Bulgarian Journal of Agricultural Science, 18 (No 4) 2012, 493-500
Agricultural Academy

MEASUREMENTS OF FOETAL GROWTH VIA TRANSABDOMINAL ULTRASONOGRAPHY DURING FIRST HALF OF PREGNANCY AT EWES FROM SYNTHETIC POPULATION BULGARIAN MILK

N. METODIEV[1]*, D. DIMOV[2], I. RALCHEV[2] and E. RAICHEVA[1]
[1] Institute of Animal Science, Department Sheep Breeding, BG - 2230 Kostinbrod, Bulgaria
[2] University of Forestry, Faculty of Veterinary Medicine, BG - 1756 Sofia, Bulgaria

Abstract

METODIEV, N., D. DIMOV, I. RALCHEV and E. RAICHEVA, 2012. Measurements of foetal growth via transabdominal ultrasonography during first half of pregnancy at ewes from synthetic population Bulgarian milk. *Bulg. J. Agric. Sci.,* 18: 493-500

The aim of present work was to determine values of some measurements of foetal growth via transabdominal ultrasonography during first half of pregnancy at ewes from Synthetic Population Bulgarian Milk. Two parallel experiments were carried out with ewes, which were in different gestational phases. The first experiment was carried out with 19 ewes, conceived in three consecutive days. Three consecutive ultrasound observations in 10 days intervals were done, on days 40-42, 51-53 и 61-63 after conceiving. The following measurements were taken: head diameters: biparieatal (BPD) and occipito-nasal (ONL), Placentome size: width and length. The second experiment was carried out with 17 ewes, conceived in the same day. The examination days were 25, 36 and 46 after artificial insemination. It was measured: Day 25: diameter of embryonic vesicle; length of embryo Day 36: crown-rump length; Day 46: crown-rump length; head diameters: biparieatal (BPD) and occipito-nasal (ONL); placentome size: width and length. All measurements were in millimeters (mm). The effect of gestational phase on foetal growth is analysed by one way ANOVA. The coefficients of correlation (r) and determination (R^2) between gestational phase and embryofetal measurements were established by standard regression. The gestational phase influence significantly (($P<0.001$) all taken measurements. The mean values for BPD were11.16 mm on 40-42 gestational days and reached 21,08 mm on 61-63 days, and for ONL -15.17 mm on 40-42 days and reached 32.94 mm on 61-63 days. The size of palcentomes varied in wide range for all taken measurements. On days 40-42 the mean values were: length- 13.67 mm and width – 10.06 mm, and reached on 61-63 days mean length – 33.02 mm and mean width – 21.33 mm. It was established high, positive and significant correlation between head diameters and gestational age (r=0.93*** и r=0.93***). It was obtained moderate values of the coefficient of determination for the size of placentomes and gestational age (R^2= R^2= 0.46 и R^2= 0.47). The embroyfetal length was 11.08 mm on day 25, 26.03 mm on day 36 and 51.79 mm on day 46. It was established high, positive and significant correlation between CRL and gestational age (r= 0.84***).

Key words: ultrasound, ewe, foetal measurements, gestational age

Introduction

The real-time B-mode ultrasonography is the earliest, most accurate, safest, fastest and most economical method of pregnancy diagnosis in sheep at farm level (Ganie et al., 2009). There are two approaches, that is use to study the female reproductive tract of small ruminants – transrectal and transabodminal, and the ap-

* *Corresponding author: n_metodiev@abv.bg*

proach, that would be choose depends on diagnosis, that is needed, probes and size of flocks (Kähn, 2004). The author, Kähn, (2004), recommends for early pregnancy diagnosis transrectal approach as more suitable until 35-th gestational day, the two approaches between 35-th and 70-th day of pregnancy, and during the second half of pregnancy, the transabodminal approach is more appropriate to use because of the better visualization of uterus.

When the date of mating is unknown, monitoring fetal development allows estimation of gestational age (Gonzáles de Bulnes et al., 1998). The most practical measurements of foetal structures are (by Karen et al., 2001): embryonic vesicle; crown-rump length; fetal head diameters; thoracic diameters; foetal heart rate; placentome size. Ideally, intrauterine fetal growth rate should be defined in the same population as that evaluated (Ali and Hayder, 2007). There are no foetal measurements and growth standards for Synthetic Population Bulgarian Milk (SPBM) sheep breed.

