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EFFECT OF THE SHORT-TERM PROGESTAGEN TREATMENTS PLUS PMSG PRIOR RAM INTRODUCTION ON THE ESTRUS SYNCHRONIZATION AND THE FERTILITY OF ILE DE FRANCE EWES

N. Metodiev, E. Raicheva

Institute of Animal Science, Sheep Breeding Department,BG-2232 Kostinbrod, Bulgaria
Corresponding author – e-mail: n_metodiev@abv.bg
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Abstract: The aim of the present study was to evaluate the effect of the short-term progestagen treatments plus PMSG prior ram introduction on the estrus synchronization and the fertility of Ile de France ewes in the beginning of mating season. The study was carried out with 36 pure-bred ewes (aged 4-6 years) during April 2009. The ewes were divided in 3 groups (n=12 for each group): group I – vaginal sponge impregnated with 30mg FGA for six days, as at the time of placement of sponge 125 μg cloprostenolum was put i.m At the time of removal of the sponge 250 UI PMSG was put i.m.; group II– the same as the I group, but without cloprostenolum treatment: III group – control. The teasers were introduced to ewes 24h after the sponge removal and ewes in estrus was inseminated artificially. The following parameters were studied: effect of estrus synchronization (EES) ewes in estrus was recorded twice daily for the first 6 days after sponge removal; onset, end and duration of estrus for group I and II, fertility (at first estrus) and fecundity (calculated after lambing). The data for fertility and fecundity for the control group was obtained as ewes were fertilized during 30 days from the begging of the breeding. A significant effect of both schemes to estrus synchronization was determined F=33,33***, P<0,001). The EES, fertility and fecundity for group I, II and III were – 91,66%, 63,64% and 142,0%; 91,66%, 45,45% and 140,0 %; 8,33%, 91,66% and 140,0% respectively. The mean onset, end and duration of estrus was 51,27 h, 77,45 h and 27,27h for the I group and 57,82h, 87,27h and 29,45 h respectively for the II group. We conclude that the scheme applied for the first group is better to use for estrus synchronization in the beginning of mating season.

Key words: ewes, estrus, progestagens, short treatment

Introduction

The synchronization of fertilities and births of the ewes are main elements of the reproductive management in sheep breeding. Synchronization of estrus allows control and shortening of lambing and kidding, with synchronization of weaning and uniform batching of animals to slaughter; it also allows more efficient use of labor and animal facilities *(Abecia et al., 2011)*. The methods of estrus synchronization (ES) can be classified as natural (non-hormonal) and pharmacological (hormonal) *(Tyankov et al., 2000; Wildeus, 2000; Dankó, 2003)*. The introduction of ram or rams (the ram effect) to isolated anoestrus ewes leads to the next reproductive reactions: increase of pulsatile secretion of LH, which may end with LH surge followed by ovulation *(Knight et al., 1978; Oldham and Cognié 1980; Martin et al., 1983; Ungerfeld et al., 2004)*. The ram effect can be achieved without prior isolation of ewes from rams *(Cusha et al., 1992)*. The ram effect is applicable in breeding season too, as the introduction of rams to cyclic ewes stimulates an increase in pulsatile LH secretion, independent of ewe genotype or stage of the estrous cycle *(Hawken et al., 2007)*.

The ram effect could be used in combination with traditional progestagens treatments to shorten the interval to estrus and to improve estrus synchronization during breeding season *(Ungerfeld and Rubianes, 1999a)*. The traditional progestagen treatments for ES in small ruminants are with intravaginal sponges impregnated with progestagen (flurogestone acetate FGA or medroxyprogesterone acetate MAP) inserted over periods of 9 to 19 days and used in conjunction with PMSG, particularly for out-of-season breeding, injected at the time of sponge removal or 48 hours prior to sponge removal *(Wildeus, 2000)*. Long-term progestagen treatments effectively synchronized estrus, but with variable fertility *(Menchaca and Rubianes, 2004)*. For the last 15 years an alternative methods for ES in small ruminants , named short-term progestagen treatment (consisting of 5-7 days progestogen priming) were developed *(Menchaca and Rubianes, 2004)* Short-term MAP priming before introducing rams may be used with results similar to those achieved by traditional long-term priming (*Ungerfeld et al., 2003)*.

The aim of the present study was to evaluate the effect of the short-term progestagen treatments plus PMSG prior ram introduction on the estrus synchronization and the fertility of Ile de France ewes in the beginning of mating season.

