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DIAGNOSTICS OF EARLY PREGNANCY IN STARA ZAGORA DAIRY SHEEP BREED

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Summary

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The study was performed on 70 sheep from the Bulgarian Stara Zagora Dairy breed, aged 2–5 years and weighing 60–65 kg. The sheep were artificially inseminated after synchronization of the oestrus by vaginal sponges containing flurogestone acetate and PMSG. At post insemination days 20, 27 and 35, a transabdominal ultrasonographic test for pregnancy was performed with the Aloka SSD 500 Micrus equipment and a 5 MHz linear transducer. The accuracy, sensitivity and specificity of the method were evaluated. An accuracy of 87.1% at post insemination day 27 that increased up to 98% at post insemination day 35 was detected. It was found out that the increase in uterine lumen diameter and the size of the embryo with detection of cardiac activity were reliable criteria for ruling out the occurrence of early embryonal, resp. foetal death.

Key words: early pregnancy, sheep, ultrasonography

INTRODUCTION

The early and precise detection of pregnancy in sheep is especially important from an economical point of view. The separation of flocks into pregnant and non-pregnant permits a scheduling of the technology of breeding, reduction of reproductive diseases and optimization of the live weight of mothers and neonates (Fowler & Wilkins, 1984; Bretzlaff *et al.*, 1993; Haibel, 1990). In the veterinary practice, the commonest method for detection of pregnancy is the real-time B-mode ultrasonography (echography) (Karen *et al.*, 2001).

Using the transabdominal method, Gearhart *et al.* (1988) detected without doubt pregnancy by day 25 on the basis of enlarged uterine lumen and the embryo whereas Kähn *et al.* (1993) reported an accuracy of 95%, sensitivity of 95% and specificity of 91% between post insemination days 22 and 90. In German Merino Mutton sheep, Kaulfuss *et al.* (1996a) reported an accuracy of 80% by post insemination day 21 and 100% by post insemination day 60. Schric & Inskeep (1993) showed an embryo size of 6 ± 1 mm; 10 ± 2 mm and 25 ± 2 mm by post insemination days 20, 25 and 35, respectively in local ovine crossbreds. According to most authors (Kähn *et al.*, 1992; Kaulfuss *et al.*, 1996b), the precise diagnosis in the early stage of the pregnancy should be based not only on the detection of an enlarged uterine lumen but on the presence of an embryo as well in order to eliminate the false positive findings. Furthermore, they reported that the approach

and the frequency of used transducer were of primary significance for the accuracy, sensitivity and specificity of the method. In the available literature, there are no data about the use of ultrasonography in local sheep breeds for detection of pregnancy and monitoring of embryofoetal development.

The aim of the present study was to examine the possibilities of transabdominal ultrasonography for detection of early pregnancy and to follow out the changes occurring in the enlarging uterine lumen and the embryo as parameters for confirmation of pregnancy in The Stara Zagora dairy sheep breed.

MATERIALS AND METHODS

The study was carried out on 70 sheep from the Bulgarian Stara Zagora dairy breed and its crossbreds, aged 2–5 years, weighing 60–65 kg, fed and reared under uniform conditions, subjected to the standard immunoprophylactic and antihelminthic regimens.

The experiment was performed during the oestral season (in September). Depending on the mating regimen of rams, the synchronization of the oestrus was done by groups: group I – n=25; group II – n=25 and group III – n=20. For this purpose, vaginal sponges containing 40 mg flurogestone acetate (Chronogest, Intervet, Holland) were applied at September 14, 24 and 30 in groups I, II and III, respectively. Twelve days afterwards, the sponges were removed and the sheep were intramuscularly treated with 500 UI PMSG (Folligon, Intervet, Holland). A programmed artificial insemination was performed 55 h after the removal of tampons using 0.2 mL fresh semen containing 80×10^6 motile spermatozoa (Bonev, 1997). The first post insemination day was accepted as the beginning of the pregnancy. All animals were submitted to a ultrasonographic study via the transabdominal approach at post insemination days 20, 27 and 35. An ultrasonic equipment Aloka - SSD 500, Micrus with a 5 MHz linear transducer (probe) was used. The findings were visualized at a Mitsubishi-P91E printer. The pregnancy was detected using the following parameters: presence of an enlarged uterine lumen with amniotic fluid and presence of an embryo. The diameter of the uterine lumen and the size of the embryo (cm) were measured in 15 animals with a singleton pregnancy.

The validation of the diagnostic method was done using the formulas of Baronet & Vaillancourt (1984). The following indices were determined: Accuracy (number of confirmed diagnoses ÷ total number of diagnoses), sensitivity (number of confirmed positive diagnoses ÷ total number of positive diagnoses) and specificity (number of confirmed negative diagnoses ÷ total number of negative diagnoses).

