



SYNCHRONIZATION PROTOCOLS IN SPANISH MERINO SHEEP: REDUCTION IN TIME TO ESTRUS BY THE ADDITION OF ECG TO A PROGESTERONE-BASED ESTRUS SYNCHRONIZATION PROTOCOL

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Abstract

An appropriate management of reproduction allows ewes to breed at a more intensive rhythm, more compatible with economic efficiency of farms. Hence, the objectives of the present work were to evaluate a traditional estrus synchronization protocol (based on medroxyprogesterone acetate-impregnated intravaginal sponges combined or not with equine chorionic gonadotropin) on a Merino sheep herd during the breeding season, in order to determine the best working protocol for this breed. A total of 277 sheep, blocked by age (200 ewes and 77 ewe lambs) were randomly allocated to four groups, two of ewes (E and Ee) and two of ewe lambs (L and Le). All females received 60 mg MAP (medroxyprogesterone acetate) sponges on the first day. At the day of removal the animals of groups Ee and Le received 450 IU of eCG (equine chorionic gonadotropin). Mean estrus synchronization rate was 92.06% and similar among groups. Time to estrus was shorter for Ee (46.93±12.44 h) and Le (44.34±10.76 h), when compared to treatments without eCG (60.60±20.46 h and 58.68±17.72 h for E and L, respectively; $P < 0.05$ for both). Pregnancy rates were 71.35 and 72.86% for ewes and ewe lambs, respectively, and similar among groups. Lambing rates were lower in ewes (1.36) than in ewe lambs (1.85) with no significant effect of eCG. The study demonstrates the efficacy of the synchronization method with MAP and the reduction in the time to estrus induced by eCG in Spanish Merino ewes and ewe lambs during the breeding season.

Key words: Spanish Merino sheep, age, estrus synchronization, reproductive response, eCG

In the last decades different reproductive control methods have been developed, allowing significant progress improving the production of commercial herds. From the research conducted by Robinson (1965), different synthetic progesterone ana-

logues are used in the regulation of the estrous cycle in small ruminants during or outside of the breeding season (Titi et al., 2010; Awel et al., 2009; Martemucci and D'Alessandro, 2011; Abecia et al., 2012). The most used protocol is based on intra-vaginal sponges impregnated with medroxyprogesterone acetate (MAP) and fluoro-progesterone acetate (FGA) during 12 or 14 days, followed by the administration of equine chorionic gonadotropin (eCG) at device withdrawal (Wildeus, 2000; Boscós et al., 2002; Quintero-Elisea et al., 2011).

In numerous studies in small ruminants a high degree of estrus synchronization has been reported (>80%) with the use of 60 mg-MAP impregnated sponges (Wil-deus, 2000; Simonetti et al., 2002; Dogan and Nur, 2006). Indeed, the distribution of the time to estrus obtained varies depending on whether eCG is included or not and on animal category (Das et al., 2000; Viñoles et al., 2001; Dogan and Nur, 2006). When fertility from natural estrus is compared to that induced by progestagens, reports show lower results which are attributed to different causes, such as alterations in ovulation quality, hormonal release patterns and sperm transport (Simonetti et al., 2000; Ungerfeld and Rubianes, 2002; Zeleke et al., 2005). Nevertheless, in fixed-time artificial insemination systems, estrus and ovulations outside normal ranges (50–60 h) are not usually accounted for and, as a consequence, a certain percentage of females are actually not fertilized (Cline et al., 2001; Dogan and Nur, 2006; Olivera-Muzante et al., 2011).

The use of eCG aims to improve estrus synchronization, follicular maturation, ovulation rate and fertility, with doses ranging from 300 to 600 IU, according to breed, animal weight and time of the year (Boscós et al., 2002; Dogan and Nur, 2006; Olivera-Muzante et al., 2011). Additionally, a superovulatory effect has been described on the induced estrus and the consequent prolificacy increment (Zeleke et al., 2005; Azawi et al., 2010; Quintero-Elisea et al., 2011). The efficacy of treatments depends on extrinsic and intrinsic factors. Extrinsic factors include the active principle of the drug, time of the year in which treatment is applied, the dose applied, duration and protocol design (Moeini et al., 2007; Knights et al., 2011; McCap-pin and Murray, 2011). Intrinsic factors include breed and age. All of these factors, whether isolated or as a group, define the ovarian status at the moment of applying the synchronization protocols and, as a consequence, the responses (Viñoles et al., 2005; Karetá et al., 2006; Scaramuzzi et al., 2006).

