Organic breeding in Sarda ewes: utilization of the ram effect in an artificial insemination program

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Abstract

Current study assesses the ram effect as an alternative to conventional hormonal treatment for estrus synchronization prior to artificial insemination. Two groups of 50 anestrus Sarda ewes were induced to ovulate with adult rams (RE group) or synchronized using intravaginal sponges impregnated with progestogen and equine chorionic gonadotropin (eCG) (PRO group). Ewes in the RE group were isolated from rams for 6 weeks. After the isolation period, 5 vasectomized rams were introduced into the flock at a ratio of 1 ram/10 females (day 0) to induce estrus and ovulation. Ewes in both experimental groups were inseminated via the cervical route with fresh semen (400 million spermatozoa/0.25 ml) 24 h after the onset of estrus (day 15-24). The pregnancy rate, assessed by ultrasound 35 days

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after insemination, was 48.9% (24/49) and 43.47% (22/50) for sheep in RE and PRO groups respectively. Lambing rates were identical to pregnancy rates in both groups, while the prolificacy rate was 120% (59/49) and 130% (65/50) in RE and PRO groups respectively. No significant differences were found between the 2 groups. The data supports the conclusion that the ram effect can be used successfully to synchronize estrus in organic farming operations.

Keywords: Fresh semen, artificial insemination, ram effect, progestogen treatment.

Introduction

Traditionally, sheep AI programs need hormonal treatments to induce and synchronize estrus cycles, making it possible to inseminate a large number of females at the same time either in or out of the breeding season. There are several treatments used for this purpose; most of them require the application of intravaginal devices containing synthetic progestogens for 12-14 days coupled with an i.m. injection of eCG at the end of treatment (Abecia et al., 2012).

In spite of the advantages associated with hormonal synchronization, there are many drawbacks like disturbance in the luteinizing hormone (LH) secretion patterns (Leyva et al, 1998; Viñoles et al., 2001), altered follicular dynamics (Berlinguer et al., 2007; Vilariño et al., 2011), production of eCG antibodies (Hervé et al., 2004) and impaired sperm motility inside the female reproductive tract (Manes et al., 2016) thus reducing the possibility of achieving a good synchronization between the time of ovulation and the time of spermatozoa reaching the ovum. Additionally, Gatti and Ungerfeld (2012) reported that intravaginal devices decrease sexual attractiveness in ewes due to alterations in the vaginal

flora, thus, limiting the effectiveness of a teaser ram in detecting ewes in estrus. Under certain specific circumstances, some of these drawbacks associated with the conventional progestogen/eCG protocol can be overcome by shorter periods of progestogen impregnation (down to 6-8 days; Menchaca and Rubianes, 2004) or by resorting to treatments with prostaglandin F2α (Menchaca et al., 2004).

Due to the disadvantages of hormonal treatments on animal health and performance, as well as the risk to human health from the accumulation of steroids in tissues and environmental contamination because of residual progesterone in devices, the European community has restricted the use of progestogens in domestic animals by the Normative 2008/97/CE.

An alternative to the use of hormonal synchronization is to inseminate during natural estrus hence reducing the drawbacks of progestogen residues. However, for commercial use, this requires a certain degree of estrus synchronization, which could be obtained by the use of the ram effect for those sheep breeds with low seasonal patterns of reproductive activity or during the transition phase between anestrus and the breeding season (Jorre De St Jorre et al., 2014; Martin G, 2014). According to Martin, G. (2009) and Dattena et al., (2012), the degree of estrus synchronization in the flock as a result of the application of the ram effect allows the use of AI in commercial flocks, especially in organic farming conditions. Despite critics that the initial synchrony broadens quickly, the degree of synchrony that is offered by the ram effect presents opportunities for far more precise management of the production system, particularly for the application of artificial insemination, ultrasound, focus feeding during pregnancy, and the management of birth (Martin, 2014; Ben Khlil et al., 2017).