The aim of present work was to determine values of some measurements of foetal growth via transabdominal ultrasonography during the first half of pregnancy at ewes from Synthetic Population Bulgarian Milk.

Materials and Methods

Two parallel experiments were carried out with ewes from SPBM, which were in different gestational phases. The dates of artificial inseminations were known, as the day of first insemination was Day 1 of pregnancy. All ewes were between 2.5 to 6.5 years old, clinically healthy during the experiments. All ewes were inseminated artificially with non-diluted semen from the first signs of estrus (detecting by teasers) to its end, in intervals of 12 hours. It was used Eickemeyer Medic 2000 device and sector probe with frequency 5 MHz. It was used transabodminal approach in right abdominal wall, in the area between pecten ossis pubis and last rib. An ultrasonic jelly was put on the probe, before attaching it to the skin.

Experiment 1

The experiment was carried out with 19 ewes, conceived in three consecutive days. All ewes were conceived as that was established previously by non-return method. Three consecutive ultrasound observations in 10 days intervals were done, on days 40-42, 51-53 и 61-63 after conceiving. All measurements were taken after freezing the images with built-in electronic callipers. Where two fetuses were predicted and measured, but after the lambing, the prognosis was untrue, we made mean measurements. During the second observation from the series, one ewe was missed to observe.

The following measurements were taken:

Head diameters (image 1): biparieatal (BPD) and occipito-nasal (ONL).

Placentome size (image 2): width and length.

BPD - head width, determined from a symmetrical image of the skull (by Sergeev et al., 1990).

ONL – head length, determined from the top of os nasale to the end of os occitopitale.

Size of placentomes. After the 40-th day of gestation, the pacentomes of ewes are cup-shape (Doizé et al., 1997). It was measured its width and length. Normally (but not in all cases), one placentome from each ewe was measured and that was the first placentome that was imaged on the screen.

Experiment 2

The experiment was carried out with 17 ewes, conceived in the same day (16 ewes were with induced synchronized estrus after two injections, given in 9 days apart, of synthetic analog of prostaglandin F2α - alfaprostolum (0.5 ml Alfabedyl CEVA ANIMAL HEALTH) and 1 ewe with natural estrus). The ewes were not examined by non-returned method for pregnancy diagnosis. Three consecutive ultrasound observations in 10 days intervals were done, too. The examination days were 25, 36 and 46 after artificial insemination. According to Hafez, (1993), the stage of embryo is to day 34 after gestation, so in first series of observations measurements were for embryo, after that it was measured fetus. Where two fetuses were predicted measured, but after the lambing the prognosis was untrue, we made mean measurements. In some cases in the first series of observations (Day 25), we imaged embryonic vesicles without embryo inside.

The following measurements were taken:

Day 25: diameter of embryonic vesicle (image 3);

