

meiotic spindle) if those oocytes were exposed in an wrong window of exposure. Therefore, it can be expected that, a chronic exposure of woman to environmental estrogens may affect spindle formation and chromosome segregation *in vivo* as well. Our hypothesis is that the *in vitro* oocyte maturation system could contribute for an integrated testing strategy (ITS) for screening chemicals that affects female fertility. Furthermore, by using ovaries from slaughterhouse waste, it could also contribute to reduce the number of experimental animals being used for those studies. We have selected compounds with a known mechanism of action (i.e. cycloheximide, estradiol, DES, cadmium chloride) to be tested in this system. As the first step to investigate this proof of principle, we cultured bovine oocytes, harvested from slaughterhouse ovaries, in a M199 media at 39°C during 22 h, under 5%CO₂ and in the presence of the following concentrations of cycloheximide (CHX), a known protein synthesis inhibitor: 0.75; 0.60; 0.45; 0.30; 0.15 and 0 µM. The toxicological endpoint was the ability of the oocyte to reach the metaphase II stage (i.e. the final stage of maturation). In addition, we investigated the general cytotoxicity of CHX by using MTT-test. Our results show no general cytotoxicity for CHX in all of the concentration used. The meiosis was inhibited in a dose-response manner and the EC50 for CHX was 0.55µM, much lower than the concentration used to block protein synthesis in somatic cells, indicating the high sensitivity of the oocyte to express toxicity. The following step is to extend this investigation to the other selected compounds and to combine functional parameters with the use of proteomics and transcriptomics to obtain mechanistic insights in oocyte maturation and the effect of chemicals hereon. It is expected that markers derived, along with functional parameters, can form a powerful assay for the *in vitro* prediction of reproductive toxicity. In conclusion, we do believe that the use of the IVM assay can grant a powerful and physiological model in an ITS of effects of chemicals on female fertility. Research supported by TNO Research Program on Hazardous Substances and Occupational Safety; and The Netherlands Toxicogenomics Center.

275. Calbindin-D9k Expression in GH3 Cells Is a Biomarker of Xenoestrogenic Potential of Parabens. Thuy T.B. Vo and Eui-Bae Jeung. Chungbuk National University, Cheongju, Republic of Korea

The potential adverse effects of parabens were reported in both *in vivo* and *in vitro* systems, but the molecular mechanism(s) and long-term consequences of parabens exposure are largely unknown. In this study, we further examined the induction of an estrogenic biomarker gene-calbindin-D9k (CaBP-9k) to investigate the estrogenic activity of parabens (methyl-, ethyl-, propyl-, isopropyl-, butyl-, and isobutylparabens) in the rat pituitary GH3 cell line. Following 24 h exposure, significant increases in CaBP-9k transcript and protein were observed depending on the concentration treated and the linear length of the alkyl chain from methyl- to isobutyl parabens, whereas co-treatment with fulvestrant, a pure antiestrogen, largely reversed the paraben-induced expressions of CaBP-9k mRNA and protein in GH3 cell line. To better understand the mechanism(s) of CaBP-9k induction by these endocrine disrupting compounds, we measured the levels of estrogen receptor (ERalpha) and progesterone receptor (PR) expression following parabens exposure. In the GH3 cells, a great increase in PR mRNA and protein was observed in a concentration-dependent manner after parabens treatment. Paraben-induced expression of CaBP-9k was effectively blocked in the presence of antagonist of 17beta-estradiol (fulvestrant). To confirm whether progesterone receptor signaling is involved in parabens derived induction of CaBP-9k mRNA and protein, we treated GH3 cells with antiprogesterone (mifepristone). In the study, parabens-induced up-regulation of CaBP-9k was completely reversed by mifepristone. Taken together, these results indicate that CaBP-9k may be induced by parabens via the PR-involved pathway in addition to ERα pathway in GH3 cell line.

276. Potential Estrogenic Effect(s) of Parabens at the Neonatal Stage of an Immature Female Rat Mode. Kyung-Chul Choi and Eui-Bae Jeung. Chungbuk National University, Cheongju, Republic of Korea

This study was performed to examine the estrogenic effects of parabens on hormonal responsiveness and on the morphology of reproductive tissues during a critical developmental stage of female rats. Two hundred immature female Sprague-Dawley rats (n = 10/group) were orally treated with methyl-, ethyl-, propyl-, isopropyl-, butyl-, and isobutylparaben from postnatal day 21 to 40 in a dose-dependent manner based on our previous study [62.5, 250, and 1000 mg/kg of body weight (BW) per day]. A high dose of methyl- and isopropyl paraben (1000 mg/kg of BW per day) resulted in a significant delay in the date of vaginal opening and a decrease in length of the estrous cycle. In measurements of organ weight and body weight, we observed significant weight changes (i.e., decreased in ovaries, kidneys and increased in adrenal glands, thyroid glands, liver) conversely, body weight was not altered following paraben treatment. In all groups exposed to paraben treatment, histological analysis of the ovaries from the immature rats revealed interstitial cell disorders, decreased corpora lutea, an increase in the number of cystic follicles, and thinning of the follicular epithelium, which occurred in a dose-dependent manner. In addition, morphological study of the uterus revealed the myometrial dysplasia such as myometrial hyperplasia in the highest dose of propyl- and isopropyl paraben and in all dose of butyl- and isobutyl parabens. We also observed a significant decrease in serum estradiol and T4 concentrations in methyl-, ethyl-, propyl-, isopropyl-, and isobutylparaben-treated groups (P < 0.01 and 0.05). Taken together, long-term exposure to parabens can produce suppressive effects on hormonal responsiveness and can disrupt the morphology of reproductive target tissues during this critical stage of development in immature female rats.

