Fec, *CA5A* and *CLSTN2* genes and their function during sheep ovulation: a review

Delgado-Tiburcio, Guadalupe A.[1]; **Cortez-Romero, Cesar**[1, 2*]; **Pro-Martínez, Arturo**[1]; **Hernández-Marín, José A.**[3]; **Gallegos-Sánchez, Jaime**[1]

[1] Colegio de Postgraduados, Recursos Genéticos y Productividad-Ganadería, Campus Montecillo, Carretera México-Texcoco km. 36.5, Montecillo, Texcoco, C.P. 56264, Estado de México, México.
[2] Colegio de Postgraduados, Innovación en Manejo de Recursos Naturales, Campus San Luis Potosí, Iturbide No. 73, Salinas de Hidalgo, C.P. 78600, San Luis Potosí, México.
[3] Universidad de Guanajuato, Departamento de Veterinaria y Zootecnia, División de Ciencias de la Vida, Campus Irapuato-Salamanca. Exhacienda El Copal, km 9, Carretera Irapuato-Silao, Irapuato, C.P. 36824, Guanajuato, México.
* Correspondence: ccortez@colpos.mx

ABSTRACT
Objective: To describe the function of *Fec*, *CA5A* and *CLSTN2* genes during ovulation in ewes.
Design/methodology/approach: A search and analysis of scientific information related to *Fec*, *CA5A* and *CLSTN2* genes in sheep was performed.
Results: *Fec*, *CA5A* and *CLSTN2* genes are involved at the ovarian level; ewes carrying the first gene were found to have increased ovulation rate, folliculogenesis and granulosa cell differentiation. *CA5A* stimulates an increased follicular rate and plays an important role in pre-implantation. While *CLSTN2* has activity in ovarian development and growth; it also can interact with other genes involved in follicular maturation, granulosa cell differentiation and development of the ovarian follicle.
Limitations on study/implications: Ewes carrying these genes increase the prolificacy rate in the flock.
Findings/conclusions: The expression of these genes acts synergistically in the ovulatory process, enhancing the ovulatory response by contributing to endocrine, paracrine, and molecular synchronization, so that the maturation of the oocyte occurs, leading to ovulation.

Keywords: genes, *Fec*, *CA5A*, *CLSTN2*, sheep.

Citation: Delgado-Tiburcio, G. A., Cortez-Romero, C., Pro- Martínez, A., Hernández-Marín, J. A., Gallegos-Sánchez, J., (2023). *Fec*, *CA5A* and *CLSTN2* genes and their function during sheep ovulation: a review. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i2.2517

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: November 12, 2022.
Accepted: February 15, 2023.
Published on-line: April 12, 2023.

Agro Productividad, 16(2). February. 2023. pp: 103-111.

INTRODUCTION

Sheep are one of the most important livestock species in the world due to their productive and reproductive potential. For this reason, one of the main objectives of selection and genetic improvement programmes is to identify animals with the best productive characteristics (Cardona-Tobar *et al.*, 2020). Reproductive efficiency is a variable that allows the productivity of the flock to be evaluated; it is determined by ovulation rate, embryo development, fertility rate; among others, which help to determine the profitability

Image by Mahmoud Ahmed at Pixabay

of sheep production systems. The development of an oocyte depends on a combination of paracrine and autocrine factors acquired during folliculogenesis in synchrony with oocyte growth and differentiation, giving it the ability to restart meiosis and support the early stages of embryonic development (Torres-Osorio *et al.*, 2019; Figure 1). Ovulation rate is genetically regulated by the combined action of a group of genes with small effects and, in some cases, by the action of a gene with a larger effect. Genetic variants related to fertility in sheep are referred to as *Fec*, and the different alleles are assigned a letter or the initials of the breed in which the variant was discovered (Luna and Alonso, 2014).

