

trifuged at 500 g to remove blood and granulosa cell contaminations. After rehydration, we processed for 2-D PAGE, and Coomassie blue and silver stainings prior to the analysis of the differential protein expressions using ImageMaster™ 2D Platinum software.

Results: In this study, numerous protein spots were identified as being differently expressed in follicular fluids in normal and RSA patients. Seven candidate proteins (1 transcription factor, 1 Zinc finger protein, 1 Tro αH, 1 apolipoprotein, 1 apolipoprotein E precursor, and 2 novel proteins) were identified using matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALD-TOF-MS) or peptide sequencing (ESI-Q-TOF-MS/MS). Isolation of these proteins may delineate general health during pregnancy and a better understanding of their cellular functions for maintaining normal pregnancy.

Conclusions: The molecular mechanisms for differential protein expressions have to be elucidated for the prognosis of the pregnancy of individuals with higher risk. Using two-dimensional PAGE and mass spectrometry, all proteins showing increased or decreased expression in RSA will be identified. These proteins are able to use as clinical biomarkers in RSA.

P-16 Cellular Localization and Intensity of Id3 mRNA in Ovaries of Adult Cycling Rats

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Background & Objectives: Inhibitor of DNA binding protein (Id) is another category of mammalian helix-loop-helix (HLH) protein. Four members of the Id family, Id1 to Id4, have been identified in mammalian cells. Id proteins act as positive regulators of cell growth and are required for cell cycle progression in cell line. This study was performed to examine the expression pattern of Id 3 mRNA during folliculogenesis in cycling rat ovary induced by PMSG.

Method: Probes for in situ hybridization studies were made by RT-PCR. All PCR products were cloned into pGEM-T Easy Vector (Promega Corp.). Anti-sense and sense cRNA probes were prepared by means of in vitro transcription using Sp6 or T7 RNA polymerase. Hybridization was carried out with the ³⁵S-labeled RNA probe (1×10⁷ cpm/ml) in a solution. The hybridization signal was estimated on a scale of 1+ to 4+; +, silver grains sparse, but positive hybridization; ++, silver grains are numerous but do not cover the cell type in question; +++, silver grains are very numerous and begin to merge in some places; +++++, silver grains are very dense and form a near uniform mass above the cell type in question.

Results: The intensity of the signals showed 2+ or 3+ in oocytes of primordial and primary follicle in no-treatment and PMSG treated ovaries, however, there were no signals in dominant or atretic follicle. The signals showed +/- or 1+ in granulosa cells in primordial or primary follicles regardless of PMSG treatment, however, the intensity of the signal in secondary and dominant follicles showed 2+ and 4+, respectively. There were no signals in theca-interstitial or externa cells during folliculogenesis. The most interesting

thing is that the expression sites are switched from oocyte to granulosa cells during folliculogenesis.

Conclusions: Id3 gene may have pivotal roles in oocyte growth during early follicular stages and in granulosa cell proliferation during late follicular stages. Further studies are needed to evaluate the function of this gene during ovarian folliculogenesis.

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부고환내 정자의 체외채취법: 임신을 유도하는 새로운 방법

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Background & Objectives: 폐쇄성 정로장애로 인한 무정자증은 남성불임의 주요원인이며 이러한 환자들에게 임신의 기회를 줄 수 있는 방법으로 여러가지 방법들이 시행되고 있으나 적용의 제한성, 술기의 복잡성의 약점을 가지고 있다. 이에 본 연구자들은 부고환정자를 보다 효율적으로 채취하는 새로운 방법을 시도하여 그 효용성을 평가하였다.

Method: 1995년부터 2005년 4월까지 총 63예의 폐쇄성 정로장애로 인한 무정자증 환자를 대상으로 하였다. 환자의 평균나이는 36세였으며 모든 환자에서 부고환내 정자의 체외채취법을 이용하여 시험관내 인공수정 및 선택적으로 난자내 정자주입법을 시행하였다.

Results: 평균 정자채취율은 96.8%였으며 채취된 평균 총정자수는 $42 \times 10^6/\text{ml}$, 운동성정자는 $8.7 \times 10^6/\text{ml}$ 였으며 7.3 pellet을 동결보존하였다. 부부당 임신성공률은 77.7% (49/63), 주기당 임신성공률은 47.7% (64/134)였고 총 35례의 분만으로 49명의 아이를 출산하였다.

Conclusions: 임신을 간절히 원하는 폐쇄성 정로장애 환자에서 부고환내 정자의 체외채취법은 새로운 임신유도방법으로 평가할 수 있다.

P-18 Proteomic Profiling of Polycystic Ovary Syndrome (PCOS): Identification of Highly Expressed Proteins

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Background & Objectives: The goal of this study was to identify potential protein markers in polycystic ovary syndrome (PCOS) that is a heterogeneous disorder characterized by chronic anovulation and hyper-