CIGARETTE SMOKING EXPOSURE AND ITS EFFECT ON CONVENTIONAL SEMEN PARAMETERS

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ABSTRACT

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Cigarette smoke has been associated with many cancers and other health conditions and there is concern about the possible negative effects of smoking on semen parameters and male reproduction. This study reports on the effect of cigarette smoking on semen parameters from data collected as part of the Fish and Infertility Study (FINS), a cross-sectional NIH study undertaken to evaluate the environmental factors on measures of male infertility. 603 men between the ages of 18 and 60 were recruited from couples presenting at two infertility clinics in Michigan. Participants filled out a detailed questionnaire on lifestyle factors and provided semen, blood, and urine specimens. Data from the FINS study was analyzed to assess smoking exposure and other lifestyle variables that may negatively affect sperm parameters. We found significant and increased odds of low normal sperm morphology between participants who were "Ever Smokers" versus "Never Smokers" (OR=1.61, p=0.032, CI= 1.043, 2.496). No significant associations were found between cigarette smoke exposure and total sperm count, sperm concentration, semen volume, or motility. When total sperm count and sperm concentration were assessed, exercise was shown to have a significant negative effect on both semen parameters, while work stress seemed to function as a protective factor. We also detected a protective association between the wearing of boxers and sperm motility.

To my incredible family and loving husband whose encouragement and continuous support made this accomplishment possible.

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INTRODUCTION

A substantial proportion of men of reproductive age worldwide smoke.[1] Cigarette smoking varies across regions, a staggering 23.4% of men in the United States take part in smoking. Cigarette smoke has been associated with many cancers including bladder, oral cavity, kidney, stomach, pancreas, and it has been causally linked to lung cancer.[2] Smoking has also been correlated with an increased risk of coronary heart disease and stroke.[3,4,5] A number of elements have caused concern about the possible negative effects of smoking on semen parameters and male reproduction. The considerable prevalence of male smokers, the many adverse health conditions caused by smoking and the fact that cigarette smoke contains more than 30 chemical agents known to be mutagens, aneugens, or carcinogens add to the significance of this issue.[1]

Alarmingly, semen quality has been declining in industrialized counties in the past half century.[6, 7, 8, 9] A 2010 Spanish cross-sectional study found that sperm count and sperm concentration may have declined in Spanish men over the last decade.[10] Another cross-sectional study found that in the general Danish population, only one in four men had optimal semen quality and 25% of men will most likely face a prolonged waiting time to pregnancy if they intend to father a child in the future and another 15% are at risk of the need of fertility treatment.[11] Moreover, a French retrospective study conducted with data from IVF clinics throughout 1989-2005 found a 32.3% decrease in sperm concentrations, with projects that indicated that concentration for a 35 year old man went from an average of 73.6 million/ml in 1989 to 49.9 million/ml in 2005.[12]

Fertility rates have drastically declined in most European countries to below replacement level of the population.[13, 14] Furthermore, diminished sperm quality has been linked to decreased fecundity, which may in turn affect fertility rates.[15] Various studies on the effects of cigarette smoking on semen parameters have been implemented, but the scientific community has not yet reached a clear consensus. There are several reasons why a consensus on the topic has not yet been reached, many of which appertain to limitations in study designs. Small sample size, use of infertile study population, and use of questionnaires to obtain exposure status are a few of the drawbacks in previous studies.

Nonetheless, cigarette smoking is a voluntary activity that is overwhelmingly detrimental to one's health. Given the declining fertility rates in the general male population, if cigarette smoking does have unfavorable effects on male reproduction and fertility, it is important that public health measures are implemented to promote smoking cessation with regards to improving male reproduction, especially for males with existing infertility issues.

BACKGROUND

Cigarette smoking has increased worldwide throughout the past few decades.[16] Considering the fact that cigarette smoke is a carcinogen and a somatic cell mutagen, there is plausible concern that paternal smoking may cause adverse reproductive outcomes.[17]

Reproductive capacity is dependent on multiple physiological and genetic functions, and there is a multitude of ways in which cigarette smoking could have an detrimental effect on male reproduction.[1] A number of mechanisms could describe the effects of compounds found in cigarette smoke on male reproduction: harmful effects of cadmium and other heavy metals[18], mutagenic effects caused by aromatic hydrocarbons[19], decreased accessibility to hemoglobin due to carbon monoxide[20], accrual of radioactive particles in the testes[21], and pernicious effects of nicotine.[22] Essentially, cigarette smoke could have a possible cytotoxic effect on sperm or a reduction in sperm number and sperm functionality. Likewise, smoke from cigarettes may cause sub-fertility by altering hormone levels. Testosterone levels may be reduced, elevated or unchanged, and estradiol levels are largely found to be elevated in smokers.[23] Smoking may also damage the testes and other parts of the reproductive tract, leading to impaired spermatogenesis.[1]

Semen quality depends on multiple measures and is a good representation of sperm production and function, and even fertility to some extent. The usual measures of semen quality include total sperm count (the total number sperm per ejaculate), sperm

concentration (the number of sperm/ml of ejaculate), sperm volume (total volume of ejaculate), sperm morphology (the evaluation of sperm shape and size; frequently reported as the percentage of abnormally/normally shaped spermatozoa), and sperm motility (the analysis of sperm movement, commonly quantified as the percentage of motile sperm). Many of these measurements have been associated with reduced fertility. Namely, a low total sperm count, a low percentage of sperm motility and a high percentage of abnormally shaped sperm.[24]

Various research studies have been conducted concerning the effect of cigarette smoke on semen parameters. However, a clear consensus has not yet been reached. Several studies have demonstrated that cigarette smoke has a negative impact on conventional semen parameters. In contrast, a number of studies have concluded that an association between cigarette smoking and its effect on semen variables does not exist. For the purpose of this thesis, studies that were most current (no older than 1990) and relevant were reviewed. Google Scholar was used in the search for papers using a variety of amalgamations of the phrase "the effects of cigarette smoking" and following words or terms: "sperm", "semen parameters", "sperm quality", "sperm count", "sperm concentration", "semen volume", "sperm motility", and "sperm morphology". Sometimes the word "semen" was used instead of "sperm". Finally, more specific searches were also executed in order to identify studies on the effects of caffeine, alcohol, stress, and exercise on semen parameters.

Epidemiologic Studies

For this review, we found three cross-sectional studies, two case-control studies, one study that was not categorized, and a systematic review on smoking and male reproduction.

Cross-Sectional Studies

Trummer et al. implemented a cross-sectional study to compare semen parameters and hormone concentrations of infertile smokers and infertile non- and ex-smokers.[25] 1,104 men with infertility for ≥1 year were separated into three groups: 517 nonsmokers, 109 ex-smokers, and 478 smokers. Ex-smokers were defined as men who stopped smoking ≥ 6 months prior to examination for infertility. Smokers were defined as men who had smoked cigarettes for >6 months and were still smoking. In this paper "infertility" was undefined. Cigarette smoking exposures were assessed via questionnaire and semen analyses were performed following World Health Organization (WHO) guidelines (Table 1). An increase in testosterone levels was observed in smoking subjects. All smoking subjects were also urged, via free verbal counseling and mailed anti-smoking information, to cease smoking in order to improve fertility prior to a second semen analysis. However, significantly more non-smokers than smokers returned for the second semen analysis, which led Trummer to conclude that only a few idiopathic infertile smokers were able to quit smoking and that smoking did not affect conventional semen parameters.

A cross-sectional study by Kunzle et al. revisited the effects of cigarette smoking on main sperm variables by using a large cohort of men from an infertility clinic.[26] The study population consisted of 655 smokers and 1,131 non-smokers. The criteria defining

"smokers" was strict (> 1 cigarette /day). Smoking and health histories were obtained using questionnaires and semen analyses were performed by established methods. The study exhibited an association between cigarette smoking and a significant decrease in normal forms of sperm, sperm density (-15.3%), total sperm count (-17.5%), and total number of motile sperm (-16.6%). It was concluded that cigarette smoking was associated with reduced semen quality and that those who have borderline semen quality who wish to have children could benefit from smoking cessation.

A cross-sectional study by Sepaniak et al. used a cohort of men from an infertility clinic and the study population consisted of 57 non-smokers and 51 smokers.[27] As with many studies, smoking status was self-reported and obtained by questionnaire. In this study, however, a CO test was also used to confirm participant's cigarette smoke exposure. Sepaniak et al. did not find a significant difference in conventional parameters between smokers and non-smokers.[27] They did, however, find a significant difference in the DNA fragmentation rate between smokers and non-smokers ($p < 0.01$; 32% versus 25.9%, respectively). Sepaniak concluded that damaged sperm DNA could lessen the chances of a successful pregnancy by damaging embryonic development and implantation, or by increasing the rate of spontaneous abortion. Ultimately, smoking cessation was recommended for anyone considering biological children in the future.

Case-Control Studies

Two case-control studies found that cigarette smoking adversely affected semen functions and fertility. The first was a study set in the department of obstetric and gynecology of a tertiary general hospital.[28] The study population was comprised of 218

infertile men (cases), and 240 fertile men (controls) whose wives were pregnant at the time of the study. Smoking exposure status and health histories were collected through a questionnaire obtained by a trained interviewer. Semen collections were performed at the subject's own home and brought into the hospital within one hour of collection. Semen analyses were completed according to WHO guidelines. The study found that semen parameters of all cases were significantly poorer than that of the controls and that the risk of infertility is associated with smoking (crude OR 2.82, 95% CI 1.93-4.13; adjusted OR 2.96; 95% CI 1.98-4.42). The second case-control study took place in Shandong Province, China.[29] The cases consisted of 110 non-smokers and 191 smokers, and the controls were comprised of 61 fertile non-smokers who had one or more children. The smokers were divided into subgroups according to the amount and the duration of smoking. Smoking status was obtained by direct interview, and semen parameters were examined using WHO guidelines. The results revealed that semen volume, density, viability, and forward progression were much lower in the medium, heavy and long-term smokers, respectively, than in non-smokers ($P< 0.01$). Similarly to Chia et al.[28], the researchers of this case-control study[29] concluded that cigarette smoking affected semen quality in the population of men in the study.

Other Studies

Sofikitis et al. examined the effects of smoking on testicular function, semen quality and sperm fertilizing capacity. [30] In this study, 77 men undergoing left testicular biopsy for surgical correction of a left inguinal hernia were divided into groups. Group 1 was composed of 49 smokers, age 21-38, who smoked more than 20 cigarettes per day

for longer than 3 years. Group 2 was comprised of 28 men, age 18-44, who had never smoked. Finally, group 3 consisted of 9 men from Group 1 who stopped smoking 8-12 months postoperatively. Smoking exposure was obtained pre- and 6 months postoperatively through a questionnaire, independently confirmed by the subject's wives or partners, and re-affirmed through a nicotine/cotinine/trans-3-hydroxycotinine assessment. Several semen samples were collected postoperatively and a standard semen analysis was performed. The study revealed that testosterone levels in the left testicular vein, left testicular androgen-binding protein secretion rate (in vitro), sperm motility, percentage of morphologically normal spermatozoa, sperm morphometric parameters and outcome of sperm function tests were significantly lower ($p< 0.05$) in smokers than in nonsmokers. Since testosterone and androgen-binding protein have important roles in normal spermatogenesis and the epididymal sperm maturation process, respectively, Sofikitis et al. postulated that reduced levels of the aforementioned factors may lead to a decrease in sperm fertilizing capacity. The study also demonstrated that sperm motility and morphology significantly improved in the 9 smokers who ceased smoking (Group 3). These findings suggested a causal relationship between smoking and a decrease in sperm motility and function.

