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Original Contribution

INFLUENCE OF GESTATIONAL PHASE AND PREGNANCY TYPE ON ECHOGRAPHIC DETERMINATION OF FOETAL NUMBER IN SHEEP

S. Yotov

Department of Obstetrics, Reproduction and Reproductive Disorders,
Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

This study examines the influence of gestational phase and pregnancy type on foetal number in sheep from wool, dairy, and meat breeds. 227 animals of the Trakia Merino breed (n=60), Blackhead Pleven breed (n=105), and Ile de France breed (n=62) were studied using echography. All sheep were tested once between the 20th and 50th gestational day by inserting a 5MHz linear probe in transrectal echography. Embryos and the resulting foetuses were counted. Birth data were compared and resulted in determining accuracy of method; and results controlled by statistical analyses. We show here that echography can be used in determining the best time for foetus enumeration, which was from Day 41-50 of pregnancy. The accuracy was the highest with singleton pregnancies, and was significantly reduced with increasing foetal number. In conclusion the gestational phase and the pregnancy type influence significantly the echographic determination of the foetal number in sheep.

Key words: sheep, pregnancy, echography, foetuses, number

INTRODUCTION

The establishment of the foetal number is a prerequisite for choosing a proper feeding regime, protecting the animals from toxaemia in the later stages of pregnancy, and reducing losses from dystocia (1,2).

The primary tendencies are directed towards finding a method with high accuracy in the early stage of pregnancy, and possibilities for diagnostics of embryo-foetal death rate (3,4). The introduction of echography in the reproduction of sheep offers new options in that direction, and it is met with steadily increasing interest (5-7).

According to Kähn et al. (8), echographic determination of the foetal number via the transrectal approach is possible before the 20th day of pregnancy. Diagnosis is based on clear visualization of anechoic embryonic vesicles. Davey (9) found greater amounts of foetal fluid in twin than in singleton pregnancies. According to Kaulfuß et al. (5), however, counting the embryos after the 26th day is the best criterion to determine their precise number.

Gearhart et al. (10) found a significant ($p<0.005$) influence of the gestational phase on the correct establishment of the foetal number. Using a transrectal approach and 5 MHz linear probe, Zipper et al. (11) counted the foetuses in gestational days 17–69 and determined the highest accuracy (89.1%) in the period between the 35th – 46th days.

Karen et al. (6), however, established 69% accuracy in the period between 43rd – 56th gestational days, going as high as 71.6% within the 76th- 87th day of pregnancy via transabdominal echography. Šlósarz et al. (7) reported accuracy of 83% on the 41st day of pregnancy. According to Kandil et al. (12), diagnosing the foetal number is very difficult in the later stages, due to the constantly growing foetal size.

Diagnostics of singleton pregnancies is easier to perform, while counting the foetuses in multiple pregnancies can lead to errors if one foetus is missed. In other cases, it is possible to count a single foetus twice (13).

Fowler and Wikins (14) reported that 22% of multiple pregnancies were initially diagnosed as singleton by examination of pregnant animals in the inguinal area. Šlósarz et al. (7) established 83.3% accuracy in twin and 50% in triplets on the 41st day of gestation. Apart from that, the accuracy of the method could be influenced by embryonic death and abortions (6).

* **Correspondence to**: *Stanimir Yotov, Reproduction and Reproductive Disorders, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria, stanrad@abv.bg*

Results related to echographic establishment of the foetal number are based on sheep breeds or crosses from various regions of the world. This could explain the significant inconsistencies of the method, reported by the various authors, and suggests adapting the method to the specific examined animals. The issue of the influence of the pregnancy type and the gestational phase in different sheep breeds has not been properly addressed. There is no data on sheep breeds reared in Bulgaria.

The aim of this study was to explore the influence of the gestational phase of examination and the pregnancy type on the echographic determination of the foetal number in sheep from wool, dairy and meat breeds.

MATERIALS AND METHODS

In our experiments, 227 animals, of the Trakia Merino breed (n=60), Blackhead Pleven breed (n=105), and Ile de France breed (n=62) were included. The sheep were 1.6–7 years of age with body weight of 40–82 kg, their feeding and housing regimens were in accordance with the technilogical requirements for the different breeds, all immuno-prophylactic and anti-parasitic procedures were promptly performed.

