Agriculture and Food

AIMS Agriculture and Food, 9(2): 472–482.
DOI: 10.3934/agrfood.2024027
Received: 09 January 2024
Revised: 10 April 2024
Accepted: 10 April 2024
Published: 19 April 2024

http://www.aimspress.com/journal/agriculture

Research article

Effects of different reproduction management protocols on the reproduction efficiency of three indigenous Greek sheep breeds

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Abstract: The aim of this study was to evaluate the effects of two different methods of controlling ovine reproduction (ram effect and a combination of eco-friendly substances) in three Greek indigenous sheep breeds, namely Florina, Karagouniko, and Chios. 180 ewes, aged 2–3 years old, were used, equally divided between the three Greek sheep breeds. Each breed was divided in two equal experimental groups: In group ECO (combination of eco-friendly substances), a dose of Gonadotropin-releasing hormone (GnRH) was administered at day 0, followed by an intramuscular injection of prostaglandin seven days later and another dose of GnRH at day 9. In group ME, sexually active males were introduced to the females that have been isolated for 3 months. The experiment was conducted in three consecutive years: 2020, 2021, and 2022. Average estrus expression and fecundity in all three examined breeds was comparable, with the exemption of Florina ewes treated with the eco-friendly substances in the first year, a result that could be attributed to the strong seasonal pattern in estrus expression of the Florina breed. The average length to estrus expression in days was significantly lower in the eco-friendly method than the procedure using male effect and the prolificacy was higher for the Chios sheep than the Florina and the Karagouniko breeds. Overall, both methods for the induction and synchronization of estrus (male effect and PGF2a administration) could be used in sheep reproduction with promising results. Nevertheless, the male effect seems to be more efficient in indigenous breeds with long anestrus periods.

Keywords: sheep; reproduction; prostaglandin; male effect

1. Introduction

Among farm animals, ruminants are of particular interest because they can produce food on non-arable lands and transform resources not used for human consumption, such as grass and fodder, into edible products [1]. Small ruminants like sheep and goats in particular, enact a crucial role for the economy of millions of people worldwide, providing meat, milk, skin, wool, and fiber for centuries [2]. Small ruminants have been extremely important specifically for the Mediterranean region, representing the only region in the world where they account for roughly one-third of the total ruminant population [3].

Traditionally, sheep farming has been the most important sector of Greek livestock farming, mainly due to environmental, social, religious, and economic factors. According to Eurostat, in 2020, the population of sheep reared in Greece was approximately 7.7 million head, occupying the 4th position amongst the European Union’s sheep flocks[4]. Sheep are multi-purpose animals and can utilize the poor forages of the mountainous regions [5], making them the ideal animal for farming in many marginal communities throughout Greece.

In contrast to northern Europe, the main food product of sheep in Greece is milk instead of meat. In 2020, according to Food and Agriculture Organization of the United Nations (FAO), Greece was the top sheep milk producer in Europe (almost 950.000 tons), being the only European country producing more ovine than cow's milk [6]. Most of this milk is designated for cheese production, since Greece possesses 23 protected designations of origin (PDO) cheeses, with the frontline being feta cheese.

The Greek sheep farming is the predominant traditional livestock production system, adapted to the special characteristics of the regional microclimate. It is performed in lands that are unsuitable for cultivation or any other agricultural operation [7]. The farms are extensive, small-sized and family-owned, with a low level of mechanization in most cases [8]. Traditional breeding and management practices are followed resulting to a pronounced seasonal character of a 5–6 months productive duration. Specifically, the interval for sheep reproduction lies between September and mid-March [9], reducing the availability of fresh milk in some periods of the year.

Naturally, sheep in temperate climates present a seasonal character of breeding, controlled mainly by the photoperiodism [9]. Consequently, most of the milk production is concentrated over a period of a few months (early winter to late spring), and after the milk production ends, the animals enter a non-productive period (anestrus/anovulatory period). Although this natural mechanism was induced to protect offspring from the winter, it creates an organizational challenge to both farmers and industry as the spotted differences in production over the year are uneconomical.