277. Evidence That Mammary Gland Infection/Injury During Pregnancy in Dairy Cows May Have a Negative Impact on Size of the Ovarian Reserve in Their Daughters. James J. Ireland, Danielle M. Scheetz, Fermin Jimenez-Krassel, Joseph K. Folger, George W. Smith, Francesca Mossa, and Alexander C.O. Evans. Michigan State University, East Lansing, MI, USA; University College Dublin, Dublin, Ireland

The effect of disease on maternal environment, which has a crucial role in embryo development and subsequent health of offspring, has not been examined extensively in cattle. Therefore, the purpose of this study was to determine if a persistent mammary gland infection such as mastitis and correspondingly a chronically high (> 200,000 units) somatic cell count (SCC) in milk of pregnant dairy cows has a potential negative impact on size of the ovarian reserve (total number of healthy follicles and oocytes in ovaries) and potentially fertility of their offspring. This study was conducted at Green Meadow Farms Inc. in Elsie, MI and used 192, 12-mo-old Holstein heifers. Each animal was injected with 2 injections of prostaglandin F2 alpha (PG) spaced 11 d apart. To assess relative size of the ovarian reserve in each heifer, a single blood sample was removed 4 d after the 2nd PG injection and assayed for concentration of anti-Müllerian hormone (AMH), and ovarian ultrasonography was used to determine number of follicles ≥ 3 mm in diameter and ovary size. Results confirm previous observations that AMH concentrations were highly variable among animals but positively correlated (P < 0.001) with both ovary size and number of antral follicles. To determine if a chronic mammary gland infection during pregnancy of dairy cows was associated with alterations in AMH concentrations and correspondingly size of the ovarian reserve in their offspring, we next tested whether number of SCC measurements > 200,000 for each heifer's dam was associated with alterations in the serum AMH concentration in each heifer. Number of SCC measurements > 200,000 per individual cow is an accurate index of recurrent udder infections, injury and/or inflammation. A total of 5 to 7 measurements of SCC were made for each dairy cow beginning 2 mo before pregnancy and continuing throughout pregnancy. Number of SCC measurements > 200,000 ranged from 0 to 5 per individual cows, thus cows were separated into groups based on their number of SCC measurements > 200,000. Cows with 4 or 5 SCC measurements > 200,000 were ~2 years older (P < 0.01, 6.1 vs 4.4 yrs old) and tended (P < 0.09) to produce less milk compared with cows that had 0 to 3 SCC measurements > 200,000. Results showed that the daughters of cows with 4 and 5 SCC measurements > 200,000 also had markedly lower (P < 0.02) AMH concentrations as young adults compared with daughters of the cows with 0 to 3 SCC measurements > 200,000. Although mechanisms are unclear, our results imply that a chronic mammary infection or injury during pregnancy of older cows (as predicted by a high number of SCC measurements > 200,000) is associated not only with low milk production, but also with diminished size of the ovarian reserve and perhaps reduced reproductive performance (fertility and reproductive lifespan) of their daughters. Research supported by USDA-NRI 2004-01697, 2007-01289 to JJI.

278. The Endocrine Disruptor TCDD Modulates microRNA Expression in Preimplantation Mouse Embryos and Spheroids Attachment on Human Endometrial Epithelial Cells In Vitro. Kai-Fai Lee, Wei-Min Liu, Hilda Tsang, Tsz-Yan Cheung, Suranga P. Kodithuwakku, William S.B. Yeung, and Chris K.C. Wong. The University of Hong Kong, Hong Kong, China; Hong Kong Baptist University, Hong Kong, China

The endocrine disruptor (ED) is an exogenous substance that acts on the endocrine system and modulates normal physiological functions of the body. Although EDs such as 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) affect normal reproductive function in humans and affects the growth and reproductive functions in rodents, the underlying mechanism that modulates these changes remains unclear. Accumulating evidence suggested preimplantation embryo development is controlled by concerted expression of microRNAs (miRNA) that regulate mRNA stability and protein translation. We hypothesized that EDs affect pre-implantation embryo development by modulating miRNA expression, as well as attachment (an initial step on implantation process) of embryo onto endometrial epithelium. Treatment of mouse zygote with 10nM TCDD did not affect the blastulation rate of mouse embryo developed *in vitro*. Yet, analysis of the expression of 238-plex miRNA by RT-PCR at the blastocyst stage revealed 9 and 11, up- and down-regulated miRNA (>2-fold changes; p<0.05) between the TCDD-treated and control embryos. RT-PCR confirmed the differential expression on some of the selected miRNA including miR-133a and 199a expression in single blastocyst. Computation analysis (picTar) identified putative implantation-related targets for these miRNAs including cadherin, integrin and GSK3beta. An *in vitro* choriocarcinoma (Jeg-3 and BeWo) and endometrial epithelial (Ishikawa and RL95-2) cells co-culture system was established and used to study the effect of TCDD on spheroids attachment. The receptor of TCDD, aryl hydrocarbon receptor (AhR), as well as estrogen receptor (ERalpha) alpha and (ERbeta) beta were detected in all the cell lines. TCDD dose-dependently (1pM - 10nM, for 24 hours) induced CYP1A1 (a biomarker gene for TCDD exposure) transcript and protein expression in these cell lines. In addition, TCDD dose-dependently suppressed attachment of BeWo spheroid onto the RL95-2 monolayer (from 82% to 53%, n=6). Yet, TCDD has no effect on cell proliferation. Our results are the first demonstrating that ED modulates the miRNA expression of preimplantation embryos, and may in turn affect the normal expression of genes that are important for embryo attachment and implantation *in vitro*. This research was supported in part by a RGC Collaborative Research fund (CRF) Group Research Project (HKBU 1/CRF/08).