Bulgarian Journal of Veterinary Medicine, 2017, **20**, No 3, 276–280
ISSN 1311-1477; DOI: 10.15547/bjvm.944

Short communication

EFFICIENCY OF TRANSRECTAL ULTRASONOGRAPHY FOR PREGNANCY DIAGNOSIS OF ILE DE FRANCE EWES DURING THE FIRST MONTH OF GESTATION

N. METODIEV[1], D. DIMOV[2], I. RALCHEV[2] & E. RAICHEVA[1]

[1]Department of Sheep Breeding, Institute of Animal Science, Kostinbrod, Bulgaria; [2] Faculty of Veterinary Medicine, University of Forestry, Sofia, Bulgaria

Summary

Metodiev, N., D. Dimov, I. Ralchev & E. Raicheva, 2017. Efficiency of transrectal ultrasonography for pregnancy diagnosis of Ile de France ewes during the first month of gestation. *Bulg. J. Vet. Med.,* **20**, No 3, 276–280.

The aim of the present study was to test transrectal real-time B-mode ultrasonography for pregnancy diagnosis in ewes from the Ile de France breed on day 16, 23 and 30 after fixed-time artificial insemination. The experiment was carried out with 22 multiparous pure-bred Ile de France ewes aged 2–5 years. Diagnosis of pregnancy was based on the presence of enlarged uterine lumen with amniotic fluid and visualisation of embryo. The accuracy (Ac %), sensitivity (Se %) and specificity (Sp %) of the technique were determined. The gestational phase influenced significantly ($P<0.01$) the accuracy of the transrectal ultrasonography approach for pregnancy diagnosis. At day 16 the accuracy was 50.0%, whereas at day 23 and at day 30 it reached 72.73% and 90.91% respectively.

Key words: early pregnancy diagnosis, ewe, Ile de France, transrectal ultrasonography

The intensive sheep management and the widespread application of controlled breeding techniques, such as artificial insemination and out-of season breeding, increase the need for an accurate and practical test for early pregnancy diagnosis (Karen, 2003). 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 (Ganaie *et al.,* 2009). There are two approaches used to study the female reproductive tract of small ruminants – transrectal and transabdominal, and the approach of choice depends on diagnosis that is needed, probes and size of flocks (Kähn, 2004).

The transrectal approach is more suitable for pregnancy diagnosis till the 35th day of gestation (Kähn, 2004). Its accuracy at these early stages is low, so in practice, it is better to wait until days 32–

34, when the efficiency reaches 85–100% depending on operator experience (Gonzales-Bulnes *et al.*, 2010).

In our previous works (Metodiev *et al.,* 2010; 2012; 2014; Metodiev, 2013) we have used transabdominal approach and sector probe with frequency 5 MHz in ewes from the Synthetic population of the Bulgarian milk sheep (SPBM). We have done a series of examinations from the 25th till the 84th gestation day first to diagnose early pregnancy and second – to determine values of some foetal growth measurements.

Numerous studies (Vassilev *et al.*, 2004; Bonev *et al.*, 2005; Yotov *et al.*, 2005; Yotov, 2005; 2008a,b; 2012; Maslev, 2008) in Bulgaria have been carried out to diagnose pregnancy diagnosis by ultrasound imaging in four breeds – Pleven Blackhead, Trakia Merino, Ile de France and Karakachan. Yotov (2012) noticed that before the 25th gestation day in ewes from Ile de France breed, the visualisation of embryos and their counting were more difficult and the examination - more time consuming.

The aim of the present study was to test transrectal real-time B-mode ultrasonography for pregnancy diagnosis of Ile de France ewes on days 16, 23 and 30 after fixed-time artificial insemination.

The experiment was carried out in May 2013, with 22 multiparous purebred Ile de France ewes, aged 2–5 years, raised in the experimental farm of the Institute of Animal Science – Kostinbrod. Estrus synchronisation was induced by two intramuscular injections of the synthetic analogue of prostaglandin F2α, given 9 days apart (13 ewes with 2.0 mg alfaprostol (1.0 mL Alfabedyl, Ceva Animal Health) and 9 ewes with 125 μg cloprostenol (0.5 mL Synchromate, Bremer Pharma, Germany). All ewes showed estrus (tested by teasers) by the 46th hour after the second treatment and were inseminated artificially in fixed time, with fresh, non-diluted semen, at a dose of 0.2 mL on 48 h and 56 h. Only ejaculates with volume ≥0.5 mL and motility 70% were used.