Merinos are the most internationally known Iberian sheep. The Merino sheep originated in the Iberian Peninsula and shows a mixed aptitude, which currently produces the milk used to prepare high quality cheese ("Torta del Casar", "Queso de la Serena" and "Queso de los Pedroches") whose production is strictly regulated by legislation protecting Designation of Origin. Merinos on the other hand are characterized by their fine wool (Pedrosa et al., 2007) and by their high quality meat lambs named "Cordero de Extremadura, CORDEREX" (Cambero, 1999; Juárez et al., 2007). The differences in reproductive issues among sheep breeds are well known (Ranilla et al., 1994). The different responses to the different reproductive hormones used normally for synchronization have been described (Abecia et al., 2011, 2012). Likewise, it is well known that there is a clear link between reproductive and productive performance in all animal production species (El-Saied et al., 2006). Hence,

for producers exploiting this old and highly genetically valuable ovine breed it is important to optimize efficiency in order to guarantee sustainability of their farms.

Therefore, the objective of the present study was to determine estrus occurrence and distribution after a traditional estrus synchronization protocol (MAP intravaginal sponge with or without eCG) in a herd of Spanish Merino, controlling the age of the ewes, in order to determine the best synchronization methods specifically for this breed.

Material and methods

Experimental animals

A total of 200 ewes, 2.5 to 5.5 years old, weighing between 45 and 55 ± 9.70 kg, body condition scores (BCS) between 3.0 and 3.5 on a 1–5 scale (Russel, 1991) and 77 ewe lambs, 12 to 15 months old, $40\text{--}45 \pm 8.92$ kg with a similar BCS, sexually mature and clinically healthy of the Spanish Merino breed, were used. In this study animals were housed in one flock in the facilities of a private farm located in Écija (latitude $5^{\circ}04'45''\text{O}$, longitude $37^{\circ}32'28''\text{N}$) in Seville, Spain, during November which implied a median length of 10.06 hours a day, with a decreasing rate of the photoperiod of 1.73 min/day until the 21st of December. The animals were managed under the same conditions. They were fed with 600 g/head/day pelleted concentrate mixture with 14% crude protein (corn, wheat bran and unhulled cotton seed) and clover hay and wheat straw were provided. Water and a mineral salt lick were provided *ad libitum*. The management of the animals did not change throughout the entire experimental period.

The experiment was carried out according to the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 86/609 about the protection of animals used in experimentation. To visualize the reproductive health status in the ewes and the sexual maturity in ewe lambs, females were scanned by transrectal ultrasonography with an 8 MHz linear probe (Aquila, Esaote-Pie Medical, Maastricht, The Netherlands). Ewe lambs with ovaries with corpora lutea were considered sexually mature. Twenty-eight Merino rams, sexually mature with proven fertility, clinically healthy, ages between 2.5 and 4.0 years old, and weighing between 55 and 65 kg were used.

Estrus synchronization

Ewes and ewe lambs were treated with 60 mg-MAP intravaginal sponges (Sin-crogest®, Ovejero Lab, Leon, Spain). Prior to insertion, sponges were impregnated with antibiotics (framicetin sulphate 40 mg and sulathiazol 960 mg, Ovejero Lab, Leon, Spain) and inserted in the vagina after thorough cleaning of the vulvar perineum. Sponges were placed into the vaginal canal as far as possible by means of an applicator previously disinfected with a 5% solution of polvidine (betadine, Meda Pharma SA, Spain). Sponges remained in the females until day 12 at which time an injection of 450 IU of eCG (Serigan®, Ovejero Lab, Leon, Spain) was administered i.m. (Groups Ee and Le) or not (Groups E and L).

Females were allocated randomly to one of four treatment groups without previous administration of any other hormonal product after the last lambing:

Group E (n=100): ewes received a MAP sponge.

Group Ee (n=100): ewes received a MAP sponge + 450 IU of eCG, i.m.

Group L (n=39): ewe lambs received a MAP sponge.

Group Le (n=38): ewe lambs received a MAP sponge + 450 IU of eCG, i.m.

The synchronization of the animals was performed in two phases. The first phase included the half of each group (Group E n=50; Ee n=50; L n=20 and Le n=18) and the rest of the ewes and lambs were synchronized during the second phase. The interval between the two phases was 15 days. Females of E and L groups received placebo intramuscular injection of saline solution.

Estrus detection and mating

The rams were randomly assigned to the groups. After sponge withdrawal females were placed with rams for four days, at a 5:1 female:male ratio. Rams were painted with special colored pencils at the preputial region and on the brisket. Thrice daily (morning, afternoon and night), at regular intervals, mounting and standing behavior was observed and marked females were separated from the flock. Percentage of females with detected estrus (ER) was calculated in the following intervals to evaluate time to estrus (TE): 24–31, 32–39, 40–47, 48–55, 56–61, 62–69, 70–77, 78–85, 86–93 and 94–102 hours after sponge withdrawal.

