

Comparison of artificial insemination methods in sheep using semen from *Ovis g. musimon*

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ABSTRACT - Reproductive biotechnologies find application both in conservation of endangered species and to improve production from domestic animals. The cross between Mouflon and domestic sheep could be exploited to produce suckling lamb, a foodstuff with high organoleptic qualities and that could meet modern consumer demand. Mouflon semen was collected from two adult males using an artificial vagina during the sexual season. 80 ewes were synchronized by insertion of intravaginal sponges (40 mg FGA), and by an intramuscular injection of 400 IU of PMSG at sponge removal. 40 ewes (Group A) were inseminated into the cervix using refrigerated semen (+4°C, 0.25 ml and 800 million spermatozoa per dose) 56 hours after the sponge removal. 40 ewes (Group B) were inseminated into the uterus using the laparoscopic technique 54 hours after sponge removal using cryopreserved and later thawed semen (0.25 ml and 400 million spermatozoa). Chi-squared test showed values significantly higher ($P<0.01$) in fertility rate in Group A (47.5% vs 32.5%). In both groups prolificity rate was similar (1.1%). In conclusion, intracervical method with the use of fresh semen, gives better reproductive performances, and it is easy to apply for obtaining cross lamb on a large scale. On the other hand, result regarding laparoscopic-intrauterine technique could be considered good and the method is very useful because it allows to use semen in a rational way and in a longer lapse of time.

KEYWORDS: Mouflon; Sarda sheep breed; suckling lamb; artificial insemination.

INTRODUCTION - Lamb meat is a common food in many countries of the Mediterranean area. In Sardinia, lambs of the local breed “Sarda”, are slaughtered at a low weight and at few weeks of age, and this foodstuff gained PGI (geographical indication) in 2001, with the name “Agnello di Sardegna” (European Union, 2001). Ovine meat is seen as an alternative to beef and other intensively-reared meats (Rubino et al., 1999; Adnøy et al., 2005) and this demands has the potential to increase farm income. Productive performance has been improved in many sheep farming situations all over the Mediterranean, by crossing dairy sheep breeds with meat ones (Sañudo et al., 1998; Gutierrez et al., 2005; Rodriguez et al., 2006). Sardinia lacks local meat sheep breeds and land adaptation of non-autochthonous sheep breeds wasn't successful in the past (Boyazoglu et al., 1979; Sanna et al., 2001) because of the tough climate (Boyazoglu et al., 2001). However, together with Corsica, Sardinia is the only territory where the European Mouflon (*Ovis gmelini musimon*) lives wild. Mating between wild Mouflon and domestic sheep, occurs in natural environment, and the crossbred was first recognised in the first century B.C., by Plinius Mayor. Nowadays we can propose this ancient and natural genetic source as a typical meat product. The great development of new techniques in the field of animal reproduction, and above all innovative artificial insemination methods, are useful both to achieve genetic improvement in domestic species and also to safeguard endangered wild species. The aim of this study was to evaluate two methods of artificial insemination, used to obtain the crossbred lamb Mouflon x Sarda sheep.

MATERIALS AND METHODS - The entire experiment was conducted in accordance with the European Community regulation regarding the protection of animal used for experimental and other scientific purposes (European Union, 1986). The trial was carried out in a commercial farm located in Sardinia. Among the animals of the flock, made up of 400 sheep and 20 rams, all registered in the official herd book of the Sarda sheep breed, 80 multiparous ewes (3 ± 0.7 years old) were randomly