A 1996 large-scale systematic review examined the effects of smoking and male reproduction. The review found that cigarette smoking was associated with modest reduction in sperm quality including sperm concentration, motility, and morphology. Associations between male smoking and sperm concentration and motility were stronger among healthy men (volunteers and sperm donors) than men from infertility clinic populations. However, despite modest reductions in sperm quality, the review did not

show a reduction in male fertility in association with paternal smoking. It concluded that although smokers as a group may not experience reduced fertility, men with questionable sperm quality who wish to have children may benefit from quitting smoking, since several studies indicated a potential for improved semen quality after smoking cessation.[1]

Cotinine Studies

Various studies have focused on cotinine levels in relation to semen parameters and most found a significant association between cotinine concentrations and semen parameters. A cross-sectional study of 35 smoking students and 30 non-smoking students in a university in Taiwan concluded that cotinine may decrease male fertility by inhibiting density, reducing total progressively motile sperm count, and increasing the percentage of abnormal sperm.[31] Similarly, a case-control study of 107 fertile and 103 sub-fertile men found a small but statistically significant correlation between cotinine concentration in seminal plasma and the percentage of abnormal sperm morphology, but not for other semen parameters.[32] A prospective study of 23 patients with normal seminal parameters, 11 smokers, and 12 non-smokers concluded that cotinine levels were correlated highly with the number of cigarettes smoked per day.[33] Yet another study focused on the effects of cotinine on sperm fertilizing capacity in vitro and found that cotinine concentrations of 400 or 800 ng/ml exerted detrimental effects on sperm, motility, membrane function, and the ability to undergo capacitation.[34] Thus, using an unbiased measure of smoking, adverse effects of smoking on sperm were still detected.

Summary

Two out of three cross-sectional studies did not find an association between cigarette smoking and conventional sperm parameters.[25, 27] However, two of the studies did recommend smoking cessation for men considering having children in the future.[26,27] Both of the case-control studies reviewed found a significant inverse association between cigarette smoking exposure and sperm quality.[28, 29] A study by Sofikitis et al. suggested a causal relationship between smoking and a decrease in sperm motility and function.[30] Finally, Vine's systematic review concluded that smokers as a group may not experience reduced fertility, but men with questionable sperm quality who wish to have children may benefit from quitting smoking.[1]

Commentary

One of the most substantial issues with many of the studies on this subject is the problem of achieving the correct time-order between smoking exposure and its effect on sperm quality. Determining that a particular exposure precedes the onset of an outcome is one of the most important requirements of procuring a truly causal relationship. Most of the studies reviewed were case-control or cross-sectional in design and it is impossible to establish an inception cohort in both of these types of studies. In the case of cross-sectional studies, both the exposure and the outcome are evaluated at the same time. Comparably, in case-control studies, the outcome is evident, but the exposure status of the case has to be obtained retrospectively. Essentially, neither study type is sufficient in obtaining a correct time order between the exposure and the outcome. This is an obvious weakness in the Chia et al. and Zhang et al. case-control studies on cigarette smoking and

sperm quality in which they selected cases and controls and retrospectively acquired the participant's exposure status.[28,29] Securing an inception cohort was also a problem in Trummer, Sepaniak, and Kunzle's cross-sectional studies on cigarette smoking and sperm quality.[25, 26, 27] The studies all collected smoking exposures by administering questionnaires close to collection of the semen samples. A prospective cohort is the optimal study design in the attainment of an inception cohort. This type of study allows the exposure to appear first, and the possible outcome to surface following the exposure. This cause and effect relationship is the hallmark of a causal association.

Noticeable deficiencies in a number of studies regard the way in which smoking exposure status was obtained. In a few of the studies smoking exposure was obtained by simply "asking" the subject or by administering a questionnaire.[25,26,28,29] This method of acquiring exposure status causes a definite complication: the subject could fabricate his smoking status and provide a false answer, or he could miscalculate the number of cigarettes smoked. Overall, this method could lead to bias and will not supply the researchers with the most accurate exposure status. A more desirable way of obtaining smoking exposure status was used by Sepaniak et al., in which subjects self-reported their smoking status and also completed a CO test to confirm their smoking status.[27] Cotinine level testing was also used in studies by Chen et al.[31], Wong et al.[32], Zenzes et al.[33], and Sofikitis et al.[34]; all studies which found a significant association between cotinine concentrations and semen parameters. Although CO testing is a simple improvement on the sole use of questionnaires, it does not always correctly detect different levels of

smoking exposure. CO also has a very short half-life of 1-4 hours, so it would not be a good measure of actual smoking exposure unless the subject smoked close to the testing interval.[35] A better measurement of smoking exposure would be testing nicotine levels in hair, as each centimeter of hair represents more than one month of exposure.[36]

A number of studies that were reviewed did not fully describe the method in which sperm parameter measurements were obtained or the way in which their samples were analyzed. According to WHO guidelines, samples should be assessed within 1-2 hours of ejaculation.[37] For example, Trummer and Sofikitis do not mention the time frame in which samples were analyzed.[30, 25] It is possible that analyzing samples after the correct time interval could lead to loss of sample quality and this type of measurement error may lead to a decrease in internal validity. The best course of action in this case would be to analyze all samples within 1-2 hours after collection. This would guarantee that the differences observed in the outcome measurements were not affected by the time between collection and analysis. Additionally, many studies have failed to mention who performed the sample analyses. It is important to know if one lab technician analyzed the samples or if many technicians performed the task. Using multiple technicians, assuming that there may be some extent of measurement error (as they are different people), could in turn possibly lower the internal validity of the study.

Some studies also had a large window of time during which the semen samples could be obtained. Such is the case in Kunzle et al.'s cross-sectional study in which semen samples were obtained by ejaculation after 2-7 days of abstinence.[26] According to WHO guidelines, semen samples should be collected after a minimum of 48 hours and no

longer than 7 days of sexual abstinence[37], but the possible differences in abstinence length between subjects may decrease the internal validity of the study by introducing measurement error. Although it may be difficult, an improvement may be to decrease the time interval during which samples may be obtained from 2-7 days to 3-5 days of sexual abstinence. This may improve the internal validity of the study by minimizing any effects based on different lengths of abstinence.

Generally, strength of an association between cigarette smoking exposure and sperm quality has not been demonstrated very well. In the studies that exhibited no association, there was an obvious lack of evidence to make a significant causal inference between the exposure and the outcome. However, a significant effect size was demonstrated by Kunzle et al., which showed a 15.4%, 17.5% and 16.6% decrease in sperm density, count, and motility, between smokers and non-smokers.[26] A large or significant effect size is very helpful in establishing a causal association.

Another evident similarity between many studies has been the failure to demonstrate a dose-response relationship between smoking and quality of semen parameters. For instance, Chia, Trummer, Kunzle, and Sepaniak did not show a dose-response relationship between the exposure and the outcome.[28, 25, 26, 27] However, Zhang et al. did demonstrate a negative correlation between the amount and duration of cigarette smoking and sperm density, viability, and forward progression.[29] Exhibiting a dose-response relationship between an exposure and an outcome is very important in achieving a causal association and makes the case for the causal relationship much stronger.

Because of the many factors that are involved with smoking, the majority of studies have not demonstrated great specificity. Since there are many other factors that may cause harm to sperm quality, it is rather difficult to profess that cigarette smoking exposure will undoubtedly have adverse effects on semen parameters. Cigarette smoking has many detrimental outcomes and is therefore not specific to the outcome of sperm quality. Specificity has also been very difficult to establish because of the lack of large effect sizes and the problems with study design. If an exemplary study design is able to show a large effect size, one could declare a high specificity in the magnitude of the association.

A number of studies on this topic lacked external validity as much of the research regarding semen parameters is accomplished at fertility clinics. The men who are usually present at these clinics have reason to believe that their fertility may be compromised and are therefore not a good generalization of the rest of the population. Although the motive for gathering study cohorts from infertility clinics is understandable, it may compromise the external validity of the study. In addition, most studies did not define 'infertility', which may affect and contribute to the differences in results.

Finally, it should be pointed out that Vine's systematic literature review did not cover many of the studies reviewed for this thesis and thus may not be the best representation of a recent systematic review.[1] However, the results very do exemplify the many disparate conclusions reached concerning the association of cigarette smoking and sperm quality, as there is very low consistency on this issue. Some studies have found no association between the mentioned exposure and outcome[28, 29], while others have found a significant association.[30, 25, 26, 29] As more studies on the subject matter

are completed, an agreeable consistency between study conclusions would aid in forming a causal association.

METHODS

Objectives

This study reports on the effect of cigarette smoking on semen parameters from data collected as part of the Fish and Infertility Study (FINS) project, a cross-sectional NIH study undertaken to evaluate the impact of environmental factors on measures of male infertility. Although FINS was not aimed toward the collection of cigarette smoke exposure data, that information was collected as part of the environmental factors and subsequently analyzed for this report. The five outcome measures evaluated were: total sperm count, sperm concentration, volume, motility, and morphology. The primary objective of this study was to compare the semen parameters of men who reported exposure to cigarette smoking and those who did not by evaluating three smoking variables: “Current Smoker”, “Ever Smoked”, and “Hours of Monthly Passive Smoking Exposure”. Secondary objectives were to evaluate the effect of other lifestyle factors on semen parameters of study subjects.

Study Design

Data collected for the NIH sponsored FINS study was analyzed for the purposes of this report. The FINS project was undertaken to investigate the relationship between measures of male reproductive health and exposure to environmental factors such as organochlorines and other heavy metals. Subject data for the FINS study was collected through self-reported questionnaires and included information regarding demographics and other life style factors. Outcome variables were obtained through semen analyses. This report uses the initial semen analysis parameters and relevant demographic and exposure data, as reported in the FINS datasets.

Study Population

Prior to conducting the study, institutional review board (IRB) approval was obtained from Michigan State University, Wayne State University, and the Michigan Department of Community Health. Informed consent protocols gathered via the mentioned institutions were followed in the recruitment and data collection from study participants. Between 2002 and 2006, two infertility clinics in Michigan (University Women's Care (UWC), an affiliate of the Detroit Medical Center, Wayne State University, Detroit, MI; and Grand Rapids Fertility and IVF, PC, in Grand Rapids, MI were used as the location for subject recruitment. Men between the ages of 18 and 60 years old were recruited from couples presenting for infertility testing at the two mentioned clinics. Invitations to participate in a project studying the impact of sport-caught fish consumption and other environmental factors on fertility were presented to all men of couples presenting for infertility at the clinics. Men fitting the following medical conditions were excluded for the study: diabetes, thyroid or adrenal disorder, genetic disorders related to fertility, testicular cancer, unilateral orchiectomy, or use of hormonal therapy. 603 men participated in the study.

Data Collection

Men who wished to participate in the study were properly enrolled using a consent form and asked to provide semen, blood, and urine specimens. Each participant was required to complete a detailed 50-page questionnaire administered by a trained interviewer and study recruiter. The questionnaire inquired about basic demographics, medical and occupational histories, and other factors of interest. Extensive information on lifestyle and other environmental factors was also obtained and included the

following: smoking history, alcohol and caffeine consumption, underwear type, stress at work and home, exercise, height, and weight.

Analysis of Semen Specimens

Each participant was asked to donate two semen specimens for analysis. However, not all participants complied with this request. Semen analysis was performed at the andrology laboratories at each study site and results were reported according to the WHO guidelines.[38] Total sperm count, concentration, initial motility, Kruger's strict morphology[39], and semen volume were used in the statistical analyses. Considering the non-uniformity of the number of semen specimen donations per participant, our analysis only used the first semen analysis performed at each study site's laboratory for each subject. This sample commonly coincided with the semen analysis carried out closest to the time of the questionnaire intake.

