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Effect of lifestyle exposures on sperm aneuploidy

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Abstract. Lifestyle exposures including cigarette smoke, alcohol, and caffeine have all been studied in relationship to male reproductive health. Over the years the focus has primarily been on semen quality and/or fertility. More recently, literature evaluating direct adverse effects of lifestyle exposures on sperm chromosomes and chromatin has grown due to concern that induced damage could be transmitted to offspring causing transgenerational health effects. In this paper we present a new analysis that summarizes published studies of smoking effects on sperm chromosome number and demonstrates a statistically significant increase in sperm disomy among smokers compared to nonsmokers ($P < 0.001$). In addition, new data on the effect of alcohol intake on sperm chromosome number are presented showing a rate ratio of 1.38 (95% CI 1.2, 1.6) for XY frequency in sperm of alcohol drinkers compared to nondrinkers.

Effect of lifestyle exposures on sperm aneuploidy

Lifestyle exposures including cigarette smoke, alcohol, and caffeine have all been studied in relationship to male reproductive fitness (reviewed by Stillman et al., 1986; Vine, 1996; Marinelli et al., 2004). The majority of published research has focused on semen quality and/or fertility. Some examples from the past two decades include Kulikauskas et al. (1985), Dikshit et al. (1987), Rantala and Koskimies (1987), Marshburn et al. (1989), Saaranen et al. (1989), Lewin et al. (1991), Bolumar et al. (1996), Curtis et al. (1997), Guzick et al. (2001), and Zitzmann et al. (2003). In addition, there is a growing body of literature evaluating direct adverse effects of lifestyle exposures on sperm chromosomes and chromatin because of concern that induced damage could be transmitted to offspring and result in transgenerational health effects (Cicero, 1994; Ji et al., 1997; Shen et al., 1997, 2000; Zenzes et al., 1999; Virro et al., 2004). Recent progress in this area of male-mediated effects follows, in part, from the Human Genome Project and associated development of molecular genetic techniques that allow improved detection, measurement, and interpretation of abnormalities of chromosomes and chromatin within sperm cells.

The majority of publications to date that have investigated lifestyle-induced chromosome changes in human sperm have utilized fluorescent in situ hybridization (sperm-FISH). The sperm-FISH methods detect and quantify chromosome complements in ejaculated human sperm cells. The methods are well established and can easily be applied in clinical or research laboratory settings (Perreault et al., 2003). Ability to detect induced changes in sperm cell chromosome numbers is important. For humans, aneuploidy is identified in at least 5% of all clinically recognized pregnancies (Lamb and Hassold, 2004) and in approximately one third of all pregnancy losses. The majority of aneuploid liveborns are attributed to parental gametes carrying abnormal numbers of chromosomes (Hassold and Hunt, 2001). In addition, approximately 1% of all conceptions may be triploid and likely aborted prior to pregnancy recognition (Dimmick and Kalousek, 1992). It has been estimated

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that 2% or more of all sperm have missing or additional chromosomes (Hassold and Hunt, 2001). Thus, capacity to detect induced changes in chromosome number in ejaculated sperm cells by sperm-FISH is a significant advancement for the field of human reproductive toxicology and is contributing important risk information related to paternal exposures.

Individual chromosome characteristics may affect susceptibility to meiotic aneuploidy (Warburton and Kinney, 1996; Egozcue et al., 2000) and result in heightened risk for these chromosomes following exposure to aneugens. As discussed in previous publications, acrocentric chromosomes or chromosome pairs with a single chiasma during meiosis are reported to be at greater risk of malsegregation. This has been demonstrated for the sex chromosomes (Shi et al., 2001) and chromosome 21 (McInnes et al., 1998; Martin and Rademaker, 1999; Soares et al., 2001). The concept is important when looking for trends across studies in terms of risk for sperm aneuploidy associated with lifestyle exposures because conclusions drawn for any specific exogenous exposure will be limited to parallel subsets of chromosomes evaluated.

