

consistently expressed throughout the oviduct and at all stages of the estrous cycle. By contrast, the ratio of LF to SF3 varied by region of the tube, with more SF3 towards the fimbria and more LF towards the isthmus. The epithelium of the oviduct is primarily composed of multi-ciliated cells and secretory cells, with more ciliated cells towards the fimbria and more secretory cells towards the isthmus. The RT-qPCR results therefore suggested the possibility that a greater proportion of LF Prlr was present on secretory cells and a greater proportion of SF3 Prlr on ciliated cells. Using antibodies raised against intracellular peptide regions specific to the LF (aa 309-325) and SF3 (aa 281-296), both receptor isoforms were localized by immunofluorescence to apical regions of both epithelial cell types, but the presence of receptors on cilia (clearly demonstrated by 3D reconstruction and rotation) complicated analysis of relative fluorescence by microscopy. Only the LF Prlr signals via Stat5 and so it was anticipated that Stat5 activation could serve as a substitute marker of the relative presence of LF Prlr. Following *in vivo* intraperitoneal injection of PRL (5 µg/g, 30 min), activated Stat5 was localized to epithelial cells at the base of, and in between, mucosal folds, thereby suggesting a further regional distribution to receptor distribution. For the fimbrial region only, which is where HGSC is thought to arise, expression of both the LF and SF3 Prlr changed as a function of the stage of the estrous cycle, with highest mRNA expression at diestrus/proestrus. Ongoing work includes flow cytometry of epithelial subpopulations and spatial analysis of gene expression.

## Reproductive Endocrinology

### OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

#### *The Effects of Adiponectin on Infertile Women Undergoing IVF/ICSI Treatment and on Human Granulosa Cells*

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Adiponectin, one of the most abundant adipocyte-secreted protein, has been involved in female reproductive regulation. This study aimed to 1) compare serum adiponectin levels in various phases of *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) treatment including Phase I (the beginning of gonadotropin stimulation), Phase II (around 8 days after gonadotropin stimulation), and Phase III (on the day of ovum pick-up) between success and unsuccessful subjects; 2) compare follicular fluid (FF) adiponectin levels between success and unsuccessful groups; 3) compare serum adiponectin levels among different phases of IVF/ICSI treatment in success and unsuccessful groups; 4) compare the levels of adiponectin between serum and FF; and 5) investigate the effects of adiponectin on mRNA expressions of follicle stimulating

hormone receptor (*FSHR*) and *CYP19A1* (aromatase) in human granulosa-like tumor cell line (KGN) (n=3). In the human study, recruited participants (n=30) with age of 26-40 years were enrolled between April 2018 - May 2019. Blood samples were collected at Phases I, II, and III while FF samples were collected at Phase III. Adiponectin levels were comparable between success and unsuccessful subjects in both serum (all phases) and FF (Phase III). Furthermore, serum adiponectin levels were comparable among Phase I, II, and III in success and unsuccessful groups. In Phase III, serum adiponectin showed positive correlations with serum adiponectin in Phase I and II and serum FSH in Phase I. Interestingly, adiponectin levels in FF were significantly lower than serum at Phase III in unsuccessful pregnancies but were comparable in successful pregnancies. Moreover, FF adiponectin had a negative correlation with serum LH at Phase III in success subjects. In the KGN cell study, adiponectin had no effects on *FSHR* and *CYP19A1* (aromatase) mRNA expression compared with control. In conclusion, high adiponectin levels in serum compared to FF might contribute to unsuccessful IVF/ICSI treatment.

## Reproductive Endocrinology

### OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

#### *The Effects of Leptin and Irisin on Steroidogenic Enzyme Gene Expression in Human Ovarian Granulosa Cells - Initial Studies*

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Fertility and energy metabolism are closely associated, and the cytokines produced by the adipose and muscle tissue play a role in this association. Leptin, predominantly produced by the white adipose tissue, and irisin, produced by the brown adipose and skeletal muscle tissues, are cytokines that are important in balancing energy metabolism. This study aimed to investigate the effects of leptin and irisin on steroidogenic enzyme gene expression in human ovarian granulosa cells *in vitro*. Granulosa cells were retrieved and isolated from ovarian follicular fluid during *in vitro* fertilization (IVF) procedures. Cells were placed in primary *in vitro* cultures and treated with increasing concentrations of leptin (25, 50, 100, 200, and 400 ng/ml) or irisin (125, 250, 500, 1,000, and 2,000 ng/ml) for 24, 48, and 72 hours. mRNA expression levels of *CYP11A1*, *CYP19A1*, *CYP21A2*, *HSD3B1*, and *HSD17B3* were measured by qRT-PCR analysis. Leptin treatment of granulosa cells resulted in significant upregulation of *CYP21A2* mRNA levels, while irisin significantly downregulated mRNA levels of *CYP11A1*, *CYP19A1*, and *HSD3B1*. Taken together, these early experiments demonstrate that leptin and irisin may affect steroid hormone production in the ovary by targeting the gene expression of key steroidogenic enzymes. Additional experiments are in progress.