CAFFEINE

This is a compilation of abstracts of articles identified during the preliminary toxicological evaluation of evidence on the developmental and reproductive toxicology of caffeine. Caffeine (CAS# 58-08-2) is a psychoactive compound found naturally in or added to numerous products such as coffees, teas, chocolate, soft drinks and over-the-counter pharmaceuticals.

Compiled are abstracts from developmental and reproductive epidemiologic and animal toxicity studies and other relevant investigations. This information was used in a screen to select appropriate chemicals for presentation to the Developmental and Reproductive Toxicant Identification Committee as possible candidates for Committee consideration. The criterion for passing this screen is the existence of two or more analytical epidemiologic studies judged to be of adequate quality that reported increased risk of adverse developmental or reproductive outcomes. The epidemiologic studies report on developmental and reproductive sequelae related to environmental exposures to caffeine. Based on a review of abstracts of the following studies, the chemical passed the epidemiologic screen.

- Thirty-two epidemiologic studies of caffeine reporting increased risk of adverse developmental or reproductive outcomes were identified, thirty of which were analytical studies of adequate quality. One meeting abstract reporting increased risk of adverse developmental or reproductive outcomes was also identified. Eighteen epidemiologic studies reporting no increased risk of adverse developmental or reproductive outcomes were identified, as well as two studies with unclear findings and three related studies.

- Fifty-two animal studies of caffeine reporting reproductive or developmental toxicity were identified, as well as five studies reporting no reproductive or developmental toxicity. Twelve studies with unclear outcome were identified, as well as sixty-three related articles and meeting abstracts. Twenty studies without abstract were identified.

Due to the large number of abstracts available, a summary table of the information contained in the abstracts of the epidemiologic studies of caffeine is provided and only studies published within the last ten years are included.

Contents

I. Epidemiologic DART Studies

A. Studies reporting increased risk of adverse developmental or reproductive outcomes

[*]Association of cytochrome P450 1B1 polymorphism with first-trimester miscarriage.
Karypidis AH, Soderstorm T, Nordmark A, Granath F, Cnattingius S, Rane A.
Fertil Steril. 2006 Nov;86(5):1498-503.

OBJECTIVE: To determine whether the cytochrome P450 1B1 (CYP1B1) Val432Leu polymorphism is associated with risk of miscarriage. We also analyzed the possible interaction between this polymorphism and caffeine intake. DESIGN: The population-based case-control study included 507 women with miscarriage in the first trimester of pregnancy and 908 controls with a normal first-trimester pregnancy. The controls were frequency matched to cases. The material was analyzed taking maternal age, smoking habits, alcohol intake, caffeine intake, fetal karyotype, nausea, and vomiting into consideration. SETTING: University hospital and primary care facility. MAIN OUTCOME MEASURE(S): CYP1B1 Val432Leu genotype frequencies in cases and controls. RESULT(S): Carriers of the CYP1B1 432 Val/Val genotype were at a higher risk of miscarriage in the first trimester of pregnancy (odds ratio = 1.46; 95% confidence interval, 1.02-2.08). We also found a significant interaction between genotype and caffeine intake. CONCLUSION(S): CYP1B1 Val432Leu polymorphism is associated with first-trimester miscarriage, and it may also modify the risk among coffee drinkers.

Environmental contaminant levels and fecundability among non-smoking couples.
Cole DC, Wainman B, Sanin LH, Weber JP, Muggah H, Ibrahim S.
Reprod Toxicol. 2006 July;22:13-9.

OBJECTIVE: To investigate the effects of low level maternal and paternal persistent contaminant exposures on fecundability among couples from the general population. METHODS: About 41 couples having their first pregnancy completed questionnaires and provided blood samples for analysis of metals, organochlorine pesticides, and polychorinated biphenyls. Associations of personal consumption and contaminant measures for mothers, fathers, and couples overall were analyzed through fecundability odds ratios (fOR, probability of pregnancy per month in more versus less exposed) in multivariable analyses. FINDINGS: Couples with higher reported caffeine consumption (couple consumption > or =111 drinks/month, fOR 0.25, 95% CI, 0.10, 0.63) and higher mercury concentrations in maternal blood (>1.2 microg/L or 0.24 ppm in hair, fOR 0.22,

[*] denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

95% CI, 0.07, 0.72) had lower fecundability, after adjustment for intercourse frequency. CONCLUSION: Reduced fecundability at levels below the mercury reference dose warrants further research and prudent reduction in persistent toxic substances exposure among women and men of reproductive age.

*** Risks of repeated miscarriage.**
George L, Granath F, Johansson AL, Olander B, Snattingius S.
Paediatr Perinat Epidemiol. 2006 Mar;20:119-26.

There is a lack of well-designed epidemiological studies of possible risk factors for repeated miscarriage. In this Swedish population-based case-control study, we investigated the association between sociodemographic and anthropometric factors, obstetric history and life-style factors, with respect to the risks of first-trimester repeated miscarriage. Information on maternal characteristics was collected through in-person interviews. Plasma blood samples were analysed for cotinine and folate concentrations. Adjusted odds ratios (OR) with 95% confidence interval [CI] were used to estimate the relative risk of repeated miscarriage. The risks of repeated miscarriage were increased for women aged > or = 35 years (adjusted OR 2.9 [95% CI 1.4, 5.8]), as well as for women aged < or = 24 years (OR 2.8 [95% CI 1.1, 6.8]). Women with a history of at least one preceding miscarriage prior to the two index pregnancies, women reporting prolonged time to conceive, and women with a history of myoma, faced a more than fourfold increased risk of repeated miscarriage. Smokers were at an increased risk of repeated miscarriage (OR 2.1 [95% CI 1.1, 4.1]). Among non-smoking women with high caffeine intake, there was an increased risk of repeated miscarriage, whereas there was no such association among smokers. Low plasma folate levels were not associated with increased risks.

*** Maternal caffeine consumption and fetal death: a case-control study in Uruguay.**
Matijasevich A, Barros FC, Santos IS, Yemini A.
Paediatr Perinat Epidemiol. 2006 Mar;20:100-9.

The objective of this study was to examine the association between caffeine intake during pregnancy and fetal mortality in Montevideo, the capital city of Uruguay, taking into account several potential confounding factors. A population-based case-control study was conducted between 1 August 2002 and 31 December 2003. A total of 382 cases and 792 controls were recruited. Cases consisted of women hospitalised with a medically confirmed diagnosis of spontaneous antepartum fetal death, in all maternity hospitals during the study period. Antepartum fetal death was defined as a fetal death in which the attending doctor certified that the death occurred prior to the onset of labour. Fetal deaths

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

were included if they were of at least 20 weeks' gestational age or weighed >350 g. Controls were women who had a live, vigorous and term adequate-for-gestational-age newborn. Multiple gestations and fetuses/newborns with evident congenital malformations were excluded. Only a small proportion of the mothers (8.1% of the cases and 9.5% of the controls) did not consume caffeine during pregnancy. Among consumers, mate drinking was the most frequent source of caffeine in both cases and controls. After controlling for mother's and her partner's education, history of abortions and/or fetal deaths, vomiting/nausea during the first trimester of gestation and attendance for prenatal care, the category of mean caffeine intake of > or = 300 mg/day showed a significantly increased risk of fetal death (OR 2.33 [1.23; 4.41]) compared with no caffeine consumption during pregnancy. The study also found that less-educated women, mothers who did not attend for prenatal care and women with a history of abortions and fetal death were at an increased risk of fetal death. As mate drinking is highly consumed among pregnant women in Uruguay, the association found with fetal death makes it a preventable risk factor.

[*] **Coffee and fetal death: a cohort study with prospective data.**
Bech BH, Nohr EA, Vaeth M, Henriksen TB, Olsen J.
Am J Epidemiol. 2005 Nov;162(10): 983-90.

The authors conducted a cohort study within the Danish National Birth Cohort to determine whether coffee consumption during pregnancy is associated with late fetal death (spontaneous abortion and stillbirth). A total of 88,482 pregnant women recruited from March 1996 to November 2002 participated in a comprehensive interview on coffee consumption and potentially confounding factors in pregnancy. Information on pregnancy outcome was obtained from the National Hospital Discharge Register and medical records. The authors detected 1,102 fetal deaths. High levels of coffee consumption were associated with an increased risk of fetal death. Relative to nonconsumers of coffee, the adjusted hazard ratios for fetal death associated with coffee consumption of 1/2-3, 4-7, and > or =8 cups of coffee per day were 1.03 (95% confidence interval (CI): 0.89, 1.19), 1.33 (95% CI: 1.08, 1.63), and 1.59 (95% CI: 1.19, 2.13), respectively. Reverse causation due to unrecognized fetal demise may explain the association between coffee intake and risk of fetal death prior to 20 completed weeks' gestation but not the association with fetal loss following 20 completed weeks' gestation. Consumption of coffee during pregnancy was associated with a higher risk of fetal death, especially losses occurring after 20 completed weeks of gestation.

[*] denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

***Caffeine intake, CYP1A2 polymorphism and the risk of recurrent pregnancy loss.**
Sata F, Yamada H, Suziki K, Saijo Y, Kato EH, Morikawa M, Minakami H, Kishi R.
Mol Hum Reprod. 2005 May;11(5):357-60.

Some case-control studies have demonstrated that caffeine intake and high CYP1A2 activity increase risks of recurrent pregnancy loss (RPL) but the multifactorial effect is obscure. To investigate whether susceptible women who have more caffeine intake are at high risk of RPL, a case-control study of 58 cases with two or more RPL and fertile 147 controls was performed. The association between daily caffeine intake together with CYP1A21F (AA versus CA and CC) genotype and RPL was assessed. Without consideration of the genotype, there were no significant differences of the RPL risk in proportion to daily caffeine intake [less than 100 mg (reference); 100-299 mg: odds ratio (OR), 1.29; 95% confidence interval (CI), 0.66-2.50; 300 mg or more: OR, 1.82; 95% CI, 0.72-4.58; P for trend, 0.20]. However, the RPL risk significantly increased only among women who had homozygous CYP1A21F alleles with a dosage effect of daily caffeine intake [less than 100 mg (reference); 100-299 mg: OR, 1.94; 95% CI, 0.57-6.66; 300 mg or more: OR, 5.23; 95% CI, 1.05-25.9; P for trend, 0.03]. It was demonstrated for the first time that an increase in caffeine intake deteriorates the fecundity among susceptible women.

*** Consequences of smoking and caffeine consumption during pregnancy in women with type 1 diabetes**.
Khoury JC, Miodovnik M, Buncher CR, Kalkwarf H, McElvy S, Khoury PR, Sibai B.
J Matern Fetal Neonatal Med. 2004 Jan;15(1):44-50.

OBJECTIVE: To test the hypothesis that, in women with type 1 diabetes, prenatal smoking and caffeine consumption during pregnancy are associated with an increased risk of adverse maternal and perinatal outcomes. METHODS: A secondary analysis of data on pregnant women with type 1 diabetes from an interdisciplinary program of Diabetes in Pregnancy. Women were interviewed monthly, by a trained non-medical member of the research team, using a standardized questionnaire, to ascertain daily smoking habits and caffeine consumption. RESULTS: Smoking and caffeine information were available on 191 pregnancies, 168 progressing beyond 20 weeks of gestation. Early pregnancy smoking (OR 3.3, 95% CI 1.2, 8.7) and caffeine consumption (OR 4.5, 95% CI 1.2, 16.8) were associated with increased risk of spontaneous abortion when controlling for age, years since diagnosis of diabetes, previous spontaneous abortion, nephropathy and retinopathy. Smoking throughout pregnancy was significantly associated with decreased birth weight and prolonged neonatal hospital stay. Smoking throughout pregnancy (OR 0.2, 95% 0.1, 1.0) and caffeine consumption after 20 weeks

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

(OR 0.3, 95% CI 0.1, 1.0) were associated with reduced risk of pre-eclampsia. CONCLUSIONS: Caffeine consumption during early pregnancy, regardless of glycemic control, increases the risk of spontaneous abortion. Smoking throughout pregnancy and caffeine consumption are associated with reduced risk of pre-eclampsia.

* **Maternal consumption of coffee during pregnancy and stillbirth and infant death in first year of life: prospective study.**

Wisborg K, Kesmodel U, Bech BH, Hedegaard M, Henriksen TB.
BMJ. 2003 Feb 22;326(7386):420.
Comment in:
BMJ. 2003 Jun 7;326(7401):1268-9; author reply 1269.
BMJ. 2003 Jun 7;326(7401):1268; author reply 1269.
BMJ. 2003 Jun 7;326(7401):1268; author reply 1269.

OBJECTIVE: To study the association between coffee consumption during pregnancy and the risk of stillbirth and infant death in the first year of life. DESIGN: Prospective follow up study. SETTING: Aarhus University Hospital, Denmark, 1989-96. PARTICIPANTS: 18 478 singleton pregnancies in women with valid information about coffee consumption during pregnancy. MAIN OUTCOME MEASURES: Stillbirth (delivery of a dead fetus at > or =28 weeks' gestation) and infant death (death of a liveborn infant during the first year of life). RESULTS: Pregnant women who drank eight or more cups of coffee per day during pregnancy had an increased risk of stillbirth compared with women who did not drink coffee (odds ratio=3.0, 95% confidence interval 1.5 to 5.9). After adjustment for smoking habits and alcohol intake during pregnancy, the relative risk of stillbirth decreased slightly. Adjustment for parity, maternal age, marital status, years of education, occupational status, and body mass index did not substantially change the estimates of association. There was no significant association between coffee consumption and death in the first year of life after adjustment for smoking habits during pregnancy. CONCLUSION: Drinking coffee during pregnancy is associated with an increased risk of stillbirth but not with infant death.

---

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

[*] **Does caffeine and alcohol intake before pregnancy predict the occurrence of spontaneous abortion?**
Tolstrup JS, Kjaer SK, Munk C, Madsen LB, Ottesen B, Bergholt T, Gronbaek M.
Hum Reprod. 2003 Dec;18(12):2704-10.

BACKGROUND: Consumption of caffeine and alcohol is suspected to affect pregnancy outcome. Use of both stimulants is widespread and even minor effects on fetal viability are of public health interest. METHODS: We performed a nested case-control study using prospective data from a population-based cohort comprising 11088 women aged 20-29 years. From this cohort, women who experienced either a spontaneous abortion (n = 303) or who gave birth (n = 1381) during follow-up [mean time: 2.1 years (range: 1.6-3.4)] were selected. Associations between self-reported exposures to caffeine and/or alcohol at enrolment and spontaneous abortion were analysed by means of logistic regression. RESULTS: Compared with women with a pre-pregnancy intake of <75 mg caffeine per day, the adjusted odds ratio (95% confidence interval) for spontaneous abortion was 1.26 (0.77-2.06), 1.45 (0.87-2.41), 1.44 (0.87-2.37) and 1.72 (1.00-2.96) for a pre-pregnancy intake on 75-300, 301-500, 501-900 and >900 mg caffeine per day respectively (P = 0.05 for trend). A pre-pregnancy intake of alcohol was not a predictor for spontaneous abortion. CONCLUSIONS: A high intake of caffeine prior to pregnancy seems to be associated with an increased risk of spontaneous abortion, whereas a low-to-moderate alcohol intake does not influence the risk.

[*] **High caffeine consumption in the third trimester of pregnancy: gender-specific effects on fetal growth.**
Vik T, Bakketeig LS, Trygg KU, Lund-Larsen K, Jacobsen G.
Paediatr Perinat Epidemiol. 2003 Oct;17(4):324-31.

It has been suggested that a high caffeine intake in pregnancy may be a risk factor for fetal growth retardation. We have tested this hypothesis in a population-based case-control study. Caffeine intake among 111 mothers of small-for-gestational-age (SGA) infants (56 boys, 55 girls) was compared with the intake among 747 mothers of non-SGA infants (368 boys, 379 girls). Food records for 3 days were collected in the second (week 17-20) and in the third (week 33) trimester, and caffeine intake from coffee, tea, soft drinks and chocolate was calculated and dichotomised as low or high, based upon the median value. Mothers of SGA infants had higher mean intake of caffeine [281 +/- 210 (SD) mg/day] in the third trimester than mothers of non-SGA infants (212 +/- 150 mg/day; P < 0.001). The risk of SGA birth was nearly doubled if the mother had a high rather than a low caffeine intake in the third trimester [odds ratio (OR) 1.8; 95% confidence intervals (CI) 1.2, 2.5]. The increased risk was mainly found in boys (OR 2.8;

---

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

95% CI 1.5, 5.2), and not in girls (OR 1.2; 95% CI 0.7, 2.1). The increased risk for boys persisted after adjustment for cigarette smoking alone, or for smoking and various other SGA risk factors together. Our results suggest that a high caffeine intake in the third trimester may be a risk factor for fetal growth retardation, in particular if the fetus is a boy.

*** The effect of caffeine consumption and nausea on the risk of miscarriage.**
Giannelli M, Doyle P, Roman E, Pelerin M, Hermon C.
Paediatr Perinat Epidemiol. 2003 Oct;17(4):316-23.

Evidence for a harmful effect of caffeine intake on risk of miscarriage (spontaneous abortion) is inconsistent and nausea during pregnancy has been claimed to explain any association seen. The objective of this analysis was to determine whether caffeine consumption both before and during pregnancy influenced the risk of miscarriage in a group of pregnant women in the UK. We examined the association with maternal caffeine intake in a case-control study of 474 nulliparous women. Participants were recruited during the years 1987-89 from the Royal Berkshire Hospital in Reading and from a large group practice situated within the hospital's catchment area. Cases were 160 women with a clinically diagnosed miscarriage and controls were 314 pregnant women attending for antenatal care. Information on coffee/tea/cola consumption and potential confounders was collected by interview and caffeine content was assigned to individual drinks according to published data on caffeine content of beverages. Compared with a maternal caffeine intake of < 151 mg/day, we found evidence that caffeine consumption > 300 mg/day doubled the risk of miscarriage. Adjusted odds ratios were 1.94 [95% CI 1.04, 3.63] for 301-500 mg/day and 2.18 [95% CI 1.08, 4.40] for > 500 mg/day. This effect could not be explained by nausea in pregnancy. Nausea appeared to be strongly independently associated with a reduced risk of miscarriage (test for trend P < 0.0001). There was no evidence that prepregnancy caffeine consumption affected the risk. Our results indicate that high caffeine consumption during pregnancy (>300 mg/day), in particular coffee consumption, is an independent risk factor for increased risk and nausea is an independent protective factor for a lower risk of miscarriage.

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

*** Maternal characteristics and lifestyle factors and the risk of delivering high birth weight infants.**
Orskou J, Henriksen TB, Kesmodel U, Secher NJ.
Obstet Gynecol. 2003 Jul;102(1):115-20.

OBJECTIVE: To identify factors associated with an increased risk of giving birth to infants weighing more than 4000 g and to study whether changes in these factors over time can explain the increasing proportion of high birth weight infants over the last decade. METHODS: Our analyses included 24,093 pregnancies of nondiabetic women with information on potential risk factors for high birth weight: maternal prepregnancy weight, height, age, parity, smoking habits, alcohol and caffeine intake, marital status, educational level, gestational age, and infant gender. Information was obtained from questionnaires completed during pregnancy and birth registration forms at the Department of Obstetrics and Gynaecology, Aarhus University Hospital, Aarhus, Denmark, from 1990 to 1999. RESULTS: We found a statistically significantly increased risk of giving birth to infants weighing more than 4000 g for women with high prepregnancy weight and height, parity greater than 2, gestational age greater than 42 weeks, and male infant gender and for nonsmokers. Women with a low caffeine intake or 10 or more years of education were also at statistically significantly higher risk. The variation found in birth weight over the past 10-year period was explained by changes in maternal prepregnancy weight, height, smoking habits, educational level, and caffeine intake over the same period. CONCLUSION: Risk factors associated with a higher proportion of high birth weight infants may be clinically significant and have an impact on public health. High birth weight increases the risk of adverse outcomes of delivery as well as the risk of childhood morbidity.

***Cigarette, alcohol, and caffeine consumption: risk factors for spontaneous abortion.**
Rasch V.
Acta Obstet Gynecol Scand. 2003 Feb;82(2):182-8.

OBJECTIVE: To study the association between cigarette, alcohol, and caffeine consumption and the occurrence of spontaneous abortion. METHODS: The study population consisted of 330 women with spontaneous abortion and 1168 pregnant women receiving antenatal care. A case-control design was utilized; cases were defined as women with a spontaneous abortion in gestational week 6-16 and controls as women with a live fetus in gestational week 6-16. The variables studied comprise age, parity, occupational situation, cigarette, alcohol, and caffeine consumption. The association between cigarette, alcohol, and caffeine consumption was studied using logistic

---

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

regression analyzes while controlling for confounding variables. In addition stratified analyzes of the association between caffeine consumption and spontaneous abortion on the basis of cigarette and alcohol consumption were performed. RESULTS: Women who had given birth twice or more previously had increased odds ratio (OR), 1.78 (1.27-2.49), whereas women who were students had decreased OR, 0.55 (0.34-0.91) for having spontaneous abortions. Regarding lifestyle factors, the adjusted ORs among women who consumed 5 units or more alcohol per week or 375 mg or more caffeine per day were 4.84 (2.87-8.16) and 2.21 (1.53-3.18), respectively. Women who smoked 10-19 cigarettes and 20 or more cigarettes per day did not have significantly increased ORs for having spontaneous abortions, after adjusting for other risk factors. CONCLUSION: Consumption of 5 or more units alcohol per week and 375 mg or more caffeine per day during pregnancy may increase the risk of spontaneous abortion.

***Association of maternal caffeine consumption with decrements in fetal growth.**
Bracken MB, Triche EW, Belanger K, Hellenbrand K, Leaderer BP.
Am J Epidemiol. 2003 Mar 1;157(5):456-66.

Whether caffeine consumption during pregnancy represents a fetal hazard remains uncertain. The authors report on a large prospective study designed to examine this question. In 1996-2000, 2,291 mothers with singleton livebirths in Connecticut and Massachusetts were evaluated after their first prenatal visit and were questioned about caffeine consumption and important confounding factors. Urine samples were provided to analyze urinary caffeine, cotinine, and creatinine levels. Mothers were followed throughout pregnancy to monitor changes in consumption. Pregnancy outcomes were obtained from medical records. Self-reports of caffeine consumption in the first and third trimesters were not associated with intrauterine growth retardation, low birth weight, or preterm delivery. For every 1 mg/g creatinine increase in urinary caffeine, risk of intrauterine growth retardation was essentially unchanged (odds ratio (OR) = 0.96, 95% confidence interval (CI): 0.85, 1.08). In contrast, a 0.005 mg/g creatinine increase in urinary cotinine significantly increased risk (OR = 1.003, 95% CI: 1.001, 1.005). Mean birth weight was reduced by reported caffeine consumption (-28 g per 100 mg of caffeine consumed daily, 95% CI: -0.10, -0.46, p = 0.001) but not mean gestational age. Decaffeinated coffee did not increase risk for any perinatal outcome. This small decrease in birth weight, observed for maternal caffeine consumption, is unlikely to be clinically important except for women consuming >/=600 mg of caffeine daily (approximately six 10-ounce (1 ounce = 28.3 g) cups of coffee).

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

***A prospective study of the effects of female and male caffeine consumption on the reproductive endpoints of IVF and gamete intra-Fallopian transfer.**
Klonoff-Cohen H, Bleha J, Lam-Kruglick P.
Hum Reprod. 2002 Jul;17(7):1746-54.

BACKGROUND: This study evaluated the timing and amount of caffeine intake by women and men undergoing IVF and gamete intra-Fallopian transfer (GIFT) on oocyte retrieval, sperm parameters, fertilization, multiple gestations, miscarriage, and live births. METHODS: A prospective study of 221 couples was conducted in Southern California between 1993 and 1998. "Usual" caffeine intake during lifetime and 1 year prior to attempt, caffeine intake during the week of the initial clinic visit, as well as intake during the week of the procedure, was evaluated from beverages (coffee, soda, tea) and chocolates. RESULTS: Not achieving a live birth was significantly associated with 10.7)] "usual" female caffeine consumption [adjusted odds ratios (95% confidence intervals): 3.1 (1.1, 9.7) and 3.9 (1.3, 11.6) for intake of >2-50 and 50 mg/day, compared with 0-2 mg/day] and consumption during the week of the initial visit [2.9 (1.1, 7.5) and 3.8 (1.4, female compared with 0-2 mg/day, although caffeine use was low. Infant gestational age decreased by 3.8 (-6.9, -0.7) or 3.5 (-6.7, -0.3) weeks for women who consumed >50 mg/day of caffeine "usually" or during the week of the initial visit. The odds of having multiple gestations increased by 2.2 (1.1, 4.4) and 3.0 (1.2, 7.4) for men who increased their "usual" intake or intake during the week of the initial visit by an extra 100 mg/day. Caffeine intake was not significantly associated with other outcomes. CONCLUSIONS: This is the first IVF/GIFT study to report any effect of caffeine on live births, gestational age, and multiple gestations. If these findings are replicated, caffeine use should be minimized prior to and while undergoing IVF/GIFT.

*** Maternal serum caffeine metabolites and small-for-gestational age birth.**
Klebanoff MA, Levine RJ, Clemens JD, Wilkins DG.
Am J Epidemiol. 2002 Jan 1;155(1):32-7.

To determine whether the third-trimester maternal serum concentration of paraxanthine, caffeine's primary metabolite, is associated with delivery of a small-for-gestational age infant (birth weight less than the 10th percentile for gestational age, gender, and ethnicity) and whether this association differs by smoking, the authors studied 2,515 women who participated in the Collaborative Perinatal Project from 1959 to 1966. The women provided a third-trimester serum sample and had been controls for a nested case-control study of spontaneous abortion. The mean serum paraxanthine concentration was greater in women who gave birth to small-for-gestational age infants (754 ng/ml) than to appropriately grown infants (653 ng/ml, p = 0.02). However, the linear trend for

---

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

increasing serum paraxanthine concentration to be associated with increasing risk of small-for-gestational age birth was confined to women who also smoked (p = 0.03). There was no association between paraxanthine and fetal growth in nonsmokers (p = 0.48). Adjustment for maternal age, pre-pregnant weight, education, parity, ethnicity, and the number of cigarettes smoked per day did not alter the results substantially, although the p value for trend among smokers increased to 0.07. The authors conclude that maternal third-trimester serum paraxanthine concentration, which reflects caffeine consumption, was associated with a higher risk of reduced fetal growth, particularly among women who smoked.

* **Caffeine metabolism and the risk of spontaneous abortion of normal karyotype fetuses.**
Signorello LB, Nordmark A, Granath F, Blot WJ, McLaughlin JK, Anneren G, Lundgren S, Ekbom A, Rane A, Cnattingius S.
Obstet Gynecol 2001 Dec;98(6):1059-66.

OBJECTIVE: To investigate whether the rate of caffeine metabolism influences spontaneous abortion risk. METHODS: We studied 101 women with normal karyotype spontaneous abortions and 953 pregnant women at 6-12 gestational weeks. Participants reported on caffeine intake and provided urine for phenotyping cytochrome P4501A2 (CYP1A2) activity and blood for genotyping N-acetylation (NAT2) status. We calculated odds ratios (OR) and 95% confidence intervals (CI) to evaluate the association between each of the two metabolic indices and spontaneous abortion risk and also the potential interaction between caffeine intake and metabolic activity on such risk. In calculating the associations between the metabolic indices and risk of spontaneous abortion, we had 80% power to detect an OR of 2.1, with a Type I error of 0.05. RESULTS: Slow acetylators had a nonsignificantly increased risk for spontaneous abortion (OR 1.36, 95% CI 0.84, 2.21) and recurrent spontaneous abortion (OR 2.51, 95% CI 0.81, 7.76). In contrast, low CYP1A2 activity was associated with a significantly decreased risk for spontaneous abortion (OR 0.35, 95% CI 0.20, 0.63). Caffeine was a risk factor for spontaneous abortion among women with high, but not low, CYP1A2 activity (OR 2.42, 95% CI 1.01, 5.80 for 100-299 mg/day; OR 3.17, 95% CI 1.22, 8.22 for 300 mg/day or more, among women with high CYP1A2 activity). CONCLUSION**:** The findings indicate that high CYP1A2 activity may increase the risk of spontaneous abortion, independently or by modifying the effect of caffeine. The results regarding NAT2 are less conclusive but suggest that slow acetylators may be at elevated risk of spontaneous abortion.

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

*** The associations of maternal caffeine consumption and nausea with spontaneous abortion.**
Wen W, Shu XO, Jacobs DR Jr, Brown JE.
Epidemiology. 2001 Jan;12(1):38-42.

To examine whether maternal caffeine consumption is associated with the risk of spontaneous abortion, we analyzed data from a population-based prospective study. The study population comprised 575 women delivering singleton livebirths and 75 women who had spontaneous abortions. The subjects were predominantly white, middle-class women enrolled before pregnancy. Study participants were traced to delivery of a liveborn, singleton infant or a spontaneous abortion. Of the 71 women who did not experience nausea, 29.6% had a spontaneous abortion, compared with 7.2% of 514 women who did experience nausea. Maternal caffeine consumption before pregnancy, or in women without nausea, did not increase the risk of spontaneous abortion, whereas maternal caffeine consumption during the first trimester after nausea started might increase risk of spontaneous abortion (risk ratio = 5.4, 95% confidence interval = 2.0-14.6 for caffeine consumption > or = 300 mg per day compared with < 20 mg per day). These results suggest that maternal caffeine consumption during pregnancy may influence fetal viability in women with nausea.

*** Effect of maternal smoking and coffee consumption on the risk of having a recognized Down syndrome pregnancy.**
Torfs CP, Christianson RE.
Am J Epidemiol. 2000 Dec 15;152(12):1185-91

To evaluate the possible effects of maternal smoking and caffeine or coffee consumption on the occurrence of a recognized pregnancy with Down syndrome, the authors analyzed data from a case-control study of 997 liveborn infants or fetuses with Down syndrome ascertained in California from 1991 to 1993 and 1,007 liveborn controls without a birth defect. Interviews with mothers covered demographic information, pregnancy, and medical history, with detailed questions on the use of tobacco, alcohol, and caffeinated beverages. All analyses were age-adjusted. High alcohol consumption (> or =4 drinks/week) in the first month of pregnancy was associated with reduced risk for a recognized Down syndrome conceptus (odds ratio (OR) = 0.54; 95% confidence interval (CI): 0.34, 0.85). Maternal smoking during the periconceptional period was not associated with risk of recognized Down syndrome (OR = 1.04; 95% CI: 0.79, 1.37), but maternal consumption of four or more cups of coffee per day was inversely associated (OR = 0.63; 95% CI: 0.41, 0.96). In multivariate analysis, a significant interaction between coffee drinking and smoking was observed. The inverse association remained

---

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

only for nonsmoking mothers who drank four or more cups of coffee per day (OR = 0.48; 95% CI: 0.28, 0.82). These results suggest that among nonsmoking mothers, high coffee consumption is more likely to reduce the viability of a Down syndrome conceptus than that of a normal conceptus.

*** Caffeine intake and the risk of first-trimester spontaneous abortion.**
Cnattingius S, Signorello LB, Anneren G, Clausson B, Ekbom A, Ljunger E, Blot WJ, McLaughlin JK, Petersson G, Rane A, Granath F.
N Engl J Med. 2000 Dec 21;343(25):1839-45.

BACKGROUND: Some epidemiologic studies have suggested that the ingestion of caffeine increases the risk of spontaneous abortion, but the results have been inconsistent. METHODS: We performed a population-based, case-control study of early spontaneous abortion in Uppsala County, Sweden. The subjects were 562 women who had spontaneous abortion at 6 to 12 completed weeks of gestation (the case patients) and 953 women who did not have spontaneous abortion and were matched to the case patients according to the week of gestation (controls). Information on the ingestion of caffeine was obtained from in-person interviews. Plasma cotinine was measured as an indicator of cigarette smoking, and fetal karyotypes were determined from tissue samples. Multivariate analysis was used to estimate the relative risks associated with caffeine ingestion after adjustment for smoking and symptoms of pregnancy such as nausea, vomiting, and tiredness. RESULTS: Among nonsmokers, more spontaneous abortions occurred in women who ingested at least 100 mg of caffeine per day than in women who ingested less than 100 mg per day, with the increase in risk related to the amount ingested (100 to 299 mg per day: odds ratio, 1.3; 95 percent confidence interval, 0.9 to 1.8; 300 to 499 mg per day: odds ratio, 1.4; 95 percent confidence interval, 0.9 to 2.0; and 500 mg or more per day: odds ratio, 2.2; 95 percent confidence interval, 1.3 to 3.8). Among smokers, caffeine ingestion was not associated with an excess risk of spontaneous abortion. When the analyses were stratified according to the results of karyotyping, the ingestion of moderate or high levels of caffeine was found to be associated with an excess risk of spontaneous abortion when the fetus had a normal or unknown karyotype but not when the fetal karyotype was abnormal. CONCLUSIONS: The ingestion of caffeine may increase the risk of an early spontaneous abortion among non-smoking women carrying fetuses with normal karyotypes.

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

*** Maternal serum paraxanthine, a caffeine metabolite, and the risk of spontaneous abortion.**
Klebanoff MA, Levine RJ, DerSimonian R, Clemens JD, Wilkins DG.
N Engl J Med. 1999 Nov 25;341(22):1639-44.

BACKGROUND: Whether the consumption of caffeine during pregnancy increases the risk of spontaneous abortion is controversial. Prior studies have determined caffeine consumption by questionnaire. We used a biologic marker, such as serum paraxanthine, a metabolite of caffeine, to measure the dose of caffeine. METHODS: In a nested case-control study, we measured serum paraxanthine in 591 women who had spontaneous abortions at less than 140 days' gestation and in 2558 matched women from the same clinic who gave birth to live infants at 28 weeks' gestation or later and who had serum drawn on the same day of gestation as the women who had abortions. The women were enrolled in the Collaborative Perinatal Project during the period from 1959 to 1966, and serum paraxanthine was measured over 30 years later. RESULTS: A total of 487 women who had spontaneous abortions (82 percent) and 2087 controls (82 percent) had quantifiable serum paraxanthine concentrations. However, the mean serum paraxanthine concentration was higher in the women who had spontaneous abortions than in the controls (752 vs. 583 ng per milliliter, $P<0.001$). The odds ratio for spontaneous abortion was not significantly elevated in the women who had serum paraxanthine concentrations of 1845 ng per milliliter or lower, corresponding to the 95th percentile of the matched women. However, the adjusted odds ratio for spontaneous abortion among women with serum paraxanthine concentrations higher than 1845 ng per milliliter, as compared with women who had concentrations below 50 ng per milliliter, was 1.9 (95 percent confidence interval, 1.2 to 2.8). CONCLUSIONS: Only extremely high serum paraxanthine concentrations are associated with spontaneous abortion. This suggests that moderate consumption of caffeine is unlikely to increase the risk of spontaneous abortion.

*** Associations between maternal decaffeinated and caffeinated coffee consumption and fetal growth and gestational duration.**
Eskenazi B, Stapleton AL, Kharrazi M, Chee WY.
Epidemiology. 1999 May;10(3):242-9.