Induction of aneuploidy in sperm cells also reflects innate susceptibility characteristics of the host male. It is known that some men carry high baseline frequencies of aneuploid sperm cells (Robbins et al., 1993; Rubes et al., 2002) and these men may be more vulnerable to induction of aneuploidy in response to environmental exposures. Finally, susceptibility in any man is a function of competing, interacting, or additive effects related to age, nutrition, stress, workplace and recreational exposures, as well as a myriad of other health factors that can affect normal meiosis and increase the risk for aneuploid sperm.

All of the above make epidemiological investigation of lifestyle exposures and sperm aneuploidy complex. Though it is relatively easy in research studies to obtain and evaluate ejaculated sperm cells using sperm-FISH methods, interpretation of data is often a challenge. Variations across study populations, study conditions, and chromosomes evaluated make it difficult to reconcile findings. However, there do appear to be trends for smoking and alcohol intake.

The purpose of this paper is to review published data on human sperm aneuploidy associated with exposure to the lifestyle factors cigarette smoking, caffeine, and alcohol intake and report new data on a workplace cohort of 69 men in northern China demonstrating an effect of alcohol intake on meiosis I errors. Because these particular lifestyle behaviors often occur together, that is, men who smoke cigarettes may also use alcohol and/or caffeine, and because each of the exposures has been reported to have effects on semen quality, attention is given to all three as potential confounders for each other.

Sperm-FISH studies of smoking

Cigarette smoke and/or its metabolites contain mutagens, carcinogens and other toxicants. Smoking has been associated with fragmented sperm DNA (Shen et al., 1997, 2000), DNA/protein adducts (Zenzes et al., 1999), and sperm from smoking fathers has been shown to contain benzo-[a]pyrene diol epoxide-DNA adducts that were subsequently transmitted to the embryo (Zenzes et al., 1995, 1999). Published studies designed specifically to look at effects of smoking on human sperm aneuploidy are rare (Robbins et al., 1997; Rubes et al., 1998; Shi et al., 2001). The few publications specifically targeted to smoking effects on sperm aneuploidy are supplemented by several informative reports where smoking data is reported secondary to the main focus of the work (Härkönen et al., 1999; Naccarati et al., 2003). General lack of definitive smoking information related to induction of sperm cell aneuploidy in the large body of published human sperm-FISH literature reflects, in part, reduction in funding for studies of health effects from smoking in favor of funding smoking cessation research. In addition, in studies of other main effects, researchers have been conscientious in restriction of study groups to nonsmokers as a means of controlling potential confounding, or matching smoking exposure across exposure groups of the main effect (for example, Padungtod et al., 1999, in a study of occupational pesticide exposure report 69% of the pesticide-exposed and 69% of the control group were current smokers). It is also possible that bias against submitting/publishing negative study results has led to fewer reports in the literature specifically addressing smoking and sperm aneuploidy.

A study by Robbins et al. (1997) investigated smoking effects on sperm aneuploidy within the context of potential confounding or interaction effects of alcohol and caffeine use. These three lifestyle behaviors often occurred together in USA populations prior to the new millennium. A subset of men (n = 45) enrolled in a cross-sectional study designed specifically to evaluate multiple aspects of semen quality related to lifestyle, workplace, and environmental exposures (n = 88) were evaluated using sperm-FISH. Recruitment occurred in North Carolina, USA, through newspaper adds. All smokers had smoked for at least one year prior to enrollment and nonsmokers were defined as men who did not smoke currently and had not smoked more than 100 cigarettes total in their lifetime. Light smokers (1–19 cigarettes per day) and nonsmokers were enrolled to match the age distribution of heavy smokers ($\geq$20 cigarettes per day). In addition to questionnaire data on smoking status, exposure was measured as urinary cotinine with the benefit of assessment of environmental tobacco smoke exposure. Of the original 88 men enrolled, only those who had adequate cell numbers remaining after conventional semen analysis were included in the sperm aneuploidy study. Because of this, all men evaluated for aneuploidy had baseline sperm counts greater than 32 million/ml whereas 10% of the original 88 men had counts less than 20 million/ml. Numerous publications have shown associations between oligospermia and increased sperm aneuploidy (e.g., Levron et al., 2001; Martin et al., 2003; Schmid et al., 2003; Liu et al., 2004), thus, use of a study population with counts of greater than 32 million/ml should have reduced any potential bias from low sperm count effect.