65 The ram effect could thus be considered as a useful and suitable tool for out-of-season
66 estrus induction, especially because its cost is negligible, and hormones are avoided. This
67 concept is in keeping with the global consumer demand for products under “clean, green
68 and ethical” farming standards (Martin and Kadokawa, 2006; Martin, 2009). Therefore, we
69 aimed to test an AI program using the “ram effect” to induce and synchronize estrus cycles
70 in Sarda sheep during late seasonal anestrus.

71 **Materials and methods**

72 This study was conducted on a private farm located in Sassari (Sardinia, Italy 40°44’6” N)
73 between March (mid-anestrus) and May (end of anestrus). The maximum and minimum
74 average annual temperatures ranged between 21 and 11°C. The average annual rainfall in
75 the study area is 573 mm.

76 **Experimental groups**

77 A flock of 100 milking ewes was selected and divided into two groups: ram effect group
78 (RE) (n=50) and progestogen synchronization group (PRO) (n=50). Ewes were between 2
79 and 5 years old and with a median body condition score of 2.6 ± 0.3 (on scale 1 to 5) and
80 weighted 43.4 kg ± 2.9. All the ewes had successfully lambed by the end of November of
81 the previous year. Average milk production per sheep was 120 liters and the duration of
82 lactation was 180 days. The natural grazing land consisted of a mixture of subterranean
83 clover, Italian ryegrass and spontaneous barley. All ewes received a daily supplementation
84 of 450-600 g of commercial cereal concentrate. The amount of concentrate fed differed
85 according to body condition score and level of milk production. All the selected animals

were confirmed to be in anestrus by repeated transrectal ultrasonography to check for the presence or absence of a corpus luteum (Gonzalez de Bulnes et al., 2000).

Ram effect protocol

At the end of March, the females in the RE group were isolated from any contact with the males (sound, sight and smell) for 6 full weeks (Jorre de St Jorre et al., 2012). Five sexually experienced (at least 2 years mating experience) adult Sarda rams were vasectomized at the end of March and isolated in a shed at least 500 m away from the females. The distance of separation between males and females was much less compared to other ram effect protocols (Ungerfeld et al., 2008). However, the hilly topography of the farm increased isolation between the two groups, avoiding any possible transmission of sociosexual stimuli from males to females. These males were used to induce the "ram effect" by inducing ovulations and synchronizing estrus (Fabre-Nys et al., 2016) and as "teasers" to precisely detect the onset of estrus.

During the second week of May, the vasectomized rams were introduced (day 0) and kept in the experimental flock at a ratio of 1 male per 10 females. The males were withdrawn from the flock on the evening of day 14, and reintroduced to check estrus, under the control of an expert operator, on day 15. Estrus was checked on 4 occasions (8:00 AM, 12:00 PM, 4:00 PM, 8:00 PM) for 30 min each time. Estrus checking was repeated daily until day 25. Two or three teasers were introduced into the flock simultaneously during the 30 min contact period.

106 The females were considered to be in heat when they showed estrus behavior (ram-ewe
107 seeking activity, fanning of the ewe's tail, and ewe immobilization) and were mounted by
108 the teaser.

109 **Progesterone synchronization protocol**

110 Estrus cycles of the females in the PRO group were synchronized during the second week
111 of May by the insertion of an intravaginal sponge containing ~~25~~ 20 mg fluorogestone
112 acetate (Chronogest ®, Intervet, Milan, Italy) for 12 days. Ewes received an i.m. injection
113 of 400 IU of ~~PMSG~~ eCG (Chrono-Gest PMSG ®, Intervet, Milan, Italy) at sponge removal
114 to synchronize ovulation. Twenty-four hours after sponge removal, ewes were placed in
115 pens of 10 animals each and tested to determine the onset of estrus behavior at 4 different
116 times by visual observations with the introduction of a vasectomized ram for 30 min.

