Polish Journal of Veterinary Sciences Vol. 21, No. 2 (2018), 353–359

DOI 10.24425/122604

Original article

Effects of subcutaneous melatonin implants and short-term intravaginal progestagen treatments on estrus induction and fertility of Kivircik ewes on seasonal anestrus

C. Yilmazer[1], M. Cevik[2*], A. Kocyigit[3]

[1] Republic of Turkey Ministry of Food, Agriculture and Livestock, 59000, Tekirdag, Turkey
[2] Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Ondokuz Mayis University, 55200, Samsun, Turkey
[3]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Cumhuriyet University, 58140, Sivas, Turkey

Abstract

The aim of this study was to compare the efficacy of estrus induction and fertility by using subcutaneous melatonin (MEL, T1) and short-term intravaginal medroxyprogesteronacetate plus pregnant mare serum gonadotropin treatments (sMAP+eCG, T2) in ewes on seasonaly anestrus. In this study, 105 mature clinically healthy Kivircik ewes in anestrus season and 4 rams were used. After synchronization applications, ewes exhibiting estrus signs were hand-mated with rams known to be fertile. Blood samples were collected at different times in order to determine progesterone (P4) concentrations. Results showed that estrus manipulation protocols induced significant improvement in pregnancy rate. All the fertility results obtained with the sMAP+eCG or MEL groups were similar, in seasonal anestrus. The efficacy duration of P4 in the MEL group was longer than that in short-term progestagen group. Plasma P4 concentrations was significantly different between the first (I) and last (III) measurement days ($p<0.01$). Increase in P4 concentration in T2 group was faster than that in T1 group, and blood P4 concentrations at higher levels could successfully be achieved by using any of the protocols in this study during the seasonal anestrus. In conclusion, according to the results obtained, the hormone application groups received very high estrus response. In addition, the twin ratio was found to be higher in T1 group compared to those determined in the other groups (T2 and Control group). Furthermore, plasma P4 concentrations and high birth rates were obtained in ewes in T1 and T2 groups. These procedures can be considered a good alternative to traditional procedures due to its flexibility under field conditions.

Key words: ewes, fertility, melatonin, short-term progesterone, seasonality

Correspondence to: M. Cevik, e-mail: cevikm@omu.edu.tr, tel.: +90 533 461 24 26, fax: +90 362 457 69 22

Introduction

Reproduction in ewes follows a seasonal pattern, which leads to variations in the availability of products throughout the year. To fulfil consumer's demands for milk and meat all year round, out-of-season breeding is required. Seasonal anestrus reduces reproductive efficiency and hinders productivity. Due to the seasonal nature of estrus cyclicity in ewes, exogenous 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). The estrus cycle can be manipulated by maintaining the luteal phase using melatonin implants, progesterone and analogues (Blaschi et al. 2014). It is well known that fertility was related positively to concentrations of progesterone during the treatment, probably as a result of more appropriate patterns of follicular development (Johnson et al. 1996, Fleisch et al. 2012). Release of progesterone from the sponges declines over time. Therefore, a short term treatment provides higher average concentrations of progesterone during the treatment period. Such treatments (5-8 days) have shown to be effective during anestrus season (Ataman et al. 2006, Dogan and Nur 2006) and breeding season (Ozturkler et al. 2003, Ustuner et al. 2007). Menchaca and Rubianes (2004) obtained 75% pregnancy rate with ram mating after the application of intravaginal sponges containing MAP for six days associated with a dose of 400 IU eCG at the time of sponges withdrawal during seasonal anestrus. Similarly, Dogan and Nur (2006) obtained 76.5% pregnancy rate with fixed-time artificial insemination after the use of the MAP-eCG protocol during non-breeding season in Kivircik ewes.

Several researchers demonstrated that melatonin given by injection, oral administration, or vaginal or subcutaneous implantation can advance the breeding season in ewes (Rajkumar et al. 1989, Zuniga et al. 2002, Gomez et al. 2006). The authors explain this by 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, especially improvement of the estrus synchronization and reproductive performance is very important to success in anoestrus ewes. In this context, it is important to know that determines the most successful and effective method. The objective of the present study was to compare the efficiency of short-term intravaginal progestagen sponges plus eCG administration and subcutaneous melatonin implants administration for 35 days to induce estrus on the reproductive performance and litter size in Kivircik ewes during the non-breeding season.

