Critical Functions of Ovarian BMPs in Folliculogenesis and Ovulation

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One of the major goals of reproductive research is to understand how follicle growth and development are regulated. In the mechanism, precise control of folliculogenesis is critical for dominant follicle development and thus for determining fertility. Alterations in the process are known to cause ovarian dysfunction and infertility. In the mammalian ovary, folliculogenesis starts when primordial follicles leave the resting pool and enter the growth phase. From there, the early growing follicles undergo a developmental process including a dramatic course of cellular proliferation and differentiation. In the past, many of the events that take place during the process have been uncovered but most of our knowledge is restricted to certain aspects of differentiation that occur during the final phases of folliculogenesis. The pituitary-derived gonadotropins, FSH and LH, are crucial endocrine regulators in the process to evoke dominant follicle formation in the ovary. An important concept to emerge in the past two decades is that growth factors produced by the follicle itself modulate, either amplify or attenuate, gonadotropin actions. In contrast, control mechanisms governing the earlier phases of follicular development that are gonadotropin-independent remain poorly understood. The current challenge is to understand how specific local factors exert control of follicle function and how these modulations are integrated into the overall pattern of ovary physiology.

Of all the growth factors locally produced in the ovary, the members of the TGF-β superfamily figure most prominently in the regulatory events of folliculogenesis. The role of inhibins, activins and TGF-βs in reproduction has been studied extensively over the last 15 years. However, there were no reports on the role of BMPs in the mammalian ovary until 1999 when we reported the first compelling evidence demonstrating the existence of an intrinsic ovarian BMP system replete with BMP ligands, receptors and novel biological functions. Since that finding it has become clear that the BMP system plays important roles in the regulation of ovarian function, evidenced by the ability of BMPs to control granulosa cell proliferation and cytodifferentiation, as well as oocyte development.

A major breakthrough in this field occurred when it was found that two naturally occurring strains of sheep called Inverdale and Hanna, which exhibit higher ovulation rates and litter sizes than their wild type counterparts, are heterozygous carriers of point mutations in the oocyte-specific factor, BMP-15. Specifically, the Inverdale mutation ($FecX^{I}$) is a T-A transversion at nucleotide 92 of the *bmp15* gene which substitutes a Val with an Asp at residue 31 of the mature protein. In Hanna ewes ($FecX^{H}$), a C-T transition at nucleotide 67 of the *bmp15* gene

replaces a Glu by a stop codon at amino acid residue 23 of the mature domain of BMP-15, thus resulting in the synthesis of a very short peptide, which is highly unlikely to be biologically active. Surprisingly, homozygous carriers of the Inverdale and Hanna *bmp15* mutations are infertile with streak ovaries and a block in the primary stage of folliculogenesis.

The importance of BMP-15 in sheep fertility was further confirmed by subsequent studies that identified two additional naturally occurring *bmp15* point mutations, $FecX^{G}$ and $FecX^{B}$, that resulted in the same phenotype as the Inverdale and Hanna ewes. Genetic and experimental studies have shown that growth and differentiation factor-9 (GDF-9), the closest homologue of BMP-15, also plays a necessary role for folliculogenesis in sheep. Specifically, a mutation in the *gdf9* gene, $FecG^{H}$, causes similar phenotypes as the BMP-15 mutations, with heterozygous carrier ewes exhibiting increased fertility and homozygous carrier ewes being sterile. Also, immunization of ewes against BMP-15 or GDF-9 results in infertility or superfertility depending on the immunization protocol. Collectively, these findings suggest that, in sheep, BMP-15 as well as GDF-9 play an important role in promoting the transition of follicles through the early stages of folliculogenesis, while restraining the transition of follicles to the dominant preovulatory stage. Thus, BMP-15 is a central player in the determination of ovulation quota and litter size in ewes.

Critical roles of BMP-15 in female fertility have also been demonstrated in humans. Specifically, Di Pasquale and colleagues identified a BMP-15 mutation in women that is associated with hypergonadotropic ovarian failure due to ovarian dysgenesis. The mutation is an A-G transition at position 704 of the *bmp15* gene and results in a non-conserved substitution of a tyrosine with a cysteine at amino acid residue 235 of the proregion of the BMP-15 proprotein. The patients with the BMP-15 mutation were sisters who inherited the mutation from their father. Interestingly both patients have streak ovaries: a characteristic phenotype observed in the homozygous, but not heterozygous, BMP-15 mutant ewes.

Our laboratory has accumulated an extensive body of data using recombinant BMPs including BMP-4, -6, -7, -15 as well as GDF-9 that establish important biological roles for these factors in regulating ovarian function. In this lecture I will provide the recent advances in our understanding of the functions of BMPs in female reproduction.