

## Comparison of foetal growth in singleton and twin pregnancies by B-mode and Doppler ultrasonography in Karya ewes

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Received: 31.08.2015 • Accepted/Published Online: 17.03.2016 • Final Version: 02.11.2016

**Abstract:** Foetal growth and haemodynamic features were investigated in singleton and twin pregnancies in Karya ewes with B-mode/ Doppler ultrasonography. Nineteen healthy singleton (n = 9) and twin (n = 10) pregnant Karya ewes were used. Foetal biometric and haemodynamic measurements were made at 15-day intervals on the 29 fetuses during pregnancy. Biparietal head diameter (BPD), thoracic diameter (TD), umbilical cord diameter (UCD), foetal heart rate (FHR), peak systolic velocity (PSV), end diastolic velocity (EDV), pulsatility index (PI), and resistance index (RI) were recorded. The BPD was higher in twins on days 55 and 130 of pregnancy (P < 0.05), and the TD was higher in singletons on day 115 of pregnancy (P < 0.01). UCD was higher in twins on day 55 of pregnancy (P < 0.01). On day 130 of pregnancy, PSV and EDV were higher in twin fetuses (P < 0.05). PI and RI were lower in twins on day 40 of pregnancy (P < 0.05). Foetal growth and umbilical artery blood flow show some differences depending on the numbers of fetuses and the gestational stage. Additionally, BPD, TD, and FHR were reliable for estimation of gestational age during twin pregnancies.

**Key words:** Foetal growth, umbilical artery, Doppler ultrasonography, twin pregnancy, Karya ewe

### 1. Introduction

Early detection and monitoring of single and multiple pregnancies by using foetal ultrasonography are very important for maternal health and foetal outcome. The use of ultrasonography minimises the slaughter rate of pregnant animals, facilitates optimal feeding for ideal birth weight, and reduces feed cost in females carrying a singleton (1-4). Moreover, the pregnant ewe model being used in numerous human biomedical studies and ultrasound examination of fetuses in the intrauterine environment emphasises the multidisciplinary importance of this method (5,6).

Various results concerning the determination of gestational age and normal foetal growth rate from measurements of some biometric parameters have been recorded for different ewe and goat breeds (6-12). These studies have provided important results for flock management systems by allowing the grouping of pregnant animals at different gestational stages or with signs of approaching labour.

Foetal heart rates (FHR) have been extensively analysed to describe normal development over the course of pregnancy, especially in singleton pregnancies. Abnormal

heart rate patterns are suggestive of foetal distress (13). Although it is well recognised that FHR decreases during the course of pregnancy in farm animals (3,11,14-16) except in goats (16), there are no data available on the FHR curve through singleton and multiple pregnancies in domestic animals.

Obstetric Doppler ultrasound provides foetal haemodynamic information in real time (recording of the vascular pulsatility and resistance in foeto-maternal vessels) that has important clinical implications for foetal surveillance. In Doppler examinations, umbilical artery (UA) resistance is associated with intrauterine growth restriction (IUGR), congenital anomalies, and other adverse foetal outcomes (17-19). Although several ultrasound studies have compared Doppler indices among human singletons and multiple fetuses (20,21), such studies have not resulted in routine applications in veterinary medicine. A multiple pregnancy is known to differ from a singleton pregnancy with respect to the mean duration of pregnancy and the mean birth weight of the infants (22). Thus, the possibility in differences in foetal Doppler data in multiple pregnancies should be considered. There are some data regarding singleton and

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multiple pregnancies from human medicine (21,23,24), but only one report in veterinary practice (16). In the current study, the authors sought to compare biometric parameters for single and twin foetuses, in addition to UA Doppler measurements throughout the pregnancy, in healthy Karya ewes.

