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Study on estrus synchronization in Lacaune sheep during the anestrus season

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Abstract

This study evaluated the reproductive outcomes of estrus synchronization in Lacaune ewes during the anestrus season. A total of 318 ewes were treated with intravaginal sponges containing 60 mg medroxyprogesterone acetate (MPA) for 14 days, followed by an injection of 250 IU of lyophilized pregnant mare serum gonadotropin (PMSG) (Gonaser, HIPRA). The first artificial insemination was carried out 48 hours after sponge removal, followed by a second insemination approximately 5 hours later. The overall conception rate was 81.1%, with a biological prolificacy of 1.72 lambs per lambing ewe. Multiple pregnancies accounted for 64.5% of gestations. The abortion rate was 3.7%. Method of mating significantly influenced fertility and prolificacy, while ewe age had no notable effect. The results demonstrate that applying an appropriate hormonal protocol enables highly effective breeding of Lacaune ewes during the anestrus season.

Keywords: Lacaune ewes, estrus synchronization, anestrus season, artificial insemination, prolificacy, reproductive performance

INTRODUCTION

Sheep farming in Bulgaria is a traditional sector of livestock production, especially significant in mountainous and semi-mountainous regions. In recent years, there has been a growing interest in high-yielding foreign breeds, among which the French dairy breed Lacaune stands out. This breed demonstrates excellent adaptability to Bulgarian conditions and provides both high milk yield and good reproductive traits. According to Sevov et al. (2025a), in a large-scale study involving 10,783 Lacaune ewes from selected flocks in Bulgaria, over 68% of the animals produced between 250 and 400 liters of milk per lactation, with the highest yield recorded in the fifth and sixth lactations. In addition to its dairy performance, Lacaune breed also shows good meat characteristics. In another study, Sevov et al. (2025b) reported a slaughter yield of 45.6% in 35-day-old male lambs, significantly higher than the 40.8% yield observed in 85-day-old

lambs. These results emphasize the breed's dual-purpose potential – both milk and meat – when properly managed under an intensive production system.

Estrus synchronization is a key tool for the intensification and regulation of the reproductive process in sheep farming. It allows lambing periods to be adjusted in response to market demand for milk and lambs. In dairy-oriented farms, such as those raising Lacaune sheep, there is an interest in ensuring year-round production of sheep milk in order to meet market needs. This makes farms applying synchronization protocols more competitive in comparison to those relying on natural breeding.

Regardless of the season, timely and proper preparation of ewes for the breeding campaign has a significant impact on conception rates, prolificacy, and the duration of the breeding and, consequently, the lambing campaign, determining its success (Zlatarev, 2001). Estrus synchronization protocols in sheep are based on various approaches. The

literature describes protocols of different durations. In studies comparing 6-day and 12-day progestagen treatments (Maslev et al., 2010), it was established that a 12-day duration of intravaginal sponge application provided better results compared to the 6-day treatment. Husein et al. (2007) reported that the use of a progesterone analogue for 4 or 12 days, combined with eCG injection on the day of sponge removal, resulted in similar outcome, although treatment with gonadotropins was mandatory after short-term progesterone application. Ataman et al. (2009) applied intravaginal sponges impregnated with 30 mg and 40 mg FGA for 12 days and ear implants containing 3 mg norgestomet for 9 days. They found that conception rates were higher in the groups treated with 30 mg FGA and the implants (93.3%). This supports the claim of Stzimsnik et al. (2002) that higher concentrations of post-ovulatory progesterone prepare the uterus too early to receive the fertilized ovum, which results in embryonic loss due to an inadequate uterine environment. Therefore, both very short and excessively long administration of exogenous progesterone and its analogues can lead to an inadequate physiological response and, consequently, to lower reproductive outcomes. Koyuncu & Alticekic (2010) demonstrated that subcutaneous administration of PMSG is preferable to intramuscular application, as it leads to increased conception rates, prolificacy, and twinning.

The objective of the present study was to investigate the outcomes of estrus synchronization in Lacaune ewes during the anestrus season.

MATERIALS AND METHODS

Data were collected from an estrus synchronization program conducted on 318 ewes which were simultaneously synchronized. Of these, 81 ewes underwent a natural breeding following a return to estrus. The synchronization procedure was carried out during the anestrus season, in April 2022. The animals were managed under an intensive, group-housing system. The farm has a dairy production orientation, with the ewes organized into groups and the reproductive management aimed entirely at ensuring year-round lactation. The animals were fed with high-quality complete mixed rations.

