ADJUSTMENT OF ECHOGRAPHY AND LAPAROSCOPIC INSEMINATION TO THE REPRODUCTIVE PARTICULARITIES OF PLEVEN BLACKHEAD SHEEP

G. B. BONEV[1], N. Y. VASSILEV[2], M. A. DIMITROV[2], S. A. YOTOV[2] & P. PENCHEV[3]

[1]Department of Genetics, Breeding and Reproduction, Faculty of Agriculture; [2]Department of Obstetrics, Gynaecology and Andrology, Faculty of Veterinary Medicine; Trakia University; [3]Firm "Select"; Stara Zagora, Bulgaria

Summary

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The study was performed on 50 ewes from the Pleven Blackhead milk sheep breed aged 10–12 months and weighing 40–48 kg in November and December. Prior to the experiment, all animals from the herd were submitted to transabdominal echographic study with Aloka SSD 500 Micrus equipment and 5 MHz linear transducer and pregnant sheep were excluded. The synchronization of oestrus in non-pregnant sheep was done with intravaginal sponges containing 40 mg flurogestone acetate (Chronogest). The laparoscopic insemination was performed with cryopreserved semen of Awassi breed on the 58th hour after removal of sponges. By post insemination days 20 and 27, the ewes were studied echographically for early diagnostics of pregnancy. During the first echography, positive diagnosis was made in 19 animals (38%) on the basis of enlarged uterine lumen. By the 27th day, pregnancy was registered in 38 sheep (76%) on the basis of enlarged uterine lumen and visualization of embryo. At parturition, the positive diagnoses made during the second examination were confirmed.

Key words: echography, laparoscopic insemination, pregnancy, sheep

INTRODUCTION

Reproductive technologies, including the echographic examination and laparoscopic insemination, could promote the level of genetic progress in sheep breeding. They are promising, but yet, their potential is not fully employed (Nicholas, 1996). The main application of echography combined with laparoscopy is for intrauterine insemination with cryopreserved semen especially in private production farms, where sexually active rams are allowed in herds prior to the beginning of reproductive campaign. The early echographic diagnostics of pregnancy is the commonest means for separating pregnant ewes from these eligible to hormonal treatment and laparoscopic insemination. This is necessitated with regard to the achievement of a high fertility and productivity and because of the high price of hormonal treatment, cryopreserved semen and the intrauterine insemination procedure in itself.

In practice, the real-time B-mode echography is the most commonly used for early detection of pregnancy (Karen *et*

al., 2001). The method allows diagnostics of pregnancy within post insemination days 12–25 via both transrectal and transabdominal approaches (Kaulfuss *et al.*, 1999).

The insemination at an early age is a great challenge in sheep breeding because of its positive economical effect. It allows to obtain marketable production (offspring and milk) on the average an year earlier. This aim is achieved by application of the three contemporary reproductive methods: oestrus synchronization, laparoscopic insemination and echographic detection of early pregnancy. Their efficacy in the established Bulgarian Pleven Blackhead sheep breed is not adequately studied. There are studies upon the potential for monitoring of reproductive cycles in local coarsewool sheep breeds (Bankov *et al.*, 1989) but they did not come to an end because at that time, the fine wool sheep breeding was a priority for Bulgaria. Both Bulgarian (Bankov *et al.,* 1989) and foreign (Chemineau *et al.*, 1991) investigators determined a various breed-related reactivity to hormonal preparations, recommending to adjust the doses and the routes of administration for each breed (Bonev, 2003).

The aim of the present experiment was to study the potential of use of these methods in the milk Pleven Blackhead sheep breed and creation of a protocol allowing their early insemination at the age of 10–12 months.

MATERIALS AND METHODS

Animals

The experiment was performed on 50 ewes from the Pleven Blackhead breed, aged 10–12 months and weighing 40–48 kg, placed under uniform conditions of rearing and feeding (closed premises − 62 m^2, yards −72 m^2, grazing − up to 6 hours per day, natural light and concentrate − 0.400 kg per animal daily, treatment against helminths, immunoprophylaxis). Preliminarily, the animals were submitted to echographic study for detection of a possible pregnancy.

Synchronization of the oestrus

The trial was done in November and December. A hormonal protocol including vaginal sponges with active substance 40 mg florogestone acetate (Chronogest, Intervet International, Boxmeer, Holland) was used. The sponges were removed fourteen days after their placement and pregnant mare serum gonadotropin − PMSG (Folligon, Intervet International, Boxmeer, Holland) was intramuscularly applied at a dose of 300 IU.

Laparoscopic insemination

The experimental sheep were deprived from feed and water for 12 hours prior to the insemination. The laparoscopic procedure was done 58 hours after sponge removal with instruments Storz (Germany) and auxiliary materials IMV (France). Frozen semen of the Awassi breed, imported from Hungary, was employed. Each dose had a volume of 0.25 mL, motility over 30% and living spermatozoa counts of 40×10^6. The semen from one paillette was injected into the lumen of the respective uterine horn for achieving a higher fertility percentage.

Echographic diagnostics of pregnancy

All animals were submitted to transabdominal echographic examination (Fowler & Wilkins, 1984) with Aloka SSD 500 Micrus equipment and 5 MHz linear transducer on post insemination days 20 and 27. A positive diagnosis of pregnancy

was made when an enlarged uterine lumen with amniotic fluid were seen and the embryo could be visualized.