Many strategies have been used to either premature or to extend the reproduction period, including the *ram effect* and the use of exogenous hormones (mainly progestogen sponges or melatonin implants) [6]. In general, the hormonal treatments are considered practical and easy-to-use but raise concerns lately, regarding their effects on human health and their environmental footprint [10]. With respect to these concerns, a more eco-friendly procedure has also been used to control the induction of ovulation and the synchronization of estrus in sheep [11]. The main component of this method is prostaglandin (PGF2a), a luteolytic agent that can be easily administered by intramuscular injection and is metabolized by the lung [12]. In accordance with male effect, the use of PGF2a is also considered a clean and ethical method [13] but its effectiveness depends on the presence of a corpus luteum, and thus it has to be applied during the breeding season [14]. More recent data support that marker or genotype-assisted selection schemes could result to animals that are less inclined to the reproduction seasonality [15].

In Greece, the ram effect and the use of exogenous hormones are the two main strategies used for the synchronization of estrus in sheep. The PGF2a method is either not well known among farmers or considered to be less efficient than the other two [16]. Therefore, the scope of the present study was the evaluation of the effects of two different methods/treatments of controlling ovine reproduction (ram effect and a combination of eco-friendly substances) in three Greek indigenous sheep breeds, namely Florina, Karagouniko, and Chios.

2. Materials and methods

2.1. Ethics approval of research

All the experiments were conducted according to Directive 2010/63/EU (http://data.europa.eu/eli/dir/2010/63/2019-06-26) on the protection of animals used for scientific purposes.

2.2. Breeds' description

Chios sheep breed is one of the most productive dairy-purposed Greek breeds with an annual milk production of 180–250 kg. The average bodyweight of adult rams and ewes is about 80 and 55 Kg, respectively, while the mean prolificacy is about 200 offsprings per birth [17].

The Florina sheep breed is characterized as a dual-purpose animal (i.e., reared for both milk and meat). The average milk yield per ewe and season is around 110 Kg while the average bodyweight of adult rams and ewes is about 82.5 and 60 Kg, respectively [18]. The Florina breed expresses estrus with an intense seasonal pattern, namely from mid-summer up to late autumn, whereas Chios reproduction, although reproductively seasonal too, is influenced to a lesser extent by seasonality, expressing estrus earlier in spring and early summer as well, hence almost throughout the year.

Finally, Karagouniko sheep, originating from Thessaly, Greece, is a breed mostly reared for milk production and one of the most bred breeds in Greece. Mature ewes weigh approximately 65 kg and mature rams weigh approximately 75 kg. Their milk production is approximately 190 kg annually and their multiplicity is 1.3. Like Florina, Karagouniko is characterized by intense reproduction seasonality levels.

2.3. Experimental design

The experimental procedures were carried out in the summer of three consecutive years i.e., 2020, 2021 and 2022. 180 ewes were used, equally divided between the three Greek sheep breeds, Chios breed (n = 60), Florina breed (n = 60), and Karagouniko (n = 60). The ewes were 2–3 years old, with the two formers (Chios and Florina) bred at the Research Institute of Animal Science of the Hellenic Agricultural Organization ELGO-DEMETER in Paralimni Giannitson and the latter (Karagouniko) in a commercial farm in Karditsa (Central Greece). The selection of the animals participating in the experiments was based on the pedigree data of the Institute, dated 20 years ago, to avoid using animals that were relatives. Similarly, regarding the commercial farm (i.e. a well-organized flock), special attention was given by the farmer to avoid closely related animals' mattings.

Each breed was divided into two experimental groups of 30 ewes each, and each group to 3 sub-groups of 10 ewes each, with 1 ram assigned at each sub-group. In group ECO, a dose of GnRH (Receptal®, MSD Animal Health, UK) was administered at day 0, followed by an intramuscular injection of prostaglandin (EstrumateTM, MSD Animal Health, UK) seven days later and another dose of GnRH at day 9. In group ME , sexually active males were introduced to the females that had been isolated for 3 months by visual contact from males at a geographic distance more than 500 m. After the treatments, female and male sheep remained together for two more months to identify return to estrus.