All sheep were tested once via transrectal echographic approach in the period from the 20^{th} to the 50^{th} gestational day, using an "Aloka" SSD 500 Micrus device (Aloka Co. Ltd, Tokyo, Japan) and a linear probe with a frequency of 5 MHz. Echographic findings were documented using an echographic printer Mitsubishi P91 E (Tokyo, Japan). An echographic gel (Eco-supergel, Forli, Italy) was used.

For easier manipulation of the probe, it was previously lubricated with echographic gel, and fixed in a special metal adapter if necessary. After manual cleaning of the rectal ampule from faecal matter, the probe was inserted into the rectum, rotated to the left and right at an angle of 90^0 and moved forward towards the abdomen.

The number of embryos, respectively fetuses, was counted. To eliminate diagnostic errors, we used the device's B/B regime of operation. After comparing with the data from birth records, we determined the correct and incorrect diagnoses and calculated the method's accuracy on this basis (correct diagnoses / total number of diagnoses x 100). The influence of the factors gestational phase and pregnancy type on the method's accuracy was taken into consideration.

The results were processed by the statistical software StatSoft (Microsoft Corp. 1984-2000 Inc.). We used the statistical packages ANOVA, non-parametric analysis for proportion comparison by the t- criterion of Student.

RESULTS

Influence of the gestational phase of examination on the accuracy of the echographic method

From the 18 examined Trakia merino sheep, in the gestational phase 20^{th} – 30^{th} day, the foetuses were correctly counted in 12, and incorrectly in 6 of the cases. For the period 30^{th} – 40^{th} day, out of 22 diagnoses, 18 and 4 were respectively correct and incorrect. For 20 examined animals in the period 41^{st} – 50^{th} day, the echographic diagnosis was confirmed in 18, and unconfirmed in 2 of the cases (**Table 1**).

For sheep of the Pleven blackhead breed, in the 20^{th} – 30^{th} day of gestation, out of 55 echographic diagnoses, 34 were confirmed, and 21 were not. For the gestational phase 31^{st} – 40^{th} day, out of 25 diagnoses, 16 were correct, and 9 – incorrect. For the period of the 41^{st} – 50^{th} day of pregnancy, out of 25 cases, 24 diagnoses were confirmed, and 1 was not.

In the Ile de France group, out of 15 examined sheep in the gestational phase between the 20^{th} and 30^{th} days, foetuses were counted correctly for 8 and incorrectly for 7 animals. In the next phase of pregnancy (31^{st} – 40^{th} days), out of 25 echographic diagnoses, 20 were confirmed at birth, and 5 were not. In the third period between the 41^{st} and 50^{th} day, out of 22 diagnoses, 18 were correct, and 4 were incorrect.

For all three breeds, for 88 animals in the gestational phase between the 20^{th} and 30^{th} day, the foetal number was determined correctly for 54 and incorrectly for 34 sheep.

For the period of 31^{st} – 40^{th} day of pregnancy, out of 72 diagnoses, 54 were correct, and 18 were not. Examining the animals in the 41^{st} – 50^{th} day of pregnancy showed that out of 67 cases, 60 were diagnosed correctly, and 7 – incorrectly.

Table 1: *Echographic diagnoses and accuracy of the method of determination of the foetal number in sheep, depending on the gestational phase (n=227)*

Breed/Parameters	**Gestational Phase**		
Trakia Merino sheep	***20-30 days***	***31-40 days***	***41-50 days***
All diagnoses (n)	*18*	*22*	*20*
Correct diagnoses (n)	*12*	*18*	*18*
Incorrect diagnoses (n)	*6*	*4*	*2*
Accuracy (%)	*67a**	*81b**	*90c***
Blackhead Pleven sheep	**20-30 days**	**31-40 days**	**41-50 days**
All diagnoses (n)	55	25	25
Correct diagnoses (n)	34	16	24
Incorrect diagnoses (n)	21	9	1
Accuracy (%)	62	64b*	96c**
Ile de France sheep	**20-30 days**	**31-40 days**	**41-50 days**
All diagnoses (n)	15	8	7
Correct diagnoses (n)	25	20	5
Incorrect diagnoses (n)	22	18	4
Accuracy (%)	53a**	80	81c**
Total	**20-30 days**	**31-40 days**	**41-50 days**
All diagnoses (n)	88	72	67
Correct diagnoses (n)	54	54	60
Incorrect diagnoses (n)	22	18	7
Overall accuracy (%)	62	75b*	90c*