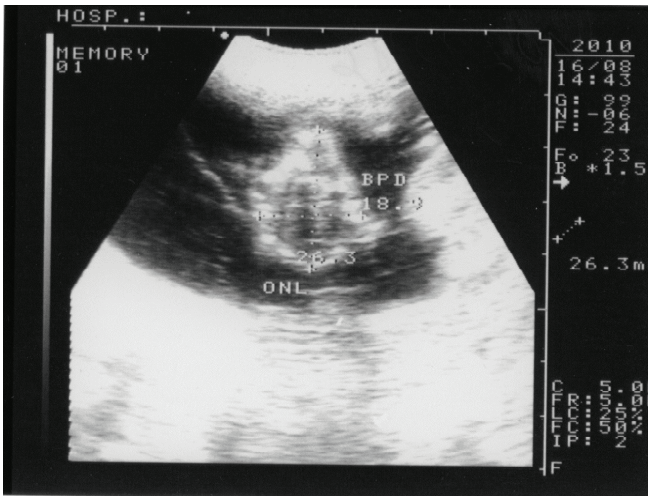

Image 1. Head dismetres (BPD and ONL) at gestational age 51-53-th days

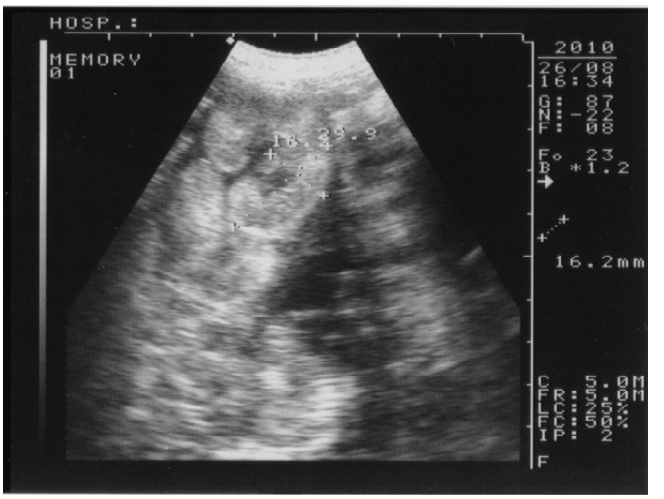

Image 2. Placentome at gestational age 61-63-th days

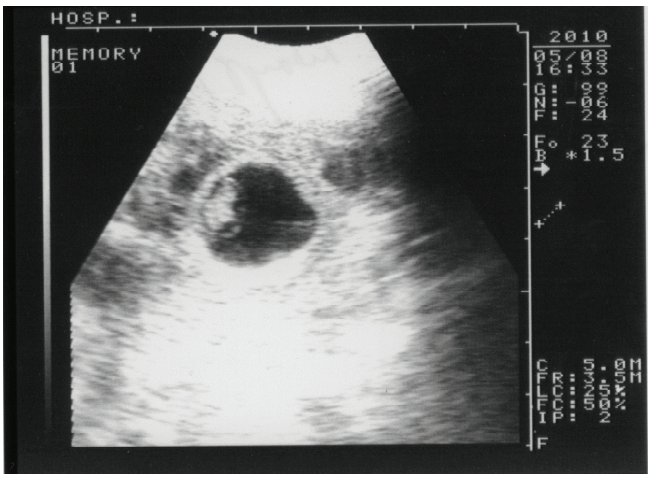

Image 3. Embryonic vesicle with embryo inside, gestational age 25-th day

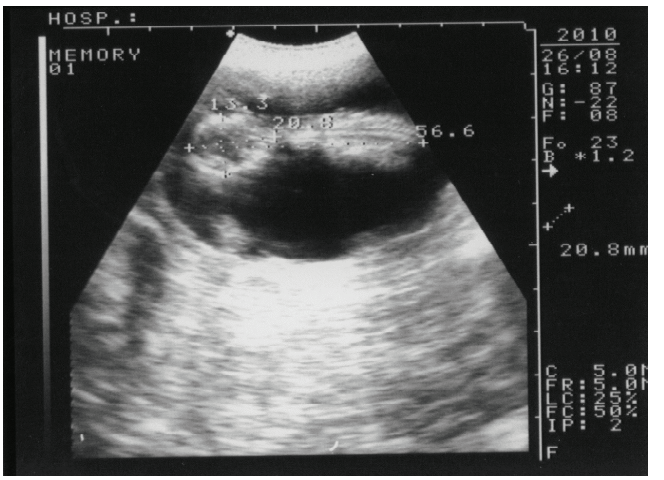

Image 4. CRL at gestational age 46-th day

length of embryo

Day 36: crown-rump length

Day 46: crown-rump length (image 4); head diameters: biparieatal (BPD) and occipito-nasal (ONL);

placentome size: width and length.

Statistical analysis

All measurements were in millimeters (mm). The results were presented in means, standard errors and variation coefficients. The effect of gestational phase on foetal growth is analysed by one way ANOVA. T-statistics was used to compare differences between different observations of each measurement.

The coefficients of correlation (r) and determination (R^2) between gestational phase and embryofetal measurements were established by standard regression with computer program EXCEL, 2003 and package Data Analysis. The correlation relationships were classified as: $r \leq 0.3$ – low correlation; r from 0.3 to 0.5 – moderate correlation; r from 0.5 to 0.7 – considerable; $r \geq 0.7$ – high correlation. The significance of the coefficients of correlation was analyzed by t-criteria of

Student. The effectiveness of regression equations was determined by values of R^2 and the significances of the regressions by the values of F-criteria.

