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Comparative Data about Estrus Induction and Pregnancy Rate on Lacaune Ewes in Non-breeding Season after Melatonin Implants and Intravaginal Progestagen

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ABSTRACT

Backgroung: Seasonal anestrus in ewes reduces reproductive efficiency and hinders productivity. Exogenous hormones can be used to facilitate the induction and synchronization of estrus in the anestrus period. The aim of this study was to evaluate the pregnancy rate in ewes after the treatment with different hormones and fixed time artificial insemination.

Methods: The ewes were randomly divided in 4 (n=4) groups: Melatonin group (Group 1), Melatonin and Medroxyprogesterone group (Group 2), Medroxyprogesterone group (Group 3) and control group (Group 4). Pregnancy rate, estrus induction rate and litter size were determined and compared.

Result: The estrus induction rate in our study was 100% for the first 3 groups and 6.25% for the 4th group. The pregnancy rate was between 4.16% (group 4) and 95.83% (group 2). For the others groups the values were 89.58% for the first group and 91.67%, for the 3rd group. The prolificacy rate was between 1 (control group) and 1.71 (2nd group), with intermediate values for the other 2 groups: 1.21 for 1st group and 1.27 for the 3rd group.

Key words: Ewes synchronization, Medroxyprogesterone, Melatonin, Reproductive efficiency.

INTRODUCTION

Seasonal anestrus in ewes reduces reproductive efficiency and hinders productivity. Hormones can be used to facilitate the induction and synchronization of estrus in the anestrus period (Carlson *et al.* 1989; Forcada *et al.* 1999; Chemineau *et al.* 2008; Jackson *et al.* 2014; Yilmazer *et al.* 2018). Hormone-based protocols have been widely employed to increase the reproductive performance and productivity of sheep. The estrus cycle can be manipulated by maintaining the luteal phase using progesterone (P4) and analogues (progestagens) and/or by interrupting the luteal phase with prostaglandin F2α.

The follicular phase can also be altered with equine chorionic gonadotropin (eCG) administered around the time that P4 exposure ceases. This increases the occurrence and speed of follicular development and ovulation, thereby improving the fertility rate following insemination (Abecia *et al.* 2011; Vilariño *et al.* 2013; Blaschi *et al.* 2014). Several researchers demonstrated that melatonin injection, oral administration, vaginal or subcutaneous implantation can advance the breeding season in ewes (Gomez *et al.* 2006; Yilmazer *et al.* 2018). Yilmazer *et al.* (2018) related the effect of melatonin on the corpus luteum and its ability to increase progesterone concentrations during the luteal phase and to support embryo development.

Therefore, improvement of the estrus synchronization and reproductive performance is very important for success in anestrus ewes. In this context, it is important to know the most successful and effective method. The opportunity to use artificial insemination (AI) is advantageous in the ovine industry, as it significantly speeds up the progression of genetic merit and synchronizing the lactation period of groups of dams that can later be managed as a batch, in order to overcome the need to detect estrus or schedule natural mating (NM) when having problems with the ram or the reproductive performance of females (Vallejo *et al.* 2019). Artificial insemination (AI) was the first important biotechnology applied to improve the genetics of farm animals. It allows the rapid and massive diffusion of desirable

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characteristics of males with high productive potential (Gibbson *et al.* 2019). Different artificial insemination (AI) methods have been described according to semen deposition in ovine species and sperm storage method-by vaginal deposition of semen, cervical (intracervical deposition of semen) or intrauterine (laparoscopic technique) deposition (Alvarez *et al.* 2019, Riesco *et al.* 2021).