The design of the investigation by Robbins et al. (1997) allowed simultaneous evaluation of alcohol intake, caffeine intake, and smoking for confounding or interaction effects. This was important because smoking more than 20 cigarettes per day was associated with sperm aneuploidy for multiple chromosomes, however, after controlling for age, alcohol and caffeine intake, only disomy X remained suggestive with a *P*

Table 1. Studies reporting significant associations between sperm aneuploidy and cigarette smoke exposure

No. men	Cigarettes/ day	% X:Y chromosomes	Significant findings	Probes	Sperm scored	Hybridization efficiency	Potential confounder addressed	Reference
		Total set:	% Disomy X:	3 color X, Y, 18	>9,805/donor for each probe	>99%	(Caucasian, USA), age 19–35 yrs, alcohol; caffeine, known mutagens, semen quality, abstinence interval	Robbins et al., 1997
10	20–45	49.9 sd 1.7 X 49.4 sd 1.7 Y	0.03[a]					
7	1–19		0.01					
28	0		0.02					
			% Disomy Y:	3 color X, Y, 8	10,000/donor for each probe	99.84%	(Czech, Teplice), age 18 yrs, caffeine, metals/solvents, abstinence interval	Rubes et al., 1998
10	1–20	48.7 X 51.3 Y	0.045					
15	0	48.7 X 51.3 Y	0.02					
			% Disomy 1:	2-color 1,7	10,000/donor for each probe	98.7%	(Danish, Denmark), age 29–49 yrs, alcohol, sperm concentration before exposure to fungicide	Härkönen et al., 1999
9	1–30	N/A	0.159					
19	0		0.105					
			% Disomy 13:	3-color X, Y, 1 2-color 13, 21	≥9,990/donor for each probe	>99%	(Chinese, China), age 24–36 yrs, alcohol, known mutagens, semen quality	Shi et al., 2001
10	20–40	49.34 X[b] 50.19 Y[b]	0.19					
11	2–15	49.76 X 49.77 Y	0.21					
10	0	49.73 X 49.46 Y	0.07					
		Total set ratio:	% Disomy (X+Y)	3 color X, Y, 2	>6,279/donor for each probe	>99%	(Italian, Tuscany), age 22–41 yrs, alcohol, semen quality, styrene	Naccarati et al., 2003
16	1–20	1.04 ± 0.08	0.17					
15	0		0.09					
			% Disomy X, Y	3-color X, Y, 18	>7,979/donor for each probe	>99%	(Chinese, China), age 26–35, alcohol, blood boron level	Current study
18	11–40	51.46 X 47.88 Y	0.017 XX18 0.039 YY18					
21	1–10	51.22 X 48.13 Y	0.021 XX18 0.027 YY18					
30	0	51.21 X 48.13 Y	0.021 XX18 0.028 YY18					

[a] $P = 0.05$ using logged urinary cotinine as measure of smoking exposure in Poisson regression.
[b] $P = 0.008$ from expected 50:50 using Chi Square.

value of 0.07 (Table 1). Using logged cotinine as a more specific index of exposure to toxicants in cigarettes, and simultaneously evaluating alcohol and caffeine, disomy X remained significant (P value = 0.05). Use of cotinine as a biological marker of smoking exposure was a strength in this investigation. Other strengths include attention in the analyses to potential interscorer effects and confounders such as age, days since last ejaculation, and conventional semen parameters. In the final analyses, disomy X in Poisson regression models of cotinine level controlling for age, alcohol, and caffeine intake was the only statistically significant numerical abnormality detected in sperm cells related to smoking exposure.

