P13 Analysis of Proteome Expressed in Follicular Fluid of Polycystic Ovary Syndrome (PCOS) patients

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Objectives: The goal of this study was to identify potential protein markers involved in polycystic ovary syndrome (PCOS) that is a heterogeneous disorder characterized by chronic anovulation and hyper-androgenism, which affects 5-10% of women of reproductive age.

Materials and Methods: Follicular fluids from normal and PCOS patients were examined for quantitative differences in protein expression using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Spot detection was accompanied by using ImageMaster™ 2D Platinum software. More than 30 candidate proteins were identified using matrix-assisted laser desorption/ ionization-time of flight-mass spectrometry (MALD-TOF-MS) or Nano-LC MS/MS. Western blotting with the respective antibody to assess the protein values in the samples examined the expression levels of the individual proteins.

Results: Seven protein spots were identified as being significantly overexpressed in follicular fluids from patients with PCOS. The expression of these proteins was increased from 1.4- to 3.5-fold as compared with normal patients. Western blot analysis confirmed that the expression level of these proteins was higher in follicular fluid from PCOS patients than those from normal controls. Semiquantitative RT-PCR analysis revealed that mRNA level was also increased in granulose cells of PCOS patients compared with normal samples.

Conclusions: Analyses of 2D-PAGE and mass spectrometry were able to identify proteins showing increased expression in PCOS. The association of these proteins with clinical variables and understanding the regulation of their expression will aid in determination of their potential use as biomarkers in this syndrome.

Key words: Biomarker, Follicular fluid, Polycystic ovary syndrome, MALDI-TOF-MS, Two-dimensional polyacrylamide gel electrophoesis

P14 Androgen Receptor in Sertoli Cell Is Essential For Germ Cell Nursery and Junctional Complex Formation in Mouse Testes

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Objectives: To examine the role of androgen receptor (AR) in Sertoli cells (SC).

Materials and Methods: We used Sertoli cell-specific androgen receptor knock-out (S-AR$^{-/y}$) mouse to evaluate the chronological changes of seminiferous tubules and the molecular mechanisms of SC androgen/AR signals on spermatogenesis.

Results: Testis morphology began changing as early as postnatal day (PD) 10.5 in wild-type (WT) but those in S-AR$^{-/y}$ did not. After puberty (PD 50), the SC nuclei of WT testes migrated to the basal area of the seminiferous epithelium; however, in S-AR$^{-/y}$ testes, SC nuclei were disarranged and dislocated. Electron microscopy further showed an obvious duplication of basal lamina of the seminiferous epithelium in S-AR$^{-/y}$ testes at PD 50 as compared to WT. Using quantitative RT-PCR analyses, the expression of SC gene profiles were compared in PD 10.5 testes. In S-AR$^{-/y}$ testes, the expression levels of 1) Vimentin was significantly increased and laminin α5 was significantly decreased in PD 10.5, which contributed to functional defects in cytoskeletons and basement membrane component production of SC leading to cell morphology deterioration and thus affecting the integrity of seminiferous epithelium; 2) claudin-11, occludin and gelsolin were significantly decreased in PD 10.5, which contributed to functional defect in intact junctional complex formation of SC leading to impairment of the integrity of the blood-testis barrier; 3) Cacna1a, tPA, transferrin and eFABP were significantly decreaseed in PD 10.5, which contributed to functional defect in production and/or secretion of specific proteases, transport proteins and paracrine factors of SC leading to impairment of its germ cells nursery functions.

Conclusions: Loss of AR specifically in SC could affect 1) structure support elements of SC leading to impaired normal supportive function for movement of developing germ cells; 2) junction complex formation and basement membrane development of SC leading to impaired integrity of the BTB; (3) SC specific proteases, transport proteins, and paracrine factors production and/or secretion, leading to impaired SC nursery functions for developing germ cells.