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ARAŞTIRMA

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Assessment of Fetal Growth by B-mode and Doppler Ultrasonography in Rabbit during Pregnancy

Fetal biometric parameters and Doppler parameters in blood flow of the umbilical artery during pregnancy in rabbits were evaluated. Ten healthy New-Zealand white female does were studied. B-mode and Doppler ultrasonographic examinations were performed at 3-4 d intervals from 9 to 30 days of pregnancy. The measurements of crown rump length (CRL) biparietal diameter (BPD), trunk diameter (TD) were recorded from the most caudal of the fetus, positioned in the left uterus horn. Additionally, fetal heart rate (FHR), arterial pulsatility and resistance indices (PI and RI) were recorded from the fetal umbilical artery. Of biometric parameters, BPD values were the most correlated with the gestational age ($r= 0.981$). CRL and TD values were in high positive correlation with the gestational age ($r= 0.972$). Lower negative correlations between FHR, PI, RI and gestational age ($r= -0.562$), ($r= -0.383$) and ($r= -0.730$) were recorded, respectively. On the 20th day of pregnancy, umbilical artery PI was stable but RI decreased significantly ($P<0.01$), after then, its curve was stable until parturition. This work provides additional information that might be useful when evaluating and monitoring fetal growth. The 20 days of pregnancy might concern as a threshold stage for umbilical artery indices in the pattern of rabbit pregnancy.

Key Words: Fetal growth, umbilical artery, doppler indices, rabbit

Tavşanlarda Gebelik Boyunca B-mod ve Doppler Ultrasonografi Yardımıyla Fötal Gelişimin İncelenmesi

Tavşanlarda gebelik boyunca fötal biometrik parametreler ve umbilikal arter kan akımına ait Doppler parametreleri incelendi. On adet sağlıklı Yeni Zelanda ırkı dişi tavşan kullanıldı. Gebeliğin 9-30 günleri arasında 3-4 gün aralıkla B-mod ve Doppler ultrasonografik muayeneler yapıldı. Sol kornunun en kaudalindeki fetus üzerinde baş sağrı uzunluğu (BSU), biparietal baş çapı (BPÇ), göğüs çapı (GÇ) değerleri kaydedildi. Ek olarak, a. umbilikalis üzerinden fetal kalp atım sayısı (FKAS), pulsatil (PI) ve resistans (RI) indeks ölçümleri kaydedildi. Biometrik parametreler arasında BSU değeri gebelik yaşı ile yüksek korelasyonu gösterdi ($r= 0.981$). Yine BSU ve GÇ değerleri ile gebelik yaşı arasında yüksek pozitif korelasyon kaydedildi ($r= 0.971$). Fetal kalp atım sayısı, PI ve RI değerlerinin gebelik yaşı ile daha düşük negatif korelasyon gösterdiği ($r= -0.562$), ($r= -0.383$), ($r= -0.730$) tespit edildi. Gebeliğin 20. gününden sonra PI sabit kalırken, RI ise belirgin şekilde düşüş gösterdi ($P<0.01$) ve sonrasında doğuma kadar stabil kaldı. Bu çalışma sayesinde fetal gelişimin değerlendirilmesi ve monitorizasyonunda kullanılabilecek yeni veriler sağlanmıştır. Tavşan gebeliklerinde 20. günün umbilikal arter indeksleri açısından eşik noktası olabileceği düşünülmektedir.

Anahtar Kelimeler: Fötal gelişim, umbilikal arter, doppler indeksi, tavşan

Introduction

Use of rabbits in human reproductive studies has been increasing particularly about reproduction in the last decades (1-4). They are considered as an ideal animal model because of similarity to that of human maternal-fetal units and, also because its size is suitable for using conventional veterinary scanners (5). Although there are several studies on sonographic results of rabbit pregnancy (6-8), the conclusive results of the monitoring fetal growth via ultrasound (6, 9, 10) are limited.

Beside B-mode ultrasonography, obstetric Doppler applications have been used in different species for monitoring fetal well-being noninvasively (11-13). By recording of the pulsatility and resistance in the fetal-maternal vessels, the hemodynamic parameters that have an important clinical implication for fetal surveillance can be obtained. Some specific changes in uteroplacental and fetal arteries related to intrauterine growth restriction (IUGR) are clearly recorded in the rabbit models (4, 14, 15). These studies mainly carried on as an experimental study for human medicine. But Doppler examinations in fetal assessment have not been routinely used in clinical work in the veterinary practice on rabbits. However, it was a well known risk that after palpation of rabbits in pregnancy diagnosis, maternal and also fetal health may deteriorate.