Pregnancy detection

Thirty days after males were withdrawn, pregnancy was confirmed by transrectal ultrasonography with an 8 MHz linear probe (Aquila, Esaote-Pie Medical, Maas-tricht, The Netherlands). Pregnancy rate (PR) was calculated as total pregnant per synchronized sheep. Lambing rate (LR), calculated as total lambs per lambing, was recorded for all animals.

Statistical analysis

The effects of treatment on the 2×2 factorial scheme were studied in the case of continuous variables (TE and LR) by one-way analysis of variance (ANOVA) followed by Duncan test used to identify different groups. Concrete variables (ER and PR) were compared with a Chi square test. Significant differences were considered at $P < 0.05$.

Results

Reproductive parameters (TE, ER, PR and LR) observed in the different experimental groups are shown in Figure 1. There were no significant differences among groups neither in the case of ER nor in the PR of animals, with the average estrous rate following MAP sponge being 92.06%. PR was 71.35 and 72.86% for ewes and ewe lambs, respectively, and similar among groups. Average time to estrus was sig-

nificantly reduced with the addition of eCG (60.60 ± 20.46 h vs. 46.93 ± 12.44 h for E and Ee and 58.68 ± 17.72 h vs. 44.34 ± 10.76 h for L and Le, respectively; $P < 0.05$ for both). No effect of eCG was found in the lambing rate, but age significantly affected the LR, with lambs lambing more lambs/lambing (1.36 vs. 1.85 for E and L, respectively; $P < 0.05$).

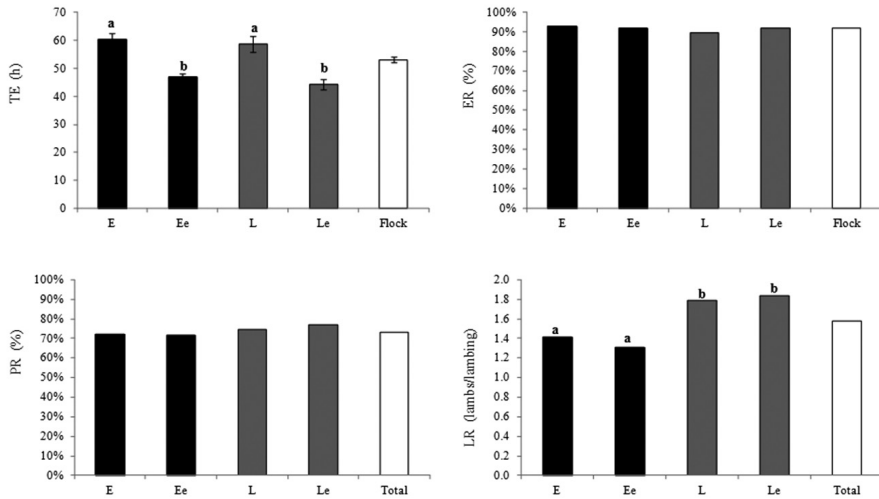


Figure 1. Time to estrus (TE, hours after MAP withdrawal), estrus rate (ER, %), pregnancy rate (PR, %) and litter size (LS, lambs/lambing) of Spanish Merino ewes (E) and ewe lambs (L) synchronized with 60 mg medroxyprogesterone acetate sponges for 12 days and treated with (groups Ee and Le) or without (groups E and L) eCG at sponge removal. Bars with different letters differ ($P < 0.05$)

Table 1. Estrus distribution in ewes and ewe lambs treated with 60 mg MAP impregnated sponges with or without 450 IU of eCG

Estrus detection intervals (hours)	Estrus distribution (%)			
	ewes		ewe lambs	
	Group E (n=100)	Group Ee (n=100)	Group L (n=39)	Group Le (n=38)
24–31	5.38	9.78*	5.71	11.43*
32–39	17.20	15.22	11.43	22.86*
40–47	7.53	23.91*	8.57	31.43*
48–55	10.75	25.00*	14.29	17.14
56–61	11.83**	15.22	20.00**	11.43*
62–69	13.98	6.52*	17.14	5.71
70–77	12.90	4.35**	8.57	0.00**
78–85	5.38	0.00*	5.71	0.00*
86–93	8.60	0.00*	5.71	0.00*
94–102	6.45	0.00*	2.86	0.00*
N/R (n)	7	8	4	3

Values with distinct superscripts differ: * within female age category (ewes vs. ewe lambs) and ** within experimental groups [with eCG (Ee and Le) vs. without eCG (E and L)] ($P < 0.05$).

N/R: number of females that showed no signs of heat.

Interval of time on estrus distribution is recorded in Table 1. There was a significant difference ($P<0.05$) between the groups that received eCG (Ee, and Le) and those that did not receive the hormone (E and L), indicating a reduction in the time to onset of estrus induced by eCG effect.