All ewes were in good health, in anestrus period, and at the same lactation period. They were kept inside the barn for the whole duration of the experiment, in pens measuring approximately 25 m^2 per group of 10 ewes and were fed with the same ration. The ration consisted of alfalfa hay, wheat straw, and concentrate. The average amount of each feedstuff was 0.2, 1.0, and 1.5 kg/ewe/day for the wheat straw, alfalfa hay, and concentrates, respectively. The concentrates consisted of maize grain, barley, wheat middling, sunflower meal, soybean meal, and mineral and vitamin premix. The average feed offered (g/ewe/day) and concentrate ingredients (g/kg) of the experimental groups are presented in Table 1.

Table 1. Average feed offered (g/ewe/day) and concentrate ingredients (g/kg) of the sheep breeds throughout the experimental period.

	Florina	Chios	Karagouniko
Average feed offered (g/ewe/day)			
Wheat straw	200	200	200
Alfalfa hay	1000	1000	1000
Concentrate	1500	1500	1500
Concentrate ingredients (g/kg)			
Maize	344	344	344
Barley	200	200	200
Wheat middling	100	100	100
Sunflower meal	160	160	160
Soyabean meal	155	155	155
Premix vitamins and minerals	41	41	41

For each year of the experiment, a different set of ewes was used. The experimental design with the respective days of application of each treatment is presented in detail in Table 2.

2.4. Measurements

The traits of reproduction that were estimated in all groups of ewes were: (1) the percentage of ewes that expressed estrus, (2) the days after male introduction that each female expressed estrus, (3) the percentage of females that gave birth (fecundity), (4) the gestation length. and (5) the number of offsprings per female (prolificacy). Traits 1 and 2 were estimated through visual observation of the receptibility of the ewes to adult, fertile rams that have been used in previous mattings.

2.5. Statistical analysis

The percentages of females that expressed estrus and gave birth were analyzed using the chi-square test. The days after males' introduction that each female expressed estrus, the gestation length and the prolificacy were submitted to a multivariate ANOVA treating the breed and the treatment as fixed factors. Pair wise comparisons were tested at 0.05 significance level with Tukey's honest test and the results are presented as least square means ± standard error of estimation (pooled SEM). All statistical analyses were performed using STAT GRAPHICS software.

Table 2. Annual experimental design.

	Year 1 (2020)		Year 2 (2021)		Year 3 (2022)	
Groups	ME	ECO	ME	ECO	ME	ECO
Male introduction	15/06/2020	-	22/07/2021	-	20/07/2022	-
1st dose of GNRH	-	30/06/2020	-	22/07/2021	-	20/07/2022
PGF2a	-	07/07/2020	-	29/07/2021	-	27/07/2022
2nd dose of GNRH	-	09/07/2020	-	31/07/2021	-	29/07/2022
Male introduction	-	10/07/2020	-	01/08/2021	-	30/07/2022
Male separation	03/08/2020	03/08/2020	16/09/2021	16/09/2021	20/09/2022	20/09/2022

3. Results

3.1. Breed x treatment interaction per year

The results of the three consecutive years of the experiment are presented in Table 3. For the first year, when using the male effect, 93% of Florina ewes, 95% of Chios ewes, and 98% of the Karagouniko ewes expressed estrus and 90%, 93.3%, and 94% of them gave birth, respectively. The average days after male introduction for estrus expression was 20.2 days for the Florina and 19.6 days for the Karagouniko, while for the Chios, the respective figure was smaller (13.3 days, $P < 0.05$). Gestation length was not different between groups while the Chios ewes had higher prolificacy than the other two breeds (1.89 for Chios, 1.44 for Florina, and 1.53 for Karagouniko). After using the eco-friendly substances, the Florina and the Karagouniko ewes expressed estrus and gave birth at significantly lower proportions than the Chios ($P < 0.05$). Gestation length was not different between groups while the same applied also for the prolificacy (Table 3).

