst mare (191.35 ± 3.97) ($P < 0.001$). As a result; it has been concluded that the measurement values of echotexture parameters (MGV, HET, CON) may be important reference criteria when early pregnancy and endometrial cyst are distinguished in mare, which can often cause misconception.

Keywords: Early pregnancy, Endometrial cyst, Echotexture, Mare

Kısraklarda Ultrasonografik Uterus Ekotekstür Değerlendirmesinde Erken Gebe ve Endometriyal Kist Ayırımı

Öz

Sunulan çalışma kısraklarda erken gebelik ile endometriyal kistli uterusun ultrasonografik görüntüsünün bilgisayar destekli analiz programları ile ayırmını ortaya koymak amacıyla yapıldı. Çalışmada erken gebe ve endometriyal kistli olmak üzere toplam 124 safkan Arap kısrak kullanıldı. Kısraklar erken gebelik dönemlerine (Grup I, n=12 (13 günlük gebelik); Grup II, n=15 (14 günlük gebelik); Grup III, n=29 (15 günlük gebelik); Grup IV, n=17 (16 günlük gebelik); Grup V, n=12 (17 günlük gebelik) ve endometriyal kist (Grup VI, n=39) olmak üzere altı gruba ayrıldı. Ultrasonografik muayene sırasında kaydedilen ve daha sonra bilgisayara aktarılan uterus görüntülerinin ortalama gri değeri (MGV), heterojenitesi (HET) ve kontrasti (CON) ölçüldü. Ekotekstür parametrelerinden MGV değeri gebe kısraklarda (sırasıyla; 55.20 ± 4.95 , 58.31 ± 3.88 , 56.23 ± 1.88 , 54.15 ± 3.47 ve 56.08 ± 2.94), endometriyal kistli kısraklardan (42.71 ± 0.71) daha yüksekti ($P < 0.001$). Diğer ekotekstür parametresi olan HET değerleri gebe kısrakta (sırasıyla; 20.75 ± 1.53 , 19.23 ± 1.39 , 21.42 ± 0.88 , 22.37 ± 1.32 ve 22.20 ± 1.09), endometriyal kistli kısraklardan (15.06 ± 0.44) daha yüksekti ($P < 0.001$). CON değerleri ise gebe kısraklarda (sırasıyla; 152.83 ± 7.02 , 144.02 ± 8.18 , 141.03 ± 4.83 , 145.93 ± 4.70 ve 154.65 ± 4.86), endometriyal kistli kısraklardan (191.35 ± 3.97) düştü ($P < 0.001$). Sonuç olarak; kısraklarda sıklıkla yanılıguya sebep olabilecek erken gebelik ile endometriyal kistin ayırt edilmesinde, ekotekstür parametrelerinin (MGV, HET ve CON) ölümsel değerlerinin önemli referans kriterleri olabileceği sonucuna varılmıştır.

Anahtar sözcükler: Erken gebelik, Endometriyal kist, Ekotekstür, Kısrak

INTRODUCTION

Real-time B-mode ultrasonography is one of the most

widely used diagnostic methods for monitoring reproduction in farm animals, which was first used in mares ^[1] in 1980 and later in cows ^[2,3]. Ultrasonography is also an important



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diagnostic tool used routinely in the physiological and pathological detection of early pregnancy, follicular changes in the ovaries and ovarian cysts and reproductive areas such as tumors, cysts, infections in the genital organs [4,5].

Ultrasonographic imaging is based on the ability of tissues to reflect high-frequency sound waves and this reflection depends on tissue density [6,7]. The images are displayed in a two-dimensional, gray scale based on the location and strength of the echoes from the tissue interfaces [8]. A two-dimensional ultrasonographic image is a matrix of square picture elements (pixels) varying in gray scale values ranging from 0 (absolute black) to 255 (absolute white) [6,7,9]. The ultrasonographic image of a tissue varies depending on the histological structure of the tissue and is called echotexture. Computer-assisted analysis (computer algorithm) allows an object to be evaluated objectively and provides determination of visual analysis subjectivities, allowing quantitative assessment of the intensity of each pixel in an image [10]. In recent years, computer-assisted ultrasonographic image analysis techniques have been used in the evaluation of genital organs including testicles [11], ovarian follicles [12], corpus luteum [12-14] and uterus [15]. The echotextural changes in the endometrium are used in the field of reproduction in veterinary medicine [16-18]. Computer assisted image analysis removes individual observation bias by allowing quantitative evaluation. The mean gray value (MGV), heterogeneity (HET) and contrast (CON) of the parameters obtained from the ultrasonographic images define the uterine echogenic image changes [19]. In computer-assisted analysis, images are obtained in three ways; point measurement, linear-time series analysis, and regional surface analysis [6]. When the images obtained in the studies were measured in computer environment, the target tissue was divided into four equal quadrants according to the point measurement and a small circular area was selected from these regions and average numerical data was formed [16,19-24].

