—Technical Note—

Progesterone Plus PMSG Priming in Seasonally Anovulatory Lactating Sarda Ewes Exposed to the Ram Effect

Luca TODINI1), Alessandro MALFATTI1), Olimpia BARBATO2), Silva COSTARELLI3) and Alessandro DEBENEDETTI2)

1)Dipartimento di Scienze Ambientali, Sezione di Produzioni Animali, Università di Camerino, 62024 Matelica, 2)Dipartimento di Scienze Biopatologiche ed Igiene delle Produzioni Animali e Alimentari, Università di Perugia, 06100 Perugia and 3)Istituto Zooprofilattico Sperimentale dell’Umbria e delle Marche, Italy

Abstract. The aim of this trial was to evaluate the effectiveness (fertility and lambing) of priming with a single injection of progesterone plus PMSG in anovulatory lactating Sarda ewes subjected to the ram effect (RE) in spring. Thirty ewes (P4 group) were i.m. injected with 30 mg progesterone and 500 IU PMSG 36 h before ram introduction (d 0). This treatment was compared to a 12-day treatment with fluorogestone acetate intravaginal sponges that was followed by injections of 350 IU PMSG upon sponge withdrawal (FGA group, n=30). All ewes responded to RE, showing plasma progesterone concentrations >1 ng/mL between d 6 and 12 (FGA) or 6 and 9 (P4). Eighty-nine percent of the P4 ewes conceived at first ovulation, and 11% conceived following a short estrus cycle. Lambings occurred on d 150.4 ± 3.9, and the lambing rate was 100%. The fertility of the FGA ewes was 83% for the induced ovulation and was 7% for the second ovulation after a normal cycle. The FGA ewes lambed on d 149.8 ± 4.4, and the lambing rate was 83%. Two abortions were recorded for the FGA ewes, which had higher prolificacy than the P4 group (2.2 ± 0.8 vs. 1.8 ± 0.4, respectively; P<0.05). Both fertility and the lambing rate were high in both groups, with a high degree of estrus synchronization, and there were no significant differences between the groups. We concluded that priming of lactating Sarda ewes in spring with P4+PMSG before RE is an effective and competitive method (cheaper and more practical than FGA+PMSG) of inducing fertile ovulations in these ewes.

Key words: Estrus induction, Fertility, Progesterone priming, Prolificacy, Sheep

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The “ram effect” (RE) is a natural and inexpensive method for control of the reproductive activity in sheep [1]. The sudden introduction of rams to seasonally anovulatory ewes induces ovulations without estrus within 2 to 4 days. This silent ovulation is followed by either an ovarian cycle of normal duration (16–17 days) and a second ovulation associated with estrus behaviour or by an ovarian cycle of short duration (6 days) followed by a second silent ovulation. In this latter case, ovulation and estrus occur after a cycle of normal duration [2, 3]. With “deep” anoestrus the percentage of short cycles is high, and anoestrus can follow the induced ovulations. The “depth” of anoestrus for a given flock in a given period could be measured as the percentage of females spontaneously ovulating, and it determines a wide range of variations in female responsiveness to RE [4]. RE is very effective in the Merinos and Mediterranean breeds of ewes, which show a shorter anoestrus period that is less deep.
compared with breeds at higher latitudes (reviewed in [5]). Lamblings in autumn are followed by longer lactations, and female lambs are preferred for substitution rate. Nevertheless, due to the poor responsiveness of ewes during the deep anoestrous season, births often do not occur early enough to have lambs with sufficient body weight for slaughter at Christmas. Furthermore, short ovarian cycles result in scattered lambing because estrouses occur 18–21 and 24–27 days after introduction of rams. A pretreatment with 20 mg of progesterone (P4) on the day of ram introduction or 3 d before prevents short cycles, so that the first silent ovulation is followed by a normal cycle, with a single peak of estrus 18–21 days after the introduction of rams [6–10]. Estrus behaviour occurs at the first induced ovulation if ewes are treated with progestagen, such as fluorogestone acetate (FGA), using subcutaneous implants or intravaginal sponges [6, 11]. In this case, the percentage of ewes induced to ovulate and the degree of synchronization are improved by gonadotropins (as pregnant mare serum gonadotrophin: PMSG) administered at the end of the progestagen treatment [3].

A single P4 injection would have several advantages compared with the use of vaginal sponges: it is less expensive than progestagen, it requires only one manipulation of ewes instead of two, and it reduces milk losses due to the withdrawal period.

The aim of the present trial was to evaluate the effectiveness of priming anovulatory lactating Sarda ewes exposed to rams in spring with a single injection of natural P4 plus PMSG in terms of fertility and lambing in comparison with the traditional 12-day FGA intravaginal sponge plus PMSG treatment.

