

**Research Article** 

# Effect of Different Progesterone Protocol and Low Doses of Equine Chorionic Gonadotropin (eCG) on Oestrus Synchronization in Arabian Ewes

F. Khalilavi<sup>1\*</sup>, M. Mamouei<sup>1</sup>, S. Tabatabaei<sup>1</sup> and M. Chaji<sup>1</sup>

<sup>1</sup> Department of Animal Science. Faculty of Animal and Food Science. Ramin Agricultural and Natural Resources University, Mollasani, Ahvaz, Iran

Received on: 11 May 2016 Revised on: 5 Sep 2016 Accepted on: 15 Sep 2016 Online Published on: Dec 2016

\*Correspondence E-mail: fatemehkhalilavi@yahoo.com © 2010 Copyright by Islamic Azad University, Rasht Branch, Rasht, Iran Online version is available on: www.ijas.ir

#### ABSTRACT

This experiment was conducted to determine the reproductive performance of Arabian ewes treated with short and long-term progesterone devices in addition to low doses of equine chorionic gonadotropin (eCG) during the anoestrus season. A total of 36 ewes were divided into three groups: in group I vaginal sponges (60 mg medroxy progesterone acetate; (MAP)) were applied and removed after 6 days; in group II, vaginal MAP sponges were removed 12 days following insertion, while group III served as control group. The first two groups were intramuscularly injected with of 300 IU eCG, following sponge removal. Parameters such as oestrus response rate, time to onset of oestrus, duration of oestrus, pregnancy, lambing and fecundity rates were evaluated. Blood samples were collected one day before sponge insertion and two days after sponge insertion and on day of oestrus. There were significant differences between the group I and II with control group regarding the plasma estradiol and progesterone levels. There were no significant differences in oestrus response, time to onset of oestrus, pregnancy, lambing and fecundity rates between groups I and II (P>0.05). However, differences were significant when these two treatment groups were compared with the control group. In group I, duration of oestrus was significantly higher than group II (P<0.05). In addition, other factors in group I was numerically greater than group II (P>0.05). It was concluded that shortterm sponge treatment (6 days) had better performance when compared with the long-term sponge treatment (12 days) in Arabian ewes.

KEY WORDS anoestrus season, Arabic ewes, MAP sponge, reproductive performance.

# INTRODUCTION

Iranian Arabi sheep have a poor reproductive performance. Therefore, increasing Arabi sheep productivity by increasing lambing frequency and fecundity is considered important in the development of Arabi sheep production in Iran. On the other hand, the induction of oestrus outside the breeding season is important in supplying the market for poulterer. Oestrus synchronization is a valuable management tool that has been successfully employed to enhance reproductive efficiency (Hashemi et al. 2006). The ability to synchronize the time of breeding and lambing and the achievement of high fertility at first service is highly beneficial for poulterer. Several methods have been used for oestrus synchronization that including natural progesterone, synthetic progesterones, melatonin, prostaglandin F2 $\alpha$  and isolated ram introduction (Bastan, 1995; Godfrey et al. 1999; Wildeus, 1999; Iida et al. 2004). Intravaginal devices impregnated with progesterone have been used to induce oestrus and ovulation in ewes (Godfrey et al. 1999; Ungerfeld and Rubianes, 2002) during breeding and nonbreeding seasons. These devices are usually inserted over a period of 9-14 days that used in conjunction with eCG, particularly for the non-breeding season in ewes, injected at time of sponge removal or 48 h prior to sponge removal (Wildeus, 1999; Ungerfeld and Rubianes, 2002). However, recent studies showed that progesterone priming as short as 5-6 days is as effective as the long-term priming to induce oestrus with acceptable pregnancy rates during the non breeding season in sheep (Knights *et al.* 2001). The aim of this study was to compare effectiveness of long-term and short-term progesterone treatments combined with low dose of eCG application for oestrus induction out of the breeding season.