In mares, endometrial cysts are fluid-filled formations that can be seen anywhere on the normal or chronically inflamed endometrium [25]. They have glandular (endometrial) or lymphatic structure, depending on their characteristics. Glandular cysts are found in the lamina propria and are typically smaller (1-10 mm) in size and exhibit multifocal distribution in one or more areas of the uterus. Lymphatic cysts are typically larger (>10 mm) and are usually isolated at one or two loci. Ultrasonography or hysteroscopy should be used to identify both types of cysts [26]. These types of cysts in the mare uterus can easily be confused with early or twin pregnancies in ultrasonographic examination and pregnancies may even be accidentally terminated during manipulation of uterine cyst in singleton pregnant mares that are mistaken for twin pregnancy. Furthermore, the inability of the embryonic vesicle to move freely in the uterine horns in the presence of cysts cannot reduce the secretion of prostaglandin F_{2α} and reveal the failure of

maternal diagnosis in pregnancy [27], impaired implantation can lead to insufficient blood flow to the conceptus and inadequate availability of nutrients resulting in early loss of embryo [27]. For this reason, early identification of uterine cysts and their discrimination from pregnancy are important in terms of fertility. Despite the fact that literature [25-27] studies conducted in this respect have differentiated early pregnancy (13-17 days) and endometrial cysts with periodic clinical and ultrasonographic examinations, the measurement and discrimination of these images by computer-assisted analysis programs has not done previously which manifest deficiency in this topic.

This study aimed to analyze the echotexture changes of uterine tissue by computer analysis program and to inform the veterinarians as an adjunct clinical modality in distinguishing early pregnancy in mares from endometrial cysts that can be confused with the former condition.

MATERIAL and METHODS

Ethics Statement

This study was approved by the Animal Ethics Committee of the University of Harran (Number: 2018/001/01-03).

Selection of the Animals

The present study was carried out on the mares brought to the Veterinary Clinics of Faculty of Veterinary Medicine, Harran University, for pregnancy and genital organ examination during breeding season. This area is located at 37°10'N - 39°03'E and 518 m higher from sea level. In this research, a total of 124 thoroughbred Arabian mares clinically healthy, with age interval of 7.6 and 14.69 years were used. Mares with early pregnancy and endometrial cysts are included in the study. Mares were divided into six groups according to stage of early pregnancy as Group I, n=12, (13th day pregnancy); Group II, n=15, (14th day pregnancy); Group III, n=29, (15th day pregnancy); Group IV, n=17, (16th day pregnancy); Group V, n=12, (17th day pregnancy) and (Group VI, n=39) with endometrial cyst.

Ultrasound Examination

The examination of genital organs or early pregnancy in mares brought to the clinic was performed with Portable B-mode 5 MHz linear probe transrectal ultrasonograph (SonoSite Edge II Vet®, Proodian Medical Equipment LLC, Highland Heights, Ohio, United States). During examinations, images of mares with endometrial cyst and early pregnancy were recorded in the memory sticks. Pregnancy and endometrial cyst images were recorded for evaluation during the breeding season and 13-17 days after the natural mating date in these mares (examination-1). Mares included in this study were then taken to the second examination on the 30th day, and those found to have embryo and fetal heartbeat were considered pregnant (examination-2). In the evaluation of recorded image

analysis, records of mares with early pregnancy findings and endometrial cyst obtained from the first examination (examination-1) were transferred to the computer. Ultrasonographic settings (depth, gain, focus, brightness) were kept unchanged throughout the entire study while images were taken. All ultrasonographic examinations were performed by the same person.