Materials and Methods

In our previous work *(Metodiev et al., 2010)* we concluded that Ile de France ewes from the flock of IAS-Kostinbrod normally cycled during the investigated season of the year (spring, April-May). So we designed the experiment

to start in the beginning of mating season. We used "mating season", not estrus season, because we didn't know the real bounds of anestrus and estrus for concrete flock and breed as whole for the conditions of Bulgaria.

The study was carried out with 36 pure-bred ewes (aged 4-6 years) during April 2009, raised in the experimental farm of Institute of Animal Science – Kostinbrod, Bulgaria. During the experimental period, natural light/day ratios were 13/11. The ewes were clinically health, body condition score – 3,0-3,5, average weight 77,8 kg (between 71-95 kg) kg and all ewes had normal lambings for the last lambing season. The ewes were sheared after the end of experiment and they were transported to hill pasture in area Zlatusha and were returned before lambing. The ewes were divided in 3 groups (n=12 for each group): group I (PGPgPMSG – vaginal sponge impregnated with 30mg FGA (Synchropart®, CEVA SANTE ANIMALE, France) for six days, as at the time of placement of sponge 125 μg cloprostenolum (Oestrofan ®, BIOVETA, Czeck Republic) was put i.m. At time of removal of sponge 250 UI PMSG (Synchropart® PMSG, CEVA SANTE ANIMALE, was put i.m.; group II (PgPMSG) – the same as the group I, but without cloprostenolum treatment: group III – control. During the remaining of sponge all ewes were kept in pen and fed hay and silage ad libitum plus 200-300g of concentrated mix. Before and after the sponge period, they grazed on pasture for 2-3 hours. Up to this moment the rams were raised separately from the ewes, in a pen near ewe's pen. The teasers were introduced to ewes 24h after sponge removal and ewes in estrus were artificially inseminated with fresh, non-diluted semen, with dose 0,2 ml. Only ejaculates with the next parameters were used: volume ≥ 0,5 ml and motility 70%. The insemination started at the time of detected estrus and continued to the end of estrus, at 12-hours interval.

The following parameters were studied: effect of estrus synchronization (EES) - ewes in estrus were recorded twice daily for the first 6 days after sponge removal; onset, end and duration of estrus for groups I and II; fertility (at first estrus) and fecundity (calculated after lambing). The data for fertility and fecundity for control group was obtained as ewes were fertilized during 30 days from the begging of the breeding. Also, the distribution of manifesting the first estrus for the control group was determined. Fertility and fecundity were calculated after lambing. Fertility is defined as the ratio of the number of ewes pregnant to the number of ewes, exposed to artificial insemination at first estrus. Fecundity is defined as the number of born lambs from pregnant ewes (included all born lambs – live and dead). One ewe from control group aborted, so we included the data about it only for fertility, but not for fecundity.

The data was calculated by the methods of variation statistic, using the computer program EXCEL, Microsoft Office, 2003. The significance of EES was estimated by F-creation of Fisher and the significance of mean differences between groups was estimated by Student's t- test. The data for

onset end and duration of estrus for groups I and II were presented with mean and SE. The fertility and fecundity were presented in percentage

Results

Estrus synchronization was influenced significantly by the applied schemes (F= 33,33, P < 0,001) (Table 1.) At both treated groups 11 from 12 ewes or 91,66 % manifested estrus till 6-th day after the beginning of mating season. The differences between group I and control, and between group II and control were highly significant (Figure 1). The mean onset of estrus for the group I was 51,27 h and 57,82 for the group II. For the most of the ewes from both treated groups the estrus started from 36h but in both groups there were ewes with delayed estrus (136h for group I and 120 h for group II, Figure 2). The mean duration of estrus was similar to both groups - 27,27 h and 29,45 h respectively for group I and II. The mean end of estrus was 77,45 h for group I and 87,27 h for group II.

The distribution of first manifested estrus of the control group is presented in Figure 3. A surge of ewes with manifested estrus was emerged between 24 and 30 after the beginning of mating was (7 from 12 ewes or 58,33% from all ewes)

The fertility of experimental groups (Table 3) varied in wide ranges –it was lowest for group II– 45,45%, and the highest for control group – 91,66%. The difference between group II and control was significant (P< 0,05), while between group I and control and group I and group II - not significant. The fecundity was similar for all groups.