117 **Semen preparation and artificial insemination**

118 Semen was prepared at the Laboratory of Biotechnologies of Reproduction at DIRPA-
119 AGRIS Institute, located 15 km from the farm. Five Sarda adult rams (3-6 years old) with
120 proven fertility were used for semen collection. Semen was collected once a day by
121 artificial vagina. Mass activity (wave motion or motility score) in undiluted semen was
122 assessed by examining a drop of semen on a warm stage using a phase contrast microscope
123 (x 100 magnification; samples were scored from 0–5 where 5 indicated rapid swirling
124 motion and 0 denoted absence of motion). Concentration (number of spermatozoa/ml) was
125 determined using a spectrophotometer that was calibrated to measure sheep sperm
126 concentration at 550 nm (Accucell R; IMV, Paris, France). Four microliters of fresh semen
127 were diluted in 3996 µl of physiological saline solution. Only ejaculates with an acceptable

mass activity higher than 3 and a concentration higher than 3 x 10^9spermatozoa/ ml were used.

For the fresh semen preparation, ejaculates were first diluted at a concentration of 1.6 x 10^9 spermatozoa/ml at 38°C using skimmed milk-based extender according to the method of Mara et al. (2005). After dilution, semen was cooled to 15°C within 30 min. and then loaded into 0.25 ml plastic straws at a concentration of 400 million spermatozoa each with 3.5 of mass activity score.

Artificial insemination was carried out "on estrus" with fresh semen within 7 hours after preparation. To avoid any effect of ram fertility on conception rates, insemination straws from each ram were randomly allocated to ewes in both RE and PRO groups. The AI was performed by the cervical route 24 h after estrus detection in both the RE and PRO groups. All inseminations were carried out by the same experienced operator.

Pregnancy diagnosis and control of lambing

Pregnancy diagnosis was performed on day 35 after the last insemination by abdominal ultrasonography using a MINDRAY DP-30 (China) ultrasound scanner with a 5 MHz transducer. Non-pregnant females were assigned to a fertile ram for natural mating while pregnant females were maintained in accordance with the farm's standard management until lambing. During the lambing period, ewes were checked daily, and the date of lambing recorded.

Statistical analysis

148 Data on estrus synchronization, pregnancy, lambing and prolificacy rates for sheep
149 inseminated with fresh semen and induced to breed using either the ram effect or hormonal
150 synchronization treatment were analyzed by Chi-Square test (Pandis, 2016). Statistical
151 significance was set at $P < 0.05$.

152 **Results**

153 A total of forty-nine sheep (98%) in the RE group were detected in estrus between 15 – 24
154 days after introduction of the ram (Fig.1). A total of fifty sheep (100%) in the PRO group
155 were detected in estrus between 24 h and 48 h after sponge removal (Fig. 2). All sheep
156 detected in estrus in both groups were inseminated.

157 No statistically significant differences were found in terms of pregnancy (48.9% and
158 43.5%), lambing (48.9% and 43.5%) or prolificacy rates (120% and 130%) between RE
159 and PRO groups respectively (Table 1).

160 **Discussion**

161 Estrus response of ewes induced by the ram effect in this study was similar to results
162 reported by various other researchers (Evans et al., 2004; Ungerlfed et al., 2008; Hawken
163 and Beard, 2009; Mayorga et al., 2010) and the distribution of estrus was relatively typical
164 of a classic response to sudden introduction of rams. However, the onset of estrus after ram
165 introduction started slightly earlier (day 15) when compared to previously cited studies.
166 Among factors involved in an early onset of estrus we can hypothesize that the ovarian
167 response is affected by the growth status of the largest follicle present and the concentration
168 of estradiol at the moment of the ram reintroduction thus promoting a shorter latency to the
169 onset of estrus (Fabre-Nys et al., 2015; 2016). In this study, Sarda breed ewes showed an

earlier onset of estrus possibly because they are more sensitive to estradiol concentration compared to other breeds (Chanvallon et al., 2011). On the other hand, the percentage of sheep exhibiting estrus induced by the exogenous hormonal treatment during the end of anestrus was similar to previous reports for the same time of the year for Sarda breed (Cappai et al., 1998; Branca et al., 2000).

The females in the RE group showed a relative (48.9%) increase ($p=0.25$) in pregnancy rates compared to females in the PRO group; this finding can be explained by the fact that synchronization with progestogens has been associated with alterations in the uterine environment thus compromising embryo implantation and development (Gonzales-Bulnes et al., 2005). Although no significant differences were found between groups, it is important to note that with the current ram effect protocol, dairy Sarda ewes can be successfully inseminated with fresh semen via the cervical route while protocol costs and veterinary consultancy are reduced since hormone treatments are not required. This result is in agreement with Donovan et al. (2004) who also found similar pregnancy rates between ewes inseminated under natural estrus or progestogen treated estrus.