Comparison of method's accuracy during the different gestational phases:
a – 20-30 days vs. 30-40 days
b – 30-40 days vs. 41-50 days
c – 20-30 days vs. 41-50 days
*Level of significance: * $p < 0.05$; ** $p < 0.01$*

While determining the method's accuracy in relation the pregnancy phase, the lowest values were registered in the period 20^{th} – 30^{th} day – 67%, 62% and 53%, respectively for the wool, dairy and meat-type breeds. In the next gestational phase, 31^{st} – 40^{th} day, the accuracy increased up to 81%, 64%, and 80%, respectively for wool, dairy and meat breeds. Significant differences ($p<0.05$ and $p<0.01$) in the accuracies for the first two phases of examination were established for merino and meat breeds. In the period 41^{st} – 50^{th} day, the accuracy was highest – 90%, 96%, and 81%. Significant differences ($p<0.05$ and $p<0.01$) in comparison to the first period were established for all three breeds. Comparing the accuracy from the second and third periods, significant differences ($p<0.05$) were found between wool and dairy sheep breeds.

The overall accuracy of the method increased with the advancement of the gestational phase. The highest overall accuracy (90%) was registered in the 41^{st} – 50^{th} day period. It was significantly higher ($p<0.05$) than the established 62% and 75%, respectively for the 20^{th} – 30^{th} day and 31^{st} – 40^{th} day of pregnancy. No significant influence of the breed on the method's accuracy in determining the foetal number was found out.

Influence of the pregnancy type on the accuracy of the echographic method of determination of foetal number

After registering the results from the births of Trakia merino sheep, from a total of 37 singleton pregnancy diagnoses, 27 were established as correct, and 10 – incorrect. Out of 19 diagnoses for twin pregnancy, 13 were correct, and 6 – incorrect whereas out of 4 diagnoses for triple pregnancy, 1 was correct, and 3 were incorrect (**Table 2**).

In Pleven blackhead sheep, out of 80 animals with singleton pregnancies, the confirmed diagnoses were 56, while 24 were not confirmed.

Out of 20 cases of twin pregnancies, 13 were confirmed and 7 were unconfirmed. Out of 5 diagnoses for triplets, only 1 was correct, and 4 were incorrect.

For Ile de France breed sheep, 37 animals were determined as with singleton pregnancies, 19 – with twin and 6 with triple pregnancies. The correct and incorrect diagnoses in those cases were, respectively, 31 and 6; 11 and 8; and 2 and 4.

From a total of 154 cases of single-foetus (**Photo 1**), 58 of double-foetus (**Photo 2**), and 15 of triple-foetus pregnancy (**Photo 3**), confirmed diagnoses were, respectively, 104, 37, and 4, while unconfirmed were 50, 24, and 11.

Table 2: Echographic diagnoses and their accuracy in determining the foetal number in sheep, depending on the pregnancy type (n=227)

Breed/Parameters	**Gestational Phase**		
Trakia Merino sheep	***Singleton***	***Twin***	***Triplet***
All diagnoses (n)	*37*	*19*	*4*
Correct diagnoses (n)	*27*	*13*	*1*
Incorrect diagnoses (n)	*10*	*6*	*3*
Accuracy (%)	*73a**	*78b***	*25c***
Blackhead Pleven sheep	**Singleton**	**Twin**	**Triplet**
All diagnoses (n)	*80*	*20*	*5*
Correct diagnoses (n)	*56*	*13*	*1*
Incorrect diagnoses (n)	*24*	*7*	*4*
Accuracy (%)	*70a**	*65b***	*20c***
Ile de France sheep	**Singleton**	**Twin**	**Triplet**
All diagnoses (n)	*37*	*19*	*6*
Correct diagnoses (n)	*31*	*11*	*2*
Incorrect diagnoses (n)	*6*	*8*	*4*
Accuracy (%)	*84a**	*58b**	*33c****
Total	**Singleton**	**Twin**	**Triplet**
All diagnoses (n)	*154*	*58*	*15*
Correct diagnoses (n)	*114*	*34*	*4*
Incorrect diagnoses (n)	*50*	*24*	*11*
Overall accuracy (%)	*74a**	*64b**	*27c**

Comparison of method's accuracy for the different pregnancy types:
a – singleton vs. twin pregnancy
b – twin vs. triple pregnancy
c – singleton vs. triple pregnancy
*Level of significance: * $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$*

