cAMP was associated with greatly increased histone H3 acetylation in FUER (6.6 fold) and proximal promoter region (3 fold) but not in AhR binding region, suggesting that FUER chromatin remodeling mediates cAMP induction.

356 METHOXYCHLOR-INDUCED ATRESIA WORKS THROUGH THE BCL-2 PATHWAY.

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The organochlorine pesticide methoxychlor (MXC) has been shown to cause ovarian atresia (apoptosis of antral follicles). Bcl-2 (an anti-apoptotic protein) and Bax (a pro-apoptotic protein) are both important apoptotic factors in the ovary, and it is thought that the ratio of pro- to anti-apoptotic proteins is a determining step in whether follicles undergo atresia. Previous studies conducted in our laboratory have shown that MXC induces antral follicle atresia without changing serum levels of estradiol, follicle-stimulating hormone and luteinizing hormone. Since MXC induces antral follicle atresia without altering pituitary-gonadal hormones, our hypothesis is that MXC targets antral follicles directly through either Bcl-2 or Bax in the ovary. In order to test this hypothesis, CD-1 mice (39 days old) were treated with 64mg/kg MXC or sesame oil (vehicle), ovaries were collected during estrus and processed for immunohistochemical analysis of Bcl-2 or Bax proteins. Further, transgenic mice that overexpress Bcl-2 and mice deficient in Bax and their wild-type littermates were treated with either 64mg/kg MXC or vehicle, and their ovaries were collected during estrus and processed for morphological analysis of antral follicle atresia. Our results show that immunohistochemical staining for Bax was stronger in the MXC-treated mice, but Bcl-2 levels remain unchanged as compared to vehicle-treated mice. In addition, our results show that Bcl-2 overexpressing mice and Bax deficient mice were protected from MXC induced-atresia. There were significantly more healthy antral follicles in the MXC-treated Bcl-2 overexpressing mice than in the MXC-treated wild-type mice (Bcl-2 overexpressers = 903 ± 123, wild-type = 540 ± 72; n=7-9, p=0.03). MXC-treated Bax deficient mice had similar levels of atresia as the vehicle-treated Bax deficient mice (31% vs. 27%, respectively). Collectively, these data suggest that MXC induces antral follicle atresia *via* a mechanism that involves Bcl-2 and Bax. (Supported by NIH HD38955 and T32 ES07263-13).

357 *IN VITRO* FOLLICLE ASSAY ALLOWS GONADAL RISK ASSESSMENT FOR BENZODIAZEPINE.

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It is currently not clear whether chronic use of benzodiazepines can affect female fertility. *In vitro* studies suggest that acute high doses of diazepam (DZ) interfere with the meiotic process. Follicles exposed *in vitro* to chronic low doses of DZ had a reduced survival rate and poor oocyte quality. To investigate efficacy & specificity of the follicle culture system, 3 benzodiazepins with different receptor specificity were tested. Flumazenil (FL) binds to receptors in the CNS while 3-chlorodiazepam (CD)to peripheral receptors, diazepam (DZ) to both. Mouse preantral follicles were cultured singly for 12 days to the preovulatory stage, in α-MEM with FBS, FSH, LH. At day 12 an ovulatory stimulus (hCG) was added. Follicles were continuously exposed to FL, CD or DZ to 5, 10 or 15 µg/ml. Follicle growth, steroid secretion, oocyte quality were concurrently analysed. At all doses tested FL-exposure had no impact on folliculogenesis, steroid production or on oocyte maturation. CD acted similarly as DZ. Both drugs decreased follicle survival and altered steroid production dose dependently. At 15 µg/ml only 25 % of the follicles survived the 12-day culture period. CD blocked meiosis reinitiation while oocytes exposed to DZ started meiosis but progression was disturbed. Analysis of spindle formation revealed an increased aberration rate in the MII-oocytes exposed to CD and DZ but not to FL. At a dose of 5 µg/ml, cytogenetic analysis revealed a diploidy-rate of 15% and 29% for DZ and CD respectively. Five µg/ml FL had no effect on chromosome constitution. In accordance to their receptor binding specificity, benzodiazepines differ in their effects on ovarian function. FL was not cytotoxic during folliculogenesis while CD and DZ induced cytotoxicity and had an aneugenic effect on oocytes. The follicle culture system is a relevant *in vitro* model to study effects of NCE on the ovarian function and will be helpful to assess female fertility *in vitro*.

