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Research Article

The Assessment Of Early Stage Corpus Luteum Ultrasonography In Pregnant Goats

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ABSTRACT

Backround /Aim: The aim of the present study was to evaluate the corpus luteum images of the goats biometric and hemodynamically and to investigate the relationship between corpus luteum (CL) size and time-dependent changes with luteal arterial resistance during the early pregnancy stage.

Material and Methods: For this purpose, 94 B-mode and 63 Pulsed Doppler CL images taken from 21 healthy pregnant goats aged 1-5 years were examined. The mean number of CL, maximum CL diameter (mm) and the resistance indices (RI) of the luteal vessels were determined in these images taken by transrectal ultrasonography on 7th, 14th and 21st days of pregnancy.

Results: The mean numbers of corpus luteum that can be visualized on days 7th, 14th and 21st of pregnancy were 1.81±0.16; 1.38±0.11 and 1.15±0.08. The mean maximum diameter and luteal blood flow resistance values of luteal tissues were measured as 13.23±0.49 mm / 0.43±0.03; 16.04±0.76 mm / 0.33±0.02 and 16.98±0.41 mm / 0.34±0.02 respectively. It was observed that the mean corpus luteum diameters increased from the first week up to the 21st day (P<0.05) and luteal vessel resistance values decreased (P<0.05). It was also seen that these significant changes which were more prominent in the progressive phase of the corpus luteum were more slightly et the 21st day of pregnancy. There were low positive correlations between the mean corpus luteum diameter and RI values at the 7th and 21st days (r=0.12; r=0.27); but low negative correlation (r=-0.18) at the days of 14th days of pregnancy. It was observed that there was a low negative correlation (r =-0.15) between the gestational age and the vascular resistance.

Conclusion: As a result, it was seen that pulsed Doppler sonography can be applied practically for the evaluation of corpus luteum functions in goats during the first three weeks of pregnancy. Moreover, RI values below 0.50 were similar to the results in pregnant women. However, it can be thought that there may be low success rate of the pregnancy diagnosis and estimation of the gestational age regarding to the low correlation rates between resistance values and luteal tissue diameters / gestational age.

Key Words: Pregnancy, corpus luteum, resistance, goat

Gebe keçilerde erken dönem korpus luteum ultrasonografisinin değerlendirilmesi

ÖZET

Özbilgi/Amaç: Sunulan araştırmada, keçilerde erken gebelik döneminde korpus luteum görüntülerinin biometrik ve hemodinamik açıdan değerlendirilmesi ve korpus luteumun büyüklüğü ve zamana bağlı değişimin luteal arteriel direnci ile olan ilişkisinin araştırılması amaçlandı.

Materyal ve Metot: Bu amaçla, 1-5 yaşlarında 21 sağlıklı gebe keçiden elde edilen toplam 94 adet B-mod ve 63 adet Pulsed Doppler korpus luteum ultrasonografi görüntüsü incelendi. Gebeliğin 7, 14 ve 21. günlerinde transrektal ultrasonografi ile görüntülenebilen ortalama korpus luteum sayısı, maksimum korpus luteum çapı (mm) ve korpus luteumda perfüzyonu sağlayan damarlara ait rezistans (RI) değerleri belirlendi.

Bulgular: Gebeliğin 7, 14, ve 21. günlerinde ortalama korpus luteum sayıları sırasıyla 1.81±0.16; 1.38±0.11 ve 1.15±0.08 olarak kaydedildi. Luteal dokulara ait ortalama maksimum çap ve luteal kan akım direnç değerleri ise sırasıyla 13.23±0.49 mm / 0.43±0.03; 16.04±0.76 mm / 0.33±0.02; 16.98±0.41 mm / 0.34±0.02 olarak ölçüldü. Ortalama korpus luteum büyüklüklerinin ilk haftadan başlayarak 21. güne kadar arttığı (P<0.05), luteal damar resistans değerlerinin ise azaldığı gözlendi (P<0.05). Yine korpus luteumun progressif fazında daha belirgin olan bu değişimlerin gebeliğin 21. gününde daha hafif seyrettiği gözlendi. Ortalama korpus luteum çapı ile damar resistans değerleri arasında gebeliğin 7 ve 21. günlerinde düşük pozitif (r=0.12; r=0.27) ve 14. gününde ise düşük negatif korelasyon (r=-0.18) vardı. Gebelik yaşı ve damar direnci arasında ise düşük düzeyde negatif bir korelasyon olduğu (r=-0.15) gözlendi.