Computer-Assisted Image Analysis

The obtained images were transferred to the computer for evaluation of the digital echotexture. The images were saved as PNG file type with a resolution of 640x480 pixels. Computer analysis was generated using a special software (ImageJ 1.42q; NIH, USA-Image Processing and Analysis Java). The relevant program gives a qualitative value between 0-255. When measurement analysis was performed, polygonal boundaries were created covering every region of the uterus as 'relevant region' by avoiding fluid accumulation in uterus and myometrium, and MGV, HET, CON measurements were made (*Fig. 1, Fig. 2*). Our study was done according to regional surface analysis which was [28] previously performed to determine the echotexture in dog ovaries. Edematous regions of myometrium were avoided during analysis. All uterine tissues were selected in polygonal style and the mean numerical data were obtained. It is thought that this method would give more reliable results compared to the analysis of whole tissue.

Statistical Analysis

Statistical analyses were performed using the SPSS software version 22. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Simirnov/Shapiro-Wilk's test) to determine whether they are normally distributed or not. Descriptive analyses were presented using means and standard deviations for normally distributed MGV, HET and CON variables. One-way ANOVA was used to compare these parameters among the early pregnancy and endometrial cyst groups. Levene test was used to assess the homogeneity of the variances. An overall P-value of less than 0.05 was considered to show a statistically significant result. When an overall significance was observed, pairwise post-hoc test was performed using Tukey's test. The data in the section of table, graph and results are expressed as mean \pm standard deviation.

RESULTS

The MGV, HET and CON values were analyzed with 95% confidence in evaluating echotexture from images taken from uterine tissue. As a result of the analysis, a significant difference was found between the all group ($P<0.001$) (*Table 1*). The MGV values of the echotexture parameters were significantly different between the endometrial cyst group (Group VI) and the 13 days pregnancy group (Group