Data Analysis

Information from the questionnaire was summarized in Excel files and statistical analysis was performed using SAS software (version 9.2). Using the WHO criteria[38], the following semen parameters were classified as abnormal or low (per ejaculate): total sperm count ≤ 40 million, sperm concentration ≤ 20 million/mL, motility $\leq$50% forward progression, morphology ≤ 4% normal forms, and semen volume ≤ 2 mL (Table 1). Body mass index was calculated from each participant's reported height and weight using the formula: BMI = (weight in Lbs * 703) / (height in inches)^2 (Table 2). Three separate smoking exposure variables were used to assess exposure in a unique and comprehensive manner. The question "Do you smoke now?" was used to determine the current smoking

status of the subjects. In order to increase subject population in the smoking data, the categorized variable "Ever Smoked" (1= Yes, 0= No) was created, merging any evidence of past smoking into one inclusive variable. "Hours of monthly exposure passive cigarette smoking" was categorized into three categories in order to examine a dose-response effect. To contribute to a more meaningful analysis, all additional continuous variables were turned into appropriate categorical variables, with exception of subject weight, height, age (Table 3).

Each semen parameter was regarded as an outcome variable and dichotomized as 1= abnormal 0=normal using the WHO cut-offs. General linear modeling was used in identifying difference in means of semen parameters between smoking variables and all other demographics variables. Using logistic regression models and univariable analyses, all variables, including all three smoke exposure variables, were individually assessed for significance with each semen parameter. All variables with a p-values < 0.20 in the univariable analyses were deemed significant and included in multivariable logistic regression models for each of five separate semen outcomes and three smoke exposure variables. Finally, full multivariable logistic regression models were applied to examine the relationship between each smoke exposure variable and sperm parameter. All multivariable logistic regression models were controlled for study site, education level, annual income, age, race, and BMI, and factors with $p< 0.05$ were regarded as significant (Table 4).

RESULTS

603 men participated in this study. The mean age of the participants was 34 years old. The mean BMI was slightly below what is defined as obese (Table 5 and Figures 1-2). 74% of the subjects were Caucasian, 20% were African American, and 7% were categorized as 'other' (Table 6). Almost half of the participants reported having a college or post-college degree and nearly 30% reported having an annual income of > $90,000. (Table 6) About half of the participants had previously fathered children; some through assisted reproductive technologies and others on their own.

244 participants replied to a question framed as "Do you smoke now?" 49% identified as current smokers and 51% identified as current non-smokers. The created smoke exposure category "Ever Smoked" indicated 42% 'Ever Smokers' and 58% 'Never Smokers'. With regard to second hand smoke exposure in the past month, 19% of the respondents reported having no exposure, while 43% reported having < 10 hours and 38% reported having ≥10 hours (Table 7).

The minimum, maximum, median, mean, and standard deviation values for semen parameters within the study population are listed in Table 8 and depicted in Figures 3-7. Based on the WHO semen parameter guidelines we found the following frequency of abnormal semen parameters within our population: 22% low total sperm count, 25% low concentration, 34% low volume, 41% low motility, and 58% low normal morphology (Table 9). In the general linear modeling, significant associations were found between high second hand smoke exposure and low total sperm count ($p=0.001$) and low volume ($p=0.002$) (Table 10). African Americans had significantly lower values of total sperm count, concentration, and volume, compared to Whites and Others (Table 11). In general,

those who were obese had lower sperm concentration and percent normal motility than those who were not. Those with less education had lower values of total sperm count ($p=0.025$) and sperm concentration ($p=0.017$). However, those indicating their education level as 'high school graduate' had a significantly higher percent normal morphology than all others ($p<0.001$). Participants with higher income had generally higher values of total sperm count, concentration, and volume, although not significantly. There was a significant positive association between higher daily coffee consumption and increased sperm concentration ($p=0.033$) (Table 12). Inversely, there was a significant negative association between increased coffee consumption and percent normal motility ($p=0.024$). Similar inconsistent associations were also found for daily hot tea, iced tea, and pop. However, when caffeine intake was regarded as a whole, there were no associations between weekly caffeine consumption and any of the semen parameters. Our findings also suggested a significant association between increased monthly beer consumption and higher semen count and concentration ($p=0.014$ and $p=0.013$, respectively). Conversely, increased liquor consumption was associated with decreased semen volume ($p=0.035$). When alcohol consumption was analyzed as a whole there were no significant associations with any of the semen parameters (Table 13). Likewise, there were no significant associations between the semen parameters, exercise, and underwear type (Table 14). However, 'no work stress' and 'moderate to severe work stress' were significantly associated with lower total sperm count ($p=0.022$), with those experiencing 'moderate to severe' work stress having higher total sperm count levels. The same pattern is seen in home stress, although not significantly (Table 14).

Using full logistic multivariable regression models, and including all variables significant at P< 0.2 in the previous logistic univariable models, we found that current and ever smoking status did not have any significant effect on low total sperm count (Tables 15 and 16). Compared to those who reported zero hours of monthly passive smoking, those who reported ≥10 hours of passive cigarette smoke exposure had 2.7 times the odds of having lower total sperm count (p=0.053) (Table 17). However, when the model for low total sperm count was analyzed using current smoking and passive smoking exposure as covariates, neither smoking exposure was significant (Table 18). In general, work stress seemed to have a protective effect on total sperm count. Compared to those who reported no stress at work, those who did experience work stress were much less likely to have low total sperm count (Tables 15 and 18). Conversely, those who reported exercising had more than twice the odds of having low total sperm count (Tables 15, 16, 17, and 18). We found no association between any of the smoke exposure variables and low sperm concentration. Our results were mixed with regard to the effects of work stress and exercise on low sperm concentration (Tables 19, 20, and 21). Compared to those who reported zero hours of monthly passive smoking, those who reported ≥10 hours of passive cigarette smoke exposure had 1.5 times the odds of having lower semen volume (p=0.04) (Table 24). However, when the model for low semen volume was analyzed using current smoking and passive smoking exposure as covariates, neither smoking exposure was significant (Table 25). Overall, cigarette smoking exposure did not seem to have any effect on semen volume or motility (Tables 22-28). We did find that, compared to those who wore briefs, participants who reported wearing boxers were significantly lower odds of having low sperm motility (Tables 26, 27, and

28). Finally, “Ever Smokers” were significantly more likely to have low normal sperm morphology than “Never Smokers” (Table 30).

DISCUSSION

For this thesis, self-reported cigarette smoke exposure among men presenting at two Michigan infertility centers was analyzed in order to assess its effects on five semen parameters: total sperm count, concentration, volume, motility, and morphology. We found a small, but significant, association between low normal sperm morphology in participants who were "Ever Smokers" versus "Never Smokers". No significant associations were found between cigarette smoke exposure and sperm count, concentration, semen volume, or motility. When total sperm count and sperm concentration were assessed, exercise was shown to have a significant negative effect on both semen parameters, while work stress seemed to function as a protective factor. We also detected a protective association between the wearing of boxers and sperm motility.

Our study found a significant association between "Ever Smoked" status and low normal sperm morphology. Study participants who had smoked were significantly more likely to have low normal sperm morphology than those who reported never smoking. Chia et al. demonstrated results along similar lines in their case-control study of 640 consecutive male partners of couples trying to conceive.[26] Cases referred to the male partner of couples unable to conceive. Controls were defined as those of proven fertility whose wives were pregnant at the time of providing the semen. The study found that participants who smoked were 2.82 times more likely to be "infertile" than those who did not. They concluded that participants who smoked cigarettes were significantly more likely to have lower normal sperm morphology and in turn more likely to experience infertility.[26]

Unlike previous studies on the effects of cigarette smoke exposure and multiple semen parameters, our analysis only identified significance between "Ever Smoking" and decreased normal sperm morphology. Our analysis also examined the effects of passive cigarette smoking, but did not find a significant association between second hand smoke exposure and semen parameters. A recent second-hand smoke study in mice found that sidestream tobacco smoke induces mutations in mouse sperm. Although the study did not report specifically on semen count, it did suggest that paternal exposure to passive cigarette smoke may have reproductive consequences.[40] To our knowledge, the effects of passive cigarette smoke exposure on semen parameters have not been studied in humans.

We also performed multivariable logistic modeling on two other smoking variables to assess current smoking exposure and ever smoking exposure. The former variable was available in our dataset. However, only 244 of our 603 study participants had chosen to answer the question. In order to increase the chances of detecting an association between cigarette smoke exposure and semen parameters, we decided to increase the sample size of the smoke exposure variable by creating an additional variable: "Ever Smoked". The "Ever Smoked" variable was created by merging all evidence of prior smoking into one inclusive variable. Subsequently, the new variable increased our sample size and we discovered that 42% of our participants had smoked sometime in their life and 58% had not.

Our univariable logistic modeling demonstrated a significant association between "Current Smoking" and low semen volume and motility, and "Ever Smoked" and low sperm morphology. However, the full multivariable models only showed a significant

association between ever smoking and decreased normal sperm morphology. The lack of significance in the effect of current smoking status and semen quality is similar to that found by a cross-sectional study by Trummer et al.[25] and a study by Sepaniak et al.[27]. Trummer's assessment of 517 nonsmoker, 109 ex-smokers, and 478 smokers found that there were no significant differences between the conventional semen parameters and the smoke exposure status of their participants. Our results were similar to Trummer's in a number of ways. Like Trummer, we were able to analyze current smokers and non-smokers. Moreover, our "Ever Smoked" variable was able to capture ex-smokers. Both our study and Trummer's found no significance between current smoking status and semen parameters. However, contrary to Trummer's results, our study analysis did demonstrate a significant association between "Ever Smoking" exposure and decreasing normal sperm morphology. Our findings were also similar to those from the study by Sepaniak et al. Although our study population was much larger than that of Sepaniak's 57 non-smokers and 51 smokers, our study design was the same and current smoking status was self-reported. To Sepaniak's benefit, however, a CO test was also used to confirm participant's cigarette smoke exposure. Overall, our results were similar in that neither analysis identified a significant association between current smoking exposure and semen parameters.

Our lack of significance between current smoking status and semen parameters contrasted with two case-control studies by Zhang et al. and Chia et al. The former study revealed that current smoking among 110 non-smokers and 191 smokers did affect semen quality and concluded that, compared to non-smokers, semen volume, density, viability, and forward progression was significantly lower in medium, heavy, and long-term

smokers.[29] Chia et al. also found that current cigarette smoking significantly affected all semen parameters and that the risk of infertility was associated with smoking.[28] Both studies differed from ours in study design and results, but were similar in obtaining current smoking status through questionnaire.

The multivariable logistic modeling also revealed a number of other significant associations between exposure variables and semen parameters. We found that, compared to those who reported experiencing "no work stress", those who did were significantly less likely to have low total sperm count and concentration. When analyzed by annual income, our results revealed that as annual income increased, so did work stress. However, given our initial univariable analysis of mean semen parameters, we discovered that those with higher annual income were generally more likely to have increased total sperm count, concentration, and volume. We propose that work stress is simply a mediator variable that explains the relationship between income and semen parameters. i.e. those men with higher paying, more secure jobs may have less work stress than those with lower paying jobs. In addition, those who reported having "no work stress" may have been without a job and answered the question as having no stress at work.

