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TESTING AN EXPERIMENTAL PROTOCOL FOR INDUCING ESTRUS IN THE NON-BREEDING SEASON IN TURCANA SHEEP

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ABSTRACT

The aim of the study was to test the efficiency of a non-breeding season estrus induction protocol in sheep. The experimental protocol involved the use of intravaginal sponges with FGA, followed by the intramuscular administration of PGF2α and GnRH. The study was carried out in a microfarm in the western part of Romania, on 20 sheep of the Turcana breed, in the months of May-August 2023. The parameters monitored and analyzed were: the rate of loss of sponges, the rate of establishment of vaginitis, the total percentage of ewes in estrus and the pregnancy rate. The values of the estrus induction and synchronization rate in the non-breeding period in the Turcana breed sheep was 80.00%, respectively the gestation rate was 87.50%. Based on the results obtained, we can conclude that the estrus synchronization protocol using intravaginal sponges containing progesterone combined with the intramuscular administration of PGF2α and GnRH was effective in inducing estrus in sheep, in non-breeding season, noting a high reproductive efficiency.

KEY WORDS: *estrus, FGA, sheep, GnRH, Turcana.*

INTRODUCTION

The synchronization and induction of estrus in sheep is an applied reproductive biotechnology technique that allows the sheep in a flock to go into heat at the same time the ewes can be subjected to natural mating or artificial insemination. The benefits of the synchronization and induction of estrus in sheep is a valuable method that leads to the control of productivity at the level of sheep farms by increasing the installation rates of pregnancies and calving rates, increasing the number of lambs.

Estrus synchronization in sheep is based on the manipulation of the estrous cycle. Since prostaglandins intervene in the natural induction of estrus, methods and schemes for inducing and synchronizing estrus based on these progestogenic substances have been proposed and are being proposed (Boscos *et al*., 2002). Techniques using

intravaginal sponges are generally applied to small ruminants (sheep, goats), the use of intravaginal sponges with progestogens is associated with the additional administration of other hormones: prostaglandin F2α (PGF2a), equine chorionic gonadotropin (eCG), pregnant mare serum gonadotropin (PMSG) (Dias *et al*., 2001).

There are studies that used intravaginal sponges containing progesterone (medroxyprogesterone acetate) followed by the intramuscular administration of PMSG as a method of inducing estrus in sheep non-breeding period. The results of the studies indicate the high efficiency of these treatments in inducing and stimulating reproductive activity in sheep non-breeding season (Suarez *et al.*, 2006; Gallab *et al.*, 2008). The effectiveness of the proposed treatment scheme may be due to synthetic progesterone that accelerates the mechanism of follicular growth and development, the role of PMSG being to stimulate follicular growth, but also to increase the rate of ovulation and fertility (Dogan *et al*., 2006). The intramuscular administration of PMSG after the application of intravaginal sponges with progesterone determines the induction and synchronization of estrus, high conception rate and high percentage of multiple births (Abdulkareem, 2002).

The administration of gonadotropins (eCG) stimulates follicular growth and increases the rate of ovulation and fertility, also inducing closer synchronization of ovulation (Dogan and Nur, 2006). Also noteworthy is the role of eCG that can modify growing follicles and their transformation into antral follicles, stimulate granulosa cell multiplication and prevent follicular atresia in most domestic animals (ZareShahneh *et al*., 2008). Injection of eCG after treatment with progesterone, causes an increase in the response to enter estrus, an increase in the conception rate and the percentage of multiple births (Abdulkareem *et al.*, 2014).

Estrus induction and synchronization schemes have been proposed and applied that use PGF2α along with vaginal sponges with FGA. Thus, in the study conducted by Ataman and the team of researchers, injections of PGF2α and 400 IU PMSG were used after vaginal sponges with FGA and an estrus induction rate of 80% was reported, outside the breeding season (Ataman *et al.*, 2009).