Rubes et al. (1998), as part of a larger study investigating air pollution in the Czech Republic, investigated cigarette smoking effects on sperm aneuploidy in a subset of 25 men. Men were invited to participate in the study when they presented for military registration and thus were all aged eighteen. They lived locally in the Teplice district and were sampled within a four-day period to give equivalent ambient air pollution exposures. No differences were found in the smokers (n = 10; 20 cigarettes/day) and nonsmokers (n = 15) in terms of self-reported occupational exposures, health status, passive smoking exposure, age at first seminal emission, wearing of briefs, sports activity, and/or physical examination results. Strengths of this study include the strict age restriction, cotinine levels to validate self-reported smoking information, and careful, thorough statistical analyses with attention to multiple potential confounders. It was determined that the smoking effects could not be completely disassociated from the effects of alcohol in this Czech cohort as all the smokers also consumed alcohol. However, according to the authors, "regression of aneuploidy frequency against cotinine levels was slightly more significant ($P = 0.1$) than alcohol ($P = 0.4$) suggesting that cotinine levels are associated more closely with aneuploidy than alcohol is with aneuploidy." In any case, smokers were reported to have statistically significantly more YY disomy than nonsmokers (Table 1).

Shi et al. (2001) looked specifically at cigarette smoking effects on sperm aneuploidy in men from Hefei, Anhui, Peoples Republic of China. Ten nonsmokers were compared to twenty-one smokers divided into two groups: 11 smoked less than 20 cigarettes per day, 10 smoked 20 or more cigarettes per day. Exposure to smoking was assessed by self-report. None of the men reported drinking alcohol, health problems, genetic disease, or exposure to known mutagens. The authors found a decrease in X bearing sperm from the expected 50:50 ratio for smokers of ≥ 20 cigarettes per day ($P = 0.008$) and increased

Table 2. Published sperm aneuploidy estimates associated with cigarette smoke exposure

No. men	Cigarettes /day	Disomy									Reference
		X (std)	Y	(X+Y)	1	7	8	13	18	21	
10	20–45	0.03 (0.04)[a]	0.02 (0.03)	0.05					0.05 (0.03)		Robbins et al., 1997
7	1–19	0.01 (0.01)	0.01 (0.01)	0.02					0.04 (0.04)		
28	0	0.02 (0.01)	0.01 (0.01)	0.03					0.03 (0.03)		
10	20	0.06 (0.008)	0.05 (0.006)[b]	0.11			0.07 (0.01)				Rubes et al., 1998
15	0	0.06 (0.006)	0.02 (0.003)	0.08			0.05 (0.006)				
3	20–30				0.2 (0.07)[c]	0.07 (0.04)					Härkönen et al., 1999
6	1–19				0.14 (0.05)	0.03 (0.01)					
19	0				0.11 (0.03)	0.05 (0.04)					
10	20–40	0.05 (0.06)	0.05 (0.09)	0.10				0.21 (0.19)[b]		0.23 (0.19)	Shi et al., 2001
11	2–15	0.04 (0.04)	0.04 (0.03)	0.08				0.22 (0.19)		0.14 (0.09)	
10	0	0.05 (0.03)	0.02 (0.02)	0.07				0.07 (0.03)		0.18 (0.05)	
16	1–20			0.17 (s.e. 0.023)[c]							Naccarati et al., 2003
15	0			0.09 (s.e. 0.023)							

[a] $P = 0.05$ logged cotinine.
[b] $P < 0.001$.
[c] $P = 0.02$.

Table 3. Smoking effect on disomy across four published studies and six autosomes (n = 57 smokers and 72 nonsmokers). Two-way ANOVA is based on the sample size, mean and standard deviation of each smoking level reported by the four studies (Robbins et al., 1997, Rubes et al., 1998, Härkönen et al., 1999 and Shi et al., 2001). One factor is smoking which has three levels: nonsmoker, light smoker, and heavy smoker. The other factor is autosome which has 6 levels: disomy 1, 7, 8, 13, 18, and 21. The main effect of smoking, autosome, and the interaction term smoking*autosome are examined accordingly.