Table 1. Value of F-criteria for the effect of estrus synchronization (EES)

Source of Variation	df	F
Between Groups	2	33.33
Within Groups	33	
Total	35	

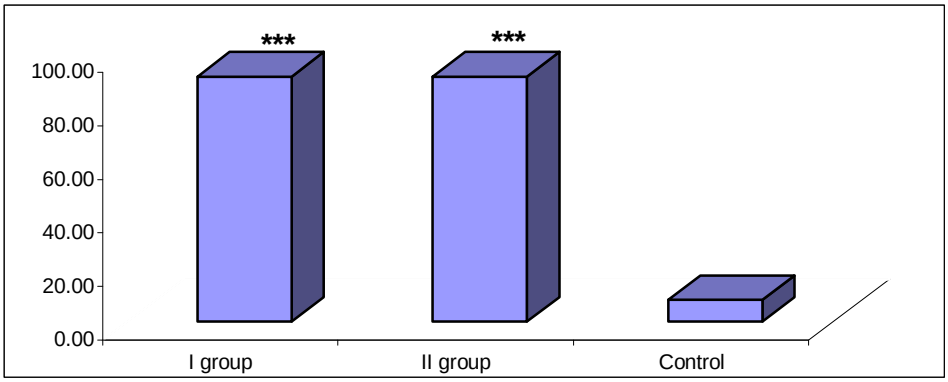

Figure 1. Percentage of ewes with manifested estrus for the first 6 days from the beginning of mating season
(Note: Significant differences *** at P<0,001 between group I and control, and between group II and control)

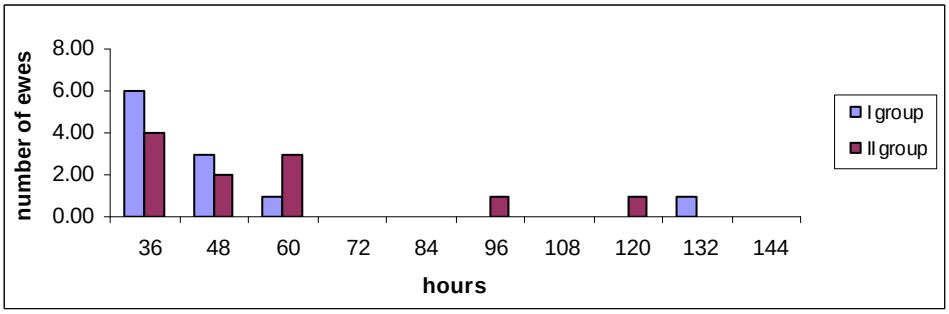

Figure 2. Onset of estrus of I and II groups

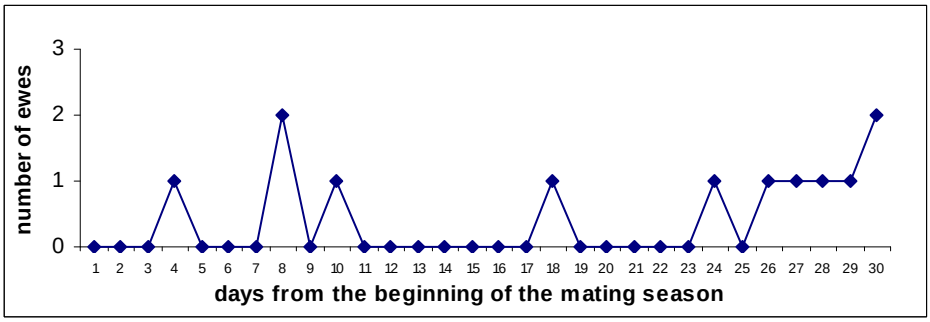

Figure 3. Distribution of manifesting of the first estrus for the control group

Table 2. Values for onset, end and duration of estrus for I and II groups

Groups	Onset			End			Duration		
	Mean ± SE	Min	Max	Mean ± SE	Min	Max	Mean ± SE	Min	Max
I	51,27±8,42	36	132	77,45±8,46	60	158	27,27±2,34	24	48
II	57,82±8,22	36	120	87,27±7,94	60	144	29,45±4,67	12	72

