

## SYNCHRONIZATION OF ESTRUS AND FERTILITY IN SHEEP OF THE NORTHEASTERN BULGARIAN FINE–WOOLEN BREED – SHUMEN TYPE BY DIFFERENT PROGESTOGEN TREATMENTS AND DIFFERENT DOSES OF PMSG

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### ABSTRACT

The aim of the present study was to test three protocols for synchronization of estrus and fertility in merino sheep. 3 experimental groups were formed, depending on the duration of progestagen treatment (FGA sponges) and PMSG dose: Group 1 (n = 12) – 12 days + 500 UI. Group 2 (n = 12) – 12 days + 250 UI. Group 3 (n = 11) – 5 days (at the time of sponge placement, sheep were treated with a synthetic analogue of PGF2 $\alpha$ ) + 500 UI. On the three groups, PMSG was injected at the time of sponge removal.

At the 48th hour of sponge removal, the highest number of ewes in the estrus was in the Group 2–12 sheep, while in the others it was 9 (Group 1) and 6 (Group 3). The highest values for fertility and fecundity were obtained in sheep from Group 1–41.66% and 160.0%.

**Key words:** sheep, estrus, progestagens, PMSG.

### Introduction

Synchronization of ewes' estrus and births are essential elements in reproductive management in sheep farming. The most common method of oestrus synchronization in small ruminants is by using synthetic progestogens through (intra-)vaginal sponges (reviews by Wildeus, 1999, Danko, 2003; Menchaca & Rubianes, 2004; Abecia et al., 2011; Gonzalez–Bulnes et al., 2020). In the modern veterinary pharmaceutical industry, two types of progestogens are used to impregnate sponges–flurogestone acetate (FGA) and medroxyprogesterone acetate (MAP – medroxyprogesterone acetate).

In traditional estrus synchronization protocols, progestogen-impregnated intravaginal sponges (fluorogestone acetate FGA or methylacetoxyprogesterone MGA) stay for a period of time similar to the life span of the cyclic corpus luteum – on average 12-14 days (with variations from 9 to 16), and they are placed regardless of the phase of the cycle or the follicular status of the ovaries at the time of treatment (Menchaca and Rubianes, 2004). According to the new concepts of follicular growth (the wave pattern, and that each follicular wave occurs every 5–7 days), alternative short-term progestagen treatments were developed in sheep and goats, consisting of inducing a 5–7 day progesterone/progestagen background (Menchaca and Rubianes, 2004).

After stopping the action of progestagens, with the aim of better fertilization and higher fertility, it is necessary to stimulate and synchronize the ovulation of the treated animals. The most widely used in practice is pregnant mare serum gonadotropin (PMSG, eHG). The dose of PMSG and the success rate of the treatment depends on many factors: breed, estrous or anestrus season, physiological, health and body condition, number of treatments on the respective animal.

The aim of the present study was to test three protocols for synchronization of estrus and fertility in sheep of the breed Northeastern Bulgarian fine-woolen – Shumen type through different progestogen treatment and different doses of PMSG.

## Materials and methods

### *Animals*

The experiment was carried out with 35 sheep aged 2–5 years of the breed North-Eastern Bulgarian fine-woolen – Shumen type in June/July 2021. The ewes were raised in the experimental farm of the National Center for Agriculture – Targovishte. The ewes were fed on pasture with a supplemented feed of 0.150 kg/per head/per day of concentrated mix. The sheep in the experimental groups were equal in age, live weight (65–70 kg), body condition (OTC = 3.0–3.5), clinically healthy and normally lambed in the last lambing campaign. Depending on the length of the stay of the sponges and the dose of PMSG, 3 experimental groups were formed:

Group 1 (n = 12) – 12 days stay of the sponges (30 mg FGA (Synchropart®, CEVA SANTE ANIMAL) and treatment of PMSG at a dose of 500 IU at the time of sponge removal (Synchropart® PMSG, CEVA SANTE ANIMAL). Group 2 (n = 12) – 12 stay of the sponges (30 mg FGA (Synchropart®, CEVA SANTE ANIMAL) and treatment of PMSG at a dose of 250 IU at the time of sponge removal (Synchropart® PMSG, CEVA SANTE ANIMAL). Group 3 (n = 11) – 5 days stay on the sponges (30mg FGA (Synchropart®, CEVA SANTE ANIMAL) and PMSG at a dose of 500 IU at the time of sponge removal (Synchropart® PMSG, CEVA SANTE ANIMAL). At the time of sponge placement, the sheep were treated with a synthetic analogue of PGF<sub>2</sub>α – Alfabedyl CEVA ANIMAL HEALTH (ad.v. alfaprostolum) at a dose of 0.5 ml.

