

EFFECT OF THE *FecX^R* ALLELE ON FOLLICLE SIZE AND OOCYTE COMPETENCE ASSESSED BY LOPU AND IVP OF SHEEP EMBRYOS

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In several sheep breeds, different mutations in the *BMP15* gene which lead to increased ovulation rate in heterozygous ewes when compared with wild-type animals have been found. One of these polymorphisms is the *FecX^R* allele in the Rasa Aragonesa breed. Although the exact mechanism by which these mutations increase ovulation rate is not fully known yet, the reduced activity of the BMP signalling system would lead to smaller antral follicles with fewer granulosa cells and altered sensitivity to gonadotropins, leading to higher ovulation rates. As the proportion of competent oocytes is widely accepted to increase along with follicular size, in the present study we aimed to investigate the effect of the *FecX^R* allele on follicle number and size, as well as its potential effect on oocyte competence.

With these aims, 8 heterozygous (R+) and 8 wild-type (++) ewes were subjected to 2 laparoscopic ovum pick-up (LOPU) trials (4 sessions per trial; 2 with and 2 without FSH). Collected COCs were subjected to in vitro maturation, fertilisation and culture. Day 7 and 8 blastocysts were fresh transferred to assess their viability by their ability to sustain pregnancy to term.

A total of 1673 follicles were aspirated, yielding 995 oocytes (mean collection rate, 59.5%). Genotype did not affect the total number of aspirated follicles per ewe and session (10.4 and 10.2 in R+ and ++ untreated ewes, 17.4 and 14.3 in R+ and ++ FSH-treated ewes, respectively), but mean follicular size was significantly reduced in R+ ewes (4.1 and 4.3 in R+ and ++ untreated ewes, 4.3 and 5.1 in R+ and ++ FSH-treated ewes, respectively; $P < 0.01$). Cleavage rate ranged from 84.0% to 87.8%, with no differences between genotypes. No significant differences were observed in the rate of day 7 blastocysts between R+ and ++ groups (27.0% and 21.4% in untreated ewes, and 31.3% and 33.7% in FSH-treated ewes, respectively), or in day 8 blastocysts (32.5% and 25.6% in untreated ewes, and 37.7% and 36.8% in FSH-treated ewes, respectively). Fertility of the recipients and survival rate of transferred embryos were similar in both genotypes. Overall fertility was 77.1% at 25 days post transfer determined by PAG, 66.7% at 30 days by ultrasonography, and 56.3% at lambing. Overall in vivo survival rate of the transferred embryos was 38.5% (37/96).

In conclusion, the presence of the *FecX^R* allele led to reduced follicular size without affecting the number of punctured follicles per ewe and session. However, despite oocytes from R+ ewes came from smaller follicles, they demonstrated to be as competent as those from wild-type ewes for embryo IVP, with no differences between genotypes in survival rate at birth of the transferred embryos.