**Polycystic ovary syndrome (PCOS)** is a disorder caused by a defect of the ovarian cells. This defect results in synthesis of excess androgen and related clinical and biochemical symptoms such as infertility, obesity, and hirsutism.1 The Steroidogenic acute regulatory protein (StAR) has been implicated as a cause of PCOS because it is necessary for cellular synthesis of steroids.2,3 StAR initiates the steroidogenesis process by transporting cholesterol - the precursor for steroids - within the mitochondrial membrane of cells.2 Studies have shown that StAR is overexpressed in the ovarian cells of women with PCOS and rodent models of PCOS3,4, yet *what specific factors regulate this expression are still unknown.*

The **objective** of this study is to determine how other factors regulate StAR and proper testosterone levels. Rats will be used for this study because several previous prenatally androgenized models have displayed phenotypes very similar to humans while remaining relatively inexpensive.5,6 I **hypothesize** that transcription factors, such as GATA-4, will directly interact with the StAR promoter resulting in activation of the gene. This hypothesis is based on research that suggested that overexpression of GATA-4 can regulate the StAR gene in ovarian cells.5 The **long-term goal** of this research is to determine which transcription factors regulate StAR expression.

Aim 1: **Determine what sequences within the StAR promoter are conserved and contribute to testosterone levels in ovaries**

**Approach**: BLAST searches of StAR homologs and next-generation sequencing of rat lines will be performed to find StAR promoter sequence variants of conserved regions that correlate with differential testosterone levels in the ovaries of rats. CRISPR/Cas9 will be used to introduce sequence mutations of conserved regions into rat ovarian cell lines to observe effects of sequence variants on StAR expression using RT-PCR.

**Hypothesis**: I hypothesize that mutating conserved regions of the StAR promoter will result in differential expression of StAR and testosterone levels.

**Rationale**: Evaluating phenotypes associated with variation within the StAR promoter allows for the confirmation that alterations of the StAR promoter impact regulation of StAR expression and testosterone levels.

Aim 2: **Identify differentially regulated genes in StAR mutants that are important for testosterone levels in ovaries**

**Approach**: A genome-wide RNAi screen will be performed in rat ovarian cell lines and compared to controls to identify genes that impact StAR expression and testosterone levels in the ovaries. RT-PCR will be used to determine if prenatally androgenized rats display differential expression of any of the identified candidate genes.

**Hypothesis**: I hypothesize that one or more of the candidate genes identified in the RNAi screen will be differentially regulated in prenatally androgenized rats.

**Rationale**: Identifying which genes impact StAR expression and testosterone levels after knock-down with RNAi allows for confirmation that these genes are involved with regulating StAR. Identifying differentially regulated candidate genes in prenatally androgenized rats connects them with the PCOS phenotype specifically.

Aim 3: **Identify direct protein interactions with the StAR promoter and protein that impact testosterone levels within ovaries**

**Approach**: Chromatin immunoprecipitation (ChIP) and Co-immunoprecipitation (Co-IP) will be used to identify proteins that bind the StAR promoter or protein directly. Potential binding proteins will be based on candidate genes identified in Aim 2. CRISPR/Cas9 will then be used to mutate previously determined conserved regions of the StAR promoter and ChIP and Co-IP will be performed once again to determine if the previously identified proteins still bind the StAR promoter or protein itself.

**Hypothesis**: I hypothesize that mutating conserved regions of the StAR promoter will result in a lack of protein binding on the StAR promoter.

**Rationale**: Identifying proteins that bind the StAR promoter and have already been determined to be differentially expressed in StAR mutants will support their role in regulating StAR at the level of transcription as transcription factors. Identifying proteins that bind StAR directly will serve as a good foundation for future studies into other regulatory factors of StAR at the post-translational level.

Works Cited

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