Results

Experiment 1

The obtained values of F–criteria showed, that the gestational phase had highly significant effect on all measurements (P<0.001) (Table 1). The biparietal diameter had mean value 11.16 mm on 40-42 gestational days and reached 21.08 mm on 61-63 days, as the differences between 40-42 days and 51-53 days and between 51-53 and 61-63 were highly significant (P<0.001) (Table 2). The occipitonasal diameter had mean value15.17 mm on 40-42 gestational days and reached 32,94 mm on 61-63 days, as the differences between 40-42 days and 51-53 days and between 51-53 and 61-63 were highly significant (P<0.001) (Table 2). There were established positive, highly by values and significant coefficients of correlation (r= 0.93*** и r= 0.92***) (Figures 1 and 2) about head diameters – BPD and ONL respectively. The size of placentomes varied in wide range for all taken measurements (Table 2). On days, 40-42 the mean values were length- 13.67 mm and width – 10.06 mm, and reached on 61-63 days mean length – 33.02 mm and mean width – 21.33 mm. For all measurements the differences between 40-42 days and 51-53 days and between 51-53 and 61-63 were highly significant (P<0.001) (Table 2).

Experiment 2

The gestational phase influence significantly ((P<0,001) on embroyfetal length (or CRL), as the F-criteria was presented on table 1. The embroyfetal length was 11.08 mm on day 25, 26.03 mm on day 36 and 51.79 mm on day 46, as the differences between days 25 and 36, and 36 and 46 were highly significant (Table 3.) There was established highly significant positive correlation (r= 0.84, P<0.001) between CRL and gestational age (Figure 5.) The mean value of the size of embryonic vesicle was - length 22.38 ± 0.92 mm and width – 17.62 ± 0.85 mm. Because of the small size of the screen, we could not study the following development of the vesicle. Nevertheless, the size of the vesicle gave the additional information about development of the early pregnancy.

Discussion

Experiment 1

The obtained high values of coefficients of correlation and determination for head diameters (BPD - r= 0.93, P<0.001, R^2 = 0.87, P<0.001; ONL r= 0.92, P<0.001, R^2 = 0.85, P<0.001) (Figures 1 and 2) were in conformity with those that were obtained from other researchers. Haibel and Perkins (1989) established coefficient of determination R^2 = 98.63% to Suffolk ewes that were studied in period 43-96 gestational days. Sergeev et al. (1990), reported high positive correlation (r = 0.957) for BPD measured at ewes crosses between Booroola x South Australian Merino. Gonzáles de Bulnes et al. (1998), in study with Manchega ewes obtained high coefficient of correlation for BPD (r = 0.96), measured between days 32-90 and high coefficient of correlation for ONL, measured between days 38-91. High coefficient of determination (R^2= 0.94) between gestational days 60-120 obtained Greenwood et al. (2002) to ewes crosses Suffolk x (Finn Landrace x Dorset). Gunduz et al. (2010) established high coefficient of determination for BPD at Kivircik ewes, measured in the period of 8-th to 20-th gestational week - (R^2= 0.955) for singles and (R^2= 0.937) for twins.

The authors gave advantages to one or another diameter as more accurate for prediction of gestational age. Kelly and Nehman (1989), recommended ONL as more accurate, but Haibel and Perkins (1989), Sergeev et al. (1990) and Gonzáles de Bulnes et al. (1998) said that the BPD was the most representative parameter for prediction during second third of pregnancy. In addition, Sergeev et al. (1990) said that the head length proved more difficult to measure.

The obtained results in our study showed, that independently using of both diameters was suitable for prognosis of gestational age. In both measurements the correlation coefficients were high and variation was low (VC were between 7.95% и 13.96%).

Our observations for size of placentomes coincided to those of на Gonzáles de Bulnes et al., (1998). The authors reported that it was existed a great variation of

Table 1
Values of F-criteria for the effect of gestational age on fetal measurements

Source of variation	BPD			ONL			Placentome, length			Placentome, width			CRL		
	DF	MS	F	DF	MS	F	DF	MS	F	DF	MS	F	DF	MS	F
Between Groups	2	677.61	263.87 ***	2	2195.901	232.15 ***	2	807.48	41.96 ***	2	682.19	56.86 ***	2	5001.62	366.57 ***
Within Groups	76	2.56		76	9.46		57	19.21		57	11.99		37	13.64	
Total	78			78			59			59			39		