Materials and Methods

Trials were conducted on a farm located in a hill area (400 m above sea level) in Tuscany (Central Italy, 42°N). Sixty pluriparous Sarda ewes were utilized. The ewes lambed in autumn-winter, lambs were slaughtered at 30–60 days of age, and the ewes were at the end of lactation when the trial began (>120 days post-partum). Rams were kept away (sight, sound, smell) from females for about 2 months. The animals grazed in a natural pasture and were offered a flushing (300 g/h/day concentrates) from 2 weeks before until 3 weeks after ram introduction. Thirty randomly chosen ewes (FGA group) received intravaginal sponges containing 40 mg fluorogestone acetate (Cronogest Spugne, Intervet, Milan, Italy) for 12 days. At sponge withdrawal the ewes were i.m. injected with 350 IU PMSG (Folligon, Intervet, Milan, Italy). The other 30 ewes (P4 group) were injected i.m. with 30 mg P4 (Gestone Pabyrn, AMSA Farmaceutici, Rome, Italy) and 500 IU PMSG. Thirty-six h later (day 0: April 21), rams previously proven fertile (3 to 6 years of age with a mean weight of 66 kg) were introduced and remained with ewes for the following 40 days. Each ram was allocated to 6 ewes (3 of each group) in separated pens, and the animals were fed alfalfa hay ad libitum plus concentrate. The animals also had free access to water and mineral blocks containing oligoelements and vitamins. They were routinely dosed with an anthelmintic in early spring (about 3 weeks before the beginning of the trial) and autumn. Blood samples were collected in evacuated tubes containing EDTA by jugular venipuncture 15, 10 and 5 days before the start of treatments and every 3 days from day 0 to 27 after introduction of rams. Samples were centrifuged (2,500 g for 20 min), and plasma samples were aspirated and stored at −20°C until assay for P4 concentration by radioimmunoassay [12]. P4 was extracted from plasma with ethyl ether, and the efficiency of the extraction procedure was monitored by addition of a tracer amount of [3H] progesterone. The efficiency of the ether extraction ranged from 82 to 95%. Extraction was conducted using 0.2 ml plasma, and each sample was assayed in duplicate. The assay sensitivity was 0.08 ng/ml for a sample volume of 200 ml of plasma. The intra- and inter-assay coefficients of variation were 7 and 11%, respectively. Pregnancy status was checked at day 70 by transabdominal ultrasonography (Falco 200, Pie Medical, Maastricht, The Netherlands) using a 5.0 MHz linear array transducer.

Fertility and lambing rates were compared using the chi-squared test, and prolificacy and lambing dates were compared using ANOVA. The degree of synchronization of lambing dates was analyzed by the variance ratio test or F-test [13].
Results

The plasma progesterone concentrations indicated that all the ewes were in anoestrus before the start of the experiment. Two ewes in the P4 group were lost before the end of the trial, and their data were discarded.

Table 1 shows the results obtained from plasma P4 concentrations (Fig. 1), ultrasonographic findings (data not shown), and lambing distribution (Fig. 2).

All the P4 ewes showed plasma P4 values > 1 ng/ml on day 6 and/or 9. The P4 ewes showed 100% fertility within the second ovulation after RE. Eighty-nine percent of ewes conceived at the first ovulation, and 11% conceived at estrus following a short cycle. Multiple lambings did not occur in the P4 ewes.

All the FGA-treated ewes responded to the treatment and had plasma P4 values > 1 ng/ml between day 6 and 12. Two FGA ewes experienced abortions in the second half of their pregnancies. Three FGA ewes did not conceive in the second ovulation and did not lamb. Among the FGA ewes there were 2 quadruple and 5 triple births. The farmer observed that FGA ewes with multiple lambings displayed weakness during the peripartum period. Synchronization of lambings did not differ between treatments.

Discussion

There is usually a strong correlation between the percentage of ewes spontaneously cycling and the percentage of ewes ovulating in response to RE [4, 14]. In the present study, none of the ewes were cycling, but the response of the ewes to both treatments in terms of induced ovulation was 100%,
with luteal activity within 9 (P4 group) or 12 days (FGA groups) after introduction of rams. The overall fertility within the second ovulation was over 94%. The lambing rate and degree of synchronization were also good. Short cycles were observed in only 3 ewes in the P4 group, and they were all followed by fertile ovulations. Many previous studies have reported the suppression of short cycles by P4 priming without gonadotropin [6, 8–11, 15, 16]. Estrus behaviour at ram-induced ovulation can be obtained without gonadotropin by P4 or progestagen priming for several days [17]. A single P4 injection (50 mg)+PMSG+RE in lactating Comisana sheep (Sicilian milk-type breed) resulted in high fertility [18]. Malfatti et al. [19] administered progesterone with and without PMSG during the non-breeding season in Ile-de-France ewes (more strictly seasonal) in Central Italy. They observed a better luteal activity on d 8 in P4+PMSG+RE ewes than in P4+RE ewes, whose results in turn were very similar to those obtained in a group exposed to RE only.

Twin and multiple lambings are undesired by the breeders of Sarda ewes because of the low survival rate of the newborns, low commercial value of the lambs, weakness and lower milk yields by the ewes. Prolificacy was significantly higher in the FGA ewes than in the P4 ewes. It seems that a longer treatment with progestagen made the ovaries of the ewes more responsive to the actions of the gonadotropin than the single P4 injection. An alternative hypothesis may be that overstimulation by the higher dose of PMSG may have resulted in failure of a larger number of follicles to ovulate. In any case, a great care must be taken with the use of PMSG in order to avoid excessive prolificacy in this breed of sheep.

In conclusion a single P4 injection gave similar results in terms of fertility, lambing rate, and estrous synchronization as were obtained in intravaginal sponge-treated ewes. P4+PMSG priming before RE was an effective and competitive method (cheaper and more practical than FGA+PMSG) of inducing ovulations in lactating Sarda ewes during seasonal anestrus.

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