# MATERIALS AND METHODS

#### **Experimental design**

This study was conducted during the non-breeding season (April-August) at the research farm of Ramin Agriculture and Natural Resources University, Ahwaz, Southern Iran. A total of 36 multiparous, 2-5 years old Arabic ewes were used in the present study. The ewes were randomly assigned to 3 groups; (I) intravaginal sponge containing 60 mg medroxy progesterone acetate (MAP) for 6 days (n=12), (II) intravaginal sponge containing 60 mg medroxy progesterone acetate (MAP) for 12 days (n=12) and (III) control group (without hormonal treatment) (n=12). Ewes were placed in a single open front barn and fitted with medroxy progesterone acetate (MAP, 60 mg, ESPON-JAVET®, Hipra, Spain) for 6 and 12 days and subsequently devices removed. Immediately after sponge removal, 300 IU of eCG (GONASER®, Hipra, Spain) was intramuscularly injected to the treated ewes. Oestrus was detected with six fertile Arabian rams (one ram:six ewes). The ewes were checked for signs of oestrus after sponge removal. The reproductive variables measured in experimental groups were: oestrus response= number of ewes showing oestrus / total ewes treated in each group  $\times$  100 (Akoz *et al.* 2006), time to onset of oestrus (h) (when ewes allowed a ram to mount and this was registered as the onset time of oestrus), duration of oestrus, pregnancy rate= number of pregnant ewes / number of ewes showing oestrus and mated in each group  $\times$  100 (Zeleke *et al.* 2005), lambing rate= number of ewes lambing / number of pregnant ewes in each group × 100 (Bacha et al. 2014) and fecundity rate= number of lambs born / number of mated ewes in each group (Bacha et al. 2014).

## Blood sampling and hormone assay

Blood samples were collected on the one day before sponge insertion, two days after sponge insertion and day of oestrus. All blood samples were collected via jugular venipuncture into heparinized tubes and centrifuged at 3000 rpm for 15 min, thereafter plasma was stored at  $_{-}$  20 °C until assayed.

Progesterone concentrations were measured using an ELISA kit (Monobind®; USA) with 0.1 ng/mL sensitivity. The plasma estradiol concentration was measured by ELISA kit (DRG International, GmbH, USA) with 0.625 pg/mL sensitivity.

#### Statistical analysis

Data were analyzed as a completely randomized design with the GLM procedure of SAS (1998). Data for oestrus responses, pregnancy rate, lambing rate and fecundity rates were analyzed using chi-square test. Time to onset of oestrus, duration of oestrus and plasma estradiol and progesterone concentrations were analyzed by using PROC GENMOD.

All results are given as mean  $\pm$  SEM. Mean values were compared by the Duncan test. The values of less than 0.05 (P<0.05) were declared significant.

# **RESULTS AND DISCUSSION**

## **Oestrus responses**

The effect of short and long-term application of MAP sponge on oestrus response, oestrus onset and duration of oestrus in Arabian ewes are gave in Table 1. No significant differences in percentage of oestrus response and time to onset of oestrus were found between the groups I and II. However, differences in oestrus response rate and time to onset of oestrus were found to be significantly different when these groups were compared with the control group (P<0.05).

This study shows that short-term sponge treatment results in higher duration of oestrus when compared to the other two groups (Table 1; P<0.05). To our knowledge, this is the first report, which compares the effects of 300 IU eCG in Arabian ewes synchronized by long and short-term progesterone sponge.

The use of intravaginal sponge devices with eCG were found to be efficient methods for oestrus induction and synchronization in ewes during the non-breeding season. In the present study behavioral oestrus was detected in 11/12 (91.67%) and 10/12 (83.33%) ewes in response to short and long-term progesterone treatment, respectively. Both the long- and short-term treatment with progesterone devices result in a high percentage of females shown sign of oestrus.