Our analysis indicated that, compared to those who did not exercise, those who reported exercising were much more likely to suffer from low total sperm count and concentration. Literature on exercise and its effect on semen parameters are mixed and have mostly relied on very small populations. One study on 10 long distance competitive cyclist and 10 controls found that cyclists had significantly lower percent normal morphology, but did not see a significant difference in other semen parameters.[41]

Another study found that, compared to controls, some endurance-trained subjects had reduced gonadotropin releasing hormones and lower total sperm count, decreased motility, and decreased percent normal morphology.[42] Most recently, a prospective cohort study of 2,261 men attending one of three IVF clinics in the Boston area during 1993-2003, found that none of the semen parameters were significantly associated with regular exercise. However, compared with no regular exercise, bicycling ≥5 hours per week was associated with low sperm concentration and low motility (OR 1.92 and 2.05, respectively).[43] Because most studies on this subject, with exception of our study and the mentioned recent prospective study, had a very small number of participants, they may not have enough power to detect significant association, or to come to meaningful conclusions.

Finally, our multivariable modeling did reveal a significant association between underwear type and sperm motility. Compared to those who wore briefs, those who wore boxers were significantly less likely to have low sperm motility. This seems to support the theory of tight fitting undergarments, high scrotal temperature, and its negative impact on various semen parameters. Similar to studies on exercise and semen parameters, literature on the impact of underwear type on sperm is mixed. One pilot study evaluated the effect of fit of underwear on sperm production in two healthy males. The participants alternated wearing tight fitting briefs and loose fitting boxers in an ABAB withdrawal design study in which the conditions lasted three months and were alternated twice to results in a one year study. The results showed that semen parameters gradually decreased in tight conditions and increased in loose conditions.[44] Another

case-crossover study in 97 consecutive men presenting for evaluation of primary clinical subfertility found no significant association between underwear type, scrotal temperatures, and semen parameters.[40] Still another case-control study found that men who wore tight underpants or trousers were significantly more likely to present with dyspermia (OR 1.9 and 1.6, respectively).[45]

This study has a number of strengths that deserve mention. The dataset used for this analysis contained a large number of individuals with complete data on a number of lifestyles factors including demographics, caffeine and alcohol intake, exercise, and most importantly cigarette smoking habits. The large sample size increased the statistical power of our analyses and the comprehensive lifestyle factors allowed us to create complex statistical models, utilizing the variety of exposure variables and the five semen outcome variables. The multivariable logistic regression models enabled us to evaluate multiple risk factors, while allowing for control of confounding variables. In addition, our dataset included very complete information on hours of monthly passive cigarette smoke exposure, a factor that has rarely been studied. Moreover, because of the variety of questions on cigarette smoke exposure, we were able create a third variable to assess participant's history of ever smoking.

This study does have some limitations. The first is that the study population consisted of men presenting to two infertility clinics. This study population is not representative of normal healthy men and the use of this population could limit the generalizability of the findings and lead to external validity issues. However, about half of the subjects had fathered children previously. The design of this study was cross-sectional, which may lead to some inherent time-order issues between the exposure

variables and the outcome measures. Additionally, all study data, sans the semen parameters, were obtained through self-reporting on lengthy questionnaires which may result in recall bias. The dataset used for this study was not designed for an analysis of cigarette smoking exposure and semen parameters, and less than half of the total study population revealed their current smoking status. Even then, we cannot be sure of the presence of bias in their responses associated with stigma of cigarette smoking. Indeed, we created a third smoke exposure variable to assess "Ever Smoking" in order to obtain a larger number of responses on cigarette smoke exposure. Lastly, although we attempted to be as consistent as possible, the cut-off points and categorizations of some lifestyle factors were set arbitrarily based on our data points. It is reasonable to assume that different categorizations or cut-offs may have the possibility of leading to other results.

In summary, this study analyzed the effects of cigarette smoke exposure on five semen parameters. We found that those who reported ever smoking had higher odds of low normal sperm morphology (OR=1.61, p=0.032, CI= 1.043, 2.496). Our analysis also revealed trends for a protective effect of work stress and low total sperm count and concentration, and also of boxer wear and low sperm motility. Lastly, we suggest the implementation of future prospective cohort studies with large study populations geared toward studying cigarette smoke exposure, namely passive exposure, and its effects on semen parameters.

APPENDIX

Table 1. Classification of low or abnormal semen parameters.*

Semen Parameter	**Considered Low/Abnormal**
Total Sperm Count	≤ 40 Million
Sperm Concentration	≤ 20 Million/mL
Semen Volume	≤ 2 mL
Percent Motile Sperm	≤ 50% Forward Progression
Percent Morphologically Normal Sperm	≤ 4% Normal Forms

* According to WHO criteria.[31]

Table 2. Body Mass Index (BMI) categories and calculation.

BMI	Weight Category
18.5-24.99	Not Overweight/Obese
25.0-29.99	Overweight
30.0-39.99	Obese
≥ 40.00	Morbidly Obese

$$BMI = \frac{\text{weight (Lb)} * 703}{\text{height (in)}^2}$$

Table 3. Variables used in analyses, by unit and type.

Variable	Units	Type of Variable
Study Site	Detroit/Grand Rapids	Categorical
Age	Numerical	Continuous
Weight	Lb	Continuous
Height	Inches	Continuous
BMI	Normal-Morbidly Obese	Continuous
Race	White/Black	Categorical
Education	Education Level	
Annual Income	U.S. Dollars	Categorical
Smoke Now	Yes/No	Categorical
Ever Smoked	Yes/No	Categorical
Second Hand Smoke Exposure	Hours/Month	Categorical
Total Sperm Count	No. of Sperm per Ejaculate	Continuous
Sperm Concentration	No. of Sperm/mL of Ejaculate	Continuous
Semen Volume	mL	Continuous
Percent Motile Sperm	Percent (%)	Continuous
Percent Morphologically Normal Sperm	Percent (%)	Continuous
Low Total Sperm Count	1/0	Categorical
Low Sperm Concentration	1/0	Categorical
Low Semen Volume	1/0	Categorical
Low Motile Sperm	1/0	Categorical
Low Normal Morphology	1/0	Categorical
Smoke Now	Yes/No	Categorical
Ever Smoked	Yes/No	Categorical
Coffee Consumption	Cups/Day	Categorical
Hot Tea Consumption	Cups/Day	Categorical
Iced Tea Consumption	Cups/Day	Categorical
Pop Consumption	12 OZ Cans/Day	Categorical
Weekly Caffeine Intake	None-High	Categorical
Beer Consumption	Beers/Month	Categorical
Wine Consumption	Glasses of Wine/Month	Categorical
Liquor Consumption	Shots of Liquor/Month	Categorical
Mixed Drink Consumption	No. Mixed Drinks/Month	Categorical
Weekly Alcohol Intake	None-High	Categorical
Exercise	Yes/No	Categorical
Home Stress	None-Severe	Categorical
Work Stress	None-Severe	Categorical
Underwear Type	Boxers/Briefs/Other	Categorical

Table 4. Outcomes and exposure used in multivariable logistic regression models.*

Model Name	Outcome Variable	Exposure Variables
1a: Low Total Sperm Count and Smoke Now	Low total sperm count	Smoke now, home stress, work stress, exercise
1b: Low Total Sperm Count and Ever Smoked		Ever smoked, home stress, work stress, exercise
1c: Low Total Sperm Count and Second Hand Smoke Exposure		Second hand smoke exposure, home stress, work stress, exercise
1d: Low Total Sperm Count, Smoke Now, and Second Hand Smoke Exposure		Smoke now, second hand smoke exposure, home stress, work stress, exercise
2a: Low Sperm Concentration and Smoke Now	Low sperm concentration	Smoke now, home stress, work stress, exercise, weekly caffeine intake
2b: Low Sperm Concentration and Ever Smoked		Ever smoked, home stress, work stress, exercise, weekly caffeine intake
2c: Low Sperm Concentration and Second Hand Smoke Exposure		Second hand smoke exposure, home stress, work stress, exercise, weekly caffeine intake
3a: Low Semen Volume and Smoke Now	Low semen volume	Smoke now
3b: Low Semen Volume and Ever Smoked		Ever smoked
3c: Low Semen Volume and Second Hand Smoke Exposure		Second hand smoke exposure
3d: Low Semen Volume, Second Hand Smoke Exposure, and Smoke Now		Second hand smoke exposure, smoke now
4a: Low Sperm Motility and Smoke Now	Low sperm motility	Smoke now, underwear type
4b: Low Sperm Motility and Ever Smoked		Ever smoked, underwear type
4c: Low Sperm Motility and Second Hand Smoke Exposure		Second hand smoke exposure, underwear type
5a: Low Normal Sperm Morphology and Smoke Now	Low normal sperm morphology	Smoke now, work stress
5b: Low Normal Sperm Morphology and Ever Smoked		Ever smoked, work stress
5c: Low Normal Sperm Morphology and Second Hand Smoke Exposure		Second hand smoke exposure, work stress

* All models were controlled for study site, education level, annual income, age, race, and BMI. Factors with $P < 0.05$ were regarded as significant.

Table 5. Demographic characteristics of participants.

Demographic Characteristics	N	Min	Max	Median	Mean	Standard Deviation
Study Site						
Grand Rapids	208	-		-	-	-
Detroit	393	-		-	-	-
Age	594	18	60	34	34.13	5.85
Weight (Lb)	602	123	465	200	208.85	46.06
Height (In)	603	62	83	71	70.66	3.07
BMI	602	16.68	55.13	27.82	29.33	5.88

Table 6. Socio-economic characteristics of participants.

Socio-Economic Characteristics	N	Frequency	%
Race	600		
White		437	73.83
Black		120	20.00
Other		43	7.17
Education	603		
Elementary		7	1.16
Some High School		31	5.14
High School Graduate		97	16.09
Some College		200	33.17
College or Post-College Graduate		268	44.44
Annual Income	574		
≤$29,999		70	12.20
$30,000- ≤$44,999		69	12.02
$45,000- ≤$59,999		99	17.25
$60,000- ≤$74,999		86	14.98
$75,000- ≤$89,999		83	14.46
≥$90,000		167	29.09

Table 7. Smoking characteristics of participants.

Smoking Exposure	N	Frequency	%
Smoke Now	244		
No [a]		120	50.82
Yes [b]		124	49.18
Ever smoked	603		
No [c]		350	58.04
Yes [d]		253	41.96
Second Hand Smoke (Monthly)	598		
None		112	18.73
<10 Hours		259	43.31
≥10 Hours		227	37.96

[a] *Replied "No" to "Do you smoke now?"*

[b] *Replied "Yes" to "Do you smoke now?"*

[c] *Did not reply "Yes" to any of the following questions: "Have you smoked >100 cigarettes (5 packs) in your lifetime?", "Have you smoked cigarettes in the last twelve months?", or "Do you smoke now?"*

[d] *Replied "Yes" to any of the following questions: "Have you smoked >100 cigarettes (5 packs) in your lifetime?", "Have you smoked cigarettes in the last twelve months?", and "Do you smoke now?"*

Table 8. Semen parameters of participants.

Semen Parameters	N	Min	Max	Median	Mean	Standard Deviation
Total Sperm Count (10^6)	575	0	948.50	117.25	159.92	149.48
Sperm Concentration (10^6/mL)	575	0	364.00	43.80	59.27	54.50
Semen Volume (mL)	577	0.02	12.00	2.90	2.93	1.52
Motile Sperm (%)	571	0	93.00	54.00	51.35	16.16
Morphologically Normal Sperm (%)	535	0	22.00	4.00	4.60	4.02

Table 9. Semen parameters of participants classified by "Normal" or "Abnormal".*

Semen Parameters	N	Frequency	%
Total Count (10^6)	575		
Normal		451	78.43
Abnormal [a]		124	21.57
Concentration (10^6/mL)	575		
Normal		433	75.30
Abnormal [b]		142	24.70
Volume (mL)	577		
Normal		381	66.03
Abnormal [c]		196	33.97
Motility (%)	571		
Normal		340	59.54
Abnormal [d]		231	40.46
Normal Morphology (%)	535		
Normal		224	41.87
Abnormal [e]		311	58.13

* According to WHO criteria.[31]

[a] Total sperm count ≤ 40 million was considered abnormal

[b] Sperm concentration ≤ 20 million/mL was considered abnormal

[c] Semen volume ≤ 2 mL was considered abnormal

[d] Sperm motility ≤ 50% forward progression was considered abnormal

[e] Sperm morphology ≤ 4% normal forms was considered abnormal

Table 10. Univariable analysis depicting mean semen parameters and cigarette smoke exposure.

