

Innovations in the selection program of the UPRA-Grupo Pastores in Rasa aragonesa sheep breed

L. Riaguas¹, E. Fantova¹, equipo Veterinario Oviaragón¹, J.H. Calvo^{2,3}, J.L. Alabart², J. Folch², B. Lahoz², J.J. Jurado⁴, M.A. Jimenez⁴ and M. Serrano⁴

¹Oviaragón-Grupo Pastores, Ctra. Cogullada s/n, 50014 Zaragoza (Spain)

²Unidad de Producción y Sanidad Animal, CITA, Instituto Agroalimentario de Aragón – IA2 – (CITA-Universidad de Zaragoza), 50059 Zaragoza (Spain)

³ARAID, 50018 Zaragoza (Spain)

⁴Departamento de Mejora Genética Animal, INIA, 28040 Madrid (Spain)

Abstract. The Cooperative Oviaragón-Grupo Pastores carries out since 1994 a selection program for prolificacy in Rasa Aragonesa sheep, with 490,337 ewes at present. Sires of higher estimated breeding values are produced within the scheme by MOET, increasing the performance of this technology through the measurement of plasmatic Anti-Müllerian Hormone (AMH) in ewe embryo donors, an endocrine marker of the ovarian activity. Within this program a natural genetic prolific variant was found in 2007, the *FecX^R* allele of the *BMP15* gene. Its effect on prolificacy leads to an increase of 0.35 lambs/lambing ewe when compared with non-carrier ewes, with an additive effect over the standard hormonal treatments used in farms. Recently, new variants in *MTNR1A* gene associated to reproductive seasonality have been detected in Rasa aragonesa breed. Non-linked SNPs in the promoter and exon 2 have been detected which decrease the length of the non-cycling period (periods with three or more consecutive weekly-sampled progesterone concentrations lower than 0.5 ng/ml from January to August) as much as 30 and 53 days, respectively. In the same way, the allele located in exon 2 was also associated to an increase of 15% of oestrus cycling months (based on oestrus records). Due to their productive interest, a controlled program for the outreach of *FecX^R* and *MTNR1A* alleles has been developed. Finally, a polygenic selection program for maternal capacity is being carried out. The selection program goes on with a combined polygenic selection for prolificacy, maternal capacity and dissemination of *FecX^R* and *MTNR1A* alleles.

Keywords. Ovine – Selection program – Prolificacy – fertility.

Innovations dans le programme de sélection de l'UPRA-Grupo Pastores dans la race Rasa aragonesa

Résumé. La Coopérative Oviaragón-Grupo Pastores mène depuis 1994 un programme de sélection pour la prolificité sur la race ovine Rasa aragonesa avec donnés enregistrées sur 490337 brebis à l'heure actuelle. Les béliers à tester pour son valeur génétique sont produits par un Programme MOET, étant le rendement de cette technologie augmentée à travers l'analyse de l'Hormone plasmatique Anti-Müllérienne (AMH) dans les brebis donneuses, un marqueur endocrinien de l'activité ovarienne. Dans ce programme une variante génétique naturel prolifique a été découverte en 2007 (*FecX^R*, allèle du gène *BMP15*). Son effet sur la prolificité conduit à une augmentation de 0,35 agneaux/brebis agnelant par rapport aux brebis non-porteuses, ayant un effet additif sur les résultats des traitements hormonaux standard utilisés dans les troupeaux. Récemment, des nouvelles variantes dans le gène *MTNR1A* associé à la saisonnalité de la reproduction ont été détectés dans la race Rasa aragonesa. Des SNPs non liés ont été détectés au promoteur et à l'exon 2 en diminuant la durée du période d'anoestrus jusqu'à 30 et 53 jours, respectivement. (périodes dont les concentrations de progestérone sont inférieures à 0,5 ng/ml au cours de trois semaines consécutives ou plus, de janvier à août). De même, l'allèle situé dans l'exon 2 est également associée à une augmentation de 15% de mois avec manifestation d'oestrus (basé sur la détection de chaleurs). En raison de leur intérêt productif, un programme contrôlé de diffusion de l'allèle *FecX^R* et *MTNR1A* ont été mis au point. Finalement, un programme de sélection polygénique de capacité maternelle est en cours. En conclusion, le programme de sélection continue avec l'association d'une sélection polygénique pour prolificité, capacité maternelle et la diffusion des allèles *FecX^R* et *MTNR1A*.

