



## Comparative study of short term and long term protocols for progesterone based estrus synchronization in ewes during breeding season

A A MALIK<sup>1</sup>✉, M R FAZILI<sup>1</sup>, A KHATUN<sup>1</sup>, H ATHAR<sup>1</sup>, M NAIKOO<sup>1</sup>, H M KHAN<sup>1</sup>,  
R A SHAH<sup>1</sup> and M SHABIR<sup>1</sup>

*Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir,  
Shuhama, Srinagar, Jammu and Kashmir 190 006 India*

Received: 28 October 2020; Accepted: 5 October 2021

### ABSTRACT

The study was designed to compare the effect of length of progesterone treatment (6 d vs 10 d vs 14 d) on synchronization efficiency and fertility response in multiparous ewes. The crossbred ewes (30) were equally allotted to three groups designated as short (6 days), medium (10 days) and long-term (14 days) treatment. All the ewes were injected PGF<sub>2α</sub> 250 µg, intramuscularly 24 h before sponge removal. After the sponge removal ewes were kept with breeding rams for tupping upto 72 h. Although the onset of estrus was more precise in short and long term groups, however overall estrus response rate was highest in short treatment group (90%) followed by long treatment group (70%) and medium treatment group (60%). The pregnancy rates were non-significantly higher for short and medium treatment groups than long treatment group. The progesterone values in pregnant ewes, decreased significantly from day pre-treatment to day 0 followed by significant increase from day 0 to day 10, 17 and 35. In conclusion, short term (6 d) intravaginal progesterone treatment may replace the traditional long term treatment (14 d) for induction of estrous during breeding season. However, the results should be validated in larger number of animals before recommending the protocol.

**Keywords:** Estrous synchronization, Ewes, Fertility, Length of progesterone treatment

In sheep, the estrus and ovulation synchronization technologies are mainly based on the control of corpora lutea life-span (Martemucci and D'Alessandro 2011). Progestagen analogues are widely used for this purpose (Emsen and Yaprak 2006). Most of the recent studies have focussed on the duration of the progestagen-based synchrony treatments (Marteniz-Ros *et al.* 2018, Silva *et al.* 2021). In these protocols, the intra-vaginal devices containing different types of progestagens are retained for 6 to 14 days. They may also be combined with PMSG or PMSG plus PGF<sub>2α</sub> (Zelege *et al.* 2005). Fixed timed AI (FTAI) following estrous synchronization with progestagens results in conception rates varying from 71.4% (Vilariño *et al.* 2013) to 84.6% (Knights *et al.* 2011).

The short term protocols (5–7 days) have been successful in inducing and synchronizing estrous in sheep during the breeding as well as non-breeding seasons (Vinoles *et al.* 2001, Marteniz-Ros *et al.* 2019, Bolard *et al.* 2020). Although progesterone priming for 2 days is sufficient to ensure normal luteal function, but for progression of the follicular growth, the retention of the sponges for 4–5 days

Present address: <sup>1</sup>Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Srinagar, Jammu and Kashmir. ✉Corresponding author e-mail: malikasloob@gmail.com

are needed (McLeod and Haresign 1984). Reducing the period of sponge placement may maintain higher P<sub>4</sub> levels on removal of pessaries and may also reduce the chance of vaginal contamination. Although long periods (12 to 14 days) protocols involving progestagen administration provide good synchronization rates in cyclic and acyclic ewes but variable fertility rates have been obtained (Koyuncu and Alticekic 2010). Although the long-term exposure to progestagens extends the phase of follicular dominance but leads to the ovulation of aged oocytes. The protocol is therefore less efficient than short-term progestagen treatment (Viñoles *et al.* 2001). The additional benefit of short-term progesterone protocols is its ability to synchronize females in a short period of time. However, with short-term progesterone/CIDR protocols, inconsistency in estrus response and increased interval to estrus had been reported (Abecia *et al.* 2011).

The inconsistencies in the results reported in available literature and difference of opinions put forth by researchers signify the need for development of a standard hormone-based protocol that could produce efficient and consistent outcome. The present study was therefore planned to assess the effect of the length of a progesterone treatment using intravaginal sponges on synchronization and fertility of crossbred ewes during breeding season.