These results indicate that using short term MAPimpregnated intravaginal sponges as a source of exogenous progesterone administered in non breeding season, had excellent effects on induction of oestrus in Arabian ewes. Knights *et al.* (2001) reported that short-term treatment of ewes with progesterone before ram introduction was adequate to induce fertile oestrus.

V	Ι	II	III
variable/treatments	(short term MAP)	(long term MAP)	(control)
(Mean±SE)	n= 12	n= 12	n= 12
Response of oestrus (%)	91.67 <sup>a</sup> (11/12)	83.33 <sup>a</sup> (10/12)	16.66 <sup>b</sup> (2/12)
Onset of oestrus(h)	44.73±4.49 <sup>a</sup>	45.62±3.76 <sup>a</sup>	78±4.57 <sup>b</sup>
Oestrus duration (h)	27.27±1.4ª	17.38±1.8 <sup>b</sup>	-
Pregnancy rate (%)	$54.44{\pm}0.45^{a}$	50±0.63ª	$0^{b}$
Lambing rate (%)	$66.66 \pm 0.16^{a}$	$80{\pm}0.17^{a}$	-
Fecundity rate	$0.36{\pm}0.12^{a}$	$0.4{\pm}0.13^{a}$	-

 Table 1
 Effects of short and long-term medroxy progesterone acetate (MAP) sponge treatment on oestrus performance, pregnancy, lambing and fecundity rates in Arabian ewes during anoestrus season

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SE: standard error

Also, Ungerfeld and Rubianes (2002) reported that shortterm progesterone treatment was adequate to induce fertile oestrus and no difference in oestrus response was observed when anoestrus ewes were primed for 6 or 14 days, with intravaginal sponge treatments. No significant difference was observed in oestrus response between treatment with long and short-term progesterone sponge in this study, which concurs with Ustuner *et al.* (2007), Ungerfeld and Rubianes (2002).

However, Vinoles et al. (1999) reported that the oestrus response following sponge withdrawal was significantly higher in long-term than in short-term-treated ewes. This disparity may be due to differences of variables, including breed of ewes, month of years, latitude and management. Incidence of oestrus in ewes treated with either synthetic progesterone or natural progesterone in conjunction with PMSG varied from 47% (Robinson et al. 1967) to 85% or greater (Akoz et al. 2006; Timurkan and Yildiz, 2005; Zeleke et al. 2005; Hashemi et al. 2006). The oestrus response in the two group was comparable to that previously reported by Beck et al. (1993); Ozturkler et al. (2003) and Ataman et al. (2006) in short-term and Ozyurtlu et al. (2010); Ozyurtlu et al. (2011); Vinoles et al. (1999); Moradi Kor and Ziaei (2012); Koyuncu and Ozis Alticekic (2010) in long term treatment with progesterone device. All ewes exhibited behavioral oestrus from 28 to 78 h following sponge withdrawal. In previous studies reported the onset of oestrus to occur within 6-120 h following progesterone device removal (Freitas et al. 1996; Romano, 1998; Greyling and Van der Nest, 2000).

Koyuncu and Ozis Altcekic (2010) reported that 96% of ewes exhibited oestrus 48-72 h following removals of progesterone-containing implants. Intervals to onset of oestrus resembled those reported in the previous study in which ewes were treated with progesterone-300 IU eCG (Zonturlu *et al.* 2008).

In this study, the time to the oestrus onset following the withdrawal of sponge in II groups was found to be 44.73 h, lower than the 46 h reported by Ungerfeld and Rubianes (2002) during non-breeding season.

The time to the oestrus onset in the I group was found to be 45.62 h; which was higher than 40 h found by Romano (1996), Ungerfeld and Rubianes (2002) and Emsen and Yaprak (2006). Vinoles *et al.* (2001) reported that ewes treated with MAP for 6 days exhibited oestrus at 84 h and those treated for 12 days exhibited oestrus at 44 h after sponge removal. Also, Zeleke *et al.* (2005) reported that the ewes treated with FGA for 14 day showed oestrus at 41 h, which is lower than the values obtained in the present study (I=44.73 h and II=45.62 h). These differences may be explained by the differences in breed, season, location, nutrition, climate and presence of male after intravaginal devices removal.