## 2. Materials and methods

### 2.1. Animals

Thirty-eight healthy, multiparous 2- to 3-year-old Karya ewes (Chios × Kivircik crosses) were used. The ewes were housed at the experimental farm at Adnan Menderes University, Faculty of Veterinary Medicine, located in Aydın, Turkey. Oestrus was synchronised in 38 Karya ewes (average weight 36.96 kg) by using intravaginal sponges. The intravaginal sponges containing 20 mg of fluorogestone acetate were inserted into the vagina of each animal for 14 days (Chronogest; MSD Animal Health, Netherlands). Forty-eight hours before sponge withdrawal, all ewes were administered intramuscularly with 600 IU of equine chorionic gonadotropin (eCG) (Folligon; MSD Animal Health, Netherlands) and 75 µg of cloprostenol (Estrumate; MSD Animal Health, Netherlands) for stimulation of oestrus and ovulation. Ram introduction was performed 2 days after sponge withdrawal. Animals in oestrus were allowed to mate twice during oestrus. The day of oestrus was designated as day 0 (day of first mating).

### 2.2. Pregnancy diagnosis, foetal counting, and monitoring via ultrasonography

All ewes were examined for pregnancy detection and foetal counting between day 30 and day 40 after mating by using real-time transrectal ultrasonography (MyLab 30-Esaote, Genova, Italy) with a 7.5-MHz linear transducer. Pregnancy was confirmed in 19 ewes, with 9 singleton and 10 twin pregnancies. For prenatal ultrasonography, foetuses were examined with the use of transabdominal ultrasonography with a 3- to 5-MHz convex transducer on days 40, 55, 70, 85, 100, 115, 130, and 145 after mating.

The ewes were examined in dorsal recumbency until 85 days of pregnancy, after which they were examined in lateral recumbency to avoid maternal aorta compression until 145 days of pregnancy. Before examination of the inguinal region, the hair on both sides was clipped and, in the advanced stage of pregnancy, the more cranial portion of the ventral abdomen was also clipped. The ewes were not sedated. Environmental stress factors were minimised during examinations (e.g., the same operator working in a silent and dimly lit room, proper restraint and handling). The transducer was covered with a copious amount of gel to eliminate air spaces. All ultrasonographic examinations were performed by the same trained operator. First, biparietal diameter (BPD), thoracic diameter (TD), and umbilical cord diameter (UCD) were recorded. Following

the biometric measurements, UA blood flow waveforms were obtained from the midcord site of the free-floating umbilical cord. The UA was visualised using colour Doppler, and then pulsed-wave Doppler ultrasonography was performed. In the twin pregnancies, the Doppler measurements recorded in both foetuses were localised caudally and cranially. The first foetus detected in twin pregnancies was considered to be the first twin; the other foetus localised cranially was recorded as the co-twin.

Recordings were obtained for at least 3 regularly consecutive arterial waveforms. Waveforms were disregarded during foetal and maternal movements or cardiac arrhythmias. In order to evaluate the blood flow waveform patterns of the UA, the FHR, peak systolic velocity (PSV), end-diastolic velocity (EDV), pulsatility (PI), and resistance index (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 s and were recessed for 1 min. All examinations lasted between 10 and 15 min.

### 2.3. Data analysis and statistics

Average data are presented as mean ± SEM. Means were compared using a paired Student's t-test and chi-squared test (Minitab-16) when appropriate. Differences were considered statistically significant at P levels of less than 0.05.

## 3. Results

### 3.1. Animals

No signs of systemic and/or genital disease were noted during the pregnancies. All parturitions occurred normally and without any assistance at day 148.84 ± 0.69 of pregnancy. In 2 ewes with twin pregnancies, the last examination was not performed because they gave birth to lambs at days 142 and 143 of pregnancy, respectively. Twenty-nine healthy lambs, 11 females and 18 males, were born. There were no stillbirths or newborn deaths after delivery.

### 3.2. Biometric parameters, FHR, and Doppler parameters

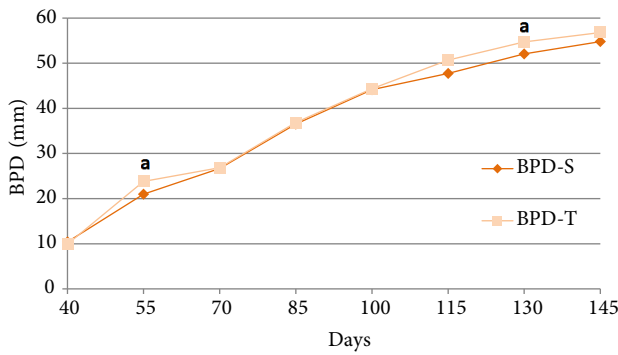
Table 1 presents the mean biometric parameters and FHR between 40 and 145 days of pregnancy in the Karya ewes. The BPD value was higher in twin foetuses than in single foetuses at days 55 and 130 of pregnancy ( $P < 0.05$ ) (Figure 1). The TD value was higher in singletons at day 115 of pregnancy ( $P < 0.01$ ) (Figure 2). When comparing UCD values between the groups, it was higher in twins at day 55 of pregnancy ( $P < 0.01$ ) (Figure 3), but there was no difference in FHR values between single foetuses and twins throughout pregnancy (Figure 4).