selected, after they were examined by a veterinarian. They were synchronized by the insertion of intravaginal progestagen-impregnated sponges (40 mg fluorogestone acetate, Crono-gest spugne, Intervet Italia), for 12 days, and by an intramuscular injection of 400 IU of PMSG (Crono-gest PMSG, Intervet Italia) at sponge removal. Two days after pessary removal they were randomly divided into two groups, Group A and B, each one of 40 sheep. Semen was collected during the breeding season (negative photoperiod, month of September) from two experienced adult European Mouflon rams (Ram 1 and Ram 2) using an artificial vagina and a teaser ewe, held in a head gate. Both the Mouflon were four years old. After collection, semen volume, concentration and motility were quantified. Semen was extended with a diluent (tris: 2.4 g/100 mL, fructose: 1.0, citric acid: 1.3, streptomycin: 0.1, penicillin: 0.1; pH 7.2; osmolarity 270-280 mOsm/kg) and motility was quantified again. The extended semen was later divided at 30 °C into 0.25 mL insemination doses and refrigerated at +4°C or frozen in liquid nitrogen (-70 °C). Frozen doses were added with 4% glycerol. The doses were kept at the same temperature (+4 and -70 °C, respectively) until use. Spermatozoa mean concentration were: 400 million per frozen dose and 800 million per refrigerated dose. Insemination was performed by means of two different techniques, and the semen of each ram was equally distributed over the groups A and B. Ewes of Group A were cervically inseminated using refrigerated semen 56 hours after the sponge removal. Semen was collected and extended the same day of the insemination. Ewes were held in a standing point and with the aid of a tubular vaginoscope equipped with a light source and a miniaturized syringe, semen was deposited at the external cervical os. Ewes of the Group B, were inseminated into the uterus using the laparoscopic technique (Mc Kelvely et al., 1985), with, cryopreserved and later thawed semen (Berlinguer et al., 2005). After AI the two groups were managed together as one group. During the morning they were allowed to graze a natural polyphytic pasture while in the evening were housed indoor receiving ad libitum darnel hay (*Lolium spp.*) and 300 g of commercial pellets per head that consisted of 18% crude protein, and 12,5 MJ ME/kg dry matter. All the animals had free access to water. Pregnancy rate (pregnant ewes/treated ewes ratio) was determined 38 days after AI by transabdominal ultrasonography examination using an Esaote Piemedical Tringa linear equipment (Esaote Europe B.V., Maastricht, The Netherlands) provided with a 5.0 – 7.5 MHz multiple frequency linear probe. At lambing time, twin lambing, number, sex, and live weight of lambs were recorded. Each lamb was also weighed 40 days after birth, at slaughtering time. Pregnancy, lambing rate and sex ratio in both the groups were compared by χ^2 -test, weights by means of ANOVA. Data are presented as mean \pm SD and differences were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION - All the results are summarized in table 1. 20 ewes belonging to Group A and 14 to Group B were diagnosed pregnant after ultrasonography. χ^2 -test shows high significance value ($P < 0.01$), with a pregnancy rate higher in Group A (20/40, 50%) than in B (14/40, 35%). At lambing time 19 and 13 ewes lambbed in Group A and B, respectively. χ^2 -test shows a high significance value ($P < 0.01$) and fertility rate was higher in Group A than in B (47.5% vs 32.5%). Lambing rate published by Berlinguer et al. (2005) using laparoscopic technique with cryopreserved semen was higher, but in this research the Mouflon semen was used on the same specie, inseminating Mouflon ewes. In Group A, 21 lambs were born (two twin lambing were recorded), and 14 in Group B (one twin lambing), so in both groups prolificity rate was similar (1.1%). Live weights at lambing were similar: 2.9 ± 0.3 Kg in Group A and 3.0 ± 0.3 Kg in Group B; also at slaughtering time, live weight had similar distribution (10.8 ± 1.3 and 10.4 ± 1.5 in Group A and B, respectively). Sex ratio was similar in both the groups. Statistical analysis indicated there was no differences in pregnancy and fertility rate, sex and weight of the lambs born from semen collected from Ram 1 in comparison to the lambs born from semen collected from Ram 2.

Table 1. Means (\pm SD) of data according to artificial insemination technique.

	Group A	Group B	χ^2 -test
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	(n=40)	(n=40)	value
Ewes diagnosed pregnant after 38 days (ultrasonography)	n =20	n =14	P<0.01
Pregnancy rate	50%	35%	
Lambs born at lambing time	n =19	n =13	P<0.01
Fertility rate	47.5%	32.5%	
Sex ratio M/F	10/9	6/7	n.s.
Live weight at birth (kg)	2.9 ± 0.4	3.0 ± 0.3	n.s.
Pre-slaughter weight, lambs 40 days-old (kg)	10.8 ± 1.3	10.4 ± 1.5	n.s.

Group A = cervical insemination with refrigerated semen; Group B = intrauterine/laparoscopic insemination with frozen and thawed semen.

n.s. = no significant

CONCLUSIONS - In conclusion, both the techniques of artificial insemination were useful to produce the crossbred lambs Mouflon x Sarda sheep, a new meat foodstuff that could be considered as a diversification of dairy sheep industry. The cervical method utilised in Group A was practical and its application was easy to obtain lambs on a large scale. Its fertility rate, higher than in Group B, indicates that the use of fresh semen could give a better reproductive performance. On the other hand, results regarding laparoscopic-intrauterine technique could be considered good and encouraging, because it allows to use semen in a rational way and in a longer lapse of time, also during long-day photoperiod exposure, when it is still possible to synchronize oestrus of the ewes by the use of intravaginal sponges and injection of PMSG, but Mouflon semen could be outside of acceptable parameters of volume, concentration and motility.

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