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Dipartimento di Scienze Veterinarie

Istituto Zooprofilattico Sperimentale della Sicilia  
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**SELECTION OF YOUNG EWE LAMBS ACCORDING TO THEIR ANTRAL FOLLICULAR COUNT: RESPONSE TO EXOGENOUS HORMONAL STIMULATION AND FERTILITY AT FIRST BREEDING SEASON.**

Torres-Rovira L<sup>1</sup>, Manca ME<sup>1</sup>, Gonzalez-Bulnes A<sup>2</sup>, Spezzigu A<sup>3</sup>, Piu P<sup>1</sup>, Gallus M<sup>4</sup>, Succu S<sup>1</sup>, Chelucci S<sup>1</sup>, Leoni G<sup>1</sup>, Berlinguer F<sup>1</sup>, Naitana S<sup>1</sup>.

<sup>1</sup>Department of Veterinary Medicine, University of Sassari, Sassari, Italy; <sup>2</sup>Department of Animal Reproduction, INIA, Madrid, Spain; <sup>3</sup>Embryo Sardegna, Technology, Reproduction, and Fertility, Perfugas, Sassari, Italy; <sup>4</sup>Department of Animal Production, AGRIS Sardegna, Bonassai, Sassari, Italy.

**Key words:** Anti-Mullerian Hormone, Antral Follicular Count, Ewe, Fertility

**Introduction:** Anti-Mullerian Hormone (AMH), Antral Follicular Count (AFC) and the response to exogenous hormonal stimulation have been used, in adults, as suitable markers to determine the ovarian reserve (1-4), to predict oocyte quality (5,6) and a wide variety of fertility indices (6-9).

This investigation aims to evaluate if animals selected according to their High or Low AFC at an early prepubertal age show different responses, in the number of follicles and AMH plasma levels, to exogenous hormonal stimulation; to verify whether differences are maintained over time until puberty; and to observe possible variations on fertility at first breeding season.

**Material and Methods:** Forty eight Sarda ewe lambs, with a mean age of  $49.77 \pm 1.15$  days-old, were classified according to the number of follicles  $\geq 2$  mm in diameter present in their ovaries into Low AFC group ( $\leq 15$  follicles) and High AFC group ( $\geq 30$  follicles). The number of  $\geq 2$  mm follicles was determined by transrectal ultrasonography with a real-time B-mode scanner fitted with a 7.5 MHz rigid laparoscopic transducer (10). At 1.5 and 6.5 month-old, one-shot intramuscular dose of 60 mg FSH was administered to all animals and the number of  $\geq 2$  mm follicles was recorded before and 24 hours after treatment along with blood sample gathering. Plasma AMH concentration was analyzed with a commercial ELISA kit. Fertility at first reproductive season was assessed by the number of animals that remained pregnant. Rams were introduced into the ewe flock from August to the end of September, then, pregnancy diagnosis was performed using transrectal ultrasonography.

**Results:** At 1.5 month of age High AFC group had a significant higher number of 2mm, 3mm and total follicles ( $\geq 2$  mm) than Low AFC group. This difference, remained after FSH administration and even became significant for  $\geq 4$ mm follicles. Follicles grew to larger sizes and follicular recruitment increased for both groups. At 6.5 month of age the High AFC group had a significantly higher number of  $\geq 4$ mm follicles. After exogenous FSH stimulation, High AFC group had higher number of follicles in each category. AMH plasma levels at 1.5 month-old were significantly higher in the High AFC group before ( $1.766 \text{ ng/ml} \pm 0.143$  for High AFC group vs  $0.357 \pm 0.143$  for Low AFC group) and after ovarian stimulation ( $1.217 \text{ ng/ml} \pm 0.143$  for High AFC group vs  $0.239 \text{ ng/ml} \pm 0.143$  for Low AFC group;  $p < 0.05$ ). Circulating AMH was positively correlated to total number of follicles  $\geq 2$  mm before ( $r = 0.65$ ) and after ( $r = 0.82$ ;  $p < 0.0001$ ) treatment. Also, initial AMH plasmatic levels were positively correlated to the number of follicles grown per each follicular category after FSH administration (2mm  $r = 0.72$ , 3mm  $r = 0.83$ ,  $\geq 4$ mm  $r = 0.39$ , total  $r = 0.85$ ;  $p < 0.01$ ). At 6.5 month of age animals had undetectable levels of AMH. Regarding fertility data, 60.87% of the High AFC ewes remained pregnant at first breeding season versus 33.33% of the Low AFC ewes ( $p < 0.05$ ).

**Conclusions:** Selection of young ewe lambs with high versus low AFC by ovarian ultrasonography can improve fertility at first breeding season. Although obvious differences in AFC tend to disappear with aging

during the prepubertal period, ovarian response to exogenous stimulation remains higher in animals with high AFC.

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