parent-of-origin imprints on haploid spermatids from exposed cultures. Based on these results, our model may be the first *in vitro* human model available to screen for male reproductive toxicants and potentially explain male factor infertility caused by environmental exposures to various chemicals such as flame retardants.

3248 Relevant Methods of Oral Exposure to E-Waste Leachate and Fracking Fluid Alters Reproductive Sperm Parameters in Male Mice

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The potential human harm from unregulated manual disassembly of electronic waste (E-waste) or fracking fluid spills could arise from exposure in soil, rivers, groundwater, and vegetation near residential sites. Both wastewater types contain chemical mixtures with the potential to adversely impact reproductive health if ingested. Thus, a laboratory study was carried out with adult B6C3F1 male mice to evaluate the persistent toxicity of e-waste leachate and fracking-produced wastewater on male reproductive development. Three groups (n=15) of 8-wk-old mice were exposed (5 d/wk/5 wk) by gavage (0.25ml/mouse) to either e-waste leachate from the Alaba Market (Lagos, Nigeria) or, via a novel mealworm (*Tenebrio molitor*) feeding route (0.25ml/2 worms) to W. Virginia fracking wastewater or deionized water. Mice were sacrificed 24 hr, 3 wk or 5 wk post-exposure, and testes, cauda epididymis, seminal vesicles, and prostate were collected and weighed. Sperm from the cauda were counted using a hemocytometer and assessed for viability, chromatin damage, and morphological aberrations. Results demonstrate that mice exposed to 10%, 25% and 50% dilutions of e-waste demonstrate a dose-dependent increase (compared to control) in sperm chromatin damage (17, 34, 68%, respectively), suggesting DNA damage, and morphological abnormalities (11, 42, 70%, respectively) that included amorphous/missing heads, misfolded, doubled, or missing tails, and cytoplasmic droplets. As some toxic metal concentrations in the e-waste leachate mixture are higher than WHO standards (e.g., As, Cd, Hg), the same metals could contribute to the observed impacts on male reproduction. Implications of this study are significant, particularly for areas where e-waste dumpsite leachate can contaminate drinking water and soil. NYU GIPH & NYU NIEHS Center.

3249 Effects of Ethylene Glycolmonomethyl Ether on the Epigenetic Related Genes in the Testis of Male Rats

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The organic solvent ethylene glycol monomethyl ether (EGME) produces testicular lesion in rats and human. In the present study, to investigate epigenetic-related gene expression in EGME testicular lesion, mRNA and miRNA array screening and real-time RT-PCR analysis were conducted using testis collected from male rats given 2000 mg/kg EGME for one day. There was death of phacytene spermatocytes from 6hr after dosing and accompanied with Sertoli cell vacuolation after 24hr. From the mRNA expression analysis, DNA methyltransferase, dnmt3a, dnmt3b, and histone demethylase, jmjd3, were increased. On the other hand, histone deacetylase, hdac4, and histone methyltransferase, symd1, were decreased by EGME. Regarding miRNA, the expression of miR-449a and miR-141 was decreased, and the expression of miR-134 was increased. For the spermatogenesis, dnmt3a and dnmt3b contribute to progress or maintain of DNA methylation and associate with proliferation of spermatogonia or meiosis of spermatocyte. One of the target mRNAs of miR-141, jmjd3, was reported to work for proliferation of spermatogonia. Decreased expression of hdac4, the target gene of miR-134, suggests to histone hypermethylation. Additionally, decrease in symd1 is expected suppression of histone methylation. Decrease in miR-449a, which is known to be highly expressed in spermatocytes and spermatid, is supposed to be disorder of meiosis. Taken together, we considered EGME affected several epigenetics-related genes and maintaining DNA methylation or histone modification and finally, these things resulted in testicular lesion.

