## FIRST RESULTS IN PORTUGAL OF ARTIFICIAL INSEMINATION BY CERVICAL ROUTE USING RAM FROZEN SEMEN ON REPRODUCTIVE PERFORMANCE

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## INTRODUCTION

Factors related with a decreased fertility using thawed semen were identified with bad seminal traits and viability after thawing, individual and breed semen susceptibility to freezing and seasonal effects. Factors mediating poor AI results with refrigerated semen are enhanced when frozen/thawed semen is used. Here are presented first results of cervical AI with frozen ram semen as influenced by breed, male and season of semen freezing in Portugal.

## MATERIAL AND METHODS

In both experiments fresh semen was diluted and packed in 0.25 mL Cassou straws  $(300 \times 10^6)$ spz/straw) and refrigeration lasted 4 h. Refrigerated semen (+4°C) was frozen by cooling until -110°C (57°C/min) and from -110°C to -180°C (2.3°C/min) in a cryochamber before LN<sub>2</sub> immersion. Only good quality ejaculates were used for freezing. Semen extender consisted of TRIS, glucose, citric acid, antibiotics, egg yolk (19%) and glycerol (8%). Oestrus synchronization was done with fluorogestone acetate (40 mg) vaginal sponges during 12 days and 500 IU of eCG i.m. at sponge removal (Intervet ®). Cervical AI was performed 55 h after sponge removal using 2 straws per ewe, with sperm motility > 40% after thawing. Experiment 1 (freezing season and ram individual effects): AI was performed in April on 273 synchronized ewes (Saloia breed) with thawed semen frozen in autumn (n=87, rams B (43) and C (44)) or winter (n=186, rams A (30), B (51) and C (105)). Fertility (lambing ewes/AI'ed) and fecundity (lambs / AI'ed) were compared between seasons (ram B and C) and among rams (autumn: B vs. C; winter: all rams) within seasons, by Mann-Whitney U test. Interactions season x ram were estimated by ANOVA-MANOVA. Experiment 2 (ewe's breed effect): A total of 62 synchronized ewes (Ille de France x Serra da Estrela x Romanov, n=36 and Merino, n=26) were AI'ed with thawed semen of 3 Merino rams (I, II and III) in May. Males I, II and III inseminated respectively 9, 11 and 16 crossbreed ewes and 12, 10 and 4 Merino ewes. Fertility and fecundity was compared among breeds (crossbreed vs. Merino) and rams as described before.

## **RESULTS AND DISCUSSION**

Experiment 1: Season of freezing (using rams B and C) did not influence fertility (18.1%) (ram B: 14.0% vs. 9.9%; ram C: 18.2% vs. 23.8%; for autumn vs. winter, respectively, p>0.05) and fecundity (24.3%) (ram B: 14.0% vs. 13.7%; ram C: 25% vs. 33.3%: for autumn vs. winter. respectively, p>0.05). Ram C showed higher fertility (p=0.038) and fecundity (p=0.039) rates than ram B only in the winter freezing season. Fertility and fecundity of Ram A (6.7% and 10%) were significantly different from those of ram C in the winter season (p<0.04). No interaction was found between season of freezing and individual rams for fertility and fecundity. Individual ram effect was detected only in winter for both variables (p<0.04). Experiment 2: Fertility was 30.6% and 0% in crossbreed and Merino ewes, respectively (p<0.002). No individual ram effect on fertility was detected in the crossbreed group (33.3%, 27.3% and 31.3 respectively for rams I, II and III; p>0.05). Although both groups of ewes were treated in the same conditions and AI'ed with same rams, not a single Merino ewe have lambed and no other factor influencing this result was identified besides breed. Results above show no interaction of season of semen freezing and individual ram competence to freezing either on fertility and fecundity. Only winter frozen semen showed individual ram differences upon fertility and fecundity. A marked negative effect of Merino ewes on fertility was detected.

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