Sonuç: Keçilerde korpus luteum fonksiyonlarının değerlendirilmesi amacıyla pulsed Doppler sonografinin de pratik olarak gebeliğin erken döneminde uygulanabildiği görülmüştür. Yine bu çalışmada 0.50'nin altında seyreden rezistans değerleri gebe kadınlardaki sonuçlarla benzerlik göstermektedir. Ancak rezistans değerleri ile luteal doku büyüklükleri ve gebelik zamanı arasındaki düşük korelasyonlar göz önüne alındığında erken gebelik tanısı ve yaşının hesaplanması noktasında başarı oranının düşük olabileceği düşünülebilir.

Anahtar Sözcükler: Gebelik, korpus luteum, rezistans, keçi

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Introduction

To detect and delineate of ovarian tissues in small ruminants, different ultrasonographic scanning technics have been replacing previous invasive methods (laparotomy / laparoscopy) due to anaesthesia and adhesion risks. Using B-mode and Doppler ultrasonography permits many experiments playing important role on luteal monitoring without causing any stress, anaesthetic and surgical complications. Results of studies revealed that the number of corpora lutea (CL), and their dimensions are suitable for assessment of luteal function in goats (Simoes et al., 2005; Simoes et al., 2007). During all luteal stages, positive correlation between serum progesterone level and luteal area was established in goats (Orita et al., 2000; Arashiro et al., 2010). Following B-mode examinations, Color or Power Doppler scans have been employed for CL to evaluate of its functional status in ewes, subjectively (Oliviera et al., 2018). In similar studies, it was also seen that subjective luteal vascularisation and luteal echogenity assessments are reliable to separate pregnant animals on day 17 and day 21 during post-breeding period (Arashiro et al., 2018; Cosentino et al., 2018). Although Balaro et al. (2017), reported that the luteal blood flow assessment by using a computerised image analysis software is more informative about the CL functionality instead of luteal biometry in cows, the quantative evaluation of luteal blood flow has not been studied before in pregnant goat.

Overall, the present study aimed to investigate, additionally to CL counts and size, the feasibility of Pulsed Doppler scans to measure luteal vessel resistance during early pregnancy in goats.

Material and Methods

The present study was carried out in Faculty of Veterinary Medicine, Aydin Adnan Menderes University, Aydin, Turkey. The permissions for all procedures performed in this research were obtained from Animal Research Ethics Committee of Aydin Adnan Menderes University (6458310172016/109). Twenty-two nulliparous Saanen goats taken to the estrus synchronization were used in the study. The goats were between 1-5 years of age and their average body weight was 28.92 kg. To ensure that goats had any abnormalities before the synchronization protocol, general clinical and gynaecological examinations were performed.

The estrus of goats was synchronized with intravaginal sponges containing 20 mg fluorogestone acetate (Chronogest CR, MSD Animal Health, Istanbul, Turkey). The synchronization protocol was performed in August, during the natural breeding season of goats in the Aydin region. The intravaginal sponges were inserted into the vagina of each goat for 11 days. Fourty-eight hours before the sponge withdrawal, 500 IU equine chorionic gonadotropin (eCG) (Folligon, MSD Animal Health, Istanbul, Turkey) and 250 mcg prostaglandin F2alpha (cloprostenol sodium) (Estrumate, MSD Animal Health, Istanbul, Turkey) were injected intramuscularly to the all does for stimulation of estrus and ovulation. Buck introductions were performed for two days after the sponge withdrawal. Animals showing estrus were allowed to mate twice with male goats known the reproductive performance before. The day of estrus was designated as day 0 of pregnancy (day of 1st mating). Pregnancy diagnosis was performed by ultrasound device (MyLab 30, Esaote, Genova, Italy) with 7.5 MHz transrectal probe between days 40-45, postcoitally. Does that visualized with gestational sacs and active fetal heart beats were recorded as pregnant in the study. In the end of study, B-mode and Doppler sonographic records of 21 goats determined as pregnant were used.

In the assessment of ovarian tissues, Doppler settings of the device were 6 cm depth, Medium WF, 1.1 PRF and % 88 gain. Goats were screened three times (7th, 14th and 21st days, postcoitally) by transrectal way. The mean of the number of CL, maximal diameter (mm) of CL, and the resistance indices (RI) of the luteal vessels were recorded from the visible CLs. All the screening procedures were performed by the same operator. For better monitorization, goats were kept on standing position and fecal pellets were removed manually before examination.