3250 Body Weights and Fertility of Male Rats Offspring after Prenatal Exposure to Bisphenol A

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Bisphenol A (BPA) can alter the hormone homeostasis and cause developmental disorders. The present study evaluated the effect of male rats' prenatal exposure to BPA on body and reproductive organs weights, fertility parameters and hormonal functions of offspring. Pregnant female rats were gavaged with BPA (1.2 and 2.4 µg/kg/day) plus a negative control during the prenatal period of six weeks. Adult first generation males (F1) were subjected to fertility assessment by mating with unexposed females to BPA. The reproductive functions of the subsequent second generation (F2) litters were investigated similarly. Hormonal levels and rat sperms qualities were investigated using an ELISA and the hemocytometer method, respectively. A significant increase was observed in number of corpora lutea, implantation sites, and post implantation loss in the females mated with the first generation offspring. Terminal body weights significantly increased as well as weights of the testes and the prostate glands across all generations. Contrarily, a significant decrease was observed in the epididymal weight, litter size, sperms count and motility. A significant decrease in follicle stimulating hormone levels across both generations F1 (51.25±1.93) and F2 (52.55±2.03) was observed. Luteinizing hormone levels showed a significant increase in the groups exposed to low doses of BPA in both generations F1 (46.53±0.15) and F2 (45.89±0.32). The plasma levels of the testosterone hormone were significantly decreased in the groups exposed to both doses of BPA of the F1 (13.06±0.28, 13.29±0.15, respectively) and the F2 (12.19±0.19, 12.74±0.23 respectively). And, only the groups exposed to low dose of BPA showed estradiol increase in both the F1 (350.73±11.51) and the F2 (259.89±2.44) generations. Prenatal exposure to environmentally relevant doses of BPA may lead to hormonal imbalances associated with body weights disruption and the male germ line leading to subsequent impairments in the offspring and their subsequent generations.

3251 Cell-Based High-Content Analysis (HCA) Reveals Differential Effects of BPA and Its Selected Analogues on Spermatogonia Stem Cells

S. Liang, L. Yin and X. Yu. *University of Georgia, Athens, GA.*

Bisphenol A (BPA), an endocrine-disrupting compound, is found to be a testicular toxicant in animal models. Bisphenol AF (BPAF), Bisphenol S (BPS) and Tetrabromobisphenol A (TBBPA), structurally similar to BPA, are introduced to the market as alternatives to BPA. However, there is limited toxicological data on the male reproductive systems of these compounds so far. In this study, we compared the time- and dose-dependent effects of BPA and its analogues on the cell viability and cell cycle alteration in spermatogonia stem cells. We observed dose-dependent decreases of cell viability using neutral red uptake assay at 24 and 48 h after treatments. The IC50 values at 48 h were 55.5, 58.5, 184.5 and 357.1 uM for TBBPA, BPAF, BPA and BPS, respectively. We established a high-throughput and high-content analysis (HCA) for the morphology and cell cycle analysis. The Hoechest 33342 staining was used to determine the nuclear size and nuclear morphology. 5-bromo-2´-deoxyuridine (BrdU) was used to label DNA synthesis for the active proliferating cells. Automated multi-channel images were acquired using ArrayScan VTI and quantified by HCS Studio 2. We found that BBAF and TBBPA significantly induced changes of nuclear shape at lower concentrations as compared to BPA and BPS, indicating a drastic condensation of chromatin. BBAF and TBBPA also significantly decreased the BrdU incorporation at non-toxicity doses at 72 h after treatments. In summary, our current study demonstrated the differential toxicity of BPA and its analogues on the spermatogonia cells. (supported by R21 OH 010473, ARDF and the UGA Startup Research funding.

3252 High Content Analysis (HCA) of Effects of Low-Dose Cadmium on DNA Damage and Cell Cycle in Spermatogonial Stem Cells

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Environmental and occupational exposures to cadmium (Cd) pose significant health risks to humans, including reproductive and developmental toxicity. Our previous studies have demonstrated that Cd at the concentration of 10 µM induced activation of stress signaling pathways, disruption ubiquitin-proteasome system and cell cycle alterations in primary rat testicular cell co-cultures. However, the testicular toxicity of Cd at environmentally relevant low doses has not been characterized. In this study, we established a high-throughput and high- content analysis (HCA) for cell cycle (Click-iT EdU and Phospho-Histone H3 Assay), DNA damage (γ-H2AX) and cytoskeletal structure (Phalloidin F-actin).

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