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In the current study, it was aimed to evaluate the fetal somatic growth and umbilical artery Doppler indices in healthy pregnant rabbits. In other words, the objective was to assess the relationships between fetal biometric/Doppler parameters and gestational age, after then to describe the changes of umbilical artery Doppler indices in the healthy pregnancies in order to establish a database for fetal monitoring in rabbit pregnancy.

Materials and Methods

Animals: In the present study, 10 healthy, sexually mature New-Zealand white female does weighing 3.6 - 4.1 kg were used. The does were housed individually in wire mesh cages under controlled light (14h light: 10h dark) and temperature (18 to 24 °C) conditions. All does were with free access to water and standard pellets. Animal handling and all procedures were performed in accordance with regulations and guidelines and with the approval of the Animal Experimental Ethics Committee of the Adnan Menderes University (050.04/2011/011). Before the start of the experiments, the does were accustomed to the environment and trained to medical manipulations for three weeks. Females were mated with sexually active bucks of the same strain and the day of controlled mating, was considered Day 0 of the pregnancy.

Pregnancy Diagnosis, and B-mod and Doppler Ultrasonographic Examinations: At 7-9th day postmate, pregnancy diagnosis was confirmed by ultrasonographical examination (MyLab 30-Esaote®, Genova, Italy) using a micro-convex probe of 8 MHz. B-mode and Doppler ultrasonographic exams were performed at 3-4 days intervals (9-13-16-20-23-27 and 30th day) throughout pregnancy. Prior to the examination, the abdominal ventral region of each doe was shaved. They were not sedated during the study. The transducer was covered with a copious amount of gel to eliminate air spaces. The measurements of CRL, BPD, TD were recorded from the most caudal of fetus, positioned in the left uterus horn. Following the biometric measurements, umbilical artery blood flow waveforms were obtained from the midcord site of the free floating umbilical cord.

Following the visualization of the umbilical artery using color Doppler; Pulsed-wave Doppler examinations were performed. Records were obtained regularly for at least three consecutive arterial waveforms. Waveforms were disregarded during fetal and maternal movements or cardiac arrhythmias. In order to evaluate the blood flow waveform patterns of the umbilical artery, the FHR, PI and RI were measured. Due to the possible thermal and cavity side effects of Doppler sound waves on tissues, Pulsed-wave examinations did not exceed 30 seconds and were recessed for 1 minute. Regarding to stress factors of the study group, all examinations were performed between 9:00-12:00 AM and 50-60 minutes totally in silent and dimly lit room, and were performed by the same trained operator.

Data Analysis and Statistics: Average data presented as mean \pm SEM. Means were compared using a paired Student's *t*-test and χ^2 test (Minitab-16) when appropriate. Regression models were fitted to evaluate the relationship between the day of pregnancy and biometric / doppler parameters which were considered to be dependent on the day of pregnancy. A 5% significance level was used.

Results

Animals: In all does, ultrasonographic examinations have accurately predicted pregnancies with no false negatives or positives. During pregnancy, no maternal disorder and fetal loss finding was recorded. Moreover, no trace having pathological Doppler findings suggestive of fetal stress (end diastolic block and /or notch formation e.g.) was recorded during the study. The does tolerated the ultrasonographic examinations well. Parturitions were observed 28 ± 0.5 d (SEM) after mating normally without any sign of stress. The does gave birth to 8 ± 0.5 live pups without assistance. There were no stillbirths or new-born deaths after delivery.

Biometric Parameters: Figure 1 presents the mean values of BPD and TD of fetuses during pregnancy in rabbits. BPD values were the most correlated with the gestational age ($r=0.981$; $P<0.001$) and were able to measurable in second and third trimester practically. TD values were high positive correlation with the gestational age ($r=0.972$; $P<0.001$). Mean while CRL values were recorded in only second trimester due to due to possibility of fitting on the screen. CRL values were high positive correlation with the gestational age ($r=0.972$; $P<0.001$).