In the second year of the experiment, for both treatments (male effect vs use of eco-friendly substances) there were no significant differences between the breeds in terms of estrus expression and fecundity (Table 3). Estrus expression in days was comparable between the groups for each treatment but significantly less for the use of eco-friendly substances in contrast to male effect. Gestation length was the same irrespective of breed or treatment while prolificacy was higher for the Chios in both treatment ($P < 0.05$).

Comparably to the second year of the experiment, in the third year, there were no differences between the breeds in estrus expression, fecundity, and gestation length, irrespective to the treatment. The days to estrus expression were fewer for the use of ecofriendly substances in comparison to the male effect in both breeds ($P < 0.05$) while prolificacy was higher for the Chios breed, especially for the male effect.

Table 3. Reproduction traits of Florina, Chios, and Karagouniko ewes after male effect (ME) or the use of ecofriendly substances (ECO) per year of the experiment.

	Florina		Chios		Karagouniko			
	ME	ECO	ME	ECO	ME	ECO	SEM	P-value
1st year (2020)								
Estrus expression (%)	93.0[a]	65.0[b]	95.0[a]	85.0[a]	98.0[a]	68.0[a]	-	0.001
Estrus expression (days)*	20.2[a]	3.1[c]	13.3[b]	2.7[c]	19.6[a]	2.8[c]	1.3	0.000
Fecundity (%)	90.0[a]	56.7[b]	93.3[a]	83.0[a]	94.0[a]	54.0[b]	-	0.002
Gestation length (days)	150.7	153.2	151.4	152.7	151.3	152.9	0.9	0.081
Prolificacy	1.44[c]	1.59[ab]	1.89[a]	1.82[a]	1.53[b]	1.68[b]	0.14	0.033
2nd year (2021)								
Estrus expression (%)	93.0	80.0	95.0	83.0	98.0	82.0	-	0.258
Estrus expression (days)*	15.4[a]	1.9[b]	15.0[a]	3.0[b]	14.3[a]	2.3[b]	1.1	0.000
Fecundity (%)	90.0	70.0	90.0	80.0	92.0	70.0	-	0.126
Gestation length (days)	151.3	151.0	149.4	152.3	151.5	151.8	0.7	0.653
Prolificacy	1.40[b]	1.29[b]	1.96[a]	1.92[a]	1.49[b]	1.33[b]	0.12	0.001
3rd year (2022)								
Estrus expression (%)	90.0	97.0	95.0	93.0	96.0	93.0	-	0.654
Estrus expression (days)*	16.6[a]	2.6[b]	13.0[a]	4.0[b]	15.5[a]	2.9[b]	1.2	0.000
Fecundity (%)	86.7	96.7	93.3	90.0	88.5	93.5	-	0.536
Gestation length (days)	149.9	151.5	148.5	152.0	150.8	152.1	0.9	0.635
Prolificacy	1.42[b]	1.66[ab]	2.00[a]	2.07[a]	1.44[b]	1.68[ab]	0.14	0.006

[a,b] Means in a row with different superscripts differ significantly ($P < 0.05$). * Estrus expression in days after male induction.

3.2. Factorial analysis

Table 4 presents a factorial analysis of reproduction traits of Florina, Chios, and Karagouniko ewes after male effect or the use of ecofriendly substances. The breed had no effect on the proportion of estrus expression, fecundity and the gestation length. On the other hand, Chios ewes expressed estrus in fewer days than the Florina ones, with the Karagouniko ewes presenting intermediate values ($P < 0.05$). At the same time, Chios ewes were more prolific than the other two breeds.

The use of ecofriendly substances significantly lowered the days from male induction to estrus expression, an observation that was evident in all three trials of the experiment. Moreover, it lowered the proportion of estrus expression as well as the fecundity of the ewes while it had no effect on gestation length and the prolificacy of the ewes ($P < 0.05$).

