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Effect of 20 mg Fluorogestone Acetate (FGA) Sponges on Reproductive Parameters of Dairy Ovine Breeds in Southern Italy

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ABSTRACT

Background: Intravaginal devices containing progestins are widely used for oestrus synchronization in sheep. Progestins give economic benefits to farmers but may have some limitations and the efficacy strictly depends on farm management. There are different devices, with different molecules (progesterone, medroxyprogesterone acetate, fluorogestone acetate), different dosages and long (12-14 days) and short-term (5-7 days) protocols. Experimental studies often include a limited number of animals and are held at different latitudes and with different system of management. To our knowledge, there are few reports in the literature on field application of the recently licensed 20 mg fluorogestone acetate (FGA) sponges in large ewe flocks, excluding the registration trials.

Materials, Methods & Results: This study was designed to evaluate oestrous synchronization in 2 different breeds. A total of 1100 Lacaune (L) and 618 Sarda (S) were assigned to 8 groups, comparing multiparous (M) and nulliparous (N) and those synchronized in November (n) and May (m). The groups were: LMm (n. 556), LNm (n. 180), SMm (n. 70), SNm (n. 32), LMn (n. 242), LNn (n. 222), SMn (n. 440) e SNn (n. 76). The intravaginal sponge was inserted for 14 days. At sponge withdrawal, eCG (400 IU, IM) was injected, and rams were joined into flocks 30 h later with a male/female ratio of 1:8. Transrectal ultrasonography was performed for pregnancy diagnosis after 30 days. Fertility parameters such as oestrus (OR), pregnancy (PR), lambing rates (LR) and prolificacy were calculated. Fisher exact test was used to compare parameters of each group with the corresponding and significance was set at $P < 0.01$. The use of intravaginal sponges in this study was easily performed in all animals. In nulliparous groups, digital insertion of the sponge, instead of the applicator, was preferred to avoid discomfort. Sponge loss was observed in 2% of treated ewes. At the removal of the sponge, mild vaginal contamination was observed in 90% of the ewes. Ultrasound finding of embryo resorption, pseudopregnancy and pyometra were found in rates under 2%. The loss of pregnancy ranged from 0 to 3.6% without significant differences among groups. The SMn group showed the best performances (0.91 OR, 0.88 PR, 0.85 LR), significantly higher than the other groups. Prolificacy was maximum in the LMn (1.68) and minimum in the SNm group (1.06), without significant differences among groups.

Discussion: The 14-day regimen based on 20 mg FGA-releasing intravaginal devices is an easy and satisfactory synchronization regimen to improve the productivity and the fertility of sheep farm, and this can be managed and optimized in different breeding conditions. In this study, Sarda breed shows a good adaption to the environment with adult ewes giving the best results in reproductive season. However young Sarda ewes were less productive especially in non-breeding season. Lacaune showed good reproductive potential, young and adult ewes responded to progestins in reproductive and non-reproductive season. It was confirmed that the reproductive performance is affected by season, age, and breed. This study encourages the breeding of indigenous breeds by implementing the management with modern technologies. The imported highly productive breeds, if well managed, can give a production less influenced by seasonal variables and age.

Keywords: ewe, sheep, Lacaune, Sarda, oestrus synchronization, progestins, intravaginal device, indigenous breeds.

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INTRODUCTION

The South of Italy is characterized by traditional and poorly productive dairy sheep farms. The introduction of highly productive breeds from other countries has represented an attempt to cope with the increase in raw materials and an overly demanding market but the effects of these introductions are not clear.

Sheep are seasonally polyoestrous and short-day breeders and the Fall is the most natural time to breed in Italy. The breeding season may be influenced by several factors, including the male effect, the presence of cycling ewes, pasture, climate, and latitude [14].

Intravaginal devices containing progestins are widely used to stimulate reproductive activity during the breeding or non-breeding season [12]. The device has been classically developed as a polyurethane sponge, impregnated of fluorogestone acetate (FGA) or medroxyprogesterone acetate. The FGA sponge has been designed to contain 45 mg [18], 40 mg [19], 30 mg [20] or, as recently licensed, 20 mg of the drug without a decrease of fertility [3,8,11,26]. The classical long-term treatment includes intravaginal insertion of the sponge for 12-14 days followed by the intramuscular injection of equine chorionic gonadotropin (eCG) at the time of sponge removal. However, short-term (5-7 days) protocols associated with prostaglandin F2alpha have been proposed [9,11,15,24].