Smoking Exposure	N	Total Sperm Count (10^6)	Concentration (10^6/mL)	Volume (mL)	Motility (%)	Normal Morphology (%)
Smoke Now						
No [a]	120	143.1	52.51	2.87	50.41	4.48
Yes [b]	124	133.2	57.95	2.66	51.79	4.79
P-Value		0.555	0.422	0.248	0.505	0.570
Ever Smoked						
No [c]	350	159.5	58.18	3.00	51.18	4.50
Yes [d]	235	160.4	60.73	2.83	51.58	4.72
P-Value		0.943	0.577	0.209	0.767	0.533
Second Hand Smoke (Monthly)						
None	112	158.51	59.79	2.86	50.04	5.04
<10 Hours	259	183.99	63.67	3.19	51.53	4.52
≥10 Hours	227	133.69	53.84	2.70	51.79	4.48
P-Value		***0.001***	0.150	***0.002***	0.639	0.479

[a] *Replied "No" to "Do you smoke now?"*

[b] *Replied "Yes" to "Do you smoke now?"*

[c] *Replied "Yes" to any of the following questions: "Have you smoked >100 cigarettes (5 packs) in your lifetime?", "Have you smoked cigarettes in the last twelve months?", or "Do you smoke now?"*

[d] *Did not reply "Yes" to any of the following questions: "Have you smoked >1000 cigarettes (5 packs) in your lifetime?", "Have you smoked cigarettes in the last twelve months?", and "Do you smoke now?"*

Table 11. Univariable analysis depicting mean semen parameters and subject demographics.

Demographic Characteristics	N	Total Sperm Count (10^6)	Concentration (10^6/mL)	Volume (mL)	Motility (%)	Normal Morphology (%)
Race						
White	437	172.02	62.08	3.08	51.31	4.70
Black	120	111.51	48.34	2.39	51.22	4.66
Other	43	168.44	59.55	2.94	52.29	3.29
P-Value		***<0.001***	0.061	***<0.001***	0.934	0.135
Age						
18-29	128	151.08	55.70	2.90	15.74	4.93
30-39	371	169.99	55.59	3.01	16.59	4.79
40-60	95	133.46	49.13	2.69	14.80	3.46
P-Value		0.094	0.419	0.211	0.076	***0.016***
BMI						
Normal	123	180.93	68.45	2.88	49.55	4.28
Overweight	262	157.98	57.59	2.97	51.50	4.72
Obese	180	161.25	59.96	2.92	53.47	4.80
Morbidly Obese	37	104.31	40.10	2.90	46.14	3.91
P-Value		0.064	***0.047***	0.962	***0.043***	0.520
Education						
Some High School	31	147.81	59.66	2.74	52.66	4.10
High School Grad	97	127.64	49.92	2.67	52.53	6.16
Some College	200	151.22	53.19	3.02	50.40	3.96
College Grad	268	179.37	67.03	2.99	51.27	4.62
P-Value		***0.025***	***0.017***	0.236	0.734	***<0.001***
Annual Income						
<$29,999	70	125.37	49.59	2.56	53.58	4.27
$30,000-44,999	69	143.41	50.65	3.00	52.53	4.52
$45,000-59,999	99	156.49	63.31	2.84	51.15	5.41
$60,000-74,999	86	184.62	67.53	2.90	50.21	5.17
$75,000-89,999	83	187.42	60.56	3.29	50.68	4.15
>$90,000	167	165.96	62.25	3.03	51.31	4.42
P-Value		0.097	0.269	0.088	0.835	0.234

Table 12. Univariable analysis depicting mean semen parameters and caffeine consumption.

Caffeine Consumption	N	Total Sperm Count (10^6)	Concentration (10^6/mL)	Volume (mL)	Motility (%)	Normal Morphology (%)
Daily Coffee						
None	240	148.64	54.81	2.87	53.12	4.86
<2 cups	176	156.83	56.46	2.98	51.90	4.20
≥ 2 cups	182	179.30	68.26	2.98	48.74	4.72
P-Value		0.115	***0.033***	0.699	***0.024***	0.272
Daily Hot Tea						
None	459	164.12	61.17	2.96	52.00	4.83
<1 cups	100	125.01	46.67	2.77	48.44	4.01
≥ 1 cups	38	199.60	68.98	3.02	52.11	3.58
P-Value		***0.019***	***0.036***	0.509	0.154	0.066
Daily Iced Tea						
None	399	175.12	63.83	2.97	52.06	4.68
<1/2 cups	142	139.20	53.56	2.88	49.62	4.28
≥ 1/2 cups	56	110.77	43.40	2.86	51.58	4.98
P-Value		***0.002***	***0.013***	0.785	0.321	0.522
Daily Pop						
None	94	168.24	58.18	2.93	49.46	4.44
<1 pop	220	157.35	59.52	3.01	52.31	4.55
≥ 1 pop	287	159.95	59.48	2.88	51.20	4.65
P-Value		0.836	0.978	0.629	0.378	0.905
Weekly Caffeine Intake						
None [a]	21	184.75	65.52	2.49	53.05	4.17
Low [b]	114	145.86	52.59	3.00	51.10	4.63
Low-Mod [c]	39	130.98	47.09	3.20	52.16	3.62
Moderate [d]	61	156.07	56.12	2.84	53.00	5.28
High [e]	338	166.74	63.14	2.94	51.07	4.58
P-Value		0.469	0.236	0.540	0.906	0.415

[a] No caffeine consumption per week

[b] 1 to < 7 caffeinated drinks per week

[c] 7 to < 14 caffeinated drinks per week

[d] 14 to < 50 caffeinated drinks per week

[e] ≥ 50 caffeinated drinks per week

Table 13. Univariable analysis depicting mean semen parameters and alcohol consumption.

Alcohol Consumption	N	Total Sperm Count (10^6)	Concentration (10^6/mL)	Volume (mL)	Motility (%)	Normal Morphology (%)
Monthly Beer						
None	199	134.39	51.09	2.83	49.22	4.40
<30 beers	331	170.30	61.30	3.02	52.31	4.50
≥ 30 beers	73	181.71	71.91	2.82	52.65	5.50
P-Value		***0.014***	***0.013***	0.346	0.091	0.127
Monthly Wine						
None	397	154.00	57.48	2.88	51.87	4.69
<10 glasses	168	175.62	63.27	3.06	50.67	4.67
≥ 10 glasses	34	155.39	61.81	2.91	50.50	3.61
P-Value		0.302	0.510	0.457	0.689	0.359
Monthly Liquor						
None	420	164.73	59.14	3.03	51.41	4.72
<3 shots	86	151.79	57.59	2.89	51.90	4.59
≥ 3 shots	94	145.19	61.12	2.57	50.61	4.18
P-Value		0.469	0.913	***0.035***	0.866	0.538
Monthly Mixed Drinks						
None	372	155.66	59.79	2.87	51.58	4.62
<4 drinks	118	177.63	58.71	3.18	49.18	4.41
≥ 4 drinks	109	154.34	58.21	2.88	52.85	4.75
P-Value		0.359	0.959	0.153	0.217	0.829
Weekly Alcohol Intake						
None [a]	132	136.77	51.35	2.92	50.02	4.25
Low [b]	116	164.32	61.58	2.93	50.79	4.42
Low-Mod [c]	131	170.31	61.84	3.03	52.79	4.71
Moderate [d]	89	181.56	64.67	2.85	50.49	4.73
High [e]	43	140.28	63.59	2.56	52.59	4.82
P-Value		0.179	0.380	0.538	0.661	0.861

[a] No alcoholic drink consumption per week

[b] 1 to < 3 alcoholic drinks per week

[c] 3 to < 7 alcoholic drinks per week

[d] 7 to < 25 alcoholic drinks per week

[e] ≥ 25 alcoholic drinks per week

Table 14. Univariable analysis depicting mean semen parameters and other lifestyle factors.

Lifestyle Factors	N	Total Sperm Count (10^6)	Concentration (10^6/mL)	Volume (mL)	Motility (%)	Normal Morphology (%)
Exercise						
Yes	250	160.24	56.88	3.03	51.29	4.69
No	270	167.30	63.84	2.85	52.25	4.47
P-Value		0.608	0.161	0.196	0.512	0.545
Home Stress						
None	67	151.89	56.88	3.02	49.35	5.03
Slight- Mod	415	168.99	62.41	2.93	51.64	4.68
Mod-Severe	117	132.38	49.91	2.85	51.56	4.07
P-Value		0.065	0.092	0.773	0.574	0.263
Work Stress						
None	53	123.59	47.97	2.82	53.22	5.04
Slight- Mod	323	177.33	64.00	3.00	52.13	4.75
Mod-Severe	199	150.71	58.12	2.88	51.26	4.46
P-Value		***0.022***	0.116	0.557	0.690	0.608
Underwear Type						
Boxers	254	169.68	60.15	2.92	52.60	4.67
Briefs	190	155.69	57.71	2.90	50.23	4.57
Other	152	165.73	60.79	2.95	50.24	4.49
P-Value		0.835	0.856	0.954	0.275	0.921