The mean overall durations of the induced oestrus period were 27.27 h and 17.38 h for I and II groups respectively. Oestrus duration in the I group was higher than the II group (P<0.05). This result was lower than the duration of oestrus as reported by Nasser et al. (2012). Nasser et al. (2012) reported the duration of oestrus was 34.4 and 36.7 hours following a short (6 days) and a long (12 days) term controlled internal drug release (CIDR) treatment, respectively, and this is consistent with the results obtained by Ustuner et al. (2007). Short duration of oestrus in this study may be explained by differences in breed, age, geographical location and lower estrogen level in the blood. Stimulation of follicular growth in the ovary by eCG together with high levels and longer duration of serum estrogen concentrations could be responsible for a prolonged duration of the oestrus period (Nasser et al. 2012).

#### Pregnancy, lambing and the number of lambs born

In this study, ewes that did not display oestrus behavior during 35 days from mating (after second oestrus period) were considered as pregnant (Moghaddam *et al.* 2012).

Results for the pregnancy, lambing and fecundity rates are presented in Table 1. No significant differences in the results for pregnancy, lambing and fecundity rates were noted between the short and long-term groups. However, the pregnancy and lambing rates were found to be significantly different in the short and long-term groups as compared with the control group (P<0.05). Pregnancy rate, as determined by ewes rebred, was 45.83% (11/24). Eleven of those 24 ewes lambed within 155 days of mating. The remaining ewes probably experienced early embryonic loss. The fertility of anoestrus ewes treated with progesterone deviese ranged from 22 to 70% (Evans *et al.* 2001). In the present study, an injection of 300 IU eCG was used in order to induce follicular growth and increase pregnancy rate. The injection of eCG at the time of oestrus, causes a higher pregnancy rates during the non-breeding season. Vinoles *et al.* (1999) obtained higher pregnancy rate after short-term treatment compared to the Long-term (12 d) treatment with PMSG at the time of vaginal sponge withdrawal.

Ustuner *et al.* (2007) and Ozyurtlu *et al.* (2011) reported that there were no significant differences in terms of pregnancy, lambing and fecundity rates between the short and long-term treatment groups. No significant difference was observed in this study which agrees with (Ustuner *et al.* 2007; Ataman *et al.* 2006; Saribay *et al.* 2011). Lambing rate in this study was 66.66 following short-term treatment and 80% following long-term treatment. It has been shown that the lambing rate in ewes treated with short-term synthetic progesterone in combination with eCG varied from 60 to 83.3% (Ozyurtlu *et al.* 2011; Ataman *et al.* 2006; Amer and Hazza, 2009).

#### **Estradiol concentrations**

Mean plasma estradiol concentrations on the day before sponge insertion were similar among ewes of the three treatment groups (Table 2). Estradiol trend is rising and falling due to follicle activity, outside of breeding season (Menegatos *et al.* 2003).

Negative feedback of estradiol on tonic center in the hypothalamus affect as much as possible to prevent the occurrence of ovarian cycle outside the breeding season (Sanchez, 2005). It can be concluded that due to high levels of estradiol and lack of optimal progesterone concentration, there is no ovarian cycle leads to ovulation in all treatments. In the most animal species oestrus behavior can be triggered by estradiol alone, but in ewes and rats, oestrus behavior is not displayed unless progesterone is present (Fajt, 2011).

Result showed that two days after initiation of the synchronization program, serum estradiol concentrations was affected by the treatments, so that use of progesterone device in non breeding season decreased estradiol concentrations in compared with the first sampling. No difference was found between the I and II groups in estradiol concentration (Table 2). Progesterone is associated with suppression of follicular growth and ovulation through exerting an inhibitory effect on the release of LH from the anterior pituitary in sheep. It inhibits the effect of estradiol secretion by granulosa cells in the ovary (Noel *et al.* 1994).