It is important to note that lambing rates were similar to pregnancy rates in both groups. This may be due to several factors. Inseminations in both group were performed with high quality semen (Lieberman et al., 2016). Checking for estrus 4 times increases the accuracy of the time of insemination with respect to the time of ovulation (Crilly et al., 2016). In sheep, 4 h of exposure to estrogens can be enough to induce estrus behavior and an LH surge (Pillon et al., 2003; Fabre-Nys et al., 1993). According to Baird (1978) and Rawlings et al. (1984) timing insemination for 24 h after the onset of estrus is optimal in relation to ovulation; a delay makes the eggs aged and lowers their quality at the time of fertilization

hence affecting their capacity to maintain the fetus during pregnancy (Gonzales Bulnes et al., 2005). The season of AI was ideal in terms of mild temperatures compared with traditional AI performed during July thus reducing the risk of embryonic losses (Dixon et al., 2007), and lastly, good farm management during the experimental period helped to get acceptable lambing rates.

Prolificacy rates were statistically similar, but PRO ewes tended to have more lambs per ewe inseminated. This is most likely a consequence of using eCG which is known to increase ovulation rate and litter size.

Conclusion

In conclusion, reproductive performance of Sarda ewes inseminated after being induced to ovulate with the ram affect is very promising, with similar estrus and pregnancy rates when compared to ewes receiving the conventional hormonal treatment. With this protocol, organic sheep farms can be “hormone free" but at the same time part they can still be part of genetic improvement programs by using the ram effect to establish fertile, grouped estrus for the purpose of artificial insemination. However, synchronization of estrus with the ram effect is not as compact as it is with conventional hormonal treatments and there is an increased labor requirement for estrus detection. Therefore, insemination programs could be longer, and this requires careful planning in the field.

Conflicts of interest

None

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Highlights

1. Sarda sheep respond to the ram effect efficiently.
2. Ram effect avoids detrimental effects of progesterone synchronization treatments.
3. Artificial insemination without the use of hormones improves sheep welfare
4. Sheep artificial insemination on organic farms is possible with the ram effect

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Abstract

The current study assesses the ram effect as an alternative to conventional hormonal treatment for estrus synchronization prior to artificial insemination. Two groups of 50 anoestrus Sarda ewes were induced to ovulate with adult rams (RE group) or synchronized using intravaginal sponges impregnated with progestogen and equine chorionic gonadotropin (eCG) (PRO group). Ewes in the RE group were isolated from rams for 6 weeks. After the isolation period, 5 vasectomized rams were introduced into the flock at a ratio of 1 ram/10 females (day 0) to induce estrus and ovulation. Ewes in both experimental groups were inseminated via the cervical route with fresh semen (400 million spermatozoa/0.25 ml) 24 h after the onset of estrus (day 15-24). The pregnancy rate,

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assessed by ultrasound 35 days after insemination, was 48.9% (24/49) and 43.47% (22/50) for sheep in RE and PRO groups respectively. Lambing rates were identical to pregnancy rates in both groups, while the prolificacy rate was 120% (59/49) and 130% (65/50) in RE and PRO groups respectively. No significant differences were found between the 2 groups. The data supports the conclusion that the ram effect can be used successfully to synchronize estrus in organic farming operations.

Keywords: Fresh semen, artificial insemination, ram effect, progestagen treatment.

Introduction

Traditionally, sheep AI programs need hormonal treatments to induce and synchronize estrus cycles, making it possible to inseminate a large number of females at the same time either in or out of the breeding season. There are several treatments used for this purpose; most of them require the application of intravaginal devices containing synthetic progestogens for 12-14 days coupled with an im injection of eCG at the end of treatment.