Despite these studies, there are few reports on field application of 20 mg FGA sponges in large ewe flocks, excluding the registration trials. This study aimed to compare reproductive parameters under this progestin treatment in 2 dairy breeds to compare the performances of an autochthonous (Sarda) and a newly introduced breed (Lacaune).

MATERIALS AND METHODS

Animals and location

The experiment was conducted in a farm in Palermo, Italy. A total of 1718 dairy ewes, aged between 1 and 5 years old, were used in the study; 1100 Lacaune (L) and 618 Sarda (S), multiparous (M) and nulliparous (N) were synchronized in November (n) and May (m). The animals were assigned to 8 groups: LMm (n 556), LNm (n 180), SMm (n 70), SNm (n 32), LMn (n 242), LNn (n 222), SMn (n 440) and SNn (n 76). Criteria

of inclusion for May and November groups were, for multiparous ewes, at least 5-month interval from the previous delivery, body condition score of 2.75 - 3.25, no history of abortion and to be clinically healthy; for nulliparous ewes, age of at least 12 months and over 80% of adult size and to be clinically healthy. Body-weight of ewes in each group was: 66 ± 2 kg (LMm), 51 ± 4 kg (LNm), 42 ± 3 kg (SMm), 34 ± 3 kg (SNm), 68 ± 2 kg (LMn), 53 ± 3 kg (LNn), 43 ± 2 kg (SMn) and 35 ± 2 kg (SNn).

Feeding and reproduction management

The animals were allowed to graze on natural pasture. When animals were kept indoors, forage and concentrate were administered following the Sementusa approach [1]. In brief, the approach included: evaluation of the vegetables available in the rotating pasture during the year and calculation of the needs of forage and concentrate; the administration of forage always before pasture even during the best season. Monthly evaluation of urea in milk was used to balance the protein intake. No pre-breeding alimentary flushing was applied in this study. At day 0, the ewes were treated with intravaginal sponges impregnated of 20 mg of FGA¹ that remained in place for 14 days; at sponge withdrawal, 400 IU of equine chorionic gonadotropin (eCG)¹ was intramuscularly injected. Rams of 3-5 years old and of proven fertility, previously sent away from the ewes for 8 weeks, were joined into the flock 30 h later with a male/female ratio of 1:8. Marking of ewes by ram was done by harness containing crayon, which is rubbed on the rump during mating. The mounted ewes were considered in standing heat. After 72 h from the sponge removal, the rams were separated. Transrectal ultrasonography was performed with a 7.5 MHz linear array transducer² for pregnancy diagnosis 30 days after mating.

Statistical analysis

Ewes were monitored until delivery, and reproductive parameters such as oestrus rate (number of ewes showing standing heat/number of ewes in the group); pregnancy rate (number of pregnant ewes/number of ewes in the group); lambing rate (number of lambed ewes/number of ewes in the group); prolificacy (number of lambs born/number of ewes delivered in the group); and lambs for treated ewe (number of lambed ewes multiplied prolificacy) were calculated. Fisher exact test was used to compare parameters

of each group with the corresponding (L vs S; M vs N; M vs N) using Socscistatistics software. Significance was set at $P < 0.01$.

RESULTS

The use of intravaginal sponges in this study was easily performed in all animals. Adult ewes had no discomfort during the insertion of the sponge with the help of the plastic applicator provided together with the device. In nulliparous groups, the presence of hymenal structures caused an obstacle to the insertion of the applicator. To avoid discomfort, digital insertion of the sponge was preferred in most nulliparous ewes. Sponge loss was observed in 2% of treated ewes, but this fact did not lead to the exclusion of the subjects as it was not possible to establish the exact moment of the loss. At the removal of the sponge, mild vaginal contamination (fluid and odour) was observed in 90% of the ewes but no negative effect on mating behaviour was evident. At pregnancy diagnosis, the animals were classified as pregnant and not pregnant. Among the latter, ultrasonographic findings of resorption, pseudopregnancy, pyometra were observed respectively in 2%, 0.3%, 0.4% sheep. These conditions were treated with a single administration of 0.05 mg of cloprostenol and never with antibiotics. Abortion was detected in about 2% of pregnant ewes but no treatment or further diagnostic investigations was performed.

The studied reproductive parameters (oestrus response, pregnancy rate, lambing rate, and prolificacy) were reported in Tables 1 and 2.