The mean concentration of estradiol in day of oestrus was similar among I and II groups and higher than control group (Table 2). One day before oestrus one or more follicles in the ovary grow rapidly, thereafter the concentration of estradiol in the venous blood increase generally about 10 to 20 pg/mL (Błaszczyk *et al.* 2004). The estradiol concentrations observed after progesterone device removal in the present study were expected to reflect estradiol secretion by the ovarian follicles. The fall in progesterone concentration stimulates estradiol secretion by the growing follicles.

#### **Progesterone concentrations**

Mean plasma progesterone concentrations on the day before sponge insertion were similar among ewes of the three treatment groups (Table 3). In non breeding season ovulation not occurs because the secretion of LH is low that not promote to development of ovarian follicles and the corpora lutea, thereafter plasma concentrations of progesterone remain very low (Chanvallon *et al.* 2008).

Tahla 2	Mean estradial-17	B concentrations (ng/m)	() ner examination at	different days of a	progesterone snonge treatment
I abit 2	Wican conautor-17	p concentrations (pg/m	L) per examination at	unificient days of	progesterone sponge treatment

<u>C</u>	I	II	III
Groups	(short term MAP)	(long term MAP)	(control)
Number of ewes	7	7	7
One day before sponge insertion	9.22±1.31ª	10.20±1.14ª	8.46±1.20 <sup>a</sup>
Two day after sponge insertion	8.35±1.46 <sup>a</sup>	8.89±1.63ª	9.1±1.22 <sup>a</sup>
Day of oestrus	11.78±2.1ª	$11.4 \pm 1.96^{a}$	8.88±1.36 <sup>b</sup>

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 3 Mean progesterone concentration (ng/mL) per examination at different days of progesterone sponge treatment					
<u>C</u>	Ι	П	III		
Groups	(short term MAP)	(long term MAP)	(control)		
Number of ewes	7	7	7		
One day before sponge insertion	$0.70{\pm}0.22^{a}$	$0.80{\pm}0.18^{a}$	$0.96{\pm}0.27^{a}$		
Two day after sponge insertion	$6.60{\pm}0.97^{a}$	6.33±0.86ª	$1.11 \pm 0.14^{b}$		
Day of oestrus	1.5±0.33ª	$1.68{\pm}0.40^{a}$	$0.98 \pm 0.31^{b}$		

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Obtained results from this experiment showed that the mean progesterone concentrations at the two days after sponge insertion were higher than first sampling and plasma progesterone levels increased with application progesterone device (Table 3). Following insertion, plasma progesterone levels increased rapidly and reached maximum values 2 days post insertion. Maximum values were  $6.60 \pm 0.97$  and  $6.33 \pm 0.86$  ng/mL for the I and II groups, respectively. Differences in maximum values were not significant (P>0.05; Table 3).

Our results are in agreement with previous studies which reported that blood plasma progesterone levels increased rapidly and reached maximum values 2 days post insertion (Husein *et al.* 1999; Husein and Kridli, 2002; Husein and Haddad, 2006).

Progesterone assays following 3rd blood sampling demonstrated that its concentrations in blood plasma declined following intravaginal progesterone devices withdrawal (Table3). Blood progesterone concentrations indicate the reproductive physiology of animals. The initial release of progesterone from intravaginal sponges increase during the first 48 h of treatment and reached maximum values, but decreases with the time. It has been described that, serum medroxy-progesterone acetate concentrations decrease with the time in about a 63% between the 2nd and 13th days after sponge-MAP insertion (Greyling et al. 1994), demonstrating that the progesterone supply from intravaginal sponges decreases with the time. The data presented above confirm that, in the day of oestrous, plasma progesterone values fall to very low levels (Husein and Haddad, 2006; Husein and Kridli, 2002; Kridli and Al-Khetib, 2006).