***Gen BMPR1B* (*FecB*)**. In 1990, Piper and Bindon observed an increase in the heritability of the number of lambs born per ewe per lamb (prolificacy) in a flock of Booroola Merino sheep and concluded that the increase could be due to the action of a gene affecting ovulation rate. It was the first time that the existence of a prolificacy gene was demonstrated, and it was later identified as a mutation in the gene encoding the Booroola or *FecB* gene, located in the Bone Morphogenetic Protein Receptor 1B (*BMPR1B*) fragment of sheep chromosome 6, which has a mutation at A746G position 249 of the protein, which changes from glutamine to arginine in animals carrying this mutation. Catalogued as an important gene with additive effect on ovulation rate, associated with high prophilicity in Booroola Merino ewes (Mulsant *et al.*, 2001; Souza *et al.*, 2001; Wilson *et al.*, 2001). In sheep, *FecB* is the main biomarker of fertility and is associated with an increase of 1.5 ovulations per estrus, which translates into an increase of 0.4 to 0.7 lambs per ewe. Bethancourt and Valerio-Mena (2022) reported a fertility of 1.17 in sheep carrying this mutation. Guo *et al.* (2018) proposed that the *FecB* gene not only affects the rate of oocytes and prolificacy, but also favours the uterine environment for correct implantation and subsequent pregnancy, concluding that ewes with this mutation (homozygotes and heterozygotes), compared to those without it, suffer changes in the sequence of amino acids

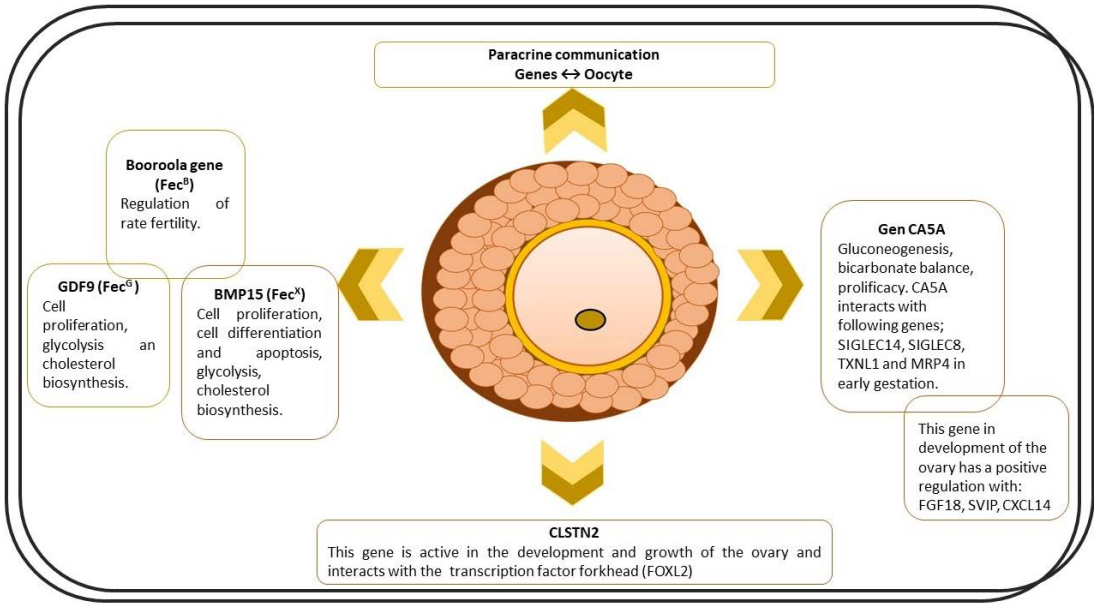

Figure 1. Autocrine, paracrine and genetic factors involved in the regulation of oocyte development.

that make up the oviductal fluid, which favours the prooxidant response in favour of the antioxidant capacity. However, Chen *et al.* (2021) point out that further research is needed on the involvement of the *FecB* gene during embryonic development and interaction with the uterus, as well as the interaction between mRNAs and long non-coding RNAs (lncRNAs) at the oviduct level in sheep. Li *et al.* (2021), identified five new mutations in the *FecB* gene (DD4, DD23, DD17, II12 and II10) in the homozygous genotype of the Chinese white ewe, and that the heterozygous genotype increases litter size at third lambing.