Finally, the trial (year) did not affect estrus expression in days, gestation length, or prolificacy. Nevertheless, in the third trial (2022), the ewes had higher proportion of estrus expression and fecundity than in the first trial (2020), while the second trial (2021) presented intermediate values ($P < 0.05$).

Table 4. Factorial analysis of reproduction traits of Florina, Chios, and Karagouniko ewes after male effect (ME) or the use of ecofriendly substances (ECO).

	Estrus expression (%)	Estrus expression (days)	Fecundity (%)	Gestation length (days)	Prolificacy
Breed					
Florina	86.7	10.1^{a}	81.7	151.5	1.52^{b}
Chios	92.2	8.4^{b}	88.3	151.5	1.95^{a}
Karagouniko	89.4	9.6^{ab}	82.2	151.9	1.53^{b}
SEM	-	0.5	-	0.3	0.06
Treatment					
ME	95.2^{a}	15.5^{a}	91.1^{a}	152.8	1.73
ECO	83.7^{b}	3.0^{b}	77.0^{b}	151.2	1.74
SEM	-	0.5	-	0.3	0.06
Year					
2020	84.4^{a}	9.9	78.3^{b}	152.0	1.68
2021	88.9^{ab}	8.8	82.2^{ab}	151.2	1.73
2022	95.0^{b}	9.0	91.7^{a}	151.2	1.80
SEM	-	0.6	-	0.4	0.07
P-value					
Breed	0.230	0.012	0.159	0.975	0.000
Treatment	0.000	0.000	0.000	0.864	0.881
Year	0.005	0.349	0.002	0.190	0.479

4. Discussion

Sheep farming in most Mediterranean countries is performed using indigenous breeds, a practice that enhances the conservation efforts of precious genetic resources and is very important for regional development [19]. Nevertheless, this practice is also associated with long anestrus periods resulting in production of dairy products only in certain times of the year [20]. In this study, an evaluation of two methods for estrus induction was performed (male effect and the use of eco-friendly substances) to investigate two Greek breeds with a strict seasonal reproductive pattern (i.e., Florina and Karagouniko) and one that is considered non-seasonal with an extensive breeding period throughout the year (Chios).

The male effect is a long-established animal manipulation method used for the induction of ovulation and the synchronization of estrus in sheep and goats [21]. It is considered clean, green, and ethical because it omits the usage of any drugs or hormones, it is environmentally friendly and does not interfere with animal welfare [22]. Its effect, however, is regulated by the depth of anestrus of ewes [23]. In most cases, higher percentages of success have been reported in breeds with short anestrus periods, like the Merino [24] or the Chios breed used in this study. In breeds with long anestrus periods, the success of the ram effect is limited when applied long before the normal breeding season and it increases in a period 2–4 weeks before the normal breeding season [23]. This was very effectively evaluated in the work of Nugent et al. [25], where Dorset ewes responded more efficiently to the effect than Hampshire ewes, and this would have also been the case for the Florina and Karagouniko breeds utilized in this study if the rams were introduced to the females earlier in spring.

The use of PGF2a is also a well-established method for the induction of ovulation and the synchronization of estrus in sheep and goats [26,27], but despite its obvious advantages in terms of environmental sustainability, it is not commonly used in Greece. The reasons for this are mainly the lack of knowledge of Greek farmers, the ability of PGF2a to provoke termination of gestation in pregnant ewes, when insufficient management data are kept, as well as the fact that it must be applied during the breeding season, while most indigenous Greek sheep breeds have large anestrus periods [16]. In the tropics, where the breeding season is continuous, early reports have demonstrated the ability of PGF2a to control the induction of ovulation in ewes [28,29] and their results were also confirmed in the more recent works of Fierro et al. [30] and Fierro, Viñoles and Olivera-Muzante [31].