Materials and Methods

The experiment was carried out according to guidelines for animal research from the National Institute of Health and all procedures on animals were approved by the Ondokuz Mayis University Ethic Committee on Animal Research in current study (approval date/number: December 2013/04-103). This study was conducted at the Catalzeytin-Kastamonu, Turkey (longitude 41°52′35.14″ North, latitude 34°13′13.07″ East) was carried out between April and July 2014. The region has an altitude of 234 m and is characterized by an annual temperature between 13 and 17°C on average. In this study, 105 mature clinically healthy Kivircik ewes in anestrus season and 4 rams were used. The body condition scores ranged from 2.5 to 4.5 with an average of 3.0 ± 0.4 (using 1–5 scale, 1-emaciated to 5-obese) (Ucar et al. 2005). All ewes had previously lambed and their last lambs had been weaned by them. Prior to the study period, rams with known fertility (according to farm records) were separated from the flock until re-introduction. All the flock was maintained under natural lighting conditions in Western Anatolia, Turkey. Water and a mineral supplement was available ad libitum. The sheep used in the study were treated with antiparasitic medication.

The ewes (n=105) were randomly divided into three groups: control (CON), melatonin (MEL, T1), and short-term progestagen (sMAP+eCG, T2). The protocols used were as follows: Group CON (n=25): the animals were kept as a control group and received no hormonal treatment. Group MEL (T1) (n=40): the ewes received one melatonin implant (Regulin®, Ceva Animal Health, France). Each of the implants contained 18 mg melatonin and were placed s.c. in the ears for a period of 35 days. The subcutaneous implants were not removed. Group sMAP+eCG (T2) (n=40): intravaginal progestagen sponges impregnated with 60 mg medroxyprogesterone acetate (MAP) (Esponjavet, Hipra, Spain) were inserted in the ewes for 7 days. Immediately after sponge removal, 350 IU of eCG (Gonaser, Hipra, Spain) was intramuscularly injected to the treated ewes. The ewes in MEL group on the 35th day after melatonin implantation and starting from the day of eCG injections in treated ewes in the sMAP+eCG group, and aproned rams were introduced to the groups, and were all visually monitored twice a day (morning and evening) for signs of estrus for 5 days. Uneasiness, increased occurrence of repeated tail wagging, frequent urination, an abnormal amount of bleating, reddish and swollen vulva and mucus under the tail were all considered true signs of estrus (Ucar et al. 2005).

The main criteria for signs of estrus were the attrac-

Table 1. Changes in reproductive parameters as a consequence of different synchronization protocols.

Reproductive Parameters	Treatments				
	Control	T1 (MEL)	T2 (sMAP+eCG)	P values	Significance
Ewes exposed (n)	25	40	40		
Oestrus response	0.08±0.05[b]	1.00±0.00[a]	0.97±0.02[a]	<0.001	**
Oestrus behaviour	0.24±0.16[b]	3.52±0.20[a]	3.60±0.20[a]	<0.001	**
Pregnancy rate	0.08±0.05[b]	1.00±0.00[a]	0.97±0.02[a]	<0.001	**
Litter size	0.08±0.05[b]	1.25±0.08[a]	1.12±0.07[a]	<0.001	**
Live litter size	0.08±0.05[b]	1.22±0.07[a]	1.05±0.06[a]	<0.001	**
Twinning rate	0.00	0.22±0.02[a]	0.10±0.01[b]	<0.001	**

T1: Melatonin, T2: short-term MAP plus eCG. Values are least Mean squares ± Standard error.
[a,b]: Different letters within row indicate significant differences between treatment groups (p<0.001:**).