Because of concern about the potential adverse effects of consuming caffeinated beverages during pregnancy, pregnant women may choose to replace caffeinated with decaffeinated coffee. In a population-based study of 7,855 livebirths in California's San Joaquin Valley, we examined the relation of maternal decaffeinated and caffeinated coffee consumption during pregnancy to measures of fetal growth and gestational

---

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

duration. Mothers answered a questionnaire in the hospital at the time of completing the birth certificate. Compared with women who drank neither decaffeinated nor caffeinated coffee, those who consumed only decaffeinated coffee showed no increased odds of small-for-gestational age birth, low birth weight, or preterm delivery, nor lowered mean birth weight or shortened mean gestational age. Women who consumed caffeinated coffee alone had an adjusted odds ratio of 1.3 [95% confidence limits (CL) = 1.0, 1.7] for preterm delivery, whereas those who consumed both caffeinated and decaffeinated coffee had an adjusted odds of 2.3 (95% CL = 1.3, 4.0). When caffeinated and decaffeinated coffee were considered as continuous variables, we found a reduction in adjusted mean birth weight of -3.0 gm per cup per week (95% CL = -5.9, -0.6) for caffeinated coffee and an increase of +0.4 gm per cup per week (95% CL = -3.7, 4.5) for decaffeinated coffee.

***Caffeine intake and pregnancy outcomes: a meta-analytic review.**
Santos IS, Victora CG, Huttly S, Morris S.
Cad Saude Publica. 1998 Jul-Sep;14(3):523-30.

Epidemiological publications on the relationship of caffeine to birth weight and duration of human pregnancy, from 1966 to 1995, were searched through Medline. Each study was treated as the stratification variable, and its weight average was proportional to the inverse of its variance. Twenty-six studies were located. Among the twenty-two studies on birth weight, eleven were on mean birth weight, nine on low birth weight (LBW), and four on intrauterine growth retardation (IUGR). Combined analysis of mean birth weight study results showed a significant decrease in birth weight of nearly 43g among newborns of the heaviest caffeine-consuming mothers. LBW, IUGR, and preterm delivery displayed significant homogeneity in the test results, indicating that a pooled estimate should not be taken as na adequate measure. The high heterogeneity of the available literature on the effects of caffeine on LBW, IUGR, and preterm delivery prevents estimation of reliable pooled estimates through meta-analysis. Further assessment of caffeine intake during pregnancy is needed in future research.

*** Moderate to heavy caffeine consumption during pregnancy and relationship to spontaneous abortion and abnormal fetal growth: a meta-analysis.**
Fernandes O, Sabharwal M, Smiley T, Pastuszak A, Koren G, Einarson T.
Reprod Toxicol. 1998 Jul-Aug;12(4):435-44

The objective was to determine the association of moderate to heavy caffeine consumption during pregnancy on spontaneous abortion and birth weight in humans. Data sources used included a computerized literature search of MEDLINE

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

(1966-July 1996); EMBASE (1988-November 1996); Psychlit I (1974-1986); Psychlit II (1987-1996); CINAHL (1982-May 1996) and manual search of bibliographies of pertinent articles. Inclusion criteria were: English language research articles; pregnant human females; case control or cohort design; documented quantity of caffeine consumption during pregnancy; control group with minimal or no caffeine consumption (0 to 150 mg caffeine/d); documented data regarding spontaneous abortion and/or fetal growth. The exclusion criteria were: case reports; editorials; review papers. The methods section of each study was examined independently by two blinded investigators with a third investigator adjudicating disagreements. Two independent investigators extracted data onto a standardized form. A third investigator adjudicated discrepancies. We compared a caffeine-exposed group (>150 mg/d) and controls (0 to 150 mg/d), using Mantel-Haenszel pooling. Of the 32 studies meeting inclusion criteria, 12 had extractable data (6 for spontaneous abortion, 7 for low birth weight, 1 common study). Mantel-Haenszel odds ratio (CI95%) was 1.36 (1.29-1.45) for spontaneous abortion in 42,988 pregnancies. The overall risk ratio was 1.51 (1.39-1.63) for low birthweight (<2500 g) in 64,268 pregnancies. Control for confounders such as maternal age, smoking, and ethanol use was not possible. We concluded that there is a small but statistically significant increase in the risks for spontaneous abortion and low birthweight babies in pregnant women consuming >150 mg caffeine per d. A possible contribution to these results of maternal age, smoking, ethanol use, or other confounders could not be excluded.

*** Coffee consumption and risk of hospitalized miscarriage before 12 weeks of gestation.**

Parazzini F, Chatenoud L, Di Cintio E, Mezzopane R, Surace M, Zanconato G, Fedele L, Benzi G.

Hum Reprod. 1998 Aug;13(8):2286-91.

In order to analyse the association between drinking coffee in pregnancy and risk of spontaneous abortion, a case-controlled study was conducted in Milan, Northern Italy. Cases were 782 women with spontaneous abortion within the 12th week of gestation. The control group was recruited from women who gave birth at term (> 37 weeks gestation) to healthy infants on randomly selected days at the same hospitals where cases had been identified: 1543 controls were interviewed. A total of 561 (72%) cases of spontaneous abortion and 877 (57%) controls reported coffee drinking during the first trimester of the index pregnancy. The corresponding multivariate odds ratios of spontaneous abortion, in comparison with non-drinkers, were 1.2, 1.8 and 4.0, respectively, for drinkers of 1, 2 or 3, and 4 or more cups of coffee per day. No relationship emerged between maternal decaffeinated coffee, tea and cola drinking in pregnancy, as well as paternal coffee consumption, and risk of spontaneous abortion. With regard to duration in years of coffee drinking, the estimated multivariate odds ratios of spontaneous abortion were, in

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

comparison with non-coffee drinkers, 1.1 (95% confidence interval (CI) 0.9-1.4) and 1.9 (95% CI 1.5-2.6) for women reporting a duration of coffee consumption < or = 10 or > 10 years. In conclusion, coffee drinking early in pregnancy was associated with an increased risk of abortion. This has biological implications, but epidemiological inference on the causality is difficult and still open to debate.

*** Caffeine intake and fecundability: a follow-up study among 430 Danish couples planning their first pregnancy.**
Jensen TK, Henriksen TB, Hjollund NH, Scheike T, Kolstad H, Giwercman A, Ernst E, Bonde JP, Skakkebaek NE, Olsen J.
Reprod Toxicol. 1998 May-Jun;12(3):289-95.

Fecundability has been defined as the ability to achieve a recognized pregnancy. Several studies on caffeine and fecundability have been conducted but have been inconclusive. This may be explained partly by lack of stratification by smoking. Furthermore, few researchers have tried to separate the effect of caffeine from different sources (coffee, tea, cola, and chocolate). Clearly, the relationship between caffeine and fecundability needs further research, given the high prevalence of caffeine intake among women of childbearing age. We examined the independent and combined effects of smoking and caffeine intake from different sources on the probability of conception. From 1992 to 1995, a total of 430 couples were recruited after a nationwide mailing of a personal letter to 52,255 trade union members who were 20 to 35 years old, lived with a partner, and had no previous reproductive experience. At enrollment and in six cycles of follow-up, both partners filled out a questionnaire on different factors including smoking habits and their intake of coffee, tea, chocolate, cola beverages, and chocolate bars. In all, 1596 cycles and 423 couples were included in the analyses. The cycle-specific association between caffeine intake and fecundability was analyzed in a logistic regression model with the outcome at each cycle (pregnant or not pregnant) in a Cox discrete model calculating the fecundability odds-ratio (FR). Compared to nonsmoking women with caffeine intake less than 300 mg/d, nonsmoking women who consumed 300 to 700 mg/d caffeine had a FR of 0.88 [95% confidence interval (CI) 0.60-1.31], whereas women with a higher caffeine intake had a FR = 0.63 (95% CI 0.25-1.60) after adjusting for female body mass index and alcohol intake, diseases of the female reproductive organs, semen quality, and duration of menstrual cycle. No dose-response relationship was found among smokers. Among males, the same decline in point estimates of the FR was present. Smoking women whose only source of caffeine was coffee (>300 mg/d) had a reduced fecundability odds-ratio (FR = 0.34; 95% CI 0.12-0.98). An interaction between caffeine and smoking is biologically plausible, and the lack of effect among smokers may be due to faster metabolism of caffeine. Our findings suggest that especially nonsmoking women who wish to achieve a pregnancy might benefit from a reduced caffeine intake.

---

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

PIP: The independent and combined effects of smoking and caffeine intake from different sources on fecundability were assessed in a national survey of 423 Danish couples. Couples were recruited to the study in 1992-95 through a mailing to 52,255 female trade union members seeking women who were 20-35 years old, lived with a partner, had no previous pregnancies, and intended to discontinue contraception in order to become pregnant. A total of 1596 cycles were included in the 6-month study and the cycle-specific association between caffeine intake and fecundability was analyzed in a logistic regression model with the outcome (pregnant, not pregnant) in a Cox discrete model. Compared with nonsmoking women with a caffeine intake less than 300 mg/day, nonsmoking women who consumed 300-700 mg/day of caffeine had a fecundability odds ratio (FR) of 0.88 (95% confidence interval (CI), 0.60-1.31), while those with a higher consumption had an FR of 0.63 (95% CI, 0.25-1.60), after adjustments for body mass index, alcohol intake, diseases of the female reproductive organs, semen quality, and duration of the menstrual cycle. No such dose-response relationship was detected among smokers. The same decline in point estimates of the FR was present was males. Smoking women whose only source of caffeine was coffee (over 300 mg/day) had a reduced FR (0.34; 95% CI, 0.12-0.98). The lack of adverse effect among smokers may be due to faster metabolism and clearance of caffeine. Overall, these findings indicate that nonsmoking women who wish to achieve pregnancy should consider reducing their caffeine intake.

*** Heavy caffeine intake in pregnancy and sudden infant death syndrome. New ZealandCot Death Study Group.**

Ford RP, Schluter PJ, Mitchell EA, Taylor BJ, Scragg R, Stewart AW.
Arch Dis Child. 1998 Jan;78(1):9-13.
Comment in:
Arch Dis Child Fetal Neonatal Ed. 1999 Mar;80(2):F159-60.
Arch Dis Child. 1998 Sep;79(3):291.

AIMS: To examine the association between maternal caffeine consumption during pregnancy and the risk of sudden infant death syndrome (SIDS). METHODS: A nationwide case-control study surveying parents of 393 SIDS victims and parents of 1592 control infants. Caffeine consumption in each of the first and third trimesters was estimated by questionnaire. Heavy caffeine intake was defined as 400 mg/day or more (equivalent to four or more cups of coffee per day). RESULTS: Infants whose mothers had heavy caffeine consumption throughout their pregnancy had a significantly increased risk for SIDS (odds ratio 1.65; 95% confidence interval 1.15 to 2.35) after adjusting for likely confounding factors. CONCLUSION: Caffeine intake has been associated with fetal harm and now SIDS. Reducing heavy caffeine intake during pregnancy could be another way to lessen the risk of SIDS. This needs confirmation by others.

---

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

*** Use of fluorescence in situ hybridization (FISH) to assess effects of smoking, caffeine, and alcohol on aneuploidy load in sperm of healthy men.**
Robbins WA, Vine MF, Truong KY, Everson RB.
Environ Mol Mutagen. 1997;30(2):175-83.

Aneuploidy is a common cause of poor reproductive outcomes in humans and is associated with severe medical problems in liveborn offspring, yet little is known about its underlying cause. A substantial amount of aneuploidy is known to be contributed by the father through cytogenetically abnormal sperm. The purpose of this cross-sectional, observational study was to investigate the potential contribution of common lifestyle exposures (smoking, caffeine, and alcohol) to the aneuploidy load in sperm from 45 healthy male volunteers 19-35 years of age. Sperm FISH (fluorescence in situ hybridization) was used to determine aneuploidy and diploidy frequencies for chromosomes X, Y and 18 across varying exposure levels of smoking, caffeine, and alcohol. Caffeine was significantly associated with increased frequencies of sperm aneuploidy XX18 and XY18, diploidy XY18-18 and the duplication phenotype YY18-18 controlling for alcohol, smoking and donor age. Alcohol was significantly associated with increased frequencies of sperm aneuploidy XX18, diploidy XY18-18 and the duplication phenotype XX18-18 controlling for caffeine, smoking and donor age. There was a suggestive, but unstable, association between smoking and XX18. Even within our truncated age range, we were able to confirm an increased risk for XX18 aneuploidy with increasing donor age. Sperm FISH proved to be a useful biomarker to detect and compare numerical cytogenetic abnormalities in human sperm cells across differing levels of exposure to smoking, caffeine, and alcohol.

*** Caffeine intake and delayed conception: a European multicenter study on infertility and subfecundity. European Study Group on Infertility Subfecundity.**
Bolumar F, Olsen J, Rebagliato M, Bisanti L.
Am J Epidemiol. 1997 Feb 15;145(4):324-34.

The effects of caffeine consumption on delayed conception were evaluated in a European multicenter study on risk factors of infertility. Information was collected retrospectively on time of unprotected intercourse for the first pregnancy and the most recent waiting time episode in a randomly selected sample of 3,187 women aged 25-44 years from five European countries (Denmark, Germany, Italy, Poland, and Spain) between August 1991 and February 1993. The consumption of caffeinated beverages at the beginning of the waiting time was used to estimate daily caffeine intake, which was categorized as 0-100, 101-300, 301-500, and > or = 501 mg. Risk of subfecundity (> or = 9.5 months) and the

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

fecundability ratio, respectively, were assessed by logistic regression and Cox proportional hazard analyses, adjusting for age, parity, smoking, alcohol consumption, frequency of intercourse, educational level, working status, use of oral contraceptives, and country. A significantly increased odds ratio (OR) of 1.45 (95% confidence interval (CI) 1.03-2.04) for subfecundity in the first pregnancy was observed for women drinking more than 500 mg of caffeine per day, the effect being relatively stronger in smokers (OR = 1.56, 95% CI 0.92-2.63) than in nonsmokers (OR = 1.38, 95% CI 0.85-2.23). Women in the highest level of consumption had an increase in the time leading to the first pregnancy of 11% (hazard ratio = 0.90, 95% CI 0.78-1.03). These associations were observed consistently in all countries as well as for the most recent waiting time episode. The authors conclude that high levels of caffeine intake may delay conception among fertile women.

Effect of caffeine intake during pregnancy on birth weight.
Vlajinac HD, Petrovic RR, Marinkovic JM, Sipetic SB, Adanja BJ.
Am J Epidemiol. 1997 Feb 15;145(4):335-8.

The aim of this study was to examine the effect of caffeine consumption during pregnancy on birth weight and its possible interaction with smoking. The sample included 1,011 women who were interviewed during their first 3 days after delivery in one of the hospitals of Belgrade, Yugoslavia. A significant reduction in birth weight was found to be associated with an average caffeine intake of > or = 71 mg per day, after adjustment for gestational age, infant sex, parity, and maternal height and weight, but only in infants born to nonsmoking mothers.

***Coffee consumption and intrauterine growth retardation in Brazil.**
Rondo PH, Rodrigues LC, Tomkins AM.
Eur J Clin Nutr. 1996 Nov;50(11):705-9.

OBJECTIVE: To examine the association between coffee consumption in pregnancy and foetal growth. DESIGN: Retrospective unmatched case-control study. SETTING: Maternidade de Campinas, Universidade Estadual de Campinas, Pontificia Universidade Catolica de Campinas, Hospital Albert Sabin. SUBJECTS: 356 mother/baby pairs who had interauterine growth retardation (IUGR) and 356 mother/baby pairs who were appropriate for gestational age (AGA). INTERVENTIONS: Newborns were classified as being IUGR according to the Lubchenco classification. Gestational age of the newborns was evaluated by the Capurro method. Coffee consumption in pregnancy was assessed by a food frequency questionnaire. Coffee consumption and a range of risk factors for IUGR were stratified and entered into a logistic regression model. The final results were

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

expressed by the attributable risk percent (AR%). RESULTS: More IUGR mothers (85.4%) than AGA mothers (70.5%) ingested coffee in pregnancy (OR = 2.45; P < 0.001). The proportion of mothers who delivered IUGR babies increased as the average consumption of coffee increased (test for trend = 31.76; P < 0.001). The tendency for heavy coffee drinkers to deliver IUGR babies remained after controlling for alcohol intake and cigarette smoking (P < 0.001). According to the logistic regression model and to the attributable risk percent (AR% = 28.0%), coffee consumption, (independent of average coffee consumption) was an important preventable cause of IUGR in this Brazilian population. CONCLUSIONS: We recommend moderation in the consumption of coffee in pregnancy, since intrauterine growth retardation increases the risk of perinatal and neonatal morbidity and mortality. Further large prospective studies evaluating the content of caffeine in the coffee consumed by this population is advised.

*** Effects of caffeine consumption on delayed conception.**
Stanton CK, Gray RH.
Am J Epidemiol. 1995 Dec 15;142(12):1322-9.
Comment in:
Am J Epidemiol. 1996 Oct 15;144(8):799-800; author reply 801.
Am J Epidemiol. 1996 Oct 15;144(8):799; author reply 801.

The authors examined the effects of caffeine consumption on waiting time to conception in the Reproductive Health Study, a retrospective study of 1,430 non-contracepting, parous women interviewed between July 1989 and June 1990 at Fishkill, New York, and Burlington, Vermont. Information was obtained on 2,501 pregnancies since 1980. Women's reported consumption of caffeinated beverages during the first month of pregnancy was used to estimate daily caffeine intake, which was categorized as none, 1-150, 151-300, and > or = 301 mg. Information on delayed conception was analyzed as a dichotomous variable (< or = 12 months delay vs. > 12 months delay), and the per cycle probability of conception (fecundability) was estimated using waiting time to conception as a continuous variable. Odds ratios of delayed conception and fecundability ratios adjusted for age, parity, smoking, last contraceptive used, infertility history, and race, were estimated by logistic regression and Cox proportional hazard models, respectively. Women who did not smoke and who consumed no caffeine were used as a reference group. The adjusted odds ratio of delayed conception for more than one year was not increased among women who consumed < or = 300 mg of caffeine daily. However, the odds ratio (OR) was 2.65 (95% confidence interval (CI) 1.38-5.07) among nonsmokers who consumed > or = 301 mg of caffeine daily. Although smoking per se was associated with a significant increased risk of delayed conception (OR = 1.77, 95% CI 1.33-2.37), no effect of high caffeine consumption was observed among women who smoked.

---

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

Fecundability was reduced among nonsmokers who consumed more than 300 mg caffeine daily (fecundability ratio = 0.74, 95% CI 0.59-0.92). Smoking reduced the fecundability ratio, but the authors observed no effect of caffeine consumption on fecundability among women who smoked. Other studies provide biologic plausibility for these findings. The authors conclude that high levels of caffeine consumption may result in delayed conception among women who do not smoke cigarettes.

B. Meeting abstracts reporting increased risk of adverse developmental or reproductive outcomes

Coffee Consumption And Cigarette Smoking During Pregnancy And Behavioural Problems In Offspring: Prospective Study Within A Danish Birth Cohort.
Ye G; Obel C; Bech B; Olsen J
J Epidemiol Community Health 2004 Aug;58(Suppl 1):A61

Objective: To study if coffee drinking and cigarette smoking during pregnancy are associated with behavioural problems during childhood. Methods: This was a follow up study carried out in Aalborg and Odense, Denmark, from 1984 to 1987 on 10916 singleton pregnant women recruited to the Healthy Habits for Two Study. In 2002, 7687 mothers participated in a follow up study and provided information on their singleton children's behavioural problems during their entire childhood. Outcome measures were the Strengths and Difficulties Questionnaire (SDQ-Dan) version for parents. Results: High coffee consumption (>=8 cups/day) during tpregnancy was associated with risk for increased SDQ scores (odds ratios, OR 2.21, 95% CI 1.67 to 2.91). Children whose mother drank eight or more cups of coffee per day during pregnancy had an increased risk for behavioural problems compared with women who did not drink coffee. After adjustment for habit changing of coffee and smoking, alcohol intake, fish intake, maternal age, marital status, education and employment, there was a slight increase in odds ratios (OR 1.43, 95%, CI 1.04 to 1.95). When we included the effects of cigarette smoking, the risk ratio rose to 4.92 (95% CI 2.38 to 10.16) among the group who had a high level of coffee consumption and smoked more than 20 cigarettes per day. Conclusion: Both high coffee consumption (>8 cups/day) and smoking during pregnancy are associated with increased risk of behavioural problems in childhood. Meanwhile, caffeine intake slightly affects the probability of behavioural disorders, but may enhance the negative effect of cigarettes.

C. Studies reporting no increased risk of adverse developmental or reproductive outcomes

Effect of reducing caffeine intake on birth weight and length of gestation: randomised controlled trial.
Bech BH, Obel C, Henriksen TB, Olsen J.
Bmj. 2007 Feb;334:409.

OBJECTIVE: To estimate the effect of reducing caffeine intake during pregnancy on birth weight and length of gestation. DESIGN: Randomised double blind controlled trial. SETTING: Denmark. PARTICIPANTS: 1207 pregnant women drinking at least three cups of coffee a day, recruited before 20 weeks' gestation. INTERVENTIONS: Caffeinated instant coffee (568 women) or decaffeinated instant coffee (629 women). MAIN OUTCOME MEASURES: Birth weight and length of gestation. RESULTS: Data on birth weight were obtained for 1150 liveborn singletons and on length of gestation for 1153 liveborn singletons. No significant differences were found for mean birth weight or mean length of gestation between women in the decaffeinated coffee group (whose mean caffeine intake was 182 mg lower than that of the other group) and women in the caffeinated coffee group. After adjustment for length of gestation, parity, prepregnancy body mass index, and smoking at entry to the study the mean birth weight of babies born to women in the decaffeinated group was 16 g (95% confidence interval -40 to 73) higher than those born to women in the caffeinated group. The adjusted difference (decaffeinated group-caffeinated group) of length of gestation was -1.31 days (-2.87 to 0.25). CONCLUSION: A moderate reduction in caffeine intake in the second half of pregnancy has no effect on birth weight or length of gestation. TRIAL REGISTRATION: Clinical Trials NCT00131690 [ClinicalTrials.gov].

Risk factors for first trimester miscarriage--results from a UK-population-based case-control study.
Maconochie N, Doyle P, Prior S, Simmons R.
Bjog. 2007 Feb;114:170-86.

OBJECTIVE: The aim of this study was to examine the association between biological, behavioural and lifestyle risk factors and risk of miscarriage. DESIGN: Population-based case-control study. SETTING: Case-control study nested within a population-based, two-stage postal survey of reproductive histories of women randomly sampled from the UK electoral register. POPULATION: Six hundred and three women aged 18-55 years whose most recent pregnancy had ended in first trimester miscarriage (< 13 weeks of gestation; cases) and 6116 women aged 18-55 years whose most recent pregnancy had progressed beyond 12 weeks (controls). METHODS: Women were questioned about socio-demographic, behavioural and other factors in their most recent pregnancy. MAIN OUTCOME MEASURE: First trimester miscarriage. RESULTS: After adjustment for confounding, the following were independently associated with increased risk: high

maternal age; previous miscarriage, termination and infertility; assisted conception; low pre-pregnancy body mass index; regular or high alcohol consumption; feeling stressed (including trend with number of stressful or traumatic events); high paternal age and changing partner. Previous live birth, nausea, vitamin supplementation and eating fresh fruits and vegetables daily were associated with reduced risk, as were feeling well enough to fly or to have sex. After adjustment for nausea, we did not confirm an association with caffeine consumption, smoking or moderate or occasional alcohol consumption; nor did we find an association with educational level, socio-economic circumstances or working during pregnancy. CONCLUSIONS: The results confirm that advice to encourage a healthy diet, reduce stress and promote emotional wellbeing might help women in early pregnancy (or planning a pregnancy) reduce their risk of miscarriage. Findings of increased risk associated with previous termination, stress, change of partner and low pre-pregnancy weight are noteworthy, and we recommend further work to confirm these findings in other study populations.

Coffee drinking and risk of preterm birth.
Chiaffarino F, Parazzini F, Chatenoud L, Ricci E, Tozzi L, Chiantera V, Maffioletti C, Fedele L.
Eur J Clin Nutr. 2006 May;60:610-3.

OBJECTIVES: We analysed the association between coffee drinking before and during the three trimesters of pregnancy and the risk of preterm birth of babies normal for gestational age (NGA) or small for gestational age (SGA). METHODS: Case-control study conducted in University clinics of North Italy. Cases were 502 women who delivered at < 37 weeks of gestation. The controls included 1966 women who gave birth at term (>or=37 weeks of gestation) to healthy infants on randomly selected days at the hospitals where cases had been identified. RESULTS: There was inverse association for coffee consumption in the third trimester of pregnancy in SGA cases compared to NGA (heterogeneity test between OR: chi1(2)=5.6811 P < 0.05). In comparison with not drinkers, all the ORs of overall intake of caffeine were closed near the unity for both SGA and NGA preterm birth. CONCLUSION: Compared with no consumption, a low consumption of coffee during pregnancy may not have significant effects on preterm birth.

[Caffeine intake and food sources of caffeine and prematurity: a case-control study].
de Souza RA, Sichieri R.
Cad Saude Publica. 2005 Nov-Dec;21(6):1919-28.

Caffeine (1,3,7-trimethylxanthine) is an alkali that easily crosses the placental barrier and can interfere in the growth and development of fetal cells and compromise fetal oxygenation. Considering the widespread consumption of foods containing caffeine in Brazil, the aim of this study was to evaluate the association between total caffeine

consumption (including its food sources) and prematurity. A case-control study of 140 cases (newborns with gestational age less than 37 weeks) and 162 controls (newborns with 37 weeks gestational age or greater) evaluated caffeine consumption during pregnancy. Intake measurement used a semi-quantitative food frequency questionnaire based on the following foods: coffee, tea, and powdered chocolate. Total caffeine consumption (including food sources) during pregnancy was not associated with prematurity, and most intakes were less than 300 mg/ day. Caffeine consumption in the present study does not support guidelines against caffeine consumption by Brazilian pregnant women.

Hypospadias and Potentially Estrogenic Exposures.
Carmichael SL, Shaw GM, Laurent C, Olney RS, Lammer EJ.
Birth Defects Res A Clin Mol Teratol. 2005 May;73(5):306.

Introduction. Estrogenic exposures may impact risk of hypospadias by interfering with the production or action of fetal androgens, which are critical to normal closure of the urethra. This study examines the association of hypospadias with exposures that may reflect maternal estrogen production or action during pregnancy. Methods. This study uses data from the National Birth Defects Prevention Study, a multi-state, population-based case-control study including data on severe hypospadias in infants with estimated dates of delivery from 1997-2000. Non-malformed, liveborn controls were selected randomly from birth certificates or birth hospitals. Data from interviews, which were completed by phone within 24 months after delivery, were available for 502 case and 1286 control mothers. Results. Bivariate logistic regression analyses suggest that nausea and vomiting of pregnancy (NVP) was associated with reduced hypospadias risk; e.g., the odds ratio for mild NVP during the second month of pregnancy was 0.6 (95% CI 0.4-1.0). Nulliparity and age 30 years were each associated with approximately two-fold increased risks. An overweight maternal body mass index (BMI) was associated with slightly increased risk (OR 1.3, 95% CI 1.0-1.9). Intake of caffeine and alcohol were not associated with hypospadias risk. Discussion. Bivariate results suggest that maternal indicators of exposure to estrogenic hormones may be associated with hypospadias risk. Potential confounding or effect modification among these factors, or by other potential covariates such as infertility, race-ethnicity and education, will be explored to improve our understanding of these preliminary findings.

Maternal coffee drinking in pregnancy and risk of small for gestational age birth.
Parazzini F, Chiaffarino F, Chatenoud L, Tozzi L, Cipriani S, Chiantera V, Fedele L.
Eur J Clin Nutr 2005 Feb;59(2):299-301.

OBJECTIVE: We have analysed the association between coffee drinking before and during the three trimesters of pregnancy and risk of small for gestational age (SGA) birth. METHODS: Cases were 555 women who delivered SGA births (ie <10th percentile

according Italian standard). The controls included 1966 women who gave birth at term (>/=37 weeks of gestation) to healthy infants of normal weight. RESULTS: In comparison with nondrinkers, the ORs for SGA birth were 1.3 (95% confidence interval, CI, 0.9-1.9) for consumption of four or more cups of coffee/day before pregnancy, and 1.2 (95% CI 0.8-1.8), 1.2 (95% CI 0.8-1.8) and 0.9 (95% CI 0.6-1.4) for consumption of three or more cups of coffee/day during the first, second and third trimester of pregnancy, respectively. CONCLUSION: These findings were consistent in women who delivered preterm and at term births and were not affected by potential confounding such as smoking.

[Birthweight and caffeine consumption] [Article in Portuguese]
Bicalho GG, Barros Filho Ade A.
Rev Saude Publica. 2002 Apr;36(2):180-7.

OBJECTIVES: To assess the association between maternal caffeine consumption during pregnancy and low birth weight, prematurity and intrauterine growth retardation. METHODS: A case-control was carried out and 354 newborns of single labor with birthweight <2,500 g (cases) and 354 with birthweight >3,000 g (controls) were analyzed. Caffeine consumption was calculated based on daily consumption of coffee, soft drinks and tea. Results were adjusted using multiple logistic regression for the following confounders: mother's age, schooling, income, marital status, skin color, parity, smoking, previous low birthweight children, mother's pre-pregnancy weight, employment status, interval between pregnancies, prenatal care and high blood pressure. RESULTS: For caffeine consumption <300 mg/day and >300 mg/day, the adjusted odds ratios for low birthweight were: 0.72 (95%IC=0.45-1.25) and 0.47 (95%IC=0.24-0.92); prematurity: 0.59 (95%IC=0.32-1.09) and 0.32 (95%IC=0.15-0.72); and intrauterine growth retardation: 1.16 (95%IC=0.45-3.01) and 0.64 (95%IC=0.20-1.98), respectively. CONCLUSION: There was no association between caffeine consumption during pregnancy and low birthweight, prematurity and intrauterine growth retardation.

Effect of caffeine exposure during pregnancy on birth weight and gestational age.
Clausson B, Granath F, Ekbom A, Lundgren S, Nordmark A, Signorello LB, Cnattingius S.
Am J Epidemiol. 2002 Mar 1;155(5):429-36.

Epidemiologic studies have been unable to conclusively evaluate whether caffeine intake during pregnancy is associated with reduced birth weight and/or fetal growth restriction. The authors conducted a prospective, population-based cohort study to investigate the effect of caffeine on birth weight, gestational age, and birth weight standardized for gestational age (birth weight ratio). Of 953 women recruited in early pregnancy in Uppsala County, Sweden, from 1996 to 1998, 873 women delivering liveborn singleton

infants were included in the analysis. Caffeine exposures were ascertained from in-person interviews at 6-12 and 32-34 completed gestational weeks, and maternal plasma was analyzed for cotinine levels as an indicator of smoking. Analysis of variance was used to estimate the effect of caffeine on birth weight, gestational age at delivery, and birth weight ratio after accounting for the effects of other covariates, such as maternal sociodemographic characteristics, plasma cotinine, and pregnancy symptoms. There were no associations between caffeine consumption and birth weight, gestational age, and birth weight ratio, neither when caffeine exposure was averaged from conception to the 32nd to 34th gestational weeks, nor when caffeine exposure was stratified by trimesters of pregnancy. These results do not support an association between moderate caffeine consumption and reduced birth weight, gestational age, or fetal growth.

Maternal caffeine intake and intrauterine growth retardation.
Grosso LM, Rosenberg KD, Belanger K, Saftlas AF, Leaderer B, Bracken MB.
Epidemiology. 2001 Jul;12(4):447-55.
Erratum in:
Epidemiology 2001 Sep;12(5):517.

This study estimates the effect of maternal caffeine consumption throughout pregnancy on fetal growth. We studied 2,714 women who delivered a liveborn infant between 1988 and 1991. Detailed information regarding coffee, tea, and soda drinking during the first and third trimesters of pregnancy was obtained. Average caffeine intake during month 1 of pregnancy was higher than for month 7 (72.4 vs 54.0 mg per day). Consumption of >300 mg caffeine per day during month 1 (adjusted odds ratio = 0.91; 95% confidence interval = 0.44--1.90) and during month 7 (adjusted odds ratio = 1.00; 95% confidence interval = 0.37--2.70) was not associated with intrauterine growth retardation. There was little evidence for any effect modification due to cigarette smoking on the caffeine associations. This study provides evidence that antenatal caffeine consumption has no adverse effect on fetal growth.

Alcohol and caffeine consumption and decreased fertility.
Hakim RB, Gray RH, Zacur H.
Fertil Steril. 1998 Oct;70(4):632-7.
Erratum in:
Fertil Steril 1999 May;71(5):974.

OBJECTIVE: To examine the effects of alcohol and caffeine on conception. DESIGN: Prospective observational study. SETTING: Healthy volunteers in two manufacturing facilities. PATIENT(S): One hundred twenty-four women who provided daily urine samples for measurement of steroid hormones and hCG, and prospective information about alcohol and caffeine consumption. MAIN OUTCOME MEASURE(S): Probability of conception per 100 menstrual cycles. RESULT(S): There was >50% reduction in the

probability of conception during a menstrual cycle during which participants consumed alcohol. Caffeine consumption did not independently affect the probability of conception but may enhance alcohol's negative effect. Women who abstained from alcohol and consumed less than one cup of coffee or its equivalent per day conceived 26.9 pregnancies per 100 menstrual cycles compared with 10.5 per 100 menstrual cycles among those who consumed any alcohol and more than one cup of coffee per day. CONCLUSIONS: This study revealed an independent dose-related negative effect of alcohol consumption on the ability to conceive. Our results suggest that women who are attempting to conceive should abstain from consuming alcohol.

Caffeine intake and low birth weight: a population-based case-control study.
Santos IS, Victora CG, Huttly S, Carvalhal JB.
Am J Epidemiol. 1998 Apr 1;147(7):620-7.

The authors conducted a matched case-control study to investigate the effects of caffeine intake during pregnancy on birth weight. From January to November 1992, in the first 24 hours after delivery, 1,205 mothers (401 cases and 804 controls) were interviewed and their newborns were examined to assess birth weight and gestational age by means of the method of Capurro et al. (J Pediatr 1978;93:120-2). The cases were children with birth weight < 2,500 g and gestational age > or = 28 weeks. Cases and controls were matched for time of birth and hospital of delivery and were recruited from the four maternity hospitals in Pelotas, southern Brazil. Daily maternal caffeine intake during pregnancy for each trimester was estimated. To assess caffeine intake, 10% of the mothers were reinterviewed at their households and samples of reported information on drip coffee and mate (a caffeine-containing drink widely used in South America) were collected and sent to the laboratory for caffeine determination through liquid chromatography. When instant coffee was reported, the weight of powder was measured using a portable scale, and caffeine intake was estimated from a reference table. Caffeine intake from tea, chocolate, soft drinks, and medicines was estimated from a reference table. Analyses were performed by conditional logistic regression. Crude analyses showed no effect of caffeine on low birth weight, preterm births or intrauterine growth retardation. The results did not change after allowing for confounders.

Differences in fertility associated with caffeinated beverage consumption.
Caan B, Quesenberry CP Jr, Coates AO.
Am J Public Health. 1998 Feb;88(2):270-4.

OBJECTIVES: The effect of caffeine consumption on fertility was examined prospectively in 210 women. METHODS: Women reported on caffeinated beverage consumption and pregnancy status monthly. Odds ratios for becoming pregnant were calculated for both high and moderate vs low consumption. RESULTS: No significant association was found for any of the caffeinated beverages except tea. Drinking one-half

cup or more of tea daily approximately doubled the odds of conception per cycle. CONCLUSIONS: These data suggest that caffeine may not be the responsible agent for variation in fertility associated with consumption of the beverages examined.