In this study, the average estrus expression and fecundity in all three examined breeds was comparable, with the exemption of Florina and Karagouniko ewes treated with the eco-friendly substances in the first year, in mid-June. This phenomenon could be attributed to the strong seasonal pattern in estrus expression of these breeds, which are usually bred from mid-summer up to late autumn [19]. These results have also been reflected in the factorial analysis, where in the first trial lower proportion of estrus expression and fecundity were found, as well as in the effect of the treatment, since in the next two years of the experiment, estrus expression and fecundity were comparable among the experimental groups. In terms of the average fecundity, similar proportions have been reported for Florina by Michailidis et al. [32], for Chios by Banos and Avdi [33] and for Karagouniko by Kouimtzis et al. [34].

The average length to estrus expression was significantly lower in the eco-friendly method than the procedure using male effect. After PGF2a administration, the ewes express estrus within 5 days while when using the male effect there are usually two picks in estrus expression among the herd, one at approximately day 18 and one around day 24 [23]. In this study, for the Florina breed, 48% of the animals expressed estrus 14–19 days after the ram induction for year one and 37% of the animals between days 20 and 29. For the second and third year of the experiment, the numbers are 70% and 19% and 54% and 31%, respectively. For Chios, in all three years of the experiment, about 50% of the ewes expressed estrus 14–19 days after ram induction and the about 20% between days 20 and 29.

According to the results of this study, the Chios sheep is much more prolific than the Florina and the Karagouniko, a result that was evident in the factorial analysis. This is a common observation and is reported by many authors [32,35]. In fact, the mean prolificacy of the Chios breed is between 1.85 and 2.00, being one of the most prolific Greek breeds, with twin lambing happening very often [36]. On the other hand, the Florina breed is categorized in the least prolific Greek breeds, with the majority of parturitions resulting in a single offspring [37].

5. Conclusions

With respect to our results, both methods for the induction and synchronization of estrus (male effect and PGF2a administration) could be used in sheep reproduction with promising results. Nevertheless, the male effect seems to be more efficient in indigenous breeds with long anestrus periods. In contrast to the hormonal methods that use progestogen sponges or melatonin implants, the male effect and PGF2a administration methods are more environmentally sustainable and therefore should be preferred. Ongoing research supports the fact that marker or genotype-assisted selection schemes could result in animals that are less inclined to the reproduction seasonality, and thus exogenous methods of reproductive manipulation are less needed.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Acknowledgments

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 'First Call for HFRI Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant' (Project Number: HFRI-FM17- 2987).

Conflict of interest

The authors stated that have no conflict of interest to declare.

References

1. Soussana JF, Tichit M, Lecomte P, et al. (2015) Agroecology: Integration with Livestock. In: *Agroecology for food security and nutrition proceedings of the FAO international symposium 18-19 September 2014,* Rome, Italy, 225–249.
2. Al-Dawood A (2016) Towards heat stress management in small ruminants—A review. *Annals Animal Sci* 17: 59–88.
3. Dubeuf JP, Hassan AA, Chentouf M, et al. (2015) The Mediterranean sheep and goat sectors between constants and changes over the last decade. Future challenges and prospects. In: *FAO-CIHEAM Network for Research and Development in Sheep and Goats* (No. 115, pp. 711). Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM).
4. Agriculture, forestry and fishery statistics—2020 edition. Available from: https://ec.europa.eu/eurostat/web/products-statistical-books/-/ks-fk-20-001.
5. Zygoyiannis D (2006) Sheep production in the world and in Greece. *Small Ruminant Res* 62: 143–147. https://doi.org/10.1016/j.smallrumres.2005.07.043
6. Lianou DT, Vasileiou NGC, Michael CK, et al. (2022) Patterns of reproductive management in sheep and goat farms in Greece. *Animals* 12: 3455. https://doi.org/10.3390/ani12243455
7. Degen A (2007) Sheep and goat milk in pastoral societies. *Small Ruminant Res* 68: 7–19. https://doi.org/10.1016/j.smallrumres.2006.09.020
8. Manolopoulou E, Aktypis A, Matara C, et al. (2018) An overview of sheep farming features and management practices in the region of south western Peloponnese and how they reflect on milk microbial load. *J Hell Vet Med Soc* 69: 759–770. https://doi.org/10.12681/jhvms.16421
9. Avdi M, Banos G, Kouttos A, et al. (2003) Sources of variation and genetic profile of spontaneous, out-of-season ovulatory activity in the Chios sheep. *Genet Sel Evol* 35: 65. https://doi.org/10.1186/1297-9686-35-1-65