Gen *GDF9* (*FecG*). In sheep, it is located on chromosome five, has a length of 1365 base pairs (bp; Bodensteiner *et al.*, 1999) this gene is expressed in the oocyte from follicular development until ovulation (Hanrahan *et al.*, 2004; Juengel *et al.*, 2013) it is involved in the proliferation and organisation of theca cells surrounding the ovarian follicle, it also promotes granulosa cell proliferation and differentiation through paracrine signalling, as well as steroidogenesis by increasing estradiol secretion (Miyoshi *et al.*, 2012; Strauss and Williams, 2019). The *GDF9* protein is secreted by the growing oocyte and acts as a paracrine factor as it is recognised by specific receptors on the cells of the oocyte cumulus complex, stimulating bidirectional communication and promoting growth and development of the ovarian follicle (McNatty *et al.*, 2005). Eleven mutations have been identified in this gene: $FecG^{T}$ (Nicol *et al.*, 2009), $FecG^{E}$ (Silva *et al.*, 2010) and $FecG^{WNS}$ (Våge *et al.*, 2013), from G1 to G8, of which the G1, G4, G6, G7 and G8 polymorphisms generate changes in the amino acid sequence of the protein they encode, G8 presenting sterility in homozygous females and increased prolificacy rate in heterozygous females (Hanrahan *et al.*, 2004). For the $FecG^{E}$ (Embrapa) variant mentioned above, homozygous ewes carrying the $FecG^{E}$ allele show increased ovulation rate (82%) and prolificacy (58%; Silva *et al.*, 2010). In Pelibuey ewes, Muñoz-García *et al.* (2021) agreed that the $FecG^{E}$ gene mutation in homozygous ewes increased ovulation rate, prolificacy rate and fecundity.

Gen *BMP15* (*FecX*). The bone morphogenic protein gene (*BMP15* or *GDF9B*), located on the X chromosome in sheep, is 1179 bp in length. The protein encoded by the *BMP15* gene is a member of the *TGFβ* family of bone morphogenic proteins (Hanrahan *et al.*, 2004), which acts through a series of signalling proteins (SMAD pathway) responsible for a wide range of physiological behaviours at the cellular level, including oocyte development and maturation, as *BMP15* is expressed in secondary ovarian follicles, stimulating the growth and differentiation of granulosa cells through homotypic connexin-37 junctions, promoting the exchange of nutrients and signals with the oocyte (Luna and Alonso, 2014).

Expression of the *BMP15* gene is a key factor in determining the rate of ovulation and fertility in mammals. In sheep, nucleotide changes in the *BMP15* gene increase ovulation rate by at least 1.0 and litter size by 0.6 (Luna and Alonso, 2014). The following mutations have been reported Fec^{XI}, $FecX^{H}$, $FecX^{G}$, $FecX^{B}$, $FecX^{L}$ and $FecX^{R}$, $FecX^{O}$, $FecX^{Gr}$, $FecX^{Bar}$ and a 17 base pair deletion in $FecX^{R}$, as well as *BMPR1B* and $FecX^{2W}$ gene, which increases the number of antral follicles (Feary *et al.*, 2007; Albarella *et al.*, 2015; Pineda *et al.*, 2018; Hernández-Montiel *et al.*, 2020). Of these, six mutations have been found in this gene that affect prolificacy: $FecX^{G}$ and $FecX^{B}$ (Hanrahan *et al.*, 2004), $FecX^{H}$ and $FecX^{I}$ (Galloway *et al.*, 2000), $FecX^{L}$ (Bodin *et al.*, 2007) and $FecX^{R}$ (Monteagudo *et al.*, 2009) $FecX^{H}$. Argüello-Hernández *et al.* (2014) found the presence of $FecX^{G}$ and $FecX^{L}$ polymorphisms

in the *BMP15* gene in Pelibuey ewes and reported an increase in double lambing, as this mutation increases ovarian sensitivity to FSH and increases the ovulation rate in ewes with this mutation. Demmers *et al.* (2011) and Lahoz *et al.* (2011) mentioned that it is a great advantage to use ewes with these mutations for lamb production. However, it has also been reported that the presence of homozygotes ($FecX^R/FecX^R$) of this variation causes sterility due to ovarian failure, which causes morphological abnormalities such as hypoplasia, leading to infertility (Lahoz *et al.*, 2011; Alabart, 2016).

The *BMP15* gene has a similar expression to the *GDF9* gene, so they are often related, as it is expressed in the oocyte as well as in the follicle and acts through a series of signalling proteins (via SMAD; Luna and Alonso, 2014; Figure 2).