Rate of caffeine metabolism and risk of spontaneous abortion.
Fenster L, Quale C, Hiatt RA, Wilson M, Windham GC, Benowitz NL.
Am J Epidemiol. 1998 Mar 1;147(5):503-10.

In a case-control study of 73 women with and 141 women without spontaneous abortion, the authors determined the activity of the three principal caffeine-metabolizing enzymes--cytochrome P-4501A2 (CYP1A2), xanthine oxidase, and N-acetyltransferase 2--by measuring levels of caffeine metabolites in urine. After examining the effect of enzyme activity and different levels of caffeine intake, they concluded that there was no evidence that an interaction between enzyme activity and caffeine intake during pregnancy resulted in risk of spontaneous abortion. In a subsample comparing 24 cases with recurrent (two or more) spontaneous abortions and 21 controls with two or more livebirths and no previous spontaneous abortions, the unadjusted odds ratio for low CYP1A2 enzyme activity (below the median) was 0.92 (95% confidence interval (CI) 0.28-3.04) compared with higher CYP1A2 activity. The odds ratio for risk of recurrent spontaneous abortion and low xanthine oxidase activity (below the median) versus higher activity was 0.37 (95% CI 0.10-1.29). Phenotypically slow acetylators (N-acetyltransferase 2 index <0.37) had an odds ratio of 1.58 (95% CI 0.48-5.13) for recurrent loss compared with rapid acetylators. Thus, some association of the latter two caffeine-metabolizing enzymes with recurrent spontaneous abortion is suggested but may also be due to chance.

Caffeinated beverages, decaffeinated coffee, and spontaneous abortion.
Fenster L, Hubbard AE, Swan SH, Windham GC, Waller K, Hiatt RA, Benowitz N.
Epidemiology. 1997 Sep;8(5):515-23.
Comment in:
Epidemiology. 1998 Sep;9(5):583-4.

We examined the relations between spontaneous abortion and the consumption of caffeine, individual caffeine-containing beverages (coffee, tea, and soda), and decaffeinated coffee in a prospective study of 5,144 pregnant women. We collected information about potential risk factors for spontaneous abortion, including consumption of caffeinated beverages and decaffeinated coffee before and during pregnancy, by interview in the first trimester. Neither total estimated caffeine nor individual caffeinated beverage consumption during the first trimester was associated with an appreciable increase in risk for spontaneous abortion. The adjusted odds ratio for consumption of greater than 300 mg per day of caffeine was 1.3 [95% confidence interval (CI) = 0.8-2.1] after adjustment for maternal age, pregnancy history, cigarette and alcohol consumption, employment, race, gestational age at interview, and marital and socioeconomic status.

The adjusted odds ratio for spontaneous abortion related to consumption of three or more cups of decaffeinated coffee during the first trimester was 2.4 (95% CI = 1.3-4.7) in the same model. Although we could not demonstrate this with available data, we suspect that this association was due to bias resulting from the relations among fetal viability, symptoms of pregnancy such as nausea, and consumption patterns during pregnancy.

Human sperm morphometry and smoking, caffeine, and alcohol consumption.
Vine MF, Setzer RW Jr, Everson RB, Wyrobek AJ.
Reprod Toxicol. 1997 Mar-Jun;11(2-3):179-84.

The purpose of this study is to determine whether sperm nuclear size, shape, and chromatin texture parameters are associated with lifestyle exposures including smoking, caffeine, and alcohol consumption. Eighty-six healthy male volunteers (ages 18-35), recruited through newspaper advertisements, provided a semen, blood, and urine sample and completed a questionnaire concerning demographic and lifestyle exposures. Sperm nuclear size, shape, and chromatin texture parameters were measured using computerized image analysis. Results indicated no associations between the sperm nuclear morphometric parameters and age, smoking, or alcohol consumption. There was weak evidence for an association with caffeine intake. In conclusion, the lifestyle factors smoking, caffeine intake, and alcohol consumption do not appear to significantly affect sperm nuclear size, shape, or chromatin texture in this study population.

Preterm delivery: effects of socioeconomic factors, psychological stress, smoking, alcohol, and caffeine.
Peacock JL, Bland JM, Anderson HR.
BMJ. 1995 Aug 26;311(7004):531-5.
Comment in:
BMJ. 1995 Aug 26;311(7004):535-6.

OBJECTIVE-- To examine the relation between preterm birth and socioeconomic and psychological factors, smoking, and alcohol, and caffeine consumption. DESIGN--Prospective study of outcome of pregnancy. SETTING--District general hospital in inner London. PARTICIPANTS--1860 consecutive white women booking for delivery; 1513 women studied after exclusion because of multiple pregnancy and diabetes, refusals, and loss to follow up. MEASUREMENTS--Gestational age was determined from ultrasound and maternal dates; preterm birth was defined as less than 37 completed weeks. Independent variables included smoking, alcohol and caffeine consumption, and a range of indicators of socioeconomic status and psychological stress. MAIN RESULTS--Unifactorial analyses showed that lower social class, less education, single marital status, low income, trouble with "nerves" and depression, help from professional agencies, and little contact with neighbours were all significantly associated with an increased risk of preterm birth. There were no apparent effects of smoking, alcohol, or caffeine on the

length of gestation overall, although there was an association between smoking and delivery before 32 weeks. Cluster analysis indicated three subgroups of women delivering preterm: two predominantly of low social status and a third of older women with higher social status who did not smoke. Mean gestational age was highest in the third group. CONCLUSIONS--Adverse social circumstances are associated with preterm birth but smoking is not, apart from an association with very early births. This runs counter to findings for fetal growth (birth weight for gestational age) in this study, where a strong effect of smoking on fetal growth was observed but there was no evidence for any association with psychosocial factors.

Maternal smoking, alcohol drinking, caffeine consumption, and fetal growth: results from a prospective study.
Shu XO, Hatch MC, Mills J, Clemens J, Susser M.
Epidemiology. 1995 Mar;6(2):115-20.
Comment in:
Epidemiology. 1995 Sep;6(5):570-1.
Epidemiology. 1996 Jan;7(1):110-11.

In a prospective study of 712 pregnancies, we examined associations between maternal smoking, alcohol, and caffeine consumption and fetal growth. We interviewed patients at entry into care [12.9 +/- 4.3 (standard deviation) weeks], and at 28 and 36 weeks of gestation. We found the expected reductions in adjusted birthweight among women who smoked throughout pregnancy: 168 gm [95% confidence limits (CL) = -326, -10] for low/moderate amounts (< or = 15 cigarettes per day); 288 gm (95% CL = -491, -84) for heavy smoking (> 15 cigarettes per day). We also found a decrease in birthweight (-179 gm; 95% CL = -364, 7) among smokers who reported quiting early in pregnancy. First trimester alcohol consumption (average: four drinks per week) was associated with a 155-gm reduction in fetal growth (95% CL = -324, 15), even after adjustment for number of cigarettes smoked. The association, observed with all types of alcohol consumption, was stronger among smokers (-270 gm) but was also present in nonsmokers (-115 gm). Caffeine consumption showed no relation to fetal growth, even among heavy consumers, although they were relatively few. This study implicates heavy maternal smoking at any point in pregnancy, including solely in the early months, and possibly moderate alcohol drinking as causes of low birthweight.

Case-control study of caffeinated beverages and preterm delivery.
Pastore LM, Savitz DA.
Am J Epidemiol. 1995 Jan 1;141(1):61-9.

Although many women reduce their caffeine consumption once they known they are pregnant, 70-80% of pregnant women still consume caffeine. To evaluate the relation between caffeine consumption and preterm delivery, a case-control study was conducted

to identify all preterm (< 37 weeks gestation) infants born to women in selected North Carolina counties from September 1988 through April 1991. Randomly selected full-term, normal-weight livebirths (matched by race and hospital) served as controls. The study population consisted of 408 cases and 490 controls. Telephone interviews with participants assessed the consumption of caffeinated coffee, tea, cola soft drinks, and noncola caffeinated soft drinks, with caffeine consumption measured by the number of daily servings of each beverage and the total milligrams of caffeine. Third-trimester caffeine consumption from all beverages combined showed a nonsignificant inverse association with preterm delivery. Both first- and second-trimester consumption of 1-150 mg/day were associated with a modestly increased risk of preterm delivery, while no association was found at higher consumption levels. Overall, these results do not support an association between caffeinated beverage consumption and preterm delivery, as is true in most previous studies.

D. Studies with unclear findings

Effects of cigarette smoking, caffeine consumption, and alcohol intake on fecundability.
Curtis KM, Savitz DA, Arbuckle TE.
Am J Epidemiol. 1997 Jul 1;146(1):32-41.

Data from the Ontario Farm Family Health Study were analyzed to determine whether smoking, caffeine, or alcohol use among men and women affect fecundability (the monthly probability of conception). In this retrospective cohort study of farm couples in Ontario, Canada, the farm operator, husband, and wife completed questionnaires during 1991-1992, yielding information on 2,607 planned pregnancies that had occurred over the previous 30 years. Fecundability ratios were calculated using an analog of the Cox proportional hazards model. Cigarette smoking among women and men was associated with decreased fecundability (fecundability ratio = 0.90, 95% confidence interval (CI) 0.82-0.98 and fecundability ratio = 0.88, 95% CI 0.81-0.95, respectively). Caffeine consumption of 100 mg or less versus more than 100 mg in women and men was not associated with fecundability (fecundability ratio = 0.98, 95% CI 0.91-1.07 and fecundability ratio = 1.05, 95% CI 0.97-1.14, respectively). Decreases were observed among women who were coffee drinkers (fecundability ratio = 0.92, 95% CI 0.84-1.00) and men who were heavy tea drinkers (fecundability ratio = 0.85, 95% CI 0.69-1.05), regardless of caffeine content. Alcohol use among women and men was not associated with fecundability. These data are consistent with previous studies of the adverse effect of tobacco on fecundability in female smokers and suggest an effect of smoking among males. Continued evaluation of coffee and tea is warranted to address constituents other than caffeine.

Maternal caffeine consumption and spontaneous abortion: a prospective cohort study.
Dlugosz L, Belanger K, Hellenbrand K, Holford TR, Leaderer B, Bracken MB.
Epidemiology. 1996 May;7(3):250-5.

We investigated the relation between caffeine beverage consumption and spontaneous abortion in 2,967 pregnant women planning to deliver at Yale-New Haven Hospital in 1988-1992. We evaluated coffee, tea, and soda drinking in the first month of pregnancy in interviews before the end of the sixteenth week of gestation. We obtained information on 98.2% of the pregnancies (including 2,714 singleton livebirths and 135 spontaneous abortions). As compared with abstention from caffeine beverages (coffee, tea, and soda), the adjusted odds ratios for spontaneous abortion associated with consumption of 1-150, 151-300, and > 300 mg caffeine daily were 0.81 [95% confidence interval (CI)) = 0.54-1.20], 0.89 (95% CI = 0.48-1.64), and 1.75 (95% CI = 0.88-3.47), respectively. Drinking > or = 3 cups of tea or coffee was associated with elevated risks of spontaneous abortion (adjusted odds ratio = 2.33, 95% CI = 0.92-5.85; and adjusted odds ratio = 2.63, 95% CI = 1.29-5.34, respectively). These results, if replicated, suggest that some ingredient (or correlate) of tea or coffee may account for some of the observed association of caffeine with spontaneous abortion. In this study, caffeine consumption is more strongly related to spontaneous abortion than alcohol or cigarette use in early pregnancy.

E. Related Articles

Stillbirth and slow metabolizers of caffeine: comparison by genotypes.
Bech BH, Autrup H, Nohr EA, Henriksen TB, Olsen J.
Int J Epidemiol. 2006 Aug;35(4):948-53.

BACKGROUND: Cytochrome P4501A2 (CYP1A2) and N-acetyltransferase 2 (NAT2) are key enzymes in the metabolism of caffeine. The polymorphism of these genes facilitates the detection of fast and slow metabolizers, and if caffeine is causally related to stillbirth, we expect slow metabolizers to have a higher risk of stillbirth at any given intake of caffeine. Gluthatione S-transferase alpha1 (GSTA1) may also be active in the metabolism of caffeine as it conjugates glutathione to aromatic amines. Our study, therefore, included analyses of the association between GSTA1 and stillbirth. METHODS: A nested case non-case study among women who participated in the Danish National Birth Cohort: 142 cases of singleton stillbirths and 157 controls of singleton live births. RESULTS: Slow oxidizer status (CYP1A2), slow acetylator status (NAT2), and low activity of GSTA1 were not individually associated with the risk of stillbirth [odds ratio (OR) = 1.06, 95% confidence interval (95% CI) 0.67-1.67, OR = 0.95, 95% CI 0.60-1.51, and OR = 1.42, 95% CI 0.88-2.28, respectively]. We did, however, observe that subjects with a combination of slow CYP1A2, slow NAT2, and low GSTA1 genes had almost a 2-fold risk of stillbirth compared with subjects with other combinations of genotypes. CONCLUSIONS: We found no link between any single genotype and the risk of stillbirth. An association between a combination of genotypes and stillbirth was

discovered. Caffeine may be causally related to stillbirth, but larger studies using Mendelian randomization are needed to verify this.

Caffeine metabolites in umbilical cord blood, cytochrome P-450 1A2 activity, and intrauterine growth restriction.
Grosso LM, Triche EW, Belanger K, Benowitz NL, Holford TR, Bracken MB.
Am J Epidemiol. 2006 Jun;163:1035-41.

Studies investigating antenatal caffeine consumption and reproductive outcomes show conflicting results, and most studies have used maternal self-reported caffeine consumption to estimate fetal exposure. This study (n=1,606) was specifically designed to test the association of caffeine and its primary metabolites in umbilical cord blood with intrauterine growth restriction (IUGR). Pregnant women were recruited from 56 obstetric practices and 15 clinics affiliated with six hospitals in Connecticut and Massachusetts between September 1996 and January 2000. In an adjusted model including caffeine only, levels in all quartiles were associated with reduced risk of IUGR. In adjusted analyses including paraxanthine and caffeine, serum paraxanthine levels in the highest quartile were associated with increased risk of IUGR (adjusted odds ratio=3.29, 95% confidence interval: 1.17, 9.22); caffeine remained protective. These conflicting findings suggest that cytochrome P-450 1A2 (CYP1A2) metabolic activity may be associated with IUGR, so the ratio of paraxanthine to caffeine was then modeled. The likelihood of IUGR increased 21% for every one standard deviation change in the ratio (adjusted odds ratio=1.21, 95% confidence interval: 1.07, 1.37), suggesting that CYP1A2 activity, and not the absolute levels of paraxanthine, influences fetal growth. No associations were observed between caffeine or any metabolites and preterm delivery.

N-acetyl-transferase phenotype and risk for preeclampsia.
Zusterzeel PL, te Morsche RH, Raijmakers MT, Roes EM, Peters WH, Steegers-Theunissen RP, Steegers EA.
Am J Obstet Gynecol. 2005 Sep;193(3 Pt 1):797-802.

OBJECTIVE: This study was undertaken to determine whether the N-acetyltransferase (NAT) phenotype contributes to the susceptibility for the development of preeclampsia. STUDY DESIGN: The NAT acetylator status was determined by measuring urinary caffeine metabolites in 134 nonpregnant women with a history of preeclampsia and in 109 control women with uncomplicated pregnancy. The chi(2) and logistic regression analyses were used for statistical evaluation of differences in acetylator status. RESULTS: Significantly more fast acetylators were found among the women with a history of preeclampsia (46.3%) than among the controls (25.4%). Fast acetylators showed an odds ratio of 2.5 (95% CI 1.4-4.3) for preeclampsia. No differences in the acetylator status were found between women with a history of preeclampsia only and those with the HELLP syndrome as well. CONCLUSION: The fast NAT acetylator

status, which may result in altered NAT detoxification capacity, is associated with preeclampsia.

F. Table of epidemiologic studies

The following table includes studies published in the past ten years. Information included in the table was taken from the study abstracts.

Study	*Population*	*Outcomes*	*Results*	*Author's Conclusions*	*Study Type*
Studies reporting increased risk of spontaneous abortion					
George et al. 2006	Swedish population based study	Repeated miscarriage	Not reported in abstract	Among non-smoking women with high caffeine intake, there was an increased risk of repeated miscarriage, whereas there was no association among smokers.	CC
Karypidis et al. 2006	507 women with miscarriage and 908 women with a normal first trimester pregnancy	Spontaneous abortion	CYP1B1 432 Val/Val genotype were at a higher risk of miscarriage OR = 1.46; 95% CI 1.02-2.08). Also reported was a significant interaction between genotype and caffeine intake	CYP1B1 Val432Leu polymorphism is associated with first trimester miscarriage, and it may also modify the risk amoun coffee drinkers.	CC
Sata et al. 2005	58 women with recurrent (≥ 2) pregnancy loss and 147 fertile controls	Spontaneous abortion	Women who had homozygous CYP1A2*1F[1] alleles. Reference: caffeine intake < 100 mg/day 100-299 mg/day OR=1.94 (95% CI: 0.57-6.66) ≥ 300mg/day OR=5.23 (95% CI: 1.05-25.9) P = 0.03 for trend	It was demonstrated for the first time that an increase in caffeine intake results in a deterioration in fecundity among susceptible women	CC
Khoury et al. 2004	191 women from a "Diabetes in Pregnancy" program (168 with ≥ 20 wks. gestation)	Spontaneous abortion Pre-eclampsia	In women with type 1 diabetes - caffeine consumption associated with increased risk of spontaneous abortion: OR=4.5 (95% CI: 1.2-16.8) Caffeine consumption after 20 weeks associated with reduced risk of pre-eclampsia: OR=0.3 (95% CI: 0.1-1.0)	Consumption of caffeine during early pregnancy increases risk of spontaneous abortion. Caffeine consumption associated with reduced risk of pre-eclampsia.	CH
Tolstrup et al 2003	303 women with spontaneous abortion and 1381 who had given birth. Selected from a cohort of 11,088 women aged 20-29 yrs.	Spontaneous abortion	Reference: Pre-pregnancy caffeine intake < 75 mg/day 75-300 mg/day OR=1.26 (95% CI: 0.77-2.06), 301-500 mg/day OR=1.45 (95% CI: 0.87-2.41), 501-900 mg/day OR=1.44 (95% CI: 0.87-2.37), > 900 mg/day OR=1.72 (95% CI: 1.00-2.96) P = 0.05 for trend	High intake of caffeine prior to pregnancy seems to be associated with an increased risk of spontaneous abortion.	NCC
Rasch 2003	330 women with spontaneous abortion at 6–16 weeks and 1168 women receiving antenatal care at 6-16 weeks	Spontaneous abortion	Caffeine consumption of ≥ 375 mg/day OR=2.21 (95% CI: 1.53-3.18)	Caffeine intake of ≥ 375 mg/day during pregnancy may increase risk for spontaneous abortion.	CC

Study	Population	Outcomes	Results	Author's Conclusions	Study Type
Signorello et al. 2001	101 women with normal karyotype spontaneous abortions and 953 pregnant women at 6-12 weeks gestation.	Spontaneous abortion	Low CYP1A2[1] activity was associated with a decreased risk of spontaneous abortion OR=0.35 (95% CI: 0.20-0.63). Among high CYP1A2 activity with caffeine intake of 100-299 mg/day OR=2.42 (95% CI: 1.01-5.80) ≥ 300 mg/day OR=3.17 (95% CI: 1.22-8.22). Slow acetylators (NAT2[2] activity) OR=2.51 (95% CI: 0.81-7.76).	High CYP1A2 activity may increase risk of spontaneous abortion independently or by modifying the effect of caffeine NAT2 Results less conclusive than those for CYP1A2.	CC
Wen et al. 2001	75 women with spontaneous abortions and 575 women delivering singleton live births.	Spontaneous abortion	Maternal caffeine intake (in first trimester after nausea started) Reference: < 20 mg/day > 300 mg/day OR=5.4 (95% CI: 2.0-14.6) Caffeine consumption before pregnancy, or in women without nausea, did not increase the risk of spontaneous abortion.	Results suggest that for those with nausea, maternal caffeine consumption during pregnancy may influence fetal viability	CH
Torfs & Christianson 2000	997 live born infants or fetuses with Down syndrome and 1,007 live born controls without a birth defect	Recognized Down Syndrome Pregnancy	During periconception period ≥ 4 cups of coffee/day OR=0.63 (95% CI: 0.41-0.96). Multivariate analyses: Among non-smoking mothers: ≥ 4 cups/coffee per day: OR = 0.48 (95% CI: 0.28-0.82).	In non-smokers high levels of coffee consumption more likely to reduce viability of a Down syndrome fetus than a normal fetus.	CC
Cnattingius et al. 2000	562 women with spontaneous abortion at 6 to 12 weeks and 953 women with no spontaneous abortion (matched by gestation week to cases)	Spontaneous abortion	Among nonsmokers Reference: maternal caffeine intake < 100 mg/ day 100-299 mg/day: OR=1.3 (95% CI: 0.9-1.8) 300-499 mg/day: OR=1.4 (95% CI: 0.9-2.0), ≥ 500 mg/day: OR=2.2 (95% CI: 1.3-3.8).	The ingestion of caffeine may increase the risk of an early spontaneous abortion among non-smoking women carrying fetuses with normal karyotypes.	CC
Klebanoff et al. 1999	591 women who had spontaneous abortions (≤ 140 days gestation) and 2558 women who gave birth to live infants at ≥ 28 weeks gestation. Final sample: 487 cases and 2087 controls	Spontaneous abortion	Reference: < 50 ng/ml < 1845 ng/ml: not significantly elevated ≥ 1845 ng/ml: OR=1.9 (95% CI: 1.2-2.8) Mean serum paraxanthine[3] concentration was higher in the women who had spontaneous abortions than in the controls (752 vs. 583 ng/ml, P < 0.001).	Only extremely high serum paraxanthine concentrations are associated with spontaneous abortion. Moderate caffeine consumption unlikely to increase risk of spontaneous abortion.	NCC

Study	Population	Outcomes	Results	Author's Conclusions	Study Type
Parazzini et al. 1998	782 cases of spontaneous abortion (≤ 12 weeks gestation) and 1543 controls who gave birth at term (> 37 wks.)	Spontaneous abortion	Coffee drinking during 1st trimester. Reference: 0 cups/day 1 cup/day = 1.2 2-3 cups/day = 1.8 ≥ 4 cups /day = 4.0 Duration in years of coffee drinking: Reference: non-coffee drinkers ≤ 10 years OR=1.1 (95% CI: 0.9-1.4) > 10 years OR=1.9 (95% CI: 1.5-2.6)	Coffee drinking early in pregnancy was associated with an increased risk of spontaneous abortion.	CC
Fernandes et al. 1998	12 studies with extractable data: 6 spontaneous abortion (42,988 pregnancies) 7 low birth weight (64,268 pregnancies) 1 common (i.e. both LBW and spontaneous abortion) study	Spontaneous abortion Low birth weight (<2500g)	Reference: 0-150 mg/day caffeine > 150 mg/day Mantel-Haenszel OR=1.36 (95% CI: 1.29-1.45) Reference: 0-150 mg/day caffeine > 150 mg/day Overall RR=1.51 (95% CI: 1.39-1.63)	Small, but statistically significant increase in the risks for spontaneous abortion and low birth weight babies in women consuming > 150 mg/day.	MA
Studies reporting no increased risk of spontaneous abortion					
Maconochie et al. 2007	603 women with spontaneous abortion at < 13 weeks of gestation and 6116 women whose pregnancy progressed past 12 weeks gestation	Spontaneous abortion	No association reported between caffeine consumption and spontaneous abortion.		CC
Fenster et al. 1998	73 women with spontaneous abortion and 141 women with no spontaneous abortion Subsample: 24 cases with recurrent (≥ 2) spontaneous abortion and 21 controls with ≥ 2 or more live births and no previous spontaneous abortion	Spontaneous abortion Spontaneous abortion (recurrent)	The authors conclude that there is no evidence that an interaction between caffeine-metabolizing enzymes (CYP1A2, xanthine oxidase[4] and N-acetyltransferase 2[2]) activity and caffeine intake during pregnancy resulted in risk of spontaneous abortion. Reference: Higher CYP1A2 or xanthine oxidase (≥ median) enzyme activity. Low (< the median) CYP1A2 activity OR=0.92 (95% CI: 0.28-3.04). Low xanthine oxidase activity (< the median) OR=0.37 (95% CI: 0.10-1.29). Reference: Phenotypically rapid acetylators (N-acetyltransferase 2 index ≥ 0.37) Phenotypically slow acetylators (N-acetyltransferase 2 index < 0.37) OR= 1.58 (95% CI: 0.48-5.13)	Some association of xanthine oxidase and N-acetyltransferase 2 with recurrent spontaneous abortion is suggested but may also be due to chance.	CC

Study	*Population*	*Outcomes*	*Results*	*Author's Conclusions*	*Study Type*
Fenster et al. 1997	5,144 pregnant women	Spontaneous abortion	> 300 mg/day of caffeine OR=1.3 (95% CI: 0.8-2.1). > 3 cups of decaffeinated coffee/day (1st trimester) OR=2.4 (95% CI: 1.3-4.7)	Suspect association is due to bias resulting from the relations among fetal viability, symptoms of pregnancy such as nausea, and consumption patterns during pregnancy.	CH
Unclear findings for spontaneous abortion					
Dlugosz et al. 1996	2,714 singleton live births and 135 spontaneous abortions. Selected from 2967 pregnant women planning to deliver at Yale-New Haven Hospital	Spontaneous abortion	Reference: 0 mg caffeine/day 1-150 mg/day OR=0.81 (95% CI: 0.54-1.20) 151-300 mg/day OR=0.89 (95% CI: 0.48-1.64) > 300 mg/day OR=1.75 (95% CI: 0.88-3.47) ≥ 3 cups/day coffee OR=2.63 (95% CI: 1.29-5.34) respectively. ≥ 3 cups/day tea OR=2.33 (95% CI: 0.92-5.85) respectively.	Results, if replicated suggest that an ingredient (or correlate) of tea or coffee may account for observed association of caffeine with spontaneous abortion.	CH
Studies reporting increased risk of low birth weight (LBW), lower mean birth weight, intrauterine growth retardation (IUGR) or preterm delivery					
Orskou et al. 2003	24,093 pregnancies, non-diabetic women.	High birth weight infants (> 4000g)	Women with low caffeine intake at statistically significant higher risk of giving birth to infants weighing >4000 g	Risk factors associated with a higher proportion of high birth weight infants may be clinically significant and have a public health impact.	CH
Vik et al. 2003	111 mothers of Small for Gestational Age (SGA) infants (56 boys, 55 girls) and 747 mothers of non-Small for Gestational Age infants (368 boys, 379 girls)	Fetal growth retardation	3rd trimester mean caffeine intake was higher in mothers of SGA infants [281 (± 210) mg/day] than in mothers of non-SGA infants [212 (± 150) mg/day] (p <0.001) High versus low caffeine intake: OR=1.8 (95% CI: 1.2-2.5). Boys: OR=2.8 (95% CI 1.5-5.2), Girls: OR=1.2 (95% CI: 0.7-2.1).	Results suggest high 3rd trimester caffeine intake may be risk factor for fetal growth retardation especially if fetus is male.	CH
Bracken et al. 2003	2,291 mothers with singleton live births in Connecticut and Massachusetts	Mean birth weight Intrauterine growth retardation	28 g decrease in mean birth weight/100 mg of caffeine intake (95% CI: -0.10 - -0.46), p = 0.001 For every 1mg/g creatinine increase in urinary caffeine: OR=0.96 (95% CI: 0.85 - 1.08) Self reports of caffeine intake were not associated with IUGR, LBW or preterm delivery	Small decrease in birth weight not clinically important except for women consuming ≥ 600 mg of caffeine daily. i.e. approximately six 10-ounce cups of coffee. (1 ounce=28.3 g)	CH

Study	Population	Outcomes	Results	Author's Conclusions	Study Type
Klebanoff et al. 2002	2,515 women of the Collaborative Perinatal Project 1959-1966	Small for gestational age fetus	3rd trimester serum paraxanthine concentration in mothers of SGA infants (754 ng/ml) was greater than in mothers of appropriately grown infants (653 ng/ml) (p = 0.02) Smokers: Linear trend for increasing serum paraxanthine concentration associated with increasing risk of SGA birth (p = 0.03). p = 0.07 when no. of cigarettes was considered. Non-Smokers: no linear trend association (p = 0.48).	Maternal 3rd trimester serum paraxanthine concentration, which reflects caffeine consumption, was associated with higher risk of reduced fetal growth particularly among smokers.	CH
Eskenazi et al. 1999	7,855 live births in California's San Joaquin Valley	Preterm delivery Mean birth weight	Reference: Women drinking neither caffeinated nor decaffeinated coffee. Just caffeinated coffee OR=1.3 (95% CI: 1.0-1.7) Caffeinated and Decaffeinated coffee OR=2.3 (95% CI: 1.3-4.0) Caffeinated coffee: -3.0 g/cup/ week (95% CI: -5.9 - -0.6) Decaffeinated coffee +0.4 g/cup/ week (95% CI: -3.7 - 4.5)	Women who consumed only decaffeinated coffee showed no increased odds for SGA birth, low birth weight, preterm delivery, lowered mean birth weight or shortened mean gestational age compared to those who did not drink coffee.	CH
Fernandes et al. 1998	See description under "studies reporting increased risk for spontaneous abortion"	Low birth weight (<2500g)	Reference: 0-150 mg/day caffeine > 150 mg/day overall RR=1.51 (95% CI: 1.39-1.63)		MA
Santos et al. 1998	26 studies: 22 birth weight, (11 mean birth weight, 9 low birth weight, 4 intrauterine growth retardation)	Mean birth weight IUGR and preterm delivery and LBW	Reference: mothers consuming lower amounts or no caffeine. Heavy consumers:significant decrease in mean birth weight of nearly 43 g Not possible to estimate reliable pooled estimates	Statistical significant decrease in birth weight of nearly 43g. Further assessment of caffeine intake during pregnancy is needed in future research.	MA
Vlajinac et al. 1997	1,011 women (interviewed during their first 3 days after delivery in Yugoslavia)	Birth weight	Significant reduction in birth weight associated with average caffeine intake ≥ 71 mg/day in non smoking mothers only		CH
Rondo et al. 1996	356 mother/baby pairs who had intrauterine growth retardation with 356 mother/baby pairs who were appropriate for gestational age.	Intrauterine growth retardation	More IUGR mothers (85.4%) than appropriate for gestational age mothers (70.5%) drank coffee in pregnancy (OR=2.45; p< 0.001) Proportion of mothers who delivered IUGR babies increased as the average consumption of coffee increased (test for trend = 31.76; p < 0.001). Attributable Risk for coffee consumption = 28.0 %	Coffee consumption was an important preventable cause of IUGR in this Brazilian population. Recommend moderation in consumption of coffee during pregnancy	CC

Study	Population	Outcomes	Results	Author's Conclusions	Study Type
Studies reporting no increased risk of LBW, lower mean birth weight, IUGR or preterm delivery					
Bech et al. 2007	1207 pregnant women drinking at least 3 cups of coffee/day, recruited before 20 weeks gestation 568 women randomized to caffeinated coffee, 629 randomized to decaffeinated coffee	Birth weight Length of gestation	Mean birth weight of babies born to women in the decaffeinated group was 16 g (95% CI -40 – 73) higher than those born to women in the caffeinated group Adjusted difference in length of gestation (decaffeinated group-caffeinated group) was -1.31 days (-2.87 to 0.25)	A moderate reduction in caffeine intake in the second half of pregnancy has no effect on birth weight or length of gestation.	RCT
Chiaffarino et al. 2006	502 cases 1966 controls	Preterm (delivered at < 37 weeks of gestation)	"In comparison with not drinkers, all the ORs of overall intake of caffeine were closed near the unity for both small for gestational age and normal for gestational age preterm birth."	Compared with no consumption, a low consumption of coffee during pregnancy may not have significant effects on preterm birth.	CC
De Souza et al. 2005	140 cases and 162 controls	Prematurity (gestational age < 37 weeks)	Not reported in abstract	Total caffeine consumption during pregnancy was not associated with prematurity, and most intakes were less than 300 mg/day.	CC
Parazzini et al. 2005	555 cases and 1966 controla	Small for gestational age (<10th percentile)	Reference: nondrinkers of coffee 3 or more cups/days 1st trimester – OR=1.2 (95% CI: 0.8 – 1.8) 2nd trimester – OR=1.2 (95% CI: 0.8 – 1.8) 3rd trimester – OR=0.9 (95% CI: 0.6 – 1.4) 4 or more cups/day before pregnancy OR=1.3 (95% CI: 0.9 – 1.9)	These findings are consistent in women who delivered preterm and at term births and were not affected by potential confounding such as smoking.	CC
Bicalho et al. 2002	354 newborn (birth weight <2,500 g) cases, with 354 (birth weight >3,000 g) controls	Low birth weight Prematurity Intrauterine growth retardation	Caffeine consumption: < 300 mg/day OR=0.72 (95% CI: 0.45-1.25) ≥ 300 mg/day OR=0.47 (95% CI: 0.24-0.92) Caffeine consumption: < 300 mg/day OR=0.59 (95% CI: 0.32-1.09) ≥ 300 mg/day OR=0.32 (95% CI 0.15-0.72) Caffeine consumption: < 300 mg/day OR=1.16 (95% CI: 0.45-3.01) ≥ 300 mg/day OR=0.64 (95% CI: 0.20-1.98)	No association between caffeine consumption during pregnancy and low birth weight, prematurity and intrauterine growth retardation.	CC

Study	*Population*	*Outcomes*	*Results*	*Author's Conclusions*	*Study Type*
Clausson et al. 2002	873 women delivering live born singleton infants	Birth weight Gestational age Birth weight ratio	Exposure periods: 1) 6-12 and 32-34 wks. gestation 2) Average caffeine consumption from conception to 32–34th wk. Gestation 3) Stratified by trimesters of pregnancy No associations between caffeine consumption and birth weight, gestational age and birth weight ratio for any of the above exposure periods.	These results do not support an association between moderate caffeine consumption and reduced birth weight, gestational age, or fetal growth.	CH
Grosso et al. 2001	2,714 women delivering a live born infant (1988-1991)	Intrauterine growth retardation	Caffeine consumption during 1st month > 300 mg/day OR= 0.91(95% CI: 0.44-1.90) Caffeine consumption during 7th month > 300 mg/day OR=1.00 (95% CI: 0.37-2.70)	The study provides evidence that antenatal caffeine consumption has no adverse effect on fetal growth.	CH
Santos et al. 1998	1,205 mothers: 401 cases (< 2,500 g and ≥ 28 weeks) and 804 controls matched for time of birth and hospital of delivery.	Low birth weight Preterm birth Intrauterine growth retardation	Crude analyses showed no effect of caffeine intake on low birth weight, preterm births or intrauterine growth retardation. The results did not change after allowing for confounders.		CC
Peacock et al. 1995	1513 white women booking for delivery	Preterm birth (< 37 weeks)	There were no apparent effects of caffeine intake on the length of gestation overall.		CS
Shu et al. 1995	712 pregnancies	Fetal growth	Caffeine consumption showed no relation to fetal growth, even among heavy consumers, although they were relatively few.		CH
Pastore & Savitz 1995	408 cases: preterm (< 37 weeks) infants and 490 controls (randomly selected, full-term, normal-weight live births) matched by race and hospital)	Preterm delivery	Both 1st and 2nd trimester consumption of 1-150 mg/day of caffeine associated with a modestly increased risk of preterm delivery. No association was found at higher consumption levels. 3rd trimester caffeine consumption from all beverages combined showed a non-significant inverse association with preterm delivery	Overall, these results do not support an association between caffeinated beverage consumption and preterm delivery	CC

Study	Population	Outcomes	Results	Author's Conclusions	Study Type
Studies reporting increased risk of fetal death, stillbirth or infant death					
Matijasevich et al. 2006	382 cases with a confirmed diagnosis of spontaneous antepartum fetal death and 792 control women with a live, term, and adequate for gestation age infant	Fetal death (at leaset 20 weeks gestational age or weighing >350 g.	Reference: no caffeine consumption Mean caffeine intake of > or = 300 mg/day showed a significant increase in risk of fetal death OR 2.33 (1.23 - 4.41)	As mate drinking is highly consumed among pregnant women in Uruguay, the association found with fetal death make is a preventable risk factor.	CC
Bech et al. 2005	88,482 pregnant women	Fetal death	Reference: nonconsumers of coffee Adjusted hazard ratios ½-3 cups/day - 1.03 (95% CI: 0.89 – 1.19) 4-7 - 1.33 (95% CI: 1.08 – 1.63) > or = 8 - 1.59 (95% CI: 1.19 – 2.13)	Consumption of coffee during pregnancy was associated with a higher risk of fetal death, especially losses occurring after 20 completed weeks of gestation.	CH
Wisborg et al. 2003	18,478 singleton pregnancies in women	Stillbirth (delivery of dead fetus at ≥ 28 weeks gestation) Infant death (death of live infant during first year of life)	Reference: 0 cups coffee/day ≥ 8 cups/day Unadjusted OR=3.0 (95% CI: 1.5-5.9) See description under studies reporting no increased risk for Stillbirth/Infant Death	Drinking coffee during pregnancy associated with increased risk of stillbirth but not with infant death.	CH
Klonoff-Cohen et al. 2002	221 couples undergoing IVF and gamete intra-fallopian transfer	Live births Gestational age Multiple gestations	Reference: female caffeine consumption of 0-2 mg/day Not achieving a live birth "Usual" consumption: >2-50 mg/day OR= 3.1 (95% CI: 1.1-9.7) >50mg/day OR = 3.9 (95% CI: 1.3-11.6) Week of the initial visit consumption: >2-50 mg/day OR=2.9 (95% CI: 1.1-7.5) >50mg/day OR=3.8 (95% CI: 1.4-10.7) Infant gestational age (at birth) decrease "Usual" consumption >50 mg/day OR = -3.8 (95% CI: -6.9 - -0.7) wks Week of the initial visit consumption: > 50 mg/day OR = -3.5 (95% CI: -6.7 - -0.3) wks. Multiple gestations In males 100 mg/day increase in: "usual" intake OR = 2.2 (95% CI: 1.1 – 4.4) Week of the initial visit consumption OR = 3.0 (95% CI: 1.2 – 7.4)	If results replicated caffeine use should be minimized prior to and during IVF/GIFT	CH

Study	Population	Outcomes	Results	Author's Conclusions	Study Type
Studies reporting no increased risk of fetal death, stillbirth or infant death					
Wisborg et al. 2003	See description under "Studies reporting increased risk in stillbirth/infant death"	Infant death (death of live infant during first year of life)	No significant association between coffee consumption and death in first year of life.		CH
Studies reporting no increased risk of birth defects					
Carmichael et al. 2005	502 case and 1286 controls mothers	Hypospadias	Not reported in abstract	Intake of caffeine and alcohol were not associated with hypospadias risk.	CC
Studies reporting increased risk of decreased fecundability or delayed conception					
Cole et al. 2006	41 couples having their first pregnancy	Fecundability	Couple consumption > or = 111 drinks/month – fOR 0.25 (95% CI: 0.10 – 0.63)	Couple with higher reported caffeine consumption had lower fecundability.	?
Jensen et al. 1998	423 Danish couples (living with partner and no prior reproductive experience.)	Fecundability	Reference: non smoking women with < 300 mg/day of caffeine Non- smoking women: 300-700 mg/day Fecundability Odds Ratio, FOR=0.88 (95% CI: 0.60-1.31), >700 mg/day FOR=0.63 (95% CI: 0.25-1.60). Smoking women: (coffee only source of caffeine) >300 mg/day FOR=0.34 (95% CI: 0.12-0.98). No dose response among smokers	Interaction between caffeine and smoking is biologically plausible. Findings suggest that especially nonsmoking women who wish to achieve a pregnancy might benefit from a reduced caffeine intake.	CH
Bolumar et al. 1997	3,187 women at risk of becoming pregnant selected from five European countries	Subfecundity	Subfecundity in first pregnancy > 500 mg/day of caffeine: OR=1.45 (95% CI: 1.03-2.04). In smokers consuming > 500 mg/day OR=1.56 (95% CI: 0.92-2.63) Non-smokers OR=1.38 (95%CI: 0.85-2.23).	Associations consistent in all countries. High levels of caffeine may delay conception among fertile women Caffeine intake was categorized as none, 1-100, 101-300 and 301- 500, ≥ 501 mg/day.	CH
		Delayed conception	Time to first pregnancy > 500 mg/day hazard ratio =0.90 (95% CI: 0.78-1.03) i.e. an 11 % increase.		

