

OESTRUS SYNCHRONIZATION EFFICIENCY IN EWES AND RAM MATURITY EFFECT ON FERTILITY DURING SUMMER SEASON

Nevena Maksimović, Dragana Ružić-Muslić, Violeta Caro Petrović, Violeta Mandić, Marina Lazarević, Bogdan Cekić, Ivan Ćosić

Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia
Corresponding author: Nevena Maksimović, nevena_maksimovic@yahoo.com
Original scientific paper

Abstract: The purpose of this study was to investigate oestrus synchronization efficiency and ram maturity effect on fertility of ewes of MIS sheep population during summer season. Research was performed on the experimental sheep farm of the Institute for Animal Husbandry in Belgrade, Serbia. The study included 70 crossbred ewes of MIS sheep population (Pirot Pramenka, Merinolandschaf and Ile de France breed) and 3 Ile de France rams (2 young, sexually unexperienced rams and 1 mature ram). Oestrus was synchronized in all 70 ewes using progestagen impregnated vaginal sponges (30 mg fluorogestone acetate) in combination with 500 IU PMSG. Ewes were mated with rams 48 h later. Obtained fertility and productivity results were as follows: lambing rate 57.14% (40/70), gestation length 147.11, litter size 1.82, stillborn rate 15.07%, birth weight of lambs 4.29 kg and body weight of lambs at 30 days of age 13.07 kg on average. Based on the results obtained in this study it was concluded that presented oestrus synchronization and mating protocol yielded lower than expected lambing rate. The effect of ram maturity on lambing rate was significant ($P < 0.05$), as ewes mated to mature ram had higher lambing rate compared to those mated to two young unexperienced rams. Mature ram also had bigger litter size and higher stillborn rate, as well as lower birth weight of lambs.

Key words: ram, maturity, fertility, oestrus synchronization, sheep

Introduction

The use of exogenous hormones for oestrus synchronization is a common practice in intensive sheep farming. Most commonly used procedure for oestrus induction/synchronization is application of progestagens via vaginal sponges in combination with PMSG or FSH, especially outside the breeding season. Progestagens are widely used to synchronize oestrus in sheep and usually result in greater than 90% of ewes in heat in a 24-hour period and conception rate of 70–

80% (Evans et al., 2001). However, fertility results achieved after hormone induced/synchronized oestrus vary depending on a number of factors, such as season, dose of PMSG used, fertilization method, body condition and physiology status of ewes. These are mostly female factors, but male factors, such as sperm fertility and libido, are also important. However, it is difficult to determine exactly which factors, female or male, contribute to fertility results and in what amount, but both must be taken into consideration.

MIS sheep population is a meat type breed of strong constitution, good carcass conformation and good meat quality properties. It was obtained by using a complex combination crossing according to a precisely defined genetic procedure, using Pirot Pramenka, Merinolandschaf and Ile de France breeds (Petrović, 2006). Ewes of MIS sheep population reach puberty at age of 6-8 months, which classifies them as the group of early maturing populations. In regard to fertility, MIS sheep population is very good if well managed and the use of progestagens in addition to PMSG hormone appears to be effective in the induction/synchronization of oestrus and in increasing of mean litter size outside the breeding season (Maksimović et al., 2015). However, there is not enough information in the literature on synchronization efficiency and fertility in ewes of MIS sheep population.

A significant percentage of domestic yearling rams are sexually inhibited when first exposed to oestrus ewes (Price et al., 1999b). Sexually inexperienced rams or ram lambs can often exhibit orientation problems with respect to mounting behavior. Among a group of oestrus ewes, rams can also show some preference for specific females while ignoring other ewes and this accounts for some differences in the lambing distribution (Fitzgerald and Morgan, 2007).

The role of experience on sexual performance suggests that early exposure to females will increase the probability of rams becoming sexually active at an earlier age (Perkins and Roselli, 2007). According to Perkins et al. (1992), the exposure of rams to oestrous ewes can increase the levels of LH and consequently the testosterone secretion in the ram, which in turn has been suggested to positively influence the production of pheromones (Haynes and Haresign, 1987). The close contact with ewes in oestrous also increases the libido of rams (Rodriguez Iglesias et al., 1991).