Oestrus response after the treatment was highest in the SMn (0.91) and lowest in the LNn group (0.61). The SMn group registered a significantly higher rate than the corresponding group of Lacaune (LMn), the corresponding nulliparous (SNn) and May groups (SMm). The LMm group had a better performance than the respective Sarda group (SMm). The LMn group was better than the corresponding nulliparous group (LNn). Finally, among November nulliparous ewes, Sarda ewes (SNn) responded better than Lacaune (LNn). The pregnancy rate was highest again in the SMn (0.88) and lowest in the SMm group (0.54). The SMn group registered a significantly higher rate than the corresponding Lacaune (LMn) and nulliparous groups (SNn). Similarly, the LMn had values better than the nulliparous group (LNn). The lambing rate was highest again in the SMn (0.85) and lowest in the SMm group (0.53). The SMn group registered a significantly higher rate than the corresponding Lacaune (LMn), May (SMm) and nulliparous groups (SNn). The latter had better performance than the respective Lacaune group (LNn). The loss of pregnancy ranged from 0 (SNm) to 3.6% (SNn) without significant differences among groups.

Prolificacy was maximum in the LMn (1.68) and minimum in the SNm group (1.06), without significant differences among groups. In the end, the highest value of 1.16 lambs for treated ewe was obtained in the SMn group, while the lowest values were 0.59 lambs in SMm and SNm groups. The difference between SMn and SMm was significant, as well as that between LMn and LNn.

Table 1. Oestrus response, pregnancy rate, lambing rate and prolificacy in the study groups.

Study groups	N	Ewes in standing heat (oestrus rate)	Pregnant ewes (pregnancy rate)	Lambd ewes (lambing rate)	N lambs (prolificacy)	Lambs for treated ewe
LMm	556	445 (0.80) ^a	373 (0.67)	365 (0.65)	480 (1.32)	0.86
LNm	180	132 (0.73)	121 (0.67)	117 (0.65)	128 (1.09)	0.71
SMm	70	45 (0.64) ^{a,b}	38 (0.54)	37 (0.53) ^a	41 (1.11)	0.59
SNm	32	20 (0.63)	18 (0.56)	18 (0.56)	19 (1.06)	0.59
LMn	242	198 (0.82) ^{c,d}	163 (0.67) ^{a,b}	158 (0.65) ^b	265 (1.68)	1.09
LNn	222	136 (0.61) ^{d,e}	123 (0.55) ^a	120 (0.54) ^c	146 (1.22)	0.66
SMn	440	400 (0.91) ^{b,c,f}	388 (0.88) ^{b,c}	376 (0.85) ^{a,b,d}	512 (1.36)	1.16
SNn	76	60 (0.79) ^{e,f}	55 (0.72) ^c	53 (0.70) ^{c,d}	63 (1.19)	0.83

Values with the same superscript letter in the same column presented a significant difference ($P < 0.01$). Lacaune (L), Sarda (S), multiparous (M), nulliparous (N), November (n) and May (m).

Table 2. Oestrus response, pregnancy rate, lambing rate and prolificacy rate in all the Lacaune (L), Sarda (S) and L+S groups.

Study groups	N	Ewes in standing heat (oestrus rate)	Pregnant ewes (pregnancy rate)	Lambd ewes (lambing rate)	N lambs (prolificacy)	Lambs for treated ewe
L	1100	855 (0.77)	721 (0.65)	699 (0.63)	958 (1.37)	0.86
S	618	531 (0.86)	507 (0.82)	494 (0.80)	648 (1.31)	1.05
L+S	1718	1386 (0.80)	1228 (0.72)	1193 (0.70)	1606 (1.35)	0.94