The use of vaginal devices impregnated with progesterone is a method frequently used in sheep farms, the method is relatively cheap and with a high degree of availability. However, there are a number of factors that can influence the induction and synchronization of estrus, such as: breed, age, season, type of progestogenic substance and concentration, other administered hormones and their concentration, the nutritional system and nutritional status of the animal, stress, environmental factors (Amarantidis *et al.*, 2004; Kleemann *et al.*, 2006).

Although the method of using intravaginal sponges is frequently used, there are also some risks: the possibility of losing the vaginal sponge or the possibility of changing the vaginal microflora, characterized by vaginal contamination and the onset of vaginitis. Vaginitis being one of the diseases that can negatively influence the process of estrus induction, as the effects of the progestogenic substance in the sponge can decrease and the effect may not be the desired one (Martinez-Ros *et al.*, 2018).

The present study proposes a method of inducing and synchronizing estrus non-breeding period in Turcana sheep by using intravaginal sponges with FGA, followed by the intramuscular administration of PGF2α and later of GnRH.

MATERIALS AND METHODS

Animals taken in the study

The study was carried out between May and August 2023, in a sheep microfarm in the western part of Romania. 20 healthy sheep from the Turcana breed (n=20) with a weight of 46.55 ± 4.27 kg represented the experimental group. The ewes were chosen randomly from the tower; it should be mentioned that the ewes are in their second or third calving. The ewes taken in the study were separated from the rest of the flock and were subjected to the induction scheme and estrus synchronization non- breeding season. Mating was allowed to occur naturally by introducing rams into the flock. The rams belonging to the same breed were healthy and had a weight of 73.52 ± 5.81 kg.

The animal study protocol was approved by the committee of experts for ensuring the welfare of experimental animals from the King Mihai I University of Life Sciences from Timisoara No. 210/7 April 2023.

Estrus synchronization protocol

Vaginal sponges impregnated with FGA (45 mg) (SYNCRITE-45 Vaginal Sponge, Animal Health Supplies, 37 Charles St., Ascot Vale, VIC, Australia) were inserted into all sheep. Before the application of vaginal sponges with FGA, they were treated with a solution of 180 IU of penicillin, the action time of the antibiotic was 5 minutes to be able to spread evenly over the entire surface of the sponge, the introduction of the sponges into the penicillin solution was carried out for to prevent the onset of vaginitis.

Inserting the sponges into the vagina was done using the Chronogest CR applicator (Intervet International, Boxmeer, Netherlands). The day of sponge introduction was considered day 1 of the estrus synchronization and induction protocol. The sponges were removed on day 11 of the experiment. PGF2α (0.2 mg) was injected

intramuscularly on the ninth day. Each ewe was then given 8 µg of GnRH by intramuscular injection 36 hours after sponge withdrawal.

Estrus detection and monitoring

The rams were introduced into the herd in order to be able to detect the establishment of estrus and to perform the mating. Estrus induction was identified by tracking the ewes, and the onset of estrus was recorded when the ewes showed a standing reflex in response to the ram. The number of ewes in oestrus was determined 24, 36, 48, 60 hours after the administration of the synchronization treatment. Based on the observations made, the percentage of ewes in estrus was calculated in the different mentioned periods, the report was made against the total number of ewes in the study.

From the first day of the experiment, the physiological state of the sheep was monitored and it was followed if there were sheep that lost their intravaginal sponge with FGA, or if vaginitis set in. The onset of vaginitis was also followed after the withdrawal of the intravaginal sponge and after the onset of estrus. Sponge placement and removal, evaluation of sponge loss, and identification of vaginitis were always performed by the same person.

After about 60 days from delivery, with the aid of the MiniTube Sonograf (Minitüb GmbH, Germany) the establishment of pregnancy was followed.

All the information was centralized and based on them the following parameters were calculated: sponge loss rate, vaginitis installation rate, total percentage of ewes in estrus and gestation rate.