The purpose of this study was to investigate oestrus synchronization efficiency using progestagen+PMSG protocol and ram maturity effect on fertility in the ewes of MIS sheep population during summer season.

Material and Methods

Location and animals

Research was performed on the experimental sheep farm of the Institute for Animal Husbandry in Belgrade, Serbia at the end of July and the beginning of

August 2020. The study included 70 crossbred ewes of MIS sheep population (Pirou pramenka x Merinolandschaf x Ile de France) and 3 Ile de France rams. Ewes were 2 to 6 years of age, two young previously unexperienced rams (marked as ram 2 and ram 3) were both 1.5 year old and one mature ram (marked as ram 1) was 3 years old. Previous to mating, as well as after mating both rams and ewes were kept outdoors at pasture, but separately.

Experimental design

All 70 ewes were synchronized into oestrus using progestagen impregnated vaginal sponges (30 mg fluorogestone acetate, FGA, Syncro-Part, Ceva sante animale, France) in combination with pregnant mares serum gonadotropin (PMSG). Sponges remained *in situ* for 12 days. On the day of sponge removal, ewes received an intramuscular injection of 500 IU PMSG (Folligon[®], MSD Animal health). Rams were introduced to ewes 48 hours later and were kept with them for mating for about 8-10 hours/day. Ram to ewe ratio for mating was 1:3/day. Mounting activities of rams were not recorded, neither visually or with harnesses (crayons). Ram breeding soundness was considered by inspection and examination of reproductive organs.

The measured traits

The following reproductive parameters were measured: lambing rate (number of ewes lambed/number of ewes mated), stillborn rate (number of stillborn lambs/number of lambs born), gestation length, litter size (number of lambs born/number of ewes lambed), birth weight of lambs, birth weight of lambs at 30 days of age.

Statistical analysis

Statistical analysis of the experimental data was performed using the statistical package Statistica for Windows 7 (Stat. Soft. Inc.). The equality of variances of analyzed treatments was tested using the Leven's test. Lambing rates and stillborn rates were tested by Chi square analysis. Effect of ram on litter size and birth weight of lambs was tested using ANOVA and the mean comparison between rams was done by Fisher's LSD test. Analyses were performed for the significance level of 5% and 1% and the results were presented as mean \pm SEM.

Results and Discussion

Table 1 shows fertility traits (lambing rate, gestation length, litter size, stillborn rate) of ewes from synchronized oestrus and body weights of lambs at birth and 30 days of age.

Table 1. Fertility results of ewes and body weights of lambs (Mean±SEM)

Lambing rate, %	Gestation length, days	Litter size (all lambs)	Litter size (live born only)	Stillborn rate, %	Birth weight of lambs, kg	Body weight of lambs at 30 days of age, kg
57.14 (40/70)	147.11±0.14	1.82	1.55	15.07	4.29±0.13	13.07±0.46

Of the total of 70 ewes in the trial, 40 of them lambed after the synchronization of oestrus and joining with rams, representing lambing rate of 57.14%. Average gestation length was 147.11±0.14 days. Litter size was 1.82 lambs/ewe born (including stillborn lambs) and 1.55 lambs/ewe born including only live born lambs. Stillborn rate was 15.07%. Birth weight of lambs was 4.29±0.13 kg and body weight of lambs at 30 days of age was 13.07±0.46 kg on average. When compared to previous study of *Maksimović et al. (2015)* investigating the fertility of MIS sheep population after progestagen+PMSG synchronized oestrus outside breeding season, present study resulted in lower lambing rate, lower litter size, higher body weight of lambs at birth and at 30 days of age, as well as lower stillborn rate. *Maksimović et al. (2017)* also report higher lambing rate of 66.66% in MIS sheep population, although after repeated prostaglandin treatment for oestrus induction outside breeding season. *Mekić et al. (2014)* report lambing rates of 48.47% on average in Ile de France ewes after progestagen+PMSG oestrus synchronization protocol outside breeding season. *Evans et al. (2001)* state that the use of progestagen for oestrus synchronization typically results in greater than 90% of ewes in heat in a 24-hour period and conception rate of 70–80%. However, fertility results achieved after hormone induced/synchronized estrus vary depending on a number of factors, such as season, dose of PMSG used, fertilization method, body condition and physiology status of ewes. Also, male factors, such as sperm fertility and libido, are also important.