Table 2 presents the mean Doppler findings from a. umbilicalis of all foetuses. Both PSV and EDV were higher in twins on day 130 of pregnancy ( $P < 0.05$ ) (Figure 5). At day 40 of pregnancy, the PI and RI values were higher in single foetuses than in twin foetuses ( $P < 0.05$ ) (Figure 6).

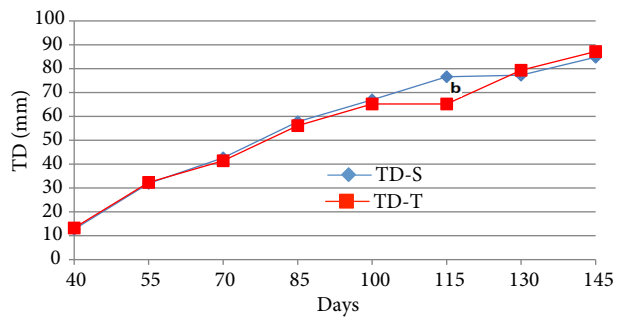
**Table 1.** The mean BPD, TD, UCD, and FHR values of single (S) and twin (T) fetuses during pregnancy.

Days	BPD-S (mm)	BPD-T (mm)	TD-S (mm)	TD-T (mm)	UCD-S (mm)	UCD-T (mm)	FHR-S (bpm)	FHR-T (bpm)
40	10.41 ± 0.33	9.92 ± 0.17	12.70 ± 0.53	13.29 ± 1.30	4.28 ± 0.26	4.28 ± 0.08	223.22 ± 3.1	226.90 ± 2.7
55	20.99 ± 0.81 <sup>a</sup>	23.79 ± 0.66 <sup>b</sup>	31.92 ± 1.00	32.31 ± 0.69	6.80 ± 0.37 <sup>c</sup>	7.94 ± 0.18 <sup>d</sup>	227.10 ± 6.1	219.3 ± 3.4
70	26.67 ± 0.63	26.83 ± 0.52	42.62 ± 1.70	41.22 ± 0.91	10.20 ± 0.38	9.48 ± 0.21	206.11 ± 2.9	215.00 ± 3.2
85	36.53 ± 0.75	36.87 ± 0.58	57.74 ± 1.30	56.16 ± 0.93	12.64 ± 0.64	12.10 ± 0.28	198.00 ± 2.4	194.40 ± 2.7
100	44.16 ± 1.60	44.35 ± 0.52	66.97 ± 1.70	65.18 ± 1.20	14.31 ± 0.66	13.61 ± 0.37	188.6 ± 3.9	191.0 ± 3.4
115	47.73 ± 0.95	50.71 ± 0.91	76.63 ± 3.1 <sup>c</sup>	65.18 ± 1.20 <sup>d</sup>	13.82 ± 0.53	14.73 ± 0.52	171.30 ± 4.4	175.50 ± 2.8
130	52.08 ± 1.20 <sup>a</sup>	54.70 ± 0.52 <sup>b</sup>	77.30 ± 3.8	79.37 ± 1.30	15.61 ± 0.45	15.66 ± 0.42	152.10 ± 3.7	147.7 ± 3.4
145	54.79 ± 1.30	56.80 ± 0.64	84.80 ± 3.3	87.19 ± 1.70	17.52 ± 0.48	16.73 ± 0.37	141.70 ± 5.4	140.5 ± 3.4

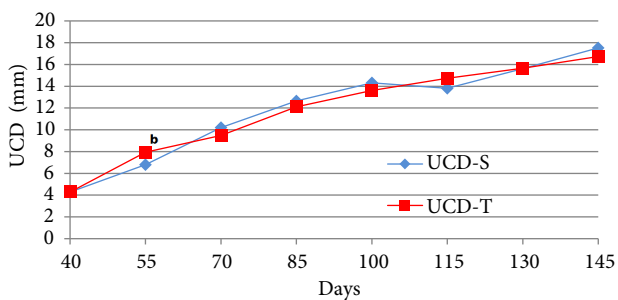
Values are represented as mean ± SEM. Results with letters (a, b, c, d) differ from those of the other groups' measurements ( $P < 0.05$ ,  $P < 0.01$ ).