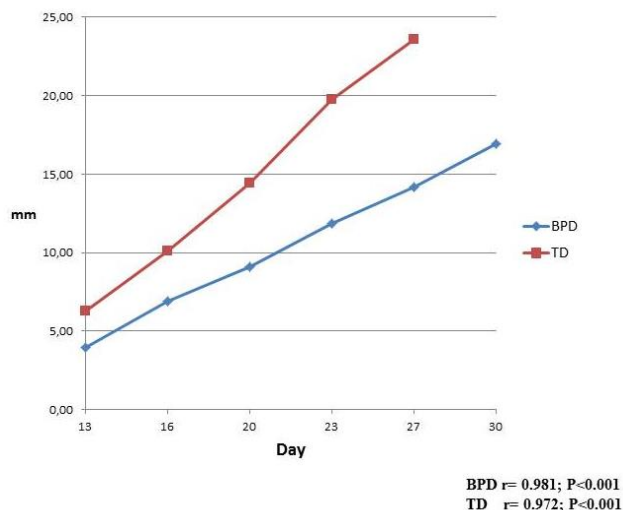


Figure 1. The mean BPD and TD values of rabbit fetuses during pregnancy

Doppler Parameters: The umbilical artery blood flow was characterized low resistance and showed systolic waveform during the second trimester of pregnancy. As of day 20, onward, diastolic waveform could be detected in the Doppler examinations (Figure 2).



Figure 2. The image of Pulsed-Doppler ultrasonography of a. umbilicalis on 20th day of pregnancy

Figure 3 presents the mean values of FHR in umbilical artery of rabbit fetuses during pregnancy. FHR increased gradually as the pregnancy progressed and the peak values were recorded on day 20 of pregnancy. After then, it decreased following two examinations and increased again after 27th days of pregnancy ($P < 0.05$). It was observed that the FHR negatively correlated with gestational age ($r = -0.562$; $P < 0.001$).

Figure 4 presents PI and RI of umbilical artery. They were negatively correlated with gestational age ($r = -0.383$; $P < 0.01$) and ($r = -0.730$; $P < 0.001$), respectively.

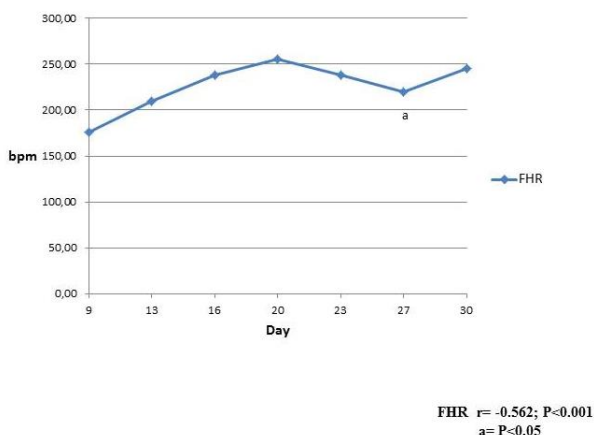


Figure 3. The mean FHR of rabbit fetuses during pregnancy

From day 20 onwards, umbilical artery RI decreased significantly ($P < 0.01$), after then it became stable until parturition. Meanwhile, PI curve has no change significantly ($P > 0.05$) during pregnancy (Figure 4).

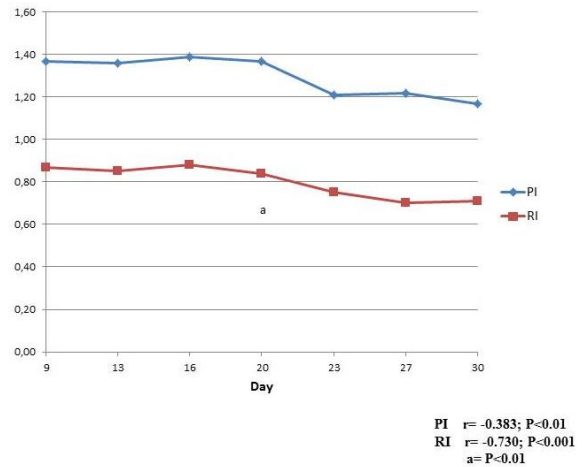


Figure 4. The mean PI and RI indices of umbilical artery of rabbit fetuses during pregnancy

Discussion

Although the time between parturition is short, pregnancy diagnosis and estimation of the gestational age should be considered important in rabbit practice. In case of lack of reports of the date of mating, transabdominal ultrasonographic examinations would be used effectively and practically in clinics and farms.