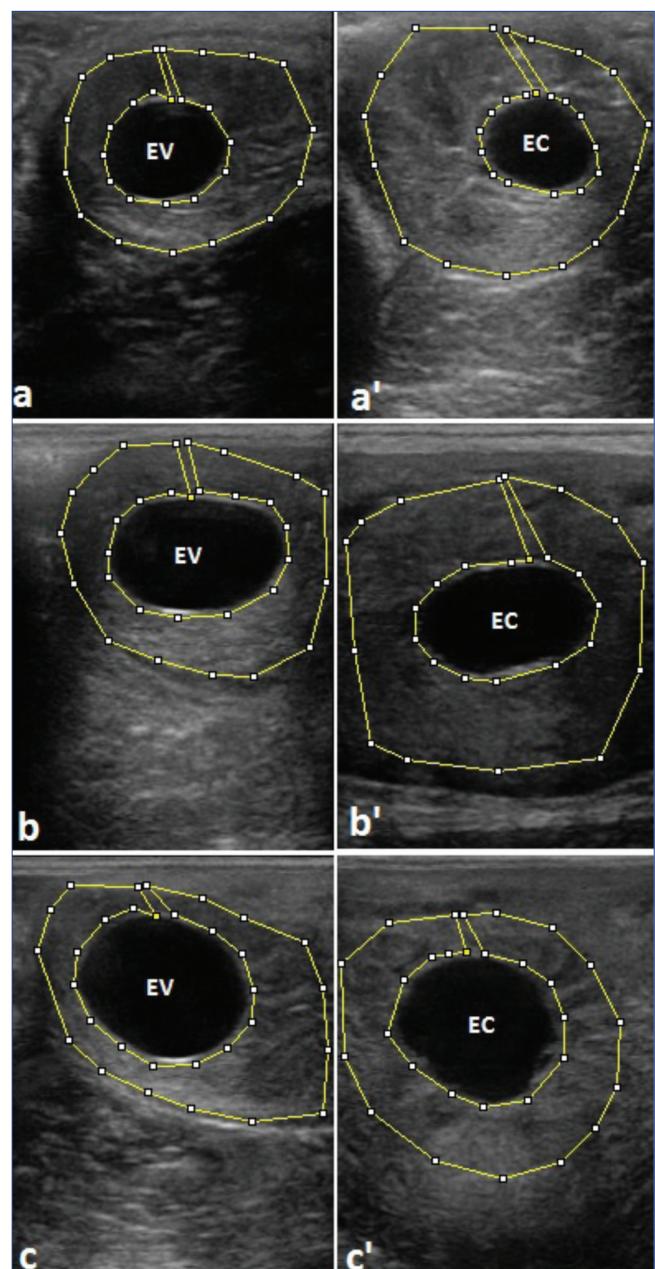


Fig 1. 13 days pregnancy (a), endometrial cyst (a'), 14 days pregnancy (b), endometrial cyst (b'), 15 days pregnancy (c), endometrial cyst (c'). EV: embryonic vesicle. EC: endometrial cyst

I) ($P<0.05$). This difference was found to be more significant ($P<0.01$) between 14 days (Group II), 16 days (Group IV) and 17 days (Group V) pregnancy and even more significant difference was found in the 15 days pregnancy (Group III) ($P<0.001$). There was no difference when the according to stage of pregnant groups were evaluated among themselves ($P>0.05$) (*Fig. 3A*). HET was statistically significant between the endometrial cyst group and the 13 days pregnancy group ($P<0.01$). This difference was found to be more significant in 15, 16 and 17 days of pregnancy ($P<0.001$). However, the difference between the 14 days pregnancy and the cyst group was not significant ($P>0.05$). There was no difference when the pregnant groups were

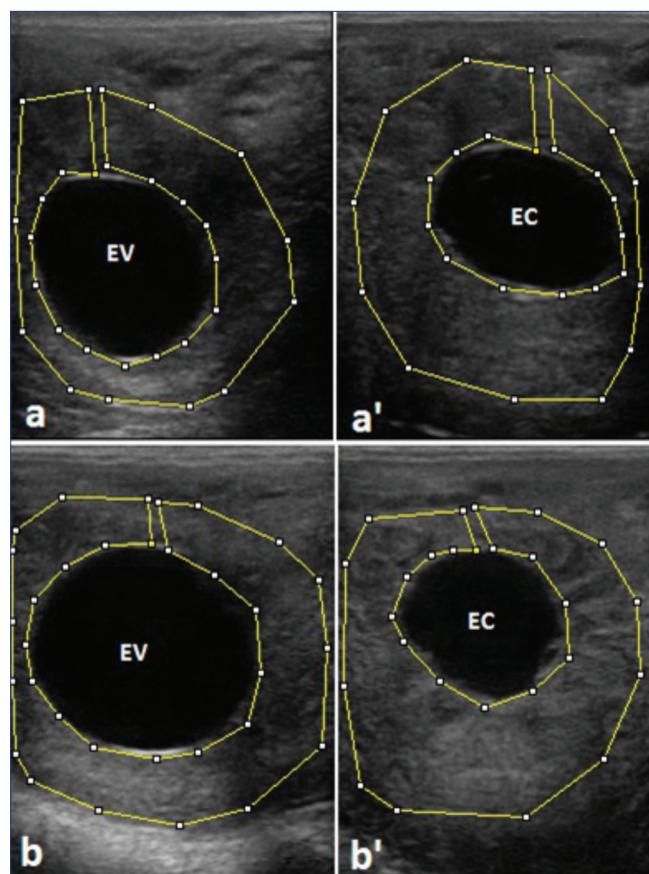


Fig 2. 16 days pregnancy (a), endometrial cyst (a'), 17 days pregnancy (b), endometrial cyst (b'), EV: embryonic vesicle. EC: endometrial cyst

evaluated among themselves ($P<0.05$) (Fig. 3B). The CON value was found statistically significant among all the other pregnant group in the endometrial cyst group ($P<0.001$). There was no difference when the pregnant groups were evaluated among themselves ($P>0.05$) (Fig. 3C).