Study	Population	Outcomes	Results	Author's Conclusions	Study Type
Stanton & Gray 1995	1,430 parous women, not currently using contraceptives	Delayed conception	Ref: non caffeine drinking women No increased effect women consuming ≤ 300 mg/day No effect of high coffee consumption among smoking women For non-smoking women ≥ 301 mg/day OR=2.65 (95% CI: 1.38-5.07).	High level of caffeine consumption may result in delayed conception among non-smoking women. Caffeine intake was categorized as none, 1-150, 151-300 and ≥301 mg/day.	CH
		Fecundability	Nonsmokers ≥ 301 mg/day FR=0.74 (95% CI: 0.59-0.92) No effect of high coffee consumption among smoking women .		
Studies reporting no increased risk of decreased fecundability or delayed conception					
Hakim et al. 1998	124 women in two manufacturing facilities	Conception	Women who abstained from alcohol and consumed less than 1 cup of coffee or its equivalent per day conceived 26.9 pregnancies per 100 menstrual cycles compared with 10.5 per 100 menstrual cycles among those who consumed any alcohol and more than 1 cup of coffee per day	Caffeine consumption did not independently affect the probability of conception but may enhance alcohol's negative effect.	CH
Caan et al. 1998	210 women	Fertility	Drinking one-half cup or more of tea daily approximately doubled the odds of conception per cycle. No significant associations were found for other caffeinated beverages.	Data suggest that caffeine may not be the responsible agent for variation in fertility associated with consumption of the beverages examined.	CH
Unclear findings for fecundability/conception					
Curtis et al. 1997	2,607 planned pregnancies occurring over 30 year period. Drawn from farm couples in Ontario, Canada	Fecundability	Reference: ≤ 100 mg caffeine/day > 100 mg/day (females) Fecundability ratio FR=0.98 (95% CI: 0.91-1.07) and (males) FR=1.05 (95% CI: 0.97-1.14). Decreases observed among coffee drinkers (female) FR=0.92 (95% CI: 0.84-1.00) and heavy tea drinkers (male) FR=0.85 (95% CI: 0.69-1.05).	Continued evaluation of coffee and tea is warranted to address constituents other than caffeine.	CH

Study	Population	Outcomes	Results	Author's Conclusions	Study Type
Studies reporting increased risk of sperm abnormalities					
Robbins et al. 1997	45 healthy male volunteers (ages 19-35)	Aneuploidy load in sperm	Caffeine was significantly associated with increased frequencies of sperm aneuploidy XX18-18 and XY18, diploidy XY18-18 and the duplication phenotype YY18-18.	Sperm FISH (fluorescence in situ hybridization) proved to be a useful biomarker to detect and compare numerical cytogenic abnormalities in human sperm cells across differing levels of exposure to smoking, caffeine and alcohol.	CS
Studies reporting no increased risk of sperm abnormalities					
Vine et al. 1997	86 healthy male volunteers (ages 18-35)	Sperm nuclear size, shape, and chromatin texture parameters	Results indicated weak evidence for an association between the sperm nuclear morphometric parameters and caffeine intake.	Caffeine intake does not appear to significantly affect nuclear size, shape or chromatin texture in this population	CS
Studies reporting increased risk of adverse post-natal outcomes					
Ye et al. 2004 Abstract only	7,687 women recruited to Healthy Habits for Two Study (1984 – 1987)	Singleton children behavioral problems during childhood measured by the Strengths and Difficulties Questionnaire (SDQ-Dan) version for parents	Reference: 0 cups coffee/day (maternal consumption during gestation) ≥ 8 cups/day (during gestation) Odds ratio for increased SDQ scores, OR=2.21 (95% CI: 1.67-2.91). ≥ 8 cups/day (maternal consumption during gestation) children had increased risk of behavioral problems	Both high coffee consumption and smoking during pregnancy are associated with increased risk of behavioral problems in childhood. Caffeine intake slightly affects the probability of behavioral disorders but may enhance the negative effect of cigarettes.	CH
Ford et al. 1998	Parents of 393 SIDS victims with parents of 1592 control infants	Sudden Infant Death Syndrome (SIDS)	Infants whose mothers had ≥ 400 mg caffeine/day or ≥ 4 cups coffee/day throughout their pregnancy: Odds Ratio for SIDS, OR=1.65 (95% CI: 1.15-2.35).	Reducing heavy caffeine intake during pregnancy could be another way to lessen the risk of SIDS. This needs further confirmation.	CC

Study type codes:

CC Case control study
CH Cohort study
CS Cross-sectional
MA Meta-analysis
NCC Nested case-control
RCT Randomized control trial

Other Abbreviations:

FR Fecundability Ratio
FOR Fecundability Odds Ratio
IUGR Intrauterine Growth Retardation
LBW Low Birth Weight
OR Odds Ratio
RR Relative Risk
SGA Small for Gestational Age

Other notes regarding table

[1] CYP1A2 is the enzyme that primarily metabolizes caffeine.
[2] N-Acetyltransferase 2 (NAT2) is an enzyme that also participates in caffeine metabolism.
[3] Paraxanthine is the primary metabolite of caffeine in the human body.
[4] Xanthine oxidase is an enzyme that participates in caffeine metabolism

Unless explicitly stated otherwise, caffeine consumption is always in units per day.
Unless explicitly stated otherwise, all effect measures: odds ratios, relative risks, fecundability ratios etc. are adjusted.

II. Animal DART Studies

A. Studies reporting developmental or reproductive toxicity

Excessive maternal caffeine exposure during pregnancy is cataractogenic for neonatal crystalline lenses in rats: a biomicroscopic and histopathologic study.
Evereklioglu C, Guldur E, Alasehirli B, Cengiz B, Sari I, and Pirbudak L.
Acta Ophthalmol Scand 2004;82(5):552-6.

PURPOSE: To investigate histologically the influence of maternal caffeine exposure during pregnancy in vivo on crystalline lenses in neonatal rats. METHODS: Experimentally naive, female Wistar-albino rats (200-220 g) were mated with adult male rats over 2 days for copulation. After confirming pregnancy with a vaginal smear method, 50 gravid rats (dams) were randomly divided into five groups (n = 10 in each), consisting of one control and four experimental groups. Groups 1, 2 and 3 experimental dams were treated with intraperitoneal (i.p.) caffeine at doses of 25, 50 and 100 mg/kg/day, respectively, during pregnancy from gestational day 9 through to day 21. Group 4 dams were treated with caffeine in distilled water in a gavage at a dose of 50 mg/kg/day. Group 5 control dams were given i.p. saline solution daily for the same period. After normal delivery, the eyes were examined by slit-lamp biomicroscopy. The neonates were then killed by decapitation at postnatal days 1 or 30 and the eyes removed for histopathologic investigation of the lenses. RESULTS: Group 1 and control eyes had normal anterior lens capsules with a single layer of anterior cuboidal epithelial cells, regularly oriented cortical and nuclear lens fibres, and a clear posterior lens capsule with no lining epithelial cells behind the equator. In the remaining groups, histopathologic findings suggesting cataractogenesis included eosinophilic degeneration, lens fibre cell swelling and liquefaction, central lens fibres with retained nuclei, and prominent epithelial cells lining the posterior lens capsule behind the equator. Moreover, some lenses in group 3 had immature cataract on slit-lamp biomicroscopic examination at postnatal day 30. CONCLUSION: Excessive maternal caffeine exposure during pregnancy had cataractogenic effects on developing crystalline lenses in newborn rat eyes, both macroscopically and histopathologically. If an appropriate dose of caffeine can be identified, caffeine-induced cataract formation may be used as a new experimental cataract model in animal studies.

High dose of caffeine administered to pregnant rats causes histopathological changes in the cornea of newborn pups.
Evereklioglu C, Sari I, Alasehirli B, Guldur E, Cengiz B, Balat Z, and Bagci C.
Med Sci Monit 2003;9(5):BR168-73.

BACKGROUND: Caffeine is frequently used during pregnancy and associated with teratogenic effects, such as low birth weight, heart and digital defects, cleft palate and abortion with fetal loss. This study investigated histopathologically the effects of caffeine

on neonatal rat cornea. MATERIAL/METHODS: Fifty pregnant Wistar-Albino rats (dams) were randomly divided into five groups, one control and four experimental. Between day 9 and 21 of gestation, group 1 dams (control, n=10) were exposed to intraperitoneal (i.p.) SF daily until delivery. Group 2 (n=10), group 3 (n=10) and group 4 (n=10) dams were treated with i.p. caffeine at doses of 25, 50 and 100 mg/kg/d, respectively, for the same period. Group 5 dams were given caffeine in distilled water in a gavage at a dose of 50 mg/kg/d during the same period. After normal delivery, the litters were killed at postnatal day 1 or 30 and the eyes were enucleated for corneal histopathologic investigation. RESULTS: Control and group 1 eyes had normal corneal epithelium, regular stromal fibers, descement membrane and monolayer inner corneal endothelium. The remaining experimental litters demonstrated changes, such as vacuolated endothelial cells with proliferation, hyperchromasia, polymorphism, endothelial cell agenesis, increased stromal mitotic activity and focal increase in corneal thickness with widely separated corneal lamellae in the injured area. These changes occurred most often in the litters treated with high doses of caffeine. CONCLUSIONS: Excessive gestational caffeine intake has been shown histopathologically to have some teratogenic effects on newborn rat cornea.

Interactions of caffeine and restraint stress during pregnancy in mice.
Albina ML, Colomina MT, Sanchez DJ, Torrente M, and Domingo JL.
Exp Biol Med (Maywood) 2002;227(9):779-85.

The maternal and developmental toxicity of combined exposure to restraint stress and caffeine was assessed in mice. On gestational Days 0-18, three groups of plug-positive females (n = 13-15) were given by gavage caffeine at 30, 60, and 120 mg/kg/day. Three additional groups received the same caffeine doses and were restrained for 2 hr/day. Control groups included restrained and unrestrained plug-positive mice not exposed to caffeine. All animals in the group concurrently exposed to 120 mg/kg/day of caffeine and restraint died during the experimental period. In the remaining groups, cesarean sections were performed on Day 18 of gestation, and the fetuses were weighed and examined for external, internal, and skeletal malformations and variations. Although maternal and embryo/fetal toxicity were observed at all caffeine doses, the adverse maternal and developmental effects were significantly enhanced in the groups concurrently exposed to caffeine and restraint. It was especially remarkable at 60 and 120 mg/kg/day. The results of this study suggest that maternal and developmental toxic effects might occur if high amounts of caffeine were consumed by women under a notable stress during pregnancy.

Maternal caffeine consumption during pregnancy does not affect preimplantation development but delays early postimplantation growth in rat embryos.
Jacombs A, Ryan J, Loupis A, and Pollard I.
Reprod Fertil Dev 1999;11(4-5):211-8.

Models for studying prenatal drug-induced intrauterine growth retardation (IUGR) have, without exception, measured growth-related factors in the postimplantation embryo, fetus or neonate. Therefore, it is not known whether effects of drug exposure on growth and metabolism begin early in the preimplantation embryo, or whether IUGR is exclusively a postimplantation phenomenon. The present study investigates whether caffeine, a drug known to induce a dose-dependent fetal IUGR, affects embryo development before and/or after implantation or is exclusively a fetal phenomenon. Preimplantation embryo assessment (with treatment from Days 2 to 4 of pregnancy) included glucose utilization, cell number evaluation and stage of development (morula to hatched blastocyst); whereas, postimplantation embryo assessment (treatment from Days 2 to 10, 10.5 or 11 of pregnancy) included somite number evaluation and extent of neural tube closure, as seen using scanning electron microscopy. Comparing control preimplantation embryos with those exposed to 30 and 60 mg kg(-1) caffeine did not reveal any effects of caffeine exposure, as assessed on Day 5 of gestation. However, postimplantation embryo development assessed on Day 12 of gestation revealed that caffeine exposure of 15 and 30 mg kg(-1) significantly reduced, at both dosage levels, somite number and the extent of neural tube closure. In addition, comparisons of control and experimental groups revealed that in the high-dose caffeine group the forebrain cavity was significantly enlarged and bounded by a reduced, irregularly aligned neuroepithelium. The findings suggest that IUGR is a phenomenon first identifiable during late postimplantation embryogenesis and continues in fetal life.

Effects of preconceptual caffeine exposure on pregnancy and progeny viability.
Pollard I, Murray JF, Hiller R, Scaramuzzi RJ, and Wilson CA.
J Matern Fetal Med 1999;8(5):220-4.

OBJECTIVE: A previous study demonstrated for the first time that a drug such as caffeine, administered prior to ovulation and genomic activation, causes a quantitative difference in growth-promoting energy utilization in a proportion of 5-day-old blastocysts. The objective of the present study was to investigate whether developmental changes induced by caffeine administered throughout the estrus cycle prior to fertilization are sustained throughout pregnancy and after birth. METHODS: Caffeine was administered to rats throughout the estrus cycle prior to fertilization, with control and experimental groups subdivided into preimplantation and postimplantation categories. Preimplantation fertilization rate was assessed on day 4 of pregnancy by a pregnancy-induced elevation in maternal plasma progesterone concentration, or by flushing each uterine horn on day 5 of pregnancy to determine the presence or absence of a litter. Postimplantation fetuses were collected on gestational day 12 or allowed to go to term.

RESULTS: Preconceptual caffeine exposure significantly reduced maternal fertility by the failure of a proportion of the litters to implant, rather than curtailing preimplantation development or postimplantation losses. Postnatal mortality between weeks 0 and 1 was elevated and the weekly incremental growth rate of the pups from week 3 through week 7 was significantly reduced in the preconceptually caffeine-treated offspring. Experimental females reached puberty at the same age as the controls but at a significantly lower body weight. Gestation length, hirthweight, litter size, sex ratio, and anogenital distance (a measure of prenatal androgenization) were not affected by preconceptual caffeine treatment. CONCLUSIONS: It was concluded that the reduced fertility rate in preconceptually caffeine-exposed rats was due to the failure of litters to implant rather than to a reduced fertilization rate, which was normal. It was further concluded that the growth rate over the neonatal and prepubertal periods of surviving pups in the caffeine-treated group was subnormal.

[Behavioral-teratological effects of caffeine on mice].
Yin W, Li H, and Peng L.
Wei Sheng Yan Jiu 1998;27(2):116-8.

Pregnant ICR mice were exposed to caffeine at levels of 0, 4, 20 and 100 mg/kg during pregnancy. The behavioral-teratological tests indicated that the retardation of development, the obstruction of early reflexes and sensation and decline of learning ability existed in the offsprings of 20 and 100 mg/kg groups. The number of the dead fetus increased in the group of 100 mg/kg caffeine treated mice. No morphological changes were found.

Teratopharmacological and behavioural effects of coffee in mice.
Ajarem JS and Ahmad M.
Indian J Pharmacol 1996;28(1):16-24.

The possible relationship between coffee exposure during pregnancy and the teratopharmacological effects on the developing neonates was evaluated in the albino mice. The body weights of pups of treated dams were affected after birth and as the pups grew, their weight gains were lower compared to control. Such effect was more significant in both prepartum and perinatal treatment group (F(6) = 5.06, P less than 0.02) than the prepartum treatment alone (F(6) = 3.12, P less than 0.05). Body hair appearance and eye opening were delayed in the treated groups and likewise, the prepartum and perinatal treatment was more effective. Almost all behavioural indices studied for 'locomotory behaviour' were affected by all doses of coffee but only in the perinatally treated groups and such effects were neither time- nor dose-dependent. In the 'tube restraint test', the latency to first bite was decreased and the number of bites was increased in male offspring. However, in female offspring, these effects were vice-versa. The body weight of these offspring remain declined even at the adult/adolescent age in

the treated groups (F(6) = 9.89, P less than 0.005). The weight of male brain remained unaffected, but of females was decreased at 2 mg/kg dose only. The weight of liver and kidney of both sexes decreased only at the lower dose levels. The protein contents of these organs were also significantly affected by coffee treatment. These results suggest that coffee intake during pregnancy should be limited since it produces significant and lasting teratopharmacological and behavioural alterations in the offspring.

Demonstration of congenital anomalies in the joints of the forelimbs and hindlimbs caused by several pharmacological agents.
Erdogan D, Kadioglu D, and Peker T.
Anat Histol Embryol 1996;25(4):263-7.

In this study, fetal joint abnormalities caused by cytosine arabinoside, caffeine, sodium salicylate, and retinyl acetate administration during pregnancy, were investigated. In the cytosine-arabinoside-administered group, complete disappearance of joint spaces in the forelimbs, and narrowing or complete disappearance of joint spaces in the hindlimbs was highly noticeable. In the caffeine group, in all forelimb joints starting from art, humeri, there were abnormal fusions in bones, together with occasional disappearance of the joint space. In hindlimbs, similar findings were observed. In the sodium salicylate group, the complete disappearance of joint space and surfaces among humerus-radius and ulna was striking, and occasional fusions in tarsometatarsal joints were also present. Severe narrowing of the same joint space in the retinyl acetate group was striking. Total disappearance of the articulation manus and carpometacarpal joints was observed, together with hindlimb joint and bone findings.

Effects on elevated plus-maze behavior of exposure to caffeine during both gestation and lactation.
Hughes RN and Loader VG.
Psychobiology 1996;24(4):314-9.

Eight to 9 months after exposure to gestational and lactational doses of either 26/45 (low dose) or 25/35 (high dose) mg/kg/day of maternally ingested caffeine (via drinking water), rats were observed in an elevated plus-maze. The highest level of caffeine exposure decreased total rearing and increased immobility, and increased entries and occupancy of the open arms. Differences between the open and the preferred enclosed arms in frequencies of entries, occupancy, and walking were also greatest for rats exposed to the highest level of caffeine, and adrenal gland weights relative to body weight were highest for males in this group. Although some of the results support earlier reports of long-lasting heightened emotional reactivity following perinatal caffeine exposure, others did not. It was suggested that preexperimental and testing procedures may have provided a situation less aversive than those in which perinatal caffeine effects have been observed previously. Treatment effects on open-arm behavior might have

arisen from impaired spatial ability.

Hormonal and histological effects of chronic caffeine administration on the pituitary-gonadal and pituitary-adrenocortical axes in male rabbits.
Ezzat AR and el-Gohary ZM.
Funct Dev Morphol 1994;4(1):45-50.

Daily administration of caffeine (30 or 60 mg/kg) to mature male rabbits for four consecutive weeks caused an increase in the plasma follicle stimulating hormone (FSH) and a decrease in the luteinizing hormone (LH). Testosterone was increased with the higher dose only while adrenocorticotrophic hormone (ACTH) was not altered by either one. These results suggest that the effects of caffeine on the two gonadotrophic hormones, FSH and LH, involve two separate pathways. The light microscope study revealed reduced sizes of the seminiferous tubules, inhibited spermatogenesis, fatty degeneration of the liver and hepatic lesions. The adrenal glands exhibited signs of stimulated steroidogenesis. It is concluded that long term intake of caffeine induces suppression of spermatogenesis mainly through inhibition of FSH release and this effect is maintained even in the presence of normal or high levels of testosterone and LH.

In utero caffeine exposure affects feeding pattern and variable ratio performance in infant monkeys.
Gilbert SG and Rice DC.
Fundam Appl Toxicol 1994;22(1):41-50.

Adult female monkeys (Macaca fascicularis) were exposed via their drinking water to 0.0, 0.15, or 0.35 mg/ml of caffeine prior to and throughout pregnancy. Caffeine exposure resulted in a dose-related increase in the number of infant deaths at parturition. Additional females were added to the control group and the original groups were rebred, which resulted in 16, 13, and 9 live infants in control, low-dose, and high-dose groups, respectively. During pregnancy the mean serum caffeine levels were 3.4 and 10.3 micrograms/ml and the mean serum theophylline levels were 6.6 and 12.9 micrograms/ml for the low-dose and high-dose groups, respectively. Infants were separated from their mothers at birth and reared in a primate nursery to facilitate evaluation of infant performance on a variety of behavioral tasks. Infant formula consumption was monitored by a computer-based system 19 hr per day until 30 days of age. At 30 days of age infants were trained to press a button for a formula reward, after which they performed on a variable ratio schedule for a 14-day period. Monitoring of feeding pattern revealed that the treated infants spent significantly more time feeding than controls. On the variable ratio schedule, the high-dose group had consistently longer pause times and longer interresponse times than the control group. The results from this study indicate that in utero exposure to caffeine and its metabolites results in altered behavioral patterns in infant monkeys.

In utero exposure to caffeine causes delayed neural tube closure in rat embryos.
Wilkinson JM and Pollard I.
Teratog Carcinog Mutagen 1994;14(5):205-11.

We have investigated the effect of caffeine on embryo growth and development. Caffeine (25 mg/kg) was administered on gestation day (g.d.) 8-9 and the embryos examined histologically 24 h after the final dose. The crown-rump length of caffeine treated embryos (1.92 +/- 0.08 mm) was significantly smaller ($P < 0.001$) than the controls (2.91 +/- 0.26 mm) as was the circumferential length (caffeine vs. controls, 3.79 +/- 0.16 mm vs. 6.03 +/- 0.61 mm; $P < 0.001$). Additional measures, such as development of the heart, eye, and limb buds, were also reduced in the caffeine treated embryos. The most striking difference between the control and caffeine treated embryos was the larger proportion of treated embryos with regions of open neural tube. This was most marked in the caudal region of the embryos where 91% of treated embryos had regions of open neural tube compared with 14% of controls. The amount of open neural tube in any individual caffeine treated embryo did not relate to the crown-rump or circumferential length of that embryo nor was the effect restricted to particular litters. These results indicate that caffeine had a significant effects on embryonic growth and development.

The effect of prenatal caffeine exposure on the enamel surface of the first molars of newborn rats.
Falster AU, Yoshino S, Hashimoto K, Joseph F Jr, Simmons WB, and Nakamoto T.
Arch Oral Biol 1993;38(5):441-7.

Timed-pregnant rats were randomly divided into three groups at day 7 of gestation. A caffeine tablet was implanted subcutaneously in the experimental group and a placebo tablet in the control group. The third group was used as surrogate dams. At birth, eight randomly selected pups born either to the experimental or control dams were placed with surrogate dams that had produced pups on the same day; these were then used in either the experimental or the control group, and so the surrogate dams raised pups that came from either the experimental or control group. At day 22 after birth, the pups were killed, and their first and second maxillary and mandibular molars were removed. They were then placed in a specially designed chamber and exposed to an acid solution. The outflowing acid solution was collected every 20 min and up to 80 min. Then the calcium, phosphorus, and magnesium contents of each fraction were measured. The enamel surfaces of untreated and acid-treated first molars of the caffeine and control groups were examined by scanning electron microscopy. Untreated teeth were powdered and separated into enamel and dentine. Pure enamel samples were examined with a Gandolfi X-ray powder camera to measure the crystallite size. At 20-, 40- and 60-min intervals, calcium and phosphorus contents of the first molars of the caffeine group were significantly higher than those of the control. The enamel surface of the untreated and

acid-treated first molars of the caffeine group had significantly different scanning microscopic appearances from those of the non-caffeine untreated and acid-treated control groups.(ABSTRACT TRUNCATED AT 250 WORDS)

Caffeine exposure in utero increases the incidence of apnea in adult rats.
Tye K, Pollard I, Karlsson L, Scheibner V, and Tye G.
Reprod Toxicol 1993;7(5):449-52.

Caffeine abuse during pregnancy may be a factor in the development of long-term breathing abnormalities. Therefore, the objective of the present study was to monitor adult breathing patterns after in utero exposure to caffeine. This was done by isolating episodes of apnea of more than 6-s duration from the breathing data as obtained by the Cotwatch breathing monitors adapted for rat use. The breathing record obtained over 6 consecutive days was expressed as daily weighted apnea-hypopnea density (WAHD) values. It was shown that administration of caffeine in moderate (30 mg/kg daily) or high (60 mg/kg daily) doses throughout gestation resulted in a significant dose-dependent increase in the WAHD value. The experimental offspring were significantly growth retarded in utero and their subsequent growth rates were also affected. The caffeine-exposed pups grew more slowly with growth plateauing at the same age, resulting in smaller adults. A link was suggested between infants with apnea of prematurity, when occurring after the first week, and an increased risk for later apnea and sudden infant death syndrome.

Caffeine-mediated effects on reproductive health over two generations in rats.
Pollard I and Claassens R.
Reprod Toxicol 1992;6(6):541-5.

The present study was designed to investigate the mechanism(s) underlying previously observed birth weight differences found in the first litter of the second (F2) generation bred from caffeine-exposed F1 females. The effect of exposure to caffeine in utero on subsequent sexual receptivity, fertility, gestation length, parturition, nesting activity, maternal behaviour, and reproductive senescence in the F1 mothers, and the viability of the F2 offspring was investigated. This information was collected by breeding control or caffeine exposed females for 8 consecutive litters. It was demonstrated that exposure to caffeine did not affect the sexual receptivity, fertility, gestation length, or maternal behaviour of the F1 females, but parturition was prolonged and the viability of the F2 generation was seriously jeopardized. Many F2 pups were born significantly larger than their control counterparts and a significant proportion of litters (after the first two litters) were wholly stillborn. It was concluded that a changed genetic program, mediated via the F2 fetus, delayed the normal progression of parturition. This, in turn, compromised the F1 mothers and caused increased mortality of their offspring. The severity of the outcome was dose dependent.

Somatic development of the infant monkey following in utero exposure to caffeine.
Gilbert SG and Rice DC.
Fundam Appl Toxicol 1991;17(3):454-65.

Caffeine has been associated with a number of reproductive and developmental effects in animals and humans. In an effort to characterize the potential effects of caffeine on the developing infant, 40 adult female monkeys (Macaca fascicularis) were randomly divided into three groups and exposed at 0, 0.15, and 0.35 mg/ml (equivalent to 0, 10-15, and 25-30 mg/kg/day, respectively) of caffeine via their drinking water, before, during, and after pregnancy. Maternal blood and milk samples were collected following parturition. Infants were separated from their mothers within 12 hr of birth and were reared in a primate nursery. Blood samples were collected during the first week of life, and body weight, somatic measurements, and food consumption data were collected throughout the first 3 years. Maternal blood and milk concentrations and infant blood concentrations of caffeine and theophylline (the major blood metabolite of caffeine in the monkey) were similar to one another. Infant half-life of these methylxanthines was longer than that of the adult but not as long as that observed in human infants. Body weights and somatic measurements of male infants were significantly reduced over the first 30 days, as were a number of initial somatic measurements in both male and female infants. These deficits were not evident after 1 year of age. There were no treatment-related effects on infant tooth eruption or milk consumption. Results from this study support previously published results from this group of monkeys as well as studies by other researchers in rodents indicating that caffeine consumption during pregnancy can alter infant somatic development.

Behavioral effects of exposure to caffeine during gestation, lactation or both.
Hughes RN and Beveridge IJ.
Neurotoxicol Teratol 1991;13(6):641-7.

Open-field behavior and latencies of emergence from a darkened chamber to a brightly lit arena were recorded at 1, 2, 4 and 6 months after birth in male and female rats that had been exposed to 26 or 45 mg/kg/day caffeine ingested by dams in their drinking water during gestation, 25 or 35 mg/kg/day during lactation or to the two low or high doses ingested during both gestation and lactation. One or both of the gestational or lactational doses reduced locomotor activity and increased defecation in the open field at all ages for males only. Rearing was decreased for both sexes by 25 mg/kg/day lactational caffeine. Numbers of rats that failed to or took longer than 1 min to emerge into the brightly lit arena were increased by 26 mg/kg/day gestational caffeine. All rats that had been exposed to either dose combination of caffeine during both gestation and lactation showed less locomotor and rearing activity, reduced tendencies to emerge within 1 min and, at 6 months of age only, more defecation in the open field. It was concluded that the effects of gestational and lactational exposure to caffeine were additive in their modification of the developing brain as reflected in decreased motor activity possibly

arising from heightened emotional reactivity to the testing situation. Hypersensitivity of males to caffeine exposure during either gestation or lactation separately seemed to diminish when exposure was increased for all rats through experience of the drug during both gestation and lactation. Possible involvement of caffeine-induced increases in adenosine receptors in the type of results obtained was discussed.

Prenatal exposure to AVP or caffeine but not oxytocin alters learning in female rats.
Swenson RR, Beckwith BE, Lamberty KJ, Krebs SJ, and Tinius TP.
Peptides 1990;11(5):927-32.

Rats whose mothers had been treated with 1 microgram of arginine vasopressin (AVP) or oxytocin (OXT), 15 mg of caffeine, or saline on days 13-19 of gestation were given training on a passive avoidance response as adults. Female rats whose mothers had been exposed to either AVP or caffeine demonstrated enhanced retention of the response. No effects were found for male rats or for exposure to oxytocin. These results suggest that prenatal exposure to AVP or caffeine produced sexually dimorphic effects on learning and that the effects are specific to the structure of AVP.

Combined effects of radiation and caffeine on embryonic development in mice.
Kusama T, Sugiura N, Kai M, and Yoshizawa Y.
Radiat Res 1989;117(2):273-81.

The combined effect of radiation and caffeine has been studied in mouse embryos. Radiation and/or caffeine were administered to ICR mice on Day 11 of gestation. Intrauterine death, gross malformation, and fetal body weight were selected as indicators of effects. Doses of whole-body gamma irradiation were 0.5 to 2.5 Gy and those of caffeine were 100 and 250 mg/kg maternal body wt. Intrauterine mortality increased with increasing radiation dose; this trend was more remarkable in combination with caffeine. Gross malformations such as cleft palate and defects of forelegs and hindlegs appeared frequently in the fetuses treated with both radiation and caffeine. Decreased fetal weight was observed even in mice treated with 0.5 Gy of radiation or 100 mg/kg caffeine. There was a linear relationship between dose and reduction of fetal weight. The fetal weight was a sensitive, precise, and easy-to-handle indicator for the effects of growth retardation. Intrauterine mortality and frequencies of cleft palate and defects of forelegs and hindlegs were higher than the sum of those induced by radiation and by caffeine separately. The results indicated that the combined action of radiation and caffeine on intrauterine death and malformations was synergistic.

Adverse pregnancy outcome in the monkey (Macaca fascicularis) after chronic caffeine exposure.
Gilbert SG, Rice DC, Reuhl KR, and Stavric B.
J Pharmacol Exp Ther 1988;245(3):1048-53.

