



DOI: 10.33188/vetheder.672255

Araştırma Makalesi / Research Article

## The vascularity of preovulatory follicle: The colour–Doppler assessment and its predictive value in the early pregnancy outcome in Arabian Mares

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### MAKALE BİLGİSİ /

ARTICLE  
INFORMATION:

Geliş / Received:

09 Ocak 20

09 January 20

Kabul / Accepted:

25 Nisan 20

25 April 20

Anahtar Sözcükler:

Gebelik

Kısırak

Preovulatör folikül

Vaskülarizasyon

Keywords:

Mare

Pregnancy

Preovulatory follicle

Vascularization

### ABSTRACT:

The aim of this study is to determine the relationship between the amount of vascularization in the preovulatory follicle wall and pregnancy establishment with colour Doppler ultrasonography. Colour Doppler ultrasonography images from 26 Arabian mares in breeding season were evaluated in the study. Mares no abnormalities in the reproductive system and mild-manner mares were handled. Preovulatory follicle (>35mm) was monitored twice in a day by transrectal B-mode ultrasonography and colour Doppler ultrasonography until the ovulation day. Follicular vascularization images which were incorporated into the study, were monitored 18 hours before the ovulation. Also amount of pixels in colour Doppler images were evaluated with computer-based image analysis program. The mares were mated naturally with a stallion. Pregnancy diagnosis was performed by ultrasonography on day 14 to day 30 after mating. As a result of ultrasonography examination, mares were divided into two groups as pregnant (n=13) and non-pregnant (n=13). The statistical difference between the amount of vascularization in the preovulatory follicle wall of pregnant mares and the amount of vascularization in the preovulatory follicle wall of non-pregnant mares was compared by t-test. As a result of the study, there were no significant differences between pregnant and non-pregnant mares in terms of area, volume and intensity units of coloured pixels in the preovulatory follicle wall (P> 0.05). In conclusion, it was observed that the quantitative evaluation of the colour Doppler images of the preovulatory follicle wall in mares in the breeding season cannot be used in the diagnosis of pregnancy.

### *Preovulatör folikülün vaskülarizasyonu: Arap kısıraklarda erken gebelik sonuçlarında renkli Doppler değerlendirmesi ve öngörülen değeri*

### ÖZET:

Sunulan makalede renkli Doppler ultrasonografi ile preovulatör folikül çeperindeki damarlaşma düzeyinin gebelik oluşumuyla ilişkisinin belirlenmesi amaçlandı. Üreme sezonundaki sağlıklı 26 adet Arap kısırakta alınan renkli Doppler ultrasonografi görüntüleri değerlendirildi. Sakin mizaçlı ve reproduktif açıdan problemi olmayan kısıraklar seçildi. Preovulatör folikül (>35mm), transrektal B-mod ultrasonografi ve renkli Doppler ultrasonografi ile ovulasyon gününe dek günde iki kere izlendi. Çalışmaya dahil edilen foliküller vaskülarizasyon görüntüleri yumurtlamadan 18 saat önce izlendi. Ayrıca, renkli Doppler üzerinden akım görülen alanlardaki piksel sayısı bilgisayar destekli görüntü analiz programı ile değerlendirildi. Kısıraklar, bir aygırla doğal aşım yoluyla çiftleştirildi. Aşımı izleyen 14-30 günlerde ultrasonografi ile gebelik muayeneleri yapıldı. Bunun neticesinde kısıraklar gebe (n=13) ve gebe olmayan (n=13) olmak üzere iki gruba ayrıldı. Gebe kısıraklara ait preovulatör folikül çeperindeki damarlaşma miktarı ile gebe olmayan kısıraklara ait preovulatör folikül çeperindeki damarlaşma miktarı arasındaki istatistiksel fark t-testi ile karşılaştırıldı. Çalışmanın sonucunda, preovulatör folikül çeperindeki renkli piksellere ait alan, hacim ve yoğunluk düzeylerinin gebe ve gebe olmayan kısıraklarda farklı olmadığı görüldü (P>0,05). Sonuç olarak, üreme sezonundaki kısıraklarda preovulatör folikül duvarına ait renkli Doppler görüntülerinin kantitatif değerlendirmesinin gebelik tanısında kullanılamayacağı görüldü.