tivity and receptivity of ewes (standing still to be mounted by the ram). Once estrus had been observed, the ewes were hand-mated by rams of known fertility (according to farm records). The mated ewes were recorded and separated from the main flock. To prevent overstraining of the rams, the sponges and melatonin implants were inserted at different times, and no more than 5 ewes were treated for ram per day. The criteria used to evaluate the efficacy of hormonal treatments were estrus response, behavior of estrus, pregnancy/lambing rates, twinning rate and litter sizes. The pregnancy rate, litter size, twinning rate and number of live offspring were recorded after parturition. The estrus response (number of ewes in estrus/total number of ewes), pregnancy rate (number of pregnant ewes/total number of ewes), litter size (number of lambs born/total number of ewes), twinning rate and number of live offspring (number of lambs born/number of pregnant ewes) were compared and evaluated in all the groups. To evaluate concentrations of plasma P4 for the treatment groups, blood samples were collected from certain number of ewes from each group on the days of (I) application with sponges or implants, (II) on the 7th day of melatonin implantation and (III) 20 days after ram introduction by puncture of the vena jugularis using vacutainers. Blood samples were centrifuged within 30 minutes of collection at 3000 g for 15 minutes. Plasma was pipetted into 1.5 mL Eppendorf tubes using sterilized plastic disposable pipettes and then stored at -20°C until assayed for P4 analysis using Electrochemiluminescence Immunoassay (ECLIA) (Roche E170, modular analytics). The test sensitivity was 0.15 ngmL^{-1} and intra-assay coefficient of variation was 3.1%. Measurement range of the progesterone kit was reported in 0.030-60.0 ngmL^{-1}. Progesterone concentrations of 1 ngmL^{-1} were considered as indicative of ovulatory activity. Ewes with P4 concentrations less than 1 ngmL^{-1} in all the three blood samples were classified as noncyclic.

Statistical Analysis

The data for the response of estrus, behavior of estrus, pregnancy rate, and litter size, twinning rate and plasma P4 concentrations of synchronization groups were investigated with Shapiro-Wilk normality test for normal distribution. Therefore, all data were accepted as normal. Data for plasma P4 concentrations were analyzed with $Y_{ij}=\mu+a_i+e_{ij}$ linear model equations, where Y_{ij} is the observation for i. treatment group and j. observation; μ is the overall means; a_i is the fixed effect of i. treatments and e_{ij} is the individual random errors for the i. treatment and j. observations. The Duncan multiple range tests was used to analyze the differences in plasma P4 concentrations between groups. Discrete data such as response of estrus, behavior of estrus, pregnancy rate, and litter size and twinning rate were executed by GENMOD procedures analyzing with the normal distributions and linked with ID functions. Comparisons between the groups were analyzed with compare structures in the GENMOD procedures. P-values were derived from likelihood ratio tests and considered as significant when <0.05/0.01. All data were presented as mean ± SEM. Data were analyzed by SAS (SAS Institute Inc, Cary, NC, 2009).

Results

There were no statistically significant differences in between the hormone treated groups for reproductive performances (p>0.05, Table 1). In the present study, all the MAP sponges remained in place until the time of withdrawal (no losses). We observed an approxi-

Table 2. Changes in plasma progesterone concentration (ng/ml) after different synchronization protocols.

Groups	n	First Measurement (I)	Second Measurement (II)	Last Measurement (III)	P values	Significance
Control	10	0.36±0.05[a]	0.33±0.02[a]	0.61±0.15[a]	>0.001	**
T1	10	0.36±0.03[a]	1.42±0.34[b]	4.46±0.48[b]	>0.001	**
T2	10	0.46±0.11[a]	1.65±0.64[b]	4.46±1.03[b]	>0.002	**

[a,b]: Means (±SEM) having different superscripts (a,b) within the same column significantly different from each other (p<0.05).

