

## Controlling the Breeding Season Using Melatonin and Progestagen in Kıvırcık Ewes

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**Abstract:** The effect of melatonin and progestagen sponge administrations separately and together on the reproductive performance at the onset of the breeding season were investigated in Kıvırcık ewes. On June 22, to 40 anoestrous and non-lactating ewes were administrated melatonin (Regulin) implants. On July 14, progestagen (Synchron) sponges were inserted in 20 of these ewes and in 20 other ewes. They were removed 14 days later and on the day of removal the ewes were given 600 I.U. pregnant mare's serum gonadotrophin (PMSG). The other 20 ewes served as controls. Rams were introduced on July 28. The lambing percentages of the ewes in the melatonin, progestagen + PMSG, melatonin + progestagen + PMSG and control groups were 85% , 90% , 95% and 75% and the twinning rates were 52.9% , 73.7%, 61.1% and 43.6%, respectively. Lambing days after ram introduction were  $156 \pm 6.24$ ,  $153 \pm 6.07$ ,  $155 \pm 7.33$  and  $164 \pm 12.03$  days in the melatonin, progestagen + PMSG, combined and control groups, and lambings were more condensed on  $17.66 \pm 3.51$  days in the treatment groups and  $33.07 \pm 2.25$  days in the control group. The mean numbers of live born lambs per ewe were  $1.47 \pm 0.51$ ,  $1.44 \pm 0.51$ ,  $1.47 \pm 0.61$  and  $1.43 \pm 0.50$ , respectively. We conclude that melatonin and progestagen treatments separately and together at the onset of the breeding season may advance and condense the rates of lambing and the breeding season can be controlled by these treatments. In addition, melatonin treatment may be a viable alternative to traditional hormonal techniques using progestagen and PMSG.

**Key Words:** Melatonin, progestagen + PMSG, anoestrus, ewes.

### Melatonin ve Progestagen Uygulamaları ile Kıvırcık Koyunlarda Üreme Mevsiminin Kontrolü

**Özet:** Melatonin ve progestagen sponj uygulamalarının birlikte ve ayrı ayrı üreme mevsimi başlangıcında kullanımlarının Kıvırcık koyunlarda reproduktif performans üzerine etkileri araştırıldı. 22 Haziranda, anöstrusta ve laktasyonda olmayan 40 koyuna melatonin (Regulin) implantlar uygulandı. Bu koyunların 20 tanesine ve başka 20 koyuna 14 Temmuzda Progestagen (Synchron) sponj yerleştirildi. 14 gün sonra sponjlar çıkarıldı ve 600 İ.U. pregnant mare serum gonadotropin (PMSG) verildi. 20 koyun ise kontrol olarak bırakıldı. 28 Temmuzda bütün koyunlara koç katıldı. Yavrulama oranı sırasıyla melatonin, progestagen + PMSG, melatonin + progestagen + PMSG ve kontrol gruplarında, % 85, % 90, % 95 ve % 75, ikizlik oranları ise sırayla % 52,9, % 73,7, % 61,1 ve % 43,6 olarak bulundu. Doğumlar koç katımından sonraki  $156 \pm 6,24$ ,  $153 \pm 6,07$ ,  $155 \pm 7,33$  ve  $164 \pm 12,03$  günlerde gerçekleşti ve doğumların tedavi gruplarında  $17,66 \pm 3,51$  gün, kontrol grubunda  $33,07 \pm 2,25$  günlerde yoğunlaştığı gözlemlendi. Koyun başına düşen canlı yavru sayısı sırasıyla  $1,47 \pm 0,51$ ,  $1,44 \pm 0,51$ ,  $1,47 \pm 0,61$  ve  $1,43 \pm 0,50$  olarak tespit edildi. Sonuç olarak üreme mevsimi başlangıcında, anöstrus koyunlarda, melatonin ve progestagen tedavileri ayrı ayrı veya birlikte kullanıldığında kuzulama oranını artırmakta ve doğumları toplulaştırmakta ve üreme mevsimi kontrol altına alınabilmektedir. Bunun yanında Melatonin ve melatonin + progestagen + PMSG uygulamaları, klasik progestagen + PMSG uygulamalarına bir alternatif olarak düşünülebilir.