The data were evaluated with One-way ANOVA test by using SPSS 22.0 (SPSS Inc. Chicago, IL, USA). In order to determine the change in values over time, statistical analysis was performed with variance analysis.

Table 1. Mean number, maximum diameter and luteal blood flow resistance of corpus luteum of pregnant goats.

Tablo 1. Gebe keçilerde korpus luteumun ortalama sayısı, maksimum çapı ve kan akım direnç değeri.

	Day 7	Day 14	Day 21
Mean number of corpus luteum	1.81±0.16 mm	1.38±0.11 mm	1.15±0.08 mm
Mean corpus luteum diameter	13.23±0.49 mm	16.04±0.76 mm	16.98±0.41 mm
Resistance Index (RI)	0.43±0.03	0.33±0.02	0.34±0.02

Results

The mean numbers of CL were 1.81±0.16; 1.38±0.11 and 1.15±0.08 at the 7th, 14th and 21st days of pregnancy (Table 1). The mean maximum diameters of CL and luteal blood flow RI were measured as 13.23±0.49 mm / 0.43±0.03; 16.04±0.76 mm / 0.33±0.02; 16.98±0.41 mm / 0.34±0.02, respectively (Table 1).

It was observed that the maximum diameter of CL was increased (P<0.05); and the luteal vessels' RI were decreased from the first week to 21st day of pregnancy (P<0.05) (Figure 1 and Figure 2). In addition, it was seen that these significant changes in progressive phase were more slightly at the 21st day of pregnancy. There were low positive correlations between the maximum luteal diameter and luteal vessels' RI at the 7th and 21st days (r=0.12; r=0.27); but negative correlation (r=-0.18) at the 14th day of pregnancy (P>0.05). Also, there was a low negative correlation (r =-0.15) between the gestational age and the vascular RI of the CL (P>0.05).

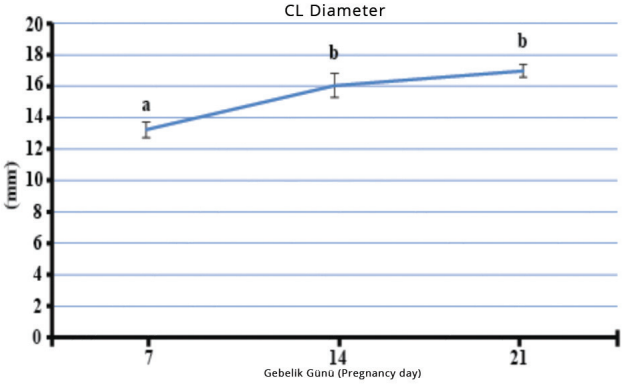

Figure 1. Mean maximum diameter of corpus luteum. Different letters (a, b) at the measurement days indicate significant differences (P<0.05).
Şekil 1. Korpus luteumun ortalama çap değerleri. Ölçümler arasındaki belirgin fark (P<0.05) farklı harflerle gösterilmiştir.

Discussion

As getting new diagnostic techniques, the evaluation of luteal functions has been changing in veterinary medicine. B-mode and Doppler, two of these new clinical approaches, are non-invasive diagnostic tools and represent real-time results. To evaluate luteal blood flow, Doppler exams are performed practically under field conditions. Several studies have focused on the detecting follicular and luteal tissues, and their morphologic and haemodynamic features (Arashiro et al., 2010; Endo et al., 2010; Kaya et al., 2017; Cosentino et al., 2018, Lasheen et al., 2018). Ultrasound images are investigated in the view of biometric, echotextural and hemodynamic analysis to find a new parameter instead of serum progesterone concentrations. To our knowledge, this is the first study reporting the pulsed Doppler findings of CL during early pregnancy stage in the pregnant goats. Although the fact that ovarian ultrasonography in goats is performed with a transrectal transducer manipulated externally (Oliviera et al., 2018) and, in the present study, goat's ovaries were able to be assessed with Pulsed Doppler ultrasonography.