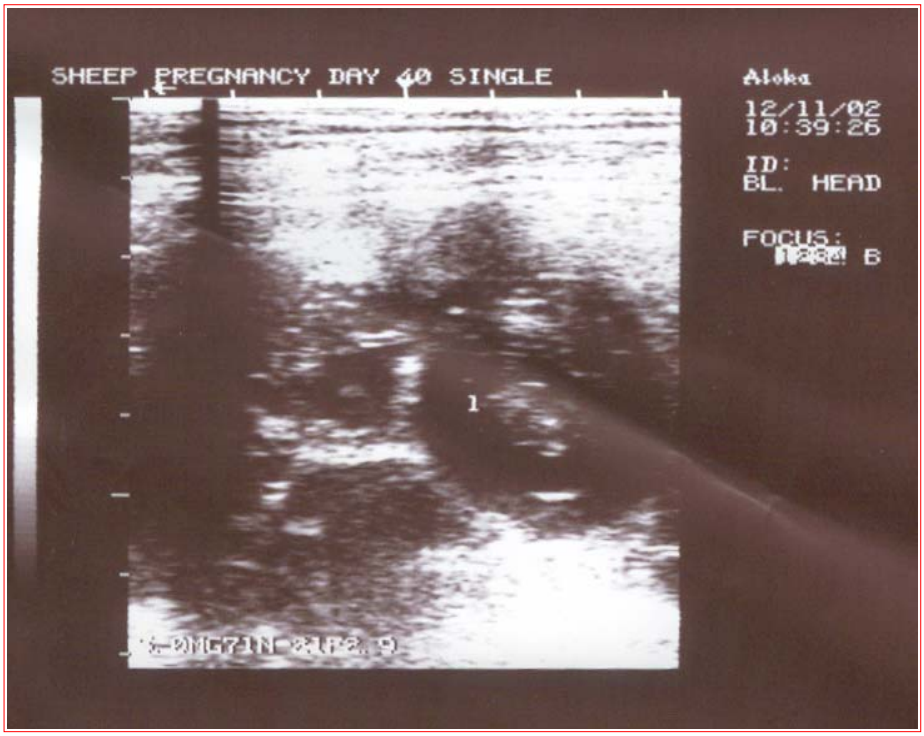

Photo 1: *Echography of sheep on day 40th of pregnancy (B-mode), single pregnancy: 1- embryo.*

For merino sheep, the highest accuracy of 73%, was established for singleton pregnancies, while the lowest (33%) – in triple pregnancies, with the difference being significant ($p<0.01$). The method's accuracy for twins, 68%, was lower ($p<0.05$) than that of singleton while significantly ($p<0.01$) higher than it was for triplets.