## CONCLUSION

In conclusion, the use of intra-vaginal MAP sponges in a 12 day + eCG regime could adequately improve the reproductive performance of ewes during the anestrous season, with the possibility of replacing it with a 6 days MAP + eCG regime with higher efficiency. Also, the results presented in this study give evidence that a short-term progesterone protocol prolongs oestrus duration when compared to a long-term treatment.

## ACKNOWLEDGEMENT

The authors gratefully acknowledge the Ramin Agriculture and Natural Resources University for research founds.

## REFERENCES

Akoz M., Bulbul M., Ataman B. and Dere S. (2006). Induction of multiple births in Akkaraman cross-breed synchronized with short duration and different doses of progesterone treatment combined with PMSG outside the breeding season. *Bull. Vet. Inst. Pulawy.* **50**, 97-100.

- Amer H.A. and Hazzaa A.M. (2009). The effect of different progesterone protocols on the reproductive efficiency of ewes during the non-breeding season. *Vet. Arhiv.* **79**, 19-30.
- Ataman M.B., Akoz M. and Akman O. (2006). Induction of synchronized oestrus in Akkaraman cross-bred ewes during breeding and anestrus seasons: the use of short and long-term progesterone treatments. *Rev. Med. Vet.* 50, 257-260.
- Bacha S., Khiati B., Hammoudi S.M., Kaidi R. and Ahmed M. (2014). The effects of dose of pregnant mare serum gonadotropin (PMSG) on reproductive performance of algerian Rembi ewes during seasonal anoestrus. J. Vet. Sci. Technol. 5, 190-198.
- Bastan A. (1995). Effect of melatonin and progesterone application on reproductive performance in akkaraman ewes (Akkaraman ırkı koyunlarda melatonin ve progesterone uygulamalarının reprodu"ktif performans u"zerine etkileri). Ph D. Thesis. Ankara Univ., Ankara, Turkey.
- Beck N.F.G., Davies B. and Williams S.P. (1993). Oestrous synchronization in ewes-the effect of combining a prostaglandin analogue with a 5-day progesterone treatment. *Anim. Prod.* 56, 207-210.
- Błaszczyk B., Udała J. And Gaczarzewicz D. (2004). Changes in estradiol, progesterone, melatonin, prolactin and thyroxine concentrations in blood plasma of goats following induced estrus in and outside the natural breeding season. *Small Rumin. Res.* 51, 209-219.
- Chanvallon A., Blache D., Chadwick A., Esmaili T., Hawken P.A.R., Martin G.B., Vinoles C. and Fabre-Nys C. (2008). Sexual experience and temperament affect the response of Merino ewes to the ram effect during the anoestrous season. *Anim. Reprod. Sci.* 37, 1110-1016.
- Emsen E. and Yaprak M. (2006). Effect of controlled breeding on the fertility of Awassi and Red Karaman ewes and the performance of the off spring. *Small Rumin. Res.* 66, 230-235.
- Evans A.C., Flynn J.D., Quinn K.M., Daffy P., Quinn P. and Madgwick S. (2001). Ovulation of aged follicles does not affect embryo quality or fertility after a 14-day progesterone estrus synchronization protocol in ewes. *Theriogenology*. 56, 923-936.
- Fajt V.R. (2011). Drug laws and regulations for sheep and goats. *Vet.* Clin. North America. *Food Anim.* 27, 1-21.
- Freitas V.J.F., Baril G. and Saumande J. (1996). Induction and synchronization of estrus in goats: the relative efficiency of one versus two fluorogestone acetate-impregnated vaginal sponges. *Theriogenology*. 46, 1251-1256.
- Godfrey R.W., Collins J.R., Hensley E.L. and Wheaton J.E. (1999). Oestrus synchronization and artificial insemination of hair sheep ewes in the tropics. *Theriogenology*. **51**, 985-997.
- Greyling J.P.C., Kotze W.F., Taylor G.J., Hangendijk W.J. and Cloete F. (1994). Synchronization of oestrus in sheep: use of different doses of progesterone outside the normal breeding season. *South African J. Anim. Sci.* 24, 33-37.
- Greyling J.P.C. and Van der Nest M. (2000). Synchronization of oestrus in goats: dose effect of progesterone. *Small Rumin. Res.* 36, 201-207.