358 METABOLIC MECHANISMS OF METHOXYCHLOR TOXICITY IN MOUSE ANTRAL OVARIAN FOLLICLES.

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The organochlorine pesticide methoxychlor (MXC) is a reproductive toxicant that targets the mammalian ovary, causing reduced fertility and persistent estrus. Antral follicles are the primary targets of MXC toxicity, while less mature follicles are not affected by MXC exposure. Previous studies indicate that antral follicles from *in vivo*-exposed mice show increased atresia and altered expression of apoptotic proteins. Although MXC toxicity in non-ovarian tissues has been attributed to cytochrome P450 (CYP) metabolism to polar compounds including bis-hydroxy MXC (HPTE), little is known about ovarian mechanisms of MXC toxicity. Our hypothesis is that MXC metabolites are responsible for antral follicle-specific toxicity, and that CYP enzymes expressed in the ovary mediate MXC metabolism to cause antral follicle toxicity. Antral follicles were isolated from immature mouse ovaries and exposed *in vitro* to MXC (0.01-100µg/mL) or HPTE (0.01-1µg/mL) for 96hrs. Follicle diameters were measured to assess growth in response to MXC or HPTE. Cultured follicles were also processed for morphological analysis of atresia, as determined by numbers of pyknotic bodies in granulosa cells. Immunohistochemistry was performed on ovaries from *in vivo* MXC-exposed mice (sesame oil, 32mg/kg MXC, 20 days) to evaluate expression of MXC metabolizing enzymes CYP3A4 and CYP2C19. MXC significantly inhibited growth of antral follicles *in vitro* compared to controls at 10 and 100µg/mL (n=25 follicles/treatment; $p<0.01$), and increased atresia over controls at 100µg/mL. HPTE did not alter follicle growth, nor did it significantly induce atresia. Yet, both CYP3A4 and CYP2C19 were present in the ovary, and induced by MXC in corpora lutea, surface epithelium and antral/preovulatory follicles. These data suggest that metabolites other than HPTE may be responsible for suppression of antral follicle growth by MXC. Also, since CYPs are expressed in the ovary, metabolism of MXC by other ovarian components may subsequently cause antral follicle toxicity. (Supported by NIH HD38955, T32 ES07263-13 and a Colgate-Palmolive Fellowship).

359 ESSENTIAL ROLE OF NRF2 IN PROTECTION AGAINST OVARIAN FOLLICLE LOSS INDUCED BY 4-VINYCYCLOHEXENE AND 4-VINYLCYCLOHEXENE DIEPOXIDE IN MICE.

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4-Vinylcyclohexene (VCH), an industrial chemical, and its metabolite 4-vinylcyclohexene diepoxide (VCD), represent a potential health hazard, because they selectively destroy oocytes in small pre-antral follicles leading to premature ovarian failure in animals. Previous studies suggest that metabolism of VCH and VCD by phase I and phase II drug-metabolism enzymes plays an important role in the ovotoxicity of these chemicals. Nrf2 is a member of the Cap ″N″ Colar bZip family of transcription factors that mediates the basal expression and induction of phase II enzymes such as NQO1. In this study, we examined the role of Nrf2-regulated gene expression in the ovotoxicity of VCH and VCD by using Nrf2 knockout mice. Immature (age, day 28) female wild-type and Nrf2-/- mice (both in B6 background) were treated with VCH or VCD using established protocols; 4 h following the final dose, ovaries were collected. Complete serial sections of ovaries were evaluated histologically for the presence of follicles. As expected, the primordial and primary follicle numbers in ovaries from wild type mice decreased significantly ($p<0.05$) following treatment with either VCH or VCD. However, the primordial and primary follicles in ovaries from Nrf2-/- mice exhibit much higher sensitive to the toxicity of VCH and VCD than those of wild type mice. Both VCH and VCD have no significant effects on growing or pre-antral follicles in either genotypes. Taken together, these results demonstrate that loss of Nrf2 function is associated with increased sensitivity to toxicity of VCH and VCD on ovary follicle development. The findings suggest that Nrf2-mediated expression of phase II genes plays an important role in detoxification of VCH and VCD, thereby protecting ovarian follicles from the ovotoxicity of the chemicals.

360 NEONATAL EXPOSURE TO GENISTEIN ALTERS OVARIAN DIFFERENTIATION RESULTING IN THE FORMATION OF MULTI-OOCYTE FOLLICLES.

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Genistein (Gen), the primary phytoestrogen in soy products, including soy-based infant formulas, was investigated for potential adverse effects on the developing ovary. We have previously shown that mice treated with Gen during the first week of life develop a dose dependent increase in the number of multi-oocyte follicles (MOF) at 19 days of age. During ovarian differentiation murine ovaries are composed of oocyte nests on day 1 which dissociate leaving predominantly single oocyte follicles by 5 days of age. To determine if Gen inhibits the breakdown of oocyte nests leading to MOF, we treated outbred CD-1 mice by subcutaneous injection on neonatal days 1-5 with Gen at 50 mg/kg/day and compared ovarian differentiation to age-matched untreated controls. Ovaries were collected on days 1-6

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