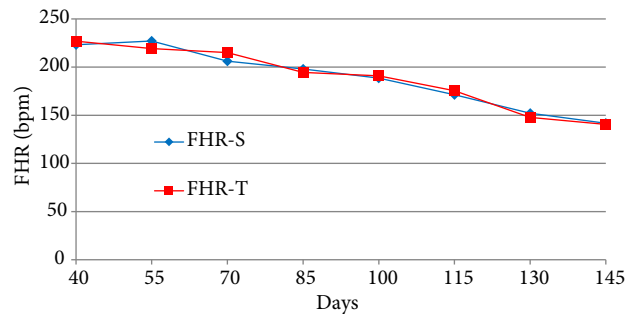
**Figure 1.** Biparietal diameters (BPD) of single and twin fetuses throughout pregnancy. Values are mean BPD of 29 fetuses from 19 ewes (9 singleton and 10 twin pregnancies). Both increase significantly during pregnancy. Values with letter (a) differ between single and twin fetuses ( $P < 0.05$ ).



**Figure 2.** Trunk diameters (TD) of single and twin fetuses throughout pregnancy. Values are mean TD of 29 fetuses from 19 ewes (9 singleton and 10 twin pregnancies). Both increase significantly during pregnancy. Values with letter (b) differ between single and twin fetuses ( $P < 0.01$ ).



**Figure 3.** Umbilical cord diameters (UCD) of single and twin fetuses throughout pregnancy. Values are mean UCD of 29 fetuses from 19 ewes (9 singleton and 10 twin pregnancies). Both increase significantly during pregnancy. Values with letter (b) differ between single and twin fetuses ( $P < 0.01$ ).

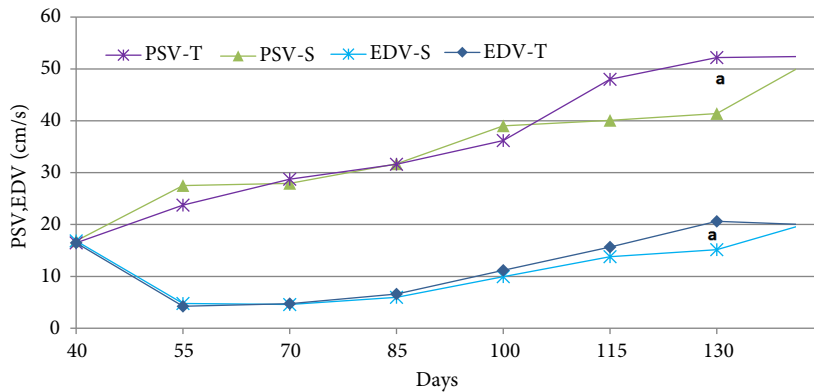


**Figure 4.** Foetal heart diameters (FHR) of single and twin fetuses throughout pregnancy. Values are mean FHR of 29 fetuses from 19 ewes (9 singleton and 10 twin pregnancies). Although there are significant decreasing trends during pregnancy, there is no difference in FHR values between study groups.

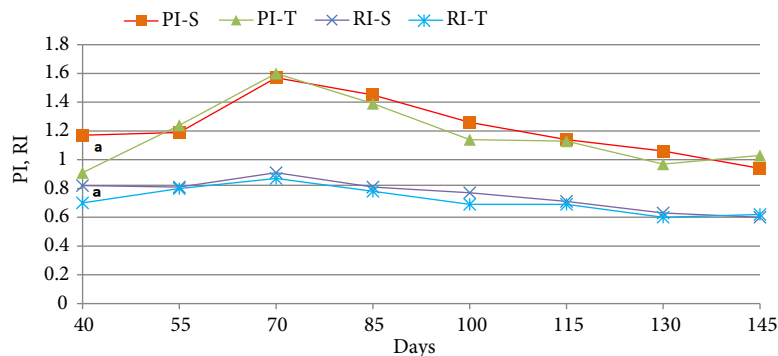
**Table 2.** The mean PSV, EDV, PI, and RI values of single (S) and twin (T) fetuses during pregnancy.