Caffeine and the related methylxanthine theophylline are consumed regularly by pregnant women. In a study originally designed to assess the neurotoxic potential of caffeine in the infant, 40 female monkeys (Macaca fascicularis) were divided into three groups and administered caffeine in their drinking water at concentrations equivalent to 0, 10 to 15 or 25 to 35 mg/kg/day of caffeine 7 days a week. After a period of adaptation to caffeine these monkeys were mated with untreated males. Reproductive failure in the form of stillbirths and miscarriages was observed in the treated groups. Subsequently, 12 control monkeys and 1 low-dose monkey were added to the study and most of the original monkeys rebred. The second round of pregnancies confirmed that the treated monkeys had an increased rate of stillbirths and miscarriages. The precise cause of death of the stillborn infants could not be determined. Maternal weight gain and infant birth weights decreased in a dose-related manner. These results indicate that in utero exposure to methylxanthines (caffeine and/or its major metabolite theophylline) adversely affects pregnancy outcome in the monkey.

Prenatal caffeine causes long lasting behavioral and neurochemical changes.
Grimm VE and Frieder B.
Int J Neurosci 1988;41(1-2):15-28.

The effects of prenatal exposure to caffeine were studied on later physical development, behavior and brain neurochemistry. Daily doses (150, 300 or 450 mg/L) of caffeine were given to rat dams during the last week of pregnancy. Prenatal caffeine exposure resulted in a number of behavioral and neurochemical changes in the offspring which were long lasting and dose related. The low dose (150 mg/L) of prenatal caffeine caused hyperactivity in an open-field. The high dose of caffeine caused learning disabilities in complex visual and auditory discrimination learning paradigms while simple motor learning or a spatial orientation task were not affected. Both male and female offspring showed some behavioral effects of caffeine exposure. The medium and high doses of caffeine resulted in weight gain that was observable as early as 35 days of age and increased progressively with age. This weight gain was associated with increased food intake. The neurochemical studies carried out at 2-3 months of age revealed an increase in choline uptake in hippocampus, mainly in the animals treated with the lower doses of caffeine and higher protein concentration (microgram/mg wet tissue) in the cortex or hippocampus of offspring exposed to the higher doses of caffeine. At 15 months of age, choline uptake in the frontal cortex was significantly reduced in the animals prenatally exposed to the 300 and 450 mg/L dose.

Male mediated caffeine effects over two generations of rats.
Pollard I and Smallshaw J.
J Dev Physiol 1988;10(3):271-81.

Caffeine exposure of a male rat prior to mating affected his progeny and the progeny of a second generation. The dose chosen, 30 mg/kg per day given orally, was approximately equivalent to a caffeine intake of 10-12 cups of brewed coffee daily. In the first (F1) generation caffeine consumption of the sires for a minimum period of 15 days prior to mating with drug naive females, caused significant fetal growth retardation of both sexes and an increased postnatal mortality of pups between weeks 1 and 2, many of which displayed characteristics of runts. Persistent caffeine effects were also found in a second (F2) generation obtained by back breeding male and female F1 offspring from control and treated groups. The F2 pups of both sexes, from the female breeding line, were born significantly heavier when compared with their control counterparts. In the male breeding line, 33% of the litters conceived were aborted in utero, and among the young F2 pups born runts were again evident. At the conclusion of the breeding for the first generation the testes of the FO sires were studied after they received caffeine for 38 consecutive days. The experimental testes showed a marked degeneration characterized by significant overall size reduction, breakdown of the germinal epithelium, accumulation of cellular debris in the lumen of the seminiferous tubules, and significant reduction in the abundance of mature spermatozoa. On ultrastructural examination there appeared to be genetic damage to the spermatozoa where nucleic cysts and pouches were seen.

Potential reversibility of skeletal effects in rats exposed in utero to caffeine.
Collins TF, Welsh JJ, Black TN, Whitby KE, and O'Donnell MW Jr.
Food Chem Toxicol 1987;25(9):647-62.

Groups of Osborne-Mendel rats, killed at three time intervals following mating, were studied to determine whether prenatal skeletal ossification delays observed following low-level caffeine administration represent transient or persistent ossification problems. Group A litters were killed on gestation day 20; group B neonates were killed on post-natal day 0; and group C pups were killed on post-natal day 6. Within each group, dose levels of 0, 0.018, 0.036 or 0.07% caffeine in distilled water were available ad lib. to groups of 30-60 dams from gestation day 0 to day 20. Average daily caffeine consumption was 24.7-29.0 mg/kg body weight for the 0.018% group, 42.7-48.8 mg/kg body weight for the 0.036% group and 70.6-75.1 mg/kg body weight for the 0.07% group. In group A litters, the mean number of viable foetuses was significantly less in the mid-dose and high-dose animals than in the controls. In the same group, the average number of foetuses per litter with at least one sternebral ossification delay was increased significantly in all treated groups and the average number of foetuses per litter with at least two sternebral variations was significantly increased in the mid- and high-dose groups. The percentages of litters containing foetuses with at least two and at least three sternebral variations and the average number of foetuses per litter with at least three

sternebral variations were significantly increased only in the high-dose group. Foetuses from the mid- and high-dose groups also had significant increases in certain skeletal defects, namely missing centra and reduced ossification of the dorsal arch. Foetuses from the high-dose group also had significant increases in bipartite supraoccipital, and reduced ossification of the hyoid, metacarpals and metatarsals. In group B, day 0 neonates from all treated groups showed a significantly increased incidence of delayed sternebral ossification (average number of foetuses per litter with one or more missing, incomplete or bipartite sternebrae). The percentages of litters containing neonates with delayed sternebral ossification were also increased significantly in all treated groups. Neonates from the 0.07% level in group B also exhibited a significant increase in the incidence of supernumerary rib bud, and in reduced ossification of the metacarpals, metatarsals and calcaneus bones. Significant increases were also seen, in group B, in the low- and mid-dose animals, respectively, in supernumery rib bud and in reduced ossification of the metatarsals.(ABSTRACT TRUNCATED AT 400 WORDS)

Caffeine induces cardiac and other malformations in the rat.
Matsuoka R, Uno H, Tanaka H, Kerr CS, Nakazawa K, and Nadal-Ginard B.
Am J Med Genet Suppl 1987;3:433-43.

At various gestational periods, caffeine was injected intra-arterially or intraperitoneally into pregnant rats. The teratogenic effects of caffeine on the fetal heart were dose dependent and detectable at relatively low concentrations. The most susceptible stage was during septation of the heart. The most common cardiovascular malformation was ventricular septal defect. Extracardiovascular anomalies, such as decreased thymic weight and degeneration of the lens, were found in all fetuses; skeletal malformations were found in some fetuses.

Effects of caffeine administered during pregnancy on fetal development and subsequent function in the adult rat: prolonged effects on a second generation.
Pollard I, Jabbour H, and Mehrabani PA.
J Toxicol Environ Health 1987;22(1):1-15.

Caffeine, when administered in moderate (30 mg/kg X d) or high (60 mg/kg X d) doses during pregnancy, was shown to cause significant fetal growth retardation of both sexes. Mortality rate at or soon after birth was significantly higher and litter size significantly lower in the litters treated with 60 mg. The subsequent growth rates were also affected. The experimental pups grew more slowly, with growth plateauing at the same age resulting in smaller adults. The male offspring when subjected to short-term stress (one session) in adulthood showed an intact emergency response, demonstrating an adequate ability to react to a sudden environmental change. A significant decrease in 3 beta-hydroxysteroid dehydrogenase (3 beta-HSD) activity, and consequent reduction in testosterone biosynthesis, in the fetal testes at d 18 and 20 of gestation was also found for

both doses of caffeine. Low 3 beta-HSD activity persisted to adulthood in the group receiving 60 mg. Lingering effects were observed in a second litter bred 8 wk after the discontinuation of caffeine consumption. In this second breeding, the offspring of both sexes from both caffeine doses were born significantly smaller when compared to the controls. Persistent effects of caffeine were also found in second-generation rats bred from females who were exposed to caffeine in utero. The pups of both sexes were born significantly heavier after a significantly longer gestation. The subsequent growth did not differ from that of the controls. It was suggested that a changed genetic program in the ovarian germ cells of the first generation and/or a changed uterine environment in the second generation may be implicated.

Effects of administering caffeine to pregnant rats either as a single daily dose or as divided doses four times a day.
Smith SE, McElhatton PR, and Sullivan FM.
Food Chem Toxicol 1987;25(2):125-33.

From day 6 to day 20 of pregnancy, rats were treated with caffeine in a total daily dose of 10 or 100 mg/kg by gavage, either as a single bolus dose or as four divided doses given at 3-hr intervals throughout the day. Controls were given distilled water at the same times. Maternal body weight and food and water consumption were reduced in the two groups receiving a total of 100 mg caffeine/kg/day and in the group given 2.5 mg/kg four times daily. Dose-related decreases in foetal weight, placental weight and crown-rump length and dose-related retardation of skeletal ossification were observed. Major foetal abnormalities, mainly ectrodactyly, were seen only in the group given 100 mg caffeine/kg in a single daily dose.

Studies on the embryotoxic risk of exposure to caffeine and ethanol during the preimplantation period in the mouse.
Spielmann H, Kruger C, Granata I, Tenschert B, and Vogel R.
Arzneimittelforschung 1987;37(7):819-22.

Pregnant mice were exposed before implantation to caffeine and ethanol to determine the dose-response relation for embryolethality during the preimplantation period. For risk estimation the embryotoxicity was evaluated at term and also 24 h after implantation. For ethanol no embryotoxic risk could be detected. Caffeine unexpectedly exhibited a high risk for embryoethality when compared to the maternal LD50. However, when taking into account realistic exposure levels an embryotoxic risk in early pregnancy can be excluded in humans for both caffeine and ethanol.

Effects of maternal caffeine ingestion on the perinatal cerebrum.
Tanaka H, Nakazawa K, and Arima M.
Biol Neonate 1987;51(6):332-9.

The cerebra of fetal rats from dams given 0.04 or 0.02% caffeine in drinking water ad libitum before and/or during pregnancy were examined on gestational day 21. A low placental weight was induced by caffeine ingestion for a long time throughout premating and pregnancy. A greater reduction in the fetal weight of the cerebrum than that of the body was observed with caffeine ingestion during pregnancy of levels of 1.5-3.0 micrograms caffeine/ml or g wet weight in dams and fetuses. In the cerebra of offspring, the levels of caffeine and theophylline did not change for 4 h after birth, and theophylline was not detected at all after intraperitoneal injection of caffeine. Thus, maternal caffeine should be warned against the fetal cerebral function.

Effect of protein malnutrition and maternal caffeine intake on the growth of fetal rat brain.
Yazdani M, Tran TH, Conley PM, Laurent J Jr, and Nakamoto T.
Biol Neonate 1987;52(2):86-92.

Pregnant dams were divided into two subgroups on day 10 of gestation. Half were fed a 20% protein diet and the other half an 8% protein diet. A second group also subdivided was pair-fed with rats of the first group. Their diet was supplemented with caffeine in amount calculated to provide daily doses of 2 mg/100 g body weight. On days 18, 20, and 22 randomly selected dams were injected with 3H-thymidine intraperitoneally and 1.5 h later their fetuses were delivered surgically in order to determine the rate of DNA synthesis along the gestation. The rest of the fetuses were delivered surgically on day 22. Pups' brains were rapidly removed and DNA, RNA, protein and 3H-thymidine uptake were studied. Average body weights of the fetuses in the caffeine-supplemented control group were smaller than those of the noncaffeine group. Effects of caffeine that were similar in both diet groups included a decrease in brain DNA content and concentration and an increase in brain protein content and concentration. However, the percent decrease and increase, respectively, was different depending on the nutritional status. DNA synthesis was not affected by malnutrition or caffeine supplementation on day 18 of gestation. Caffeine's effect on the rate of DNA synthesis was different on day 20 of gestation depending on nutritional status. Caffeine supplementation resulted in a decrease in DNA synthesis in both groups on day 22 of gestation. These data indicate that caffeine intake during pregnancy produces differential effects on fetal rat brain depending on dietary protein content.

Behavioral effects of prenatal exposure to caffeine in rats.
Hughes RN and Beveridge IJ.
Life Sci 1986;38(10):861-8.

For the first ten days of gestation, rats received daily intraperitoneal injections of 10-40 mg/kg of caffeine. Open field behavior of their fostered offspring was observed 61, 145 and 188 days after birth. While there were no obvious physical effects of the prenatal experience, at 61 days caffeine exposure led to an increase in the number of times seen walking for males only and increased ambulation (distance travelled) for both sexes. At 145 days occupancy of centre squares of the apparatus and latencies of emergence from a dark box into an illuminated arena were higher for caffeine-exposed males only. When 188 days old, rats exposed to 20 mg/kg of caffeine tended to exhibit less locomotor activity and more grooming behavior while spending more time in corners of the apparatus. Male rats prenatally exposed to 20 mg/kg of caffeine avoided the centre squares of the apparatus. It was concluded that prenatal caffeine had modified the development of mechanisms controlling voluntary motor activity in the youngest rats. However, at older ages, the prenatal effect was probably manifested as increased timidity or emotional reactivity. Males were often affected differently from females by the prenatal treatment.

Prevention of caffeine-induced limb malformations by maternal adrenalectomy.
Moriguchi M and Scott WJ Jr.
Teratology 1986;33(3):319-22.

Caffeine at high doses is a known rodent teratogen and induces limb malformations along with cleft palate in various strains of rats and mice. Fujii and Nishimura ('74) postulated that caffeine was teratogenic by virtue of catecholamine release from maternal or embryonic tissue. We tested this hypothesis by surgically removing the maternal adrenal gland on day 6 of pregnancy and then administering 175 mg/kg of caffeine intraperitoneally at 1600 h day 11 and 900 h day 12. The teratogenic effects of caffeine in adrenalectomized versus nonadrenalectomized AKR mice were assessed in day 18 fetuses. Thirty percent of the surviving offspring were malformed in caffeine-treated, nonadrenalectomized dams compared to 7% of the offspring from adrenalectomized dams. Therefore we believe caffeine teratogenesis is initiated by release of catecholamines from the maternal adrenal gland.

Effects of chronic ingestion of caffeine on mammary growth and reproduction in mice.
Nagasawa H and Sakurai N.
Life Sci 1986;39(4):351-7.

Normal mammary gland growth and reproduction were examined in C3H/HeMei mice given caffeine as drinking water (500 mg/l tap water) after weaning on day 20 of age. Caffeine ingestion had little effects on any of the day of vaginal opening, body growth, pattern of estrous cycles, mammary gland growth and reproduction except for the rearing rate on day 12 of lactation. Five out of 12 mothers given caffeine lost all pups before day 12 and resulted in 51% of the rearing rate on that day, while that of the control was 91%. The rate did not change thereafter in either group. The results indicate that the chronic heavy ingestion of caffeine would induce the high mortality of pups during early stage of lactation.

Postnatal neurobehavioral development in rats exposed in utero to caffeine.
West GL, Sobotka TJ, Brodie RE, Beier JM, and O'Donnell MW Jr.
Neurobehav Toxicol Teratol 1986;8(1):29-43.

Potential behavioral and teratogenic effects of caffeine were studied in Charles River CD albino rats. Caffeine in distilled water was given by gavage to pregnant rats (dams) at doses of 5, 25, 50 or 75 mg/kg on Days 3-19 of gestation. Concurrent controls received distilled water gavage (10 ml/kg) on the same days. Dams were allowed to deliver normally. Physical and behavioral observations were made on dams during gestation and lactation and on F1 offspring through 9 weeks of age. Caffeine decreased body weights and food intake and increased water intake in gestating dams but these effects dissipated during lactation. Spontaneous locomotor activity (PAC) and open field (OF) were increased immediately after caffeine gavage but not before. Parturition was slightly delayed. With analyses of data based on individual pups the following effects were noted. Pre- and post-weaning offspring body weights were decreased in females at 50 and 75 mg/kg and in males at 75 mg/kg. Incisor eruption was delayed in females at 5, 50 and 75 mg/kg and in males at all doses. Auditory startle developed earlier in the 5 mg/kg dose group but was delayed at 75 mg/kg for males only. Eye opening was delayed in both sexes at 25, 50 and 75 mg/kg. In females, vaginal opening was delayed at 5, 25 and 75 mg/kg and 9-week ovary weights were increased at 75 mg/kg. In postweaning males, food intake was decreased and water intake was increased with increasing dose. In males, PAC was decreased at 75 mg/kg only on Day 12. At 7 weeks of age, step-down passive avoidance was decreased at 5 and 25 mg/kg but increased at 50 and 75 mg/kg, and at 8 weeks of age, shuttlebox active avoidance was decreased with increasing dose. Maternal and offspring behaviors were only weakly correlated. Correction for litter effect in developmental data yielded fewer significant results and only at 50 and 75 mg/kg. The issue of whether it is always appropriate to correct for "litter effect" is discussed.

Effects of prenatal caffeine administration on offspring mortality, open-field behavior and adult gastric ulcer susceptibility.
Glavin GB and Krueger H.
Neurobehav Toxicol Teratol 1985;7(1):29-32.

Pregnant rats were given caffeine (0.0%, 0.017%, 0.034% or 0.05%) in their drinking water throughout gestation. Offspring were cross-fostered to non-caffeine-treated mothers at birth. A dose-related increase in offspring mortality was observed at 24 hr and at 10 days post partum. Prenatal caffeine exposure did not significantly influence open-field ambulation or defecation when tested at 48, 68, or 196 days of age. A significant dose-related increase in restraint-stress gastric ulcer susceptibility was detected at 200 days of age. Offspring from rats treated with 0.05% caffeine during pregnancy, developed significantly more frequent and significantly more severe gastric lesions than did offspring from control rats or from rats prenatally exposed to 0.017% and 0.034% caffeine. Prenatal caffeine exposure may: (1) predispose organisms to increased gastric disease susceptibility as adults and (2) interfere with neonatal feeding ability and thereby produce infant mortality.

Perinatal caffeine treatment: behavioral and biochemical effects in rats before weaning.
Peruzzi G, Lombardelli G, Abbracchio MP, Coen E, and Cattabeni F.
Neurobehav Toxicol Teratol 1985;7(5):453-60.

Administration of drinking water containing 0, 0.02%, 0.04% and 0.08% of caffeine to female rats throughout gestation and lactation affects several behavioral parameters in the offspring. Righting reflexes, swimming ability development, motor coordination and muscle tone were affected. The activity of these animals, as measured with an open-field test at weaning (i.e., at the end of the treatment), was reduced. The effects observed were dose-dependent. However, for righting reflexes the dose-dependency was direct (the highest dose producing maximal effects), whereas in all the other tests, the dose-dependency was inverse, the lowest dose producing maximal effects and the highest dose producing no effects. This might reflect the presence of subclasses of receptors having different affinities for adenosine, mediating opposite effects and antagonized by caffeine. On the other hand, perinatal caffeine effects are certainly not mediated by blockade of phosphodiesterases, since cAMP levels at the end of the treatment were dose-dependently reduced. This study shows therefore that administration of caffeine to rat dams is able to influence the neurobehavioral development of the offspring. Moreover, all the doses utilized and corresponding to 27, 58 and 108 mg/kg, were able to produce all or some of the mentioned effects, indicating that further testing with doses lower than 27 mg/kg is required to find a dose which does not affect the offspring.

Behavioral and physical development of rats chronically exposed to caffeinated fluids.
Butcher RE, Vorhees CV, and Wootten V
Fundam Appl Toxicol 1984;4(1):1-13.

Coffee and caffeine solutions were administered as the sole source of fluid to male and female Sprague-Dawley rats (F0) beginning 60 days before breeding and continuing until the litters (F1) from these animals were weaned. Treatments were administered as 100% brewed coffee (COF-100), and a 25% dilution of coffee (COF-25), together with solutions of caffeine in water that paralleled the caffeine content of the coffee groups, 0.056% caffeine (CAF-100) and 0.014% (CAF-25). Controls received measured amounts of plain water (CNL) and another group received vitamin A (40,000 IU/kg) on Days 7-20 of gestation (positive control treatment). During pregnancy all groups receiving COF and CAF consumed significantly more fluid than CNLs. Offspring from the COF-100 and CAF-100 dams were significantly lower in weight than CNLs. No abnormalities of reproductive performance were observed. Of 10 preweaning tests, COF-100 and CAF-100 litters displayed delayed incisor eruption, delayed swimming development, and altered activity. On 7 postweaning measures, these groups showed decreased running wheel activity and increased open-field ambulation and/or defecation. The CAF-25 group, by contrast, showed an increase in running wheel activity. Vitamin A (Vit-A) offspring showed multiple effects, including delayed incisor eruption, increased pre- and postweaning open-field activity, and reduced running wheel activity. COF and CAF produced effects on tests for psychoteratogenesis that appear consistent with the morphological consequences (delayed development) known to be associated with pre- and neonatal administration of caffeine, alone or in coffee, at high doses. The data indicate that most of the behavioral effects observed from caffeine exposure were consistent with the expected effects of concurrent administration of this agent, while the postweaning exposure effects suggest a longer-term change in activity. No effects of caffeine were found, however, on measures of learning, memory, or motoric functioning.

Comparative toxicities of dietary caffeine and theobromine in the rat.
Gans JH.
Food Chem Toxicol 1984;22(5):365-9.

Caffeine, incorporated into pulverized Purina Rat Chow at a concentration of 0.5%, was fed to male Sprague-Dawley rats for 7 or 8 wk and the effects were compared with those of 0.8% dietary theobromine, fed to male rats for 7 wk. Both dietary methylated xanthines produced significant decreases in food consumption and body-weight gain when compared to their respective control groups. Food consumption of caffeine-fed rats was 57.2% of controls and for theobromine-fed rats it was 77.9% of the respective controls. Theobromine produced significant decreases in thymus weights, with caffeine producing smaller decreases. The theobromine-fed rats showed severe testicular atrophy with extensive spermatogenic cell degeneration and necrosis, while the testes of rats fed

caffeine for 7 or 8 wk showed only scattered vacuolar degeneration of spermatogenic cells. Caffeine appears to be more potent than theobromine as an anorexic agent in rats, but to be equivalent to theobromine in its potential for inducing thymic atrophy and spermatogenic cell destruction with testicular atrophy.

Caffeine and its dimethylxanthines and fetal cerebral development in rat.
Tanaka H, Nakazawa K, Arima M, and Iwasaki S .
Brain Dev 1984;6(4):355-61.

The relationship between the distribution and pharmacokinetic behavior of caffeine and its dimethylxanthines in pregnant rats and fetuses and fetal cerebral development was compared in four groups with different modes of oral caffeine ingestion by the mothers. During the premating period and pregnancy, female Wistar rats were divided into 0.04% caffeine (C) and water (W) groups, respectively. When the groups are expressed as W or C before mating-W or C during pregnancy, the fetal body weight was low in the three caffeine-treated groups (W-C, C-W and C-C) and the fetal cerebral weight was the lowest in the W-C group. The mean concentration of caffeine or metabolites in maternal plasma, maternal liver, placenta and fetal cerebrum on gestational day (g d) 21 was increased in the W-C group compared to in the C-C group. The concentration of caffeine in fetal cerebrum was increased but that of metabolites was not, compared to the concentration of caffeine or metabolites in the placenta. Radioactivity in fetal cerebrum after intraperitoneal injection of 14C-caffeine was higher in the W-C group than in the other three groups. After intravenous injection of caffeine the apparent volume of distribution of caffeine in maternal plasma was markedly decreased in the W-C group, and the plasma molar concentration ratio of theophylline to caffeine was significantly increased in both the W-C and C-C groups. The adverse effect of maternal caffeine ingestion on the fetal cerebrum may be associated with the decreased apparent volume of distribution of caffeine in maternal plasma and the high caffeine content of fetal cerebrum.

A study of the teratogenic potential of caffeine ingested in drinking-water.
Collins TF, Welsh JJ, Black TN, and Ruggles DI.
Food Chem Toxicol 1983;21(6):763-77.

Caffeine dissolved in drinking-water was available ad lib. to Osborne-Mendel rats at dose levels of 0, 0.007, 0.018, 0.036, 0.07, 0.10, 0.15 or 0.20% during days 0-20 of gestation. The corresponding daily caffeine intakes were 0, 10.1, 27.4, 50.7, 86.6, 115.8, 160.9 and 204.5 mg/kg body weight. Dosages of 160.9 and 204.5 mg/kg were associated with decreased implantation efficiency, increased resorptions and decreased mean numbers of viable foetuses. Numbers of runts were significantly increased after dosages of 115.8-204.5 mg/kg/day. Foetal body weight and length were decreased and oedematous foetuses were increased at dosages of 86.6-204.5 mg/kg/day. Contrary to results seen after gavage studies, caffeine available ad lib. in drinking-water produced no dose-related

gross anomalies. Only two animals with missing or hypoplastic nails were produced, both in the 160.9-mg/kg group. Sternebral ossification deficiencies were increased at all dose levels except 10.1 mg/kg/day. Skeletal ossification deficiencies were increased in a dose-related manner at the four highest dose levels. Caffeine given by water bottle produced ossification deficiencies similar to those seen after intubation, but at higher dosages.

Caffeine-induced limb malformations: description of malformations and quantitation of placental transfer.
Scott WJ Jr.
Teratology 1983;28(3):427-35.

Caffeine was administered intraperitoneally to CD-1 mice on days 11 and 12 of pregnancy at doses of 80-250 mg/kg. A dose-related pattern of malformations was seen that included mainly cleft palate, limb malformations, and hematomas. Many of the limb malformations were examined in preparations stained for cartilage and bone and a number of unique structural arrangements were found. As in previous studies, an asymmetric response was seen, the left limbs being affected more often than the right. Transplacental passage of caffeine was also studied. Caffeine and many metabolites pass into the embryo and attain concentrations slightly below those in maternal plasma. A peak caffeine concentration of 1 mM is attained after a teratogenic dose, which is at least an order of magnitude greater than that of any of the metabolites.

Adverse effect of maternal caffeine ingestion on fetal cerebrum in rat.
Tanaka H, Nakazawa K, and Arima M.
Brain Dev 1983;5(4):397-406.

This study was undertaken to determine whether maternal caffeine ingestion is or is not a risk factor in fetal cerebral development using experimental rat models. Pregnant rats of the Wistar strain were given 0.04% caffeine in drinking water before and/or during pregnancy for various numbers of days. Control rats received water for the same periods. There was no reduction of maternal body weight, fetal body weight or fetal total brain weight. Low fetal cerebral weight and placental weight were observed when dams were given caffeine before mating for long times and/or throughout pregnancy. DNA, RNA and protein contents per cerebrum were also reduced in fetuses from dams given caffeine throughout pregnancy or for the last 6 gestational days. Cerebral DNA and protein contents as expressed per wet weight were higher and significantly lower respectively in the fetuses from dams given caffeine throughout pregnancy when compared to controls. Activity of thymidine kinase was not significantly decreased in caffeine-treated fetuses. There was a positive correlation between maternal serum and fetal cerebral caffeine levels. Additionally a negative correlation between maternal caffeine levels and fetal survival rates which decreased in litters from dams given caffeine throughout pregnancy was demonstrated. Our rat model indicates maternal caffeine ingestion during pregnancy

is associated with reduction of fetal cerebral weight and protein content without reduction of body weight.

Studies on the teratogenic effects of different oral preparations of caffeine in mice.
Elmazar MM, McElhatton PR, and Sullivan FM.
Toxicology 1982;23(1):57-71.

Caffeine in doses up to 250 mg/kg per day in drinking water or up to 150 mg/kg per day in sustained release pellets was administered to pregnant mice. Apart from a low incidence of cleft palate, in the 50 mg/kg and 150 mg/kg caffeine pellet groups no gross abnormalities were observed which were attributable to caffeine treatment. The most important effect observed was a reduction in fetal weight. Retarded ossification particularly of the supraoccipital bones was observed in fetuses when caffeine (150 mg/kg) was administered in drinking water but not when the same dose was given as a sustained release pellet. Analysis of caffeine blood level data showed that the total exposure from the pellets was greater than from the drinking water. It would thus appear that the effect on the supraoccipital bones is an indirect one mediated through reduced food and water intake of the dams when caffeine is administered in drinking water.

Coffee consumption during pregnancy: subsequent behavioral abnormalities of the offspring.
Groisser DS, Rosso P, and Winick M.
J Nutr 1982;112(4):829-32.

Offspring of rats fed coffee during pregnancy had reduced body, liver, and brain weight at birth. By 30 days postnatally these animals had recovered in size but exhibited increased locomotion, decreased grooming time, and decreased time spent with a novel object. Offspring of dams fed decaffeinated coffee demonstrated reduced liver weight at birth and similar behavioral characteristics at 30 days of age.

Caffeine: effects of acute and chronic exposure on the behavior of neonatal rats.
Holloway WR Jr.
Neurobehav Toxicol Teratol 1982;4(1):21-32.

The behavioral responses of 1- and 10-day-old rats to caffeine, a central nervous system stimulant, were determined using several behaviors readily exhibited by the neonatal rat. In pups of both ages activity as well as attachment latencies in the on-nipple suckling test increased, while weight gain and attachment frequencies in on-mother and on-nipple suckling tests decreased. In addition, the home orientation of 10-day-old rats was disrupted. Similar effects were found in 1-day-old pups exposed to theophylline. Long term exposure to caffeine during gestation (1-day-old pups) or on days 1--9 of lactation

(10-day-old pups) increased the pups' activity levels and altered the activity increase observed following an acute caffeine challenge. The absence of a differential response to theophylline indicated these changes were specific to caffeine. These results indicate that biologically relevant behaviors can be used to assess behavioral alterations in the neonate which arise from prenatal or early postnatal exposure to toxic substances.

Blood levels of caffeine and results of fetal examination after oral administration of caffeine to pregnant rats.
Ikeda GJ, Sapienza PP, McGinnis ML, Bragg LE, Walsh JJ, and Collins TF.
J Appl Toxicol 1982;2(6):307-14.

Pregnant FDA-strain Osborne-Mendel rats were administered repeated doses of caffeine by oral intubation (gavage) and by administration in the drinking water (ad libitum sipping). When [1-methyl-14C]caffeine was administered at a dosage of 80 mg per kg per day by ad libitum sipping on days 12 to 15 of gestation, the amounts of radioactivity in blood were variable; the highest level on day 12 was 0.2% of the dose per ml of blood. The highest blood level of caffeine observed during a 24-h sampling period averaged 5.7 micrograms ml-1. When [14C]caffeine was administered by gavage at a dosage of 80 mg kg-1 on day 12, the blood level of radioactivity reached a peak of 0.4% of the dose per ml of blood and declined rapidly thereafter. The highest amount of caffeine observed in blood averaged 63.1 micrograms ml-1, 1 h after gavage. The overall blood elimination half-life of radioactivity in pregnant rats treated by gavage was 2.6 h, and the half-life of caffeine in blood was 1.7 h. The levels of radioactivity in the fetus and maternal muscle per unit weight were comparable after each method of administration. A comparison of autopsy results from both groups indicated that resorptions were increased when compared with rats that did not receive caffeine; this effect was more marked in the gavage group than in the ad libitum sipping group. Ectrodactyly was observed only in offspring of the gavage group. The incidences of ectrodactyly or resorptions did not appear to be directly related to nutrition or fluid intake.

Effects of maternal caffeine ingestion on neonatal growth in rats.
Dunlop M and Court JM.
Biol Neonate 1981;39(3-4):178-84.

When caffeine (1,3,7-trimethylxanthine) was introduced into the diet of rats throughout pregnancy and lactation at levels of consumption of 10 mg/kg/day, offspring of successive pregnancies showed growth reductions. This finding was not accompanied by teratogenic effects. However, following four pregnancies severely reduced offspring growth and neonatal mortality was demonstrated. The birthweights of these offspring were 72.5% of control. This study mimicked the mode of intake and quantities of caffeine consumed in many societies.

Gestational caffeine modifies offspring behaviour in mice.
Sinton CM, Valatx JL, and Jouvet M.
Psychopharmacology (Berl) 1981;75(1):69-74.

Dams from two strains of mice, BALB/c C57BR were tested during gestation with caffeine, at doses of about 60, 80 and 100 mg/kg/day, in their drinking water. The resulting offspring were behaviourally tested over a 6-month period commencing at age 9 months. When compared with controls, mice from dams that had received caffeine demonstrated longer latencies in a passive avoidance test, and differences were also noted for female C57BR offspring in activity and habituation measures. Having controlled as far as possible for post-natal maternal and environmental effects, the most likely conclusion is that caffeine has a direct pharmacological action on the foetus, and should therefore be classed as a behavioural teratogen in mice.

Increased sleep time in the offspring of caffeine-treated dams from two inbred strains of mice.
Sinton CM, Valatx JL, and Jouvet M.
Neurosci Lett 1981;24(2):169-74.

Dams from two inbred strains of mice (C57BR and BALB/c) were treated with caffeine in solution in their drinking water during gestation. Doses of caffeine used corresponded to about 60, 80 or 100 mg/kg/day; controls received tap water. The offspring (as adults) revealed a significantly increased sleep time following caffeine treatment, but primarily as slow wave sleep in the males of the BALB/c strain and paradoxical sleep in the females of the C57BR strain. BALB/c females and C57BR males were relatively unaffected. These results, and in particular the sex differences, are discussed in terms of a possible central site of action of caffeine.

Effect of caffeine on rat offspring from treated dams.
Aeschbacher HU, Milon H, Poot A, and Wurzner HP.
Toxicol Lett 1980;7(1):71-7.

Pregnant Sprague-Dawley rats were given caffeine at 1.0, 0.5 and 0.25 g/kg diet during gestation and lactation. At birth, half of the pups from control and treated rats at each dose level were exchanged and cross fostered. Two litters were produced by each animal from each of the experimental groups. Caffeine at dietary concentrations of 0.5 and 0.25 g/kg throughout gestation and lactation had no significant effect on birth weight, litter size or development. There was also no effect at these doses following treatment during either gestation alone, or lactation alone. At 1.0 g/kg there was a slight reduction of birth weight, as well as a trend towards lower weight gain in litters from dams fed the test diet throughout gestation and lactation.

Testicular atrophy and impaired spermatogenesis in rats fed high levels of the methylxanthines caffeine, theobromine, or theophylline.
Friedman L, Weinberger MA, Farber TM, Moreland FM, Peters EL, Gilmore CE, and Khan MA.
J Environ Pathol Toxicol 1979;2(3):687-706.

Experiments were designed to determine the effects of feeding the methylxanthines caffeine, theobromine, or theophylline to 4- to 6-week-old males rats at a dietary level of 0.5 percent for periods ranging from 14 to 75 weeks. In the first two experiments, Osborne-Mendel rats were fed the test substances alone or in combination with sodium nitrite to test the hypothesis that these amines might nitrosate in vivo to produce toxic nitrosamine compounds. The compounds failed to produce neoplastic or preneoplastic lesions, but a significant positive finding was the occurrence of severe bilateral testicular atrophy with aspermatogenesis or oligospermatogenesis in 85-100 percent of the rats fed caffeine or theobromine. In a third experiment the methylxanthines were fed to Holtzman rats for 19 weeks to determine whether testicular atrophy would be induced in a second strain of rat. The testicular effects were similar to those in Experiments I and II but were more pronounced. Caffeine and theobromine induced testicular injury in nearly all rats. Theophylline induced severe testicular atrophy in 14 percent of the rats, mild to moderate atrophy in 71 percent, and had no effect in 15 percent. The relative testicular toxicity of the methylxanthines was caffeine, most potent; theobromine, slightly less potent; and theophylline, considerably less potent. Somewhat variable atrophic changes of the accessory sexual organs (epididymis, prostate, and seminal vesicles) accompanied the testicular changes. Cytogenetic analysis of testes from caffeine- or theophylline-treated rats revealed a significantly reduced number of mitotic cells in the caffeine-treated group. Plasma testosterone concentrations were significantly elevated in the theobromine group and somewhat elevated in the caffeine-treated group; this correlated morphologically with an apparent hyperplasia of interstitial cells in severely atrophied testes in these groups. Plasma cholesterol concentrations were significantly increased in the caffeine and theobromine groups. Possible sites and mechanisms of action of the methylxanthines in the induction of testicular atrophy and impaired spermatogenesis are discussed.