DISCUSSION

The precise diagnosis of early pregnancy in mares during breeding season is critical for breeders. Although rectal palpation is widely used for diagnosis of pregnancy, it can be confused with endometrial cyst since it is not possible to palpate the embryonic vesicle [29]. It is important to monitor the embryonic vesicle without inner cell mass and embryo in the management of clinical diagnosis of early pregnancy in mares. Although the diagnosis of a product of conception is routinely detected by transrectal ultrasonography between 14–18 days after ovulation in mares, it is not possible to diagnose an abnormal vesicle because ultrasonographic appearance of the inner cell mass or embryo development cannot be obtained at this stage. By transrectal ultrasonography, embryo in the embryonic vesicle can be detected earliest at day 20 ovulation and the embryonic heart beat can be seen between 24–25 days [29]. Examination was done in mares between days 13 and 18 and embryonic heartbeats were observed parallel to the growth of the embryonal sac in the repeated examination. However, there was no change in vesicle size and appearance in the mares with endometrial cyst. In studies conducted on endometrial cysts, the incidence of cysts increased with age; the most commonly affected mares were reported to be over 10 years old [30,31]. The incidence of endometrial cysts in the fertile and subfertile mares has been reported to be between 13 and 22% [32]. In some studies, encountered 55.5% of endometrial cysts in ultrasonographic examinations routinely performed on 310 mares, ranging in age from 8 to 20 years [33]. In another study, a total of 95 cysts were found in 58 of 259 healthy mares, ranging in age from 3 to 22 years. While 73.1% of these cysts belonged to the mares older than 14 years, 29.1% belonged to mares between 7–14 years of age [32]. The number of cystic structure in mares varies from 1 to

Table 1. The difference between the average MGV, heterogeneity and contrast values of the uterus of endometrial cystic and early pregnant mares

Groups	N	MGV	Heterogeneity	Contrast
		X±Sx	X±Sx	X±Sx
Group I (13 th day pregnancy)	12	55.20±4.95 ^a	20.75±1.53 ^a	152.83±7.02 ^a
Group II (14 th day pregnancy)	15	58.31±3.88 ^a	19.23±1.39 ^b	144.02±8.18 ^a
Group III (15 th day pregnancy)	29	56.23±1.88 ^a	21.42±0.88 ^a	141.03±4.83 ^a
Group IV (16 th day pregnancy)	17	54.15±3.47 ^a	22.37±1.32 ^a	145.93±4.70 ^a
Group V (17 th day pregnancy)	12	56.08±2.94 ^a	22.20±1.09 ^a	154.65±4.86 ^a
Group VI (Endometrial cyst)	39	42.71±0.71 ^b	15.06±0.44 ^b	191.35±3.97 ^b
P value (one way ANOVA)		P=0.000 ***	P=0.000 ***	P=0.000 ***

^{a,b}The letters on the table are statistically different. A significant difference was found between the six group averages for the related parameter variables ($P<0.001$), ANOVA: Analysis of variance. Values in the table mean the mean±SEM