Source	SS[a]	DF[a]	MS[a]	F statistics	*P* value
Smoke	0.1914	2	0.0957	13.15	<0.001
Autosome	0.6767	5	0.1353	18.60	<0.001
Smoke*autosome	0.1972	10	0.0197	2.71	0.004
Error	1.2368	170	0.0073		
Total	2.3021	187			

[a] SS: Sum of Squares; DF: Degrees of Freedom; MS: Mean Square.

chromosome 13 aneuploidy in sperm cells of both light and heavy smokers compared to nonsmokers ($P < 0.01$ and $P < 0.0001$ respectively). A very important finding in this study was the smoking associated increase in inter-donor heterogeneity for disomic sperm across all chromosomes evaluated (X, Y, 13, 21) suggesting that some men may be especially susceptible to induction of sperm aneuploidy by smoking (Table 1). This has great implications for public health and deserves further evaluation as to etiology of this underlying smoking associated susceptibility.

Two additional published studies contain enough detail on cigarette exposure within the study groups to comment on sperm aneuploidy effects (Table 2). In an investigation of agricultural fungicide exposures, Härkönen et al. (1999) list out data on sperm aneuploidy and numbers of cigarettes smoked prior to the pesticide exposures. Thirty-two healthy Danish farmers between the ages of 29 and 49 years were evaluated. After controlling for age, alcohol intake, and sperm concentration, the authors noted a significant association between smoking and chromosome 1 disomy as well as sperm cells diploid for chromosomes 1 and 7. Naccarati et al. (2003) in a study of occupational exposure to styrene and its effects on sperm aneuploidy in subjects selected for normal semen parameters, reported a statistically significant increase ($P = 0.018$) in sex chromosome disomy (X+Y) in smokers (n = 16) compared to nonsmokers (n = 15) after adjusting for styrene and alcohol (Table 1). The researchers also evaluated chromosome 2 but reported no smoking effects on this chromosome.

What can be concluded from these papers about the effect of smoking on sperm aneuploidy? If the study groups are matched as near as possible on smoking exposure (number of cigarettes smoked per day by self-report), trends are seen (Table 2). Although depicting disomy frequencies by smoking categories is somewhat misleading as it can not show the adjusted values (that is, the frequency after controlling for confounding by age, alcohol, etc. that yielded statistically significant findings in modeling within individual studies), it is still instructive. When aneuploidy frequency is summed across sex chromosomes (disomy X plus disomy Y), the four studies evaluating sex chromosomes show a statistically significant increase in disomy (X+Y) among smokers after controlling for study ($P < 0.001$). Similarly, when disomy is summed across the six autosomes, a statistically significant increase in autosomal disomy among smokers is seen after controlling for chromosome ($P < 0.001$, Table 3). At this time, published findings for autosomes are limited to single laboratory reports per chromosome and, as suggested by Shi et al. (2001), confirmatory studies on the same autosome by multiple labs are needed. Finally, the finding of a significant smoking induced heterogeneity in sperm aneuploidy frequency compared to nonsmokers (Shi et al., 2001) begs further research into the question of underlying individual susceptibility to ciga-

rette induced nondisjunction. Subsets of men at increased risk from cigarette smoking could benefit from targeted public health smoking cessation messages.

Sperm-FISH studies of alcohol

Chronic alcohol abuse leads to testicular atrophy, feminization, and infertility in alcoholic men (Van Thiel et al., 1980). Mechanisms postulated for adverse testicular effects are lipid peroxidation (Rosenblum et al., 1985), changes in secretory function of Sertoli cells (Zhu et al., 1997), changes in hormone levels (Woolveridge et al., 1999), and induction of apoptosis (Eid et al., 2002; Hu et al., 2003). Bielawski et al. (2002) suggest alcohol may produce transgenerational effects by decreasing cytosine methyltransferase mRNA levels and affecting normal imprinting. Kagan-Krieger et al. (2002) suggest paternal alcohol consumption may affect offspring through genetic changes in sperm DNA, decreasing numbers of sperm produced resulting in a less healthy subpopulation available for fertilization, and/or altering the chemical composition of seminal fluid giving less protection for the ejaculated sperm cells.

