Animal Models Demonstrating the Importance of Androgens for Early Stages of Follicle Maturation

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Lately there has been a lot of interest towards the role of androgens in regulating follicular development and female fertility. In fact, over the years our understanding of androgen effects on follicular development and female fertility has undergone considerable change. While for decades, androgens were considered detrimental to normal folliculogenesis, associated with poor health and reduced fertility in women, in recent years, however, a new concept has emerged suggesting that sufficient androgen signaling through the androgen receptor is necessary for normal follicle development and function. Consequently, it is now increasingly realized that, likely, a critical balance exists between essentiality of androgens in normal follicular development and their detrimental effects in hyper-androgenic conditions that regulates female fertility. While androgen excess embellishes follicular development and dysfunctional formation of antral follicles leading to polycystic ovary syndrome (PCOS), low androgen levels may also negatively impact female fertility. Despite these studies and development of various hyper-androgenic and genetic animal models as well as our understanding of hormonal changes in hyper- or hypo-androgenic patients the underlying mechanism of androgen actions regulating ovarian physiology and female fertility is not clearly understood. In fact till date no androgen-specific genes have been identified in the ovary, which is a huge limitation in understanding how androgens may regulate follicular physiology under normal as well as in pathophysiological conditions. The effect of androgens on female fertility is an emerging field in reproductive science at the interface of endocrinology and gynecology. This talk will summarize the direct physiological actions of androgens in regulating follicular development, follicular atresia and epigenetic modulation with respect to ovarian physiology and female fertility and provide insights to the molecular and/or signaling basis of these androgen actions.

Through generation of an ovarian granulosa cell (GC)-specific androgen receptor (AR) knockout (ARKO) mouse model, we have pioneered a new concept that critical androgen actions through ARs in GCs are absolutely essential for normal ovarian function and female fertility (*Molecular Endocrinology 2010*). Androgen functions are mediated by both “genomic/nuclear” and “non-genomic/extra-nuclear” actions of ARs. Our studies demonstrate that the physiological effects of androgens in GCs involve a synergistic action between these two AR signaling pathways (*JBC 2010, Steroids 2011, JCI 2012, PNAS 2014, Journal of Endocrinology 2014*). We have found that androgen signaling in GCs promotes pre-antral follicle growth and development to antral follicles by increasing follicle stimulatory hormone (FSH) receptor protein levels, which augments FSH-mediated follicle growth and development (*PNAS 2014*). Additionally we have uncovered that androgens attenuate follicular atresia through a synergistic action between AR-induced nuclear and extra-nuclear signaling pathways in GCs by enhancing the expression of a micro-RNA (*miR-125b)*, which in turn suppresses expression of pro-apoptotic proteins (*PNAS 2014*). In fact as a proof of concept we have shown that low doses of exogenous androgens enhance gonadotropin- induced ovulation in mice, further demonstrating the critical role that androgens play in follicular development and fertility (*PNAS 2014*).

Intriguingly, although ARs are transcription factors, surprisingly few direct transcriptional targets of ARs have been identified in the ovary. Instead, genes identified so far by microarray analysis in PCOS patients, in prenatal androgenized animal models, or in AR null mouse models, do not have androgen-regulated elements (AREs) in their promoters. In the last couple of years an emerging body of evidence suggests that steroid hormones may also alter gene expression through epigenetic modifications that represents an independent avenue for hormones to coordinate gene regulation from its classical pathway. We therefore posed that, instead of regulating genes containing AREs, androgens may have more global effects on ovarian gene expression. Regulation of gene expression is controlled at a number of different levels, one of which is the accessibility of the transcription machinery to the genes and their controlling elements. This accessibility is dictated broadly by the degree of chromatin compaction, which is influenced in part by the Polycomb group proteins (PcG). The PcG protein EZH2 (enhancer of zeste homolog 2) promotes histone H3 lysine 27 trimethylation (H3K27me3) that restrict the ability of cofactors and enhancers to bind specific DNA sequences and subsequently cause gene silencing. Accordingly, inhibition of EZH2 removes the H3K27me3 repressive mark, thereby creating a conducive environment for gene expression. We have uncovered a novel mechanism in GCs whereby androgens regulate EZH2 thereby promoting gene expression. We find that androgens

through the PI3K/Akt pathway and induction of a micro-RNA, *miR-101* expression inhibit EZH2 activity and expression, respectively, thereby removing the H3K27me3-repressive mark from the promoter region of *RUNX*1, a luteinizing hormone (LH)-induced transcription factor essential for ovulation. This androgen-mediated epigenetic modulation enables the LH-induced transcription machinery to access the *RUNX1* promoter region to induce *RUNX1* expression.

Translational Relevance: This presentation offers an insight about androgen actions in the follicle and its physiological effects related to follicular development and female fertility. The data presented here will provide a mechanistic understanding for various clinical data in the recent years showing a potential benefit of androgen priming prior to fertility treatment in women undergoing IVF. Moreover these studies form the basis towards expanding current infertility treatments from only the last 2 weeks into earlier stages of follicle maturation.