**How to cite this article:** Uçmak ZG, Kurban İ, Uçmak M: The vascularity of preovulatory follicle: The colour–Doppler assessment and its predictive value in the early pregnancy outcome in Arabian Mares. *Veteriner Hekimler Derneği Dergisi*, 91(2): 104-109, 2020, DOI: 10.33188/vetheder.672255

## 1. Introduction

Mares are seasonally polyestrous breeder which they usually reach puberty at two years old. The recommended first insemination age is around three years. Cyclic activity may persist until the age of 20 years (2).

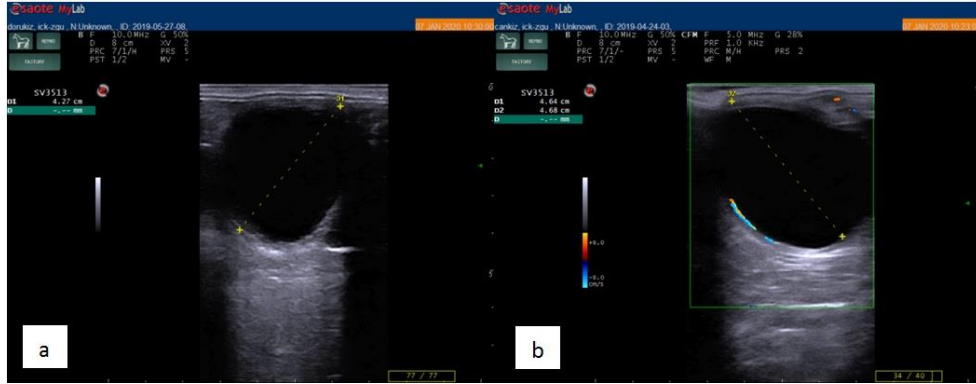
B-mod ultrasonography is utilized routinely in veterinary gynecology to achieve the knowledge on follicular wave, embryonic migration, changes in ovulation, uterine infections, ovarian cysts and tumors, gender determination (4). However, ultrasonographic examination enables the reliable information about the reproductive situation and function of uterus and ovaries in mare genital organs (10). Because 5 MHz probe has a higher resolution than a 3 or 3.5 MHz probe in the ultrasonographic examination of ovaries in the mare, evaluation of the structures on ovaries (follicle or corpus luteum) become more suitable (9). Doppler ultrasonography is also a noninvasive and practical tool to evaluate the function and hemodynamic structure of the organs in different animal species (5). Colour-Doppler ultrasonographic imaging and Doppler ultrasonography provide distinctively different approaches for assessing the vascular system of reproductive organs. In colour-flow mode, different colours are represented on the screen display according to the direction of blood flow to the face of the transducer. The extent of colour can be estimated by percentage of a tissue with colour signals or can be calculated by computer, based on number of coloured pixels (8). In literature review, there are studies for predicting the fertility in horses and cattles by examining vascular perfusion of the preovulatory follicle via Doppler ultrasonography (11, 12). Siddiqui et al. (12) investigated the relationship of vascular perfusion of the wall of the preovulatory follicle to in vitro fertilization and embryo development in heifers. As a result, they supported positive correlation between greater vascular perfusion of the wall of the preovulatory follicle and in-vitro fertilization and embryo development. Varughese et al. (15) stated that vascularization of follicle could be a successful predictor of fertility in dairy cows and also it was hypothesized that cows with a highly vascularized follicle ( $>550 \text{ pixel}^2$ ) underwent a normal pregnancy. Altermatt et al. (3) compared ovarian and follicular vascularity related to age of the mares. Although they supported older mares have greater follicular vascularity than younger mares, follicle growth was not found associated with vascularity. However, Ginther et al. (6) investigated the relationships of follicle versus oocyte maturity to ultrasound morphology, blood flow, and hormone concentrations of the preovulatory follicle in mares.