### *Test for estrus and artificial insemination*

At 48 hours after removal of the vaginal sponges, the ewes were examined for estrus by an estrus detector (Draminski Ltd). Sheep with electrical resistance  $\leq 350$  units (according to the instructions of the manufacturer and our previous experiments) were considered to be in estrus. Sheep with estrus boundary values (360–440 units) as well as those outside estrus (over 450 units) were also recorded. Immediately after testing with the estrus detector, each ewe was artificially inseminated, regardless of the presence or absence of estrus. The artificial insemination was deeply vaginal, with diluted semen (diluent for semen of a ram, produced in NCZ – Targovishte, in a ratio of 1:3) in a dose of 0.2 ml. Six sexually active rams aged between 2.5 and 5.5 years were used for artificial insemination and only ejaculates with the following parameters volume  $\geq 0.5$  ml and motility 70% were used. From the 16th to the 19th day after the first artificial insemination, the sheep from the three experimental groups were examined with teasers for the presence of absence of estrus. Sheep with clinically manifested estrus were artificially inseminated.

### *Studied parameters*

The following parameters were studied:

- Estrus synchronization rate at 48 hours after sponge removal – number of ewes in estrus / total number of sheep x 100.
- Number of ewes, that came in estrus after 16th to the 19th day after the first artificial insemination (ewes with repeated estrus – ERE)
- Ewes, that were in estrus after sponge removal and did not come in estrus after 16th to the 19th day, but remained barren (barren ewes – BE).
- Fertility after first artificial insemination – lambed ewes / inseminated ewes x 100.

- Fertility of the ERE – lambed ewes / ewes in estrus x 100.
- Fecundity – aborted, live-born and stillborn lambs / lambed ewes x 100.

Fertility and fecundity were reported after the end of the lambing campaign. The results were presented in the number of sheep or lambs (n) and in percentages (%) for each studied parameter.

## Results

At the 48th hour of sponge removal, the highest number of ewes in the estrus was in the Group 2–12 sheep or 100.0% while in the others it was 9 (75.0%) (Group 1) and 6 (50.0%) (Group 3).

The highest values for fertility and fecundity were obtained in sheep from Group 1 – 41.66% and 160.0% (Table 2). The lowest fertility had Group 2 – 16.66%. The values of fecundity were similar to all studied group – between 150–160%.

The number of ewes, that came in estrus after 16th to the 19th day after the first artificial insemination, was the highest in Group 2–7 ewes, while to other groups the number was 5 (Group 1) and 4 (Group 3). (Table 3). The fertility of that ERE ewes was the highest in Group 1 – 89%, and lowest in Group 3 – 50.0%. The fecundity was the same in for all ewes – one lambs from one ewe or 100%.

**Table 1: Number of ewes, that came or not in estrus and mean vaginal electrical resistance of the ewes from the experimental groups**

Group	Number of ewes with vaginal electrical resistance $\leq$ 350 UNITS (Ewes in estrus)	Number of ewes with boundary values for estrus (360-440 UNITS).	Number of ewes that were not in estrus (over 450 UNITS).
Group 1 (n=12)	9	1	2
Group 2 (n=12)	12	0	0
Group 3 (n=11)	6	0	5

**Table 2: Fertility and fecundity after first artificial insemination**

Group	Fertility		Fecundity	
	n	%	n	%
Group 1 (n=12)	5	41,66	8	160,0
Group 2 (n=12)	2	16,66	3	150,0
Group 3 (n=11)	4	36,36	6	150,0

**Table 3: Number of ERE and BE. Fertility and fecundity of ERE**

Group	Number of ERE	Fertility		Fecundity %	Number of BE
		Number of ewes n	%		
Group 1 (n=12)	5	4	80,0	100	2
Group 2 (n=12)	7	4	57,14	100	2
Group 3 (n=11)	4	2	50,0	100	0

In all three groups there were ewes that did not come in estrus after 16th to the 19th day after the first artificial insemination, but remained barren – 2 (Group 1 and 2) and 3 (Group3).