Three consecutive ultrasound examinations were done on day 16, day 23 and day 30 after the artificial insemination. The examinations were done in the morning between 9.00–11.00 AM and last feeding of the animals was during 5.00–6.00 PM on the previous day. Ultrasound examinations were conducted in standing position of ewes. First the ampulla recti was evacuated from faeces, after that the ultrasound probe, covered with gel, was inserted with the help of a cradle into the rectum. The equipment included digital portable ultrasound system ALOKA Pro-Sound 2 (Aloka Co., Ltd.) supplied with electronic linear transducer UST 5820, with frequency of 7.5 MHz. Diagnosis of pregnancy was based on the presence of enlarged uterine lumen with amniotic fluid (for day 16, day 23 and day 30) and visualisation of embryo (day 23 and day 30). Validity of detection of pregnancy was verified through lambing dates, as normal pregnancy lasted average 150±7 days and the day of AI was accepted as day 1 of pregnancy.

The following diagnoses were given during the study: true positive (pregnant), false positive (non-pregnant), true negative (non-pregnant), and false negative (pregnant). The test parameters were determined (Yotov, 2005): accuracy (Ac %) – number of true diagnoses/total number of diagnoses; sensitivity (Se %) – number of true positive diagnoses/total number of positive diagnoses; specificity (Sp %) – number of true negative diagnoses/ total number of negative diagnoses.

Table 1. Number of diagnoses and values of the parameters obtained on the studied days

		Days of pregnancy		
		day 16	day 23	day 30
Diagnoses	true positive	5	8	10
	false positive	4	2	0
	true negative	6	8	10
	false negative	7	4	2
Parameters	accuracy, %*	50.0	72.73	90.91
	sensitivity, %	41.67	66.67	83.33
	specificity, %	60.0	80.0	100.0

* Cochran's Q = 8.714, level of significance P<0.01.

The significance of the accuracy of this technique for pregnancy diagnosis at different gestational days was tested with Cochran's Q test with the statistical software SPSS 19. Cochran's Q test is a non-parametric test, which is used when the studied groups are more than 2, the groups are related, and data are binomial (1 – correct diagnosis and 0 – false diagnosis).

The gestational phase influenced significantly (P<0.01) (Table 1) the accuracy of the transrectal approach of ultrasonography for pregnancy diagnosis. At day 16 the accuracy was 50.0%, whereas at days 23 and 30 it reached 72.73% and 90.91% respectively. The first sign of pregnancy was enlarged uterine lumen with amniotic fluid. A similar finding was observed by Gonzales-Bulnes *et al.* (1998) with 7.5 MHz probe on day 12.8 after insemination in Machega dairy ewes. Yotov (2008a) reported a breed-related specificity of this first sign of pregnancy – in Trakia Merino sheep this sign was established with 20.0% and 60.0% accuracy at days 12 and 20, while in Pleven Blackhead and Ile de France breeds this sign could be found at day 20, with accuracy 40.0% and 20.0% respectively.

Our results were different from those of Yotov (2008a). We suppose that this difference was due to individual characteristics of ewes within the breed, because the age, breed, and ultrasound technique were the same. The effect of operator in that case also could be excluded, because in both experiments the operators were well experienced.

Visualisation of the conceptus may be performed after day 19 in sheep (Gonzales-Bulnes *et al.,* 1998). Yotov (2008a) firstly found conceptus at day 20 with accuracy 40% in Trakia Merino breed, whereas in Ile de France sheep the first visualisation of embryo was possible at day 25 with the same accuracy 40%. At day 23 we obtained accuracy of 72.73% in early pregnancy diagnosis, as all true positive diagnoses were based on visualisation of embryos. In our previous study (Metodiev, 2013), we achieved accuracy of 94.12% in early pregnancy diagnosis (by enlarged uterine lumen and embryo presence) with transadbomial ultrasound and 5 MHz sector probe in SPBM ewes, which in our belief was breed-specific. It is therefore suggested that despite the numerous studies in that field, it is necessary to carry out more researches to establish the deviations of the criteria of early pregnancy diagnosis within the breed.

The other two parameters – sensitivity and specificity, also increased their values and at day 30 they were – 83.33% and

100% respectively. Our results for day 23 and day 30 were in correspondence to those, obtained from the other authors. The sensitivity and specificity increased with progression of the pregnancy and ranged between 65.0 % and 87.0 % at days 25 to 50, depending on the breed, age and parity of the ewes, experience of the operator and the technique of the examination (Karen *et al.*, 2001). Yotov (2008a) obtained 81.8% sensitivity and 69.2% specificity between gestation days 20–30 in Ile de France ewes.

On the basis of the obtained results, we share the opinion of Martinez *et al.* (1998) that the pregnancy diagnosis with this technique should be carried out after day 24. At day 23 we obtained an accuracy of 72.73%, sensitivity – 66.67% and specificity – 80.0%.