In farm animals, recent researches with colour Doppler ultrasonography were usually associated with follicular maturation (6, 11, 12). Therefore, investigation of the vascular perfusion of the wall of preovulatory follicle in mares from the point of pregnancy formation was aimed in the presented study.

## 2. Material and Methods

Fourty Arabian mares (age, 8-15 years; weight, 350-430 kg) were examined during the breeding season and twenty-six of them included to the study. The mares had free access to grass hay, water, and trace-mineralized salt. Mild-mannered mares which had no abnormalities in the reproductive system were handled. For this purpose, transrectal ultrasonography and cytobrush were performed to eliminate the mares with endometritis. Also, mares were selected according to have had at least one pregnancy in their breeding history.

The selected mares were scanned daily to detect the follicle reached 35mm by B-mode ultrasonography (Esaote Pie Medical, Genoa, Italy) (Figure 1a). Thereafter preovulatory follicle (35mm) was monitored twice in a day until ovulation occurred. Any hormone administration was not performed to move ovulation to an earlier time. Follicular vascularization images which were incorporated into the study, were monitored 18 hours before the ovulation. Colour Doppler measurements were performed by using Esaote MyLab Five Vet (Esaote Pie Medical, Genoa, Italy) equipped with a 7.5 MHz linear-array transducer (Figure 1b). Also, follicular diameter on the other ovary was measured to be greater than 27 mm by B-mode ultrasonography.



**Figure 1: (a)** Measurement of preovulatory follicle diameter, **(b)** Colour Doppler ultrasonography image of preovulatory follicle.

**Şekil 1: (a)** Preovulatör folikül çapının ölçülmesi, **(b)** Preovulatör follikülün renkli Doppler ultrasonografi görüntüsü

The mares were subjected to natural breeding with a stallion. Pregnancy diagnosis was performed by transrectal ultrasonography on day 14 to day 30 after mating. The mares with early pregnancy loss were not included to the study. As a result of ultrasonography findings, mares were divided into two groups related to pregnancy occurrence. Follicle diameters (mm) belonging to pregnant and non-pregnant mares were calculated during the ultrasonography imaging. Also, area of coloured pixels (A red, A blue, A mix; cm<sup>2</sup>) were measured with the computer-based image analysis program (Pixelflux, Chameleon-Software GmbH, Münster, Germany). Additionally, volume (V red, V blue, V mix) and intensity (I red, I blue, I mix) of coloured pixels were determined. For this purpose, the stored Doppler images (three images of the blood flow area belonging to the preovulatory follicle) were used for evaluation of vascularization on the follicle wall. Thus, the whole follicle was chosen as a region of interest (ROI). Then, the coloured pixels inside the ROI were calculated to reach the values of area, volume and intensity; respectively (Figure 2).



**Figure 2:** Imaging of the coloured pixels inside the ROI.

**Şekil 2:** ROI içindeki renkli piksellerin görüntüsü

SPSS 13.0 packet program was used for the statistical analyses. The association between area, volume, intensity of coloured-pixels in follicular wall and pregnancy was determined by t-test. Pearson correlation test was used to determine the correlation between areas of colour pixels. The significance level was accepted as  $P < 0.05$ .

Ethics approval for the study was obtained from the Unit Ethics Committee (Foundation: University of Istanbul – Cerrahpasa, Faculty of Veterinary Medicine, Unit Ethics Committee, Date: 01/08/2019, Number: 2019/35).

### 3. Results

The groups were composed with equal numbers in terms of presence of pregnancy (n=13). The mean values of preovulatory follicle diameter in pregnant and non-pregnant mares were 44.26±1.30 mm and 44.23±0.92 mm; respectively. The areas of coloured-pixels (A mix, A red, A blue) on the follicular wall were evaluated in pregnant and non-pregnant mares. According to the evaluation of colour Doppler parameters, A mix was positively correlated with A red (P=0.000) and A blue (P=0.004). Also, volume and intensity of coloured pixels were detected with the computer-based image analysis program (Pixelflux, Chameleon-Software GmbH, Münster, Germany). As a result of this, areas, volumes and intensities of coloured-pixels were not significantly associated with the presence of pregnancy (P>0.05) (Table 1).