Table 3. Fertility and fecundity of the three experimental groups

Group	Fertility, %	Fecundity,%
I	63.64	142
II	45.45*	140
III	91.66	140

Note: Significant differences * at P< 0,05 between group II and control group

Discussion

The obtained results from our experiment showed excellent response to applied schemes for estrus synchronization. The value of F-criteria (F= 33,33, Table 1) and highly significance (P<0,001) between experimental groups and control group for induced estrus for first 6 days after ram introduction (Figure 1) confirm this statement. These results are similar to those obtained *Ungerfeld and Rubianes (1999a,b)*, *Viñoles et al. (2001), Ustuner et al. (2007), Karaca et al. (2009); Martemucci and D'Alessandro (2011)* about short-term progestagen treatments at the same reproductive season (late anestrous or breeding season). *Ustuner et al. (2007)* used the same products as we and studied different schemes. Ustuner et al., 2007 used the same products as we and studied different schemes. They obtained from scheme: sponge for 6 days + PMSG in dose 300 UI at sponge removal, estrus synchronization 83,3%. *Karaca et al. (2009)*, when used FGA sponge for 7 days, as 1 day before sponge removal injected PGF2α and PMSG in dose 400 UI obtained estrus response 88,8%. These two studies were carried out in Turkey with Awassi *(Ustuner et al., 2007)* and Tahivora *(Karaca et al., 2009)* in the beginning of breeding season. Turkey is a neighbor country to Bulgaria, so we obtained similar results to theirs. *Martemucci and D'Alessandro (2011)*, studied at cross-breed pluriparous Altamurana ewes different schemes with short-term FGA treatments. At scheme 100 μg ICI, Day 0) + FGA (40 mg, 5 days) + eCG (200 IU i.m. s.r., Day 5) they obtained estrus response 92,3%, but in schemes :FGA (40 mg, 5 days) + $PGF_{2\alpha}$ (100 μg i.m. s.r. (Day 5) + eCG (200 IU i.m. s.r., Day 5) and $PGF_{2\alpha}$ (100 μg ICI, Day 0) + FGA (40 mg, 5 days) + GnRH 100 μg given i.m. 30 h after s.r. results are 86,7% and 66,7% respectively.

The onset of estrus (Figure 2, Table 2) after sponge withdrawal was 51,27 h for group I and 57,82 h for group II. The onset is similar to both group, but as it

is seen on Figure 2 , the group I has earlier onset. This may be due to induced luteolysis with cloprostenolum at the time of sponge placement and so all the females will maintain similar and adequate serum levels of exogenous progestogen during the treatment. This technique was described by Beck et al., 1993. Our results for onset of estrus are similar to this reported from *Ungerfeld and Rubianes (1999a).*They report middle onset of estrus 58,4h. *Ungerfeld and Rubianes (1999b), Martemucci and D'Alessandro (2011)* reported earlier onset of esturs – between 36,0h and 43,4h for their experimental groups. Other authors report more hours to reach estrus after sponge withdrawal - *Viñoles et al. (2001) - 84*,8 h and *Ustuner et al. (2007)* – 70,8 h. So at the same or similar treatments, different results were observed. These results show that the onset of estrus depends on various factors, mainly season (estral or anestral), ovary status, individual reaction of every ewe to treatment, breed, body condition Regarding duration of estrus and end of estrus, our results were shorter to those reported from *Ustuner et al. (2007).*

The distribution of manifesting the first estrus for the control group (Figure 3) showed that 33,3 % (4/12) had first heat for the first 10 days of starting the mating campaign. We suggest that these ewes already started to cycle, because they didn't react according to literature established reaction of ewe to the ram effect Ewes generally ovulate in response to ram introduction within 54 h *(Oldham et al., 1978)*, but the first ovulation following introduction of rams usually is not accompanied by behavioral estrus *(Oldham and Cognié, 1980; Nugent et al., 1988)*. In some ewes there is an initial short luteal phase of 4-5 days, then a second ovulation without any signs of estrus followed by a luteal phase of normal duration *(Ungerfeld et al., 2004)*. According to *Martin et al. (1986)*, there are differences of ovarian response at ewes which could lead to two surges of manifesting synchronized estrus induced by the ram effect- first is between 17-20 days and the second is between 21-25 days after ram introduction. The other ewes reacted in the mentioned order. We suggested that most of them (7/12) had second silent ovulation and elongated cycle, as the frame of ewe cycle is 14-19 days, but 10 % ewes could have longer or shorter cycle *(Todorov, 2008)*.