Table 2 shows effect of ram on lambing rate, litter size, stillborn rate and birth weight of lambs.

Table 2. Effect of ram on fertility results and birth weight of lambs (Mean±SEM)

Parameters	Lambing rate, %	Litter size	Stillborn rate, %	Birth weight of lambs, kg
Ram 1	75 ^a	2.22 ^a ±0.25	25 ^a	3.81 ^A ±0.15
Ram 2	47.83 ^b	1.91 ^{ab} ±0.28	4.76 ^b	4.25 ^A ±0.2
Ram 3	47.83 ^b	1.18 ^b ±0.12	7.69 ^b	5.52 ^B ±0.22

^{a, b} - Column means with different superscript letters differ significantly at P<0.05

^{A, B} - Column means with different superscript letters differ significantly at P<0.01

As presented in Table 2, the effect of ram on all studied parameters was significant; 75% of ewes conceived and lambed with older, mature ram, compared to 47.83% of ewes that lambed after being mated to each of two young previously unexperienced rams ($P < 0.05$). Mature ram also had bigger litter size and higher stillborn rate, as well as lower birth weight of lambs. Higher rates of stillbirths are common in more numerous litters, as well as lower birth weights are (Berger, 1997; Maksimović *et al.*, 2015). With the use of exogenous hormones there is a greater chance for intense ovarian activity and ovulation of greater number of oocytes that will potentially be able to become fertilized. Highly prolific sheep breeds normally have higher mortality rates of lambs at birth compared to less fertile breeds, because of the more numerous litters (Berger, 1997).

It is common for young rams to give lower fertility results when first introduced to breeding. Price *et al.* (1994) point out that intermittent exposure of ram lambs to estrous ewes early in life can improve their sexual performance as yearlings. In later study, Price *et al.* (1999a) state that direct contact with females was necessary for enhanced sexual performance in young rams. Authors have found that 59% of the rams (age 7-8 months) reared without contact with females failed to become active during their first sexual performance test. In contrast, the same study showed that only 15% of the rams reared with direct contact with females were inactive during their first sexual performance test. Also, 38% of the rams that had fence-line contact with ewes was sexually inactive during their first test. High percentage of domestic yearling rams are sexually inhibited when first exposed to ewes in estrous, however, most of these rams will eventually mate with ewes after repeated or prolonged exposure to females (Katz *et al.*, 1988; Price *et al.*, 1999b). In the study of Price *et al.* (1999b), only 56% of tested rams successfully mated with estrous ewes on first exposure. Stellflug and Lewis (2007) conclude that early exposure of 7–8 months old ram lambs to oestrus ewes improves sexual performance in serving capacity tests at 16–19 months of age in most rams. However, some authors did not find clear link between maturity and previous experience of rams to fertility results in ewes. Kenyon *et al.* (2007) found that two-tooth rams previously unused and mature rams previously used for mating are a better option than unused ram hoggets to maximize the pregnancy rate of ewe hoggets, but that it was unclear why lower conception rates were observed in ewe hoggets joined with mature rams which had been used previously. Authors suggested that as a result, the advantage of using mature rams may have been underestimated.

With the practice of hand mating within restricted time it is ideal to have one on one ewe to ram ratio. When given a choice to choose between more females, rams tend to mate with same ewe/s more than once and not pay attention to others (Tilbrook *et al.*, 1987; Fitzgerald and Morgan, 2007), or they chase after all of them at once and become exhausted, which is common in first time breeders, or get intimidated by females, also common in young rams. Therefore, one ram to

three ewes ratio for limited time period mating that was used in this study could have contributed to less fertilized ewes and lower lambing rate.

However, it is difficult to determine exactly which factors, female or male, contributed to fertility results and in what amount, but both must be taken into consideration.