**Table 1:** Area, Volume and Intensity of coloured pixels according to the presence of pregnancy (Mean values ± Std. Error)

**Tablo 1:** Gebelik varlığına göre renkli piksellerin alanları, hacimleri ve yoğunlukları (Ortalama değerler ± Std. Hata)

	Pregnant mares (n=13)	Non-pregnant mares (n=13)	Significance (P)
A mix (cm <sup>2</sup> )	0.03 ± 0.00	0.02 ± 0.00	0.545
A red (cm <sup>2</sup> )	0.05 ± 0.14	0.04 ± 0.10	0.351
A blue (cm <sup>2</sup> )	0.01 ± 0.03	0.02 ± 0.00	0.666
V mix (cm/s)	2.02 ± 0.22	1.92 ± 0.28	0.777
V red (cm/s)	1.57 ± 0.25	1,94 ± 0.28	0.340
V blue (cm/s)	2.47 ± 0.29	1.90 ± 0.41	0.272
I mix (cm/s)	0.00 ± 0.00	0.00 ± 0.00	0.957
I red (cm/s)	0.00 ± 0.00	0.00 ± 0.00	0.559
I blue (cm/s)	0.00 ± 0.00	0.00 ± 0.00	0.515

*A mix: Area mix, A red: Area red, A blue: Area blue, V mix: Volume mix, V red: Volume red, V blue: Volume blue, I mix: Intensity mix, I red: Intensity red, I blue: Intensity blue.*

### 4. Discussion and Conclusion

Ultrasonographic examination of the ovaries provides to monitorize the follicular and luteal changes in mares (10). As the researchers (10) reported, development of preovulatory follicle was evaluated by transrectal B-mod ultrasonography. Diameter of preovulatory follicle ranges between 40mm and 50mm in mares. Its shape states from spherical to conic before the ovulation (9). In line with the previous report, mean of preovulatory follicles were 44mm in diameter in the presented study.

Doppler USG is a technique that helps to collect data about the vascularization level and flow characteristics of the tissue. The current map, direction and velocity can be determined in colour Doppler. The oncoming currents to the probe are coded in red colour and the receding currents from the probe are coded in blue colour (5). In accordance with the researchers' review (5), the current map of vascularization was imaged by colour Doppler ultrasound. Also, Acosta et al (1) used colour Doppler ultrasonography to evaluate the vascular perfusion of dominant follicle in mares during the transition seasons. Unlike with the previous report, preovulatory follicle was evaluated in terms of vascularization by using colour Doppler USG in this study.

However, it was reported that power Doppler is an enriched version of colour Doppler which enable for low-flow tissues such as ovaries (5). Silva et al (13) investigated the relationship between vascularity of the preovulatory follicle and establishment of pregnancy in mares by spectral Doppler USG. They hypothesized that blood flow to the preovulatory follicle was positively correlated with pregnancy establishment (13). In contrast with Silva et al (13), vascularization area of the preovulatory follicle wall was determined by colour Doppler measurements in the presented research. However, opposite results were obtained in relationship between vascularization area of preovulatory follicle wall and pregnancy establishment. It was conceived that colour Doppler measurements were not delicate enough to evaluate the preovulatory follicle wall like power Doppler USG or spectral Doppler USG as Erdoğan (5) reported.

Moreover, pregnancy establishment is related with maternal recognition and regular secretion of ovarian and placental hormones in mares. Concisely, it is indicated that pregnancy is associated with immunology and endocrinology (14). Although evaluation of preovulatory follicle by power and spectral Doppler USG could give a clue on time of ovulation and pregnancy occurrence rate, significant results were not obtained by colour Doppler USG findings related to pregnancy establishment. As the researchers (14) stated that it was thought to be because pregnancy establishment is associated with not only quality of preovulatory follicle but also it was related with maternal immunology and endocrinology.

In conclusion, it is not sufficient to estimate the early pregnancy outcomes of mares by determining vascularization in the preovulatory follicle wall by colour Doppler USG alone.

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