The fertility for groups I and II was lower to the control group, as the differences between group II and control is significant The high fertility for the control group (91, 66%) showed that the shearing and transport haven't got stress effect to fertility. Our results confirm that the use of progestagen devices, such as intravaginal sponges lead to lower conception rates than nonhormonal natural services, due to alternations in patterns of LH release, in quality of ovulations and/or in sperm transport and survival in the female reproductive tract *(Abecia et al., 2011)*

Despite of these, some authors *(Ungerfeld, 1999b; Viñoles et al., 2001; Ustuner et al., 2007; Karaca et al., 2009; Martemucci and D'Alessandro (2011)* reported higher fertility (over 80%) after short –term treatments, but all of these were after natural mating, not after artificial inseminations. *Martemucci and*

D'Alessandro (2011) in their study made second experiment with short –term progestagen treatments and fix intrauterine AI with frozen semen on 52 or 60 h after sponge removal and obtained fertility between 40% to 60% for the different groups.

The prolificacy was the same to the three experimental groups. The applied schemes for estrus synchronization didn't affect litter size.

Conclusion

In conclusion the scheme: cloprostenolum injected at sponge insertion +sponge with FGA for six days + 250 UI PMSG at sponge removal is more suitable for estrus synchronization. This scheme could be applied when artificial insemination is required. This is mainly to intensive breeding or to realize a breeding strategy. At extensive breeding natural stimuli are better to use.

Uticaj kratkotrajnih tretmana progestagenom + pmsg pre uvođenja ovna u reprodukciju na sinhronizaciju estrusa i plodnost ovaca il de frans rase na početku sezone pripusta

N. Metodiev, E. Raicheva

Rezime

Cilj ovog istraživanja bio je da se oceni efekat kratkotrajnog progesteron tretmana uz korišćenje PMSG-a pre uvođenja ovnova na sinhronizaciju estrusa i fertilitet Ile de France ovaca na početku sezone parenja. Istraživanje je sprovedeno sa 36 čistokrvnih ovaca (starosti 4-6 godina) tokom aprila 2009. Ovce su bile podeljene u tri grupe (n=12 u svakoj): I grupa– vaginalni sunđer impregniran sa 30 mg FGA tokom 6 dana, u vreme postavljanja sunđera dato je 125 µg cloprostenolum i.m. U vreme odstranjivanja sunđera dato je 250 UI PMSG i.m.; II grupa – ista kao i I grupa, ali bez cloprostenolum tretmana: III grupa – kontrolna. Ovnovi-razdraživači su uvedeni kod ovaca 24h po uklanjanju sunđera i ovce u estrusu su veštački osemenjene. Ispitivani su sledeći parametri: Efekat sinhronizacije estrusa (EES) - ovce u estrusu praćene su dva puta dnevno prvih šest dana nakon uklanjanja sunđera; početak, kraj i trajanje estrusa u I i II grupi; plodnost (u prvom estrusu) i plodnost (izračunat nakon jagnjenja). Podaci o fertilitetu i fekunditetu u kontrolnoj grupi dobijeni su pošto su ovce bile oplođene tokom 30 dana od početka gajenja. Podaci su obrađeni kompjuterskim programom EXCEL (2003) varijaciono statističkom metodom i analizom varijanse ANOVA. Utvrđen je signifikantan uticaj obe šeme na sinhronizaciju estrusa (F=33,33***,

P<0,001). EES, fertilititet i fekunditet u I, II i III grupi bili su – 91,66%, 63,64% i 142,0%; 91,66%, 45,45% i 140,0 %; 8,33%, 91,66% i 140,0% respektivno. Prosečno vreme početka, kraja i trajanja estrusa bilo je 51,27 h, 77,45 h and 27,27h u I grupi i 57,82h, 87,27h i 29,45 h u II grupi respektivno. Zaključujemo da je šema primenjena u i grupi bolja za upotrebu u sinhronizaciji estrusa na početku sezone parenja.

References

ABECIA J.A., FORCADA F., GONZÁLES-BULNES A. (2011): Pharmaceutical control of reproduction in sheep and goats. Vet. Clin. Nort. Am.Food Anim. Pract., 27, 1, 67-79.

BECK N.F.G., DAVIES B., WILLIAMS S.P. (1993): Oestrus synchronization in ewes: the effect of combining a prostaglandin analogue with 5-day progestagen treatment. Anim. Prod., 56, 207-210.

CUSHA W.T., BRADFORD G.E., STABENFELDT G.H., BERGER Y.M., DALLY M. R. **(**1992): Ram influence on ovarian and sexual activity in anestrus ewes: effects of isolation of ewes from rams before joining and date of ram introduction. J. Anim. Sci., 70, 1195-1200.

