P-2 Effects of Development and Viability of Pig Oocytes Matured in a Protein-Free Medium Containing PVA, PVP and pFF

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Background & Objectives: This study was conducted to develop a serum-free, defined medium of IVM of pig oocytes. Base medium of the North Carolina State University (NCSU)-23 media with supplemented with polyvinylalcohol (PVA), polyvinylpyrrollidone (PVP) and porcine follicular fluid (pFF) were used as base media.

Method: In-vitro maturation and fertilization were performed according to the modified procedures of Funahashi et al. Pig oocytes from abattoir-derived ovaries were matured in TCM199 media containing pyruvic acid, gentamycin, L-cysteine, B-estradiol, FSH and supplemented 0.1% PVA, 0,1% PVP and 10% pFF for 40h at 39℃, 5% CO_2. Fertilized oocytes were cultured in glucose-free NCSU 23 supplemented with 5 mM sodium pyruvate, 0.5 mM sodium lactate and the same concentrated supplements for 2 days. Maturation, fertilization, morula and blastocyst formation were examined at 40 h after IVM and at 12, 48 and 144 h after IVF, respectively. Morphology of oocytes and cleaved cells were stained by Hoechst 33342.

Results: Maturation rate of pig oocytes in IVM media containing PVA (82.4%) and pFF (89.4%) were significantly higher ($p<0.05$) than that of PVP (78.6%). Cleavage rate after IVF of PVP (64.1%) was significantly lower ($p<0.05$) than that of PVA (73.0%) and pFF (77.2%) supplements. In vitro development rates to morulae and blastocyst on PVP (54.0%) were also significantly lower ($p<0.05$) than that of PVA (63.0%) and pFF (69.0%) supplements.

Conclusions: It may be concluded that PVA and pFF can be substituted for FCS in medium for culturing pig oocytes; however, it can not be completely substituted for BSA in the in vitro culture of the embryos. And PVP was considered on limited use for culture in the media.

P-3 Induction of ATF4 and Heat Shock Proteins by Estrogen and Estrogen Mimics in Mouse Testes

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Background & Objectives: In testis Leydig cells as well as germ cells expresses aromatase and estrogen receptor, suggesting important of estrogen in steroidogenesis as well as steroidogenesis. Activating transcription factor 4 (ATF4) is a basic leucinezipper transcription factor that is a member of the ATF/cAMP responsive element-binding (CREB) protein family. ATF4 is expressed in a wide variety of organs,

including the brain, heart, liver, spleen, kidney, lung, thymus, testis and regulates cell proliferation and differentiation of in a broad number of tissues. Basically, steroid hormones may have a direct effect on cellular stress because heat shock genes is activated by a number of steroids. The heat shock protein forms heteromeric complexes together with steroid-receptor. Moreover, ATF4 and molecular chaperones are candidate genes for upstream and down stream genes in the steroid response. To date however, gene battery mediating the physiological effect of estrogen has been uncovered in testis. Recently, estrogen mimicking chemicals have been reported to affect various aspects of male reproduction in animals including human. In an effort to elucidate molecular mechanism of regulation of spermatogenesis by estrogen mimics as well as natural estrogen, we examined the expression of ATF4, HSP70.1 and HSP70.3 mRNA in adult mouse testes following bisphenolA and/or 17 beta estradiol treatment.

Method: Adult male mice was treated with bisphenolA (oral administration of 20, 200, 1000 mg/kg B.W./day for 4 weeks) and/or 17beta estradiol (single i.p. injection of 300 ng/head). Testes were sampled 24h after the last dosage. Semiquantitative RT-PCR was optimized for each gene primers. Mean while immunohistochemical localization of ATF4 in testis was analyzed in control and drug treated mice.

Results: Result showed significant induction of ATF4 mRNA in estrogen or BPA-treated mouse testis. Parallel analysis of HSP70.1 and 70.3 mRNA revealed similar pattern of expression. In immunohistochemical analysis, ATF4 was found seminiferous tubule as well as interstitium of normal adult testis. Interestingly, ATF4 immunoreactivity among seminiferous tubule was heterogenous acccoring to spermatogenic cycle. Among the germ cells, immunoreactivity of ATF4 was largely found in pachytene spermatocytes. Sertoli cell showed strong immunoreactivity of ATF4.

Conclusions: These results suggest that ATF4 is a transcription factor possibly mediates the response to steroid in germ cells as well as somatic cells in testis. Similar induction of ATF4 and HSP70 by natural estrogen and BPA suggests that they may be involved in endocrine disruption by estrogen mimicking chemical such as BPA in testis.

P-4 생쥐의 자궁에서 밀착결합 유전자 Junctional Adhesion Molecule-1 (JAM-1)의 발현

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Background & Objectives: 상피조직의 apical side에 형성되는 밀착결합 (Tight Junction, TJ)은 혈액-조직 사이의 확산장벽을 형성하여 조직 특이적 특수 환경 조성에 중요한 역할을 한다. 밀착결합은 occludin, claudins 등 integral membrane protein과 ZO-1, JAM 등의 plaque protein으로 구성되며 세포질 골격 및 다양한 신호전달 분자와 복합체를 형성한다. 따라서 다양한 조직에서 세포 내외부의 신호에 반응하여 그 구조와 기능이 역동적으로 조절된다. 자궁내막은 생식주기와 착상을 위한 준비과정 동안 주로 난소 스테로이드의 영향 하에 구조 및 기능적 분화를 진행한다. 자궁내막에 존재하는 상피와 혈관내피세포에서 발현되는 밀착유전자들은 특히 착상의 준비와 진행에 필요한 환경 조성에 중요한 역할