Table 2
Measurements in Experiment 1

Days	40-42			51-53			61-63		
Measurement	n	mm Mean ± SE	VC, %	n	mm Mean ± SE	VC, %	n	mm Mean ± SE	VC, %
BPD	27	11.16 ± 0.17	8.15	24	16.63 ± 0.26 A***	7.95	28	21.08 ± 0.42 B***	10.63
ONL	27	15.17 ± 0.26	8.96	24	25.98 ± 0.48 C***	9.01	28	32.94 ± 0.85 D***	13.63
Placentome, length	23	13.67 ± 0.65	22.68	18	24.19 ± 1.18 E***	20.75	19	33.02 ± 1.1 F***	14.51
Placentome, width	23	10.06 ± 0.48	23.24	18	17.0 ± 0.74 G***	18.47	19	21.33 ± 1.08 H***	22.08

Note: *** - - Significant differences at P<0,001: A – Days 51-53 to Days 40-42; B Days 61-63 to Days 51-53; C - Days 51-53 to Days 40-42; D - Days 61-63 to Days 51-53 E - Days 51-53 to 40-42; F - Days 61-63 to 51-53 G - Days 51-53 to Days 40-42; H – Days 61-63 to Days 51-53;

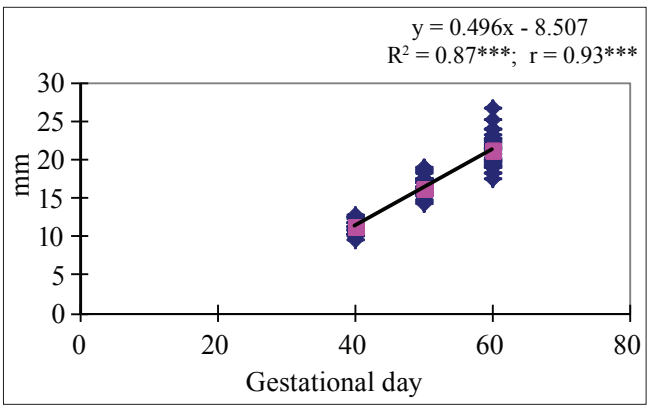

Fig. 1. Linear regression relationship between gestational day and BPD

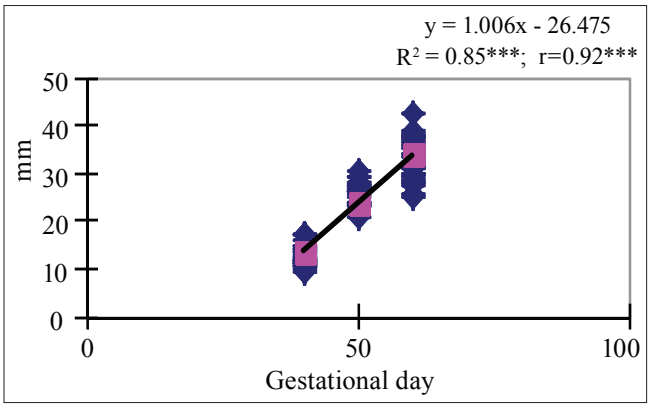

Fig. 2. Linear regrssion relationship between gestational day and ONL

Table 3
Measurements in Experiment 2

Day	25					36					46				
Measurement	n	Mean ± SE, mm	VC, %	Min., mm	Max., mm	n	Mean ± SE, mm	VC, %	Min., mm	Max., mm	n	Mean ± SE, mm	VC, %	Min., mm	Max., mm
Embryonic vesicle, length	17	22.38 ± 0.92	16.98	16.1	27.7										
Embryonic vesicle, widht	17	17.62 ± 0.85	19.86	10.4	22.9										
Embryofetal length (CRL)	13	11.08 ± 0.59	19.22	7.3	14.6	16	26.03 ± 0.71 A***	10.95	21.4	31.1	11	51.79 ± 1.73 B***	11.06	42.7	63.1
BPD											13	13.88 ± 0.45	11.6	11.9	17
ONL											13	20.31 ± 0.66	11.77	16.7	24.9
Placentome, length											11	19.13 ± 1.03	17.88	14.2	24.4
Placentome, width											11	12.44 ± 1.11	29.66	7.4	19.7

Note: *** - Significant differences at P<0,001 : A – Day 36 to Day 25 ден; B Day 46 to Day 36

size of placentomes in the same foetus. It was known, that the number and weight of placentomes varied between two uterine horns and between females and breed of ewes (Alexander, 1964). The palcentomes, that we measured, were chosen accidentally, as we took those that first appeared on the screen.