## MATERIALS AND METHODS

*Location and management:* The present study was conducted at Mountain Research Centre for Sheep and Goats (MRCSG), Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, SKUAST-Kashmir located in Srinagar, Kashmir, India (34°08'N 74°28'E). The treatment was initiated with the onset of the breeding season; Autumn season (September–November) in this temperate zone. Thirty healthy multiparous cross bred ewes (NARI-Swarna ram × non-descript ewes) weighing 36.41±4.25 kg with body condition scores ranging from 2.5–3.5 were used. They were in third or fourth parity. All the ewes were maintained under uniform management conditions. Animals were left outdoor during the day and housed in closed pens at night. They grazed on green natural pastures from 9:00 AM to 3:00 PM upto November 15. Thereafter, 1.5 kg hay (oats + sorghum) and 300 g concentrate feed (Agrofeed Industries J&K Ltd) were provided daily to each ewe upto 31<sup>st</sup> January. Subsequently, the concentrate feed was increased 600 g/day/pregnant ewe. Free access to drinking water was ensured throughout.

*Experimental design:* Crossbred ewes (30) were randomly allotted equally (10) to three treatment groups, i.e. short, medium and long-term. All the ewes were subjected to similar estrus synchronization protocol but the length of time the intravaginal progesterone sponges were retained varied among the groups. Progesterone impregnated intravaginal sponges (AVIKESIL-S, CSWRI, Avikanagar, India) were introduced into the vagina on day 1 with using a speculum and introducer. The vaginal sponges were removed after 6, 10 and 14 days in ewes belonging to the different groups respectively. All the ewes received PGF<sub>2</sub>α (Cloprostenol Sodium, Pragma™, Intas, Ahmedabad, India) 250 µg intramuscularly 24 h prior to the sponge removal. Immediately after removal of sponges, all the ewes were kept with three proven breeding rams at a ewe ram ratio 10:1 upto 72 h for tupping. Wool colour was applied every evening on the brisket region of the rams in the evenings. The ewes were observed for breeding marks every morning and evening. They were assumed to be in estrus and tupped if more than 60% of the rump was coloured.

*Pregnancy diagnosis:* The pregnancy was confirmed on day 45 post-tupping using a real time B-mode ultrasonography machine (esoate MyLab™40 VET) equipped with a 3.5 MHz sector array transducer.

*Blood sampling:* The blood samples were collected on day of start of the treatment, the day of insemination/tupping (day 0), day 10, day 17 and day 35 post tupping. Samples (8.0 ml) were collected by jugular venipuncture into 15 ml centrifuge tubes without anticoagulant. The tubes were immediately stored in ice and placed in slanting position for 1–2 h. Serum was harvested by centrifugation at 1,500 × g at 4°C for 15 min. Serum was removed from tubes with micropipette and stored in stored 2 ml storage vials in duplicate at –20°C pending further analysis.

*Reproductive performance:* Reproductive performance was evaluated on the basis of following parameters.

*Time to onset of estrus (TOE)/ Interval to estrus:* It was recorded as the time elapsed after sponge removal to when the ewe was first spotted in estrus. It was counted as within 24 h, 48 h and 72 h.

*Estrus response rate (ERR)/Estrus rate:* No of ewes displaying estrus/ no of synchronized ewes. The total number of ewes per group that exhibited estrus within 72 h was calculated as ERR.

*Pregnancy rate:* Number of ewes found pregnant on day 45/total number of ewes tupped.

*Lambing rate:* Number of ewes lambed/ total number of ewes tupped.

*Prolificacy rate:* Total number of lambs born/ Total number of ewes lambed.

*Blood hormone analysis:* The serum samples were estimated for progesterone, nitric oxide and ascorbic acid concentrations. The serum concentrations of progesterone were measured using solid phase competitive enzyme immunoassay kits obtained from Calbiotech Inc (Cordell Ct., El Cajon, CA). Sensitivity of the assay was 0.22 ng/ml; intra-assay and inter-assay variation coefficients were 5.36% and 9.68%, respectively.

*Statistical analysis:* The data obtained in the study were analyzed using standard statistical procedures (Snedecor and Cochran 1994) using statistical software SPSS version 20. The data obtained in respect of time to onset of estrus, estrus response rates, pregnancy rates, lambing rates and prolificacy was analyzed by chi-square test. In this study, since the number of non-pregnant animals was only one therefore statistical analysis was done by Z-test calculator for a single sample by comparing the single sample mean with the population mean. However, variation in means at different days within pregnant and non-pregnant groups was analyzed by One way-ANOVA. The Post-hoc analysis was performed using Duncan's multiple range test. All the data are presented in the tables as mean±SEM. The level of significance was set as P<0.05.