REFERENCES

Bonev, G., N. Y. Vassilev, M. A. Dimitrov, S. A. Yotov & P. Penchev, 2005. Adjustment of echography and laparoscopic insemination to the reproductive particilarities of Pleven Blackhead sheep. *Bulgarian Journal of Veterinary Medicine,* **9**, 61–65.

Ganaie, B. A., M. Z. Khan, R. Islam, D. M. Makhdoomi, S. Quresh & G. M. Wani, 2009. Evaluation of different techniques for pregnancy diagnosis in sheep. *Small Ruminant Research,* **85**, 135–141.

Gonzáles de Bulnes, A., J. Santiago-Moreno & A. López-Sebastián, 1998. Estimation of fetal development in Manchega dairy ewes by transrectal ultrasonographic measurements. *Small Ruminant Research,* **27**, 243–250.

Gonzáles de Bulnes, A., P. Pallares & M.Z.Vazquez, 2010. Ultrasonographic imaging in small ruminant reproduction. *Reproduction in Domestic Animals,* **45 (Suppl. 2)**, 9–20.

Kähn, W., 2004. Ultrasonography in sheep and goats. In: *Veterinary Reproductive Ultrasonography*, ed. W. Kähn, Schlütersche Verlagsgesellschaft, Hannover, pp. 187–210.

Karen, A., 2003. Pregnancy diagnosis in sheep. PhD Thesis, Clinic for Large Animals, Faculty of Veterinary Science, Üllő, Hungary.

Karen, A., P. Kovács, J. F. Beckers & O. Szenci, 2001. Pregnancy diagnosis in sheep. Review of the most practical methods. *Acta Veterinaria Brno,* **70**, 115–126.

Martinez, M., P. Bosch & R. A. Bosch, 1998. Determination of early pregnancy and embryonic growth in goats by transrectal ultrasound scanning. *Theriogenology,* **49**, 1555–1565.

Maslev, Ts., Ts. Hristova, I. Stankov & B. Georgiev, 2008. Study of the capacities of the ultrasonography for early pregnancy diagnostics in ewes. In: *Proceedings from Scientific Conference "80 years Agrarian Science in Rodopi",* Smolyan, Bulgaria, pp. 103–105 (BG).

Metodiev, N., I. Ralchev, D. Dimov & E. Raicheva, 2010. Effectiveness of transabdominal ultrasonography for early pregnancy diagnosis and determination fetus number of ewes. In: *Proceedings of Scientific Conference "Tradition and the Present of veteRinary Medicine",* Faculty of Veterinary Medicine, University of Forestry, Sofia, Bulgaria, pp. 91–97.

Metodiev, N., D. Dimov, I. Ralchev & E. Raicheva, 2012. Measurements of foetal growth via transabdominal ultrasonography during first half of pregnancy at ewes from synthetic population Bulgarian milk. *Bulgarian Journal of Agricultural Science,* **18**, 493–500.

Metodiev, N., 2013. Main reproductive traits and biotechnological methods for control of the reproduction at ewes from Synthetic population Bulgarian milk. PhD thesis, IAS-Kostinbrod (BG).

Metodiev, N., D. Dimov, I. Ralchev & E. Raicheva, 2014. Measurements of fetal

growth via transabdominal ultrasonography during mid-pregnancy. *Bulgarian Journal of Agricultural Science,* **20**, 721–725.

Vassilev, N., G. Bonev, M. Dimitrov, S. Yotov & S. Laleva, 2004. A non-genetic approach for promoting reproduction in sheep from the Ile De France breed. *Bulgarian Journal of Veterinary Medicine,* **7**, 45–49.

Yotov, S., 2005. Diagnostics of early pregnancy in Stara Zagora dairy sheep breed. *Bulgarian Journal of Veterinary Medicine* **8**, 41–45.

Yotov, S., M. Dimitrov & N. Vassilev, 2005. Early pregnancy diagnosis and examination of embryofoetal growth in the local ovine breed. *Bulgarian Journal of Agricultural Science,***11**, 119–124.

Yotov, S., 2008a. Early pregnancy diagnosis and fetal number and sex determination in sheep. PhD Thesis, Trakia University, Stara Zagora (BG).

Yotov, S., 2008b. Influence of gestational phase and pregnancy type on determination of foetal number in sheep. *Trakia Journal of Sciences,* **6**, 88–94.

Yotov, S., 2012. Ultrasound diagnostics of late embryonic and foetal death in three sheep breeds. *Journal of Veterinary Advances,* **2**, 120–125.

Paper received 13.07.2015; accepted for publication 12.11.2015

Correspondence:

Nikola Metodiev, DVM
Department of Sheep Breeding,
Institute of Animal Science,
Kostinbrod, Bulgaria
e-mail: n_metodiev@abv.bg