Conclusion

Based on the results obtained in this study it can be concluded that presented oestrus synchronization protocol of using progestagen+PMSG during summer months subsequently followed by mating of ewes through single natural service, yielded lower than expected lambing rate. Ram maturity had significant effect on lambing rate as ewes mated to mature ram had higher lambing rate compared to those mated to two young unexperienced rams. Mature ram also had bigger litter size and higher stillborn rate, as well as lower birth weight of lambs. Ram to ewe ratio of 1:3 may have also contributed to lower conception and lambing rate. Perhaps 1:1 to 1:2 ram to ewe ratio would be better when young rams are used for limited time breeding. Fertility results are influenced by both management and animals and therefore further studies are needed in order to determine what contributes the most. Also, modification of oestrus synchronization protocol and usage of different hormone levels to ensure better oestrus response for MIS sheep population can be considered. Use of teaser rams to detect ewes in heat and prolonged mating time could ensure higher conception rates. Also, concept of ram maturity and previous experience effect on mating abilities and fertility results should be further investigated.

Efekat sinhronizacije estrusa i uticaj zrelosti ovna na plodnost ovaca tokom letnje sezone

Nevena Maksimović, Dragana Ružić-Muslić, Violeta Caro Petrović, Violeta Mandić, Marina Lazarević, Bogdan Cekić, Ivan Ćosić

Rezime

Cilj istraživanja bio je da se ispita efikasnost sinhronizacije estrusa i uticaj zrelosti ovna na plodnost ovaca MIS populacije tokom letnje sezone. Ispitivanja su sprovedena na eksperimentalnoj farmi Instituta za stočarstvo u Beogradu, Srbija. U ogled je bilo uključeno 70 plotkinja MIS populacije (dobijene složenim kombinacijskim ukrštanjem pirotske pramenke, virttemberg i il de frans rase ovaca)

i tri il de frans ovna, od kojih su dva mlada priplodnjaka prvi put uvedena u priplod i jedan zreo ovan sa prethodnim iskustvom u reprodukciji. Estrus je sinhronizovan kod svih 70 ovaca upotrebom progestagenskih vaginalnih sunđerica (30 mg FGA) u trajanju od 12 dana i u kombinaciji sa 500 i.j. SŽK. Plotkinje su pripuštene za parenje sa ovnovima nakon 48 h od aplikacije SŽK. Ostvareni su sledeći rezultati: stopa jagnjenja 57,14% (40/70), dužina bremenitosti 147,11, indeks jagnjenja 1,82, stopa mrtvorodenja 15,07%, masa jagnjadi na rođenju 4,29 kg i masa jagnjadi u uzrastu od 30 dana 13,07 kg, prosečno. Uticaj zrelosti ovna na stopu jagnjenja bio je statistički značajan ($P < 0,05$) pri čemu su se plotkinje koje su se parile sa zrelim ovnom sa prethodnim iskustvom u reprodukciji ojašnjile u većem broju u poređenju sa plotkinjama koje su parene sa mladim, reproduktivno neiskusnim ovnovima. Zreo ovan je takođe imao i veći broj rođene jagnjadi, veću stopu mrtvorodenja, kao i manje porođajne mase jagnjadi. Rezultati plodnosti se nalaze istovremeno pod uticajem samih životinja kao i menadžmenta u proizvodnji, a dodatna istraživanja potrebna su da bi se utvrdilo u kolikoj meri koji faktori doprinose. Fokus bi trebalo staviti na eventualnu modifikaciju protokola sinhronizacije estrusa da bi se osigurala efikasnija pojava estrusa. Upotreba ovnova probaća za detekciju ovaca u estrusu, kao i produžetak vremena trajanja pripusta ili dvokratni pripust mogli bi osigurati bolju stopu koncepcije. Takođe, trebalo bi dodatno ispitati koncept uticaja zrelosti i prethodnog reproduktivnog iskustva ovna na sposobnost parenja i rezultate plodnosti.

Ključne reči: ovnovi, zrelost, plodnost, sinhronizacija estrusa

Acknowledgments

This research was supported and financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia No. 451-03-68/2020-14/200022.

References

BERGER Y.M. (1997): Lamb mortality and causes – A nine year summary at the Spooner agricultural research station. Proceedings of the 45th Annual Spooner Day. Department of animal science, University of Wisconsin – Madison, 33-40.
EVANS A.C.O., FLYNN J.D., QUINN K.M., DUFFY P., QUINN P., MADGWICK S., CROSBY T.F., BOLAND M.P., BEARD A.P. (2001): Ovulation of aged follicles does not affect embryo quality or fertility after a 14-day progestagen estrus synchronization protocol in ewes. *Theriogenology* 56, 923–936.