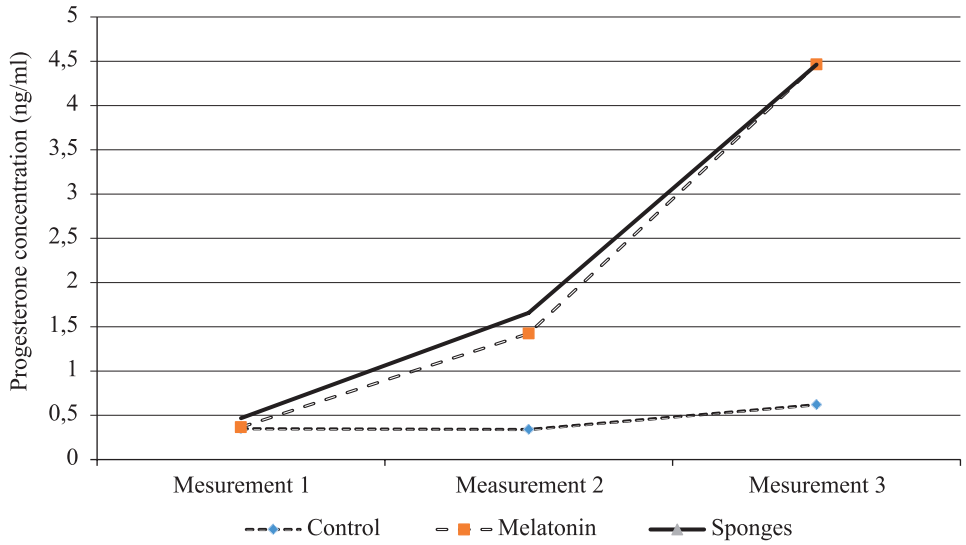

Fig. 1. Plasma progesterone concentration (P4) changes after different synchronization applications.

mately 100% estrus response in ewes in all the hormone-treated groups. We achieved an approximately 8% estrus (only 2 ewes) in the control group. This discrepancy could be due to the fact that the control group ewes might have been influenced by the inclusion of rams for the treatment groups. Indeed, some of control group ewes could have been sexually stimulated by the rams (through combined effects of visual, auditory and olfactory factors) and this situation led them to show estrus. The number of newborns was greater in sMAP+eCG and MEL groups than the control group (p<0.01). However, live litter size and litter size did not differ significantly between the hormone treated groups (p>0.05, Table 1). Although, the occurrence of twinning rates was significantly (p<0.001) higher in the T1 group (0.22 ± 0.02) compared to both the T2 (0.10 ± 0.01) and control groups (0.00). We believe that this situation could be attributed to the stimulatory effect of melatonin upon the corpus luteum.

Mean plasma P4 concentrations in ewes synchronized subcutaneously with melatonin and short-term intravaginal progestagen are summarized in Table 2. In the present study, plasma P4 levels in the T1 group were detected 6.56 ngmL^{-1}, while in the T2 group 10.43 ngmL^{-1} were measured as highest concentration. Initial plasma P4 concentrations on day first measurement was basal between the groups and averaged 0.39 ± 0.04 ngmL^{-1} (<1 ngmL^{-1}), indicating absence of cyclicity and seasonal anestrus. Differences in P4 concentrations on the days of treatment with sponges and implants were not significant between the groups (p>0.05). As shown in Fig. 1, P4 concentrations increased gradually until day 20 both in the T1 and T2 groups. At insert removal (II) higher P4 values were measured in the treatment groups compared to the control group (p<0.05). On day 20 (III) T1 and T2 groups showed higher P4 concentrations than those in the control group (p<0.05). Especially in the hormone treated groups, P4 concentrations measured on day 20 did not influence the percentage of ewes lambing and litter size to service period (p>0.05). Mean plasma P4 concentrations between the first measurements (I) and the last measurement days (III) differed significantly among all the groups (p<0.01) (Table 2). The ewes with lower P4 profiles after 20 days were determined as non-pregnant. Although, for all the hormone-treated groups, the concentrations (4.46 ± 1.03 and 4.46 ± 0.48 ngmL^{-1}) at the end of synchronization (on

day III) were markedly higher ($p<0.01$) than those (0.61 ± 0.15 ngmL^{-1}) in the control group. Changes in the level of progesterone offered a more stable situation in the MEL group, and also fluctuations in hormone levels, remained in the expected range as well. However, the efficacy period of progesterone in the MEL group was longer than that in sMAP+eCG group. An increase in plasma P4 concentrations in this group occurred faster than the MEL group. Therefore, mating in this group began earlier than in the MEL group. According to our results, plasma P4 concentrations at higher levels could successfully be achieved by using any of the protocols in this study during the seasonal anestrus.

Discussion

This study investigated the effects of short-term progestagen and melatonin applications on the induction of oestrous and some of the reproductive parameters in natural anestrus ewes. The results obtained demonstrated that estrus response, estrus behavior, pregnancy rates and lambing rates did not significantly differ between melatonin or/and short-term progesterone applications, except for twinning rate.