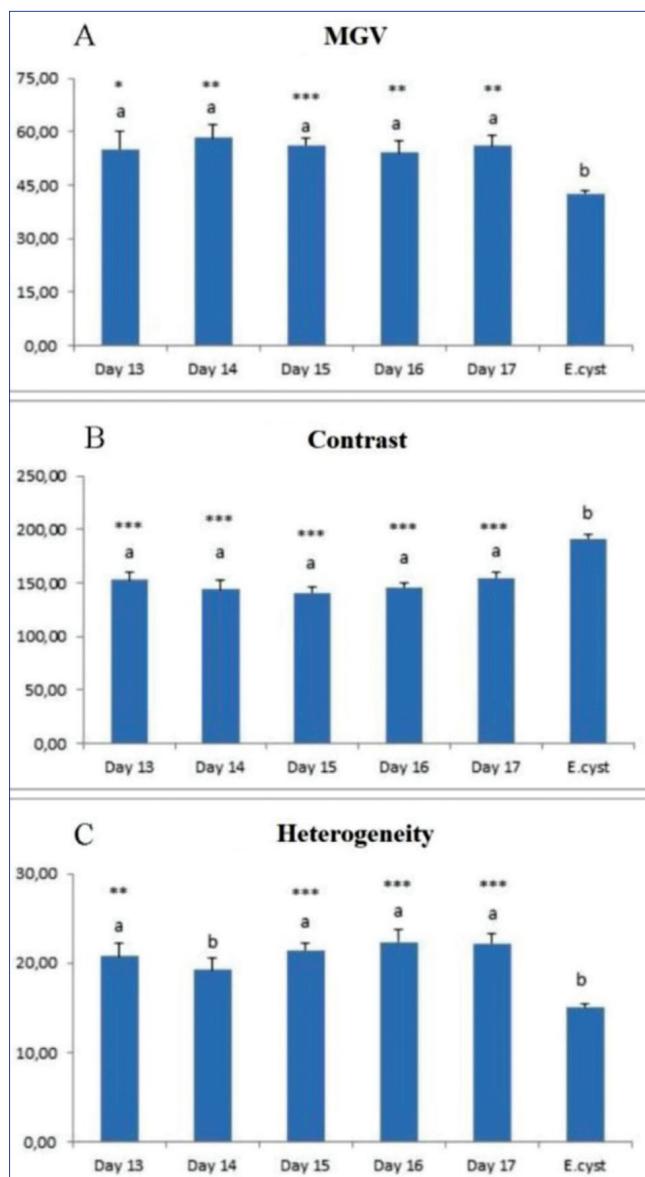


Fig 3. Mean gray value (MGV) (3A), heterogeneity (3B) and contrast (3C) in pregnant and endometrial cyst groups. Values in the column values the mean \pm SEM

A: ^{a,b} The letters on the graph are statistically different. *^{****} Differentiation of endometrial cysts compared to early pregnancy periods, * P<0.05, ** P<0.01, *** P<0.001; B: ^{a,b} The letters on the graph are statistically different. *** Differentiation of endometrial cysts compared to early pregnancy periods, *** P<0.001; C: ^{a,b} The letters on the graph are statistically different. *** Differentiation of endometrial cysts compared to early pregnancy periods, ** P<0.01, *** P<0.001

7 per mare with 3 to 48 mm in diameter which increases with age [32,34]. In this study, mares between 5 and 18 years of age were used and both fertility and predisposition to cyst development were achieved, and the prevalence of endometrial cysts (localization to a single uterine region) by ultrasonographic examinations and localization of cysts was found to be consistent with the literature data. While endometrial cysts can be found anywhere within the uterus or cervix, they are more common near the bifurcation area of the horns and in the uterus [35]. In a retrospective study of 55 mares treated for cysts, 33.8% of the cysts were found

endometrium in the uterine tissue, and 16.4% were found in the endometrium in the conjunctival region of the uterine horns [36]. Two other studies conducted on 48 and 259 mares, respectively, reported that the most common location of cysts is the junction of the uterine horns and body of the uterus [30,32]. In our study, the vast majority of cysts were found in the endometrium and only one of them was in the junction of the horns. Twins have also been encountered during the study, but they have not been evaluated.

B-mode ultrasonography computer-assisted image analysis has been used for evaluation of uterus [37] and ovaries [20,23,38-40]. In order to determine the relationship of embryonic fixation with echotexture parameters, a significant increase in uterine echotexture was demonstrated in the MGV measurements on day 21 after mating in cattle [16,41], on day 15-16 in mares [42], on day 16 in goats [19] and in pigs [17]. In the mares [43] and in cows [16], echotextural changes in the endometrial tissue were reported to be associated with peripheral concentrations of ovarian hormones. It has been reported that uterine echotexture values increased during the pre-estrus and pre-ovulation period [41] in the heifers and decreased in the mares [42]. In the goats, computer-assisted analysis measurements for reproductive organs and luteal function were established [19,44,45]. Studies on echotextures in farm animals have been carried out on sexual cycle follow-up, pregnancy, evaluation of genital organs and monitoring ovarian hormones. Increased echotexture parameters in early pregnancy were reported in mares, but endometrial cyst and early pregnancy, which constitute significant problems, have not been evaluated for echotextural changes. In the present study, increased uterus echotexture in early pregnancy was compatible with the previous studies and uterus echotexture was found to be statistically significantly higher in pregnancies between 13-17 days compared to mares with endometrial cysts. This has led to the conclusion that the uterine echotexture may have been increased in pregnancy due to endometrial folding, which begins to increase during early pregnancy and to the effect of the incoming blood flow on vascular permeability [29].

As a result, endometrial cysts that may lead to confusion in mares during early pregnancy can be distinguished from early pregnancy by analyzing their MGV, HET and CON of echotexture parameters.

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