Testicular atrophy and impaired spermatogenesis in rats fed high levels of the methylxanthines caffeine, theobromine, or theophylline.
Weinberger MA, Friedman L, Farber TM, Moreland FM, Peters EL, Gilmore CE, and Khan MA.
J Environ Pathol Toxicol 1978;1(5):669-88.

Experiments were designed to determine the effects of feeding the methylxanthines caffeine, theobromine, or theophylline to 4- to 6-week-old male rats at a dietary level of 0.5 percent for periods ranging from 14 to 75 weeks. In the first two experiments, Osborne-Mendel rats were fed the test substances alone or in combination with sodium

nitrite to test the hypothesis that these amines might nitrosate in vivo to produce toxic nitrosamine compounds. The compounds failed to produce neoplastic or preneoplastic lesions, but a significant positive finding was the occurrence of severe bilateral testicular atrophy with aspermatogenesis or oligospermatogenesis in 85-100 percent of the rats fed caffeine or theobromine. In a third experiment the methylxanthines were fed to Holtzman rats for 19 weeks to determine whether testicular atrophy would be induced in a second strain of rat. The testicular effects were similar to those in Experiments I and II but were more pronounced. Caffeine and theobromine induced testicular injury in nearly all rats. Theophylline induced severe testicular atrophy in 14 percent of the rats, mild to moderate atrophy in 71 percent, and had no effect in 15 percent. The relative testicular toxicity of the methylxanthines was caffeine, most potent; theobromine, slightly less potent; and theophylline, considerably less potent. Somewhat variable atrophic changes of the accessory sexual organs (epididymis, prostate, and seminal vesicles) accompanied the testicular changes. Cytogenetic analysis of testes from caffeine- or theophylline-treated rats revealed a significantly reduced number of mitotic cells in the caffeine-treated group. Plasma testosterone concentrations were significantly elevated in the theobromine group and somewhat elevated in the caffeine-treated group; this correlated morphologically with an apparent hyperplasia of interstitial cells in severely atrophied testes in these groups. Plasma cholesterol concentrations were significantly increased in the caffeine and theobromine groups. Possible sites and mechanisms of actions of the methylxanthines in the induction of testicular atrophy and impaired spermatogenesis are discussed.

Mitigation of caffeine-induced teratogenicity in mice by prior chronic caffeine ingestion.
Terada M and Nishmura H.
Teratology 1975;12(1):79-87.

Pregnant A/J female mice, which had drunk tap water or a 0.05% caffeine solution for 8-19 weeks after weaning, were each injected sc with 150 or 250 mg/kg caffeine once on day 13 of gestation. After 150 mg/kg caffeine the frequencies at term of fetal death, external malformation, and subcutaneous hematomas were significantly lower in the caffeine- than water-drinking group. After 250 mg/kg caffeine the frequency of fetal death but not of malformations and hematomas was lower in the group with caffeine pretreatment. These findings were explained by assuming that long-term ingestion of caffeine induced and increased rate of degradation of caffeine administered during pregnancy.

B. Studies reporting no developmental or reproductive toxicity

Prenatal effects of caffeine and restraint stress in mice.
Colomina MT, Sanchez DJ, Esparza JL, and Domingo JL.
Proc Soc Exp Biol Med 1999;220(2):106-11.

The maternal and developmental toxicity of combined exposure to restraint stress and caffeine was assessed in mice. On Day 9 of gestation, six groups of pregnant mice were treated (p.o.) with a single dose of 30, 60, or 120 mg/kg of caffeine. Immediately after caffeine administration, three of these groups were subjected to restraint for 14 hr. Control groups included unrestrained and restrained pregnant mice not exposed to caffeine. An additional group of animals (unrestrained and not exposed to caffeine) was deprived of food for 14 hr. A two-way (caffeine dose x restraint) analysis of variance revealed an overall effect (reduction) of restraint and caffeine exposure on maternal body weight gain and food consumption on gestation Days 9-11. Significant reductions were also observed in body weight at termination and corrected body weight change of dams concurrently exposed to 120 mg/kg of caffeine and restraint. By contrast, no significant effects of caffeine, restraint, or caffeine plus restraint on embryo/fetal development were noted. The doses of caffeine administered here are much higher than those usually consumed by the general population. Under the current experimental conditions, caffeine alone or combined with restraint stress was not embryotoxic or teratogenic in mice.

Reproduction study of caffeine administration to male Osborne-Mendel rats.
Whitby KE, Collins TF, Welsh JJ, Black TN, O'Donnell M, Gray GC, Green S, and West WL.
Food Chem Toxicol 1986;24(4):277-82.

The potential for caffeine, administered twice daily by gavage in a total dose of 40 or 80 mg/kg/day, to adversely affect the reproductive performance of male rats was investigated. Treatment was continued through 3 wk of serial mating; mating and dosing were terminated concurrently, after which the sires were autopsied and their testes weighed. Controls were treated similarly with distilled water. Significant dose-related differences were detected for sire body weight, but not for testes weight. The majority of the significant effects on the offspring were for those born to the low-dose sires. Statistically significant differences were sporadically detected for the number of pups born and their body weights and survival; however, these differences were not consistently detected in either a dose- or temporal-related fashion. Thus, caffeine appears to have little potential to produce adverse reproductive effects when administered by gavage to male rats at the levels tested in this study.

The effects of maternal carbohydrate (sucrose) supplementation on the growth of offspring of pregnancies with habitual caffeine consumption.
Dunlop M, Court JM, and Larkins RG.
Biol Neonate 1981;40(3-4):196-8.

Reduced offspring growth was found following introduction of caffeine into the normal diet of rats during pregnancy and lactation. When maternal caffeine (10 mg/kg/day) was consumed together with supplementary sucrose (7 g/kg/day) the expected offspring growth reduction attributed to caffeine did not occur. It is concluded that maternal nutritional status may determine the outcome of caffeine exposure at low concentrations which mimic human usage.

Caffeine concentrations in mice plasma and testicular tissue and the effect of caffeine on the dominant lethal test.
Aeschbacher HU, Milon H, and Wurzner HP.
Mutat Res 1978;57(2):193-200.

Large groups of male Swiss mice received per os on average 100 mg caffeine per kg body weight per day for 1 or 8 weeks. The dominant lethal test was designed to achieve maximum sensitivity in order to detect any possible mutagenic effect. No mutagenic induction of dominant lethals, pre-implantation egg loss or depression of the fertility of females, caused by caffeine at the dose levels administered were observed. The half life of caffeine, which was between 2.5 and 3 h, was similar in plasma and testicular tissue. It was concluded that caffeine did not accumulate in the testicular tissue of mice. The maximum concentration of caffeine found was below 10 microgram/g testicular tissue, which is about a 100 times lower than concentrations that cause chromosome aberrations in cultured mammalian cells.

Chemical induction of sperm abnormalities in mice.
Wyrobek AJ and Bruce WR.
Proc Natl Acad Sci U S A 1975;72(11):4425-9.

The sperm of (C57BL X C3H)F1 mice were examined 1, 4, and 10 weeks after a subacute treatment with one of 25 chemicals at two or more dose levels. The fraction of sperm that were abnormal in shape was elevated above control values of 1.2-3.4% for methyl methanesulfonate, ethyl methanesulfonate, griseofulvin, benzo[a]pyrene, METEPA [tris(2-methyl-l-aziridinyl)phosphine oxide], THIO-TEPA [tris(l-aziridinyl)phosphine sulfide], mitomycin C, myleran, vinblastine sulphate, hydroxyurea, 3-methylcholanthrene, colchicine, actinomycin D, imuran, cyclophosphamide, 5-iododeoxyuridine, dichlorvos, aminopterin, and trimethylphosphate. Dimethylnitrosamine, urethane, DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane], 1,1-dimethylhydrazine, caffeine, and calcium cyclamate did not induce elevated levels of

sperm abnormalities. The results suggest that sperm abnormalities might provide a rapid inexpensive mammalian screen for agents that lead to errors in the differentiation of spermatogenic stem cells in vivo and thus indicate agents which might prove to be mutagenic, teratogenic, or carcinogenic.

C. Studies with unclear outcome

Interactions in developmental toxicology: combined action of restraint stress, caffeine, and aspirin in pregnant mice.
Colomina MT, Albina ML, Sanchez DJ, and Domingo JL.
Teratology 2001;63(3):144-51.

BACKGROUND: Stress can result in an increased use of substances such as caffeine and aspirin. The effect of maternal stress on concurrent exposure to caffeine and aspirin on prenatal development was assessed in mice. METHODS: On gestational day 9, mice were assigned to three treatment groups orally exposed to caffeine (30 mg/kg), aspirin (250 mg/kg), or a combination of caffeine (30 mg/kg) and aspirin (250 mg/kg). Three additional groups of pregnant animals received similar caffeine and aspirin doses and were immediately subjected to restraint for 14 hr. Control groups included unrestrained and restrained pregnant mice not exposed to caffeine or aspirin. All dams were euthanized on gestational day 18. Live fetuses were evaluated for sex, body weight, and external, internal, and skeletal malformations and variations. RESULTS: A single oral dose of caffeine or aspirin did not cause significant maternal toxicity. However, coadministration of these drugs with restraint produced some adverse maternal effects (i.e., reduction in maternal weight gain and food consumption on gestational days 9-11). In relation to embryo/fetal toxicity, the incidence of some skeletal defects was significantly increased after exposure to caffeine, aspirin, or maternal restraint, and their binary and ternary combinations. CONCLUSIONS: Although caffeine and aspirin were given in a single dose in this study, the results suggest that prenatal stress could slightly exacerbate the maternal and developmental toxicity of the combination of these drugs in mice.

Combined effects of caffeine and alcohol during pregnancy on bones in newborn rats.
Case TS, Saltzman MJ, Cheuk J, Yazdani M, Sadeghpour A, Albrecht D, Rossowska MJ, and Nakamoto T.
Res Exp Med (Berl) 1996;196(3):179-85.

The combined effects of caffeine and alcohol on mineral contents of fetal mandibles and femurs were studied. Pregnant rats were divided into four groups: group 1, control; group 2, caffeine; group 3, alcohol; and group 4, caffeine-plus-alcohol. Alcohol (1.0 g ethanol/kg body weight) was intubated twice daily, beginning at day 9 of gestation. Caffeine (2 mg/100 g body weight) was given as a dietary supplement. Groups 1 and 2

were intubated with isocaloric sucrose solution. At birth, randomly selected pups were killed and the mandible and femur were removed and dried. Ca, P, Mg, Zn and hydroxyproline in these bones were measured. Notwithstanding the dams' intake of caffeine and alcohol administered separately, the present results suggest that the combination of caffeine and alcohol exhibited the most detrimental effects.

Effects of prenatal exposure to alcohol plus caffeine in rats: pregnancy outcome and early offspring development.
Hannigan JH.
Alcohol Clin Exp Res 1995;19(1):238-46.

The factors determining susceptibility to fetal alcohol syndrome (FAS) are not fully understood. We used an animal model of alcohol-related birth defects to assess the coteratogenic potential of caffeine as a risk factor in FAS. Rats were exposed prenatally to alcohol (approximately 15 g/kg/day) with or without caffeine (approximately 84 mg/kg/day) from gestation days 6 through 20 via liquid diet. All control groups were pair-fed to the alcohol-exposed groups. In addition, some controls had free access to lab chow and water. Prenatal exposure to alcohol or caffeine reduced both maternal weight gain during pregnancy and birth-weight of offspring. The combination of alcohol plus caffeine produced an additive effect in reducing birthweight and synergistic effects in increasing postnatal offspring mortality. Prenatal alcohol exposure had a significant negative impact on several developmental indices, including grip strength and negative geotaxis. Prenatal caffeine exposure did not affect maturational measures and did reduce offspring serum levels of the zinc-dependent enzyme alkaline phosphatase. This study in rats demonstrated that caffeine can exacerbate some of the effects of alcohol on prenatal development, specifically reduced birthweight, litter size, and postnatal survival, but that caffeine does not appear to alter prenatal alcohol-induced delays in early postnatal maturation of survivors. The relative impact of intralitter birthweight rank on developmental outcome was also assessed.

Relationship of prenatal caffeine exposure and zinc supplementation on fetal rat brain growth.
Yazdani M, Fontenot F, Gottschalk SB, Kanemaru Y, Joseph F Jr, and Nakamoto T.
Dev Pharmacol Ther 1992;18(1-2):108-15.

Pregnant rat dams were divided into four groups on the 3rd day of gestation. Group 1 dams were fed a 20% protein diet as controls. Dams of group 2 were fed a 20% protein diet supplemented with zinc (0.6 g ZnCl2/kg diet). Group 3 dams were fed a 20% protein diet supplemented with caffeine (2 mg/100 g body weight) and dams of group 4 were fed a 20% protein diet supplemented with both caffeine and zinc. Fetuses were surgically delivered on day 22, and brains were removed and analyzed for alkaline phosphatase activity, protein, zinc, cholesterol and DNA concentrations. Fetal brain caffeine levels, as well as maternal and fetal plasma caffeine levels, were determined in caffeine-

supplemented groups. The body weight of group 4 and brain weights of groups 3 and 4 were higher than those of groups 1 and 2. Alkaline phosphatase activity of group 3 was less than that of group 1. The brain zinc concentration of group 2 was higher than in the other groups, but that of group 4 was less than that of group 1. The present study indicated that the supplementation of caffeine to the maternal diet decreased zinc levels in the fetal brain, and the addition of extra zinc to this diet did not return the zinc level to that of the control level as we had expected. In addition, the supplementation of caffeine and zinc together increased the body weights of the fetuses compared to the controls, but the addition of only one of these substances had no effect, suggesting that the combination of caffeine and zinc may have unique effects on fetal growth.

Sex-and age-dependent effects of prenatal exposure to caffeine on open-field behavior, emergence latency and adrenal weights in rats.
Hughes RN and Beveridge IJ.
Life Sci 1990;47(22):2075-88.

Pregnant rats were provided with drinking water containing 0, 0.23 or 0.3 mg/ml of caffeine throughout gestation. These concentrations gave rise to daily doses of 0, 28 and 36 mg/kg. Open-field behavior and latencies to emerge from a darkened chamber were observed in offspring at regular intervals from 1 to 8 months after birth. The main results revealed increases in open-field locomotor and rearing activity with 28 but not 36 mg/kg/day. The opposite pattern characterized emergence latency. These changes were more typical of male rats particularly when older. Combining the present results with those of an earlier study by the authors strengthened the curvilinear trends observed and led to the conclusion that, low doses of prenatal caffeine increase activity and decrease emotionality. Higher doses may have the opposite effects to the point that the significant differences from control subjects reported earlier can occur. When 8 months old, female but not male rats prenatally exposed to 36 mg/kg/day of caffeine had significantly heavier adrenal glands than controls.

Effects of maternal caffeine with zinc intake during gestation and lactation on bone development in newborn rats.
Sasahara H, Yamano H, and Nakamoto T.
Arch Oral Biol 1990;35(6):425-30.

On day 9 of gestation, pregnant dams were randomly divided into 3 groups. Dams of group 1 were fed a 20% protein diet as a control. Dams of group 2 were fed a 20% protein diet supplemented with caffeine. Dams of group 3 were fed a 20% protein diet supplemented with caffeine and zinc. The amount of caffeine added to the maternal diet was 2 mg/100 g body weight; the amount of zinc was 0.6 g/kg of diet. At birth, pups were mixed within each group, and 8 randomly selected pups from each group were assigned to each dam of the respective group and were continuously fed the same diet. On day 15,

the pups were killed and cranial bones, mandibles and femurs removed. The bones were measured, and the mineral content of the mandibles and femurs was determined. Although there were no differences in the dimensions of the cranial bones among the groups, the measurements and mineral content of the mandibles and femurs were consistently affected by the caffeine in the diet. On the other hand, supplementation of the caffeine-added diets with zinc led to greatly improved bone development, reaching values up to or beyond control levels. Thus zinc supplementation of a caffeine diet given to the dams during gestation and lactation can favourably influence the otherwise impaired bone development of their offspring.

Effects of caffeine intake during gestation and lactation on bones of young growing rats.
Schneider PE, Miller HI, and Nakamoto T.
Res Exp Med (Berl) 1990;190(2):131-6.

The objective of this study was to evaluate the extended effect of caffeine intake received during gestation and lactation on the mandible and femur of rats. Timed-pregnant dams were divided into two groups. Dams of group 1 were fed a 20% protein diet throughout the experimental period from day 9 of gestation. Dams of group 2 were also fed a 20% protein diet, supplemented with caffeine (1 mg/100 g of body weight). Upon delivery, 8 pups were assigned to each dam, and the dams were continued on their respective diets. At weaning (day 22 postnatally), only male rats were selected. Pups of both groups were fed a 20% protein diet without caffeine. At day 56 postnatally the rats were killed. Mandibles and femurs were removed and the following parameters analyzed: weight, physical dimension, volume, and Knoop microhardness. Caffeine intake during gestation and lactation resulted in an impairment of femur growth and development and to a lesser extent mandibular growth and development. The early effects of caffeine in the maternal diet were lasting, as noted by the lack of recovery of the offspring even after changing to a caffeine-free diet for an extended time after weaning.

Effect of teratogenic exposure on the developing brain: research strategies and possible mechanisms.
Grimm VE.
Dev Pharmacol Ther 1987;10(5):328-45.

Some of the problems associated with human behavioral teratology are reviewed and research strategies necessary for the elucidation of the possible prenatal bases for the later occurrence of Learning Disabilities (LD) are suggested. An animal model for LD was created by exposure of female rats during pregnancy or during lactation to diazepam, monosodium glutamate, caffeine, or maternal stress. The gestation period most sensitive to these prenatal insults was established, and some of the neurochemical and histological correlates of the resulting discrimination learning deficits were investigated.

Effects of combinations of maternal agents on the fetal cerebrum in rat--ethanol or caffeine with X-irradiation in utero.
Tanaka H, Iwasaki S, Arima M, and Nakazawa K.
Brain Dev 1985;7(1):10-20.

Fetal cerebral development influenced by maternal ethanol or caffeine either singly or in combination with X-irradiation was investigated in rat. Female Wistar rats were given 20% ethanol, 0.04% caffeine and water during the premating period and pregnancy, and 0.03% vitamin E only during pregnancy. Pregnant rats were X-irradiated with 100R or sham-irradiated on gestational day 13. Ethanol-treatment alone much reduced the fetal body and cerebral weights, and X-irradiation alone resulted in great reductions in weight and DNA concentration in the fetal cerebrum. The reduction in body weight with ethanol exceeded that with X-irradiation, therefore, the addition of X-irradiation had no effect on that of ethanol. The reduction in cerebral weight on X-irradiation exceeded that with ethanol, thus the addition of ethanol had only a slight effect on that with X-irradiation. The decrease in body and cerebral weights and the increase in lipid peroxide (LP) formation on caffeine-treatment and the decrease in cerebral weight and the increase in LP on vitamin E-treatment were inhibited by X-irradiation as compared to the combined effects of the other drink treatments. The increase in placental weight and the decrease in cerebral weight on ethanol-treatment and the decrease in placental, body and cerebral weights on caffeine-treatment, which findings were covered by the addition of X-irradiation, became much clearer on single drink treatment. Independently of X-irradiation, ethanol-treatment resulted in increased fetal mortality and LP, and decreased body weight. These results suggest that the combined effects of maternal agents on live fetuses should be investigated as to whether they act independently of or dependently with each other and how the effects appear either singly or mixed.

A reproduction/teratology study of brewed and instant decaffeinated coffees.
Nolen GA.
J Toxicol Environ Health 1982;10(4-5):769-83.

Sprague-Dawley rats of both sexes were given, in place of their drinking water, full-strength or 50 or 25% dilutions of either brewed or instant decaffeinated coffees for about 6 mo from weaning. These levels are equivalent to the human consumption of about 50, 25, or 12 cups of coffee per day. Controls were given either distilled water, or full-strength or a 25% solution of regular brewed coffee. The rats were bred twice after 91 d to assess reproduction and teratogenicity. The parent animals given decaffeinated coffees and 100% regular coffee drank less than the rats given water, while the rats given 25% regular coffee drank more. No effects on body weight gain or feed efficiency were seen, except that the group given 100% regular coffee gained significantly less weight than the water controls. None of the coffee treatments had a significant effect on reproductive characteristics such as conception rate, number born, or number weaned. During the second pregnancy, no significant effects from the coffee treatments were seen on early embryotoxicity measured in dams sacrificed on d 13 of pregnancy or fetal toxicity in

dams sacrificed on d 21. No significant fetal abnormalities due to any of the coffee treatments were observed in either soft-tissue or skeletal examinations, although there was a significant increase in unossified sternebrae in the fetuses from dams given the full-strength regular coffee.

Acute studies to investigate the mechanism of action of caffeine as a teratogen in mice.
Elmazar MM, McElhatton PR, and Sullivan FM.
Hum Toxicol 1981;1(1):53-63.

1 In Charles River CD1 mice, a single dose of 100 mg kg-1 caffeine injected intraperitoneally on day 14 of pregnancy caused a low incidence of cleft palate in the fetuses. 2 Single oral doses of caffeine of 200 and 300 mg kg-1 but not 100 mg kg-1 on day 14, caused cleft palate in some of the fetuses, but was clearly toxic to the dams. 3 Oral doses of caffeine up to 300 mg kg-1 on day 14 of pregnancy did not reduce utero-placental blood flow, placental transfer function, or amniotic fluid volume. 4 An oral dose of 100 mg kg-1 caffeine induced a marked stimulation of adrenocortical secretion producing plasma corticosterone levels of 1248 +/- 129 microgram per 100 ml by 2 h and with elevated levels persisting more than 8 h. 5 It is suggested that the elevated plasma corticosterone is the cause of the cleft palate induced in mice by caffeine. Since corticosterone is a known cleft palate inducer in mice but not in man these results do not predict a hazard from normal caffeine consumption in man.

Increased sleep time in the offspring of caffeine-treated dams from two inbred strains of mice.
Sinton CM, Valatx JL, and Jouvet M.
Neurosci Lett 1981;24(2):169-74.

Dams from two inbred strains of mice (C57BR and BALB/c) were treated with caffeine in solution in their drinking water during gestation. Doses of caffeine used corresponded to about 60, 80 or 100 mg/kg/day; controls received tap water. The offspring (as adults) revealed a significantly increased sleep time following caffeine treatment, but primarily as slow wave sleep in the males of the BALB/c strain and paradoxical sleep in the females of the C57BR strain. BALB/c females and C57BR males were relatively unaffected. These results, and in particular the sex differences, are discussed in terms of a possible central site of action of caffeine.

D. Related articles and meeting abstracts

Chronic intake of caffeine during gestation down regulates metabotropic glutamate receptors in maternal and fetal rat heart.
Iglesias I, Leon D, Ruiz MA, Albasanz JL, Martin M
Amino Acids. 2006; 30(3):257-66.

Caffeine is the most widely consumed substance in the world which antagonizes adenosine effects. Adenosine acting through A(1) receptors inhibits glutamate release which binds to metabotropic glutamate receptors (mGluRs). Recently, we have shown that maternal caffeine intake during gestation causes down-regulation of A(1) and metabotropic glutamate receptors in the brain of both rat mothers and fetuses. In the present work we provide evidence that caffeine also affects receptors in hearts, causing a decrease in mGluRs from both maternal and fetal hearts. A decrease in G(q/11) and PLC beta(1) proteins level was also observed in both tissues. However, phospholipase C activity was only affected in fetal heart, being significantly decreased. These results suggest an in vivo cross-talk mechanism between adenosine and glutamate receptors in peripheral tissues. Therefore, special attention should be paid to caffeine ingestion during gestation.

Caffeine in the milk prevents respiratory disorders caused by in utero caffeine exposure in rats.
Bodineau L, Saadani-Makki F, Jullien H, Frugiere A
Respir Physiol Neurobiol. 2006; 150(1):94-8.

Consequences of postnatal caffeine exposure by the milk on ponto-medullary respiratory disturbances observed following an in utero caffeine exposure were analysed. Ponto-medullary-spinal cord preparations from newborn rats exposed to caffeine during gestation but not after the birth display an increase in respiratory frequency and an exaggeration of the hypoxic respiratory depression compared to not treated preparations. These data suggest that tachypneic and apneic episodes encountered in human newborns whose mother consumed caffeine during pregnancy are due in large part to central effect of caffeine at the ponto-medullary level. Both baseline respiratory frequency increase and emphasis of hypoxic respiratory depression are not encountered if rat dams consumed caffeine during nursing. Our hypothesis is that newborn rats exposed to caffeine during gestation but not after the birth would be in withdrawal situation whereas, when caffeine is present in drinking fluid of lactating dams, it goes down the milk and is able to prevent ponto-medullary respiratory disturbances.

Influence of caffeine on the mRNA expression of type III iodothyronine deiodinase and thyroid hormone receptor in BeWo cell culture and rat placenta.
Saito S, Hamajima S, Abiko Y
J Pharmacol Sci 2005;97(Suppl 1):251P

Although chronic caffeine exposure during pregnancy has been shown to affect fetal growth, adverse effects of caffeine on embryogenesis are not only well understood, but also controversial. We employed cDNA micro array technology in an attempt to identify to what extent, if any, caffeine could possibly alter gene expressions in the cytotrophoblast-like cell line BeWo. We found that thyroid hormone receptor and type III iodothyronine deiodinase mRNA expressions in BeWo cells were up-regulated by caffeine, suggesting that the chronic exposure during the gestational period could exert an influence on embryogenesis. We then focused to study the responsive gene expressions in placentas of pregnant rats fed a diet supplemented with caffeine (2 mg/100 g body weight) during gestation, and analyzed the gene expressions using tested real-time PCR, or PCR/Southern blot hybridization technique. Significantly increased gene expressions of the receptor and the deiodinase were shown in caffeine-treated placentas. It is conceivable that caffeine influences on the pathway for maternal-fetal thyroid hormone transmission and the thyroid state of the fetus.

Chronic caffeine or theophylline intake during pregnancy inhibits A1 receptor function in the rat brain.
Leon D, Albasanz JL, Ruiz MA, Martin M
Neuroscience 2005;131(2):481-9.
The aim of this work was to study whether caffeine or theophylline chronically consumed during pregnancy affect inhibitory adenylyl cyclase pathway mediated by adenosine, in rat brain of both mothers and full-term fetuses. Immunoblotting analysis revealed a significant decrease in alphaGi(1,2) subunit level (27-29% in mothers, 15-18% in fetuses), associated with a significant increase in the mRNA level coding alphaGi(1) in both maternal and fetal rat brain (12-22%) after methylxanthine intake. No significant differences in alphaGi(3) level were detected in any case. On the other hand, forskolin- and forskolin plus guanosine-5'-O(3-thiotriphosphate) tetralithium salt-stimulated adenylyl cyclase activity was significantly decreased (30-36%) in maternal brain. Moreover, adenylyl cyclase inhibition elicited by N(6)-cyclohexyladenosine, specific adenosine A(1) receptor agonist, was also significantly decreased in caffeine- (40.5%) and theophylline- (55.0%) treated mothers, suggesting a desensitization of adenosine A(1) receptor/adenylyl cyclase pathway in maternal brain. However, no significant differences were detected in fetal brain between control and treated animals. Therefore, caffeine or theophylline chronic intake during pregnancy differently modulates inhibitory adenylyl cyclase pathway mediated by adenosine in maternal and fetal brain causing a loss of the system responsiveness only in maternal brain but down-regulating Gi(1) protein in both mother and fetus brain.

Effect of chronic gestational treatment with caffeine or theophyllineon Group I metabotropic glutamate receptors in maternal and fetal brain.
Leon D, Albasanz JL, Ruiz MA, Iglesias I, Martin M
J Neurochem. 2005; 94(2):440-51.

Pregnant rats were treated throughout the gestational period with either caffeine or theophylline, and its effect on the metabotropic glutamate receptor (mGluRs) signal transduction pathway was studied in both maternal and fetal brain. In maternal brain, radioligand binding assays showed that chronic treatment with methylxanthines caused a significant decrease in the total number of mGluRs. This decrease was accompanied by an increase in receptor affinity. Immunodetection showed that mGluR1a and phospholipase C beta1 (PLCbeta1) were significantly decreased in response to chronic methylxanthine treatment, whereas alphaG(q/11) was not affected. A loss was also detected of PLC stimulation mediated by (S)-3,5-dihydroxyphenylglycine (DHPG), a selective Group I mGluR agonist, suggesting desensitization of the mGluR/PLC pathway. In fetal brain, a loss in total mGluRs was observed in fetuses from mothers treated with caffeine or theophylline, without variation in receptor affinity. A decrease in mGluR1a, alphaG(q/11) and PLCbeta1 levels was also observed in response to treatment. However, changes detected in this immature tissue were not associated with variations in PLC activity. These results suggest that chronic caffeine or theophylline treatment down-regulates several mGluR/PLC transduction pathway components in both maternal and fetal brain, causing a loss of receptor responsiveness only in maternal brain.

Maternal caffeine intake impairs MK-801-induced hyperlocomotion in young rats.
da Silva RS, Hoffman A, de Souza DO, Lara DR, Bonan CD
Eur J Pharmacol. 2005; 509(2-3):155-9.

Here we have investigated the effects of maternal caffeine intake (1 g/l) on MK-801-induced hyperlocomotion in rat pups. Animals submitted to caffeine treatment during the gestational and lactational period were separated in two groups: caffeine-treated group (up to 21 days old) and washout group (caffeine treatment up to 7 days old). MK-801 (0.25 mg/kg, i.p.) promoted hyperlocomotion in control rats, but this stimulatory effect was significantly decreased in caffeine-treated and washout groups. The permanent effect after caffeine withdrawal suggests durable or adaptive changes during neurodevelopment, mainly on adenosine receptors or neurotransmitter systems modulated by adenosine, such as the glutamatergic system.

Effects of caffeine on the developmental potential of in vitro matured, aged and denuded ovine oocytes.
Maalouf W, Lee JH, Campbell KH
Hum Fertil (Camb) 2005;8(2):129-30

Introduction. Oocyte aging is characterized by a decrease in the activities of MPF and MAPK kinases, a decrease in development and an increase in polyspermy. Caffeine, a protein phosphatase inhibitor, prevents the decline and in fact increases oocyte kinase activities (Lee, I.-H., & Campbell, KH.S. (2004). Reprod. Fertil. and Develop., 16, 125 abst). We examined the effects of caffeine treatment on the development of aged and denuded ovine oocytes fertilized in vitro. Methods. Cumulus oocyte complexes were matured in TCM-199 supplemented with 10% FCS, 5 ug/mL FSH, 5 ug/mL LH, 1 ug/mL estradiol, 0.3 mM sodium pyruvate and 100 um cysteamine. At 24 hr, oocytes were either fertilized (A) or stripped of their cumulus cells by vortexing, and incubated for a further 6 h in the presence (B) or absence (C) of 10 mM caffeine in maturation medium. All oocytes were fertilized as previously described (Sinclair, KD. et al. (1999) J. Reprod. Fertil., 116, 177 - 86). Briefly, frozen thawed ram semen pellets were used at a final concentration of 2.5 - 3.5 x 10(6) sperm/mL. Twenty-four hours after fertilization presumptive zygotes were washed and cultured in SOF media supplemented with 3 mg/ml BSA for 2 days, then cultured- for another 5 days in SOF supplemented with 10% FCS, all at 5% CO2, 5%O2 and 90%N2 at 39°C. Results and discussion. Cleavage rates were not different between groups (69%, 74% and 73%). Development to blastocyst decreased on oocyte aging, caffeine treatment prevented this decline (32%, 30% and 13%, respectively ($P < 0.05$)) The total cell numbers in blastocysts were not statistically different (92.4 +/ - 5.2, 84.7 +/ - 3.7 and 80.4 +/- 5.8). Polyspermy rates increased on oocyte aging, caffeine did not significantly reduce polyspermy (2%a, 10%ab and20%b). In conclusion, caffeine treatment can prevent the decline in developmental potential of ovine oocytes matured and aged in vitro.

Teratogenic Potential of Citrus aurantium.
Hansen DK, Wall KS, White G, Pellicore LS
Birth Defects Res A Clin Mol Teratol 2006;76(5):385

Manufacturers have recently begun to use extracts of Citrus aurantium (also known as bitter orange, Seville orange, or zhi shi) as a replacement for ephedra in dietary supplements marketed for weight loss. Since these products may be consumed by women of child-bearing age, it is important to know if there are any adverse fetal effects. The purpose of this study was to examine the teratogenic potential of Citrus aurantium extract in rats. Groups of 25 adult female Sprague-Dawley rats were mated overnight with males, and the finding of a sperm plug was counted as GD O. An extract that contained approximately 6% synephrine was gavaged daily from GD 3-20 at 0, 10, 25, 50 or 100 mg of synephrine/kg body weight. Other animals were treated with 50 or 100 mg synephrine/kg of an extract that contained approximately 95% synephrine. An additional

group of animals was treated with 25 mg/kg caffeine, and a final group was dosed with 50 mg synephrine/kg body weight using the 6% synephrine extract and 25 mg caffeine/kg body weight. Animals were sacrificed on GD 21. In total, 7 animals died during treatment; four of these deaths appeared to be due to gavage errors. The other three maternal deaths may be related to treatment; these deaths occurred in the two 95% synephrine extract groups and the group which received the combination treatment. There were no differences among the groups in the number of pregnant rats, average fetal weight/litter, average male fetal weight/litter, or average female fetal weight/litter. Citrus aurantium extracts did not alter the number of implants, the number of live implants/litter or the number of non-live implants/litter; caffeine alone also had no effects on these parameters. However, the combination of Citrus aurantium extract and caffeine did increase the number of non-live implants/litter with a corresponding decrease in the number of live implants/litter. The only litter that was completely resorbed was also in the group treated with the combination of Citrus aurantium extract and caffeine. Few gross or visceral malformations were observed in any of the groups. Skeletal defects have not yet been completely evaluated. These data suggest that Citrus aurantium extracts alone do not produce maternal or fetal toxicity but may produce toxicity in combination with caffeine.

In vitro evaluation of the quality and fertilizing capacity of boar semen frozen in 0.25 ml straws.
Pelaez J, Breininger E, Alegre B, Pena FJ, Dominguez JC
Reprod Domest Anim 2006; 41(2):153-61.

Twenty-two boar ejaculates were frozen in 0.25 ml straws using a controlled cooling rate, then evaluated in vitro in order to assess: (i) the extent to which a range of semen evaluation parameters accurately characterize sperm quality, (ii) the value of quality assessment in the characterization of long-term sperm survival and fertility and (iii) the suitability of the cryopreservation protocol used for yielding semen with good quality and fertilizing capacity. Motility with or without caffeine, plasma membrane integrity (PMI) evaluated with both propidium iodide (PI) and Hoechst 33258, and acrosome morphology were studied, the ejaculates being then classified into five quality groups. A thermoresistance test and a homologous in vitro fertilization test were applied to selected ejaculates of these groups. Caffeine-stimulated motility and PMI evaluated with PI provided better estimations of semen quality than the other tests of motility, PMI, or acrosome morphology, but this quality assessment could not reveal differences in fertilizing capacity or thermoresistance among ejaculates. Over 43% spermatozoa survived cryopreservation in 19 of the 22 ejaculates, with inter-boar and inter-ejaculate variability in the freezing success being observed. The fertilizing capacity, however, was seriously affected by the process regardless of the semen quality. It is concluded that caffeine-stimulated motility and PMI evaluated with PI give accurate information on sperm quality, but important aspects to the valuation of semen such as thermoresistance and fertilizing capacity are not revealed by this quality study. Moreover, the approach of

selecting suitable protocols of cryopreservation does not appear to be sufficient for guaranteeing systematically good quality and fertilizing capacity in the frozen-thawed semen.