- Hashemi M., Safdarian M. and Kafi M. (2006). Estrous response to synchronization of estrus using different progesterone treatments outside the natural breeding season in ewes. *Small Rumin. Respon.* 65, 279-283.
- Husein M.Q. and Haddad S.G. (2006). A new approach to enhance reproductive performance in sheep using royal jelly in comparison with equine chorionic gonadotropin. *Anim. Reprod. Sci.* **93**, 24-33.
- Husein M.Q., Kridli R.T. and Humphrey W.D. (1999). Effect of royal jelly onestrus synchronization and pregnancy rate of ewes using flourogestone acetate sponges. J. Anim. Sci. 77(1), 431-438.
- Husein M.Q. and Kridli R.T. (2002). Reproductive responses following royal jelly treatment administered orally or intramuscularly into progesterone-treated Awassi ewes. *Anim. Reprod. Sci.* 74(2), 45-53.
- Iida K., Kobayashi N., Kohno H., Miyamoto A. and Fukui Y. (2004). A comparative study of induction of estrus and ovulation by three different intravaginal devices in ewes during the non-breeding season. J. Reprod. Develop. 50, 63-69.
- Knights M., Maze T.D., Bridges P.J., Lewis P.E. and Inskep E.K. (2001). Short-term treatment with a controlled internal drug releasing (CIDR) device and FSH to induce fertile estrus and increase prolificacy in anestrus ewes. *Theriogenology*. 55, 1181-1191.
- Koyuncu M. and Alticekic S. (2010). Effects of progesterone and PMSG on estrous synchronization and fertility in Kivircik ewes during natural breeding season. *Asian Australas J. Anim.* 23, 308-311.
- Kridli R.T. and Al-Khetib S.S. (2006). Reproductive responses in ewes treated with eCG or increasing doses of royal jelly. *Anim. Reprod. Sci.* 92, 75-85.
- Menegatos J., Chadio S. and Kauskoura T. (2003). Endocrine event during the pre estruos period and the subsequent estrous cycle in ewes after estrous synchronization. *Theriogenology*. 59, 1533-1543.
- Moghaddam G.H., Olfati A., Daghigh Kia H. and Rafat S.A. (2012). Study of reproductive performance of crossbred ewes treated with GnRH and PMSG during breeding season. *Iranian J. Appl. Anim. Sci.* 2(4), 351-356.
- Moradi Kor N. and Ziaei N. (2012). Effect of PGF2 administration and subsequent eCG treatments on the reproductive performance in mature Raieni goats during the breeding season. *Asian J. Anim. Vet. Adv.* **7**, 94-99.
- Nasser S.O., Wahid H., Aziz A.S., Zuki A.B., Azam M.K., Jabbar A.G. and Mahfoz M.A. (2012). Effect of different oestrus synchronizations protocols on the reproductive efficiency of Dammar ewes in Yemen during winter. *African J. Biotechnol.* 11, 9156-9162.
- Noel B., Bister J.L., Pierquin B. and Paquay R. (1994). Effect of FGA and PMSG on follicular growth and LH secretion in Suffolk ewes. *Theriogenology*. **41**, 719-727.
- Ozturkler Y., Colak A., Baykal A. and Guven B. (2003). Combined effect of a prostaglandin analogue and a progesterone treatment for 5 days on oestrus synchronization in Tushin ewes. *Indian Vet. J.* **80**, 917-920.