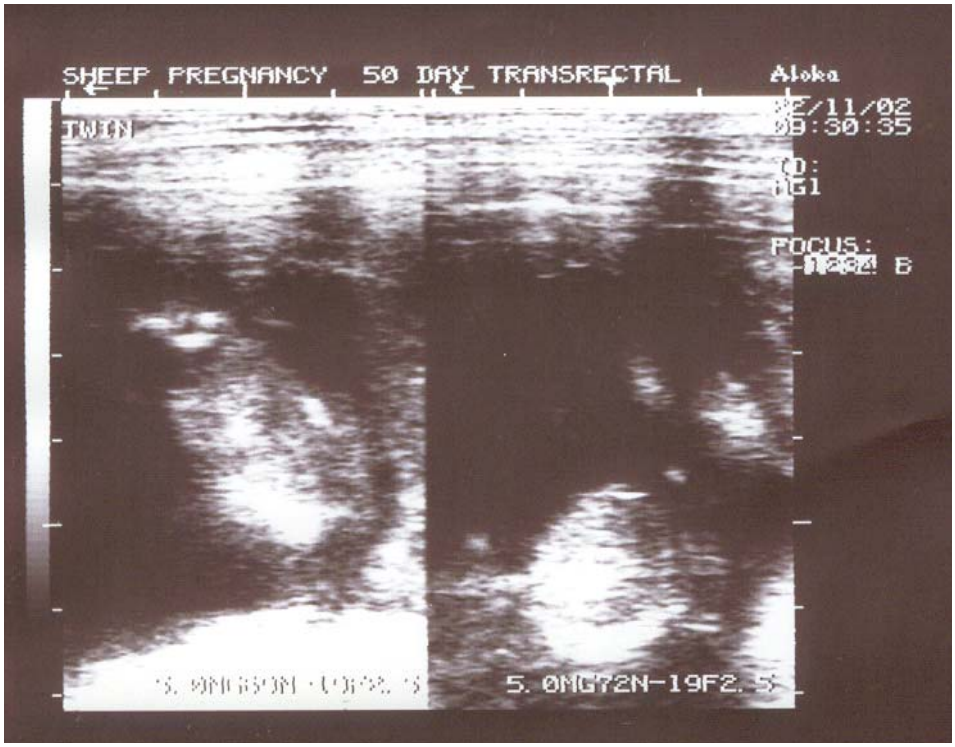

Photo 2: *Echography of sheep on day 50th of pregnancy (B-B mode), twin pregnancy: 1-2 embryos.*

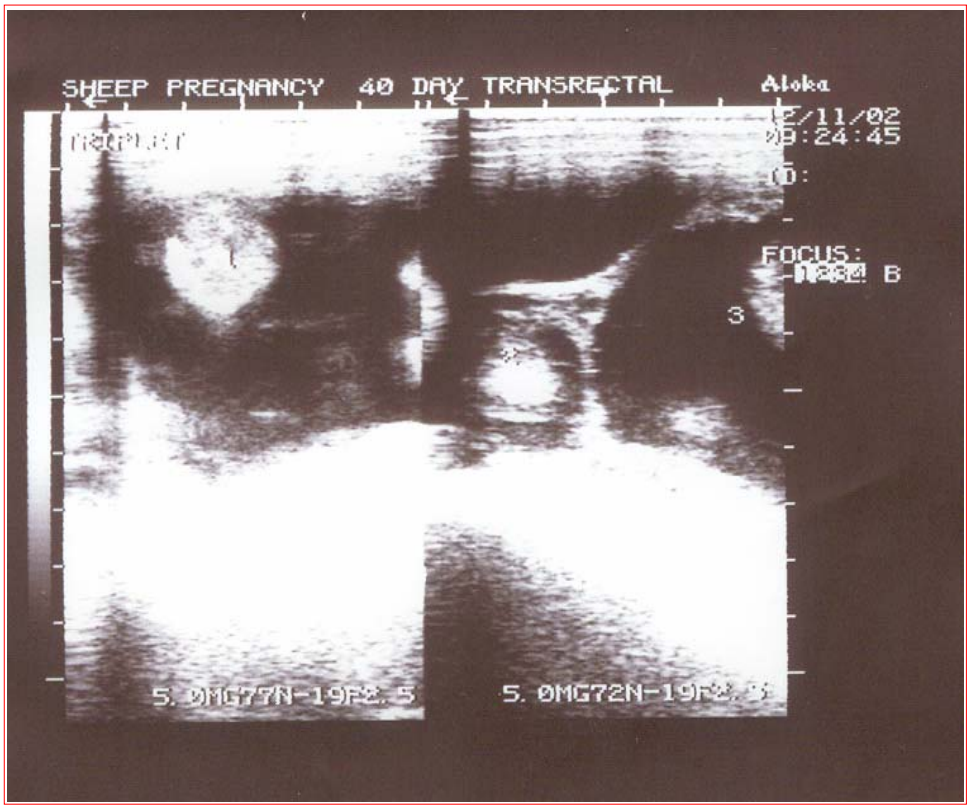

Photo 3*: Echography of sheep on day 40th of pregnancy (B-B mode), triple pregnancy: 1-3 embryos*

Data in the dairy breed were similar: 70% accuracy for singletons, 65% for twin and 20% for triple pregnancies. Significant differences in the accuracy were registered between singleton and twin ($p<0.05$), twin vs. triplets ($p<0.01$), and single vs. triplets ($p<0.01$).

Results for meat-type sheep also showed a decrease in the accuracy with the increasing foetal number. The highest accuracy of 84% was established for singleton diagnoses, 58% for twin, and the lowest accuracy of 33% – in triplets. Significant differences (from $p<0.05$ to $p<0.001$) were found out in the accuracy between the different types of pregnancy.

Data on the overall accuracy of the method confirmed the tendency observed in the different breeds that a higher number of

foetuses decreased the method's accuracy. Significant differences ($p<0.05$) were found in the accuracy (67.5%, 63.8% and 26.7%) between the different types of pregnancy.