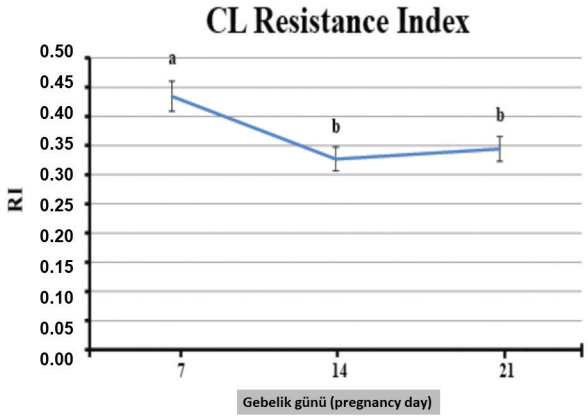

Figure 2. Mean luteal blood flow resistance of corpus luteum. Different letters (a, b) at the different measurement days indicate significant differences (P<0.05).
Şekil 2. Korpus luteum kan akım ortalama direnç değerleri. Ölçümler arasındaki belirgin fark (P<0.05) farklı harflerle gösterilmiştir.

The size, structure and steroidogenic activity of corpus luteum shows variations throughout the estrus cycle and pregnancy (Fields and Fields, 1996). Nevertheless, the corpus luteum of buffaloes could clearly be detected and measured from Day 4 to the end of the oestrous cycle (Gaur and Purohit, 2018). Arashiro et al (2010) demonstrated that the CL of the goats could be observed meanly at Day 5 of the estrus cycle. In another study, it was also observed that the corpus luteum in goats were detected with details at the 3rd days of pregnancy (Kandiel et al., 2010).

Previous reports demonstrated that there was a positive correlation between progesterone level and luteal tissue area (Arashiro, 2010) and luteal size (Orita, 2000; Green et al, 2005) in goats. Moreover, the mean of diameter and blood flow of the CL increased linearly and significantly from Day 5 of estrus till Day 13 in cows (Gaur and Purohit 2018). According to previous results, in our research, luteal diameters of pregnant goats were significantly increased between 7-14th days.

There are contradictory findings on the effect of corpus luteum number on progesterone production. Although it was stated that the P4 levels of goats with more than one CL were significantly higher than those of single CL between from 7th to 30th day of pregnancy (Jarrel and Dziuk, 1991), Arashiro et al (2010) reported that the progesterone production is not affected by the number of CL. In our study, it was found that mean CL numbers decreased in pregnant goats during the examination period. Regarding to increase of luteal sizes beyond Day 21, it may be considered that the relation with pregnancy and luteal tissue quality than account of corpus luteum on the ovaries.

With the use of the transrectal color Doppler sonography in cows, it was revealed that the progesterone levels depend primarily on the luteal blood flow (Acosta, 2004). Moreover, at the assessment luteal status, the luteal blood flow is seen more reliable than it's size (Herzog et al, 2010; especially in premature regressing CL cases (Oliviera et al 2018).

Based on these results, early pregnancy detection including luteal Doppler findings was studied (Utt et al., 2009). Although there were high variations in 15-18th days' scanning (Herzog et al., 2011), the visual evaluation of luteal blood flow is considered as high reliable predictor for non-pregnant females at the day of sponge withdrawing (Cosentino et al., 2018), or between days 17-21 (Utt et al., 2009; Siqueira et al., 2013; Pugliesi et al., 2014; Guimaraes et al., 2015; Arashiro et al., 2018). Researchers reported that the enhanced luteal blood flow could be related to the increased demand of nutritients and substrates of P4 by the embryo and CL, respectively, during the elongation phase (Kanazawa et al., 2016). But, all these studies were performed by using color Doppler techniques, not based on the quantitative parameters.

In recent studies, it can be seen that the semi-quantitative evaluation of luteal blood flow for luteal assessment, and early pregnancy diagnosis by using computerized image analysis program. According to Hassan et al (2019), this technique is more sensitive for evaluating of luteal function during first three weeks after artificial insemination in cows. Besides this, it was reported that the pregnancy diagnosis can be done accurately by semiquantative assessment of luteal vascularization as early as 17 days postbreeding in ewes (Dal et al., 2019).

In the end of study, low correlation rates were seen between luteal biometric and hemodynamic parameters / and gestation age. Therefore, it can be thought that there may be estimation of the gestational age between resistance values and luteal tissue diameters / gestational age.

In our knowledge, this study is the first report including quantitative assessment of luteal blood flow in pregnant goats. According to our result's, luteal blood flow resistance were below 0.5 in healthy pregnant women (Kupesic et al 2003). Up to date, there is no Pulsed Doppler findings of corpus luteum in domestic animals. Nevertheless, the fact that this applicability of transrectal Pulsed Doppler exams in goats, and the similar result between goats and women may be a step for further studies to early luteal function in pregnant goats.

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