Table 15. Logistic regression model for Low Total Sperm Count, using "Smoke Now" as the main variable (N=145).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Smoke Now			
No	Ref.		0.707
Yes	1.23	(0.416, 3.647)	
Home Stress			
None	Ref.		0.566
Slight- Mod	2.70	(0.355, 20.591)	
Mod- Severe	3.28	(0.366, 29.422)	
Work Stress			
None	Ref.		***0.014***
Slight- Mod	***0.08***	(0.013, 0.440)	
Mod- Severe	***0.07***	(0.010, 0.489)	
Exercise			
No	Ref.		***0.022***
Yes	***3.50***	(1.197, 10.260)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 16. Logistic regression model for Low Total Sperm Count, using "Ever Smoked" as the main variable (N=448).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Ever Smoked			
No	Ref.		0.945
Yes	1.02	(0.591, 1.758)	
Home Stress			
None	Ref.		0.215
Slight- Mod	1.68	(0.673, 4.186)	
Mod- Severe	2.48	(0.876, 7.009)	
Work Stress			
None	Ref.		0.118
Slight- Mod	0.38	(0.150, 0.966)	
Mod- Severe	0.38	(0.141, 1.040)	
Exercise			
No	Ref.		***0.001***
Yes	***2.63***	(1.489, 4.632)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 17. Logistic regression model for Low Total Sperm Count, using "Monthly Hours of Passive Smoking" as the main variable (N=445).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Hours of Passive Smoking (Monthly)			
None	Ref		
< 10 hours	1.70	(0.722, 4.000)	0.053
≥ 10 hours	2.69	(1.163, 6.222)	
Home Stress			
None	Ref.		
Slight- Mod	1.64	(0.652, 4.118)	0.259
Mod- Severe	2.37	(0.828, 6.763)	
Work Stress			
None	Ref.		
Slight- Mod	0.40	(0.152, 1.050)	0.147
Mod- Severe	0.37	(0.133, 1.038)	
Exercise			
No	Ref.		***0.001***
Yes	***2.55***	(1.437, 4.540)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 18. Logistic regression model for Low Total Sperm Count, using "Monthly Hours of Passive Smoking" and "Smoke Now" as main variables (N=143).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Hours of Passive Smoking (Monthly)			
None	Ref		
< 10 hours	7.58	(0.595, 96.502)	0.259
≥ 10 hours	4.11	(0.370-45.726)	
Smoke Now			
No	Ref.		0.644
Yes	1.30	(0.424, 3.997)	
Home Stress			
None	Ref.		
Slight- Mod	2.37	(0.321, 17.583)	0.691
Mod- Severe	2.44	(0.273, 21.819)	
Work Stress			
None	Ref.		
Slight- Mod	***0.08***	(0.012, 0.613)	***0.021***
Mod- Severe	***0.09***	(0.012, 0.470)	
Exercise			
No	Ref.		***0.028***
Yes	***3.40***	(1.138, 10.146)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 19. Logistic regression model for Low Sperm Concentration, using "Smoke Now" as the main variable (N=141).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Smoke Now			
No	Ref.		0.735
Yes	1.21	(0.405, 3.604)	
Home Stress			
None	Ref.		0.485
Slight- Mod	1.08	(0.119, 9.788)	
Mod- Severe	2.20	(0.199, 24.221)	
Work Stress			
None	Ref.		***0.007***
Slight- Mod	***0.04***	(0.006, 0.306)	
Mod- Severe	***0.05***	(0.007, 0.425)	
Exercise			
No	Ref.		0.150
Yes	2.22	(0.762, 5.935)	
Weekly Caffeine Intake			
None	Ref.		0.424
Low	0.19	(0.004, 8.445)	
Low-Mod	0.03	(<0.001, 2.408)	
Moderate	<0.001	(<0.001, >999.999)	
High	0.07	(0.001, 3.074)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 20. Logistic regression model for Low Sperm Concentration, using "Ever Smoked" as the main variable (N=425).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Ever Smoked			
No	Ref.		0.960
Yes	1.01	(0.589, 1.745)	
Home Stress			
None	Ref.		0.122
Slight- Mod	1.37	(0.563, 3.316)	
Mod- Severe	2.45	(0.891, 6.747)	
Work Stress			
None	Ref.		***0.006***
Slight- Mod	***0.22***	(0.085, 0.549)	
Mod- Severe	***0.28***	(0.104, 0.742)	
Exercise			
No	Ref.		***< 0.001***
Yes	***2.87***	(1.652, 4.992)	
Weekly Caffeine Intake			
None	Ref.		0.771
Low	1.13	(0.318, 4.042)	
Low-Mod	1.20	(0.253, 5.667)	
Moderate	0.59	(0.140, 2.485)	
High	0.94	(0.275, 3.185)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 21. Logistic regression model for Low Sperm Concentration, using "Monthly Hours of Passive Smoking" as the main variable (N= 422).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Hours of Passive Smoking (Monthly)			
None	Ref		
< 10 hours	1.35	(0.599, 3.025)	0.464
≥ 10 hours	1.66	(0.734, 3.757)	
Home Stress			
None	Ref.		
Slight- Mod	1.33	(0.549, 3.237)	0.108
Mod- Severe	2.48	(0.898, 6.827)	
Work Stress			
None	Ref.		
Slight- Mod	***0.23***	(0.090, 0.604)	***0.011***
Mod- Severe	***0.29***	(0.106, 0.783)	
Exercise			
No	Ref.		***<0.001***
Yes	***0.86***	(1.575, 4.777)	
Weekly Caffeine Intake			
None	Ref.		
Low	1.15	(0.323, 4.104)	0.694
Low-Mod	1.44	(0.301, 6.902)	
Moderate	0.62	(0.147, 2.583)	
High	0.89	(0.263, 2.983)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 22. Logistic regression model for Low Semen Volume, using "Smoke Now" as the main variable (N=216).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Smoke Now			
No	Ref.		0.132
Yes	1.66	(0.858, 3.209)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 23. Logistic regression model for Low Semen Volume, using "Ever Smoked" as the main variable (N=535).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Ever Smoked			
No	Ref.		0.984
Yes	0.99	(0.671, 1.478)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 24. Logistic regression model for Low Semen Volume, using "Monthly Hours of Passive Smoking" as the main variable (N=531).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Hours of Passive Smoking (Monthly)			
None	Ref		
< 10 hours	***0.81***	(0.467, 1.422)	***0.039***
≥ 10 hours	***1.48***	(0.850, 2.583)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 25. Logistic regression model for Low Semen Volume, using "Monthly Hours of Passive Smoking" and "Smoke Now" as the main variables (N=213).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Hours of Passive Smoking (Monthly)			
None	Ref		
< 10 hours	0.78	(0.272, 2.243)	0.843
≥ 10 hours	0.97	(0.362, 2.571)	
Smoke Now			
No	Ref.		
Yes	1.46	(0.733, 2.903)	0.283

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 26. Logistic regression model for Low Sperm Motility, using "Smoke Now" as the main variable (N=150).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Smoke Now			
No	Ref.		0.300
Yes	0.63	(0.268, 1.501)	
Underwear Type			
Briefs	Ref.		***0.001***
Boxers	***0.15***	(0.050, 0.424)	
Other	**0.63**	(0.228, 1.727)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 27. Logistic regression model for Low Sperm Motility, using "Ever Smoked" as the main variable (N=461).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Ever Smoked			
No	Ref.		0.874
Yes	1.04	(0.673, 1.593)	
Underwear Type			
Briefs	Ref.		***0.028***
Boxers	***0.55***	(0.335, 0.901)	
Other	***0.96***	(0.562, 1.645)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 28. Logistic regression model for Low Sperm Motility, using "Monthly Hours of Passive Smoking" as the main variable (N=458).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Hours of Passive Smoking (Monthly)			
None	Ref		
< 10 hours	0.75	(0.408, 1.362)	0.517
≥ 10 hours	0.95	(0.519, 1.748)	
Underwear Type			
Briefs	Ref.		
Boxers	***0.57***	(0.345, 0.926)	***0.039***
Other	0.97	(0.565, 1.653)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 29. Logistic regression model for Low Normal Sperm Morphology, using "Smoke Now" as the main variable (N=212).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Smoke Now			
No	Ref.		0.505
Yes	1.27	(0.628, 2.577)	
Work Stress			
None	Ref.		0.708
Slight- Mod	0.71	(0.217, 2.327)	
Mod- Severe	0.59	(0.167, 2.100)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 30. Logistic regression model for Low Normal Sperm Morphology, using "Ever Smoked" as the main variable (N= 530).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Ever Smoked			
No	Ref.		***0.032***
Yes	***1.61***	(1.043, 2.496)	
Work Stress			
None	Ref.		0.548
Slight- Mod	0.83	(0.287, 1.755)	
Mod- Severe	0.67	(0.302, 1.502)	

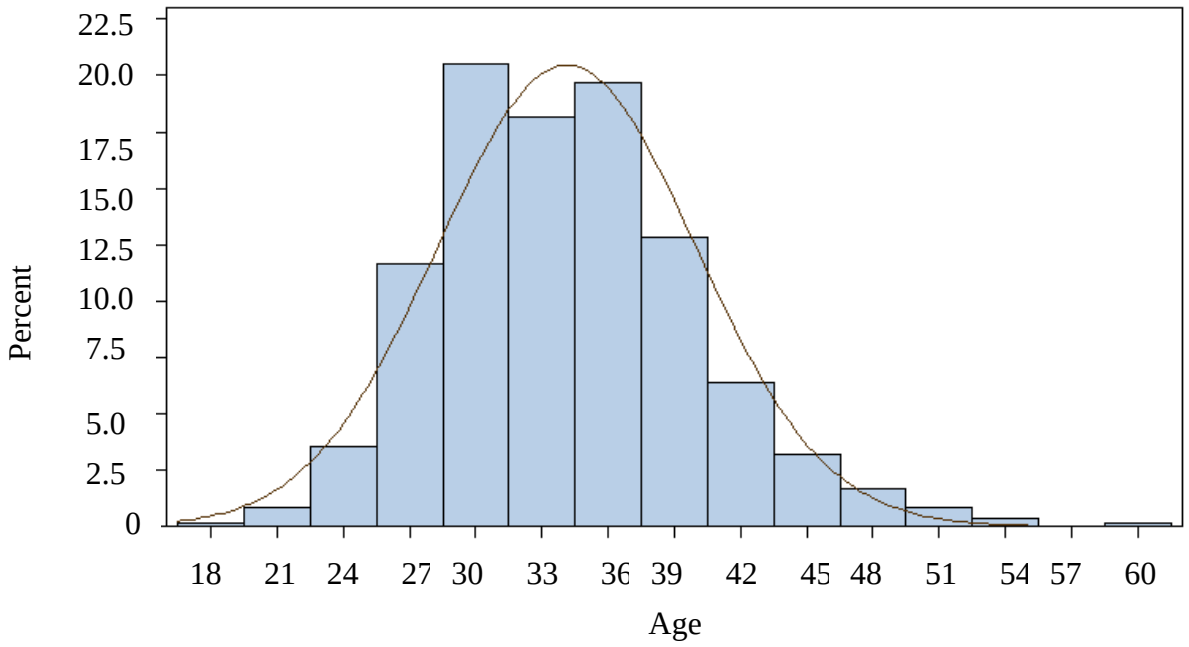
* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Table 31. Logistic regression model for Low Normal Sperm Morphology, using "Monthly Hours of Passive Smoking" as the main variable (N=526).*

Variable	Odds Ratio	95% Confidence Interval	P-Value
Hours of Passive Smoking (Monthly)			
None	Ref.		
< 10 hours	0.65	(0.359, 1.182)	0.269
≥ 10 hours	0.62	(0.338, 1.147)	
Work Stress			
None	Ref.		
Slight- Mod	0.84	(0.391, 1.792)	0.631
Mod- Severe	0.70	(0.314, 1.576)	

* Model was controlled for study site, education level, annual income, age, race, and BMI. Factors with P <0.05 were regarded as significant.

Figure 1. Age of study subjects. *



*For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Figure 2. Body Mass Index (BMI) of study subjects.

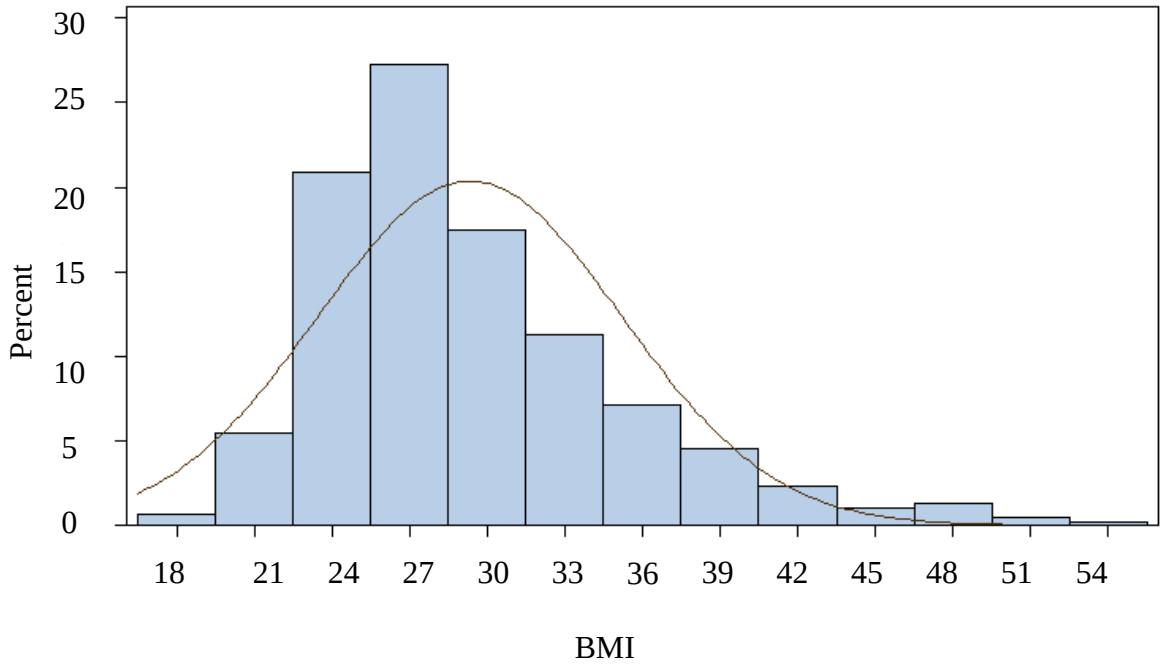


Figure 3. Total sperm count of study subjects (10^6).