The estimated population of small ruminants in Romania is near to 11.48 million (2018 statistics) with a population increase of 0.7% in comparision to the last year. Ovine livestock systems from Romania are based on open grazing systems in altitudes ranging from 1000 to 3000 m; average rainfall of 381-635 mm/ year and a temperature range from -15 to 35°C (local informations). The reproductive management in Romania, like in other countries is based on free mating programs with an average females ram ratio of 35:1, mostly maintained on natural vegetation on common grazing lands, wastelands and uncultivated (fallow) lands, stubbles of cultivated crops and top feeds (tree loppings) (Shivakumara *et al.* 2020, Khaskheli *et al.* 2020), with farmers possessed medium level of knowledge about feedings, management and breeding technologies (Verma and Sharma, 2009). Reproductive parameter-based decisions, hormonal treatments, or ultrasound pregnancy diagnosis are not current practices. While such characteristics of sheep-producing systems predominate the breeders are interested in alternative strategies that allow them to improve the reproductive efficiency of their herds (Vallejo *et al.* 2019).

The aim of this study was to evaluate the pregnancy rate in ewes after the treatment with different hormones protocols and fixed time artificial insemination.

MATERIALS AND METHODS

The study was carried out during March 2019-May 2019 in a sheep flock from Covasna country Romania (lat. 44.4267674, long. 26.102538390000063). A number of 192 ewes from Lacaune breed with the age between 1.5-5 years were synchronized with: melatonin implants (Regulin, Ceva), intravaginal sponges with Medroxyprogesterone (Ovigest, Hipra) and PMSG (Folligon, Intervet) and, after that, were artificial inseminated by intracervical method. The rams (n=3) with known fertility (according to farm records) were separated from the flock until re-introduction.

The ewes were randomly divided in 4 groups: melatonin group (Group 1), medroxyprogesterone group (Group 2), melatonin and medroxyprogesterone group (Group 3) and control group (Group 4). The protocol of hormonal synchronization was different for each group (Table 1). Group 1 (n=48) received in 17 March (day 0) one implant which contain 18 mg of Melatonin, the implants being placed subcutaneously (SC) in the ears. The implants were not removed and in 23 April (day 37) the ewes received 500 IU, intramuscular (IM) injection of PMSG. Group 2 (n=48) received in 17 March (day 0) 18 mg of Melatonin, in 11 April (day 25) the animals were treated with 60 mg of Medroxyprogesterone intravaginal sponges and 12 days later (day 37) the sponges were removed and 500 IU of PMSG were administered by I/M injection. Group 3 (n=48) received in 11 April (day 25) 60 mg of Medroxyprogesterone intravaginal sponges and 12 days later (day 37) the sponges were removed and 500 IU of PMSG I/M injection was administered at each sheep. The animals for group 4 (n=48) were kept as a control group and received no hormonal treatment.

In the interval between 51-56h after the PMSG injection, the ewes which showed signs of estrus (restlessness, vagging of tail, slightly swollen vulva, moist and reddish cervical external os) were artificial inseminated by intra-cervical method. For artificial insemination, we used cooled semen collected from 3 rams witch had higher body weight and with a high sperm quality. The semen was collected by the artificial vagina method in the morning of the A.I day. A 1:1 dilution of the sperm was performed at the temperature of 38°C. After the first dilution, the semen samples were objectively evaluated for volume, consistency, wave motion (0-5 scale), density and percentage of motile spermatozoa (0-100%). After evaluation, a second dilution of the semen samples was performed at room temperature and finally the samples were diluted in 1:10. After 15 minutes from the second dilution, the semen was stored at 4°C. The artificial insemination was performed with 0.25 ml of semen by intra-cervical method. At 28 days after the insemination the pregnancy diagnosis was performed using the ultrasonographic method with a linear probe using the frequency of 5 mHz. After the parturition season, the litter size was established.

The raw data was fed into Microsoft excel spread sheet and descriptive statistics and analysis of variance were performed with Two-Way ANOVA test. Least significant differences (LSD) was used to determine the significance of the differences between the average value of the lots and the control for p values less than 0.5 (0.5, 0.1, 0.01). For establish the differences between the treated groups were performed multiple comparisons by Duncan test.