Although a number of researchers have published effects of alcohol intake on conventional semen parameters (reviewed by Marinelli et al., 2004), studies designed specifically to address effects on human sperm chromosome number are extremely rare. In a case-control study, Kagan-Krieger et al. (2002) did not find an association between paternal alcohol consumption self-reported and Turner syndrome in their offspring. Robbins et al. (1997) in a sperm-FISH study related to three lifestyle behaviors (smoking, caffeine intake, and alcohol effects) looked at alcohol drinking in a group of 45 men. The researchers first categorized men into three equally sized groups according to alcohol intake based on the distribution in the data: fewer than 6 drinks per week, 6–14 drinks per week, and 14 or more drinks per week. The categorization was not optimal and resulted in the middle group demonstrating the least amount of disomy for chromosomes X, Y, and 18 and the baseline group demonstrating the highest frequency of disomy for XY. However, when the self-reported number of alcohol drinks per week was evaluated as a continuous variable, a significant linear association was found between increasing alcohol intake and chromosome X disomy ($P = 0.04$) after adjusting for age, logged urine cotinine, and caffeine intake. The original artificial group assignments were misleading and unable to detect the dose-response effect. No other published studies designed specifically to evaluate alcohol drinking and sperm aneuploidy were identified. Similar to published data on cigarette smoking, however, insight into alcohol effects on sperm aneuploidy can be gleaned from investigations of a variety of main effects where researchers provide information on alcohol as a potential confounder (Rubes et al., 1998; Härkönen et al., 1999; Padungtod et al., 1999).

In a study by Rubes et al. (1998) where smoking effects on sperm aneuploidy were investigated in 18-year-olds, smokers reported significantly greater intake of alcohol than nonsmokers ($P < 0.0001$). In fact, all the smokers in the study also drank alcohol so the finding of an increase in disomy among smokers for chromosomes Y and 8 could not be disentangled from alcohol. In an occupational study by Padungtod et al. (1999), men exposed to pesticide in a production factory (n = 20) were compared to controls in a textile factory (n = 23). Smokers were equally distributed between the exposed and control groups but alcohol use was higher in the exposed group (23%) compared to the control group (13%). The authors do not report adjusting for alcohol in the analysis so a portion of the increased rate ratio of 1.51 (95% CI 1.04, 2.20) for sperm aneuploidy in the pesticide-exposed workers may also include an effect of alcohol as well. Härkönen et al. (1999) reported a significant negative association between alcohol intake and sperm aneuploidy for chromosomes 1 and 7 among a cohort of 30 agricultural workers prior to pesticide exposures but no statistically significant findings related to alcohol intake after exposure. The authors suggest the inconclusive findings may reflect moderate dose as alcohol consumption for the drinkers averaged six drinks/week, comparatively lower than the self-reported drinking behavior reported in the studies by Robbins et al. (1997) and Rubes et al. (1998).

Table 4. Effect of alcohol intake on XY disomy in study of 69 boron workers and controls[a]

Parameter	Dependent variable XY18		
	Estimate	Standard Error	*P* Value
Smoke (1 = Yes; 0 = No)	0.0064	0.0698	0.9274
Alcohol (1 = Yes; 0 = No)	0.3206	0.0702	<0.0001
Age	–0.0209	0.0148	0.1572
Blood boron	0.0154	0.0332	0.6418

[a] Poisson regression ANOVA table.

In addition to these published works, a recent evaluation of men in Liaoning Province, PR China, provides new evidence for an alcohol effect on sperm aneuploidy. Sixty-nine men were enrolled as part of a study investigating effects of boron exposure on semen quality. Detailed information on general and reproductive health, diet, lifestyle exposures, and work history were collected as well as workplace dust samples, blood, urine, stool, semen, food and drink specimens. Although detailed information on smoking was collected, effect on sperm aneuploidy could not be adequately assessed because 97% of the men reported exposure to environmental tobacco smoke at home and/ or work. However, variability in alcohol intake was noted (n = 38 nondrinkers of alcohol, n = 21 beer drinkers, n = 10 beer + white wine drinkers). In Poisson regression modeling (Table 4), after controlling for age, self reported cigarette smoking, and blood levels of boron (the main effect being evaluated in the study), men who reported drinking alcohol demonstrated significantly greater numbers of sperm disomic for XY ($P < 0.001$, rate ratio of XY frequency in alcohol drinkers compared to nondrinkers 1.38, 95% CI 1.2, 1.6). The ability to detect this clear alcohol effect may reflect regional characteristics of the alcohol drinks. White wine averages 50% alcohol in this part of NE China and was consumed along with beer by over 15% of the study participants at an average rate of ~5 liters per week. Beer (ranges 4–6% alcohol in this region of China) was con-