Mots-clés. Ovine – Programme de sélection – Prolificité – Fertilité.

I – Introduction

Rasa aragonesa is a Mediterranean autochthonous sheep breed from the northeastern Spain, mainly reared in extensive or semiextensive farming systems and oriented to meat production. Improvements in farms efficiency is possible by genetics, nutrition and management approaches. In this context, the Cooperative Oviaragon-Grupo Pastores carries out since 1994 a selection program for prolificacy in Rasa aragonesa, with 490,337 ewes at present, since the number of lambs born per lambing ewe is a critical point in the efficiency and viability of these farms (Pardos *et al.*, 2008). Artificial Insemination (AI) with fresh semen at 15 °C is used for the connection of the flocks and diffusion of the genetic improvement (133,000 AI with a mean fertility of 55% since the beginning of the program). Prolificacy is considered a difficult trait because of its low polygenic heritability ($h^2 = 0.034$; Jurado *et al.*, 2008). In Rasa aragonesa, phenotypic prolificacy is 1.37 lambs/birth (16th genetic evaluation made in 2016, unpublished). However, new selection objectives, as maternal ability, or marker-assisted selection based on major genes are being developed to increase the selection efficiency.

In particular, this paper focuses in some results on innovations in the selection program of the UPRA-Grupo Pastores in Rasa aragonesa sheep breed.

II – Multiple ovulation and embryo transfer (MOET)

This selection scheme exploits multiple ovulation and embryo transfer (MOET) to produce ovine embryos with identified sex in combination with *PrnP* genotype determination. In the selection scheme to improve prolificacy in the Rasa aragonesa sheep breed, the males to be tested are produced by a MOET programme, using the ewes of higher genetic value as donors. In the period from 1998 to 2013, a total of 331 flushings have been performed in 213 donor ewes. Ewes were treated with FGA sponges and superovulated with 8.8 mg of oFSH (Ovagen) in eight decreasing doses (2 x 1.32; 4 x 1.1 and 2 x 0.88 mg) at 12h intervals. Since 2008, Ovagen was replaced by Folltropin. Intrauterine insemination (200×10^6 spermatozoa/ewe) with fresh semen from selected rams was carried out 51h after sponge withdrawal. A total of 3076 embryos were obtained (2037 morphologically viable), 1551 of which were fresh-transferred to FGA+eCG synchronized recipients (two morulae or blastocysts per recipient). A total of 931 lambs were born (470 males), 226 of which were suitable to be tested (Folch *et al.*, 2015). The efficiency of the MOET program has been influenced by many factors, such as the improvement of the techniques, the number of flushings per donor, the genetic value of the donor or the ovulation rate of the recipient. Anti-Müllerian hormone (AMH) has been demonstrated to be a good predictor of the ovarian response to gonadotropins in several mammalian species. We aimed to test its usefulness in the MOET program, where the performance is widely known to be low mainly due to the variable ovarian responses to FSH treatments. Two plasma AMH samples were obtained from each ewe and session at the time of the first FSH injection (T0) and 4 days before (T-4), and were analyzed using the AMH equine ELISA kit (AnshLab, Webster, TX, USA). The AMH concentrations as well as the other MOET-related variables were highly variable between individuals. The AMH at T0 was highly correlated with the total number of corpus luteum (CL) ($r=0.70$; $p<0.05$). In conclusion, plasma AMH concentrations measured before the FSH treatment could be used to predict the ovulatory response of donor ewes, and so to improve the efficiency of MOET programs (Lahoz *et al.*, 2015). More data are necessary to assess the individual repeatability as well as the relationship of AMH with other MOET-related variables, in order to establish a reliable protocol to be implemented in farms before selecting and moving the donor ewes. Moreover, selecting at the same time offspring of the preferred sex will make more efficient the MOET selection scheme for increasing prolificacy. In this sense, sex determination in ovine embryos using a duplex PCR has been applied with an efficiency in sex de-

termination of 95 and 98% when more than two and more than three cells were sampled from compact morulae, respectively (Dervishi *et al.*, 2008; Dervishi *et al.*, 2011). The total time required for the genetic test, was less than 4 h. This rapid sex determination using *AMEL* and *PRNP* genes allows transferring sexed fresh embryos in MOET and IVF (In Vitro Fertilization) programs to make them more efficient.