RESULTS AND DISCUSSION

The estrus induction rate in our study was 100% for the treated groups and 4.16% for the control group, all the animals from the groups with treatment have manifested estrus and from the control group just 3 animals showed signs of estrus. All the animals with estrus signs were artificially inseminated.

The pregnancy rate is representing in the Table 2. For the first group, the pregnancy rate was 89.58%, from 48 animals which were artificial inseminated and in 43 cases the pregnancy was confirmed upon ultrasound exam at 28 days. For group 2 the pregnancy rate was 95.83%, from 48 animals which were artificially inseminated in 46 cases the pregnancy has been confirmed. The third group had a pregnancy rate of 91.67%, from 48 animals which were artificially inseminated in 44 cases the diagnosis of gestation

Table 1: Protocol of synchronization for each group.

	Day 0	Day 25	Day 37	Day 39
Group 1	Melatonin 18 mg	-	PMSG 500 IU	A.I
Group 2	Melatonin 18 mg	Medroxy progesterone 60 mg	PMSG 500 IU	A.I
Group 3	-	Medroxy progesterone 60 mg	PMSG 500 IU	A.I
Group 4	-	-	-	A.I

Table 2: Percent of gestation and the difference from the control group.

	Hormonal treatment	Gestation %	LSD% for p=5%,1%, 0,1%	Signification
G1	Melatonin + PMSG	89.58	85.38	***
G2	Melatonin + Medroxy progesterone + PMSG	95.83	91.67	***
G3	Medroxy progesterone + PMSG	91.67	87.42	***
G4	Control group	4,16	Ct	Ct

LSD=Least significant differences (LSDp0.5=11% , LSDp0.1=14.19%, LSDp0.01=18.33%) Ct=control group.

was a positive one. In the control group, the pregnancy rate was 4.16% and from 48 animals of this group, 3 animals exhibited signs of estrus. All these 3 animals were artificially inseminated and in 2 ewes the pregnancy has been confirmed. Significant differences were observed between the treated groups and the control group (Table 2).

After the lambing period, in case of group 1 from 43 pregnancies were obtained 58 lambs, in 11 cases was a twin gestation and in 2 cases were obtained triplets, in the rest 30 cases 1 lamb was obtained for each sheep. The percent for twin gestation was 25.58%, for the gestation with triplets was 4.65% and the rest 69.76% were simple gestation. From second group were obtained 82 lambs, 20 (43.47%) single gestations, 16 (34.78%) twin gestations and 10 (21.73%) gestations with triplets. In case of the 3th group 61 lambs were obtained, 28 lambs (63.63%) from single gestations, 30 lambs (34.09%) from twin gestations and 3 (2.27%) from triple gestation. At the control group were obtained 2 lambs, no one twin or triple gestation. The litter size for the treated groups was 1.21 for group1, 1.71 for group 2, 1.27 for group 3 and 1 for control group.

The results of our experiment showed that the treatments with progestagens and/or melatonin in association with PMSG are a good option for inducing the estrus period at ewes in out of breeding season. The performed treatments induced ovarian activity and estrus signs at all the animals (100%) from group 1, group 2 and group 3. The rate of estrus response was higher than the rate reported in another studies, Blaschi *et al.* reported in (2014) a rate of exhibiting estrus with values in the interval between 75.0%-95.7% for different groups of ewes treated with progestagens for different periods of time. Essam *et al.* (2016), related the estrus response 100% at Rahmani Egyptian Ewes during the non-breeding season for the groups treated with progesterone for 6 and 8 days respectively 90% and 87.5% for the ewes treated for 14 and 6 days. In control group the estrus response was significantly lower in our study compared with the treated groups, just 6.25% of ewes were observed with estrus signs. In sheep and goats, the introduction of males in a flock of anestrus females provokes an increase in LH pulsatility followed by synchronized ovulations (Philippe *et al.* 2006.), this phenomenon, called “male effect”, works well in Merino sheep for induction of fertile estrus in the non-breeding season; however, in other breeds, it is less effective (Fleisch *et al.* 2015). In a study (Menegatos *et al.* 2003; Fleisch *et al.* 2015) performed at the Lacaune sheep in spring, the results indicate that reproductive activity is not strictly seasonal as reported earlier (Abecia *et al.* 2002). Higher values for the rate of estrus response for the control group were reported in the previous studies (Yildiz *et al.* 2004; Essam *et al.* 2016).