## RESULTS AND DISCUSSION

Among the synchronized ewes, estrus was noted in 73.3% (22/30) over the 72 h observation period following the removal of intravaginal sponges. The onset of estrus was more precise in short and long treatment groups. In these groups, 50% ewes showed estrus upto 48 h after sponge removal. In medium treatment group, only 40% ewes were detected in estrus during this period (Table 1). No significant (P>0.05) difference was observed between the groups in percentage of ewes exhibiting estrus. However, highest estrus response rate was observed in short treatment group (90%) followed by long treatment group (70%) and medium treatment group (60%) (Table 1). The pregnancy rates were non-significantly (P>0.05) higher for short and medium treatment groups than long treatment group. The lambing rate (90%) was similar in short and long treatment group. The prolificacy rate differed non-

Table 1. Time to onset of estrus (TOE) and estrus response rate (ERR) in crossbred ewes subjected to estrous synchronization

Treatment group	Time to onset of estrus				Overall ERR (%)
	Treated ewes (N)	From sponge withdrawal up to 24 h	Between 24–48 h	Between 48–72 h	
Short	10	30.0 (3/10)	20 (2/10)	40.0 (4/10)	90
Medium	10	30.0 (3/10)	10 (1/10)	20.0 (2/10)	60
Long	10	20.0 (2/10)	30 (3/10)	20.0 (2/10)	70
		26.7 (8/30)	20 (6/30)	26.7 (8/30)	73.3% (22/30)

Table 2. Fertility parameters (Pregnancy, Lambing and Prolificacy rate) of crossbred ewes subjected to estrous synchronization

Treatment group	Fertility parameters			
	Treated ewes (N)	Pregnancy rate (%)	Lambing rate (%)	Prolificacy rate (%)
Short	10	100 <sup>a</sup>	90 <sup>a</sup>	144.44 <sup>a</sup>
Medium	10	100 <sup>a</sup>	100 <sup>a</sup>	140.00 <sup>a</sup>
Long	10	90 <sup>a</sup>	90 <sup>a</sup>	133.33 <sup>a</sup>

Means bearing different superscript within columns differ significantly (P<0.05).

significantly between the treatment groups (Table 2).

Progestagen analogues are widely used in small ruminants to induce and synchronize estrous (Emsen and Yaprak 2006). Recent studies (Marteniz-Ros *et al.* 2018, Silva *et al.* 2021) have focused on the duration of the progestagen-based synchrony treatments. In the current study, estrus was expressed following intravaginal progesterone sponge removal in 73.3% ewes, indicating its usefulness in inducing ovarian activity and estrus expression during breeding season. The average estrus response rate in the ewes included in this study is not in concurrence with the rates reported by other workers (Olivera-Muzante *et al.* 2011). However, our results are in agreement with the findings of Husein *et al.* (2007). In that study, estrus response rate of 75% was reported in ewes without eCG, but the rate enhanced to 91.7% when eCG was also incorporated in the synchronization protocols. This difference in the results may therefore be due to inclusion or exclusion of eCG in various protocols. eCG has potent FSH activity which stimulates follicular growth on the ovaries and subsequently leads to higher levels of estrogen and the occurrence of estrus within a short period of time (McNatty *et al.* 1984). The estrus response rate did not differ significantly amongst the different treatment groups included in our study.

The absence of estrus in the remaining ewes may be attributed to inadequate estradiol secretion, reflected by silent estrus. Based on the progesterone profile, ovulation occurred in all the ewes including those showing no typical estrus signs. Our results of similar estrus response rate among different treatment groups are concomitant with those of Blaschi *et al.* (2014). No significant difference in

estrus response rate was also observed when ewes were primed with intravaginal sponge treatment for 6 or 14 days (Ungerfeld and Rubianes 2002, Ustuner *et al.* 2007).

The pregnancy rates were non-significantly (P>0.05) different between treatment groups although it was higher for short (100%) and medium (100%) treatment groups than long (90%) treatment group. The lambing rate was similar between short (90%) and long (90%) treatment groups due to the pregnancy loss in one ewe in short treatment group. Similar findings of non-significant difference in pregnancy rate between such groups had been reported by Ustuner *et al.* (2007) and Ungerfeld and Rubianes (2002). The effectiveness of a 7-day-FGA-eCG treatment in synchronizing estrus and producing high pregnancy and lambing rates had been reported (Marteniz-Ros *et al.* 2019). Husein *et al.* (2007) also documented that 4-day-FGA and eCG regimen could adequately replace 12-day-FGA protocol. Other studies (Vinoles *et al.* 2001, Sareminejad *et al.* 2014) also obtained higher pregnancy rate after short term (6 day) treatment compared to the traditional 12–14 day treatment. Contrary to it, Blaschi *et al.* (2014) reported higher conception rate with 14 day norgestomet treatment compared to short and medium treatment groups.