Gen *CA5A* (Carbonic anhydras 5a). Among the candidate genes associated with prolificacy in sheep is the *CA5A* gene, a gene of the carbonic anhydrase (CA) family of zinc-containing metalloenzymes, whose main function is to catalyse the reversible conversion of carbon dioxide to bicarbonate (HCO_{3-}) and is also involved in gluconeogenesis. The *CA5A* gene plays an important role in the bicarbonate supply of many mitochondrial enzymes and is involved in biological and metabolic processes (Pokharel *et al.*, 2020). Hernandez-Montiel *et al.* (2019) showed that *CA5A* is positively expressed in the ovaries of Pelibuey ewes; they observed that the presence of the gene triggered double births compared to non-carriers who had single births. Pokharel *et al.* (2020) studied the regulation of the *CA5A* gene in the ovaries of Texel ewes and found that it was expressed in F1 crosses, leading to the hypothesis that this gene has an important function at least up to the pre-

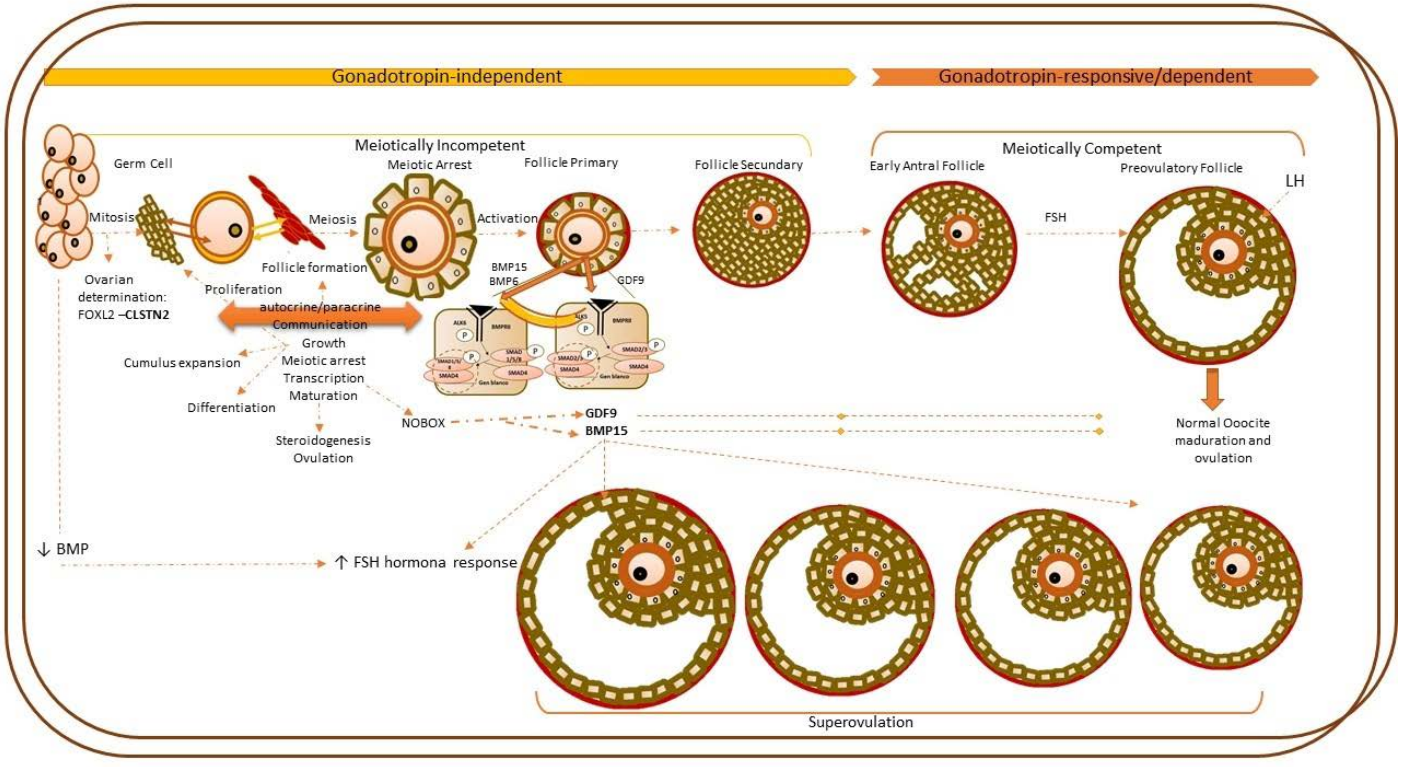

Figure 2. Folliculogenesis and oocyte development through gene expression, paracrine and autocrine (Adapted from Sanchez and Smitz, 2012).