DISCUSSION

A primary aspect of echography utilization is the combination of early pregnancy diagnostics and accurate determination of the foetal number (6,11). Although Kahn et al. (8) reported establishing of the foetal number as early as the 20th day of pregnancy by transrectal examination, we consider the criterion used in that case, the visualization of the anechoic embryonic vesicle, to be insufficiently accurate.

The echographic image, resembling anechoic vesicles can be observed in the presence of other fluids in the uterus or artifacts, which may lead to significant diagnostic error. This was the cause for the low accuracy of the method, 67%, 62%, and 53%, for the period between the 20th and the 30th gestational days, for the wool, dairy and meat breeds, respectively. The lower percentage for meat-type sheep, compared to the other two breeds, is attributed to later visualization of an objective criterion for determining the number of embryos in that phase.

The significantly established higher accuracy values ($p<0.05$ and $p<0.01$) for the period 31st – 40th day – 81% and 80% for merino and meat sheep breeds respectively, showed the significant influence of the gestational phase on the echographic establishment of the number of foetuses via transrectal approach. During that stage of pregnancy, the most accurate criterion for determining the foetal number is the visualization of an embryo, which Kaulfuß et al. (5) established after the 26th day of pregnancy. Apart from that, we believe that with the advancement of gestation age, the risk of false diagnoses related to inaccurate criteria or embryonic death decreases. If the study is performed in the period 41st – 50th day, this leads to a significant increase in accuracy ($p<0.05$ and $p<0.01$). The data showed that this phase of pregnancy was most suitable for determining the foetal number in the studied breeds of sheep. The data by Zipper et al. (11), who reached the highest accuracy of 89.1% in the period 35th – 46th day, support our attitude.

Results related to the determination of single- or multiple pregnancy showed that pregnancy type had a significant influence ($p<0.05$) on the method's accuracy for sheep of all three breeds. Similar data were obtained by Fowler and Wilkins (14) for merino and meat sheep.

The significantly higher ($p<0.05$ and $p<0.01$) accuracies of 73%, 70%, and 84% for singleton pregnancies compared to 68%, 65%, and 58% for twin pregnancies in sheep of the respective types were similar to the values reported by Gearhart et al. (1988) and Šlósarz et al. (7).

The lower accuracy for twin vs. singleton pregnancy was due to the greater share of incorrect diagnoses for the wool, dairy and meat breeds. These could be caused by embryo-foetal death that, according to Kaulfuß et al. (5), could reach up to 33.5% in the period 22nd – 31st day of pregnancy.

We submit that another reason for incorrect diagnosis in cases of multiple pregnancies could be the higher level of embryo's motor activity until the 40th day of pregnancy. It does not allow the operator to count the embryos accurately in a multi-foetal pregnancy. Moreover, the sizes of placentomas and the embryo are not significantly different in the period 31st – 40th day, which leads to a cover-up of one part of the embryos and greater diagnostic error. In their studies, Šlósarz et al. (7) also found significant differences in the method's accuracy, due to difficulties with the visualization of all foetuses.

The established low ($p<0.01$ and $p<0.001$) accuracies of 33%, 20%, and 33%, in triplets for all three examined breeds are due to inaccurate diagnoses caused by partial embryonic death. Similar discussion of death of one embryo in sheep with multiple pregnancies are encountered in the studies of Dixon et al. (15), while Karen et al. (6) attribute a greater significance to the total embryonic or foetal death.

Another problem is the double-counting of the same embryo, also registered by White et al. (13). We believe that in order to exclude that error, scanning should be performed by using the device's B/B regime, which allows for more accurate counting. The lack of significant influence of the sheep breed on the echographic method's accuracy with regard to gestational phase and pregnancy type, established by us, is also supported by the findings of Fowler and Wilkins (14).

With the pregnancy's advancement, the options for counting the foetuses through transrectal approach decrease.

The reason for that is the mutual covering of the echographic foetal images and

the impossibility for the ultrasonic rays to reach the pregnant uterus. Similar statements can be seen in the research by Kandil et al. (12) as well.

The analysis of results from the present research showed that the gestational phase and the pregnancy type influence significantly ($p<0.05$) the echographic determination of the foetal number in sheep. The most suitable time to establish the number of foetuses by echography was in the period between 41st – 50th days of pregnancy. The accuracy was the highest for single pregnancies, and decreased significantly ($p<0.05$) with the increase of the foetal number.

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