Sponge loss rate (%) = number of ewes that lost sponge/number of ewes treated with intravaginal sponge × 100,

Vaginitis rate (%) = number of ewes showing vaginitis/number of ewes treated with intravaginal sponge × 100,

Percentage of ewes in estrus (%) = number of ewes in heat/number of ewes in heat × 100,

Gestation rate (%) = number of pregnant ewes/number of mated ewes × 100.

RESULTS AND DISCUSSIONS

Based on the observations and determinations made in the sheep pen in the experimental group, the following parameters were determined to quantify the effectiveness of the proposed experimental protocol: the rate of loss of sponges, the rate of vaginitis, the total percentage of ewes in estrus and the rate of gestation. The values of these parameters of interest have centralized and are represented graphically in Figure 1-Figure 2.

The dynamics of coming into heat of the flock of sheep to which the experimental estrus induction protocol was applied was monitored and it was found that the most favorable interval of coming into heat was 36-48 hours after the intramuscular injection of GnRH, followed by the 24-36 hours' interval. From the total of 20 ewes from the Turcana breed, from the experimental group, 5.00% showed estrus in the interval 0-24 hours, 25.00% showed heat in the interval 24-36h, respectively 55.00% in the interval 36-48 hours (figure 1). Estrus induction was achieved in a total of 80.00% of the group of sheep from the Turcana breed taken in the experimental group.

The vaginal sponges impregnated with FGA remained intravaginal throughout the duration of the experiment in 95% of the total sheep in the experimental group, only one sheep lost its vaginal sponge with FGA. The sheep that lost its vaginal sponge during the 11 days of treatment was excluded from the experimental group. The installation of vaginitis was reported in 5% of the sheep after the application of the intravaginal sponge with FGA, the respective sheep was subjected to antibiotic treatment and was eliminated from the experimental group (figure 2).

After a period of 60 days from the natural breeding, the pregnancy rate was determined and it was found that it has an incidence of 87.50% (Fig. 2), a relatively high percentage.

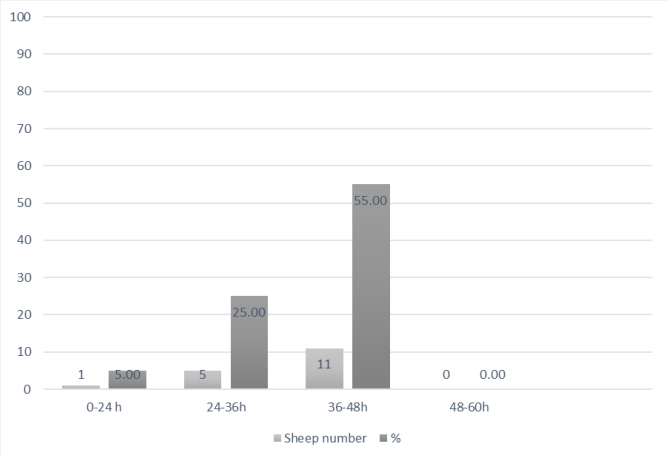

FIG. 1. Percentage of estrus ewes in different time periods (%)

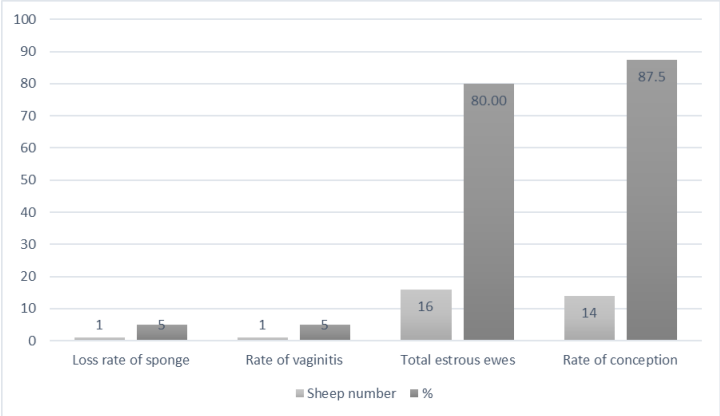

FIG 2. Reproductive performance of Turcana ewes with the estrus synchronization protocol (%)