implantation stage, since it was observed that the expression in F1 crosses of the corpus luteum showed a similar behaviour to that of the dams. This gene is associated with several genes during early pregnancy, also participates together whith other genes in the process of ovarian development in ewes (Pokharel *et al.*, 2018; Hernández-Montiel *et al.*, 2019).

Gen *CLSTN2* (Calsyntenin 2), this gene is involved in several processes of vital importance such as: lipid metabolism (Ugi *et al.*, 2014) calcium regulation, metabolic disorders such as glucose and insulin. In addition, this gene is involved in cell proliferation, differentiation, cell death, tumorogenesis, ovarian development and growth together with the transcription factor forkhead (FOXL2), follicular expression: growth and maturation of ovarian follicles, sex determination, and also participates in granulosa cell differentiation with subsequent follicle development (Pisarska *et al.*, 2011).

In Brahman heifers, it was found to be involved in the regulation of insulin and glucose levels, which are very important for cell metabolism and proper communication of the endocrine axis, resulting in carrier females achieving oocyte development and quality leading to first ovulation and puberty faster than non-carriers (Amstalden and Williams, 2014). In sheep and goats, CLSTN2 has been identified as a potential candidate gene for fertility and can be used for marker-assisted selection (Wijayanti *et al.*, 2022).

CONCLUSIONS

This review describes the genetic control of ovulatory development, the mechanisms involved in the control of ovulation rate in mammals and the interaction with other genes. These findings provide useful DNA markers for the selection of sheep with genes associated with increased prolificacy, which is a valuable genetic resource for livestock production when establishing a breeding programme to increase the profitability of the flock.

ACKNOWLEDGEMENTS

To the Consejo Nacional de Ciencia y Tecnología (CONACyT) for supporting the first author GADT as a PhD student (Grant holder 262912). To the Campus Montecillo, the Sheep and Goat Reproduction Laboratory (LaROCa) and the LGAC- Technological Innovation and Food Safety in Livestock Production of the Colegio de Postgraduados.

REFERENCES

Alabart, J.L., Lahoz, B., Calvo, J.H., Jurado, J.J., Fantova, E., Equipo Técnico de UPRA-Grupo Pastores, Folch, J. 2016. Estudios realizados y situación actual de la variante génica prolífica ROA (FecXR) de la raza ovina Rasa Aragonesa. *Arch. de Zootec. 65*(251):449–52. Doi:10.21071/az.v65i251.714.

Albarella, A., Ciotola, F., Selvaggi, M., Dario, C., Licciardi, S., Scopino, G., Frate, F., Peretti, V. 2015. Analysis of major fecundity genes in autochthonous Laticauda and Bagnolese sheep breeds. *Small Rumin. Res.* 133:118– 22. DOI: 10.1016/j. smallrumres.2015.09.005

Amstalden, M., Williams, G.L. 2014. Neuroendocrine control of estrus and ovulation, in: Bov. Reprod, John Wiley & Sons, Inc., Hoboken, NJ, USA, 203–218, https://doi.org/10.1002/9781118833971.ch23.

Argüello-Hernández, H.J., Cortez-Romero, C., Rojas-Martínez, R.I., Segura-León, O.L., Herrera-Haro, J.G., Salazar-Ortiz, J. 2014. Polimorfismos de la proteína 15 morfogénica ósea (BMP15) y su relación con el tipo de parto en la oveja Pelibuey. *Agrociencia*. 48:53-69.

Bethancourt, H., Valerio-Mena, H. 2022. Un Estudio sobre la prolificidad de ovejas mestizas y ovejas con el gen FecB (Booroola) en República Dominicana: A study on the prolificacy of crossbred sheep and sheep carrying the FecB (Booroola) gene in the Dominican Republic. *APF. 10*(2): 83-90.