DANKÓ G.N. (2003): Some practical and biotechnological methods for improving reproduction traits in sheep, www.date.hu/acta-agraria/2003-11/novotnine.pdf

HAWKEN P. R., BEARD A P., ESMAILI T., KADAKOWA H., EVANS A.C.O., BLANCHE D., MARTIN G.B. (2007): The introduction of rams induces an increase in pulsatile LH secretion in cyclic ewes during breeding season. Theriogenology, 68, 56-66.

KARACA F., ATAMAN M.B., ÇOYAN K. (2009): Synchronization of estrus with short- and long-term progestagen treatments and use of GnRH prior to short –term progestagen treatment in ewes. Small Rum. Res., 81, 185-188

KNIGHT T.W., PETERSON A. J., PAYNE E. **(**1978): The ovarian and hormonal response of the ewe to stimulation by ram early in the breeding season. Theriogenology, 10, 343.

MARTEMUCCI G., D'ALESSANDRO A.G. (2011): Synchronization of oestrus and ovulation by short time combined FGA, $PGF_{2\alpha}$, GnRH, eCG treatments for natural service or AI fixed-time. Anim. Reprod. Sci., 123, 32-39.

MARTIN G.B., SCARAMUZZI R.J., LINDSAY D.R. (1983): Effect of the introduction of rams during the anestrous season on the pulsatile secretion of LH in ovariectomized ewes. J. .Reprod. Fertil., 67, 47-55.

MARTIN G.B., OLDHAM C.M., COGNIÉ Y., PEARCE D.T. (1986): The physiological responses of anovulatory ewes to the introduction of rams-review, Livestock Prod. Sci., 15, 219-247.

MENCHACA A., RUBIANES E. (2004): New treatments associated with timed artificial insemination. Reprod. Fertil. Dev., 16, 403-413.
METODIEV N., TODOROV N., RAICHEVA E. (2010): Sexual activity and use of non-hormonal methods for synchronization of fertility and increasing litter size of ewes from Ile de France breed. J. Anim. Sci., XLVII, 15-23 (In Bulgarian).
NUGENT R.A., NOTTER D.R., BEAL W.E. (1988): Effects of ewe breed and ram exposure on estrous behavior in May and June. J. Anim. Sci., 66, 1363.
OLDHAM C.M., MARTIN G.B., KNIGHT T.W. (1978): Stimulation of seasonally anovulator Merino ewes by rams. I. Time from introduction of the rams to the preovulatory LH surge and ovulation. Anim. Reprod. Sci., 1, 283.
OLDHAM C. M., COGNIÉ Y. (1980): Do ewes continue to cycle after teasing? Proc. Aust. Soc. Anim. Prod., 13, 82.
TODOROV N. **(**2008): Feeding and care of sheep publishing house "Matkom", Sofia, 136. (In Bulgarian)
TYANKOV S., DIMITROV I., STANKOV I., SLAVOV R.,PANAYOTOV D. (2000): Sheep and goat breeding, "Abagar", Veliko Tarnovo, 384. (In Bulgarian)
UNGERFELD R., RUBIANES E. (1999a): Estrus response to the ram effect in Corriedale ewes primed with medroxyprogesterone during the breeding season. Small Rum. Res., 32, 89-91.
UNGERFELD R., RUBIANES E. (1999b): Effectiveness of short-term progestagen primings for the induction of fertile oestrus with eCG in ewes during late seasonal anoestrous. Anim. Sci., 68, 349-353.
UNGERFELD R., SUÁREZ G., CARBAJAL B., SILVA L., LACA R., FORSBERG M., RUBIANES E. (2003): Medroxyprogesterone priming and response to the ram effect in Corriedale ewes during the nonbreeding season. Theriogenology, 60, 35-45.
UNGERFELD R., FORSBERG M., RUBIANES E. (2004): Overview of the response of anoestrus ewes to the ram effect. Reprod. Nutr. Dev., 16, 479-490.
USTUMER B., GUNAY U., NUR Z., USTUNER H. (2007): Effects long and short-term progestagen treatments combined with PMSG on oestrus synchronization and fertility in Awassi ewes during breeding season. Acta Vet. Brno, 76, 391-397.
VIÑOLES C., FORSBERG M., BANCHERO G., 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.
WILDEUS S. (2000): Current concepts in synchronization of estrus: Sheep and goat. J. Anim. Sci., 77, 1-14.

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