Modulation of methotrexate-induced cytogenotoxicity in mouse spermatogonia and its transmission in the male germline by caffeine.
Palo AK, Choudhury RC
Environmental Toxicology and Pharmacology 2006; 21(3):254-9

Apart from its own controversial cytogenotoxic effects, caffeine (CAF), one of the most commonly consumed alkaloids worldwide, is found potentiative to and so also protective from the cytogenotoxic effects of numerous chemical and physical mutagens. It also has modulated the actions of several antineoplastic agents. Additionally, it has been tested as a chemopreventive of cancer and is reportedly associated inversely with different cancer risks. Therefore, in the present study, three different sub-lethal doses of CAF, 25, 50 and 100 mg/kg, were tested in mouse to assess their cytogenotoxic effects on dividing spermatogonia at 24 h post-treatment, and transmission of such effects in the male germline from the primary spermatocytes and sperm at week 4 and week 8 post-treatment, respectively. CAF was found to be weakly clastogenic to mouse spermatogonia and the effects were also found transmitted in the male germline. Interestingly, such induced effects were quantitatively related to the dose of CAF tested. On the other hand, methotrexate (MTX), an antifolate antimetabolite, is prescribed frequently for the treatment of various types of cancers. However, MTX is reportedly clastogenic. Modulation of the said three different pre-treated doses of CAF on MTX 10 mg/kg-induced cytogenotoxic effects, tested in the same experimental protocol, indicated that CAF pre-treatment was decreasing the MTX-induced clastogenicity in spermatogonia, and was lowering the concurrent transmission of such effects in the male germline of mice, significantly. Such decreases were related to the dose of CAF tested, i.e. higher the dose of CAF more was the decrease in the MTX-induced cytogenotoxic effects and in their transmission. The possible mechanisms that might have caused the manifestation of a weak clastogenic action of CAF on spermatogonia and in its transmission in the male germline, and the CAF modulation of MTX-induced cytogenotoxic effects in spermatogonia and in their transmission have been discussed.

Consequences of in utero caffeine exposure on respiratory output in normoxic and hypoxic conditions and related changes of Fos expression: a study on brainstem-spinal cord preparations isolated from newborn rats.
Bodineau L, Cayetanot F, Sådani-Makki F, Bach V, Gros F, Lebleu A, Collin T, Frugière A.
Pediatr Res. 2003;53(2):266-73.

Several aspects of the central regulation of respiratory control have been investigated on brainstem-spinal cord preparations isolated from newborn rats whose dam was given 0.02% caffeine in water as drinking fluid during the whole period of pregnancy. Analysis of the central respiratory drive estimated by the recording of C4 ventral root activity was correlated to Fos ponto-medullary expression. Under normoxic conditions, preparations obtained from the caffeine-treated group of animals displayed a higher respiratory frequency than observed in the control group (9.2 +/- 0.5 versus 7.2 +/- 0.6 burst/min). A parallel Fos detection tends to indicate that the changes of the respiratory rhythm may be due to a decrease in neuronal activity of medullary structures such as the ventrolateral subdivision of the solitary tract, the area postrema, and the nucleus raphe obscurus. Under hypoxic conditions, the preparations displayed a typical hypoxic respiratory depression associated with changes in the medullary Fos expression pattern. In addition, the hypoxic respiratory depression is clearly emphasized after in utero exposure to caffeine and coincides with an increased Fos expression in the area postrema and nucleus raphe obscurus, two structures in which it is not increased in the absence of caffeine. Taken together, these results support the idea that in utero caffeine exposure couldaffect central respiratory control.

Effect of caffeine on meiotic maturation of porcine oocytes.
Kren R, Ogushi S, and Miyano T.
Zygote 2004;12(1):31-8.

This study was conducted to evaluate the effect of caffeine on the meiotic maturation of porcine oocytes. Oocyte-cumulus complexes were collected from slaughterhouse-derived ovaries and cultured for 24, 32 or 48 h in medium 199 supplemented with 10% fetal calf serum, 10 microg/ml FSH, 50 microg/ml sodium pyruvate and 50 microg/ml gentamicin in the presence or absence of 2.5 mM caffeine. Caffeine inhibited the meiotic resumption of pig oocytes effectively after 24 h of culture, and 95.5% of oocytes were arrested at the germinal vesicle (GV) stage (control 17.8%, $p < 0.05$). Prolonged culture with caffeine up to 32 h or 48 h, however, resulted in a significant decrease in the inhibitory effect (GV: 13.8% and 8.2%). The number of oocytes at metaphase II after 48 h of culture in the presence of caffeine was significantly lower than that in the control medium (65.3% vs 94.7%, $p < 0.05$). The withdrawal of caffeine after 24 h of culture resulted in the resumption of meiotic maturation, and the oocytes reached metaphase II after 48 h. However, the ability of caffeine-treated oocytes to develop to blastocysts after artificial activation was lower than that of the control (5.5% vs 9.1%, $p < 0.05$). Caffeine treatment

significantly increased cAMP levels in the oocytes after 24 h of culture, while both Cdc2 kinase and MAP kinase activation were inhibited in the oocytes. These results suggest that caffeine, similarly to other purine derivatives, prolongs the meiotic arrest of porcine oocytes at the GV stage, perhaps by its action of increasing the cAMP level and by the suppression of Cdc2 kinase and MAP kinase activities in the oocytes.

Involvement of adenosinergic A1 systems in the occurrence of respiratory perturbations encountered in newborns following an in utero caffeine exposure. a study on brainstem-spinal cord preparations isolated from newborn rats.
Saadani-Makki F, Frugiere A, Gros F, Gaytan S, and Bodineau L.
Neuroscience 2004;127(2):505-18.

Involvement of adenosinergic A1 systems in the occurrence of respiratory perturbations encountered in newborns following an in utero caffeine exposure has been investigated on pontomedullary-spinal cord, caudal pons-medullary-spinal cord and medullary-spinal cord preparations isolated from newborn rats. According to the drinking fluid of dams (tap water or 0.02% caffeine), two groups of preparations were distinguished, no-caffeine and caffeine. In the no-caffeine group, adenosine A1 receptor activation induces a decrease in respiratory frequency (Rf) in caudal pons-medullary-spinal cord and medullary-spinal cord preparations whereas, in presence of the rostral pons, an increase is observed. A parallel Fos detection indicates that this discrepancy may be due to the excitatory action of the medial parabrachial nucleus at the rostral pontine level that surpasses inhibitory influence of the adenosine A1 receptor activation at the medullary level particularly in the ventrolateral reticular nucleus of the medulla. In caffeine group, an increase in the baseline Rf in presence of the pons and no change in medullary-spinal cord preparations have been observed. Depending on Fos detection, we assume that the medial parabrachial nucleus is the main region involved in the exaggeration of Rf. Moreover, adenosine A1 receptor activation was modified by in utero caffeine exposure with an overcharge of the Rf increase in pontomedullary-spinal cord preparations and an exaggeration of the Rf decrease in medullary-spinal cord preparations. Based on Fos detection, we link the overcharge in Rf of pontomedullary spinal cord preparations to an increase in the medial parabrachial nucleus neuronal activity. Similarly, exaggeration of Rf decrease observed without the pons is linked with a decrease in activity of the ventrolateral reticular neurons. This study brings evidence for the involvement of adenosinergic A1 systems in the occurrence of respiratory perturbations in newborns following in utero caffeine exposure and the importance of rostral pons in the adenosinergic A1 modulation of the respiratory control.

Effects of caffeine and its reactive metabolites theophylline and theobromine on the differentiating testis.
Pollard I, Locquet O, Solvar A, and Magre S.
Reprod Fertil Dev 2001;13(5-6):435-41.

A previous study in the rat (Pollard et al. 1990) established that caffeine, when administered during pregnancy, significantly inhibited the differentiation of the seminiferous cords and subsequent Leydig cell development in the interstitium. However, that study could not distinguish between the direct effects of caffeine and/or the intermediary secondary toxic effects of metabolites such as theophylline and theobromine. Because the fetus lacks the appropriate enzyme systems, clearance of toxic substances takes place via the placenta and maternal liver. Thus, a suitable in vitro system can effectively differentiate between primary and secondary drug effects. In the present study, 13-day-old fetal testis, at the stage of incipient differentiation, were cultured for 4 days in vitro in the presence of graded doses of caffeine, theophylline or theobromine. It was found that explants exposed to caffeine or theobromine differentiated normally, developing seminiferous cords made up of Sertoli and germ cells, soon followed by the differentiation of functionally active Leydig cells appearing in the newly formed interstitium. However, explants exposed to theophylline failed to develop seminiferous cords and, as a consequence, Leydig cells. In conclusion, insights obtained from different experimental methods, such as organ culture or whole organism studies, are not always identical. It may be prudent, therefore, to take into account that certain experimental techniques, despite providing valuable information, may require confirmation by other test methods in order to obtain an in-depth understanding of mechanisms of action involved.

Caffeine induces in vivo premature appearance of telencephalic vesicles.
Sahir N, Bahi N, Evrard P, and Gressens P.
Brain Res Dev Brain Res 2000;121(2):213-7.

Caffeine administered to pregnant mice during germinative neuroepithelium preparation (embryonic days 8-10) dramatically accelerated primitive neuroepithelium evagination into telencephalic vesicles, versus age-matched controls. This histologically-documented, dose-dependent effect seemed reversible during subsequent neuronal migration if caffeine exposure was discontinued. Our in vivo model provides a new tool for studying telencephalic symmetry acquisition and for identifying genes potentially involved in holoprosencephaly, a developmental disorder characterized by defective telencephalic vesicle formation.

Caffeine-induced disturbances of early neurogenesis in whole mouse embryo cultures.
Marret S, Gressens P, Van-Maele-Fabry G, Picard J, and Evrard P.
Brain Res 1997;773(1-2):213-6.

In toto mouse embryos were cultivated at embryonic day 8.5 for 26 h with 105, 310 or 620 microM caffeine; 105-310 microM correspond to concentrations transferred by the placenta of heavy caffeine consumers. Failure of neural tube closure, excessive proliferation of neuroepithelial cells and premature evagination of telencephalic vesicles were present in 50% of treated embryos. When reaching the embryonic neural tube before neuronal migration, caffeine regionally modifies the schedule and/or rate of neural cell proliferation.

Preconceptual caffeine exposure increases glucose utilization and accelerates development in the preimplantation rat embryo.
Loupis A, Ryan J, Waite K, and Pollard I.
J Matern Fetal Med 1996;5(6):321-7.

The present study was designed to investigate whether caffeine administered daily throughout the estrous cycle prior to fertilization affected the development of the subsequent preimplantation day 5 rat embryo. The viability parameters chosen for assessment were glucose utilization, cell number, and stage of embryonic development (morula to hatched blastocyst). Two independently replicated experiments were conducted. Together these experiments demonstrated that after fertilization, a proportion of affected oocytes maturing in a caffeine-perfused ovarian environment used and oxidised glucose at a significantly higher rate and were significantly more advanced developmentally compared with their litter mates or with the control counterparts. Cell number per embryo and the number of embryos recovered (litter size) remained constant, suggesting that caffeine, at the doses used, is unlikely to affect the ovulation rate or prevent fertilization. This study is significant because it demonstrates for the first time that a drug such as caffeine, when administered prior to ovulation and genomic activation, causes a quantitative difference in growth promoting energy utilization in a proportion of susceptible embryos after genome activation. A link between genomic imprinting and changed developmental program in the preimplantation embryos was suggested.

Cytogenetic effects of caffeine during in vivo mouse oocyte maturation.
Mailhes JB, Young D, and London SN.
Mutagenesis 1996;11(4):395-9.

Numerous investigators have studied the reproductive and genetic toxicity of caffeine. Caffeine has also been reported to retard meiotic progression and induce aneuploidy in

hamster oocytes in vitro. However, the ability of caffeine to induce aneuploidy in mammalian oocytes in vivo has not been reported. The objective of this study was to test the hypothesis that chemical-induced perturbations during in vivo oocyte meiotic maturation (OM) predispose oocytes to chromosome missegregation. Caffeine inhibits cAMP phosphodiesterase, which is needed for dephosphorylating p34(cdc2) kinase and initiating OM. Following superovulation, a dose of 150 mg/kg caffeine was administered to Institute of Cancer Research (ICR) female mice at various times prior to metaphase I (MI). Ovulated oocytes were collected from the oviducts and processed for cytogenetic analysis. Statistical analyses of the frequencies of hyperploid, MI, diploid, premature centromere separation and single chromatids revealed nonsignificant ($P > 0.05$) differences between the controls and each of the caffeine groups. Structural chromosome aberrations were not found. Under our experimental conditions, we rejected the hypothesis and concluded that caffeine neither retarded the rate of OM nor increased the incidence of aneuploidy in mouse oocytes. The factors responsible for the different in vivo and in vitro responses require investigation.

Comet assay studies indicate that caffeine-mediated increase in radiation risk of embryos is due to inhibition of DNA repair.
Muller WU, Bauch T, Wojcik A, Bocker W, and Streffer C.
Mutagenesis 1996;11(1):57-60.

It is well known that under specific conditions caffeine is able to enhance radiation risk of mammalian cells by a factor of approximately 1.5-2. Various mechanisms are discussed in the literature as possible explanations for this interaction. Inhibition of DNA repair plays a crucial role in the discussion, although direct evidence for this assumption is difficult to obtain. We used the "comet assay' in order to analyse the significance of repair inhibition by caffeine in the two-cell stage of mammalian gestation. Our data show that at the concentration necessary for increasing radiation risk (2 mM), caffeine effectively inhibits the restitution of radiation-damaged DNA.

The effect of caffeine on mammary gland development and milk yield in primiparous sows.
Li S and Hacker RR.
J Anim Sci 1995;73(2):534-40.

Pregnant Yorkshire gilts (n = 42) were fed caffeine (6 g/d) or served as controls from d 60 of pregnancy until d 4 postpartum to test the effect of caffeine on mammary gland development, milk yield, and feed consumption. Caffeine reduced voluntary feed intake ($P = .001$) and body weight gain ($P = .001$) of gilts from d 60 to 109 of gestation. Pig birth weight in the treated group was less than ($P = .01$) that in the control group. However, pig viability score at birth was not affected by maternal caffeine ingestion. For assessing mammary gland DNA, RNA, dry fat-free tissue (DFFT), fat, and protein

content, four sows from the caffeine group and three controls were slaughtered on the 1st d of lactation. Immediately after slaughter, mammary systems were removed, separated by gland, and dissected free of skin, muscle, and fatty pad, which had not been invaded by glandular tissue. The DNA and RNA content were evaluated in DFFT. Caffeine increased mammary RNA content (P = .023) and milk yield (P = .001) on d 1 of lactation. However, DNA, DFFT, fat, and protein were not significantly increased, although values were somewhat greater in the treatment group (approximately 82%). On d 21 of lactation, milk yield of treated sows did not differ from that of controls. The increased milk yield on d 1 of lactation was due to increased mammary epithelial cell activity and cell numbers. These results indicate that caffeine feeding can have a positive effect on porcine mammary gland development as well as milk yield.

Effects of caffeine intake during gestation and lactation on the acid solubility of enamel in weanling rats.
Schneider PE, Alonzo G, Nakamoto T, Falster AU, and Simmons WB.
Caries Res 1995;29(4):285-90.

The purpose of this study was to evaluate the effects of dietary caffeine during gestation and lactation on the acid solubility of molar teeth of weanling rats. Nineteen pregnant dams were divided into two groups. The 9 dams in the control group were fed a 20% protein diet supplemented with caffeine (2 mg/100 g BW) throughout the experiment. At birth, 8 pups were randomly assigned to each dam. Pups were killed on day 22. The 1st and 2nd molars were removed from each pup's maxilla and mandible. Four randomly selected molars from each litter were placed in a chamber and bathed with a flow of acid solution and the amount of mineral dissolved from the enamel was determined. The results showed that the amount of dissolved Ca and Mg from enamel surfaces of 1st molars from rats in the caffeine group after exposure to acid was consistently greater than that of the non caffeine group. In the 2nd molars there was no significant difference between caffeine and noncaffeine groups. Scanning electron microscopy revealed an alteration of the enamel surface of the 1st molars of the caffeine group after acid exposure. These results indicate that caffeine intake during gestation and lactation would have a deleterious effect on dental enamel of 1st molars in newborn rats.

In vitro study of teratogenic effects of caffeine on cultured rat embryos and embryonic cells.
Iwase T, Arishima K, Ohyama N, Inazawa K, Iwase Y, Ikeda Y, Shirai M, Yamamoto M, Somiya H, and Eguchi Y.
J Vet Med Sci 1994;56(3):619-21.

The teratogenic potential of caffeine was examined in vitro by a whole embryo culture system (WECS) and an embryonic cell culture system (micromas teratogen assay: MTA) in the rat. In the WECS, hyperemia of the tail, and a reduction of the placental size was

induced by caffeine at concentrations higher than 50 micrograms/ml; hypoplasia of the forelimb bud was induced at concentrations higher than 100 micrograms/ml; hematoma in the yolk sac and dysmorphogenesis of the fore- and hind-limb buds, prosencephalon and tail were induced by 200 micrograms/ml caffeine. In the MTA, even with 200 micrograms/ml caffeine, the toxicological parameters obtained by proliferation and differentiation assays of the midbrain and limb bud cells were almost the same as in the control. In conclusion, caffeine induced various morphological anomalies, but did not affect proliferation or differentiation of cells in these experimental systems.

Effects of caffeine and related methylxanthines on fetal mouse palates cultured in vitro.
Kosazuma T and Kawauchi S.
J Toxicol Sci 1994;19:175-80.

The maxillary regions of day-12.5 ICR mouse fetuses were cultured in a chemically-defined serumless medium and the effects of methylxanthine derivatives on cultured palates were studied. Explanted palates were treated for 72 hr in vitro with 0.5-2 mM caffeine (CA), 1-2 mM theophylline (TP), or 1-2 mM theobromine (TB). Although the three compounds tested did not prevent the contact of opposing palatal shelves, palatal fusion was inhibited by CA and TP at a concentration of 2 mM, and the inhibitory effect of CA was more evident than that of TP. On the other hand, TB did not exert an inhibitory effect on palatogenesis at 2 mM. Since the in vitro toxicity of the methylxanthine compounds appeared to correlate well with their in vivo teratogenic potential, the organ culture method of fetal rodent palates should be a useful tool for screening teratogenic agents, especially those causing cleft palate.

Alteration of femoral structure in later life by chronically feeding caffeine during rapid growing period in newborn female rats.
Sasahara H, Cheuk SL, Wink CS, Hashimoto K, Rossowska MJ, and Nakamoto T.
Toxicol Lett 1994;73(1):55-64.

The effects of caffeine intake in early life on bone structure later in life were studied in rats. At day 9 of gestation, dams were divided into 2 groups. Group 1 (control) received a 20% protein diet; group 2 received the 20% protein diet supplemented with caffeine (2 mg/100 g body weight). After birth pups were continuously fed their respective diets until day 93, when the diet of group 2 was replaced with a noncaffeine 20% protein diet. On day 388 animals from both groups were weighed, killed, and femora and mandibles were removed. Calcium, phosphorus, magnesium, zinc, hydroxyproline, and hexosamine concentrations were measured. Radiographs of some femora were taken and paraffin cross sections were made at the midshaft of others. Femora in the caffeine group were wider, periosteal bone area/total bone area was greater, the cross sectional area of femoral bone was smaller, and there were fewer osteocytes/bone area than in controls. Calcium,

phosphorus, zinc, and hydroxyproline concentrations in the caffeine group were less in both bones of the caffeine group. These results indicate that if animals are exposed to caffeine during the rapidly growing period, changes occur in femoral bone which are similar to those that occur with aging.

Combined effects of caffeine and malnutrition during pregnancy on suckling behavior in newborn rats.
Yoshino S, Narayanan CH, Joseph F Jr, Saito T, and Nakamoto T.
Physiol Behav 1994;56(1):31-7.

Six groups of pregnant dams were fed a 20%, 12%, and 6% protein diet with and without caffeine (2 mg/100 g b.wt.), starting on day 7 of gestation. At day 18 of gestation, randomly selected dams of each group were used to record prenatal fetal behavior. The remaining dams were continuously fed their respective diets until the birth of their pups. Upon delivery, newborn pups from the dams fed a 20%, 12%, or 6% protein diet with caffeine were placed with foster dams that the dietary regimen during gestation was a 20%, 12%, or 6% protein diet, respectively. Dams fed a noncaffeine diet, along with their newborns, were fed their respective diets until day 15. Suckling behavior tests for newborns were conducted on days 2, 8, and 15. On day 15, both nondeprived and deprived newborn rats were studied. Caffeine in combination with a malnourished diet has different effects on general activity in prenatal stages compared to postnatal stages. Our findings support the view that prenatal exposure to caffeine may produce greater effects because: a) caffeine and its metabolites pass freely into the embryo and attain a concentration slightly lower than in the maternal plasma; and b) caffeine may be poorly metabolized during pregnancy, causing an accumulation in the fetal tissues. Prenatal caffeine at the dosage we have used in combination with malnutrition may produce lasting metabolic alterations in the nervous system related to the emergence of suckling behavior and general motor activity.

Placental transfer and foetal disposition of caffeine and its immediate metabolites in the 20-day pregnant rat: function of dose.
Abdi F, Pollard I, and Wilkinson J.
Xenobiotica 1993;23(4):449-56.

The dispositions of caffeine and its immediate dimethylxanthine metabolites, theobromine, theophylline and paraxanthine were studied after a single oral dose of 5 and 25 mg/kg caffeine administered to 20-day pregnant and non-pregnant rats, respectively. 2. Peak plasma levels were reached between 1 and 3 h in all fluids and tissues studied. 3. The elimination phase, however, differed significantly between the pregnant and non-pregnant groups. For 25 mg/kg the plasma half-life (t1/2) of caffeine was significantly longer in the pregnant than the non-pregnant group; for 5 mg/kg the elimination rate of caffeine was similar in both groups. 4. AUC values were used to compare caffeine and

metabolite exposure in foetal tissues. At 5 mg/kg, peak concentrations for amniotic fluid, foetal blood, liver and kidney were not significantly different from one another. At 25 mg/kg peak levels in foetal liver and kidney were significantly less than those of foetal blood, amniotic fluid or placenta. 5. Because of the observed increase in maternal t1/2 at high dosage, a cautionary note is sounded about caffeine intake in pregnancy.

Acetazolamide with caffeine causes exencephaly in "resistant" SWV mice.
Beck SL and Urbano CM.
Reprod Toxicol 1993;7(2):123-9.

Pregnant SWV mice were treated on day 9 of gestation (PC) with 50 mg/kg of caffeine (CAFF), 200 mg/kg (LD) or 1000 mg/kg (HD) of acetazolamide (ACZM), or a combination of both agents, or on day 8 PC with both agents (ACZM + CAFF). Untreated (UNTD) and vehicle-treated (VEH) groups served as controls. The SWV strain is widely reported to be resistant to ACZM; it was resistant to ACZM or CAFF + ACZM when treated on day 9 of gestation, but a significant frequency of malformations, primarily exencephaly, was produced by ACZM + CAFF on day 8 PC. This study provides evidence that ACZM, coupled with a subteratogenic dose of caffeine can produce abnormalities in the "resistant" SWV mice, using the endpoint of exencephaly on day 8 of gestation. The mean number of ossified caudal vertebrae in day-9 treatments and ossified cervical vertebral centra in day-8 treatments were reduced. The frequency of ossification of the first cervical vertebra (C1) was reduced from 93% in UNTD to 39% in HD-ACZM day 9 PC and 69% in HD-ACZM + CAFF day 9 PC groups, and was also significantly reduced in the HD-ACZM + CAFF day-8 treated group.

Relation between rate of cell proliferation and formation of micronuclei after combined treatment with X-rays and caffeine.
Muller WU, Kasper C, and Streffer C.
Radiat Environ Biophys 1993;32(3):239-49.

We studied the effects of caffeine (2 mM), X-rays (1 Gy) and the combination of both agents on cell proliferation and formation of micronuclei in the early stages of preimplantation mouse embryos in vitro. Two-cell embryos were exposed to the agents shortly before division to the 4-cell stage. Proliferation and micronucleus production was monitored every 2 h in the 4- and 8-cell stages. A rather peculiar pattern of micronucleus formation after radiation exposure alone was observed for 8-cell embryos: those embryos that were the first to enter the 8-cell stage showed two to three times higher numbers of micronuclei per cell when compared with those embryos that entered the 8-cell stage some hours later. Studies of the kinetics of cell proliferation and of micronucleus formation in 4- and 8-cell embryos and exposure to caffeine revealed that this result could be explained by two factors: a slight asynchrony in the developmental stage at the time of exposure and the length of the interval being available for repair processes. When

caffeine was present, a third factor had to be taken into consideration: direct inhibition of repair by caffeine.

Effects of alcohol and caffeine on cultured whole rat embryos.
Fadel RA and Persaud TV.
Acta Anat (Basel) 1992;144(2):114-9.

The direct effects of ethanol and caffeine on embryogenesis were investigated using the whole rat embryo culture system. Compared to control embryos, the crown-rump length, number of somites, branchial bars, and morphological score were significantly reduced in embryos exposed to ethanol, caffeine, or both substances. Development of the craniofacial region, cardiac primordium and forelimb was delayed following ethanol treatment. Compared to the controls, the anterior neuropore lagged in development following caffeine treatment; closure of the posterior neuropore was significantly delayed in each of the treatment groups. The optic and olfactory primordia were not affected. The results indicate that alcohol and caffeine independently affect the embryo, but when combined their effects were not potentiated.

The effects of caffeine on the maxillary composition in the newborn rat.
Valdes M, Shaye R, Joseph F Jr, and Nakamoto T.
Calcif Tissue Int 1992;50(2):165-8.

The possible influence of caffeine on maxillary structure was studied. Seventeen pregnant rats at days 9 of gestation were randomly divided into two groups. The dams of group 1 received a 20% protein diet ad libitum throughout the experimental period. The dams of group 2 were pair-fed, with group 1, a 20% protein diet supplemented with 2 mg/100 g body weight (B.W.) caffeine. At birth, pups were mixed within the same group and 8 randomly selected pups were assigned to each dam and continuously fed the respective diet. On day 22, 11 male pups from the control and 12 males from the caffeine group were randomly selected, separated from the dams, and continued to be fed their respective diets. On day 44, a rubber elastic band was inserted between the first and second maxillary right molars. The size of the elastic band was increased throughout the next 5 days. Animals were sacrificed at day 49 and the composition of the maxillas was analyzed. After pulverization, organic and inorganic contents of the bones were measured. Zinc (Zn) and hydroxyproline concentration of the caffeine group showed a significant decrease when compared with those of the controls. However, Ca, P, Mg, and hexosamine concentration showed no difference between the groups. The interdental space measured occlusally and laterally with the visual method, and occlusally in histological sections showed no significant difference between the control and caffeine groups, although variation of the space in the caffeine group was less than in the control group.(ABSTRACT TRUNCATED AT 250 WORDS)

Interaction between caffeine and zinc on brain in newborn rats.
Nakamoto T and Joseph F Jr.
Biol Neonate 1991;60(2):118-26.

The purpose of this study was to determine whether adding zinc to the caffeine-supplemented diet of dams during gestation and lactation would affect brain development in newborn rats. On day 9 of gestation, dams of group 1 were fed to a 20% protein diet as a control. Dams of group 2 were fed a 20% protein diet supplemented with caffeine. Dams of group 3 were fed a 20% protein diet supplemented with caffeine and zinc. The amount of caffeine added to the maternal diet was 2 mg/100 g of body weight. The amount of zinc chloride added to diet was 0.6 g/kg of diet. At birth, 8 randomly selected pups from each group were assigned to each dam of the respective group and were continuously fed the same diet. On day 15, the pups were killed and brains were removed. Zinc, protein, DNA, alkaline phosphatase activity and cholesterol contents were measured. Milk and maternal and neonatal blood were collected to determine caffeine levels. There was a significant correlation between the milk caffeine and brain caffeine concentrations in group 3. A significant correlation between the neonatal plasma caffeine and brain caffeine concentrations was observed in groups 2 and 3. There was no correlation between neonatal brain weight and zinc content per brain in each group. The correlation between neonatal brain weight and alkaline phosphatase activity was significant in groups 1 and 3. The neonatal zinc content and concentration of group 2 was less than that of group 1. The DNA content and concentration of group 3 was greater than that of either groups 1 or 2. Supplementation of zinc to the caffeine-added diet could restore the brain zinc levels observed in brains of newborn rats.

Lasting effects of early chronic caffeine feeding on rats' behavior and brain in later life.
Nakamoto T, Roy G, Gottschalk SB, Yazdani M, and Rossowska M.
Physiol Behav 1991;49(4):721-7.

Pregnant dams were fed a 20% protein diet with caffeine (2 mg/100 g b.wt.), starting on day 9 of gestation. At birth, each dam with 8 assigned pups was fed this diet until weaning, day 22. On day 22, female rats were caged and fed this diet until day 93. Starting on day 93, the caffeine-supplemented diet was replaced with a caffeine-free, 20% protein diet until day 388. Starting on day 31, each animal was placed in a photoactivity cage, and locomotive activity was measured until day 375. On day 388, the animals were killed, and their brains were removed and divided into 7 regions. The weight, DNA, protein and zinc contents, and alkaline phosphatase activity of each region were determined. Locomotive activity of the caffeine-fed group was higher than in the noncaffeine control group. Accumulative activity scores showed 3 subgroups (high, medium, and low) in both groups at day 93. The medium activity subgroup in the caffeine group was greater than the controls from day 72 to day 93. These differences reappeared 5 weeks after cessation of caffeine supplementation and continued until day

375. The differences in activity were minimum in the high and low subgroups. Chronic caffeine intake in early life permanently affected the medium activity subgroup. Furthermore, various regions of the brain were biochemically altered in spite of the feeding of a noncaffeine diet for almost 300 days after caffeine.

Caffeine administered during pregnancy augments subsequent lactation in mice.
Sheffield LG.
J Anim Sci 1991;69(3):1128-32.

Mice were administered caffeine (500 mg/liter of drinking water) from d 1 until d 18 of pregnancy. Before and after receiving caffeine, mice were given distilled water for drinking, as were controls. Litter size, birth weight, and offspring survival were not affected by caffeine, but litter weight on d 15 of lactation was increased by caffeine 84.0 +/- 3.1 g (n = 8) in controls vs 98.3 +/- 3.7 g (n = 7) in caffeine-treated mice (P less than .05). Mammary gland cell number, measured by the DNA content of mammae, was increased by giving caffeine during pregnancy (P less than .05). On d 18 of pregnancy, mammary DNA was .47 +/- .07 mg (n = 6) in controls vs .71 +/- .11 mg (n = 6) in caffeine-treated mice. On d 15 of lactation, mammary DNA was .96 +/- .12 mg (n = 8) in control vs 1.26 +/- .11 mg (n = 7) in caffeine-treated mice. RNA increased (P less than .05) parallel to DNA. In additional experiments, litters were cross-fostered and standardized to eight pups per litter. Mice were bred and caffeine was administered as described previously. At birth, eight pups from mice treated with caffeine or as controls during the preceding pregnancy were fostered to a control or caffeine-treated mother. In these experiments, litter weight on d 15 of lactation was 82 g for control litters nursing control mothers, 84 g for caffeine litters nursing control mothers, 96 g for control litters nursing caffeine mothers, and 99 g for caffeine litters nursing caffeine mothers (n = 7 per groups, pooled SE = +/- 3.4 g).(ABSTRACT TRUNCATED AT 250 WORDS)

Prenatal effects of maternal caffeine intake and dietary high protein on mandibular development in fetal rats.
Driscoll PG, Joseph F Jr, and Nakamoto T.
Br J Nutr 1990;63(2):285-92.

The purpose of the present study was to determine the effects of caffeine on the mandibles of newborn rats whose dams were given a normal diet (200 g protein/kg diet) compared with those given a high-protein diet (400 g protein/kg diet) during gestation. A total of twenty pregnant Sprague-Dawley rats were randomly divided into four groups of five each. Starting on day 7 of gestation, groups 1 and 2 were fed on control and high-protein diets respectively, and groups 3 and 4 were pair-fed with groups 1 and 2 respectively, but with caffeine added to their diets. The caffeine supplement was 20 mg/kg body-weight. At birth, pups were killed and various measurements of their mandibles were made. The mandibular weights, calcium contents, and alkaline (EC

3.1.3.1) and acid (EC 3.1.3.2) phosphatase activities of the group given the caffeine-supplemented control diet were significantly lower than those of the corresponding unsupplemented group. Alkaline and acid phosphatase activities, collagen synthesis and hydroxyproline contents of the caffeine-supplemented high-protein group were greater than those of the corresponding unsupplemented group, whereas Ca and protein contents of the caffeine-supplemented high-protein group were lower than those of the corresponding unsupplemented group. There were no significant differences in plasma caffeine levels for either dams or pups between the caffeine-supplemented control and high-protein groups. The effects of caffeine on the development of fetal mandibles are apparently modified by different levels of maternal dietary protein.

Maternal-fetal electrocardiographic effects and pharmacokinetics after an acute i.v. administration of caffeine to the pregnant rat.
Leal M, Barletta M, and Carson S.
Reprod Toxicol 1990;4(2):105-12.

The relationship between fetal exposure and cardiovascular functional effects in the caffeine-treated pregnant rat was investigated. Caffeine (100 mg/kg) was administered intravenously to dams on day 21 of gestation. The transplacental transport of caffeine was studied by obtaining maternal and fetal blood (umbilical vein) samples at designated times after drug administration. Concurrent maternal-fetal electrocardiograms (ECGs) were measured and evaluated for caffeine-induced changes. Maternal and fetal plasma caffeine levels as well as area under the curve values were proportionally related throughout the experiment, indicative of equal exposure to caffeine. The fetal ECG exhibited more extensive changes associated with caffeine than did the dam's, but the effects were not detected in the first 30 min, suggesting a lag period for the action of caffeine on the fetal heart. The frequency of fetal ectopic beats and abnormal T waves were directly related to fetal plasma caffeine levels. Fetal ECG combined with the fetal blood microsampling technique was a practical method of testing for prefunctional effects of caffeine in the rat fetus.

Influence of caffeine administered during pregnancy on the early differentiation of fetal rat ovaries and testes.
Pollard I, Williamson S, and Magre S.
J Dev Physiol 1990;13(2):59-65.