The rabbit uterus is ultrasonographically detectable only in pregnant animals. Averagely 8 mm-diameter fluid-filled embryonic vesicles are seen as anechoic, round shape formations and uterine wall is getting thicker (7). Pregnancy diagnosis was performing apparently at days of 7 postmating by seeing vesicles which diameter 10-12 millimeters (5, 7, 8). In this study, embryonic sacs were detectable on 7th day of pregnancy, according to the results of earlier studies (5, 9). But the conclusive diagnosis was done based on signs of 9th day postmating again in order to the exclude possibility of the early embryonic losses. Moreover, it could be possible that the Doppler examinations are performed with a microconvex probe with 8 MHz from this day onward.

There have been several detailed studies on fetal somatic growth and also fetal Doppler findings in rabbit (5, 10, 16). Concerning of fetal biometric parameters results reported in previous studies, the agreement between our study's results and others were seen. Regarding the high correlations between the day of the pregnancy and the somatic growth parameters, CRL, BPD and TD are seen as very reliable parameters for determining gestational age in cases with an unknown

breeding date. In the present study, BPD was measurable throughout pregnancy and has the highest correlation value with gestational age. Therefore, it can say that BPD is the most reliable and practical biometric parameter in assessment of fetal growth in rabbit.

Fetal heart rates have been extensively analyzed to describe normal development over the course of pregnancy. It is well recognized that FHR decreases along with pregnancy in sheep and goats (17, 18), and it remains more stable in dogs (11) and cats (19). But there has been a limited FHR findings in rabbit fetuses. Polisca et al (10) observed no significant change in FHR during pregnancy. But in the present study, different FHR curve was recorded. The similar decreasing trend was only observed between 20th and 27th days. In the last examination on 30th day, FHR increased again the prior to parturition. In our opinion, this differences in FHR curve is not attribute to fetal stress related with any pathology due to minimizing of environmental stress factors and also no pathological Doppler findings suggestive of fetal stress (end diastolic block and /or notch formation e.g.) were detected during the study. Therefore, this increase seen in the last days of pregnancy can be related to physiological fetal stress that triggers the parturition process. Another results of Doppler exams revealed that the low success of the estimation of the gestational age based on FHR. In earlier studies, FHR value correlated in a wide range ($r = -0.77$ and -0.55) with gestational age during pregnancy in Saanen goats (18) and Egyptian native goats (20). Moreover, there is no data related to these correlations in rabbit fetuses. Therefore FHR, PI and RI values would be inconsistent in rabbit due to low correlations comparing with other biometric parameters.

Following the evaluation of the Doppler examinations, it is possible to say that the findings of 20th day of pregnancy can be accepted as threshold in this study. The umbilical arterial blood flow that has typically low-resistance was characterized only by systolic waveform until the 20th day. On the 20th day of pregnancy, RI decreased significantly ($P < 0.01$) after then, it was stable until parturition. Although PI value has no change significantly, its curve tended to decrease. According to the previous studies in different species (12, 21-23), diastolic waveform takes places following the decreasing of Doppler indices. The last third of the pregnancy is very important and characterized by maximum fetal growth and rate, therefore, due to the need for a higher amount of nutrient (24). In our opinion, the resistance in umbilical vessels started to decrease significantly at this stage and fetal blood flow within the umbilical cord can be seen in diastolic phase continuously. In rabbit IUGR models, fetal growth retardation findings could be seen examinations at day 21 (15). Consequently, the biometric and Doppler examinations findings should be evaluated together in the antenatal assessment of fetal growth in third trimester.

In conclusion, ultrasound monitoring with B-mode and Doppler may be a useful tool for assessment of fetal growth in the second and third trimester in the rabbit. BPD should be the first choice for estimation of gestational age and can be used as an important parameter for fetal growth retardation. Although umbilical artery blood flow shows some similarity with other species especially in the last trimester, further studies should be undertaken to evaluate the fetomaternal hemodynamic changes. The findings herein could be interest to researchers studying the rabbit reproduction and also fetal monitoring and may be used in the future.

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