sumed by almost a third of the participants at an average rate of ~3 liters per week. Thus, the alcohol content of the drinks and the large number of men self-reporting intake may be key to the ability to detect the alcohol effect in this study. There seemed to be no stigma against reporting use of alcohol, a factor that can lead to misclassification in studies in the USA. This finding of a clear alcohol effect on XY disomy in the human suggests an important area for further research.

Sperm-FISH studies of caffeine

Caffeine has the fewest citations in relationship to human sperm chromosome number when looking at common lifestyle behaviors. This is surprising because caffeine has received quite a bit of popular press in relationship to reproductive outcomes and is generally used by more men than either cigarettes or alcohol alone (Boyer, 1993). However, lack of publications may reflect difficulties in the study of caffeine due to its short half-life in the body, wide fluctuations in circulating serum levels following exposure, and large interperson variability in metabolism and elimination (Lelo et al., 1986; Balogh et al., 1992). In addition, caffeine consumption has been shown to vary considerably in individuals over time, for example related to season. Unlike smoking and alcohol, it is not often mentioned as a potential confounder in sperm-FISH publications.

In a study by Rubes et al. (1998) investigating smoking, alcohol and caffeine intake on sperm aneuploidy in 18-year-olds, the smokers reported significantly greater intake of alcohol ($P < 0.0001$) and reportedly consumed twice the amount of caffeine as nonsmokers ($P = 0.06$) although caffeine consumption was not significantly correlated with urine cotinine levels ($r = 0.3$, $P = 0.16$). Robbins et al. (1997) in their study of combined smoking, alcohol, and caffeine effects on sperm aneuploidy reported increasing numerical chromosomal abnormalities in sperm of men exposed to caffeine measured as coffee cup equivalents per day. Significant increases in sperm disomy for chromosome X ($P = 0.04$) and XY ($P = 0.0002$) were found after controlling for alcohol, age, and logged urine cotinine. It is difficult to estimate caffeine intake based on self-report because cups of coffee vary in strength related to brewing and brand (thus caffeine content), caffeine is present in many products that would not necessarily be recognized or reported during study interviews (for example, caffeine is present in many over-the-counter medicines), and variability in intake would make estimating "usual intake" a problem in semen studies collecting data over the previous months of spermatogenesis. However, given the growth and popularity of "boutique" coffee houses, it may be time to take a closer look at this beverage and its potential for male reproductive effects. Biomarkers of exposure such as paraxanthine, the main caffeine metabolite in the human, as well as assessment of genetic variability in metabolic enzymes in individual men, would be one way to approach this important unresolved question.

Conclusion

It is important to understand the potential for lifestyle exposures to induce sperm chromosome and chromatin damage because this damage has been associated with compromised fertility as well as adverse effects on offspring. Specifically, abnormalities of sperm chromosome number have been associated with the production of aneuploid embryos and liveborn children (Blanco et al., 1998; Martinez-Pasarell et al., 1999; Moosani et al., 1999; Rubio et al., 1999; Soares et al., 2001) in most but not all studies (Hixon et al., 1998). We report in this paper an analysis conducted across published studies of the effect of smoking on sperm aneuploidy that demonstrates an increased risk among smokers. Additionally, as reported by Shi et al. (2001), it appears some men may have innate susceptibility to this increased risk. Ethanol distributes equally into all body compartments, including the testes and there is both animal and epidemiological support for an effect of paternal drinking on offspring health (reviewed by Abel, 2004). We present new evidence showing an effect of alcohol on XY disomy in a cohort of 69 men in NE China. Inconsistent results in previous work may reflect lower exposure doses suggesting alcohol intake warrants further investigation. Finally, little research has been published on caffeine-associated effects on sperm aneuploidy but tools are available to address the question. Given the current popularity of coffee consumption and special coffee houses, it may be time to investigate this common lifestyle exposure in more depth.

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