III – Major genes

In 2007, some descendants of the tested rams showed an uneven increase in prolificacy unexplained by polygenic heredity, leading to the discovery of a new naturally occurring polymorphism in the bone morphogenetic protein 15 (*BMP15*), a fecundity gene with a major effect on ovulation rate (OR) in sheep. The polymorphism (*FecX^R* allele) consists of a deletion of 17 bp in the coding region of *BMP15*, located on the X chromosome, which produce an increased in prolificacy in heterozygous (R+) ewes and sterility in homozygous (RR) ewes (Martinez-Royo *et al.*, 2008). The *FecX^R* mutation, when present in heterozygosity, produces 0.44 extra ovulations in ewe lambs and 0.63 in adult ewes, that lead to an increase in prolificacy of 0.35 lambs per lambing ewe, with no adverse effect on oocyte quality, preovulatory LH surge, fertility after AI or offspring weights at birth (Lahoz *et al.*, 2011). The R+ ewes show an increased response to eCG that should be taken into account to avoid too high rates of triplets and higher order births (Lahoz *et al.*, 2011). A study was conducted to evaluate the effects of lamb (RR, R+ or ++) and maternal genotypes (R+ or ++) on birth weight, growth and meat quality traits. All lambs were classified within the normal ranges for the “Ternasco de Aragón” commercial category, and no significant differences were observed between the genotype groups. We conclude that birth weight, growth traits, light lamb carcass characteristics and meat quality traits were not affected by the maternal and lamb *FecX^R* genotype combinations studied (Roche *et al.*, 2012). The use of the *FecX^R* allele allows for a very quick increase in prolificacy that results in important economical advantages, meeting the needs of farmers to improve this parameter. However, it is essential to continue with the classical polygenic selection to prevent excessive inbreeding and to improve other productive parameters. In fact, in 2016 the estimated population of R+ ewes was approximately 15,200, showing a clear trend toward increased numbers.

Sheep breeds from the Mediterranean area show reproductive seasonality, mainly regulated by variation in the photoperiod. Maximal reproductive activity is associated with short days, with the highest percentage of ewes exhibiting ovulatory activity from August to March. This reproductive seasonality induces great variation in lamb production and, therefore, in the market price of lamb meat. Hormonal treatments are widely used in some countries to control reproductive activity out of the breeding season, but the increasing demand for hormone-free products leads to search for alternative methods such as the ram effect or the use of genetic markers. In this sense, new variants in *MTNR1A* gene associated to reproductive seasonality have been detected in two different (R+ and ++) populations in the Rasa aragonesa breed. Non-linked SNPs in promoter and exon 2 regions have been detected decreasing the length of non-cycling period (based on weekly individual plasma progesterone levels and defined as the sum of days in anoestrus, considering anoestrus those periods from January to August with three or more consecutive P4 concentrations lower than 0.5 ng/ml) as much as 30 and 53 days, respectively. In the same way, the allele located in exon 2 was also associated to an increase of 15% of oestrus cycling months (defined for each ewe as the rate of months with at least one oestrus record between January and August). The SNP located in exon 2 produces an aminoacid change, while mutations identified in the promoter region could affect binding motifs for some transcription factors. It is important to remark that mutations in the promoter region are age dependent, affecting mainly to young animals. Due to their productive interest, a controlled program for dissemination of *MTNR1A* favorable alleles is currently being developed.

IV – Maternal capacity

Selecting rams with high prolificacy estimated breeding values (EBVs) will increase lamb numbers produced by their female offspring. Then, selecting for improved maternal ability traits will ensure ewes have sufficient milk to rear their lambs, as well as maternal carebehaviour. In this sense, in 2015, a maternal capacity selection criterion in the Oviaragon-Grupo Pastores breeding program has been included, taking into account the lamb birth and weaning weights. For this second weight (weaning) it has been developed an automatic weighting equipment with electronic identification, reducing labour and improving efficiency.

V – Conclusions

A selection programme to increase prolificacy has been carried out in the Spanish sheep breed Rasa aragonesa since 1994, exploiting multiple ovulation and embryo transfer (MOET) to produce males to be tested. This selection led to the detection of a new naturally occurring polymorphism in the BMP15 gene (*FecX^R* allele) causing increased prolificacy in heterozygous (R+) and sterility in homozygous (RR) ewes compared with non-carrier (++) ewes, with an additive effect over the standard hormonal treatments used in farms. Recently, *MTNR1A* variants associated to reproductive seasonality have been detected in Rasa aragonesa breed increasing fertility in the non-breeding season. The selection program goes on with a combined polygenic selection for prolificacy, maternal capacity and dissemination of *FecX^R* and *MTNR1A* alleles.

