PAM both on the Western blots and for ICC (Immunocytochemistry) using the Vectastain ABC method. The ICC slides were blinded and reviewed by two examiners who scored the intensity of staining on a scale of 1 (no stain) to 5 (highest intensity). Western blots were quantified by densitometry. ICC data were analyzed by nonparametrics analysis while Western blots were compared by non-paired t-test.

Results: A marked decrease in an immunoband of PAM was detected by Western blot at full-term vaginal delivery compared to preterm cesarean delivery (1.98 ± 0.75 vs 4.59 ± 0.34, p < 0.05). In addition, the staining for PAM was located primarily in the region of the cytotrophoblast.

Conclusions: Three main results may be drawn from this study: (1) PAM is detected in the placenta; (2) it decreased at the time of delivery; and (3) it is located primarily in the cytotrophoblast. In conclusion, the decrease in PAM suggests that certain amidated peptides are diminished at the end of pregnancy in the placenta. The diminished peptide synthesis may contribute to events important in the delivery mechanism.

Supported by: Research fund of Chosun University, Creation and Love Women’s Hospital.

P-459


Objective: A failure of the conventional parameters to predict fertilization indicates that patients with male infertility may possess hidden abnormalities in the composition of their sperm nuclei, including damaged DNA, impaired chromatin packaging, abnormal content and ratio of protamines and histones. Quantitative and qualitative alterations in sperm chromatin may result in defective decondensation and DNA activation during fertilization. Electron microscopy and Atomic force microscopy provide important information regarding sperm inner structures but without specific chemical information. Recently X-ray microscopy (XM) was introduced for the evaluation of a single cell. The major advantage of XM over scanning electron microscope is higher contrast of details from transmission images of whole cell up to a thickness of 10 mM. Chemical state contrast caused by electron excitation into unoccupied molecular orbitals can be obtained by tuning to x-ray absorption near-edge structure (XANES) resonance. XM provides complementary information by measuring the spatial distribution of major chemical binding motifs without using any labels. We performed a pilot study of human sperm microscopy using new cryogenic scanning transmission microscope (SXTM) to examine distribution of DNA, protamines and histone in human sperm cell and obtain spatially resolved biochemical information.

Design: Human semen samples were randomly obtained from the Andrology Lab.

Materials/Methods: After centrifugation, droplet was placed on the electron microscope grid. Grid was air-dried after rinsing in deionized water and imaged in cryoSTXM. X-ray absorption near edge nitrogen spectral images was obtained. Spectra of sperm DNA, Protamines 1 and 2, and Protamine 2 precursor were obtained. Hamster histone reference spectrum was used with good approximation.

Results: We obtained a component distribution maps for DNA, histone, Protamines 1 and 2 according to nitrogen edge reference spectra (Fig. 1).

Conclusions: Soft X-ray spectromicroscopy is a powerful approach to study the spatially-resolved biochemical makeup of normal and abnormal individual sperm to better understand the changes associated with male infertility. It provides a unique combination of high spatial resolution and good chemical state sensitivity, which was already proven to be powerful in mapping protein and DNA concentration in animal sperm. Spectral differences between DNA, histone and the protamines allow spatial separation of these components of the human sperm head. Further quantitative analysis with higher spectral resolution will open interesting perspective in direct assessment of spermatogenic disorders leading to fertilization failure.

Supported by: Department of Physics.

P-460


Objective: Fertilization and embryo development potential of reconstructed oocytes after GV transfer may be greatly compromised by a lack of critical cytoplasmic factors. Human chorionic gonadotropins (hCG) and epidermal growth factors (EGF) have been demonstrated to induce final maturation of preantral follicles in-vitro. The aim of this investigation was to assess whether grafted GV-oocytes can be matured in-vitro to gain full competence for oocyte maturation, fertilization and embryogenesis.

Design: Cytoplasts and karyoplasts were prepared from mouse GV-stage oocytes developed after IVC or in-vivo stimulation. Various types of oocytes were reconstructed after fusion with different combinations of these prepared cytoplasts and karyoplasts. The competence of these reconstructed oocytes was evaluated after maturation, fertilization and embryo development in-vitro.

Materials/Methods: GV-stage oocytes can be obtained after IVC of isolated preantral follicles for 10 days or collected by puncturing murine PMSG-stimulated antral follicles. Both in-vivo and in-vitro derived GV-stage oocytes were used to prepare cytoplasts and karyoplasts by micromanipulation. Karyoplasts from IVC-derived oocytes were fused with cytoplasts from IVC-derived oocytes (Group A) or in-vivo-derived oocytes (Group D). In contrast, karyoplasts from in-vivo-derived oocytes were fused with cytoplasts from IVC-derived oocytes (Group B) or in-vivo-derived oocytes (Group C). All reconstructed oocytes were matured in-vitro and subsequently fertilized by ICSI using a Piezo-actuated unit. The developmental potential of these embryos was evaluated after 2, 3, and 4 days of culture.

Results: Results are presented in the following table:

The fusing, fertilization, and blastocyst formation rates of reconstructed oocytes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Karyo-</th>
<th>Cyto-</th>
<th># NT</th>
<th># Fused (%)</th>
<th># GV (%)</th>
<th># MI (%)</th>
<th># MII (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>IVC</td>
<td>IVC</td>
<td>62</td>
<td>47 (76)</td>
<td>13 (28)</td>
<td>10 (21)</td>
<td>24 (51)</td>
</tr>
<tr>
<td>B</td>
<td>In-vivo</td>
<td>IVC</td>
<td>97</td>
<td>78 (80)</td>
<td>16 (20)</td>
<td>27 (35)</td>
<td>35 (45)</td>
</tr>
<tr>
<td>C</td>
<td>In-vivo</td>
<td>In-vivo</td>
<td>40</td>
<td>34 (85)</td>
<td>4 (12)</td>
<td>7 (21)</td>
<td>23 (68)</td>
</tr>
<tr>
<td>D</td>
<td>IVC</td>
<td>In-vivo</td>
<td>69</td>
<td>56 (81)</td>
<td>15 (27)</td>
<td>8 (14)</td>
<td>33 (59)</td>
</tr>
</tbody>
</table>

Conclusions: We have demonstrated that IVM can serve as an alternative method to mature reconstructed GV-stage oocytes. These in-vitro matured oocytes can be fertilized and develop to blastocyst-staged embryos. Regardless of the origin of cytoplasts and karyoplasts, the potential of reconstructed oocytes for maturation (p = 0.068), fertilization (p = 0.299), and embryogenesis (p = 0.15) were similar. Our data strongly suggests that both nuclear and cytoplasmic competence can be achieved after IVC and IVM.