The placentomes of ewe became visible by ultrasound on day 32 as small nodules and after day 39 they had cup-shaped form and reached maximal size on day 74 (Doizé et al., 1997). The same authors observed significant effect of the gestational age on the placentomes size of ewes (F= 63.74, P<0.001), but with low coefficient of determination (R^2= 15.59). The authors obtained high coefficient of determination (R^2= 70.34) for placentomes size and gestationa age to Alpine goats.

In our study, the size of placentomes varied in wide range in all gestational phases, and the coefficient of determination for width and length had moderate values (R^2= 0.46) and (R^2= 0.47 (Figures 3 and 4). Our results were similar to those, obtained from Ali and Hayder (2007) at Ossimi ewes. The authors established moderate value for the coefficient of determination of the size of placentomes (R^2 = 0.38).

The obtained results for placentomes size determined that these measurements were poorer reliable

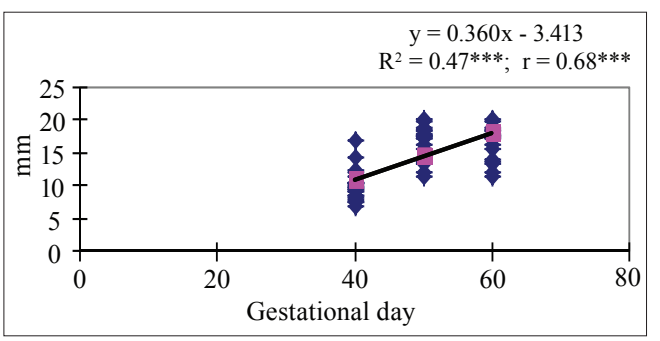

Fig. 3. Linear regression relationship between gestational day and width of palcentomes

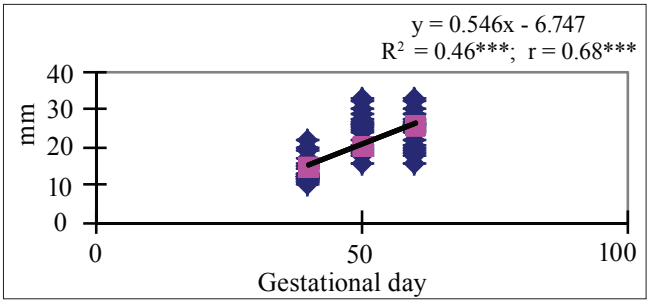

Fig. 4. Linear regression relationship between gestational day and length of placentomes

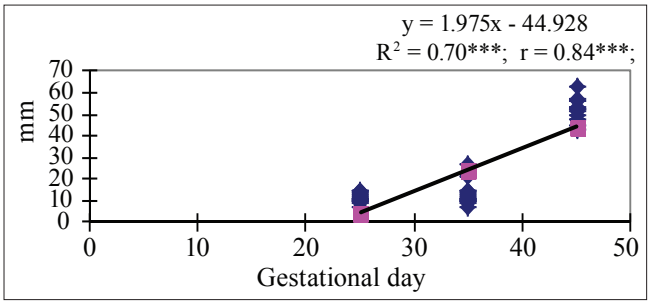

Fig. 5. Linear regression relationship between gestational day and CRL

for prognosis gestational age in comparison to head diameters. Nevertheless, they could be used as auxiliary parameter for prognosis.

Experiment 2

The established high positive correlation (r= 0.84, P<0.001) and high value for coefficient of determination (R^2 = 0.70, P<0.001) (Figure 5) were in correspondence with the results obtained from Gonzáles de Bulnes et al. (1998), Yotov et al. (2005); Ali and Hayder (2007); Godfrey et al. (2010). Gonzáles de Bulnes et al. (1998) established high positive correlation (r = 0.94) for this measurement between 19-th and 48-th gestational days. The authors determined that CRL measurement was highly reliable and representative for prognosis of gestational age. Ali and Hayder, (2007) found out high coefficient of determination (R^2 = 0.876) between 25-th and 50-th gestational days. Godfrey et al. (2010) reported that there was difference in regression in gestational days 28-th and 42-th between single and twins at ewes from next breed - Barbados Blackbelly, Dorper and St.Croix White and their crosses - coefficient of determination for singles was R^2 =0,56, but for twins was - R^2 =0.73. Such difference was not found out from Gonzáles de Bulnes et al. (1998).