One of the oldest methods for inducing ovarian activities in anestrus ewes outside the breeding season is progesterone plus gonadotropin treatments. Under ideal conditions this technique gives 95% estrus and 75% pregnancy rates (Forcada et al. 1999, Ataman et al. 2006). It has been reported that estrus rates can be changed from 81 to 100% (Wigzell et al. 1988, Haresign 1992). In accordance with the results of the researchers, we observed estrus rate of 97-100% in the hormone treated groups. Although, the present results were within range reported in previous studies (Wigzell et al. 1988, Haresign 1992), relatively higher estrus rates observed in this study may be due to the differences in breeds and managements. In our study, we achieved 8% estrus in the control group which differs from that estimated in previous reports (Ataman et al. 2006, Uslu et al. 2012). This discrepancy could be due to the fact that the control ewes might have been influenced by the inclusion of rams in the treatment groups. In addition, some of the control group ewes could have been sexually stimulated by the rams and they led them to show estrus (Fabre-Nys et al. 2015). In a study by Ataman et al. (2006), progesterone treatment for 7 days was effective to synchronize estrus in sheep during both breeding and non-breeding seasons. These results are consistent with findings obtained in this study, where better performance has been recorded following a short-term protocol of sponge insertion. According to some researchers (Ustuner et al. 2007, Karaca et al. 2009, Santos et al. 2010), the injection of ECG at the time of estrus and prior mating causes a higher pregnancy rates during the non-breeding season. In this study, an injection of 350 IU eCG in the intravaginal progestagen insertion group was used in order to induce follicular growth and increase pregnancy rate. These data are in agreement with the findings of this study. For ewes in the sMAP+eCG group, the estrus response and pregnancy rate were similar to those obtained by Ataman et al. (2006) (93.3 and 85.7%, respectively) using a GnRH-$PGF_{2\alpha}$ protocol in Akkaraman cross-bred ewes during the breeding season.

Estrus synchronization studies using melatonin and progesterone administrations have been conducted widely in ewes both in and out of the breeding season (Forcada et al. 1999, Horoz et al. 2003). Some researchers, studying the effects of melatonin upon the stimulation of ovarian activity in anoestrous ewes, argue that successful stimulation of ovarian activity is unlikely during the seasonal anestrus (Horoz et al. 2003, Uslu et al. 2012). In previous studies, pregnancy rates ranging from 40 to 100% were achieved after melatonin administration (Horoz et al. 2003, Fleisch et al. 2015). Similarly, in this study a 97% and 100% pregnancy rate was achieved in the sMAP+eCG and MEL groups, respectively. This result is in agreement with that obtained by Ozturkler et al. (2003) who achieved a synchronization rate of 93.3% using a treatment associating short-term progesterone and ECG. The increase in pregnancy rate and fertility obtained with the melatonin method of synchronization has been confirmed by several researchers (Abecia et al. 2007, Chemineau et al. 2008).

According to our study results, the number of newborns per ewes treated was greater in MEL and sMAP+eCG groups than the control group. The mean litter size obtained in our study (1.25 ± 0.08 and 1.12 ± 0.07 for MEL and sMAP+eCG groups, respectively) was comparable with that (1.1-1.9) reported in earlier studies (Forcada et al. 1999, Bonev 2012, Uslu et al. 2012). In agreement with our results, Forcada et al. (1999) reported that the melatonin implants did not increase the mean litter size of ewes that conceived at either the first or second estrus after pessary removal. In contrast to these results, some other researchers (Rajkumar et al. 1989, Haresign 1992, Horoz et al. 2003, Gomez et al. 2006, Fleisch et al. 2015) have also pointed out that melatonin increased the twinning percentage by having a positive effect on pregnancy and embryo survival rates. Differences may be due to various factors such as: (a) the time of the year (b) route of administration (subcutaneous or intravaginal), (c) breed type with fat-tail or lean-tail (Kivircik breed, as used

herein) and (d) geographic location (with relative seasonality of Kivircik reared in Western Turkey, in the latter study). In the present study, twinning rates from the melatonin group was relatively higher in comparison to those found in the short-term intravaginal progestagen administration group. As previously reported by some researchers (Zuniga et al. 2002, Abecia et al. 2007, Chemineau et al. 2008), this can be attributed to the stimulatory effect of melatonin upon the corpus luteum, thereby increasing the progesterone production during the luteal phase, which ultimately sustains the embryonic development. Some other researchers (Rajkumar et al. 1989, Haresign et al. 1992) have also pointed out that melatonin increased the twinning percentage by having a positive effect on pregnancy and embryo survival rates. In accordance with our results, it has been described by numerous researches that this was attributed to the stimulatory effect of melatonin upon the corpus luteum, thereby increasing the progesterone production during the luteal phase, which ultimately sustains the embryonic development (Zuniga et al. 2002, Abecia et al. 2007, Chemineau et al. 2008).