No statistical significant differences between the treated groups were observed in the case of conception rate, the conception rate in this study was 89.58%, 95.83%, respectively 91.67% for the group1, group2 and group 3. In case of the ewes treated with Progesterone our results were higher than the results reported in another studies. Blaschi *et al*. 2014; reported a rate of pregnancy of 83.3% for the ewes treated with progestagens for 14 days. In previous studies, pregnancy rates ranging from 85 to 100% were achieved after melatonin administration (Horoz *et al.* 2003, Emrelli *et al*. 2003). In our study the pregnancy rate for the group treated with melatonin has similar value 89.6%. Also, lower rates were reported in another studies, Baris *et al*. 2012, reported a 70% of pregnancy for a group treated with melatonin implants followed by eCG injection upon reproductive traits of fat-tailed Morkaraman ewes during suckling, anestrus season. Indeed, the pregnancy rate was higher (95.9%) when melatonin was combined with progesterone (supplemented with the eCG) and similarly results were reported by Horoz *et al.* 2003. Melatonin treatment with or without as certain mineral (Ca, Mg) and element (Cu, Fe, Mn, Zn) in sheep, can increase both fertility and prolificacy by improving luteal function and embryonic survival (Knights *et al*. 2001; Baris *et al.* 2017). However, a significant difference was observed between the treated groups and the control group where the pregnancy rate was 4.16%.

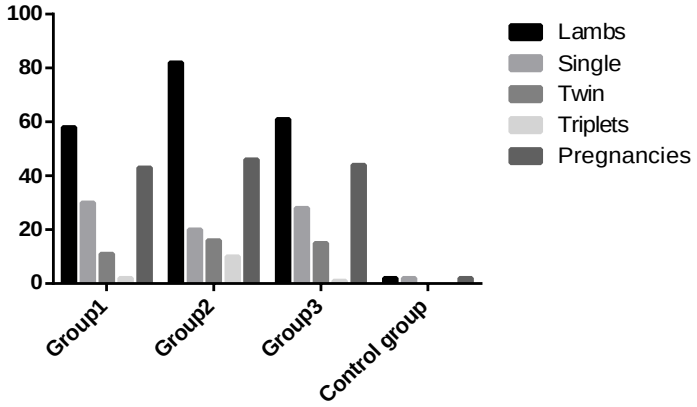

Fig 1: Number of lambs and the litter size.

After the lambing period, the number of lambs and the litter size were determined (Fig 1). Statistically significant differences were observed between the treated groups. The hormonal treatment with Melatonin, Medroxyprogesterone and eCG was found to increase significantly the litter size in our study. Treatment with eCG is known to enhance the recruitment of small follicles, to improve the synchrony of estrus, to increase ovulation rates during the breeding season and to enable the induction of estrous activity during the out of season (Abecia, 2002; Fleisch, 2013). In this study, the litter size for the treated groups has the same values with those reported in previous investigations (Knights *et al.* 2001; Fleisch *et al.* 2015, Kılıçalp and Yücel, 2020).

CONCLUSION

In conclusion, this study reports that all three protocols (Melatonin, Melatonin and Medroxyprogesterone, Medroxyprogesterone) of synchronization can be performed with good results to induce estrus activity and high percent of pregnancy during the non-breeding season for Lacaune ewes. The best results can be obtained by using the protocol with Melatonin in combination with Progesterone and PMSG. Fixed time artificial insemination with cooled semen can be used effectively to take advantage of both the genetic improvement and economic benefit after hormonal syncronisation.

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