**Anahtar Sözcükler:** Melatonin, progestagen + PMSG, anöstrus, koyun.

### Introduction

The ewe is a seasonally polyoestrous animal. Its sexual activity starts during the autumn months when day length shortens and heat decreases (1,2). The mating season in

Turkey is August and December in some regions, and June-January in others. Generally, September-November is the most suitable time for mating and insemination (3).

The importance of day length in the seasonal control of reproductive activity in the ewe has long been recognised. Decreasing day lengths were thought to stimulate the endocrine changes associated with cyclicity. Melatonin, a pineal hormone, mediates the response to changes in the photoperiod in sheep.

Melatonin levels are high during dark periods and low during light periods; probably this difference in the pattern of melatonin secretion acts as a signal indicating day length to the neuroendocrine axis (4,5).

Although sheep lamb once a year, ovulation can be induced during anoestrus and their reproduction frequency can be increased (6,7).

For many years the standard method of inducing oestrus and ovulation during anoestrus in ewes has been the use of progestagen sponges combined with pregnant mare's serum gonadotrophin (PMSG) treatment when the sponge is withdrawn. Recently, the importance of the pineal gland and its hormone melatonin in the control of the breeding season in the ewe has been established and it has been demonstrated that melatonin given by injection, oral administration, or vaginal or subcutaneous implantation can advance the breeding season in ewes (8-12). Because melatonin administration mimics the naturally occurring increase in melatonin production during the shortened day lengths in autumn, it might be expected to give more reliable and more uniform ovulation and conception than the less natural progestagen-PMSG treatment. Melatonin overcomes the effects of seasonality (4). Combined treatment gives significantly more lambs born per ewe (9).

The aim of this study was to investigate the effect of administration separately and together of melatonin and progestagen + PMSG on the onset of the breeding season and reproductive performance in Kivircik ewes.

## Materials and Methods

Eighty anoestrus, non-lactating ewes, 2-6 years old which had previously had at least one pregnancy were divided into four equal groups at the beginning of the breeding season. All ewes were run as one flock under field conditions at the Experimental Farm of the Veterinary Faculty, İstanbul.

Group 1: (n = 20) Treated with Regulin (18 mg melatonin) only

Group 2: (n = 20) Treated with Regulin and progestagen sponges (60 mg of medroxyprogesterone acetate) + PMSG

Group 3: (n = 20) Treated with progestagen sponges + PMSG only

Group 4: (n = 20) Untreated control.

On June 22, Regulin implants were administered subcutaneously near the base of the ear (groups 1 and 2). On July 14, progestagen sponges were inserted into groups 2 and 3. Sponges were removed 12 days later and ewes were injected with 600 I.U. PMSG (1,11).

Rams were introduced to all groups on July 26, after the sponges were withdrawn (11) and on the 34<sup>th</sup> day of melatonin treatment (8), and they were left for 15 days (11). The pregnancy rate and the number of lambs born per ewe were recorded by daily observation during lambing.

Pregnancy rates, twinning rates and litter size distributions were compared by  $X^2$  analysis and lambing days after ram introduction were compared by variance analysis with Duncan's test.

## Results

In the present study, the effects of melatonin (group 1), melatonin + progesterone + PMSG (group 2) and progesterone + PMSG (group 3) administrations on fertility at the beginning of the breeding season (July) were investigated. The results are presented in the Table.

The percentages of mated ewes which lambled were 85%, 95%, 90% and 75% in the melatonin (n = 20), melatonin + progesterone + PMSG (combined) (n = 20), progesterone + PMSG (n = 20) and control groups (n = 20), respectively. The twinning rates of these groups were 52.9%, 73.7%, 61.1% and 43.6%, respectively. Twinning rates were significantly higher in the second group ( $P < 0.05$ ,  $X^2 = 8.462$ ). The mean number of live born lambs per ewe were  $1.47 \pm 0.51$ ,  $1.47 \pm 0.61$ ,  $1.44 \pm 0.51$  and  $1.43 \pm 0.50$ , respectively. The intervals from the introduction of the rams to lambings were  $156 \pm 6.24$ ,  $153 \pm 6.07$ ,  $155 \pm 7.33$  and  $164 \pm 12.03$  days in the melatonin, combined, progesterone + PMSG and control groups. Lambing days after ram introduction were different in the control group ( $P < 0.05$ ). Lambings were more condensed on  $17.66 \pm 3.51$  days in the treatment groups and  $33.07 \pm 2.25$  days in the control group ( $P < 0.05$ ,  $X^2 = 4.681$ ).