In our study, mean plasma P4 concentrations were determined at insert application to confirm cyclicity (I), at insert removal to detect subluteal progesterone values in ewes treated with sMAP+eCG and MEL (II) and 20 days after ram introduction to verify pregnancy (III). Some researchers (Abecia et al. 2007, Fleisch et al. 2012) reported which is consistent with our findings that blood P4 concentration is related to the number of ovulations and corpora lutea formed on the ovaries. An increase in plasma P4 concentration in the short-term progestagen group was found to occur faster than that in the melatonin application group. Therefore, in this group matings began earlier than in the MEL group. According to our results, changes in the level of progesterone offered a more stable situation in the MEL group, and also fluctuations in the hormone levels remained in the expected range as well. Moreover, the efficacy duration of progesterone in the MEL group was longer than that in sMAP+eCG group.

Conclusion

In conclusion, the findings of estrus synchronization by subcutaneous melatonin implants and short-term progesterone plus eCG suggest that: (1) the estrus response could successfully be induced in all hormone-treated ewes, (2) markedly higher progesterone concentrations and relatively higher lambing rates were obtained in treated groups when compared to those found in the control Kivircik ewes during seasonal anestrus. These synchronization procedures can be considered a good alternative to traditional procedures due to their flexibility under field conditions.

Acknowledgments

The authors would like to thank Ondokuz Mayis University for financially supporting this study *(*PYO. VET.1904.14.001*)* and Dr. Arslan for statistical analysis.

References

Abecia JA, Valares JA, Forcada F, Palacin I, Martin S, Martino A (**2007**) The effect of melatonin on the reproductive performance of three sheep breeds in Spain. Small Ruminant Res 69: 10-16.

Ataman MB, Akoz M, Akman O (**2006**) Induction of synchronized estrus in Akkaraman cross-bred ewes during breeding and anestrus seasons: the use of short-term and long-term progesterone treatments. Revue Med Vet 157: 257-260.

Blaschi W, Lunardelli PA, Marinho LS, Max MC, Santos GM, Silva-Santos KC, Melo-Sterza FA, Baldassarre H, Rigo TR, Seneda MM (**2014**) Effects of progestagen exposure duration on estrus synchronization and conception rates of crossbreed ewes undergoing fixed time artificial insemination. J Vet Sci 15: 433-437.

Bonev G (**2012**) Effect of melatonin treatment on fertility, fecundity, litter size and sex ratio in ewes. Agri Sci Tech 4: 113-116.

Carlson KM, Pohl HA, Marcek JM, Muser RK, Wheaton JE (**1989**) Evaluation of progesterone controlled internal drug release dispensers for synchronization of estrus in sheep. Anim Reprod Sci 18: 205-218.

Chemineau P, Guillaume D, Migaud M, Thiery JC, Pellicer-Rubio MT, Malpaux B (**2008**) Seasonality of reproduction in mammals: intimate regulatory mechanisms and practical implications. Reprod Domestic Anim 43: 40-47.

Dogan I, Nur Z (**2006**) Different oestrous induction methods during the non-breeding season in Kivircik ewes. Veterinarni Medicina 51: 133-138.

Fabre-Nys C, Kendrick KM, Scaramuzzi RJ (**2015**) The "ram effect": new insights into neural modulation of the gonadotropic axis by male odors and socio-sexual interactions. Front Neurosci 9: 111.

Fleisch A, Bollwein H, Piechotta M, Janett F (**2015**) Reproductive performance of Lacaune dairy sheep exposed to artificial long days followed by natural photoperiod without and with additional progestagen treatment during the nonbreeding season. Theriogenology 83: 320-325.