Estrus synchronization was performed following a standard protocol. Day 0 of the protocol was defined as the day of insertion of the intravaginal sponges containing 60 mg medroxyprogesteron acetate (MPA) (OVIGEST®, Hipra), which were left for 14 days. Upon removal of the sponges, each ewe received an injection of 250 IU of lyophilized pregnant mare serum gonadotropin (PMSG) (Gonaser, HIPRA). The first artificial insemination was carried out 48 hours after sponge removal, followed by a second insemination approximately 5 hours later.

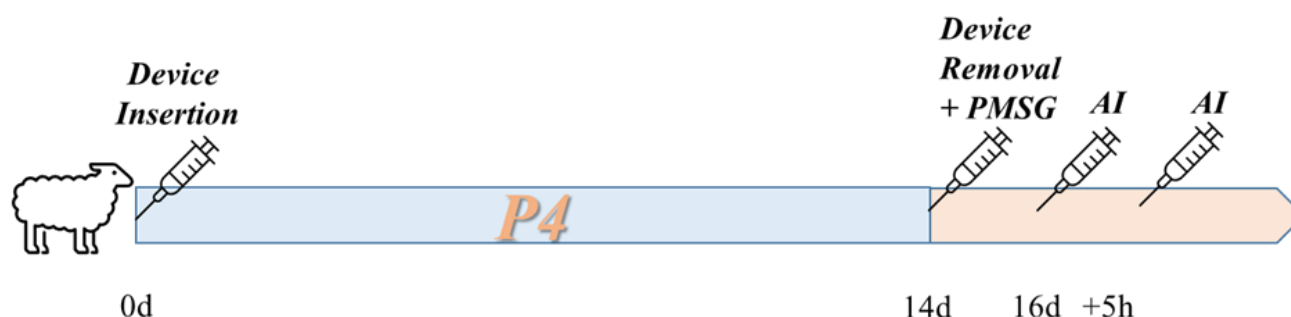


Figure 1. Protocol for estrus synchronization in ewes during the anestrus season

Artificial insemination was carried out using pre-evaluated and diluted semen according to a predetermined breeding plan. The breeding rams are reared on the farm. During artificial insemination, the ewes were inseminated by deep vaginal deposition of semen, obtained from specially prepared breeding rams housed in a separate pen. The quality of the semen was evaluated, and after dilution the semen was used for ewes' insemination.

Biological prolificacy was determined after the completion of the lambing campaign, expressed as a percentage based on the ratio of the total number of lambs born to the number of ewes that lambed. Conception rate was established following an ultrasound examination, calculated as the ratio of pregnant to non-pregnant ewes. The ultrasound examination was performed between the 40th and 50th day after insemination. Eickemeyer Medic 2000 device equipped with a 5 MHz sector transducer was used. A transabdominal approach was applied in the right inguinal region, visualizing the area above the udder between the pecten ossis pubis and the last rib. The primary ultrasound criteria for pregnancy diagnosis were monitored, including the presence of an embryo or fetus, detection of placentomes, and determination of the number of fetuses.

Data were analyzed using MS Excel 365 and IBM SPSS 21. The following statistical model was used:

$$Y_{smkl} = \mu + R_s + K_m + RM_{sm} + e_{smkl}$$

Where: Y_{smkl} – observation vector, μ – overall mean constant, R_s – effect of the insemination order ($s = 2$), K_m – category effect ($m = 2$, ewe lambs and adult ewes), RM_{sm} – random effect of insemination order within category, e_{smkl} – residual variance.

RESULTS AND DISCUSSION

In the studied group of 318 Lacaune ewes treated during the anestrus season with a

protocol consisting of 14-day MPA sponges and PMSG administration, a conception rate of $81.1 \pm 3.71\%$ was achieved. The results demonstrated high effectiveness of the applied hormonal protocol for estrus synchronization in Lacaune ewes outside of the breeding season. The conception rate of 81.1 % is comparable to data from the literature on similar protocols. For instance, Blaschi et al. (2014) reported a conception rate of approximately 83.3% following fixed-time insemination after a 14-day progestagen treatment. Higher values have been reported in other studies: Berean et al. (2021) achieved pregnancy rates of up to 95.8% in Lacaune ewes out of season by combining melatonin implants with vaginal sponges and PMSG administration. By comparison, in the absence of hormonal treatment during the anestrus period, fertility is extremely low – in control group without treatment, only 4.16% of ewes became pregnant. This highlights the importance of hormonal synchronization to overcome seasonal anestrus in ewes.