Short periods of progestagen sponge treatment for as little as 5 to 7 days have been reported to be successful in inducing/synchronizing estrus in sheep both during and outside the breeding season (Vinoles *et al.* 2001, Marteniz-Ros *et al.* 2019). Mcleod and Haresing (1984) demonstrated that progesterone priming for 2 days is sufficient to ensure luteal function in sheep, but final stages of follicular growth require 4–5 days. Reducing the sponge period may result in higher progesterone level upon removal of the pessaries and may reduce the chances of vaginal contamination. Short-term progesterone treatment can be considered as a good alternative to traditional longer duration procedures, due to its flexibility under field conditions. This protocol enhances fertility due to higher progesterone level induced by short term sponge insertion. In fact, it has been shown that low levels of progesterone reduces subsequent fertility. The explanations put forth for this reduction of fertility are impaired sperm transport and extension of the life span of the ovulatory follicle (Ozyurtlu *et al.* 2011). The prolonged progesterone treatment has an adverse effect on oocyte development. Long-term progesterone treatment results in sub-luteal progesterone level and this leads to an increase in LH pulse frequency, without any LH surge leading to

Table 3. Serum progesterone concentration (Mean±SEM) in pregnant crossbred ewes

Stage/Day	Progesterone concentration (ng/ml)
Pre-treatment	17.17±2.13 <sup>b</sup>
Day 0	0.47±0.05 <sup>a</sup>
Day 10	20.40±2.46 <sup>b</sup>
Day 17	22.51±2.70 <sup>b</sup>
Day 35	23.23±1.81 <sup>b</sup>

Means bearing different superscript (a, b) within columns differ significantly (P<0.05).

the persistence of the largest follicle (Ozyurtlu *et al.* 2011). The use of intravaginal sponges for 12–14 days was also related to the presence of purulent and fetid vaginal discharges at their removal. Short progestagen protocols are associated with lower risk of vaginitis arising because of prolonged presence of the sponge within the vagina (Manes *et al.* 2015). Predisposition to proliferation and changes in the composition of the local microbiota may result from retention of vaginal secretions (Vasconceles *et al.* 2016). These changes in the vaginal microbiota induce inflammation and infection associated with abnormal discharges leading to lower pregnancy rates (Martins *et al.* 2009).

Most of the ewes in the experiment were cyclic as indicated by the higher progesterone levels on pre-treatment day in both pregnant (17.17±2.13 ng/ml) and the non-pregnant (12.19 ng/ml) ewes (Table 3). There was no significant difference in serum progesterone concentration between the non-pregnant and the total ewes (pregnant and non-pregnant) at different days of sampling. However, within pregnant ewes, the progesterone values decreased significantly (P<0.05) from pre-treatment to day 0 (48 h after sponge removal) followed by significant (P<0.05) increase from day 0 (0.47±0.05 ng/ml) to day 10 (20.4±2.46 ng/ml). The values then increased non-significantly (P>0.05) on day 17 (22.51±2.7 ng/ml) and day 35 (23.23±1.81 ng/ml).

The mean serum progesterone concentration in pregnant ewes was significantly (P<0.05) higher on day 10, 17 and 35 than day 0. With advancing pregnancy, the values showed an upward trend. Our results are in agreement with those of Alwan *et al.* (2010) and Anghel *et al.* (2011). Values close to our findings of 14.4±1.42 ng/ml had been detected on day 25 post-tupping in pregnant sheep (Anghel *et al.* 2011). However, contrary to our results, low (4.5±0.41 ng/ml) progesterone level have been documented on day 18 in pregnant ewes (Weigl *et al.* 1975). Real hormone level deviations might exist because of differences in the breed used, to sampling and to environmental factors like climate or season (Boscos *et al.* 2003) as well as age of animal, stress and the analytical method used (Mitchell *et al.* 1999). In this study, serum progesterone concentration in both pregnant and non-pregnant sheep decreased significantly from day pre-treatment to day 0. This drop in progesterone concentration is due to the onset of estrus in these animals.

Anghel *et al.* (2011) reported 1.56±0.15 ng/ml on the day of estrus. However, in our study, the serum progesterone values were <1 ng/ml, indicative of complete luteolysis following the use of PGF<sub>2α</sub>. In pregnant ewes, the serum progesterone level increased up to day 35. Progesterone concentration in all the ewes reached high level on day 6 after ovulation. The level dropped in non-pregnant ewes on day 16 but was sustained in pregnant ewes (Strmsnik and Kosec 2002).