RESULTS AND DISCUSSION

The effect of used biotechnological methods is presented in Table 1 and 2.

Oestrus synchronization

The applied hormonal treatment with progestogens in ewes resulted in induction and synchronization of sexual cycle and ovulation and the conception rate was 76% - Table 2). These data showed that in studied sheep, the amount of exogenous progestogen in vaginal sponges induced levels, necessary for the fertilization and the survival of the ovocyte. This is valid for the preovulatory phase too, when mature ova are very sensitive to the influence of absolute, relative and variable levels of steroid hormones (McEvoy *et al.*, 1995).

The observed total fertility in treated ewes was 102% and in those that gave birth – 134.2%. Taking into account the natural biological fertility of Pleven Blackhead ewes, that is 150%, our percentage obtained in young animals (134.2%) was very high, especially in winter. Our results were higher than those of Eyal *et al.* (1978) who reported fertility rates of 60% in Awassi milk ewes and 122% in Asaf ewes, bred without oestrus synchronization, laparoscopic insemination or early detection of pregnancy. The increase in the PMSG dose over 300 IU combined with concentrate supplementation would most probably result in a higher economical productivity through enhanced biological productivity, as observed in fine wool and meat sheep breeds in Bulgaria. This hyperstimulation of ovaries, could however have a negative impact upon the physiological state of young sheep after parturition, that was reported in aforementioned breeds and that caused problems during the next breeding (Bonev, 2003). We suggest that the present experimental design and used biotechnological methods allowed us to attain optimal results and the administration of

Table 1. Diagnoses obtained from the echographic study

Diagnoses	Post insemination day 20		Post insemination day 27		Confirmed at parturition	
	number	%	number	%	number	%
Positive	19	38	38	76	38*	76
Negative	31	62	12	24	12	24
Total	50	100	50	100	50	100

* animals with abortions are counted for confirmed diagnosis

Table 2. Principal reproductive parameters in experimental animals

Total number of animals	Conception rate		Parturitions		Abortions		Productivity, %	
	number	%	number	%	number	%	% of treated	% of born
50	38	76	36	72	2	4	102	134

PMSG doses higher than 300 IU was not justified from an economical point of view.

With regard to the rate of abortions (4%), there were no differences from physiologically allowed values.

Laparoscopic insemination

Its performance several hours prior to anticipated ovulations or between hours 58 and 60 after progestogen discontinuation created favourable conditions for fertilization of ovulated ova. The conception rate of 76% from the first insemination in this experiment was very high taking into consideration that only frozen ram semen, characterized by short survival time after defrosting, was used. Stellflug *et al.* (2001) reported a conception rate of 79% from the first, but natural insemination after hormonal oestrus synchronization in ewes. Therefore the used method was highly reliable and without negative side effects for ewes.

Echographic diagnostics of pregnancy

Using echography, a positive diagnosis of pregnancy on the basis of enlarged uterine lumen was made in 19 sheep (38%) on the 20th day after the insemination (Table 1, Fig. 1) whereas 31 (62%) sheep were non-pregnant. During the subsequent examination (post insemination day 27), 38 ewes or 76% were pregnant with enlarged uterine lumen and visualization of embryo (Fig. 2) and 12 (24%) – yielded negative result. These data were confirmed later during the parturition and were similar to those obtained by Kaullfuss *et al.* (1999).

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The results obtained throughout the present study showed that the combine use of biotechnological methods, namely oestrus synchronization, laparoscopic insemination with frozen Awassi semen and
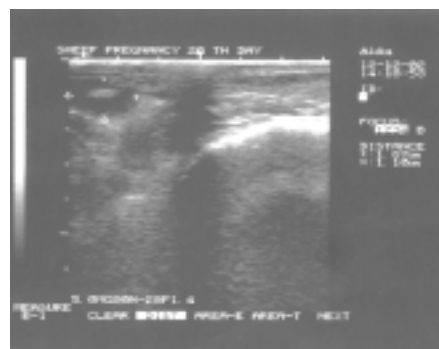

Fig. 1. Echography of pregnant Pleven Blackhead ewe (20th day). The anechoic gestation sac with echoic embryo is observed cranially to the urinary bladder.

Fig. 2. Transrectal echography of pregnant Pleven Blackhead ewe (27th day). A clear visualization of enlarged uterine lumen with hyperechoic embryo – 23.9 mm.

echographic detection of early pregnancy were prerequisites for enhanced economical efficacy and genetic progress in Pleven Blackhead sheep. Using those approaches, the fertilization, deliveries and weaning of lambs could be performed by groups. After weaning, the lactation begins at a time as well as the subsequent passing to the dry period.

CONCLUSIONS

The laparoscopic insemination with frozen semen after hormonal treatment with

vaginal sponges containing 40 mg flurogestogene acetate (Chronogest) and i.m. administration of PMSG at 300 UI performed by us, resulted in a conception rate of 76% and 102% economic productivity in Pleven Blackhead ewes.

The transabdominal echography gave best results when applied in ewes from this breed after the 27th post insemination day.

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Correspondence:

G. Bonev
Department of Genetics, Breeding and Reproduction, Faculty of Agriculture,
Trakia University
Student's Campus
6000 Stara Zagora, Bulgaria