Days	PSV-S (cm/s)	PSV-T (cm/s)	EDV-S (cm/s)	EDV-T (cm/s)	PI-S	PI-T	RI-S	RI-T
40	16,84 ± 1.4	16.46 ± 0.85	16.84 ± 1.40	16.46 ± 0.85	1.17 ± 0.05 <sup>c</sup>	0.91 ± 0.02 <sup>d</sup>	0.82 ± 0.01 <sup>a</sup>	0.70 ± 0.02 <sup>b</sup>
55	27.50 ± 2.8	23.74 ± 0.93	4.76 ± 0.32	4.24 ± 0.24	1.19 ± 0.07	1.24 ± 0.04	0.81 ± 0.02	0.80 ± 0.01
70	27.94 ± 2.0	28.73 ± 1.30	4.58 ± 0.49	4.74 ± 0.28	1.57 ± 0.14	1.60 ± 0.06	0.91 ± 0.06	0.87 ± 0.04
85	31.72 ± 2.9	31.61 ± 1.5	5.96 ± 1.1	6.58 ± 0.48	1.45 ± 0.02	1.39 ± 0.04	0.81 ± 0.01	0.78 ± 0.01
100	39.02 ± 3.0	36.20 ± 2.6	9.94 ± 1.2	11.16 ± 0.77	1.26 ± 0.09	1.14 ± 0.04	0.77 ± 0.03	0.69 ± 0.01
115	40.07 ± 3.5	48.0 ± 2.2	13.81 ± 1.8	15.66 ± 1.2	1.14 ± 0.09	1.13 ± 0.05	0.71 ± 0.04	0.69 ± 0.02
130	41.4 ± 3.7 <sup>a</sup>	52.2 ± 2.7 <sup>b</sup>	15.14 ± 2.0 <sup>a</sup>	20.62 ± 1.3 <sup>b</sup>	1.06 ± 0.07	0.97 ± 0.04	0.63 ± 0.02	0.60 ± 0.01
145	52.93 ± 2.10	52.45 ± 2.5	21.11 ± 1.7	19.84 ± 1.6	0.94 ± 0.07	1.03 ± 0.04	0.60 ± 0.02	0.62 ± 0.01

Results with letters (a, b, c, d) differ from those of the other groups' measurements (P < 0.05).



**Figure 5.** Peak systolic velocity (PSV) and end diastolic velocity (EDV) of all fetuses throughout pregnancy. Values are mean PSV and EDV of 29 fetuses from 19 ewes (9 singleton and 10 twin pregnancies). Values with letter (a) differ between single and twin fetuses (P < 0.05).



**Figure 6.** Pulsatility index (PI) and resistance index (RI) of all fetuses throughout pregnancy. Values are mean PI and RI of 29 fetuses from 19 ewes (9 singleton and 10 twin pregnancies). Values with letter (a) differ between single and twin fetuses (P < 0.05).

Except for this examination, no significant difference was recorded in Doppler indices between the 2 study groups.

#### 4. Discussion

Early and proper pregnancy scanning, estimation of gestational age, foetal counting, and the separation of pregnant ewes carrying singletons from twins are important management steps to improve flock reproduction parameters. Because of the long duration of the breeding season and variation related to time of mating in the flock, these examinations can raise productivity in the ewe enterprise. Various studies on the estimation of gestational age have provided foetal biometric correlation data in small ruminants (8,9,11,25,26). Among these studies, there is only one that has compared biometric traits of single and twin foetuses and attempted to find correlation among them. Moreover, no differences between pregnancies with single and twin foetuses were found in the measurements of the skull, trunk, abdominal cavity, and femur (25).

In the present study, it was remarkable that the BPD values of twins were higher on day 55 and day 130 of pregnancy. Regarding uterine capacity, it was expected to be lower due to the twins' somatic growth. It can be described as the transient acceleration of BPD values in twins, which follow the single foetuses' BPD values normally. In addition, the significantly higher PSV and EDV values on day 130, which were related to higher foetal blood flow, support this enhancement.