Fleisch A, Werne S, Heckendorn F, Hartnack S, Piechotta M, Bollwein H, Thun R, Janett F (**2012**) Comparison of 6-day progestagen treatment with Chronogest® CR and Eazi-breed CIDR® G intravaginal inserts for estrus synchronization in cyclic ewes. Small Ruminant Res 107: 141-146.

Forcada F, Abecia JA, Lozano JM, Ferrer LM, Lacasta D (**1999**) The Effects on Reproductive Performance in the Short and Medium Term of the Combined Use of Exogenous Melatonin and Progestagen Pessaries in Ewes with

a Short Seasonal Anoestrous Period. Vet Res Com 23: 257-263.

Gomez JD, Balasch S, Gomez LD, Martino A, Fernandez N (**2006**) A comparison between intravaginal progestagen and melatonin implant treatments on the reproductive efficiency of ewes. Small Ruminant Res 66: 156-163.

Haresign W (**1992**) Responses of ewes to melatonin implants: importance of the interval between treatment and ram introduction on the synchrony of mating, and effects on ovulation rate. Animal Prod 54: 41-45.

Horoz H, Kasikci G, Ak K, Alkan S, Sonmez C (**2003**) Controlling the breeding season using melatonin and progestagen in Kivircik ewes. Turk J Vet Anim Sci 27: 301-305.

Jackson CG, Neville TL, Mercadante VR, Waters KM, Lamb GC, Dahlen CR, Redden RR (**2014**) Efficacy of various five-day estrus synchronization protocols in sheep. Small Ruminant Res 120: 100-107.

Johnson SK, Dailey RA, Inskeep EK, Lewis PE (**1996**) Effect of peripheral concentration of progesterone on follicular growth and fertility in ewes. Domestic Anim Endocrin 13: 63-79.

Karaca F, Ataman MB, Coyan K (**2009**) Synchronization of estrus with short- and long-term progestagen treatments and the use of GnRH prior to short-term progestagen treatment in ewes. Small Ruminant Res 81: 185-188.

Menchaca A, Rubianes E (**2004**) New treatments associated with timed artificial insemination in small ruminants. Reprod Fertil Develop 16: 403-413.

Ozturkler Y, Colak A, Baykal A, Guven B (**2003**) Combined effect of a prostaglandin analogue and a progestagen treatment for 5 days on estrus synchronization in Tushin ewes. Indian Vet J 80: 917-920.

Rajkumar RR, Argo CM, Rodway RG (**1989**) Fertility of ewes given either melatonin or progestagen sponges. Vet Rec 124: 215-217.

Santos IW, Binsfeld LC, Weiss RR, Kozicki LE (**2010**) Fertility Rates of Ewes Treated with Medroxyprogesterone and Injected with Equine Chorionic Gonadotropin plus Human Chorionic Gonadotropin in Anoestrous Season. Vet Med Int, doi:10.4061/2010/978520.

SAS (**2009**) The SAS system for Windows Release 8.02. SAS Institute Inc., Cary, NC, USA. http://sas09.cs.ucdavis.edu/.

Ucar O, Kaya M, Yildiz S, Onder F, Cenesiz M, Uzun M (**2005**) Effect of progestagen/PMSG treatment for estrous synchronization of Tushin ewes to be bred after the natural breeding season. Acta Vet Brno 74: 385-393.

Uslu BA, Tasal I, Gulyuz F, Sendag S, Ucar O, Goericke-Pesch S, Wehrend A (**2012**) Effects of oestrus synchronisation using melatonin and norgestomet implants followed by eCG injection upon reproductive traits of fat-tailed Morkaraman ewes during suckling, anoestrus season. Small Ruminant Res 108: 102-106.

Ustuner B, Gunay U, Nur Z, Ustuner H (**2007**) Effects of Long and Short-Term Progestagen Treatments Combined with PMSG on Oestrus Synchronization and Fertility in Awassi Ewes during the Breeding Season. Acta Vet Brno 76: 391-397.

Wigzell S, Robinson JJ, Wallace JM, Aitken RP (**1988**) Duration of melatonin treatment and ovarian activity in ewes. Anim Prod 46: 510.

Zuniga O, Forcada F, Abecia JA (**2002**) The effect of melatonin implants on the response to the male effect and on the subsequent cyclicity of Rasa Aragonesa ewes implanted in April. Anim Reprod Sci 72: 165-174.