Table. Effects of melatonin and progesterone treatments on reproductive performance in ewes.

Treatments	Pregnancy Rates (%)	Twinning Rates (%) Ram Introduction (days)	Lambing Days after	Live Foetuses Per Ewe
Melatonin (n = 20)	85 <sup>a</sup>	52.9 <sup>b</sup>	156± 6.24 <sup>b</sup>	1.47±0.51 <sup>a</sup>
Melatonin + Progesterone + PMSG (n = 20)	95 <sup>a</sup>	73.7 <sup>a</sup>	153± 6.07 <sup>b</sup>	1.47± 0.61 <sup>a</sup>
Progesterone + PMSG (n = 20)	90 <sup>a</sup>	61.1 <sup>b</sup>	155± 7.33 <sup>b</sup>	1.44± 0.51 <sup>a</sup>
Control (n = 20)	75 <sup>a</sup>	43.6 <sup>b</sup>	164± 12.03 <sup>a</sup>	1.43± 0.50 <sup>a</sup>

\* The differences among the different letters in the same colon were significant (P < 0.05).

## Discussion

It is reported that the ovarian activities of sheep can be induced during seasonal anoestrus by various treatments to increase the lambing frequency and lambs per ewe (2,13-15). The most effective treatment for this purpose is reported to be slow releasing subcutaneous implants containing melatonin or melatonin combined with other hormones (10,14-17).

In the present study, the effects of progesterone + PMSG treatment and subcutaneous melatonin implant administration together and as a single treatment on the reproductive performance of anoestrus sheep at the beginning of the breeding season were investigated.

Some researchers (16-18) have reported that melatonin treatment could start ovarian activities in 5 weeks and achieves oestrus and ovulation. However, others (19,20) have said that this period could extend to 12-15 weeks. In our study, rams were introduced to the flock after 5 weeks of melatonin treatment and 85% pregnancy and 52.9% twinning rates were achieved.

One of the oldest methods for inducing ovarian activities in anoestrus ewes outside the breeding season is progesterone + PMSG treatments. Under ideal conditions this technique gives 95% oestrus and 75% pregnancy rates (1). However, some researchers (5) have reported fertility rates of 30-60%. This difference in fertility and ovulation rates depends on the time of year and the dose of PMSG. Ewes which come to heat by progesterone + PMSG treatment show only one oestrus and if they do not conceive they do not come to heat again (12).

When melatonin was used together with classical progesterone + PMSG, there was a marked increase in

pregnancy and twinning rates (9). By this treatment 95% pregnancy and 73.7% twinning rates were achieved.

The 85% pregnancy rate achieved by melatonin administrations in the present study is higher than the rates in some studies (10,21,22) and lower than those in others (23,24). The possible reason for lower results is the difference in sheep breeds and the earlier start of the study. Some researchers claim that ewes must be exposed to long daylight in order to get the best results from studies carried out during anoestrus (15,25). Nowak et al. (23) observed that lactation had a negative effect on early melatonin treatments.

In the group 1, to which only melatonin was administered, a twinning rate higher than that of the control group was observed. Wellace et al. (26) reported that melatonin increased the progesterone level by its luteotropic effect and this enhanced embryonic survival. Some other researchers (11,14,18,27) have also pointed out that melatonin increased the twinning percentage by having a positive effect on pregnancy and embryo survival rates. However, since it is not possible to determine the ovulation rate it is hard to determine whether this twinning effect is caused by increasing the ovulation rate or decreasing the early embryonic losses. Haresign et al. (21) claim that melatonin increases the ovulation rate; in contrast, some other researchers (20,26,28,29) state that the ovulation rate does not change but pregnancy and live foetus rates increase in the melatonin treated group. These researchers explain this by the effect of melatonin on corpus luteum and its increasing the progesterone level during the luteal phase and supporting embryo development.

In the present study the effects on fertility of single and combined administrations of melatonin and progesterone + PMSG to Kıvrıkcık ewes at the beginning of the breeding season were investigated. The best pregnancy and twinning rates were achieved with combined administrations. When a single administration was used, progesterone + PMSG gave better results.

By single and combined administrations of melatonin and progesterone + PMSG at the beginning of the breeding season, births could have been combined and the breeding season started earlier; pregnancy and twinning rates are also increased.

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