The results were statistically processed by the analysis of variance (ANOVA) and the least significance difference (LSD) as a post-hoc test (StatSoft 1984–2000 Inc., Copyright © 1990-1995 Microsoft Corp.).

RESULTS

In 70 animals studied 20 days after the insemination, an enlarged uterine lumen as an anechoic zone located cranially or ventrally to the urinary bladder was visible. This finding was absent in non-pregnant animals. On this ground, positive diagnosis of pregnancy was assumed in 59 sheep (Fig. 1), whereas 11 were determined as non-pregnant. During the next examination (day 27), 52 out of all 59

Fig. 1. Pregnancy in a Stara Zagora dairy sheep by post insemination day 20 – enlarged uterine lumen.

Fig. 2. Pregnancy in a Stara Zagora dairy sheep by post insemination day 27 – enlarged uterine lumen and embryo.

Fig. 3. Pregnancy in a Stara Zagora dairy sheep by post insemination day 35 – enlarged uterine lumen, foetus and placentomas.

sheep with positive diagnoses were evaluated as pregnant (presence of enlarged uterine lumen and embryo) (Fig. 2) and 7 gave negative diagnoses. Fifty-one diagnoses were confirmed by day 35, when a heart rate and placentomas were also clearly detected (Fig. 3) and one sheep was slaughtered and the pregnancy proved post mortem. From the initial 11 negative diagnoses, 9 were confirmed during the next ultrasonographies (by days 27 and 35) and 2 showed a positive echographic finding (pregnancy).

In studied sheep, the accuracy of the method by day 27 was 87.1% , and its sensitivity and specificity: 88.14% and 81.82% respectively (Table 1). By day 35, the accuracy was 98%. The values of uterine lumen diameter and size of the embryo by day 35 were significantly higher ($P<0.05$) than those by day 27 (Table 2).

DISCUSSION

The detection of early pregnancy in sheep via ultrasonography depends primarily on the approach, the frequency of used transducer and the experience of the investigator (Fowler and Wilkins, 1984; Karen *et al.*, 2001). The accuracy, sensitivity and specificity by day 27 observed in the Stara Zagora dairy sheep breeed were similar to those reported by Kaulfuss *et al.* (1996a) but different from those of Kähn *et al.* (1993) in Merino sheep who also used a transducer with 5 MHz frequency. The differences, in our opinion, are breed-dependent.

The early embryonal death that, according to Michels *et al.* (1998) is the highest between post insemination days 3–26 could be assumed as cause for the false positive findings. In such cases, the anechoic area within the uerus is not observed during a second examination, and when an embryo or a placentoma is present, they do not grow. The false negative

Table 1. Comparison of ultrasonographic findings with those observed at parturition in 70 sheep from the Stara Zagora dairy breed

Findings	Days post insemination			Parturient sheep
	20	27	35	
Number of positive diagnoses	59	54* (52)	54 (52)	54
Number of negative diagnoses	11	16 (9)	16 (9)	16
Accuracy (%)		87.10	98.36	–
Sensitivity (%)		88.14	98.00	–
Specificity (%)		81.22	100.00	–

The figures in brackets designate the number of confirmed diagnoses from the 20th post insemination day; * one sheep was slaughtered by day 32 and the pregnancy was proved post mortem.

Table 2. Diameter of the uterine lumen and size of the embryo in pregnant sheep from the Stara Zagora dairy breed

Parameters	Periods of study			
	Day 27		Day 35	
	n	mean±SEM	n	mean±SEM
Diameter of uterine lumen (cm)	15	2.00±0.15	15	3.29±0.11*
Size of the embryo (cm)	15	1.15±0.01	15	2.39±0.04*

* $P<0.05$ vs the values by day 27.

findings could probably be attributed to the early period of the study (day 20) when the embryo is commonly not visualized and the enlarged uterine lumen could be due to accumulation of oestral secretion or due to some kind of uterine pathology.

The observed sizes of the embryo by days 27 and 35 are similar to those, reported by Schric & Inskeep (1994) but different from the data of Chalhoub *et al.* (2001) in the local sheep breed Ideal, that is probably due to the breed difference, as stated in the studies of Kaulfuss *et al.* (1999). The values of ultrasonographic parameters, obtained in the present study allowed to assume that the increase in the uterine lumen diameter and the size of the embryo with detection of heart rate and placentoma were precise criteria for ruling out the embryonal, resp. the foetal death. Our data could be used for elaboration of an embryofoetal growth profile and for determination of the gestation age of the foetus in the studied sheep breed.

CONCLUSIONS

Transabdominal ultrasonography with a 5 MHz transducer could be used for detection of early pregnancy in the Stara Zagora dairy sheep breed and its crossbreds with accuracy of 98% after post insemination day 35. The visualization of an enlarged uterine lumen and an embryo's size with detection of fetal heart rate were the most accurate criteria for eliminating the possibility of early embryonal, resp. foetal death.

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