DISCUSSION

This study reported the reproductive performances of dairy ewes belonging to 2 diffuse breeds in Southern Italy under the in-label long-term 20 mg FGA synchronization regimen. The use of sponges may be accompanied by some benefits and complications [8]. Under progestins, lambing accommodation and labour are managed more efficiently, and nutrition and disease prevention are better targeted. Progesterone is the elective drug to treat ewes with silent estruses or in anoestrus; it is also curative for ovarian cysts and anovulation. Progestins give economic benefits to farmers that generally lasts for a period over the time of the treatment. On the other hand, progestagen-based devices may have some limitations. The efficacy depends on the quality of the farm management and an eventual failure is seen as an extra cost for the farmer. In many countries, the application of the device, the registration of the treatment, and the use of other associated drugs require a veterinary prescription and performance with a further cost. The use of hormones in the farm may be accompanied by a special registration and may be strictly monitored by the official veterinary service, having the devices withdraw periods for meat and milk despite differences among countries and devices. Finally, there is a relevant ethical feeling among people to avoid cheese, milk, meat coming from animals treated with hormones that discourage the use of such drugs. The sponges can get lost during the treatment, which lasts about 10 days, or the silk thread of the device, which normally comes out of the vulva, can get stuck deep in the vagina and make it more difficult to remove the sponge, requiring the use of a vaginal speculum. Also, the applicator, being rigid, can cause damage to the vaginal walls or, in the case of virgins, the applicator tears the hymen during insertion and consequently adhesions can develop that make it difficult to remove the sponge later. In this study, we experimented that it is better to insert the sponge in these animals with a

gloved finger. In addition, if the sponge is not inserted following hygiene rules (disinfection of the applicator, cleaning of the perineum), there is a significant risk of inducing even catarrhal vaginitis [8]. In this study, mild contamination was almost constantly observed. The resulting vaginitis was self-limiting and had no detectable effects on mating, pregnancy rate, embryo resorption and uterine infections. The addition of antibiotics on sponges should not be considered nowadays, while it is interesting the use of vaginal infusion of probiotics at the time of sponge insertion [16].

As stated, the 20 mg FGA impregnated sponge replaced, in many countries, the 30, 40 and 45 FGA sponges. In a pioneer study, synchronisation protocols with the reduction of the dose of FGA from 40 to 20 mg were shown to be sufficient in terms of follicular dynamics, ovulation, and the development of functional corpora lutea [11]. According to manufacturer registration trials 20 mg is the minimum drug load resulting in good fertility, reporting 71% of pregnancy rate and 1.69 of prolificacy in 950 dairy ewes in breeding season in France [6]. However, some authors [4,7,10] have tried cutting the sponge in half, halving the dose of the active ingredient to about 15 mg of FGA, and have obtained results that can be compared with using whole sponges. In other studies, however, lower fertility has been observed when using too low progestogen [22-24].

In a study, in Mexico, using 20 mg FGA sponges, oestrus was observed in 14 of 15 hair sheep after 30 ± 8 h and the oestrus duration was 55 ± 8 h; the pregnancy rate was 53% [3]. In Turkey, on 68 Awassi ewes, oestrus response was observed in 81% of animals after 45 h from sponge removal; oestrus duration was about 30 h, pregnancy rate (on 55 ewes) was 73%, the lambing rate was 95% and the prolificacy 1.2 [26]. The overall results of our study (Table 2) on 1718 ewes were 80%, 72%, 70% respectively for oestrus, pregnancy, and lambing rates and 1.35 for prolificacy. They agree with previous studies and differences reflect different breed, management, regimen, season, and latitude.

Prolificacy may be increased modifying the amount of eCG [2,5,17,25], especially in non-breeding season; in this study, the dose of eCG did not change throughout November and May groups. Multiple foetus pregnancies require a supplement in feeding and appropriate care during lambing and suckling period; low survival rate of new-borns and lower milk yields of the Sarda ewes have been reported [21]. For these reasons, most of dairy ruminant farms prefer managing single pregnancy [13].

Lacaune has an optimal reproductive potential, in this study, young and adult ewes responded to FGA in reproductive and non-reproductive season. Importing this breed can increase the farmer's income, but above all make it less fluctuating throughout the year. However, the use of this breed is probably only possible in some farm with advanced management. Nutritional supplement is mandatory for this breed also in presence of an optimal pasture and may explain eventual low performances also in reproductive season. Sarda breed shows best adaption to environment and gives the best results in reproductive season, young ewes must be strictly monitored because of the risk to be unproductive if badly managed [1]. This study encourages the breeding of native breeds by implementing the management with modern technologies, including hormonal stimulation. The native

species maintains a good productivity even with an average management of the farm, as it can make the most of the pastures and are more resistant to diseases and metabolic disorders.

CONCLUSIONS

The 20 mg FGA device is an easy and satisfactory synchronization regimen to improve the productivity and the fertility of sheep farm, and this can be managed and optimized in different breeding conditions. The reproductive performance is affected by season and age, by latitude and breed. The use of the drug cannot prescind from a good breeding practice.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the contents and writing of the paper.

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