In spite of the advantages associated with hormonal synchronization, there are many drawbacks like disturbance in the luteinizing hormone (LH) secretion patterns (Leyva et al, 1998; Viñoles et al., 2001), altered follicular dynamics (Berlinguer et al., 2007; Vilariño et al., 2011), production of eCG antibodies (Hervé et al., 2004) and impaired sperm motility inside the female reproductive tract (Manes et al., 2016) thus reducing the possibility of achieving a good synchronization between the time of ovulation and the time of spermatozoa reaching the ovum. Additionally, Gatti and Ungerfeld (2012) reported that intravaginal devices decrease sexual attractiveness in ewes due to alterations in the vaginal flora, thus, limiting the effectiviness of a teaser ram in detecting ewes in estrus.

Due to the disadvantages of hormonal treatments on animal health and performance, as well as the risk to human health from the accumulation of steroids in tissues and environmental contamination because of residual progesterone in devices, the european community has restricted the use of progestogens in domestic animals by the Normative 2008/97/CE.

An alternative to the use of hormonal synchronization is to inseminate during natural estrus hence reducing the drawbacks of progestogen residues. However, for commercial use, this requires a certain degree of oestrous synchronization, which could be obtained by the use of the ram effect for those sheep breeds with low seasonal patterns of reproductive activity or during the transition phase between anoestrus and the breeding season (Jorre De St Jorre et al., 2014; Martin G, 2014). According to Martin, G. (2009) and Dattena et al., (2012), the degree of estrus synchronization in the flock as a result of the application of the ram effect allows the use of AI in commercial flocks, especially in organic farming conditions.

The ram effect could thus be considered as a useful and suitable tool for out-of-season oestrus induction, especially because its cost is negligible and hormones are avoided. This concept is in keeping with the global consumer demand for products under “clean, green and ethical” farming standards (Martin and Kadokawa, 2006; Martin G, 2009). Therefore, we aimed to test an AI program using the “ram effect” to induce and synchronize oestrus cycles in Sarda sheep during late seasonal anoestrus.

Materials and methods

This study was conducted on a private farm located in Sassari (Sardinia, Italy 40°44'6" N) between March (mid-anoestrus) and May (end of anoestrus). The maximum and minimum average annual temperatures ranged between 21 and 11°C. The average annual rainfall in the study area is 573 mm.

Experimental groups

A flock of 100 milking ewes was selected and divided into two groups: ram effect group (RE) (n=50) and progestagen synchronization group (PRO) (n=50). Ewes were between 2 and 5 years old and with a median body condition score of 2.6 ± 0.3 (on scale 1 to 5), and weighted 43.4 kg ± 2.9. All the ewes had successfully lambed by the end of November of the previous year. Average milk production per sheep was 120 liters and the duration of lactation was 180 days. The natural grazing land consisted of a mixture of subterranean clover, Italian ryegrass and spontaneous barley. All ewes received a daily supplementation of 450-600 g of commercial cereal concentrate. The amount of concentrate fed differed according to body condition score and level of milk production. All the selected animals were confirmed to be in anestrus by repeated transrectal ultrasonography to check for the presence or absence of a corpus luteum (Gonzalez de Bulnes et al., 2000).

Ram effect protocol

At the end of March, the females in the RE group were isolated from any contact with the males (sound, sight and smell) for 6 full weeks (Jorre de St Jorre et al., 2012). Five sexually experienced (at least 2 years mating experience) adult Sarda rams were vasectomized at the end of March and isolated in a shed at least 500 m away from the females. The distance of

86 separation between males and females was much less compared to other ram effect
87 protocols (Ungerfeld et al., 2008). However, the hilly topography of the farm increased
88 isolation between the two groups, avoiding any possible transmission of sociosexual stimuli
89 from males to females. These males were used to induce the "ram effect" by inducing
90 ovulations and synchronizing estrus (Fabre-Nys et al., 2016) and as "teasers" to precisely
91 detect the onset of estrus.