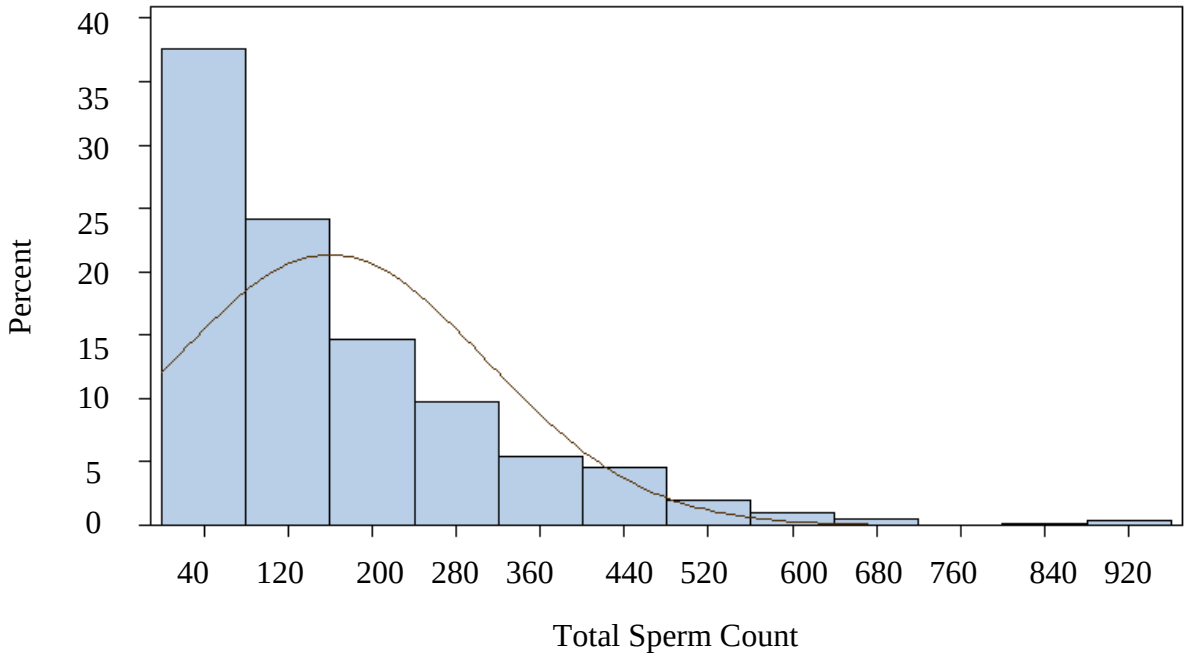


Figure 4. Semen concentration of study subjects (10^6/mL).

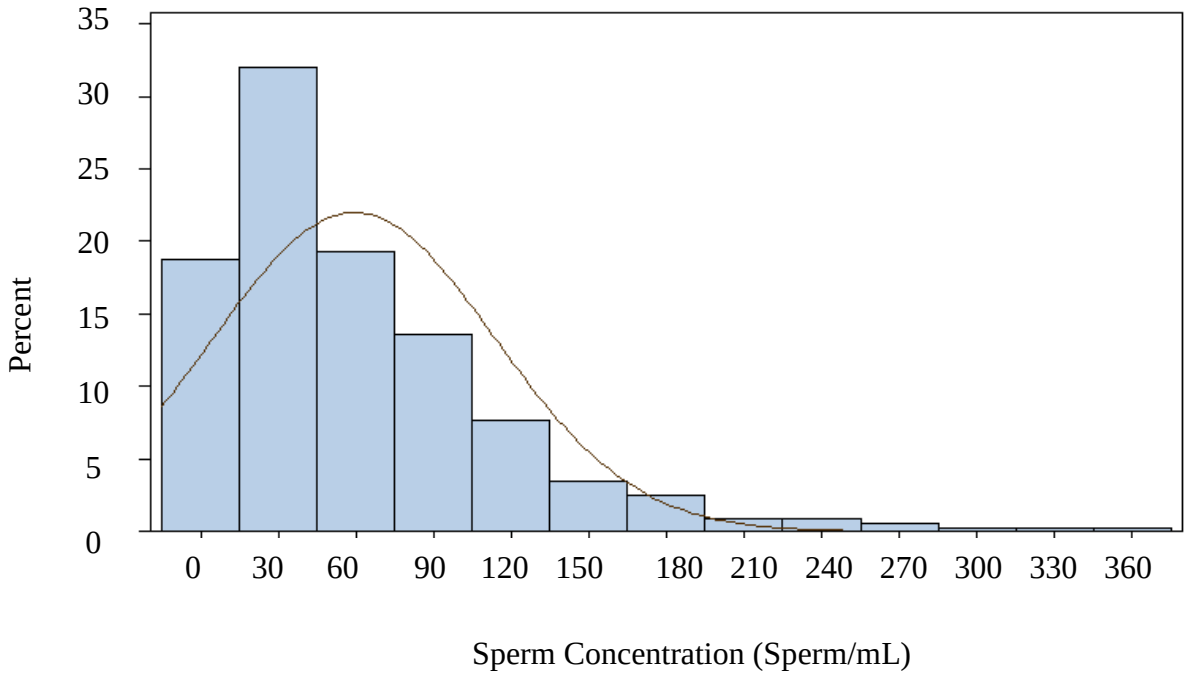


Figure 5. Ejaculate volume of study subjects (mL).

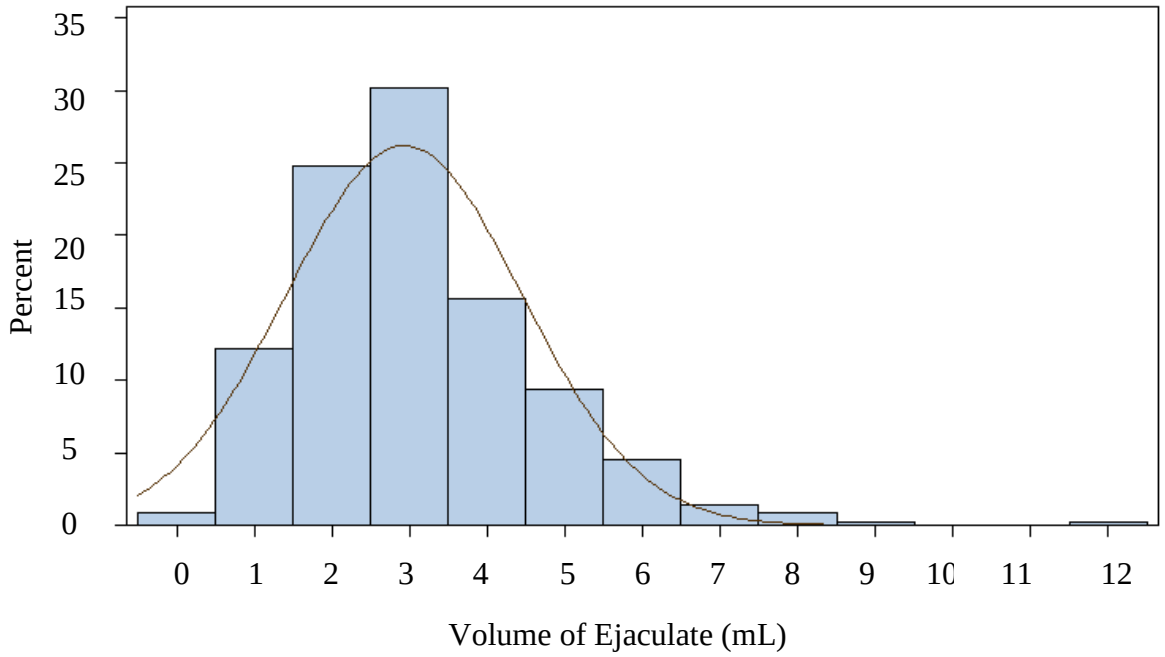


Figure 6. Percent sperm motility of study subjects.

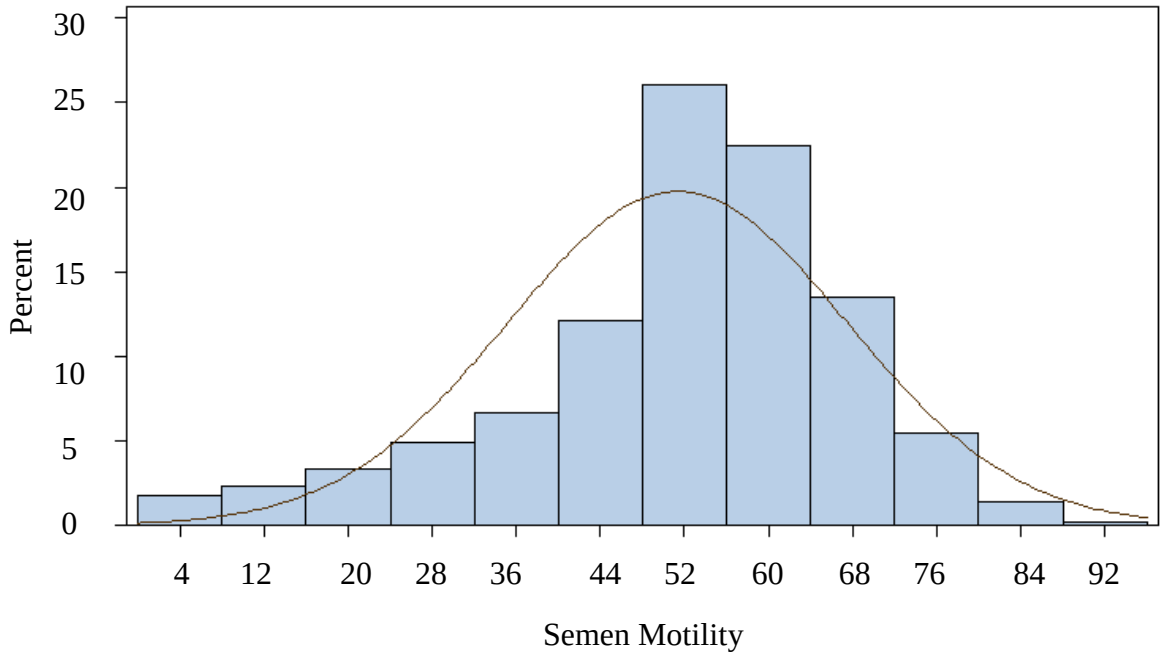
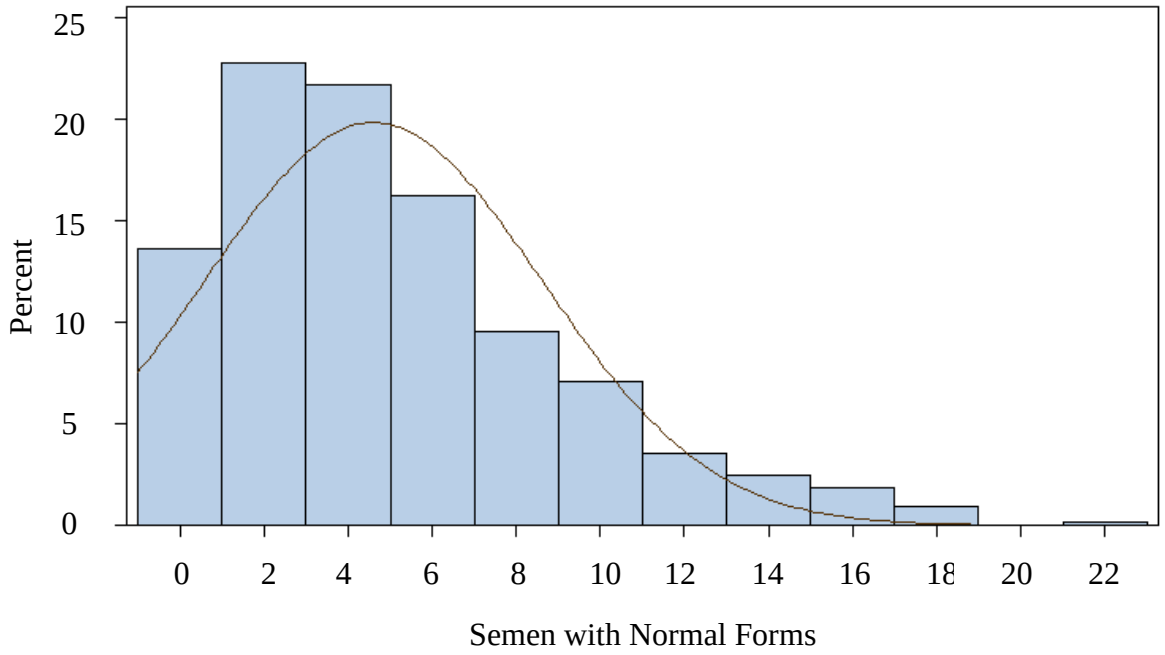