Bodensteiner, K.J., Clay, C.M., Moeller, C.L., Sawyer, H.R. 1999. Molecular Cloning of the Ovine Growth/Differentiation Factor-9 Gene and Expression of Growth/Differentiation Factor-9 in Ovine and Bovine Ovaries. *Reprod Biol. 60*(2):381-6. Doi:10.1095/biolreprod60.2.381

Bodin, L., E., Pasquale, S., Fabre, M., Bontoux, M., Morget, P., Persani, P., Mulsant. 2007. A novel mutation in the bone morphogenetic protein 15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. *Endocrinology*. 148: 393-400. Doi: 10.1210/en.2006-0764.

Cardona-Tobar, K.M., López-Álvarez, D.C., Álvarez-Franco, L.Á. 2020 Estudios de asociación genómica en ovinos de América Latina. Revisión. *Rev Mex Cienc Pecu. 11*(3):859-883. Doi: 10.22319/rmcp.v11i3.5372.

Chen, W., Li, Z., Sun W., Chu, M. 2021. Analysis of Expression Profiles of mRNA and lncRNA in Oviduct During Estrus Phase of Sheep (*Ovis Aries*) with Two Fecundity (FecB Gene) Genotypes. PREPRINT (Version 1) available at Research Square. Doi: 10.21203/rs.3.rs-119198/v1

Demmers, K.J., Smaill, B., Davis, G.H., Dodds, K.G., Juengel, J.L. 2011. Heterozygous Inverdale ewes show increased ovulation rate sensitivity to pre-mating nutrition. *Reprod Fertil Dev. 23*(7):866-75. Doi: 10.1071/RD10344

Feary, E.S., Juengel, J.L., Smith, P., French, M.C., Connell, A.R.O., Lawrence, S.B., Galloway, S.M., Davis, G.H., Mcnatty, K.P. 2007. Patterns of Expression of Messenger RNAs Encoding GDF9, BMP15, TGFBR1, BMPR1B, and BMPR2 During Follicular Development and Characterization of Ovarian Follicular Populations in Ewes Carrying the Woodlands FecX2 W Mutation 1. *Biol. Reprod*. 77: 990–998. Doi: 10.1095/biolreprod.107.062752

Galloway, S.M., McNatty, K.P., Cambridge, L. M., Laitinen, M.P.E., Juengel, J.L., Jokiranta, T.S., Ritvos, O. 2000. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nat Genet. 25*(3): 279–283. Doi: 10.1038/77033.

Guo, X., Wang, X., Di, R., Liu, Q., Hu, W., He, X., Yu, J., Zhang, X., Zhang, J., Broniowska, K., Chen, W., Wu, C., Chu, M. 2018. Metabolic Effects of FecB Gene on Follicular Fluid and Ovarian Vein Serum in Sheep (*Ovis aries*). *Int J Mol Sci.* 19: 539. Doi: 10.3390/ijms19020539

Hanrahan, J.P., Gregan, S.M., Mulsant, P., Mullen, M., Davis, G.H., Powell, R., Galloway, S.M. 2004. Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). *Reprod Biol. 70*(4): 900–909. Doi: 10.1095/biolreprod.103.023093

Hernández-Montiel, W., Collí-Dula, R.C., Ramón-Ugalde, J.P., Martínez-Núñez, M.A., and Zamora-Bustillos, R. 2019. RNA-seq transcriptome analysis in ovarian tissue of Pelibuey breed to explore the regulation of prolificacy. *Genes*. 10: 358. Doi: 10.3390/genes10050358

Hernández-Montiel, W., Martínez-Núñez, M.A., Ramón-Ugalde, J.P., Román-Ponce, S.I., Calderón-Chagoya, R., Zamora-Bustillos, R. 2020. Genome-Wide Association Study Reveals Candidate Genes for Litter Size Traits in Pelibuey Sheep. *Animals (Basel). 10*(3):434. Doi: 10.3390/ani10030434.

Juengel, J.L., Davis, G.H., Mcnatty, K.P. 2013. Using sheep lines with mutations in single genes to better understand. *Reproduction. 46*(4):111-23. Doi: 10.1530/REP-12-0509.

