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CIGARETTE SMOKE EXPOSURE AND MALE REPRODUCTIVE HORMONES IN HEALTHY MEN

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Reproductive hormones are important determinants of sperm production and male sexual function. Early animal and human research during the 1970s suggested cigarette smoking could adversely affect male hormone profiles although subsequent studies did not reach consensus. BMI, age, and alcohol intake were recognized as important covariates of this relationship in the 1980s but studies still did not reach consensus. Exposure misclassification may explain part of the variability in findings, including misclassification due to environmental tobacco smoke (ETS). The objective of this work was to use a biomarker of cigarette exposure (nicotine metabolite in blood serum) to more accurately describe effects of cigarette smoke on male reproductive hormones in a cohort of healthy working men.

Methods: This work was based on 192 men enrolled as a subset from a larger occupational health study. Each participant gave informed consent and was interviewed with a questionnaire tool about general health, work, ETS exposure, and lifestyle habits. Each was measured for height and weight and provided a single blood specimen. Blood serum was evaluated for reproductive hormones. DPC IMMULITE 1000® platform was used for the assays. Free Androgen Index (FAI) was derived (FAI= 3.467 x total testosterone / SHBG). Selected categorical characteristics (Table 1) and continuous characteristics (Table 2) of the study subjects are shown below.

Table 1 Categorical Characteristics	Smoker (n=125)	Non-smoker (n=67)	p-value
ETS exposure at home or work (%yes)	96.8	92.5	0.281
Alcohol drinker (%yes)	60.0	37.3	0.003
Pesticide Exposure in past 90 days (%yes)	19.2	20.9	0.778
Have Children (%yes)	97.3	100.0	0.552

	Smoker (n=125)		Non-smoker (n=67)		
Table 2 Continuous Characteristics	Mean	SD	Mean	SD	p-value
Age (years)	33.2	4.7	30.8	5.8	0.004
BMI (kg/m^2)	22.2	2.5	22.4	2.7	0.613
Nicotine metabolite (ng/ml)	331.0	156.1	25.1	69.3	<0.0001
FSH (mIU/ml)	4.47	2.3	3.9	1.6	0.240
LH (mIU/ml)	3.56	1.6	3.2	1.4	0.201
Free testosterone (ng/dl)	519.5	172.8	514.3	192.6	0.726
Androstenedione (ng/ml)	2.5	0.7	2.3	0.8	0.130
SHBG (nmol/l)	38.5	13.4	35.6	14.9	0.171

Results: Over 92% of all men reported environmental cigarette smoke exposure at work or home. After controlling for BMI, age, alcohol, and specific work exposure in multiple linear regression modeling, only androstenedione showed a statistically significant increase with increasing nicotine metabolite in the blood ($p<0.0001$).

Conclusion: Using a biomarker of exposure that allows modeling total cigarette exposure (smoking and indirect ETS), and controlling for important covariates, it was found that androstenedione levels increased in a dose-dependent fashion with cigarette smoke exposure in this cohort of healthy men. This confirms previous studies finding androstenedione changes associated with smoking but adds important new information related to total exposure including ETS after adjusting for important confounders. Further modeling will determine the amount of change attributed to each source of exposure.

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SPERM MIGRATION ASSAY AS MEASURE OF RECENTLY EJACULATED SPERM MOTILITY IN SPECIMENS SHIPPED OVERNIGHT

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The traditional assessment of male fecundity includes semen volume, sperm concentration, sperm morphology, sperm viability, and sperm motility. These assessments are conducted on a fresh (<1 hour) semen sample collected by masturbation. For a complete clinical analysis of an individual's fecundability, often several semen samples are assessed. Our laboratory and others have been successful in assessing reproductive health in population based studies using a single or few samples per man. These studies are usually conducted by bringing a field laboratory to a site and having fresh samples delivered to this laboratory.

Conducting a large study with men from many locations across time complicates the study design. Semen samples will have to be delivered to a central andrology laboratory by overnight mail. Special insulated mailing kits are available and pilots of such studies have been successful. Semen volume, sperm concentration, and sperm morphology can readily be conducted on sperm samples that are 24 hours old. The major loss in such a design is sperm motility.

This study was initiated to determine if an assessment of sperm motility in the fresh ejaculate could be preserved for evaluation in the specimen delivered the following day. The procedure needed to be simple and easy to do by study participants in the home without extensive training. Any chemicals used needed to be non-toxic because the procedure would be conducted in the home where family members could be accidentally contaminated.

Sperm migration assays were developed in the 1980s to study sperm motility patterns in cervical mucus. Early studies indicated that the distance traveled per unit time in mucus and other synthetic mucus like substances provided useful information about sperm motility. With the advent of computer assisted sperm analysis (CASA) systems providing sophisticated motility assessments these assays have been abandoned by most labs. It was hypothesized that the sperm migration assay straw could be placed in the freshly ejaculated semen by the study participant and then evaluated in the laboratory the next day.

Sperm migration straws were filled with hyaluronic acid (HA), plugged at one end, and sealed in a plastic bag with 100?l of the HA to ensure the HA would not evaporate from the straw end.

For these studies, CASA assessments of 17 fresh ejaculates were conducted. The migration straw was then placed in the semen sample that was still in the collection jar. The specimen was maintained in the shipping container with a freezer pack overnight to simulate overnight mail. Approximately 24 hours later the straw was evaluated. The distance traveled by the vanguard sperm was measured to the nearest mm.

Correlation coefficients (r) were calculated for the distance traveled and the various CASA measures of the fresh ejaculate. Significant correlation (pd"0.01) with r>0.60 calculated for mm traveled and percent motility; percent rapid sperm; and percent progressive sperm.

The migration straws are being implemented into a large epidemiological study of couples where sperm migration will be studied in relation to fertility. The study participants are shown a brief movie on how to place the straw into the specimen, receive written instructions, a migration straw sealed in a plastic bag and scissors.

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