Figure 7. Percent normal sperm morphology of study subjects.



BIBLIOGRAPHY

BIBLIOGRAPHY

[1] Vine MF. Smoking and male reproduction: a review. International Journal of Andrology. 1996; 19: 323-337.

[2] U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. 2004. Accessed November 27, 2009. <http://www.cdc.gov/tobacco/data_statistics/sgr/2004/index.htm>

[3] U.S. Department of Health and Human Services. Reducing the Health Consequences of Smoking: 25 Years of Progress. A Report of the Surgeon General. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. DHHS Publication No. (CDC) 89-8411. 1989.

[4] U.S. Department of Health and Human Services. Tobacco Use Among U.S. Racial/Ethnic Minority Groups-African Americans, American Indians and Alaska Natives, Asian Americans and Pacific Islanders, and Hispanics: A Report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services, CDC. 1998. Accessed December 5 2009. <http://www.cdc.gov/tobacco/data_statistics/sgr/1998/index.htm>

[5] Ockene IS, Miller NH. Cigarette Smoking, Cardiovascular Disease, and Stroke: A Statement for Healthcare Professionals From the American Heart Association Journal of American Health Association. 1997; 96(9): 3243–3247.

[6] Carlsen E, Giwercman A, Keiding N, Skakkebæk NE. Evidence for decreasing quality of semen during past 50 years. BMJ. 1992; 305: 609-13.

[7] Swan SH, Elkin EP, Fenster L. The question of declining sperm density revisited: an analysis of 101 studies published 1934-1996. Environ Health Perspect. 2000; 108: 961-6.

[8] Kaufman RB, Spitz AM, Strauss LT, Morris L, Santelli, JS, Koonin LM, and Marks JS. The decline in US teen pregnancy rates, 1990-1995. Pediatrics. 1998; 102: 1141-1147.

[9] Pearce D, Cantisani G, Laihonon A. Changes in fertility and family sizes in Europe. Popul. Trend. Spring. 1999: 33-40.

[10] Mendiola J, Jorgensen N, Minguez-Alarcon L, Sarabia-Cos L, Lopez-Espin JJ, Vivero-Salmeron G, Ruiz-Ruiz KJ, Fernandez MF, Olea N, Swan S, Torres-Cantero AM. Sperm counts may have declined in young university students in Southern Spain. Andrology. 2013: 1-6.

[11] Jorgensen N, Nordstrom UN, Jensen TK, Jensen MB, Almstrup K, Olesen IA, Juul A, Andersson AM, Carlsen E, Petersen JH, Toppari J, Skakkebaek NE. BMJ Open. 2012 Jul 2; 2(4).

[12] Rolland M, Le Moal J, Wagner V, Royere D, De Mouzon J. Decline in semen concentration and morphology in a sample of 16 609 men close to general population between 1989 and 2005 in France. Hum Reprod. 2013; 28(2): 462-70.

[13] Lutz W. Fertility rates and future population trends: will Europe's birth rate recover or continue to decline? Int J Androl 2006 February; 29(1): 25-33.

[14] United Nations. World Population Prospects: The 2006 Revision. New York, United Nations 2007.

[15] Bonde, JP, Ernst E, Jensen TK, Hjollund, NHI, Kolstad H, Henriksen TB, Scheike T, Giwerman A, Olsen J, Skakkebaek NE. The relation between semen quality and fertility. A population based study of 430 first-pregnancy planners. Lancet. 1998; 352: 1172-1177.

[16] Connolly GN. Worldwide expansion of transnational tobacco industry. Journal of the National Cancer Institute Monographs. 1992; 12: 29-35.

[17] International Agency for Research on Cancer. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans: Tobacco smokmg. IARC Mongraphs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. 1986; No. 38: 65-74.

[18] Ostergaard, K. Cadmiumkoncentrationen inyrevaev hos rygere og ikke-rygere. Ugeskr. Lag. 1977; 139: 989-991.

[19] Kier LD, Yamasak E, Ames BN. Detection of Mutagenic Activity in Cigarette Smoke Condensates. Proc. Nat. Acad. Sci. USA. 1974; No.10: 4159-4163.

[20] Kaufman DW, Helmrich SP, Rosenberg L, Meittinen OS, Shapiro S. Nicotine and carbon monoxide content of cigarette smoke and the risk of myocardial infarction in young men. N. Engl. J. Med. 1983; 309: 409-414.

[21] Ravenkolt, RT. Radioactivity in cigarette smoke. New Engl. J. Med. 1982: 307-312.

[22] Mattison, DR. The effects of smoking on fertility from gametogenesis to implantation. Environmental Research. 1982; 26(2): 410-433.

[23] Vogt, HJ, Heller WD, Borelli S. Sperm quality of healthy smokers, ex-smokers, and never-smokers. Fertil. Steril. 1986; 45: 106-110.

[24] National Research Council Subcommittee on Reproductive and Neurodevelopmental Toxicology. Chapter 7: Biologic markers of human male reproductive health and physiologic damage. In: Biological Markers in Reproductive Toxicology (eds N. Grossblatt & L. R. Paulson), pp. 83-105. National Academy Press, Washington, DC. 1989.

[25] Trummer, H., Habermann, H., Haas, J., Pummer, K. The impact of cigarette smoking on human semen parameters and hormones. Human Reprod. 2002; 17: 1554–1559.

[26] Kunzle R, Mueller MD, Hanggi W, Birkhauser MH, Drescher H, Bersinger NA. Semen quality of male smokers and nonsmokers in infertile couples. Fertility and Sterility. 2003. 79(2): 288-291.

[27] Sepaniak S, Forges T, Gerard H, Foliguet B, Bene MC, Monnier-Barbarino P. The influence of cigarette smoking on human sperm quality and DNA fragmentation. Toxicology. 2006; 223: 54-60.

[28] Chia SE, Lim SA, Tay SK, Lim ST. Factors associated with male infertility: a case-control study of 218 infertile and 240 fertile men. BJOG. 2000; 107(1): 55-61.

[29] Zhang JP, Meng QY, Zhang LJ, Mao YL, Sun ZX. Effects of smoking on semen quality of infertile men in Shandog, China. Asian J Anrol. 2000; Jun; 2: 143-146.

[30] Sofikitis, N Miyagawa I, Dimitriadis, D et al. Effects of smoking on testicular function, semen quality and sperm fertilizing capacity. J. Urol. 1995; 154: 1030-1034.

[31] Chen HW, Kuo CT. Cotinine Characterization and Quality Effect of Sperm for Smoking and Nonsmoking Students. Bull Environ Contam Toxicol. 2007; 79: 11-14.

[32] Wong WY, Thomas CM, Merkus HM, Zielhuis GA, Doesburg WH, and Steegers-Theunissen RP. Cigarette Smoking and The Risk of Male Factor Subfertility: Minor Association Between Cotinine in Seminal Plasma and Semen Morphology. Fertil Steril. 2000; 74(5): 930-5.

[33] Zenzes MT, Bielecki R, and Reed TE. Detection of benzo(a)pyreene Diol Epozide-DNA Adducts in Sperm of Men Exposed to Cigarette Smoke. Fertil Steril. 1999; 72(2): 330-5.

[34] Sofikitis N, Takenaka M, Kanakas N, Papadopoulos H, Yamamoto Y, Drakakis P, and Miyagawa I. Effects of Cotinine on Sperm Motility, Membrane Function, and Fertilizing Capacity In Vitro. Urol Res. 2000; 28(6): 370-5.

[35] Usmani ZC, Craig P, Shipton D,and Tappin D. Comparison of CO breath testing and women's self-reporting of smoking behaviour for identifying smoking during pregnancy. Subst Abuse Treat Prev Policy. 2008; 3:4.

[36] Uematsu T, Mizuno A, Negashima S, et al. The axial distribution of nicotine content along hair shafts as an indicator of changes in smoking behavior. Evaluation in a smoking cessation programme with or without the aid of nicotine chewing gum. Br J Clin Pharmacol. 1995; 39: 655-699.

[37] WHO. World Health Organization Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. 3rd ed. Cambridge: Cambridge University Press, 1992.

[38] WHO. World Health Organization Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. 4th ed. New York: Cambridge University Press, 1999.

[39] Kruger TF, Menkveld R, Stander FS, Lombard CJ, Van der Merwe JP, van Zyl JA, et al. Sperm morphologic features as a prognostic factor in vitro fertilization. Fertility and Sterility. 1986; 46: 1118-23.

[40] Munkelwitz R, and Gilbert BR. Are boxer shorts really better? A critical analysis of the role of underwear type in male subfertility. J Urol. 1998; 160(4): 1329-33.

[41] Gebreegziabher Y, Marcos E, McKinon W, and Rogers G. Sperm Characteristics of Endurance Trained Cyclists. Int J Sports Med. 2004; 25(4): 247-251.

[42] De Souza MJ, and Miller BE. The effect of endurance training on reproductive function in male runner. A 'volume threshold' hypothesis. Sport Medicine (Auckland, N.Z.) 1997; 23(6): 357-374.

[43] Wise LA, Cramer DW, Hornstein MD, Ashby RK, and Missmer SA. Physical activity and semen quality among men attending an infertility clinic. Fertility and Sterility. 2011; 95(3): 1025-1030.

[44] Sanger WG, et al. Fit of underwear and male spermatogenesis: a pilot investigation. Reprod Toxicol. 1990; 4(3): 229-32.

[45] Parazzini F, Marchini M, Luchini L, Tozzi L, Mezzopane R, and Fedele L. Tight underpants and trousers and risk of dyspermia. Int J Androl. 1995; 18(3): 137-40.