The average number of lambs born per ewe (total flock prolificacy) was 1.34 ± 0.08 , reflecting the high productivity of the flock. Out of 304 diagnosed pregnancies, 13 abortions were recorded, corresponding to an abortion rate of 3.7%. Biological prolificacy, the average number of lambs per lambing ewe, reached approximately 1.72 ± 0.063 lambs per ewe. This indicates a pronounced level of multiple pregnancies, with 64.5% of all pregnancies being multiple (Table 1). Under similar conditions of hormonal stimulation outside the breeding season, a prolificacy rate of around 1.7 lambs per ewe has also been reported by other authors (Berean et al., 2021). High -fecundity breeds like the Hu sheep can attain 1.7 lambs per ewe with optimized protocols (twinning rates around 70%) (Yu et al., 2022). This agrees with broader findings that exogenous progestagens (12–14 day devices) combined with eCG are required to induce fertile estrus in ewes during the non-breeding period (Kuru et al., 2020). Hormonal stimulation effectively substitutes for

the environmental cues of the breeding season, enabling high conception rates even when daylight length would normally preclude reproductive activity (Abecia et al., 2012).

Table 1. Mean values of the key reproductive parameters in Lacaune ewes subjected to estrus synchronization and artificial insemination

Parameter	Mean	±SE
Conception rate, %	81.06	3.706
Biological prolificacy, % (n/N)	1.724	0.063
Abortion rate, %	3.697	1.986
Prolificacy, number of lambs per group	1.340	0.084
Multiple pregnancies, % (exclude abortions)	64.54	4.818

The observed twinning rate (64.5 %) in Lacaune ewes was expectedly high. Higher twinning rate of 51.6% in Kıvrıkcık ewes was reported by Koyuncu & Altınçekiç (2010), although with a lower average conception rate of approximately 75% (during the estrous season). This difference underscores that Lacaune ewes, under proper treatment, can not only maintain fertility in non-breeding season but also express their high prolificacy,

achieving multiple births at rates on par with or exceeding those seen in naturally bred flocks.

The observed low abortion rate (3.7%) indicates good pregnancy maintenance under the applied protocol. Comparable levels have been reported in other breed. In synchronized Barki ewes using a similar protocol, Abdalla et al. (2014) reported an abortion rate of approximately 5.3% which is within the normal range. In another study using a comparable estrus synchronization scheme in Barki ewes, Abdalla et al. (2014) reported an abortion rate of 5.26%.

The percentage distribution of the offspring revealed 38% singletons, 52% twins, 9.7% triplets, and 0.3% quadruplets (Figure 2). The analysis by animal category showed that young ewes (yearlings) responded similarly to the protocol as adult ewes: the conception rate after the first insemination was 74.1% in yearlings and 71.2% in adult ewes. Following the second mating (natural mating), 81.2% of the remaining non-pregnant yearlings and 97.14% of the non-pregnant adult ewes conceived. Thus, the overall conception rate in the group reached the previously mentioned 81.1%.