92 During the second week of May, the vasectomized rams were introduced (day 0) and kept
93 in the experimental flock at a ratio of 1 male per 10 females. The males were withdrawn
94 from the flock on the evening of day 14, and reintroduced to check estrus, under the control
95 of an expert operator, on day 15. Estrus was checked on 4 occasions (8:00 AM, 12:00 PM,
96 4:00 PM, 8:00 PM) for 30 min each time. Estrus checking was repeated daily until day 25.
97 Two or three teasers were introduced into the flock simultaneously during the 30 min
98 contact period.

99 The females were considered to be in heat when they showed estrus behavior (ram-ewe
100 seeking activity, fanning of the ewe's tail, and ewe immobilization) and were mounted by
101 the teaser.

102 **Progesterone synchronization protocol**

103 Oestrus cycles of the females in the PRO group were synchronized during the second week
104 of May by the insertion of an intravaginal sponge containing 25 mg fluorogestone acetate
105 (Chronogest ®, Intervet, Milan, Italy) for 12 days. Ewes received an i.m. injection of 400
106 IU of PMSG (Chrono-Gest PMSG ®, Intervet, Milan, Italy) at sponge removal to
107 synchronize ovulation. Twenty-four hours after sponge removal, ewes were placed in pens

of 10 animals each and tested to determine the onset of estrus behavior at 4 different times by visual observations with the introduction of a vasectomized ram for 30 min.

Semen preparation and artificial insemination

Semen was prepared at the Laboratory of Biotechnologies of Reproduction at DIRPA-AGRIS Institute, located 15 km from the farm. Five Sarda adult rams (3-6 years old) with proven fertility were used for semen collection. Semen was collected once a day by artificial vagina. Mass activity (wave motion or motility score) in undiluted semen was assessed by examining a drop of semen on a warm stage using a phase contrast microscope (x 100 magnification; samples were scored from 0–5 where 5 indicated rapid swirling motion and 0 denoted absence of motion). Concentration (number of spermatozoa/ml) was determined using a spectrophotometer that was calibrated to measure sheep sperm concentration at 550 nm (Accucell R; IMV, Paris, France). Four microliters of fresh semen were diluted in 3996 µl of physiological saline solution. Only ejaculates with an acceptable mass activity higher than 3 and a concentration higher than 3 x 10^9spermatozoa/ ml were used.

For the fresh semen preparation, ejaculates were first diluted at a concentration of 1.6 x 10^9 spermatozoa/ml at 38°C using skimmed milk-based extender according to the method of Mara et al. (2005). After dilution, semen was cooled to 15°C within 30 min. and then loaded into 0.25 ml plastic straws at a concentration of 400 million spermatozoa each with 3.5 of mass activity score.

Artificial insemination was carried out "on estrus" with fresh semen within 7 hours after preparation. The AI was performed by the cervical route 24 h after estrus detection in both

130 the RE and PRO groups. All inseminations were carried out by the same experienced
131 operator.

132 **Pregnancy diagnosis and control of lambing**

133 Pregnancy diagnosis was performed on day 35 after the last insemination by abdominal
134 ultrasonography using a MINDRAY DP-30 (China) ultrasound scanner with a 5 MHz
135 transducer. Non-pregnant females were assigned to a fertile ram for natural mating while
136 pregnant females were maintained in accordance with the farm's standard management
137 until lambing. During the lambing period, ewes were checked daily and the date of lambing
138 recorded.

139 **Statistical analysis**

140 Data on oestrus synchronization, pregnancy, lambing and prolificacy rates for sheep
141 inseminated with fresh semen and induced to breed using either the ram effect or hormonal
142 synchronization treatment were analyzed by Chi-Square test. Statistical significance was set
143 at $P < 0.05$.

144 **Results**

145 A total of forty nine sheep (98%) in the RE group were detected in estrus between 15 – 24
146 days after introduction of the ram (Fig.1). A total of fifty sheep (100%) in the PRO group
147 were detected in estrus between 24 h and 48 h after sponge removal (Fig. 2). All sheep
148 detected in estrus in both groups were inseminated.

149 No statistically significant differences were found in terms of pregnancy (48.9% and
150 43.5%), lambing (48.9% and 43.5%) or prolificacy rates (120% and 130%) between RE
151 and PRO groups respectively (Table 1).