10. Dardente H, Lomet D, Robert V, et al. (2016) Seasonal breeding in mammals: From basic science to applications and back. *Theriogenology* 86: 324–332. https://doi.org/10.1016/j.theriogenology.2016.04.045
11. Deligiannis C, Valasi I, Rekkas CA, et al. (2005) Synchronization of ovulation and fixed time intrauterine insemination in ewes. *Reprod Domest Anim* 40: 6–10. https://doi.org/10.1111/j.1439-0531.2004.00534.x
12. Light JE, Silvia WJ, Reid RC (1994) Luteolytic effect of prostaglandin F2 alpha and two metabolites in ewes. *J Anim Sci* 72: 2718–2721. https://doi.org/10.2527/1994.72102718x
13. Contreras-Solis I, Vasquez B, Diaz T, et al. (2009) Efficiency of estrous synchronization in tropical sheep by combining short-interval cloprostenol-based protocols and "male effect". *Theriogenology* 71: 1018–1025. https://doi.org/10.1016/j.theriogenology.2008.11.004
14. Acritopoulou S, Haresign W, Foster JP, et al. (1977) Plasma progesterone and LH concentrations in ewes after injection of an analogue of prostaglandin F-2alpha. *J Reprod Fertil* 49: 337–340. https://doi.org/10.1530/jrf.0.0490337
15. Giantsis IA, Laliotis GP, Stoupa O, et al. (2016) Polymorphism of the melatonin receptor 1A (MNTR1A) gene and association with seasonality of reproductive activity in a local Greek sheep breed. *J Biol Res (Thessalon)* 23: 9. https://doi.org/10.1186/s40709-016-0050-y
16. Samartzi F, Fthenakis GC (2003) Control of oestrous cycle in small ruminants. *J Hell Vet Med Soc* 54: 351–361. https://doi.org/10.12681/jhvms.15345
17. Gelasakis AI, Valergakis GE, Fortomaris P et al. (2010) Farm conditions and production methods in Chios sheep flocks. *J Hell Vet Med Soc* 61: 111–119. https://doi.org/10.12681/jhvms.14880
18. Christodoulou V, Bampidis VA, Sossidou E, et al. (2007) Evaluation of Florina (Pelagonia) sheep breed for growth and carcass traits. *Small Ruminant Res* 70: 239–247. https://doi.org/10.1016/j.smallrumres.2006.03.010
19. Antonopoulou D, Giantsis I, Symeon G, et al. (2023) Association of MTNR1A and GDF9 gene allelles with the reproductive performance, response to oestrus induction treatments and prolificacy, in improved and non-improved local indigenous sheep breeds. *Reprod Domest Anim* 58: 1532–1541. https://doi.org/10.1111/rda.14468
20. Tampaki M, Koutouzidou G, Ragkos A, et al. (2022) Eco-value and public perceptions for indigenous farm animal breeds and local plant varieties, focusing on Greece. *Sustainability* 14: 11211. https://doi.org/10.3390/su141811211
21. Delgadillo JA, Gelez H, Ungerfeld R, et al. (2009) The 'male effect' in sheep and goats—Revisiting the dogmas. *Behav Brain Res* 200: 304–314. https://doi.org/10.1016/j.bbr.2009.02.004
22. Martin G, Kadokawa H (2006) 'Clean, Green and Ethical' Animal Production. Case Study: Reproductive Efficiency in Small Ruminants. *J Reprod Dev* 52: 145–152. https://doi.org/10.1262/jrd.17086-2
23. Rosa HJD, Bryant MJ (2002) The 'ram effect' as a way of modifying the reproductive activity in the ewe. *Small Ruminant Res* 45: 1–16. https://doi.org/10.1016/S0921-4488(02)00107-4
24. Martin GB, Oldham CM, Cognié Y, et al. (1986) The physiological responses of anovulatory ewes to the introduction of rams—A review. *Livest Prod Sci* 15: 219–247. https://doi.org/10.1016/0301-6226(86)90031-X
25. Nugent RA III, Notter DR, Beal WE (1988) Effects of ewe breed and ram exposure on estrous behavior in May and June. *J Anim Sci* 66: 1363–1370. https://doi.org/10.2527/jas1988.6661363x