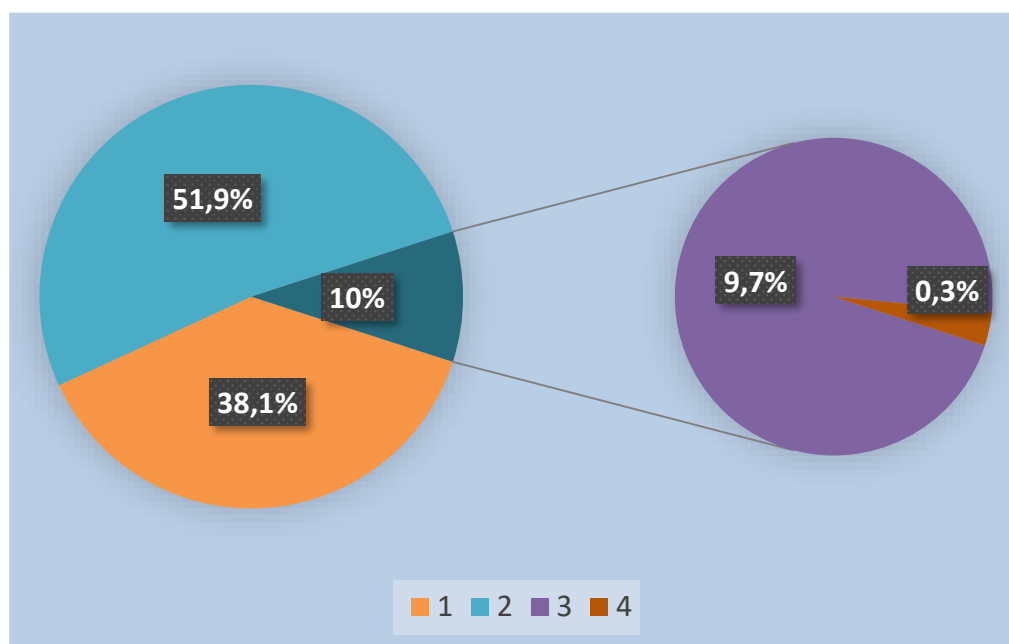


Figure 2. Percentage distribution according to litter size

Typically, younger ewes and yearlings respond less robustly than mature ewes during the non-breeding season. For example, Santos-Jiménez et al. (2022) observed that nulliparous ewes had markedly lower estrus and fertility rates than multiparous ewes under similar out-of-season treatment – only about 54% of yearlings showed estrus and 30% became pregnant, versus 100% estrus and 73% pregnancy in adult ewes (Santos-Jimenez et al., 2022). In the current study, the lack of parity effect suggests that the nulliparous were well prepared (good body condition and cycling status) and thus responded to the PMSG-based protocol as effectively as the older ewes. This outcome is supported by the overall results of the synchronization protocol in both categories and indicates that, under optimal management, nulliparous ewes Lacaune ewes can be successfully bred out of season with no penalty in conception rate relative to mature ewes.

Nulliparous ewes exhibited slightly lower prolificacy than multiparous ewes during the first insemination (1.69 vs. 1.80). This trend was reversed after the second mating, where nulliparous ewes achieved higher prolificacy (1.89) than multiparous ewes (1.51). While this may reflect individual variation and small sample sizes in the second-mating group, it indicates that younger ewes can perform well reproductively when given sufficient opportunity and support.

The abortion rate was considerably higher in nulliparous ewes (10%) compared to multiparous ones (4.79%) after the first insemination. However, no abortions were observed in either category following the second mating. This may suggest that pregnancies established via natural estrus are more stable, possibly due to better uterine receptivity and synchrony between embryo development and endometrial maturation. Additionally, the higher abortion rate in young ewes may reflect a less mature uterine environment or higher sensitivity to hormonal

fluctuations, which is consistent with some previous findings (Abecia et al., 2012).

When analyzing the total lamb output per group, multiparous ewes outperformed nulliparous ewes in both inseminations, although the difference narrowed significantly after the second. For instance, after the first insemination, multiparous ewes produced 1.21 lambs per ewe vs. 1.13 in nulliparous ones. After the second mating, this was 1.47 vs. 1.55, respectively. These values support the notion that the second-chance mating significantly boosts productivity, especially in replacement stock.

Overall, the rate of multiple pregnancies was high in both ewe categories, consistent with the biological prolificacy of the Lacaune breed. Notably, nulliparous ewes exhibited a higher rate of multiple gestations after the second mating (77.78%) compared to their first insemination (66.67%). Conversely, multiparous ewes showed a decrease in multiple pregnancies after the second insemination (48.53%) compared to the first (65.17%).

The analysis of variance presented in Table 3 highlights that the insemination method order significantly influenced key reproductive indicators – including conception rate, abortion rate, and number of lambs per ewe. These findings confirm that providing a second insemination opportunity (natural mating following artificial insemination) improves reproductive performance. In contrast, animal category (nulliparous vs. multiparous ewes) did not have a statistically significant effect on any of the analyzed traits, indicating that young, well-managed ewes responded just as effectively to the hormonal protocol as adult ones. This suggests successful adaptation of the synchronization strategy across different age groups. A tendency for interaction between insemination order and category was noted in terms of prolificacy, suggesting possible variation in litter size outcomes depending on age and timing of insemination, though not to a statistically strong degree.

