Piercing out AMH's Ovarian Function in Small Animal Models

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In women, Anti-Müllerian Hormone (AMH) produced by the granulosa cells (GC) of preantral and antral follicles acts as a natural gatekeeper of follicle growth and maintains the follicle pool throughout the reproductive age. If there is less AMH, follicles are lost faster causing diminished ovarian reserve (DOR) / premature ovarian insufficiency (POI). Similarly AMH levels are significantly high in women with polycystic ovary syndrome (PCOS) where follicles fail to grow beyond small antral stage, form cysts and never ovulate. Currently, in clinical practice, AMH is exclusively used as a diagnostic and/or prognostic marker for PCOS, DOR/POI as well as a predictor for ovarian response in fertility treatments. Absence of mechanistic insights into AMH actions or lack of knowledge of how AMH expression is regulated is a significant limitation towards using AMH as a potential therapeutic option/target. In this presentation we provide an insight into the underlying mechanism of AMH actions and the regulation of AMH expression in GCs.

Recently (*Hayes et.al, MCE 2016*), we reported that AMH is a stalling/inhibitory factor for folliculogenesis and ovulation and acts through induction of two microRNAs, *miR-181a* and *miR-181b*, which target activin-receptor2A and adenylate cyclase-9, leading to decline in FSH signaling/sensitivity, ovarian gene expression and follicular growth. Moreover, we showed that AMH pre-treatment of mice prior to superovulation improves oocyte yield and thus, AMH can, indeed, be a potential therapeutic option for women with low functional ovarian reserve. Now we find, that in primary mouse GCs, GDF9+BMP15 heterodimers significantly induces AMH expression and is much more effective at lower concentrations than GDF9 or BMP15 alone. Intriguingly, this induction of AMH expression is inhibited by FSH treatment and can also be blocked by a SMAD2/3 inhibitor, SB431542. Therefore, we propose that GDF9+BMP15 through SMAD2/3 pathway induces AMH expression and there exists a feedback loop where AMH inhibits FSH signaling while FSH in turn inhibits AMH expression.

We find that blocking FSH signaling by a PKA inhibitor, H-89 or inhibiting FSH-induced gene expression with a SF-1 (selective steroidogenic factor-1) inhibitor, SID7969543 rescues AMH expression from the inhibitory effects of FSH. Furthermore, bioinformatics analysis of FSH-induced genes revealed a transcription repressor, GIOT1 (gonatotropin-inducible ovarian transcription factor 1) that is expressed by the FSH-PKA-SF1 pathway. Our studies show that siRNA-mediated knockdown of GIOT1 mimics the effect of H-89 and SID7969543 on AMH expression. Thus, our studies suggest that FSH through PKA-SF1 pathway induces the transcription repressor GIOT1 that in turn inhibits GDF9+BMP15-induced AMH expression. These studies establish AMH as a potential therapeutic option and target for various pathophysiological conditions like DOR/POI and PCOS.

Clinical Relevance:

1. DOR patients or DOR-like condition seen in patients with post-cancer-treatment "burnout" of developing follicles caused by low AMH production can be treated with AMH or recombinant GDF9+BMP15 to slow the development of follicles and restore natural level of AMH and AMH-producing small follicles.
2. AMH/GDF9+BMP15 treatment can be used to increase the number of oocytes harvested in an induced ovulation cycle. Normally, a cohort of pre-antral follicles enters the antral stage and over the course of an ovulation cycle, one follicle generates an oocyte while others undergo atresia. In a standard induced ovulation cycle, FSH and/or other gonadotropins treatment speeds up follicle maturation and cause more than one follicle to ovulate. Therefore, AMH pretreatment prior to IVF will arrest follicles in the pre-antral follicle stage, resulting in an increased number of pre-antral follicles. When AMH treatment is stopped and ovulation is induced using FSH or other gonadotropins, all the small follicles will mature and ovulate together resulting in a substantial increase in the oocyte yield. In fact, as a proof of concept, we have shown (*Hayes et.al. MCE 2016*) that AMH pre-treatment of mice prior to superovulation improves oocyte yield and thus, AMH can, indeed, be a potential therapeutic option for women with low functional ovarian reserve.
3. PCOS involves in arrest of multiple pre-antral and small-antral follicles and high AMH levels, which continues to slow folliculogenesis creating a feedback loop. Accordingly, inhibiting AMH will allow the

follicles slowed by PCOS to mature and ovulate or die. Thus FSH can be a treatment option in PCOS as it is routinely used in the fertility clinics and the proposed studies can form the basis for a phase1 clinical trial. In fact a recent study reported that exogenous FSH could lower AMH values in PCOS patients (*Köninger et.al. Gynecol Endocrinol 2015*).