- FITZGERALD J., MORGAN G. (2007): Reproductive physiology in ram. In: Current therapy in large animal theriogenology (2nd Edition). Elsevier Inc., 617-620.
- HAYNES N.B., HARESIGN W. (1987): Endocrine aspects of reproduction in the ram important to the male effect. *World Review of Animal Production*, 23, 21–28.
- KATZ S.L., PRICE O.E., WALLACH R.J.S., ZENCHAK J.J. (1988): Sexual performance of rams reared with and without females after weaning. *Journal of Animal Science*, 33, 1166-1171.
- KENYON R.P., MOREL H.C.P., MORRIS T.S., WEST M.D. (2007): Effect of the age of rams on reproductive performance of ewe hoggets, *New Zealand Veterinary Journal*, 55, 4, 184-187.
- MAKSIMOVIĆ N., DELIĆ N., STANKOVIĆ B., HRISTOV S., CARO-PETROVIĆ V., RUŽIĆ-MUSLIĆ D., MIĆIĆ N. (2015): Oestrus synchronization efficiency and fertility in ewes of MIS sheep population during anoestrous and breeding season. *Proceedings of the 4th International Congress New Perspectives and Challenges of Sustainable Livestock Production*. October 7 – 9, Belgrade, 104-112.
- MAKSIMOVIĆ N., MILOVANOVIĆ A., BARNA T., DELIĆ N., STEFANOV R., PANTELIĆ V., TAUSHANOVA P. (2017): Effects of prostaglandin and hCG on out of season oestrous synchronization and fertility and assessment of progesterone concentration for early pregnancy diagnosis in ewes. *Comptes rendus de l'Académie bulgare des Sciences* 70, 6, 885-894.
- MEKIĆ C., PERIŠIĆ P., NOVAKOVIĆ Z. PETROVIĆ P.M., VUJIĆ R. (2014): Plodnost ovaca pri indukciji i sinhronizaciji estrusa u sezoni anestrije u zavisnosti od telesne mase tretiranih plotkinja. *Veterinarski Glasnik* 68, 5-6, 323-330.
- PERKINS A., FITZGERALD J.A., PRICE E.O. (1992): Luteinizing hormone and testosterone response of sexually active and inactive rams. *Journal of Animal Science* 70, 2086–2093.
- PERKINS A., ROSELLI E.C. (2007): The ram as a model for behavioral neuroendocrinology. *Hormones and Behavior* 52, 70-77.
- PETROVIĆ P.M. (2006): Mis sheep: Creation of meaty sheep breed. Institute for Animal Husbandry, Belgrade, pp. 43 (in Serbian, English summary).
- PRICE E.O., BORGWARDT R., BLACKSHAW J.K., BLACKSHAW A., DALLY M.R., ERHARD H. (1994): Effect of early experience on the sexual performance of yearling rams. *Applied Animal Behaviour Science*, 42, 41–48.
- PRICE E.O., BORGWARDT R., DALLY M.R. (1999a): Effect of early fenceline exposure to estrous ewes on the sexual performance of yearling rams. *Applied Animal Behaviour Science*, 64, 241–247.
- PRICE O.E., ORIHUELA A., PARTHASARATHY V., BORGWARDT R., DALLY R.M. (1999b): Yearling rams initially disinterested in estrous ewes subsequently exhibit subnormal sexual performance *Applied Animal Behaviour Science*, 64, 235–240.

RODRIGUEZ IGLESIAS R.M., CICCIOLO N.H., IRAZOQUI H., RODRIGUEZ, B.T. (1991): Importance of behavioural stimuli in ram-induced ovulation in seasonally anovular Corriedale ewes. *Applied Animal Behaviour Science*, 30, 323–332.

STELLFLUG N.J., LEWIS S.G. (2007): Effect of early and late exposure to estrual ewes on ram sexual performance classifications. *Animal Reproduction Science*, 97, 295–302.

TILBROOK A.J., CAMERON A.W.N., LINDSAY D.R. (1987): The influence of ram mating preferences and social interaction between rams on the proportion of ewes mated at field joining. *Applied Animal Behaviour Science*, 18, 173-184.

Received 29 September 2020; accepted for publication 23 November 2020