Table 2. Percentage of pregnant ewes and farm-level prolificacy according to animal category (yearlings and adult ewes) and mating method in Lacaune ewes

Parameter	Mating method	Category	N	Mean (%)	±SE
Conception rate, %	Artificial insemination	Nulliparous ewes	54	74.07	5.649
		Multiparous ewes	264	71.21	2.555
	Natural mating	Nulliparous ewes	11	81.82	12.52
		Multiparous ewes	70	97.14	4.962
Biological prolificacy, %	Artificial insemination	Nulliparous ewes	36	1.694	0.106
		Multiparous ewes	178	1.798	0.048
	Natural mating	Nulliparous ewes	9	1.889	0.212
		Multiparous ewes	68	1.515	0.077
Abortion rate, %	Artificial insemination	Nulliparous ewes	40	10.00	3.179
		Multiparous ewes	188	4.787	1.466
	Natural mating	Nulliparous ewes	9	0.000	-
		Multiparous ewes	68	0.000	-
Prolificacy, number of lambs per group	Artificial insemination	Nulliparous ewes	54	1.130	0.128
		Multiparous ewes	264	1.212	0.058
	Natural mating	Nulliparous ewes	11	1.545	0.283
		Multiparous ewes	70	1.471	0.112
Multiple pregnancies, % (exclude abortions)	Artificial insemination	Nulliparous ewes	36	66.67	8.049
		Multiparous ewes	178	65.17	3.620
	Natural mating	Nulliparous ewes	9	77.78	16.09
		Multiparous ewes	68	48.53	5.857

Table 3. Effect of some factors on conception rate and prolificacy in ewes subjected to estrus synchronization in anestrus season

Factor	F-value and level of significance				
	Conception rate, %	Prolificacy, %	Abortion rate, %	Prolificacy, number of lambs per group	Multiple pregnancies, %
Mating method	5.161*	3.536	3.464*	4.053*	0.082
Category	0.707	1.138	0.430	0.001	2.546
Mating method * Category	1.51	3.536*	0.430	0.218	2.074

Legend: * $p < 0.05$

Overall, the results underline the importance of insemination timing and management in achieving high fertility and productivity.

In the context of intensive dairy sheep farming, the opportunity to achieve year-round lambing by overcoming seasonality is of crucial importance. The Lacaune breed, selected for high milk production, demonstrates excellent reproductive performance under appropriate

management conditions. With proper hormonal stimulation, this breed can reproduce effectively out of the natural estrous season. This allows for regulation of lambing periods and subsequent lactation, production planning, and more efficient utilization of genetic potential of high-yielding sires through artificial insemination. The obtained results are consistent with current trends in the modern sheep farming. Combination of hormonal protocols and

biotechnologies, such as artificial insemination, aimed at improving reproductive efficiency (Vallejo et al., 2019).

CONCLUSIONS

The applied synchronization protocol, consisting of 14-day intravaginal sponges containing 60 mg MPA and 250 IU PMSG at sponge removal, proved highly effective during the anestrus season, resulting in a conception rate of $81.1\% \pm 3.71$. The biological prolificacy (number of lambs per lambing ewe) reached 1.72 ± 0.063 , and 64.5% of all pregnancies were multiple, including 52% twin, 9.7% triplet, and 0.3% quadruplet lambings. The average number of lambs per ewe in the flock (prolificacy) was 1.34 ± 0.08 . Abortion rate was low (3.7%), suggesting effective pregnancy maintenance under the applied hormonal protocol.

The analysis showed that mating method significantly affected reproductive outcomes. A second mating (natural service) led to improved results, particularly in multiparous ewes, where conception increased from 71.2% to 97.14%, and in nulliparous ewes — from 74.07% to 81.82%. No significant differences were found between nulliparous and multiparous ewes in terms of overall reproductive performance, indicating that well-managed nulliparous ewes can be successfully bred during anestrus, achieving conception and prolificacy rates comparable to adult ewes.

These findings support the use of hormonal synchronization protocols as a reliable method for anestrus season breeding in Lacaune dairy ewes, facilitating year-round lambing and milk production planning in intensive sheep farming systems.