Discussion

Optimizing management procedures to improve the productive and reproductive potential of Spanish Merino sheep may be extremely important for producers. The present study offers information on the specific reproductive behavior of Spanish Merino sheep after estrus synchronization with MAP sponges and eCG or not. The results of this study indicate that Merino sheep can be successfully synchronized with these protocols, and that the addition of eCG to the protocol shortens the time to estrus, without a reduction in pregnancy or lambing rate.

The use of MAP intravaginal sponges has been demonstrated as an efficient method of estrus synchronization during the ovine reproductive season. In this work, the percentage of estrus synchronization in the general herd was 92.06%. Such a high degree of synchronization has been achieved by other investigators in other breeds (Wildeus, 2000; Barrett *et al.*, 2004; Olivera-Muzante *et al.*, 2011).

After the protocols with eCG (Groups Ee and Le), the percentage of synchronization was similar between ewe lambs and ewes, which corroborates the results from other studies (Moses *et al.*, 1997; Stellflug *et al.*, 2001) where similar estrus rates were observed. However, the findings of the current study do not support previous research of Alberio *et al.* (1994) that reported lower estrus rates in ewe lambs compared to ewes. A possible explanation for these differences may be attributed to the different breeds, nutrition, time of the year, utilization and type of gonadotropin used by the different authors (Zelege *et al.*, 2005; Kareta *et al.*, 2006; McCappin and Murray, 2011); such factors have been described as having possible impacts on estrus detection (Scaramuzzi *et al.*, 2006; Knights *et al.*, 2011; Quintero-Elisea *et al.*, 2011). In fact, estrus rates of eCG-treated females in this study are similar to those previously reported (Simonetti *et al.*, 2000; Dogan and Nur, 2006).

Time to estrus in group Ee ranged between 24–31 and 70–77 h after MAP removal, whilst in lambs with eCG (Le) TE ranged between 24–31 and 62–69 h. Differences in estrus distribution between ewe lambs and ewes due to eCG utilization have been described previously (Simonetti *et al.*, 1999, 2000). Although the current results differ from some published studies (Quirke *et al.*, 1981; Ainsworth and Wolynetz, 1982) with shorter TE intervals in ewes than in lambs, they are consistent with those of Moses *et al.* (1997). In the groups without eCG (E and L), the time to estrus ranged between 24–31 h and 94–102 h. These findings are in agreement with those reported in other sheep breeds and experimental conditions (Simonetti *et al.*, 2000; Viñoles *et al.*, 2001; Dogan and Nur, 2006) in which a positive effect of eCG in both ewes and lambs is reported. A reduction of time to estrus as well as a higher concentration of

sheep showing estrus are an advantage in the reproductive management of flocks. The knowledge of these facts is important when artificial synchronization or short synchronization protocols should be implemented. These results corroborate those reported by Cline et al. (2001), Zeleke et al. (2005) or Dogan and Nur (2006).

The use of eCG has been somewhat controversial in terms of fertility. In the present study no reductions in fertility nor prolificacy were associated with eCG administration. Although these results differ from some published studies (Zeleke et al., 2005; McCappin and Murray, 2011) which reported higher fertility rates in sheep from other breeds or even a reduction when eCG was added, they are consistent with those of Simonetti et al. (2000), Ungerfeld and Rubianes (2002) or Quintero-Elisea et al. (2011).

Another important application of eCG in ovine is to increase prolificacy rates by modifying the ovulatory rate which depends on the dosage used (Boscós et al., 2002). Nevertheless, there is a great variability in responses that may be associated with breed, individuals within a breed, female category, time of the year and general animal condition (Samartzi et al., 1995; Boscós et al., 2002). Prolificacy in the eCG treated Merino sheep (Ee and Le) was similar to that reported by Simonetti et al. (2002) but lower than that described by Maxwell (1986).

Different studies clearly showed that an i.m. eCG injection at a dose recommended for estrus synchronization induced anti-eCG antibody production, which in turn produced negative effects when the next treatment was applied (Bodin et al., 1997). Animals in this study were not treated with eCG previously during the preceding years. So, no negative effects were attributed to this fact in the estrous synchronization program utilized.

Finally, age significantly affected prolificacy with higher rates in ewe lamb vs. ewes, independent of the use of eCG. Similar results were reported in different breeds (Anel et al., 2005; Zeleke et al., 2005; Fukui et al., 2010). This could be understandable based on the fact that compared with young ewes, aged ewes have increased risks of reproductive disorders and decreased rates of ovulation with quality ovulated oocytes causing this difference, which could be the case in the present study.

In conclusion, estrus of Spanish Merino ewes and ewe lambs was successfully synchronized with traditional MAP sponge protocols and the addition of eCG on the day of MAP withdrawal which resulted in a reduction of the time to estrus interval. These results will help farmers to improve the reproductive management of this Spanish sheep breed.

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