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Good quality sheep embryos produced by superovulation treatment without the use of progesterone devices

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Abstract

Multiple ovulation and embryo transfer (MOET) is a very important tool for the genetic improvement and preservation of endangered livestock. However, the success of a MOET programme highly depends on the number of transferable embryos in response to a superovulation treatment. Thus, the aim of this study was to compare the number and quality of embryos produced during natural oestrus under porcine FSH treatment without the use of progesterone devices to more traditional protocols. Forty Sarda sheep were divided into 2 groups: without sponges (WS) (n = 20) and with sponges (S) containing 40mg FGA for 12 d (n = 20) (control group); 350 I.U. of porcine FSH per sheep was administered in eight decreasing doses twice daily starting four days after estrus was detected (Day 0) in group WS and 48 h before sponge removal in group S. A single i.m. dose of 125 μg of cloprostenol was administered on Day 6 after estrus in group WS to induce luteolysis. Sheep were naturally mated 24 h after cloprostenol injection or sponge removal. Seven days after mating, an inguinal laparotomy was performed and the number of corpora lutea (CL) recorded. Embryos were recovered surgically by flushing each uterine horn. A total of 38 fresh and 22 vitrified embryos were transferred in pairs into 3 groups of recipients seven days after estrus detection: fresh embryos from group S (S-F) (n = 9), fresh embryos from group WS (WS-F) (n = 10) and vitrified embryos from group WS (WS-V) (n = 11). Data on the number of corpora lutea (CL), recovered ova and embryos (OER), and quality 1–2 and 3 embryos ($EQ_{1\text{-}2}$, EQ_3) per ewe were analyzed by ANOVA. Recovery (RR), fertility (FR) and quality 1–2 embryo ($Q_{1\text{-}2}R$) rates per treatment were analyzed by a Chi Square analysis. A Chi Square analysis was also applied to pregnancy rate (PR), lambing rate (LR) and twinning rate (TR) of fresh and vitrified embryos in order to analyze embryo transfer results. Among all superovulation variables analysed, results show statistically significant differences in mean number of CL/ ewe (9.3 ± 3.9 vs 7 ± 3.2), RR (67% vs 80 %) and FR (100% vs 80%) ($P < 0.05$) between WS and S groups respectively. There were no significant differences in PR (78%, 70% and 82%), LR (67%, 60% and 59%) and TR (71%, 71% and 44.4%) among S-F, WS-F and WS-V groups respectively. In conclusion, it is possible to produce a good number of transferable embryos during natural oestrus avoiding the use of sponges.

Keywords: MOET; Natural estrus; Superovulation; Embryo transfer

1. Introduction

Multiple ovulation and embryo transfer (MOET) biotechnology has the potential to speed genetic improvement of domestic animals and preserve the genetic resources of endangered livestock.

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Table 1
Distribution of onset of estrus.

Date	No. of animals in heat/estrus detection		Total no. of animals in heat/day	No. of animals considered for WS treatment/day*
	8 AM	*4 PM*		
Friday Oct 3/08	2	2	4	4
Monday Oct 6/08	2	2	4	4
Tuesday Oct 7/08	1	1	2	2
Wednesday Oct 8/08	1	2	3	3
Thursday Oct 9/08	7	0	7	4
Friday Oct 10/08	3	1	4	3

* Note: Given the time and facilities required to perform embryo recovery surgery, a maximum of 4 animals were selected per day.

In small ruminants, a traditional MOET programme includes the insertion of a fluorogestone acetate (FGA) or medroxy-progesterone acetate (MAP) intravaginal sponge for 12 or 14 d in order to induce and synchronize the estrous cycle and the administration of exogenous gonadotrophins beginning 2 d before the sponge removal to stimulate follicular growth [1]. It has been reported that despite the benefits of synchronization, progestagens are considered to have a negative effect on the number of the ovulations and transferable embryos in response to a superovulation treatment [2,3]. Indeed, when the treatment is not able to maintain normal physiologic levels of progesterone it is possible to have alterations in the patterns of follicular growth and dominance of large estrogenic follicles [4,5,6] as well as alterations in the process of fertilization and in the development of a good quality embryo [7,8]. Hawk et al [9] also described local effects such as impairment of sperm transport into the female genital tract. Strategies used to overcome the negative effects of progestagen treatment include: i) the insertion of a second progesterone device to maintain constant levels of this hormone during treatment [10,11], ii) supplying gonadotrophins soon after sponge removal at the time of ovulation to avoid the detrimental effects of follicular dominance [12,13] or iii) administering superovulatory treatment without the use of sponges, starting four days after natural estrus detection, making use of physiological progesterone levels [14]. However, this last choice is practical only in small scale MOET programs (4–5 donors/day), because only 6–8% of females in a flock come into estrus spontaneously each day during the breeding season [15], necessitating multiple days of estrus detection.

Thus, the aim of this study was to compare the number and quality of embryos produced during natural oestrus under superovulation porcine FSH treatment without the use of sponges to more conventional protocols.

2. Materials and methods

2.1. In vivo embryo production

The study took place during breeding season (September–December, 2008) at the Laboratory of Biotechnologies of Reproduction in DIRPA-AGRIS Sardinia located at 40°40' north latitude and 8°22' east longitude. Forty healthy Sarda ewes (from a flock of 60) between 2–5 years old were selected as donors and divided into 2 groups: without sponge (WS) (n = 20) and with sponge (S) (n = 20) (control group).

2.1.1. Donor management

2.1.1.1. Selection of without sponge (WS) group. Group WS ewes in estrus were selected (day 0) via the introduction of vasectomised rams into the flock twice daily (8:00 AM and 4:00 PM) for 30 min. The ewes were considered to be in estrus when they showed estrus behaviour and were mated by the vasectomised ram. Six days of estrus detection (excluding Saturdays and Sundays) were necessary to reach the required group total of 20 animals (Table 1).

2.1.1.2. Selection and synchronization of the with sponge (S) group. Group S animals were selected and synchronized (4 ewes/day) by insertion of intravaginal sponges containing 40 mg fluorogestone acetate (FGA) (Chronogest ®, Intervet, Boxmeer, Holland). Sponges were left in place for 12 d. After sponge removal, ewes were placed in pens of 4 animals each and tested to determine the onset of estrus behaviour by twice-daily visual observations (8:00 AM and 4:00 PM) with the introduction of a vasectomised ram for 30 min.

2.1.1.3. Superovulatory treatment. Superovulatory treatment consisted of 350 I.U. of porcine FSH (pFSH) (Folltropin®, Bioniche Animal Health, Ireland) administered in eight decreasing i.m. doses every 12 h (2 mL × 2, 1.5 mL × 2, 1.0 mL × 2 and 0.5 mL × 2) starting 4 d after estrus detection (Day 0) in the WS group, and