REFERENCES

- Abdalla, E. B., Farrag, B., Hashem, A. L. S., Khalil, F. A., & Abdel-Fattah, M. S. (2014). Effect of progestagen, PGF $_{2\alpha}$, PMSG and GnRH on estrus synchronization and some reproductive and productive traits in Barki ewes. *Journal of Agroalimentary Processes and Technologies*, 20(1), 93-101. <http://journalofagroalimentary.ro>
- Abecia, J. A., Forcada, F., & González-Bulnes, A. (2012). Hormonal control of reproduction in small ruminants. *Animal Reproduction Science*, 130(3–4), 173–179. <https://doi.org/10.1016/j.anireprosci.2012.01.011>
- Berean, D., Ergene, O., Blaga-Petrean, A., Bogdan, I., Ciupe, S., M. Cenariu, E. Páll, L.M. Bogdan (2021). Comparative data about estrus induction and pregnancy rate on Lacaune ewes in non-breeding season after melatonin implants and intravaginal progestagen. *Indian Journal of Animal Research*, 55(5), 517-521. <https://doi.org/10.18805/IJAR.B-1282>
- Blaschi, W., Lunardelli, P. A., Marinho, L. S., Max, M. C., Santos, G. M., Silva-Santos, K. C., Melo-Sterza, F. A., Baldassarre, H., Rigo, T. R., & Seneda, M. M. (2014). Effects of progestagen exposure duration on estrus synchronization and conception rates of crossbred ewes undergoing fixed time artificial insemination. *Journal of Veterinary Science*, 15(3), 433-437. <https://doi.org/10.4142/jvs.2014.15.3.433>
- Husein, M. Q., Ababneh, M. M., & 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. <https://doi.org/10.3844/ajavsp.2007.23.28>
- Koyuncu, M., & Altınçekiç, Ş. Ö. (2010). Effects of progestagen and PMSG on estrous synchronization and fertility in Kivircik ewes during natural breeding season. *Asian-Australasian Journal of Animal Sciences*, 23(3), 308-311.

<https://acikerisim.uludag.edu.tr/entities/publication/ee065a04-3271-44ae-af27-0c9d8f353e8a>

- Kuru, M., Boga Kuru, B., Sogukpinar, O., Cebi, Sen C., Oral, H., Kirmizibayrak, T. (2020). Oestrus Synchronisation with Progesterone-containing Sponge and Equine Chorionic Gonadotropin in Pirlak Ewes During the Non-breeding Season: Can Toryum Improve Fertility Parameters? *Journal of Veterinary Research*, 64 (4), 573-579. <https://doi.org/10.2478/jvetres-2020-0074>.
- Maslev, T., Hristova, T., & Stoycheva, S. (2010). Prouchvane na razlichni shemi za sinhronizatsiya na estrusa pri ovtse, Study of different protocols for estrus synchronization in sheep, *J. of Mountain Agric. on the Balkans*, 13(4), 864-870.
- Santos-Jiménez, Z., Martínez-Ros, P., Encinas, T., Morales-Cruz, J. L., Guerrero-Gallegos, H. Z., González-Avalos, R., González-Bulnes, A., & Guillén-Muñoz, J. M. (2022). Ovarian response and fertility after short-term progestagen/eCG treatments are compromised in nulliparous sheep during non-breeding season. *Veterinary Sciences*, 9(12), 663. <https://doi.org/10.3390/vetsci9120663>
- Sevov, S., Panayotov, D., Penchev, I., Kalaidzhiev, G., & Gogova, P. (2025a). A study on the milk productivity of sheep from the selection flocks of the Lacaune breed in Bulgaria. *Proceedings of the Scientific Conference with International Participation “30 Years Trakia University – Opportunities, Challenges, Achievements”*, May 16–17, 2025, Stara Zagora, Bulgaria.
- Sevov, S., Panayotov, D., Penchev, I., Kalaidzhiev, G., & Gogova, P. (2025b). A study on the slaughter performance of lambs of the Lacaune breed. *Proceedings of the Scientific Conference with International Participation “30 Years Trakia University – Opportunities, Challenges, Achievements”*, May 16–17, 2025, Stara Zagora, Bulgaria.
- Vallejo, D. A., Londoño, J. D., Yepes, Y. A., Tamayo, V., Mejía, A. F., & Maldonado, J. G. (2019). Pregnancy rates in hair sheep after Ovsynch synchronization and a combined intracervical fixed-time artificial insemination and 10-day mating period. *Veterinary World*, 12(11), 1779-1783. <https://doi.org/10.14202/vetworld.2019.1779-1783>
- Yu, X., Bai, Y., Yang, J., Zhao, X., Zhang, L., & Wang, J. (2022). Comparison of five protocols of estrous synchronization on reproductive performance of Hu sheep. *Frontiers in Veterinary Science*, 9, 843514. <https://doi.org/10.3389/fvets.2022.843514>
- Zlatarev, St. (2001). Biotehnologii za izkustveno osemenyavane na ovtse i kozi, [Biotechnologies for artificial insemination of sheep and goats]. Available online: <https://obuch.info/blgarska-akademiya-na-naukite-sekciya-reproduktivni-biotehnolo-v2.html> [in Bulgarian]