In Bulgaria, Yotov et al. (2005) established high positive correlation (r =0.79) for embryofetal length at ewes from Trakia fine-wool breed during gestational days 25-40.

The obtained results for CRL measurement gave us reason to recommend it as a parameter for prognosis of gestational age in day 25-46 form the beginning of pregnancy. The mean value for embryonic vesicle (length 22.38 ± 0.92 mm and width – 17.62 ± 0.85 mm) was similar to those ontained form Gonzáles de Bulnes et al. (1998). The author studied via transrectal ultrasound and probe with resolution 7,5 MHz the development of embryonic vesicle for 12-th to 26-th day from the beginning of pregnancy, as it enlarged its diameter from 8,00 mm in day 12 to 25,3 mm in day 26.

The information about size of embryonic vesicle could be used as additional for prognosis of gestational age.

Conclusion

It was established high, positive and significant correlation between head diameters and gestational age (r=0.93*** и r=0.93***).

The size of placentomes varied in wide range in the three-measured gestational phase (40-42, 51-53, 61-63). It was obtained moderate values of the coefficient of determination for the size of placentomes and gestational age (R^2= R^2= 0.46 и R^2= 0.47).

It was established high, positive and significant correlation between CRL and gestational age (r= 0.84***).

References

Ali, A., and M. Haider, 2007. Ultaronographic assessment of embryonic, fetal and placental development in Ossimi sheep. *Small Rum. Res*., **73:** 277-282.

Doize, F., D. Vaillancourt, H. Carabin and D. Belanger, 1997. Determination of gestational age in sheep and

goats using transrectal ultrasonographic measurements of placentomes. *Theriogenology*, **48:** 449-460.

Ganie, B. A., M. Z. Khan, R. Islam, D. M. Makhdoomi, S. Qureshi and G. M. Wani, 2009. Evaluation of different techniques for pregnancy diagnosis in sheep. *Small. Rum. Res*, **85**:135-141.

Godfrey, R. W., L. Larson, AJ. Weis and S. T. Willard, 2010. Evaluation of ultrasonography to measure fetal size and heart rate as predictors of fetal age in hair sheep. *Sheep and Goat Research Journal***, 25**: 60-65.

Gonzáles de Bulnes, A., J. S. Moreno and A. L.Sebastián, 1998. Estimation of fetal development in Manchega dairy ewes by transrectal ultrasonographic measurements. *Small Ruminant Research*, **27:** 243-250.

Gunduz, M. C., O. Turna, M. Ucmak, S. Apaydin, G. Kasikci, B. Ekiz and N. I. Gezer, 2010. Prediction of gestational week in Kivircik ewes using fetal ultrasound measurements. *Agricultural Journal*, **5** (2): 110-115.

Haibel, G. K. and N. R. Perkins, 1989. Real-time ultrasonic biparietal diameter of secong trimester Suffolk and Finn sheep fetuses and prediction of gestational age. T*heriogenoly*, **32**: 863-869.

Hafez E. S. E., 1993. Reproduction in farm animals/edited by E.S.E. Hafez – 6th ed. (USA).

Kelly, R.W., and J. P. Newnham, 1989. Estimation of gesational age in Merino ewes by ultrasound measurement of fetal head size. *Austr. J. Agric. Res.,* **40:** 1293-1299.

Kähn, W., 2004. Veterinary reproductive ultrasonography. Printed in Germany. ISBN 3-89993-005-3.

Karen, A., K. Uhlich, S. Brabant, K. Blume and O. Szenci, 2001. Pregnancy diagnosis in sheep. Review of the most practical methods. *Acta Vet. Brno*, **7**0: 115-126.

Sergeev, I., D. O. Kleemann, S. K. Walker, D. H. Smith, T. I. Grosser, T. Mann and R. F. Seamark, 1990. Real-time ultrasound imaging for predicting ovine fetal age. *Theriogenology,* **34:** 593-601.

Yotov, S., M. Dimitrov and N. Vassilev, 2005. Early pregnancy diagnosis and examination of embryofetal growth in the local ovine breed. *Bul. J. Agric. Sci*., **11:** 119-124.

Received November, 23, 2011; accepted for printing June, 2, 2012.