Lahoz, B., Alabart, J.L., Jurado, J.J., Calvo, J.H., Martínez-Royo, A., Fantova, E., Folch, J. 2011. Effect of the FecX(R) polymorphism in the bone morphogenetic protein 15 gene on natural or equine chorionic gonadotropin-induced ovulation rate and litter size in Rasa Aragonesa ewes and implications for on farm application. *J Anim. Sci. 89*(11):3522-30. Doi: 10.2527/jas.2010-3828.

Li, H., Xu, H., Akhatayeva, Z., Liu, H., Lin, C., Han, X., Lu, X., Lan, X., Zhang, Q., Pan, C. 2021. Novel indel variations of the sheep FecB gene and their effects on litter size. *Genes*, 767:145-176. Doi:10.1016/j.gene.2020.145176.

Luna, P.C., Alonso, M.R.A. 2014. Genes con efecto mayor sobre la fertilidad de ovejas:Revisión. *Rev Mex Cienc Pecu. 5*(1): 107-130. Doi: 10.22319/rmcp.v5i1.3219

McNatty, K.P., Juengel, J.L., Reader, K.L., Lun, S., Myllymaa, S., Lawrence S.B, Laitinen. M.P. 2005. Bone morphogenetic protein 15 and growth differentiation factor 9 co-operate to regulate granulosa cell function in ruminants. *Reproduction.* 129: 481-487. Doi: 10.1530/rep.1.0511

Miyoshi, T., Otsuka, F., Nakamura, E., Inagaki, K., Ogura-ochi, K., Tsukamoto, N., Makino, H. 2012. Regulatory role of kit ligand-c-kit interaction and oocyte factors in steroidogenesis by rat granulosa cells. *Mol Biol Cell. 358*:(1):18-26. Doi: 10.1016/j.mce.2012.02.011.

Monteagudo, L.V., Ponz, R., Tejedor, M.T., Laviña, A., Sierra, I. A. 2009. 17 bp deletion in the bone morphogenetic protein 15 (BMP15) gene is associated to increased prolificacy in the rasa Aragonesa sheep breed. *Anim. Reprod Sci*. 110: 139-146. Doi: 10.1016/j.anireprosci.2008.01.005.

Mulsant, P., Lecerf, F., Fabre, S., Schibler, L., Monget, P., Lanneluc, I., Pisselet, C., Riquet, J., Monniaux, D., Caballebaut, I., Cribiu, E., Thimonier, J., Teyssier, J., Bodiin, L., Cognié, Y., Chitour, N., Elsen, J.M. 2001. Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. *Proc Nat Acad Sc. U S A. 240* (98): 5104-5109. Doi: 10.1073/pnas.091577598

Muñoz-García, C., Vaquera-Huerta, H., Gallegos-Sánchez, J., Becerril-Pérez, C.M., Tarango-Arámbula, L.A., Bravo-Vinaja, Á., Cortez-Romero, C. 2021. Influence of $FecG^{E}$ mutation on the reproductive variables of Pelibuey ewes in the anestrus period. *Trop Anim Health Prod. 17*: 53(2):328. Doi: 10.1007/s11250-021-02755-7.

Nicol, L., Bishop, S.C., Pong-wong, R., Bendixen, C., Holm, L., Rhind, S.M., Mcneilly, A.S. 2009. Homozygosity for a single base-pair mutation in the oocyte specific GDF9 gene results in sterility in Thaka sheep. *Reprod Res.* 138, 921–933. Doi: 10.1530/REP-09-0193

Pineda, R., Montes, D., Hernández, D. 2018. Association of the polymorphisms FecXR, FecGH, and FecGI and non-genetic factors that affect the prolificacy of colombian creole sheep. *Indian J Sci Technol. 11*(17). Doi: 10.17485/ijst/2018/v11i17/122374

Piper, L.R., Bindon, B.M. 1990. Strategies for utilization of a major gene for prolificacy in sheep. In 2º Int. W. on Major Genes for Reproduction in Sheep. 43: 399-408.

Pisarska, M.D., Barlow, G., Kuo, F.T. 2011. Minireview: Roles of the forkhead transcription factor FOXL2 in granulosa cell biology and pathology. *Endocrinology*. 152: 1199-1208. Doi: 10.1210/en.2010-1041.