In conclusion, this study has shown that short term 6-day intravaginal progesterone therapy is as effective as its long term 14-day protocol for induction of estrous during breeding season. The protocol with several advantages needs to be evaluated in larger number of ewes before its adoption in sheep reproductive management systems.

#### ACKNOWLEDGEMENTS

The authors would like to thank Incharge, Mountain Research Centre for Sheep and Goats (MRCSG) and Division of Veterinary Clinical Complex, Faculty of Veterinary Science and Animal Husbandry, Shuhama, Srinagar for sparing the animals and allowing to use USG equipment respectively, for this study.

#### REFERENCES

- Abecia J A, Forcada F and González-Bulnes A. 2011. Pharmaceutical control of reproduction in sheep and goats. *Veterinary Clinics of North America: Food Animal Practice* **27**: 67–79.
- Alwan A F, Amin F A M and Ibrahim N S. 2010. Blood progesterone and estrogen hormones level during pregnancy and after birth in Iraqi sheep and goat. *Basrah Journal of Veterinary Research* **10**(2): 153–57.
- Anghel A, Zamfirescu S, Coprean D, Elena S and Dobrin N. 2011. Assessment of progesterone and pregnancy associated glycoprotein concentrations for early pregnancy diagnosis in ewe. *Annals of the Romanian Society of Cell Biology* **XVI** (2): 133–36.
- Blaschi W, Lunardelli P A, Marinho L S R, Max M C, Santos G M G, Silva-Santos K C, Melo-Sterza F A, Baldassarre H, Rigo T R and Seneda M M. 2014. Effects of progestagen exposure duration on estrus synchronization and conception rates of crossbreed ewes undergoing fixed time artificial insemination. *Journal of Veterinary Science* **15**(3): 433–37.
- Boscos C M, Samartzi F C, Lymberopoulos A G, Stefanakis A and Belibasaki S. 2003. Assessment of progesterone concentration using enzyme immunoassay for early pregnancy diagnosis in sheep and goats. *Reproduction in Domestic Animals* **38**(3): 170–74.
- 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 Ruminant Research* **66**: 230–35.
- Fitzgerald J A, Ruggles A, Stellflug J N and Hansel W. 1985. Seven-day synchronization method for ewes using Medroxyprogesterone acetate (MAP) and prostaglandins F<sub>2α</sub>. *Journal of Animal Science* **61**(2): 466–69.
- Husein M Q, Ababneh M M and Abu-Ruman D S. 2007. The effects of short or long term FGA treatment with or without eCG on reproductive performance of ewes bred out-of-season.

