PP34 - Regulation of AMH type 2 receptor expression by Bone Morphogenetic Protein 15 in ovine granulosa cells

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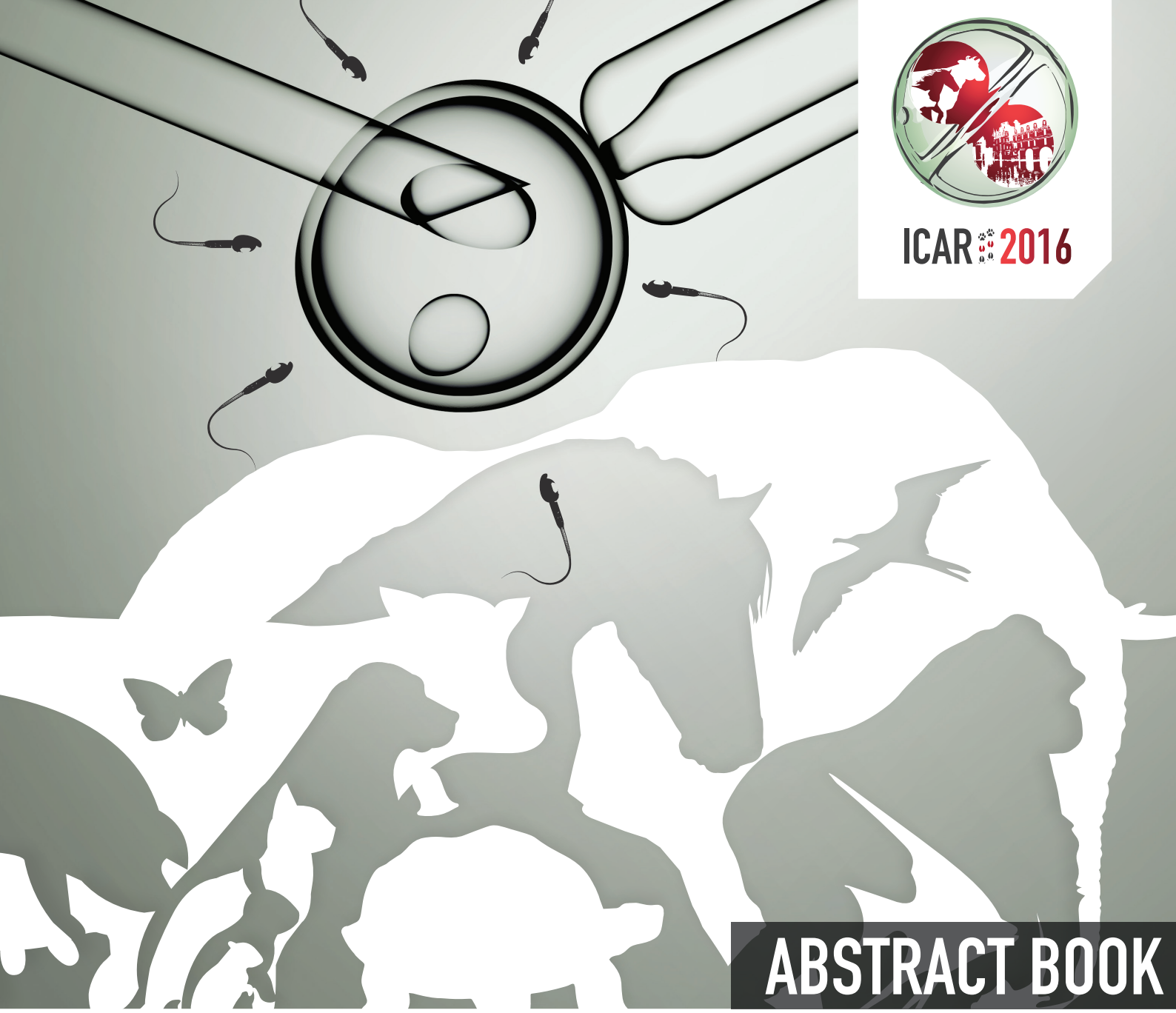
Anti-Müllerian Hormone (AMH) is known to be produced by the granulosa cells of growing small antral follicles in ovaries and to modulate importantly follicle growth and maturation. However, the mechanisms regulating the expression of AMH and its specific type 2 receptor AMHR2 are still poorly understood. A recent study indicates that AMH production by granulosa cells is regulated by the Bone Morphogenetic Proteins (BMPs), acting through the Bone Morphogenetic Receptor Type 1B (BMPR1B), and that a partial loss-of-function mutation in BMPR1B impairs AMH production in the hyperprolific Booroola ewes [1]. In this present study, we have studied the regulation of AMH and AMHR2 expression in hyperprolific ewes carrying mutations in BMP15, an oocyte-derived factor known to be one of the BMPR1B ligands.

The expression of AMH and AMHR2 was studied in heterozygous carriers of loss-of-function FecXL (in Lacaune breed) or FecXR (in Rasa Aragonesa breed) mutations in BMP15, in comparison with non-carrier wild-type ewes. For that, we recovered antral follicles of more than 1 mm in diameter by ovarian dissection. Follicles were classified according to their size and granulosa cells were recovered for quantitative RT-PCR (RT-qPCR) analyses of AMH and AMHR2 expression. Entire ovaries were fixed, embedded in paraffin and serially sectioned for AMHR2 immunohistochemistry investigations. We also tested the in vitro effects of BMP15 and other factors of the BMP family on granulosa cells from wild-type ewes by measuring the phosphorylation of Smads by Western Blotting, AMHR2 expression by RT-qPCR, and AMHR2 promoter activity by transfection of a promoter reporter construct.

In FecXL and FecXR heterozygous carrier ewes, AMHR2 expression was strongly reduced in granulosa cells whereas AMH expression was not affected. Moreover, AMHR2 immunostaining was lower in granulosa cells of mutant ewes than in wild-type ones. In vitro experiments showed that BMP4, BMP15 and BMP15 in combination with GDF9 (Growth Differentiation Factor 9) enhanced Smad1/5/8 phosphorylation, AMHR2 expression and AMHR2 promoter activity in wild-type granulosa cells, GDF9 alone being ineffective.

To conclude, both BMP15 mutations clearly affected AMHR2 expression in sheep ovaries but did not affect the expression of AMH. In vitro experiments showed a direct effect of BMP15 on AMHR2 expression through activation of the Smad1/5/8 signaling pathway and the AMHR2 promoter. It is suggested that low BMP15 and low AMH signaling in follicles of ewes carrying a loss-of-function mutation in BMP15 at the heterozygote state both contribute to enhance the sensitivity of granulosa cells to FSH, thereby increasing ovulation rate at each ovarian cycle.

[1]. Estienne A, Pierre A, Di Clemente N, Picard JY, Jarrier P, Mansanet C et al. Anti-Müllerian Hormone Regulation by the Bone Morphogenetic Proteins in the Sheep Ovary: Deciphering a Direct Regulatory Pathway. Endocinology. 2015;156:301-313



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