152 **Discussion**

153 Estrus response of ewes induced by the ram effect in this study was similar to results
154 reported by various other researchers (Evans et al., 2004; Ungerlfed et al., 2008; Hawken
155 and Beard, 2009; Mayorga et al., 2010) and the distribution of estrus was relatively typical
156 of a classic response to sudden introduction of rams. However, the onset of estrus after ram
157 introduction started slightly earlier (day 15) when compared to previously cited studies.
158 Among factors involved in an early onset of oestrus we can hypothesize that the ovarian
159 response is affected by the growth status of the largest follicle present and the concentration
160 of estradiol at the moment of the ram reintroduction thus promoting a shorter latency to the
161 onset of oestrus (Fabre-Nys et al., 2015; 2016). In this study, Sarda breed ewes showed a
162 earlier onset of estrus possibly because they are more sensitive to oestradiol concentration
163 compared to other breeds (Chanvallon et al., 2011). On the other hand, the percentage of
164 sheep exhibiting estrus induced by the exogenous hormonal treatment during the end of
165 anoestrus was similar to previous reports for the same time of the year for Sarda breed
166 (Cappai et al., 1998; Branca et al., 2000).

167 The females in the RE group showed a relative (48.9%) increase ($p=0.25$) in pregnancy
168 rates compared to females in the PRO group; this finding can be explained by the fact that
169 the synchronization with progestagens has been asociated with alterations in the uterine
170 enviroment thus compromising embryo implantation and development (Gonzales-Bulnes et

al., 2005). Although no significant differences were found between groups, it is important to note that with the current ram effect protocol dairy Sarda ewes can be successfully inseminated with fresh semen via the cervical route while protocol costs and veterinary consultancy are reduced since hormone treatments are not required. This result is in agreement with Donovan et al. (2004) who also found similar pregnancy rates between ewes inseminated under natural oestrus or progestogen treated oestrus.

It is important to note that lambing rates were similar to pregnancy rates in both groups. This may be due to several factors. Inseminations in both group were performed with high quality semen (Lieberman et al., 2016). Checking for oestrus 4 times increases the accuracy of the time of insemination with respect to the time of ovulation (Crilly et al., 2016). In sheep, 4 h of exposure to oestrogens can be enough to induce oestrus behaviour and an LH surge (Pillon et al., 2003; Fabre-Nys et al., 1993). According to Baird (1978) and Rawlings et al. (1984) timing insemination for 24 h after the onset of oestrus is optimal in relation to ovulation; a delay makes the eggs aged and lowers their quality at the time of fertilization hence affecting their capacity to maintain the foetus during pregnancy (Gonzales Bulnes et al., 2005). The season of AI was ideal in terms of mild temperatures compared with traditional AI performed during July thus reducing the risk of embryonic losses (Dixon et al., 2007), and lastly, good farm management during the experimental period helped to get acceptable lambing rates.

Prolificacy rates were statisticaly similar, but PRO ewes tended to have more lambs per ewe inseminated, probably due to the fact that synchronization treatment was combined with eCG hormone which could have a follicular stimulatory effect in treated animals.

Conclusion

In conclusion, reproductive performance of Sarda ewes inseminated after being induced to ovulate with the ram affect is very promising, with similar oestrus and pregnancy rates when compared to conventional hormonal treatment. With this protocol organic sheep farms can be “hormone free" but at the same time part of genetic improvement programs by using the ram effect to establish fertile, grouped estrus. However, synchronization of estrus with the ram effect is not as compact as it is with conventional hormonal treatments and there is an increased labor requirement for estrus detection. Therefore, AI programs could be longer and this requires careful planning in the field.