Acknowledgments

The authors are thankful to the cooperative UPRA-Grupo Pastores and to the staff of CITA de Aragón for their assistance in sample collection and analysis. These studies were supported by INIA (RTA2006-0140, RTA2013-00041, RTA2015-0090), TRACE (PET-2008-0076), INNPACTO (010000-2010 IPT-33), CDTI (CDTI-IDI-2004-0611, 2007-0880, 2012-0768, 2016-0426), FITE 2016 (I+D PLATEA, A9), Interreg V A -POCTEFA (EFA103/15-PIRINNOVI), and UE-H2020-Call: SFS-01c-2015 (iSAGE) projects and co-financed with FEADER funds.

References

- Dervishi E., Martínez-Royo A., Sánchez P., Alabart J.L., Cocero M.J., Folch J. and Calvo J.H., 2008. Reliability of sex determination in ovine embryos using amelogenin gene (AMEL). In: *Theriogenology*, 70 (2), p. 241-247.
- Dervishi E., Sánchez P., Alabart J.L., Cocero M.J., Folch J. and Calvo J.H., 2011. A Suitable Duplex-PCR for Ovine Embryo Sex and Genotype of *PmP* Gene Determination for MOET-based Selection Programmes. In: *Reprod. Domest. Anim.*, 46, p. 999-1003.
- Folch J., Alabart J.L., Lahoz B., Mozo R., Calvo J.H., Cocero M.J., Quintin F., Sevilla E., Hernández M., Ramón J., Olivera J., Echegoyen E., Sánchez P., Fantova E., Equipo Técnico Veterinario de UPRA-Grupo Pastores and Jurado J.J., 2015. Rendimiento del MOET aplicado al programa de selección de la raza aragonesa de UPRA-GRUPO PASTORES. In: *XVI Jornadas sobre Producción animal*, p. 444-446. Zaragoza, España. ISBN 978-84-606-7971-4.
- Jurado J.J., Martínez-Royo A. and Calvo J.H., 2008. Efecto fenotípico del alelo BMP15/*FecxR* en la prolificidad de la población de CarnesOviaragon S.C.L. In: *ITEA*, 104 (2), p. 149-154.
- Lahoz B., Alabart J.L., Jurado J.J., Calvo J.H., Martínez-Royo A., Fantova E. and J. Folch., 2011. Effect of *FecXR* polymorphism in the BMP15 gene on natural or eCG-induced ovulation rate and litter size in Rasa aragonesa ewes and implications for on-farm application. In: *J. Anim. Sci.*, 89, p. 3522-2530.
- Lahoz B., Alabart J.L., Sánchez P., Echegoyen E. and Folch J., 2015. Elección de las mejores ovejas donantes de embriones mediante la determinación plasmática de su AMH (HORMONA ANTI-MÜLLERIANA). In: *XVI Jornadas sobre Producción animal*, p. 447-449. Zaragoza, España. ISBN 978-84-606-7971-4.

- Martinez-Royo A., Jurado J.J., Smulders J.P., Martí J.I., Alabart J.L., Roche A., Fantova E., Bodin L., Mul-sant P., Serrano M., Folch J. y Calvo J.H., 2008.** A Deletion in Bone Morphogenetic Protein 15 Gene (BMP15) causes Sterility and increased Prolificacy in Rasa aragonesa Sheep. In: *Anim. Genet.*, 39 (3), p. 294-297.
- Pardos L., Maza M.T., Fantova E. and Sepulveda W., 2008.** The diversity of sheep production systems in Aragón (Spain): characterisation and typification of meat sheep farms. In: *Span. J. Agric. Res.*, 6, p. 497-507.
- Roche A., Ripoll G., Joy M., Folch J., Panea B., Calvo J.H. and Alabart J.L., 2012.** Effects of the FecX R allele of BMP15 gene on the birth weight, growth rate and carcass quality of Rasa Aragonesa light lambs. In: *Small Rumin. Res.*, 108(1), p. 45-53.