The validity of the use of twins' BPD values in estimation of foetal age is important clinically because it is the most correlated biometric parameter during pregnancy (26,27). After comparing the data for the 2 groups, it can be said that BPD is as reliable a parameter for estimation of foetal age during twin pregnancy as it is in singleton pregnancy. BPD values of the ovine foetuses were recorded as <20 mm in the first, between 20 and 45 mm in the second, and >45 mm in the third trimester.

Trunk diameter is another biometric parameter that is usually used in estimation of the gestational age (9,11). It allows the performance of foetometry when crown rump length and BPD are not measurable, especially in the third trimester (4,11). In the present study, there was only one significant difference between the TD values of single and twin foetuses, on day 115 of pregnancy ( $P < 0.01$ ). Therefore, TD can be considered a reliable parameter for the estimation of foetal age in twin pregnancies. Regarding all examinations in the present study, it can be concluded that TD values were <30 mm in the first, between 20 and 65 mm in the second, and >65 mm in the third trimester.

In the monitoring of foetal growth, UCD has a lower correlation with foetal age than either BPD or TD (9,27). In the current study, the UCD value of twins was higher on day 55 of pregnancy. Increased diameter of the umbilical

cord at the beginning of the second trimester can result in increased blood flow to the foetus; therefore, BPD was increased on the same day.

According to similar studies performed in ewes and goats, the FHR curve descends corresponding to the progression of pregnancy (8,11,12,16). We observed a similar decreasing trend and no difference in FHR between single and twin Karya foetuses. It can also be seen that the FHR values were above 220 bpm during the first trimester and below 190 bpm in the third trimester of these healthy pregnancies. In the results of our previous study (16), no difference was recorded between single and multiple pregnancies of Saanen goats. Regarding cardiac effects related to diseases and some stress factors, the study subjects were selected from healthy animals and environmental stress factors that can increase FHR sensitivity were minimised.

When evaluating PSV and EDV of a. umbilicalis, day 130 of pregnancy was a remarkable stage for twins in this study. Although no similar difference was detected in PI and RI, the higher BPD is considered to support somatic growth of the calvarium through increased foetal blood flow.

The PI value increased at day 55 of pregnancy in singletons and multiple pregnancies and decreased significantly at day 85 of pregnancy; its values then reached a plateau until parturition in goats (16). In our study, the PI was recorded at day 70 of pregnancy, after which a similar decreasing curve was observed. Similarly, RI curves were stable, increasing nonsignificantly until 70 days' pregnancy, and then decreased until parturition. This low-resistance uteroplacental vascular bed helped to increase the maternal blood supply for foetuses. When we examine all the results, it can be seen that the average threshold value of PI was 1.20. Accordingly, in the second trimester, PI exceeded the 1.20 value, but after the third trimester began, it was below the threshold again until delivery. Similarly, we can say that the average threshold was 0.7 for RI. After the end of the second trimester, RI values were less than 0.7, and this continued until birth.

Doppler indices have diagnostic value in foetal stress. Although no difference was observed in PI value between singletons and multiple pregnancies (21), a difference in pulsatility and resistance of UA was observed in the first third of pregnancy (day 40). In twins, both recorded indices were lower than in singletons. It can be inferred from low resistance in twin pregnancies that the difference is a compensation system for retarded foetal growth results from multiple pregnancy stress.

Advances and innovations in ultrasound have opened up new opportunities to collect data about intrauterine life. Twin or triplet foetuses should be monitored by reproductive specialists for changes in condition or signs of growth discordance or foetal stress. At the last week

of pregnancy, daily ultrasound examinations to verify foetal health and detect changes in the pregnancy allow us to better predict treatment effectiveness and impending delivery.

In conclusion, foetal growth and umbilical artery blood flow show some differences depending on the foetal number and the gestational stage in ewe fetuses. A detailed B-mode and Doppler ultrasonography may be

an advantage for foetal monitoring that might improve the antenatal management of twin pregnancy, which should be evaluated in further studies.

### Acknowledgements

This study was funded by the Adnan Menderes University Scientific Research Project (ADU-BAP) with project number VTF-10003.

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