26. Lymberopoulos AG, Boscos CM, Dellis S, et al. (2002) Oestrous synchronization under range conditions in dairy goats treated with different PGF2α doses during the transitional period in Greece. *Anim Sci* 75: 289–294. https://doi.org/10.1017/S1357729800053042
27. Al-Merestani M, Zarkawi M, Wardeh M (2003) Improving the reproductive efficiency, pregnancy diagnosis and monitoring the resumption of luteal activity in indigenous Damascus goats. *Reprod Domest Anim* 38: 36–40. https://doi.org/10.1046/j.1439-0531.2003.00394.x
28. Godfrey RW, Gray ML, Collins JR (1997) A comparison of two methods of oestrous synchronisation of hair sheep in the tropics. *Anim Reprod Sci* 47: 99–106. https://doi.org/10.1016/S0378-4320(97)00007-9
29. Godfrey RW, Collins JR, Hensley EL, et al. (1999) Estrus synchronization and artificial insemination of hair sheep ewes in the tropics. *Theriogenology* 51: 985–997. https://doi.org/10.1016/S0093-691X(99)00044-8
30. Fierro S, Olivera-Muzante J, Gil J, et al. (2011) Effects of prostaglandin administration on ovarian follicular dynamics, conception, prolificacy, and fecundity in sheep. *Theriogenology* 76: 630–639. https://doi.org/10.1016/j.theriogenology.2011.03.016
31. Fierro S, Viñoles C, Olivera-Muzante J (2017) Long term prostaglandin based-protocols improve the reproductive performance after timed artificial insemination in sheep. *Theriogenology* 90: 109–113. https://doi.org/10.1016/j.theriogenology.2016.11.031
32. Michailidis G, Pappa V, Avdi M (2008) Reproductive performance and investigation of BMPR-IB and BMP-15 gene mutation in Greek Chios and Florina sheep breeds. *Arch Zootech* 11: 24–31.
33. Banos G, Avdi M (2003) Relationship between spontaneous, out-of-season ovulatory activity and other reproductive characteristics of Chios sheep. *Livest Prod Sci* 83: 205–210. https://doi.org/10.1016/S0301-6226(03)00097-6
34. Kouimtzis SA, Belibasaki S, Doney JM (1989) Melatonin advances and condenses the onset of seasonal breeding in Greek dairy ewes. *Anim Sci* 48: 399–405. https://doi.org/10.1017/S0003356100040393
35. Theodoridis A, Ragkos A, Rose G, et al. (2018) Defining the breeding goal for a sheep breed including production and functional traits using market data. *Animal* 12: 1508–1515. https://doi.org/10.1017/S1751731117003007
36. Pappa-Michailidou V, Avdi M, Zafrakas A, et al. (1999) Prepubertal plasma FSH concentrations and their relationship with reproductive performance in three Greek breeds of sheep. *Small Ruminant Res* 33: 37–41. https://doi.org/10.1016/S0921-4488(98)00197-7
37. Triantafillidis D, Ligda C, Georgoudis A, et al. (1997) The Florina (Pellagonia) sheep breed. *Anim Genet Resour* 22: 7–13. https://doi.org/10.1017/S1014233900000961