The results of the study indicate that the estrus induction protocol applied to stimulate reproductive activity non-breeding season in Turcana sheep was efficient. These results obtained are in agreement with those previous studies that used intravaginal sponges containing FGA, followed by the intramuscular administration of PGF2α and later of GnRH, the proposed protocol being very useful in inducing reproductive activity in sheep non-breeding season.

In the case of sheep, the application of intravaginal sponges containing progestogens to induce and synchronize estrus in herds in the non-breeding season is a frequently used method, as progestogens cause the prolongation of the luteal phase (Biehl *et al.*, 2019). Finding an optimal protocol for combining the administration of sponges with gestagen hormones is a permanent concern of research teams.

However, the use of sponges in estrus induction and synchronization protocols also has some disadvantages: there is a risk of losing the sponges, especially when the exposure time is extended (Yu *et al.*, 2022), respectively the risk of vaginitis during the period after applying the sponge or after its extraction (Martinez-Ros *et al.*, 2018). In the present study, both risk situations were identified, one of the sheep lost the sponge, respectively one sheep had vaginitis during the application of the sponge, in these conditions both sheep were eliminated from the experimental group.

The rate of induction of estrus synchronization within the herd, but also the pregnancy rate was studied when applying different treatment schemes, the results that were obtained were different, being directly influenced by the treatment scheme applied, the sheep breed and the conditions climatic conditions specific to the location of the

microfarms. Thus, in Merino sheep by using intravaginal sponges with medroxyprogesterone acetate in association with the intramuscular administration of gonadotropin-releasing hormone (GnRH) 12 hours after removing the sponge, a reduction in the time of estrus appearance was identified and a higher incidence of heat (Yu *et al.*, 2022). In the case of the present study, most of the ewes in the experimental group showed the state of estrus 36-48 hours after the administration of GnRH, a relatively short time interval.

In another study carried out on sheep, a different method of stimulating and inducing estrus, respectively of increasing fertility, was considered, based on the use of vaginal sponges with progesterone for a period of 14 days, in combination with a complex of substances biologically active and eCG as a substitute for GnRH. The results of the study indicated an increase in the fertility of some sheep breeds when the dose of eCG was reduced (Skliarovet *et al*., 2021).

In another study, the effectiveness of short-term and long-term progesterone treatments on the reproductive function of sheep non-breeding season was monitored. The treatment consisted in the administration of intravaginal sponges with FGA for a period of 7 days, respectively 12 days, followed on the day of extraction of the sponges by the intramuscular administration of Triaprost tromethamine and later by PMSG. The results obtained were different depending on the time of exposure to FGA, so that the short-term treatment the estrus induction rate was 86.6% and the pregnancy rate 76.90%, and in the case of long-term treatment the estrus induction rate was 93.3% and the pregnancy rate 85.70%. Short-term treatment with progesterone has been shown to be efficient for the induction and synchronization of estrus in sheep outside the breeding season (Ataman *et al.*, 2006).

CONCLUSIONS

Finding a method of inducing and synchronizing economically favorable estrus and reproductive indices at the level of microfarms represents a challenge for many groups of researchers. The favorable effects of the methods of inducing and synchronizing estrus in sheep are evident by the short period between lambings, the increase in the pregnancy rate, the increase in the birth rate, the efficiency of lamb meat production increases and implicitly an overall economic growth at the level of the microfarm. The estrus induction and synchronization method proposed in this study, which combines the use of vaginal sponges with FGA with the administration of PGF2α and GnRH, determined an estrus induction rate non-breeding period in Turcana sheep of 80%, respectively a rate of pregnancy deception of 87.50%.

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