Ozyurtlu N., AY S.S., Kucukaslan I., Gungor O. and Aslan S.

(2011). Effect of subsequent two short-term, short-term and long-term progesterone treatments on fertility of Awassi ewes out of the breeding season. *Ankara Univ. Vet. Fak. Derg.* **58**, 105-109.

- Ozyurtlu N., Kucukaslan I. and Cetin Y. (2010). Characterization of oestrous induction response, oestrous duration, fecundity and fertility in Awassi ewes during the non-breeding season utilizing both CIDR and intravaginal sponge treatments. *Reprod. Domest. Anim.* **4**, 464-467.
- Robinson T.J., Moore N.W., Holst P.J. and Smith J.F. (1967). The evaluation of several progestogens administered in intravaginal sponges for the synchronization of estrus in the entire cyclic Merino ewe. Pp. 76-91 in Control of the Ovarian Cycle in the Sheep. T.J. Robinson, Ed. White and Bull PTY Ltd., Australia.
- Romano J.E. (1996). Comparison of fluorgestone and medroxyprogesterone intravaginal pessaries for estrus synchronization in dairy goats. *Small Rumin. Res.* 22, 216-223.
- Romano J.E. (1998). The effect of continuous presence of bucks on hastening the onset of estrus in synchronized does during the breeding season. *Small Rumin. Res.* **30**, 99-103.
- Sanchez E.J. (2005). Melatonin-estrogen interaction in breast cancer. J. Pineal. Res. 38, 217-222.
- Saribay M.K., Karaca F., Dogruer G., Ergun Y., Yavas I. and Ates C.T. (2011). Oestrus synchronization by short and long-term intravaginal sponge treatment in lactating goats during the breeding season: the effects of GnRH administrations immediately after matings on fertility. J. Anim. Vet. Adv. 10, 3134-3139.
- SAS Institute. (1998). SAS<sup>®</sup>/STAT Software, Release 6. SAS Institute, Inc., Cary, NC. USA.
- Timurkan H. and Yildiz H. (2005). Synchronization of oestrus in Hamedani ewes: the use of different PMSG dose. *Bull. Vet. Pulawy.* **49,** 311-314.
- Ungerfeld R. and Rubianes E. (2002). Short term primings with different progestogen intravaginal devices (MAP, FGA and CIDR) for eCG oestrous induction in anestrus ewes. *Small Rumin. Res.* **46**, 63-66.
- Ustuner B., Gunay U., Nur U. and Ustuner U. (2007). Effects of long and short-term progestogens treatment combined with PMSG on oestrus synchronization and fertility in Awassi ewes during the breeding season. J. Acta Vet. Brno. 76, 391-397.
- Vinoles C., Forsberg M., Banchero G. and Rubianes E. (2001). Effect of long-termand short termprogesterone treatment on follicular development and preganancy rate in cyclic ewes. *Theriogenology*. 55, 993-1001.
- Vinoles C., Meikle A., Forsberg M. and Rubianes E. (1999). The effect of subluteal levels of exogenous progesterone on follicular dynamics and endocrine patterns during the early luteal phase of the ewe. *Theriogenology*. **51**, 1351-1361.
- Wildeus S. (1999). Current concepts in synchronization of oestrus: sheep and goats. *Proc. Am. Soc. Anim. Sci.* **39**, 1-14.
- Zeleke M., Greyling L.M.J. and Schwalbach T. (2005). Effect of progesterone and PMSG on oestrous synchronization and fertility in dorper ewes during the transition period. *Small Rumin. Res.* **56**(1), 47-53.
- Zonturlu A.K., Aral F., Ozyurtlu N. and Yavuzer U. (2008). Syncronization of estrous using FGA and CIDR intervaginal pes-

saries during the transition period in Awassi ewes. J. Anim. Vet. Adv. 7, 1093-1069.