## Discussion

Wildeus (2000) summarized that the classic protocols that include 12 – 14 days progestogen sponge synchronization of estrus had high success rates of over 90% and ewes showed estrus 24 to 48 hours after sponge removal. With short-term progestagen treatments, the synchronizing effect was 80 – 100% up to 144 hours after tampon removal (Ungerfeld & Rubianes, 1999; Viñoles et al., 2001; Aköz et al., 2006; Ustuner et al., 2007; Karaca et al., 2009; Martemucci & D'Alessandro, 2011; Metodiev & Raicheva, 2011; Metodiev & Raicheva, 2014; Metodiev et al., 2018). In our previous studies with Ile-de-France sheep (Metodiev & Raicheva, 2011; Ralchev et al., 2012), we observed that the onset of estrus occurred earlier and more synchronously after sponge removal with long-term progestagen treatments compared to short-term. The situation was similar in the present study – sheep subjected to long term treatment had better results in estrus synchronization (Table 1).

In our previous study (Metodiev & Raicheva 2011) with Ile-de-France ewes to induce synchronous estrus with 6 days of progestagen treatment, we observed that in some ewes estrus occurred after 48 h of sponge removal. It was also possible that in the present study some ewes that had boundary values for estrus to come into estrus after 48 h.

In the accessible literature, there are numerous studies on the effect of synthetic progestogens on fertility after their use. Fertility varies from 0.0% to 100.0%, with the general belief being 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 (by the review of Abecia et al., 2011). Most often, the fertility varies around 50 – 60%. In addition to the above-mentioned conclusions, fertility is influenced by a number of factors, the most important of which are: the method (natural vs. artificial) and the frequency of insemination (one, two or multiple), body condition, nutrition, stress factors and others. In our previous study (Ralchev et al., 2012) we tested the effectiveness of protocols to induce synchronous estrus in Ile-de-France ewes by administering progestogens for 12 days and PMSG inserted at different times (-48 h prior or at the time of sponge removal). We obtained that the fecundity is greater in the sheep group with inserted PMSG at the time of sponge removal – 55.56%, than group with inserted PMSG – 48 hours prior sponge removal 40,0% (Ralchev et al., 2012.) In a series of our studies with short-term progestagen treatments, we achieved first-estrus fecundity rates of 22.00% to 100.0% (Metodiev & Raicheva, 2011, 2014; Metodiev et al., 2018; Metodiev, 2019, 2020<sup>1</sup>, 2020<sup>2</sup>), as variation in fecundity we supposed to be due to insemination frequency and animal body condition.

The values for the fecundity in all groups were high (150.0 – 160.0%) and exceed the biological average values of biological fecundity for the breed. The biological fecundity of sheep from the Northeastern Bulgarian fine-woolen – Shumen type varies within 125 – 135% (Stancheva et al., 2015). This showed that the application of PMSG is a reliable method of increasing the biological fertility of sheep of the North-East Bulgarian thin fleece breed – Shumenski type.

In the future, efforts should be made to improve fertility at first estrus and in general. In the **ERE**, the best fertility is in the sheep of Group 1, while in the other two it is low – about 50%. In Group 1 and Group 2 there were two sheep in each group that were in estrus after sponge removal

and did not come in estrus after 16th to the 19th day, but remained barren. This, in our opinion, is due to abortions that have gone unnoticed or underreported by shepherds.

### Conclusion

At the 48th hour of sponge removal, the highest number of ewes in the estrus was in the Group 2–12 sheep, while in the others it was 9 (Group 1) and 6 (Group 3).

The highest values for fertility and fecundity were obtained in sheep from Group 1–41.66% and 160.0%.

Based on the results of fertility and fecundity, we concluded that the protocol that consist of 12 days stay of the sponges and treatment of PMSG at a dose of 500 IU at the time of sponge was most suitable for the estrus synchronization and artificial insemination in fixed time in sheep of the Northeastern Bulgarian fine-woolen breed – Shumen type.

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