- American Journal of Animal and Veterinary Sciences* **2**(1): 23–28.
- Knights M, Ramgattie R, Siew N, Singh-Knights D and Bourne G. 2011. Effectiveness of a short-term treatment with progesterone injections on synchrony of lambing and fertility in tropical hair sheep. *Animal Reproduction Science* **126**: 70–75.
- Koyuncu M and Altýcekic S O. 2010. Effects of progestagen and PMSG on estrous synchronization and fertility in Kivircik ewes during natural breeding season. *Asian Australasian Journal of Animal Science* **23**(3): 308–11.
- Lombardo H N S, Monteiro C A S, Delhado K F, Pinna A E, Vasconcelos C O P, Nogueira L A G, Brandão F Z and Balaro M F A. 2020. Hormonal protocols for the synchronization and induction of synchronized estrus in dairy ewes kept under tropical conditions. *Acta Scientiae Veterinariae* **48**: 1751.
- Manes J, Campero C, Hozbor F, Alberio R and Ungerfeld R. 2015. Vaginal histological changes after using intravaginal sponges for oestrous synchronization in anoestrous ewes. *Reproduction in Domestic Animals* **50**: 270–74.
- Martemucci G D and Alessandro A G. 2011. Synchronization of estrous and ovulation by short time combined FGA, PGF<sub>2α</sub>, GnRH, eCG treatments for natural service or AI fixed time. *Animal Reproduction Science* **123**: 32–39.
- Martinez-Ros P, Lozano M, Hernandez F, Tirado Alejandra, Alejandro Rios-Abellan A, López-Mendoza M C and Gonzalez-Bulnes A. 2018. Intravaginal device-type and treatment-length for ovine estrus synchronization modify vaginal mucus and microbiota and affect fertility. *Animals* **8**: 226.
- Martinez-Rosa P, Gonzalez-Bulnes A, Garcia-Rosellod E, Rios-Abellana A and Astiz S. 2019. Effects of short-term intravaginal progestagen treatment on fertility and prolificacy after natural breeding in sheep at different reproductive seasons. *Journal of Applied Animal Research* **47**(1): 201–05.
- Martins G, Figueira B, Penna B, Brandão F, Vargas R, Vasconcelos C and Lilenbaum W. 2009. Prevalence and antimicrobial susceptibility of vaginal bacteria from ewes treated with progestin-impregnated intravaginal sponges. *Small Ruminant Research* **8**: 182–84.
- McLeod B J and Haresign W. 1984. Evidence that progesterone may influence subsequent luteal function in the ewe by modulating preovulatory follicle development. *Journal of Reproduction and Fertility* **71**: 381–86.
- McNatty K P, Gibb M, Dobson C, Ball K, Coster J, Heath D A and Thumley D C. 1984. Preovulatory follicle development in sheep treated with PMSG/and or Prostaglandin. *Journal of Reproduction and Fertility* **65**: 111–23.
- Mitchell L, King M, Aitken R P, Gebbie F E and Wallace J M. 1999. Ovulation, fertilization and lambing rates, and peripheral progesterone concentrations, in ewes inseminated at a natural oestrus during November or February. *Journal of Reproduction and Fertility* **115**: 133.
- Olivera-Muzante J, Fierro S, López V and Gil J. 2011. Comparison of prostaglandin and progesterone-based protocols for timed artificial insemination in sheep. *Theriogenology* **75**: 1232–38.
- Ozyurtlu N, Serhan A Y, Kuçukaslan I, Güngör O and Aslan S. 2011. Effect of subsequent two short-term, short-term, and long-term progestagen treatments on fertility of Awassi ewes out of the breeding season. *Ankara Üniversitesi Veteriner Fakültesi Dergisi* **58**: 105–09.
- Sareminejad P, Tabatabaei S, Mamouei M, Mirzadeh K and Boujarpour M. 2014. The effects of short and long term medroxy progesterone acetate (MAP) sponge treatments on reproductive performance during the non breeding season of Arabian ewes. *Iranian Journal of Applied Animal Sciences* **4**(4): 747–51.
- Silva R O, Oliveira R P M, Silva A F, Oliveira F F, Rufino J P F and Silva M L M. 2021. Effect of different protocols for estrus synchronization on reproductive performance of Santa Inês ewes under Amazon environmental conditions. *Acta Scientiarum Animal Sciences* **43**: 1–7.
- Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 8<sup>th</sup> Edition. Iowa State University Press, Ames, Iowa.
- Strmsnik M P and Kosec N C K M. 2002. Examination of estrus cycle and early pregnancy in sheep using trans-rectal ultrasonography. *Slovenian Veterinary Research* **39**: 47–58.
- Ungerfeld R and Rubianes E. 2002. Short term primings with different progestagen intravaginal devices (MAP, FGA and CIDR) for eCG-estrous induction in anoestrus ewes. *Small Ruminant Research* **46**: 63–66.
- Ustuner B, Gunay U, Nur Z and 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 Veterinaria Brno* **76**: 391–97.
- Vasconcelos C, Brandão F Z, Martins G, Penna B, Souza J M G and Lilenbaum W. 2016. Qualitative and quantitative analysis of bacteria from vaginitis associated with intravaginal implants in ewes following estrus synchronization. *Ciência Rural* **46**: 632–36.
- Vilariño M, Rubianes E and Menchaca A. 2013. Ovarian responses and pregnancy rate with previously used intravaginal progesterone releasing devices for fixed-time artificial insemination in sheep. *Theriogenology* **79**: 206–10.
- Vinoles C, Forsberg M, Banchero G and Rubianes E. 2001. Effect of long-term and short-term progestagen treatment on follicular development and pregnancy rate in cyclic ewes. *Theriogenology* **55**: 993–1004.
- Weigl R M, Tilton J E, Hauge C N, Light M R and Buchanan M L. 1975. Pregnancy diagnosis in the ewe II. Plasma progesterone levels. *Farm Research* **33**(2): 11–13.
- Zelege M, Greyling J P C, Schwalbach L M J, Muller T and Erasmus J A. 2005. Effect of progestagen and PMSG on oestrous synchronization and fertility in Dorper ewes during the transition period. *Small Ruminant Research* **56**: 47–53.