In this manuscript it is demonstrated that caffeine when administered to the rat (30 mg/kg per day) during pregnancy affected certain aspects of normal sexual differentiation of the fetal gonads. In the male fetus caffeine was shown to significantly inhibit differentiation of the interstitial tissue and Leydig cells. A significant decrease in the number of Leydig cells exhibiting 3 beta-hydroxysteroid dehydrogenase activity, and consequent reduction in testosterone biosynthesis in the fetal testes at day 15 and day 16 of gestation was

found. While the beginnings of Leydig cell function was first seen in the afternoon of the 14th day of gestation in both the experimental and control groups, the adverse effects became marked by 15 days and extreme by 16 days. With the aid of the scanning electron microscope it was observed that caffeine also had an effect on the earlier morphogenic organisation of the seminiferous cords at 13 days of gestation where the aggregation of the Sertoli cells forming the seminiferous cords, was marginally advanced in the control group. However the treated group had caught up by 14 days of gestation. In the female fetus scanning electron microscope studies revealed that in the control and caffeine treated groups the early phase of ovarian differentiation and the later 20 day ovaries were similar in morphology, tissue arrangement and overall appearance. It was also seen that chronic caffeine exposure did not affect the rate of early mitotic proliferation of germ cells, nor later in development the numbers entering meiosis. At 20 days of gestation the numbers and proportion of meiotic to atretic oocytes were comparable in the control and treated groups.(ABSTRACT TRUNCATED AT 250 WORDS)

Teratogenic drugs inhibit the differentiation of fetal rat limb buds grafted in athymic (nude) mice.
Shiota K, Uwabe C, Yamamoto M, and Arishima K.
Reprod Toxicol 1990;4(2):95-103.

Forelimb buds of day-14 rat fetuses were cut into pieces and transplanted subcutaneously into athymic (nude) mice. On the 7th, 9th, and 11th days after grafting, the nude mice were treated with various drugs including rat teratogens. On the 20th day, the grafted tissue was examined macroscopically and histologically. While control grafts showed substantial growth and tissue differentiation similar to that observed in vivo, the differentiation of grafts was significantly inhibited by the treatment with 5-fluorouracil, cyclophosphamide, hydroxyurea, cycloheximide, mitomycin C, caffeine, aspirin, retinol palmitate, all-trans-retinoic acid, and ascorbic acid. Hydrocortisone, tetracycline, and thalidomide did not adversely affect the differentiation of grafts. Thus, the susceptibility of transplanted rat limb buds was generally close to the teratologic sensitivity of rat fetuses in vivo. The heterotransplantation method of embryonic tissues may be useful as a new experimental system in developmental toxicology.

Cerebral amino acids in neonate from caffeine-drinking dam.
Tanaka H and Arima M.
Amino Acids: Chem Biol Med (Pap Int Congr Amino Acid Res) 1990;303-6.

The cerebral free amino acids in neonatal rats, from dams given 0.04% caffeine in the drinking fluid ad libitum before and/or during pregnancy throughout the lactational period, were examined on days 1, 5 and 10. Significantly reduced cerebral weight was observed on day 1. The tyrosine concentration in the cerebrum, but not that in the liver, was increased on days 1 and 5. The tyrosine level showed a positive correlation with the

caffeine level in neonatal cerebrum only on day 1. These results suggest that maternal caffeine disturbs the neonatal cerebrum through tyrosine and tyrosine hydroxylase, and then produces behavioral abnormalities in developing rats.

Maternal caffeine ingestion increases the tyrosine level in neonatal rat cerebrum.
Tanaka H and Nakazawa K.
Biol Neonate 1990;57(2):133-9.

The cerebral free amino acids in neonatal rats, from dams given 0.04% caffeine in the drinking fluid ad libitum before and/or during pregnancy throughout the lactational period, were examined on days 1, 5 and 10. Significantly reduced cerebral weight was observed on day 1 with a mean caffeine level of 7 micrograms/g wet weight. The tyrosine concentration in the cerebrum, but not that in the liver, was increased on days 1 and 5 with approximate mean caffeine levels of above 1.5-2.0 micrograms/g wet weight. The tyrosine level showed a positive correlation with the caffeine level in neonatal cerebrum only on day 1 in the group with caffeine ingestion after pregnancy. There was no significant increase in the fetal cerebral concentration of MOPEG-SO4 on day 5 with maternal caffeine. These results suggest that maternal caffeine disturbs the neonatal cerebrum through tyrosine and tyrosine hydroxylase, and then produces behavioral abnormalities in developing rats.

Various levels of maternal caffeine ingestion during gestation affects biochemical parameters of fetal rat brain differently.
Yazdani M, Joseph F Jr, Grant S, Hartman AD, and Nakamoto T.
Dev Pharmacol Ther 1990;14(1):52-61.

Pregnant dams were divided into four groups on day 10 of gestation. Dams of group 1 were fed an 20% protein diet as controls. Dams of groups 2, 3 and 4 were fed a 20% protein diet supplemented with 0.5 mg, 1 mg and 2 mg caffeine/100 g body weight of dams, respectively. Pups were delivered surgically on day 22, and their brains were rapidly removed and analyzed for DNA, protein, cholesterol, zinc and alkaline phosphatase activity. The dams' brains were analyzed for the same parameters as those of the pups. Plasma and brain caffeine levels were also determined in caffeine-supplemented groups. The pups' brains in group 2 were heavier than those in group 4. The DNA concentration of group 2 was higher than that of the other groups. The protein concentration of group 4 was higher than that of the other groups. The cholesterol concentration of group 3 and 4 was less than that of the controls. The zinc concentration of group 4 was less than that of group 2. Alkaline phosphatase activity was decreased in groups 3 and 4 compared with either controls or group 2. Dams showed no significant difference among the groups in the same biochemical parameters except for cholesterol concentration that was higher in groups 2, 3 and 4 than in the controls. Plasma and brain caffeine levels of the fetuses and plasma caffeine of the dams in group 4 were higher than

those of either group 2 or 3. It is concluded that various amounts of maternal caffeine intake exert different effects on fetal brain growth. In contrast, the effect of caffeine on the dams' brain is relatively minor.

Toxicity of various combinations of X-rays, caffeine, and mercury in mouse embryos.
Muller WU.
Int J Radiat Biol 1989;56(3):315-23.

Preimplantation mouse embryos in vitro were exposed to various doses of X-rays (0.25-2 Gy) and to different concentrations of two chemicals: caffeine (0.5-2 mM) and mercury (0.5-5 microM). X-irradiation was given first, followed immediately by exposure to the chemicals. The effects of the agents, applied either singly or in combination of two or of three, were studied using morphological, proliferative and cytogenetic endpoints (formation of blastocysts, hatching, trophoblast outgrowth, formation of inner cell mass, cell numbers, micro-nucleus frequency). The term 'enhancement in risk' was used whenever the effects observed after combined exposure (two or three agents) significantly exceeded the sum of the effects due to the component individual agents. The enhancement in risk observed after exposure to the three agents could be explained by the interactions already detected at the level of a combined exposure to only two agents. There was no increase in risk specific for the presence of all three agents.

Alteration of the effects of caffeine by prenatal stress.
Pohorecky LA, Roberts P, Cotler S, and Carbone JJ.
Pharmacol Biochem Behav 1989;33(1):55-62.

We examined the effect of prenatal stress exposure on sensitivity to caffeine using behavioral and physiological measures. Pregnant rats were handled 5 minutes daily from the 14th to 21st day of gestation. Male offspring were tested when 60 days of age in a modified open field apparatus 30 and 90 minutes after injection with caffeine (0, 10, 30 mg/kg). Caffeine increased crossover frequency and duration at the 10 mg/kg dose. Rearing frequency and duration were increased by the 10 mg/kg dose while the 30 mg/kg dose was ineffective. Gnawing was increased by caffeine, especially 90 minutes postinjection. Headpoke activity was decreased by caffeine treatment. Caffeine had no effect on defecation and urination. Gnawing activity was increased by caffeine in prenatally nonstressed animals, but was depressed in prenatally stressed animals. Prenatal stress increased sensitivity to caffeine on corner activity and rearing. The other measures were not affected differentially by prenatal stress exposure. Rectal temperature was depressed 0.75 degrees C in both prenatally stressed and nonstressed animals, by the 30 mg/kg dose of caffeine. Thus, our results indicate that prenatal stress affects sensitivity to caffeine in the adult offspring. However, the long-term effects of prenatal stress exposure are dependent on the measures employed.

Preliminary indications that functional effects of fetal caffeine exposure can be expressed in a second generation.
Sinton CM.
Neurotoxicol Teratol 1989;11(4):357-62.

Caffeine, added to the drinking water of males used for impregnation and gestant BALB/c mice such that their daily caffeine intake was 60 mg/kg, modified the passive avoidance behavior of the offspring when tested as adults. Caffeine-treated and control mice of the F1 generation were then cross-mated. The F2 generation was not exposed to caffeine but, when tested as adults, there were significant differences in passive avoidance latencies among the F2 mice. These data are a preliminary indication that effects resulting from fetal caffeine exposure in the F1 mice can be expressed in a second generation. Some cross-fostered groups of mice were tested in both the F1 and F2 generations as an initial control for postnatal maternal effects. F1 caffeine-treated mice also carried significantly smaller litters, implying that prenatal caffeine exposure could have affected the reproductive ability of these mice. It is tentatively concluded that a changed uterine environment, possibly interacting with an effect on the germ line, may be reflected in neurobehavioral effects in the second generation.

Effects of caffeine and 3-isobutyl 1-methyl xanthine on caprine milk secretion.
Brown DL and Harris DM.
J Dairy Sci 1988;71(2):513-7.

The objective of these studies was to determine if methyl xanthines can be used to alter milk production or composition in ruminants by enhancing adipose tissue mobilization. Three trials were conducted, one with intravenous caffeine infusions, one with intramuscular caffeine injections, and one with intramuscular injections of 3-isobutyl 1-methyl xanthine. Results indicate that: 1) continuous intravenous infusions of caffeine (720 mg/d) may reverse the milk fat depression of intravenously infused glucose in dairy goats; 2) intramuscular injections of caffeine (200 mg twice daily) do not reverse the milk fat-depressing effects of pelleted dairy goat diets during the 4th mo of lactation; and 3) intramuscular injections of 3-isobutyl 1-methyl xanthine (50 mg twice daily) do not consistently affect milk production of early lactation dairy goats.

Caffeine disposition in the pregnant rabbit. II. Fetal distribution of caffeine and paraxanthine during chronic maternal caffeine administration.
Dorrbecker SH, Kramer PA, Dorrbecker BR, and Raye JR.
Dev Pharmacol Ther 1988;11(2):118-24.

Twenty-three New Zealand White rabbits received a continuous intravenous infusion of caffeine during gestation. The amniotic fluid/maternal plasma concentration ratio was higher for caffeine than for its major metabolite, paraxanthine, throughout gestation, and

increased near term for both compounds. Both compounds distributed nearly homogeneously to fluids and tissues of the 29-day fetus, with mean fetal/maternal concentration ratios of 0.7 for paraxanthine and 0.9 for caffeine. The free fraction of caffeine was constant during gestation (about 0.8), while that of paraxanthine increased from 0.25 to 0.4. Similar results were observed in 3 Dutch Belted rabbits given caffeine in their drinking water and sacrificed at 29 days of gestation.

Caffeine and reduction of fetal ossification in the rat: fact or artifact?
Muther TF.
Teratology 1988;37(3):239-47.

This study was done to determine the gestational period during which the rat fetus is susceptible to reduction of skeletal ossification by caffeine. Caffeine, 100 mg/kg/day by gavage, caused the greatest reduction in ossification, as assessed by staining with alizarin red S, in fetuses exposed between day 16 to 19 of gestation, less in those treated between day 7 to 19, and markedly less in those receiving it between day 7 to 16; a single dose on day 19 had very little effect. This indicates that the fetus is most susceptible late in pregnancy. Bones in early stages of mineralization on day 20 showed a greater reduction in staining than those in later stages. Thus, caffeine appears to lower the rate of ossification rather than reduce its final extent. In the day 7 to 19 caffeine treatment group, but not in the day 16 to 19 group, maternal and fetal body weights were reduced, and 1.6% of the fetuses had aphalangia. The day 7 to 16 caffeine treatment reduced fetal body weight. This argues against an association between reduction in fetal weight and ossification. None of the treatments affected rates of resorption or litter size. A novel and important observation made is that the different caffeine treatments affected the staining by alizarin of both claws and bones in a qualitatively and quantitatively similar manner. Since claws are devoid of osteoid, this observation questions the specificity of alizarin for the assessment of the state of fetal ossification and raises doubt as to the significance of the observed action of caffeine on ossification.

Protein-energy malnutrition in rats during pregnancy modifies the effects of caffeine on fetal bones.
Nakamoto T and Shaye R.
J Nutr 1986;116(4):633-40.

The mandibles and long bones of newborn rats were analyzed for the effects of maternal caffeine consumption and protein-energy malnutrition. On d 13 of gestation, dams were randomly picked and divided into four groups. Group 1 received a 20% protein diet ad libitum. Group 2 was pair-fed with group 1 a 20% protein diet with a caffeine supplement (2 mg/100 g body wt). Group 3 received a 6% protein diet ad libitum. Group 4 was pair-fed with group 3 a 6% protein diet with caffeine. Within 8 h of delivery, all pups were weighted. Randomly selected pups were injected with 14C proline to study collagen

synthesis of bones. Other pups were injected with 45Ca to study mineralization of bones. Although the average litter size from the 20% protein groups with or without caffeine did not show much variation, fetal resorption and stillbirths were higher in litters from group 4 compared to those from group 3. The mandibular weights of pups from group 2 was less than those from group 1, whereas weight of long bones of those from group 4 was heavier. The rate of collagen synthesis and calcium content of the mandible of group 4 and 45Ca uptake of the mandible of groups 2 and 4 were greater than that of the corresponding noncaffeine group. The rate of collagen synthesis, hydroxyproline content, 45Ca uptake and calcium content of the long bones of groups 2 and 4 were greater than that of the noncaffeine groups. The findings suggest that nutritional factors and the effects of caffeine are closely interrelated in the growth and development of the fetus and bone in newborn rats.

Caffeine effects on cyclic AMP levels in the mouse embryonic limb and palate in vitro.
Schreiner CM, Zimmerman EF, Wee EL, and Scott WJ Jr.
Teratology 1986;34(1):21-7.

Caffeine is a teratogen that causes limb and palate malformations in rodents. Since the ability to raise cyclic nucleotide levels is a known biological action of caffeine, cyclic AMP levels were measured in CD-1 mouse embryonic forelimb from whole embryo culture and embryonic limb and palate cells grown in primary culture following treatment with various concentrations of caffeine (0, 1, 3, or 10 mM). In forelimb buds from whole embryo culture, a dose-dependent response was observed. Caffeine at 1 mM concentration stimulated cyclic AMP levels to 151% of control value at 60 min. Even greater stimulation of cyclic AMP occurred at higher caffeine concentrations. A dose-dependent response was seen in both limb and palate cell culture. In limb cell culture, all caffeine concentrations significantly stimulated cyclic AMP after 10 min compared to control. In palate cell culture, there was a twofold increase in cyclic AMP at the 1-mM caffeine concentration. At higher caffeine concentrations, cyclic AMP was significantly increased after 60 min. In addition, stimulation of cyclic AMP in cultured limb and palate cells by isoproterenol, a beta-adrenergic agonist, was used as a positive control. Isoproterenol stimulated a 2.5-fold greater response in the palate cells than in the limb bud cells at isoproterenol levels of 10(-5) or 10(-4) M. The increase of cyclic AMP may be influential in the process of abnormal limb or palate development.

Teratogenicity of paraxanthine (1,7-dimethylxanthine) in C57BL/6J mice.
York RG, Randall JL, and Scott WJ Jr.
Teratology 1986;34(3):279-82.

The teratogenicity of caffeine, as well as two of its three dimethylated metabolites (theobromine and theophylline), has been established in animal studies. The third

metabolite, paraxanthine, has not been reported as being tested for teratogenicity even though it is actually the major demethylated metabolite of caffeine metabolism in man. Pregnant C57BL/6J mice were treated i.p. with 175 or 300 mg/kg/day paraxanthine (1,7-dimethylxanthine) dissolved in deionized water at 4 p.m. on day 11 and 9 a.m. on day 12 of gestation. All dams were sacrificed on day 18, and fetuses were fixed for Wilson's razor blade sectioning or double-staining skeletal examination. A dose-related increase in total malformations, primarily cleft palate and limb malformations, was found. The pattern of malformations was similar to that reported for caffeine, theobromine, and theophylline, i.e., an asymmetric response with the left forelimb most often affected. A 21% resorption and a 46% malformation rate was observed at 300 mg/kg/day of paraxanthine, indicating that paraxanthine was slightly less toxic to the embryo than caffeine. Therefore, the parent compound, caffeine, as well as all three of its dimethylated metabolites--paraxanthine, theophylline, and theobromine--are teratogenic.

Caffeine disposition after oral administration to pregnant rats.
Jiritano L, Bortolotti A, Gaspari F, and Bonati M.
Xenobiotica 1985;15(12):1045-51.

Caffeine disposition was studied over 24 h in rats on the 12th day of pregnancy given 80 mg/kg of drug as a single oral dose or in four divided doses every three hours. Peak blood levels of caffeine were reached at three hours after the single dose, and at 10 h (at half the previous value) after the first of the divided doses. At the end of the experiment both caffeine and its dimethylxanthine metabolites were higher in blood, amniotic fluid and fetuses after divided doses than after the single dose. Urinary excretion over 24 h was the same for the two groups. The overall conclusions underline that caffeine per se and not its metabolites are responsible for the teratogenic effects.

Supraadditive formation of micronuclei in preimplantation mouse embryos in vitro after combined treatment with X-rays and caffeine.
Muller WU, Streffer C, and Wurm R.
Teratog Carcinog Mutagen 1985;5(2):123-31.

The influence of caffeine (0.1 or 2 mM), X-rays (0.24 Gy [= 25 R] or 0.94 Gy [= 100 R]), or of a combination of both on the formation of micronuclei in early stages of preimplantation mouse embryos in vitro was studied. X-rays as well as caffeine induced micronuclei. The dose-effect curve after irradiation was linear for the dose range measured (0-3.76 Gy; = 0-400 R). Caffeine did not induce micronuclei if the concentration was 1 mM or less; between 1 mM and 7 mM, however, there was a linear increase in the number of micronuclei. A considerable enhancement of the number of radiation-induced micronuclei was observed when irradiation of the embryos was followed by a treatment with caffeine. Not only was the sum of the single effects exceeded by the combination effects, but the combination results even lay in the range of

supraadditivity of the envelope of additivity.

Developmental and cytogenetic effects of caffeine on mouse blastocysts, alone or in combination with benzo(a)pyrene.
Spindle A and Wu K.
Teratology 1985;32(2):213-8.

Mouse blastocysts were treated with caffeine and/or benzo(a)pyrene (BP), and the effects on development and on induction of sister chromatid exchanges (SCEs) were examined. Caffeine interfered with blastocyst development in a dose-related manner. At 4 mM, the highest concentration tested, caffeine interfered with development of blastocysts to all four endpoints: hatching, trophoblast outgrowth, inner cell mass (ICM) growth, and two-layer (primary endoderm and ectoderm) differentiation of ICMs. At 2 mM, caffeine reduced the incidence of both ICM growth and differentiation but did not affect hatching or formation of trophoblast outgrowths. At 1 mM, caffeine interfered only with ICM differentiation. Cell proliferation was least sensitive to caffeine and was reduced at concentrations of greater than or equal to 2 mM. Induction of SCEs was most sensitive to caffeine exposure; an increase in SCE frequency was observed at 0.1 and 0.5 mM. When caffeine was added to cultures with BP (1 microM, a concentration that was not embryotoxic and did not induce SCEs), both embryotoxic effects and SCE frequency were increased. The enhancing effect on SCE induction was particularly marked; as little as 0.1 mM caffeine was sufficient to cause doubling of induced SCE frequencies when added to cultures with BP.

Reduction of caffeine teratogenicity in mice by inducing maternal drug metabolism with beta-naphthoflavone.
York RG, Randall JL, and Scott WJ Jr.
Teratology 1985;31(2):217-25.

The effect of stimulating maternal drug metabolism on caffeine teratogenicity was investigated in C57BL/6J (cytochrome P1-450 inducible) and AKR/J (cytochrome P1-450 noninducible) mice. The inducing agent, beta-naphthoflavone (beta-NF) in corn oil, was administered intraperitoneally (IP) to dams at 20 or 80 mg/kg/d on days 9 and 10 of gestation. Teratogenic injections of 175 mg/kg/d caffeine in deionized water were administered IP on days 11 and 12 of gestation. All dams were sacrificed on day 18 of gestation, and fetuses were fixed for razor blade sectioning and skeletal examination. Caffeine, without maternal metabolism stimulation, caused similar types and rates of malformations in both strains of mice. Inducing drug metabolism during pregnancy with beta-NF protected the embryos from the congenital toxicities of large injections of caffeine. Reductions in embryolethality, limb malformations, and hematoma formation were evident in the inducible strain but not in the strain incapable of being induced. A dosage of eighty mg/kg/d was more effective than 20 mg/kg/d beta-NF in decreasing

malformations, suggesting that stimulation of metabolism and caffeine-induced teratogenicity are inversely related. Rapid elimination of caffeine resulting from increasing drug metabolism with the concomitant decrease in toxicity would indicate that caffeine, and not a metabolite, is the toxicant.

Effects of ethanol and/or caffeine on fetal development and placental amino acid uptake in rats.
Henderson GI and Schenker S.
Dev Pharmacol Ther 1984;7(3):177-87.

The effects of ethanol alone or in combination with caffeine on fetal viability, growth and placental amino acid uptake function in the rat were examined. Compared to pair-fed control values, chronic ethanol exposure reduced fetal survival by 24%, fetal weight by 17%, along with weight decreases of fetal brain (16%), heart (31%), and kidney (48%) as compared to pair-fed control values. Placental weight was significantly increased by 17%. Concomitant caffeine intake generally exacerbated these effects with a further reduction in fetal survival, fetal body and visceral weights. Caffeine intake alone had no consistent effect on these parameters. Acute in vitro (3 mg/ml) and chronic in vivo ethanol exposure reduced placental net uptake of alpha-aminoisobutyric acid (AIB) by 32 and 45%, respectively. Neither acute in vitro (10 micrograms/ml) nor prior chronic caffeine exposure altered villous AIB uptake. Concomitant ethanol and caffeine treatment increased AIB uptake as compared to ethanol alone. However, AIB uptake continued to be reduced (by 22%) as compared to pair-fed control values.

Blood flow changes and conceptal development in pregnant rats in response to caffeine.
Kimmel CA, Kimmel GL, White CG, Grafton TF, Young JF, and Nelson CJ.
Fundam Appl Toxicol 1984;4(2 Pt 1):240-7.

Alterations in blood flow to the uterus and its contents during pregnancy have been suggested to account for the teratogenicity and/or embryotoxicity of several agents, including caffeine. Using a radioactive microsphere technique, blood flow to several maternal organs, including ovary, uterus, decidua, and chorioallantoic placenta (CAP), was measured following a single dose of 0 or 120 mg/kg caffeine by gavage to pregnant CD rats on Day 12 of gestation. At 1 or 4 hr after treatment, animals were anesthetized and strontium 85-labeled microspheres (25 micrometers diam) were infused into the left ventricle. Whole body and tissue radioactivity were determined. Maternal cardiac output (CO) and absolute flow (f1; ml/min), relative flow (f2; ml/min/g tissue), and flow as %CO (f3) to each tissue were calculated. Maternal CO was not altered. All blood flow parameters for ovaries, uterus, and bladder were reduced in treated animals at both time points except for absolute flow (f1) to the ovaries at 1 hr. Decidual changes included reduced weight at 1 hr, reduced f2 at 4 hr, and reduced f1 and f3 at both times. However,

CAP weight and blood flow wre not significantly altered by caffeine treatment. Examination of conceptuses from thse litters, and from other animals at 24 hr after treatment or at term did not reveal any significant effect of this dose of caffeine on viability, growth, or physical development. The ratio of embryo to maternal blood caffeine concentrations was approximately 1, indicating free transfer of caffeine to the embryos.(ABSTRACT TRUNCATED AT 250 WORDS)

Long-lasting tolerance to stimulatory effects of perinatal caffeine treatment.
Lombardelli G, Balduini W, Feduzi A, Peruzzi G, and Cattabeni F.
Psychopharmacology (Berl) 1984;84(2):285-6.

Pregnant rats were treated with caffeine in their drinking water at doses of 136.3 mg/kg/day during gestation and 222.2 mg/kg/day during lactation. The resulting offspring at 60 days of age (40 days after drug withdrawal) were tested in an activity monitor cage. Spontaneous locomotor activity and that induced by caffeine (10, 30, 60 mg/kg/day) were observed. Treated rats showed apparent tolerance to caffeine-induced motility. Therefore perinatal caffeine treatment seems to induce long-lasting tolerance. This finding contrasts with those previously reported for chronic caffeine treatment in adult rats, where tolerance disappears after only 2-3 weeks following drug withdrawal.

Effects of caffeine on the growth of mandible and long bone in protein-energy malnourished newborn rats.
Nakamoto T and Shaye R.
Proc Soc Exp Biol Med 1984;177(1):55-61.

Rat dams with eight pups each were divided into six groups upon delivery; the first three were fed 6, 12 or 20% protein diets, and the second three the same diets but with caffeine added in the amount of 2 mg/100 g body wt. At Day 15, randomly selected pups were injected with [14C]proline to determine collagen synthesis of mandible and long bone. Other pups were used to determine the calcium content of these bones. The body, mandibular, and long bone weight of the pups whose dams were fed the 6% protein diet with caffeine increased compared to the noncaffeine group. Calcium content of the mandible and the collagen synthesis of the long bone were also increased. However, calcium content of long bone, collagen synthesis of mandible, and hydroxyproline content of mandible and long bone showed no difference between the caffeine and noncaffeine groups. In the pups whose dams were fed the 12% protein diet with caffeine, body and mandibular weight, collagen synthesis, and hydroxyproline and calcium contents in mandibles and long bones of pups showed no difference from those of the noncaffeine group, but long bones were heavier. In the pups whose dams were fed the 20% protein diet with caffeine, the body and long bone weight and hydroxyproline and calcium contents of the long bone of pups were lower than those of the noncaffeine group. Mandibular weight, calcium content, and hydroxyproline showed no difference

between caffeine and noncaffeine animals, but collagen synthesis of the mandible was increased. Current data indicate that nutritional state and caffeine intake of the mother have a close relation to growth and development of the offspring.

Two-year toxicity/carcinogenicity study of fresh-brewed coffee in rats initially exposed in utero.
Palm PE, Arnold EP, Nick MS, Valentine JR, and Doerfler TE.
Toxicol Appl Pharmacol 1984;74(3):364-82.

Fresh-brewed regular coffee at concentrations of 25, 50, and 100% was consumed ad libitum as the sole fluid intake of F1 Sprague-Dawley rats (55 male and 55 female/group), derived from P0-treated females which were provided 50% coffee for about 5 weeks prior to copulation and throughout gestation and lactation. P0 males, P0 control females, and two groups of F1 control rats received tap water. Ten rats/sex/level were killed and examined after 1 year; survivors were killed after 2 years. Smaller mean body weights (50 and 100% coffee concentrations) occurred with increased feed and liquid consumption. Mean serum alkaline phosphatase, bilirubin, BUN, and calcium values occasionally were elevated. Serum cholesterol levels at 2 years were elevated in males (25 and 100%) and at 1 and 2 years in females (100%). Bone calcium was slightly reduced in females consuming 25 or 100% coffee for 1 year, but not after 2 years. Treatment-related increases in relative weights of lungs, kidneys, liver, and epididymides were recorded. Significantly increased mortality was observed in females receiving 50 or 100% coffee. There also was some evidence of a relationship between coffee consumption and the number of primary tumor-bearing animals; however, this finding appeared ambiguous, dependent on the assumption that tumors were the probable cause of death.

Pre- and postnatal effects of caffeine on brain biogenic amines, cyclic nucleotides and behavior in developing rats.
Concannon JT, Braughler JM, and Schechter MD.
J Pharmacol Exp Ther 1983;226(3):673-9.

To examine the perinatal effects of caffeine on pup behavior and brain neurochemistry, rat mothers were exposed to caffeine in a choice situation prenatally, postnatally, at both times or at neither time. Prenatally, caffeine-exposed mothers drank approximately 14 mg/kg/day, an amount ineffective in altering mothers' overall prenatal body weight, although it did reliably decrease birth femur length of offspring. Postnatal pup activity measures revealed that postnatal caffeine exposure depressed activity, with an additional contribution of prenatal caffeine exposure. Those effects occurred at caffeine intake levels (circa 48 mg/kg/day) which minimally affected pup body weight, body length, femur length or eye-opening. Postwithdrawal (35 days of age) biochemical determinations revealed significant postnatal effects of caffeine by depressing cyclic

AMP/"whole-brain" and elevating the cyclic GMP/cyclic AMP ratio in cerebellum. Whole-brain levels of dopamine and norepinephrine, however, were not affected by the caffeine treatments. These results suggest that activity profiles may be a more sensitive index of caffeine "toxicity" than other indices of physical development, and that cyclic nucleotides may play at least some role in the hypoactivity-inducing effects of caffeine in developing rats.

Effects of a combination of X-rays and caffeine on preimplantation mouse embryos in vitro.
Muller WU, Streffer C, and Fischer-Lahdo C.
Radiat Environ Biophys 1983;22(2):85-93.

The influence of a combination of caffeine (0.1 mM, 1 mM, or 2 mM) and X-rays (0.24 Gy, 0.94 Gy, or 1.88 Gy) on preimplantation mouse embryos in vitro was studied under different conditions. The agents were applied either singly or in combination. The embryos were irradiated in the G2-phase of the two-cell stage (28 h p. c. or 32 h p. c.) either 1 h after or immediately before application of caffeine. Caffeine was present during the whole incubation period (until 144 h p. c.). The effects on the microscopic visible development (formation of blastocysts 96 h p. c., hatching of blastocysts 144 h p. c.) and on the cell numbers of embryos at different times (48 h p. c., 56 h p. c., 96 h p. c., 144 h p. c.) were determined. We found conditions under which caffeine markedly enhanced radiation risk, i.e., under which the combination effect exceeded the sum of the single effects. This is true, in particular, for the embryonal development, for which the risk may almost be doubled, whereas the enhancement of risk is not so great for the proliferation of cells. In some cases the combination results lie even outside the envelope of additivity in the range of supra-additivity. The amount of caffeine necessary for such marked effects, however, is so high (at least 1 mM caffeine for rather long times), that it is almost impossible to reach them in vivo by consumption of caffeine-containing beverages.

Brain catecholamines and sleep states in offspring of caffeine-treated rats.
Enslen M, Milon H, and Wurzner HP.
Experientia 1980;36(9):1105-6.

Caffeine was administered in the diet to rats throughout gestation. In the 2 consecutive untreated generations, an increase of parodoxical sleep was observed at maturity. In the 1st generation, the dopamine level was markedly reduced in the locus coeruleus, wheras that of noradrenaline remained constant. The effect was less pronounced in the 2nd generation.

Mitigation of caffeine-induced fetopathy in mice by pretreatment with beta-adrenergic blocking agents.
Fujii T.
Jpn J Pharmacol 1976;26(6):751-6.

In a previous experiment, fetopathic effects of caffeine were significantly reduced by pretreatment with propranolol at dosage levels of 2.5 to 10 mg/kg. The present experiments were undertaeken to investigate the relation between time intervals of propranolol pretreatment and its effect on reducing fetopathy. Furthermore, the effect of timolol, another beta-adrenergic blocking agent, on reducing fetopathy was compared with that of propranolol. Propranolol (5 mg/kg) administered 15, 30 or 60 minutes before caffeine treatment significantly reduced the caffeine-induced fetopathy. The optimal effect was found when propranolol was given 30 minutes before caffine. The reduction in fetopathy by timolol pretreatment was comparable to that of propranolol. The results lend support to the hypothesis that the fetopathic effect of caffeine is linked with released catecholamines in material or fetal issues of mice.

Heritable chromosome aberrations in mammals after exposure to chemicals.
Leonard A.
Radiat Environ Biophys 1976;13(1):1-8.

The observation of dividing spermatocytes is routinely used to detect the induction of heritable chromosome aberrations such as reciprocal translocations in the treated animals or in their F1 offspring. 37 compounds have so far been tested for the induction of chromosome rearrangements in spermatogonia. Only 9 gave positive results. However, positive results were observed for all alkylating agents in the F1 test. From these observations it can be concluded that the spermatogonia which are the main germ cell type at risk represent a relatively safe germ cell stage.

E. Titles without abstracts

Caffeine effects on meiotic maturation in hamster oocytes in vitro.
Jagiello GM.
Reprod Toxicol 1993;7(2):183.

Interpretation of animal experiments as illustrated by studies on caffeine.
Sullivan FM, Smith SE, and McElhatton PR.
Pharmacokinetics in Teratogenesis 1987;1:123-7

[Antagonistic effects of ascorbic acid on caffeine-induced embryotoxic and teratogenic activities in rats].
Zhai SD, Chen MQ, Dong LP, and Jia XR.
Zhongguo Yao Li Xue Bao 1986;7(2):185-7.

The synergistic effects of radiation and caffeine on embryonic development in mice.
Kusama T and Yoshizawa Y.
J Radiat Res (Tokyo) 1984;25(3):225-33.

The effect of brewed and instant coffee on reproduction and teratogenesis in the rat.
Nolen GA.
Toxicol Appl Pharmacol 1981;58(2):171-83.

Evaluation of the teratogenic potential of fresh-brewed coffee and caffeine in the rat.
Palm PE, Arnold EP, Rachwall PC, Leyczek JC, Teague KW, and Kensler CJ.
Toxicol Appl Pharmacol 1978;44(1):1-16.

Accumulation of caffeine and its metabolites in rat fetal brain and liver.
Galli C, Spano PF, and Szyszka K.
Pharmacol Res Commun 1975;7(3):217-21.

Proceedings: Prevention of embryopathic effects of caffeine in mice by pretreatment with propranolol.
Fujii T and Nishimura H.
Jpn J Pharmacol 1974;24(0):s:44.

Reduction in frequency of fetopathic effects of caffeine in mice by pretreatment with propranolol.
Fujii T and Nishimura H.
Teratology 1974;10(2):149-52.

[Teratogenic effect of caffeine on the development of extremities in mice].
Bartel H and Mielczarek J.
Ginekol Pol 1973;44(5):507-13.

Effect on the offspring of repeated caffeine administration to pregnant rats.
Gilbert EF and Pistey WR.
J Reprod Fertil 1973;34(3):495-9.

[Histological studies on the effects of caffeine on the embryonic development of limbs in mice].
Bartel H and Gnacikowska M.
Folia Morphol (Warsz) 1972;31(2):193-200.

Adverse effects of prolonged administration of caffeine on rat fetus.
Fujii T and Nishimura H.
Toxicol Appl Pharmacol 1972;22(3):449-57.

Meiosis suppression by caffeine in female mice.
Jagiello G, Ducayen M, and Lin JS.
Mol Gen Genet 1972;118(3):209-14.

[Ectrodactyly caused by caffeine in rodents. Role of specific and genetic factors].
Bertrand M, Girod J, and Rigaud MF.
C R Seances Soc Biol Fil 1970;164(7):1488-9.

[Studies on the teratogenic influence of caffeine on the fetuses of white mice].
Snigorska B and Bartel H.
Folia Morphol (Warsz) 1970;29(3):353-63.

[Experimental teratogenesis: study of caffeine in mice].
Therapie 1969;24(4):575-80.

Does caffeine induce dominant lethal mutations in mice?
Adler ID.
Humangenetik 1969;7(2):137-48.

Teratogenicity of caffeine in mice related to its mode of administration.
Fujii T, Sasaki H, and Nishimura H.
Jpn J Pharmacol 1969;19(1):134-8.

Cytogenetic investigations of mutagenic action of caffeine in premeiotic spermiogenesis in mice.
Adler ID.
Humangenetik 1966;3(1):82-3.