Pokharel, K., Peippo, J., Honkatukia, M., Seppälä, A., Rautiainen, J., Ghanem, N., Hamama, T.M., Crowe, M.A., Andersson, M., Li, M.H. 2018. Integrated ovarian mRNA and miRNA transcriptome profiling characterizes the genetic basis of prolificacy traits in sheep (*Ovis aries*). *BMC Genomics*. 19:104. Doi: 10.1186/s12864-017-4400-4

Pokharel, K., Peippo, J., Weldenegodguad, M., Honkatukia, M.L. and Kantanen, J. 2020. Gene Expression Profiling of Corpus luteum RevealsImportant Insights about Early Pregnancy in Domestic Sheep. *Genes (Basel). 10*:11(4):415. Doi: 10.3390/genes11040415

Sánchez, F., Smitz, J. 2012. Molecular control of oogenesis. *Biochim Biophys Acta. 1822*(12):1896-912. Doi: 10.1016/j.bbadis.2012.05.013

Silva, B.D.M., Castro, E.A., Souza, C.J.H., Paiva, S.R., Sartori, R., Franco, M.M., Azevedo, H.C., Silva, T.A.S.N., Vieira, A.M.C., Neves, J.P., 2010. A new polymorphism in the Growth and Differentiation Factor 9 (GDF9) gene is associated with increased ovulation rate and prolificacy in homozygous sheep. *Anim Genet*. 42, 89–92. Doi: 10.1111/j.1365-2052.2010.02078.x

Souza, C.J., Macdougall, C., Macdougall, C., Campbell, B.K., McNeilly, A.S., Baird, D.T. 2001. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. *J Endocrinol.* 169: 1–6. Doi: 10.1677/joe.0.169r001.

Strauss, J.F., Williams, C.J. 2019. Ovarian Life Cycle. In J. S. R. B. A. Gargiulo (Ed.), Yen & Jaffe's Reproductive Endocrinology: Physiology, Pathophysiology, and Clinical Management: Eighth Edition (Eighth Edi, pp. 167-205.e9).

Torres-Osorio, V., Urrego, R., Echeverri-Zuluaga, J.J., López-Herrera, A. 2019. Estrés oxidativo y el uso de antioxidantes en la producción *in vitro* de embriones mamíferos. Revisión. *Rev Mex Cienc Pecu. 10*(2): 433-459. Doi:10.22319/rmcp.v5i1.3219

Ugi, S., Maeda, S., Kawamura, Y., Kobayashi, M.A., Imamura, M., Yoshizaki, T., Morino, K., Sekine, O., Yamamoto, H., Tani, T., Rokushima, M., Kashiwagi, A., Maegawa, H. 2014 CCDC3 is specifically upregulated in omental adipose tissue in subjects with abdominal obesity. *Obesity* 22:1070–1077, https://doi.org/ 10.1002/oby.20645.

Våge, D.I., Husdal, M., Kent, M.P., Klemetsdal, G., Boman, I.A. 2013. A missense mutation in growth differentiation factor 9 (GDF9) is strongly associated with litter size in sheep. *BMC Genet*. 2013, 14, 1–8. Doi: 10.1186/1471-2156-14-1

Wijayanti, D., Bai, Y., Hanif, Q., Chen, H., Zhu, H., Qu, L., Guo, Z., Lan, X. 2022. Goat CLSTN2 gene: tissue expression profile, genetic variation, and its associations with litter size. Anim Biotechnol. 18:1-10. doi: 10.1080/10495398.2022.2111311. Amstalden, M., Williams, G.L. 2014. Neuroendocrine control of estrus and ovulation, in: Bov. Reprod, John Wiley & Sons, Inc., Hoboken, NJ, USA. 203–218. https://doi.org/10.1002/9781118833971.ch23.

Wilson, T., Wu, X.Y., Juengel, J.L., Ross, I.K., Lumsden, J.M., Lord, E.A., Dodds, K.G., Walling, G.A., Mcewan, J.C., O'Connell, A.R. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. *Biol Reprod*. 64: 1225–1235. Doi: 10.1095/biolrepro