Conflicts of interest

None

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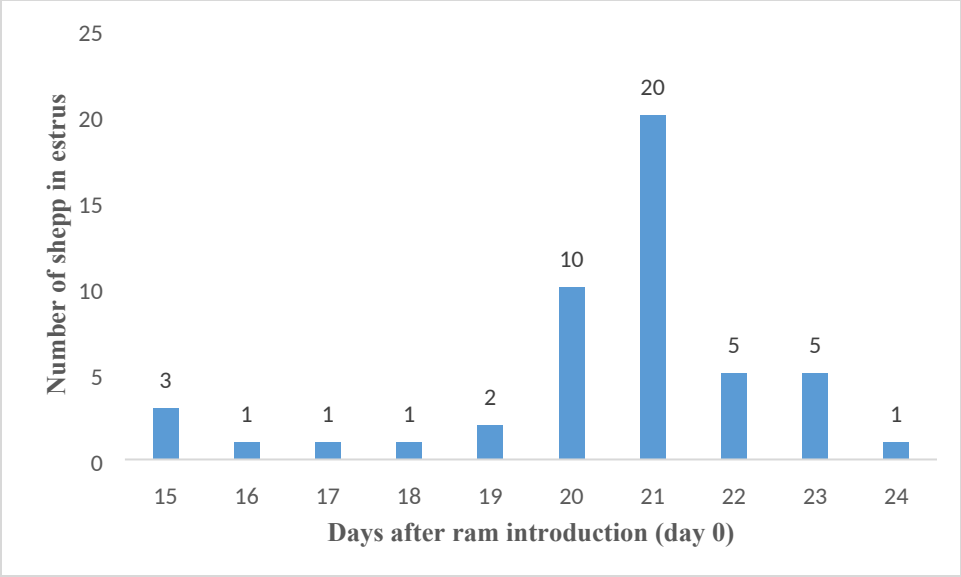

Fig. 1 Number of ewes detected in estrus between days 15 and 24 after rams' introduction (Group RE)

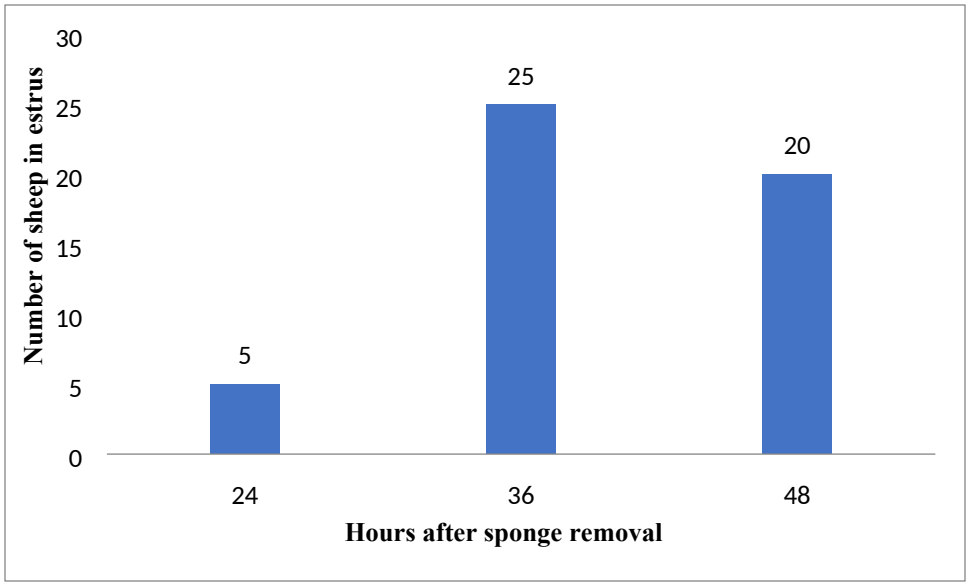

Fig. 2 Number of ewes detected in estrus after sponge removal (PRO group)

Table 1. Pregnancy and lambing rates of Sarda ewes after insemination in ewes induced to breed with Ram Effect (RE group) and with intravaginal progesterone treatment (PRO group).

Treatment group	**Ewes inseminated**	**Pregnancy rate %** **(ewes pregnant/ewes inseminated)**	**Lambing rate %** **(ewes lambed/ewes inseminated)**
RE	49	48.9% (24/49)	48.9% (24/49)
PRO	50	43.47% (22/50)	43.47% 22/50)
Total	99	46	46
Prob. > χ^2		0.25	0.61

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Dear Editor

I declare that there is not any conflict of interest in the present manuscript

Regards

Isabel Mayorga