BIOLOGY OF REPRODUCTION **61,** 651–656 (1999)

Inhalation of Mainstream and Sidestream Cigarette Smoke Retards Embryo Transport and Slows Muscle Contraction in Oviducts of Hamsters (*Mesocricetus auratus*)[1]

G. DiCarlantonio and P. Talbot[2]

Department of Biology, University of California, Riverside, California 92521

ABSTRACT

Prior experiments have shown that the functioning of hamster oviducts is impaired by in vitro exposure to cigarette smoke. To determine if cigarette smoke affects oviductal functioning in vivo, an inhalation experiment was done in which hamsters were exposed to doses of smoke similar to those received by human smokers. The effects of mainstream smoke (the bolus of smoke inhaled by active smokers) and sidestream smoke (the main component in environmental tobacco smoke) were compared. Transport of preimplantation embryos through the hamster oviduct was retarded in females inhaling doses of mainstream or sidestream smoke that produced serum cotinine levels within the range reported for women who actively or passively smoke during pregnancy. In addition, hamster oviductal muscle contraction rate decreased significantly during a single exposure of animals to either mainstream or sidestream smoke, and contraction rate failed to return to initial control values during a 25-min recovery period. Both preimplantation embryo transport and muscle contraction were more sensitive to sidestream than mainstream smoke. These data demonstrate that inhalation of doses of mainstream and sidestream cigarette similar to those received by active and passive human smokers adversely affects functioning of the oviduct and may explain the increased incidence of ectopic pregnancies reported in women who smoke.

INTRODUCTION

The public is generally aware of the harm done to the lungs and circulatory system by cigarette smoke exposure [1, 2]; however, the effects of smoke on other systems, including the reproductive organs, are not well established. Moreover, most studies involving cigarette smoke have been done using mainstream (MS) smoke, which is the bolus of smoke inhaled by active smokers. In contrast, sidestream (SS) smoke, the smoke produced at the burning end of a cigarette and the main component of environmental tobacco smoke, has received relatively little attention [3]. Numerous epidemiological studies have shown that women who smoke cigarettes experience reproductive problems including ectopic pregnancy [4], but the exact effects of smoke exposure on the reproductive organs are not well understood.

Various lines of evidence, most coming from in vitro studies, support the idea that the oviduct is a target of cigarette smoke or its components. The oviduct's normal function is to pick up the ovulated oocyte cumulus complexes, convey them to the ampulla for fertilization, and then transport the preimplantation embryos to the uterus at a precisely regulated rate for implantation [5, 6]. Factors that interfere with oocyte cumulus complex pick-up, fertilization, or preimplantation embryo transport can preclude establishment of a normal pregnancy. In vitro studies have shown that smoke solutions decrease oviductal ciliary beat frequency and oocyte cumulus complex pick-up rate in hamsters [7, 8]. The decrease in ciliary beat frequency has been attributed to cyanide, which is present in smoke solutions in sufficient concentration to retard ciliary beating [9]. The decrease in oocyte pick-up rate can occur independently of an effect on ciliary beating and appears to be related to altered adhesion between the cilia and oocyte cumulus complex [8, 10]. Prior in vivo studies have shown that exposure to smoke causes blebbing of the hamster oviductal epithelium [11] and alters contraction of oviductal muscle in humans [12] and rabbits [13]. Although these studies indicate that the oviduct is a target of cigarette smoke, no previous work has been done to determine if inhalation of cigarette smoke at doses typically experienced by human smokers alters the functioning of the oviduct in vivo.

The purposes of this study were to determine if inhalation of cigarette smoke by hamsters at doses equivalent to those received by human smokers during pregnancy affects in vivo functioning of the oviduct and to compare the effects of MS and SS smoke on oviductal functions. Specifically, we examined the effect of smoke inhalation on the rate of preimplantation embryo transport through the oviduct and on the rate of oviductal muscle contraction, which is important in regulating embryo transport [14, 15].

MATERIALS AND METHODS

Animals

Female golden hamsters (*Mesocricetus auratus*), purchased from Harlan Sprague Dawley (San Diego, CA), were maintained on a 14L:10D cycle (lights-on 0600–2000 h) in a room at 26°C as described previously [11]. Animals were given food and water ad libitum. The stage of the estrous cycle was determined by checking daily for a vaginal discharge that occurs on Day 1 of their cycle. Only females weighing 150–200 g and with at least two consecutive estrous cycles were used. To ensure correct identification of females used in the preimplantation embryo transport experiment, transponders (Biosonics, Seattle, WA) were implanted s.c. in the back of each female.

Males born in our colony were used for mating in the embryo transport experiment. In the evening of Day 4 of their cycle, females were placed in a cage containing a fertile male and examined the next morning for sperm in a vaginal smear. Mated females were housed in separate cages for the remainder of the experiment.

Media and Reagents

Earle's balanced salt solution (EBSS) was made fresh daily from a 10-strength stock solution of inorganic salts.

Accepted April 13, 1999.
Received December 29, 1998.
[1]Supported by a grant from the California Tobacco Related Disease Research Program (6RT–0039).
[2]Correspondence. FAX: 909 787 4286; e-mail: talbot@citrus.ucr.edu

Sodium bicarbonate (26.2 mM) and Hepes (25 mM) were added to a single-strength salt solution to produce EBSS-H. EBSS-H was then enriched with 0.5% bovine serum albumen (BSA, fraction-V, tissue culture tested; Sigma Chemical Company, St. Louis, MO) to produce EBSS-HA, the medium used for culture of oviducts in the muscle contraction experiment. The pH of EBSS-HA was adjusted to 7.4 at 37°C. Research cigarettes (2R1) were purchased from the University of Kentucky and used in all experiments. 2R1 cigarettes are standard-size unfiltered cigarettes that are often used in inhalation experiments to facilitate cross comparison of data among laboratories.

Effect of Smoke Exposure on Oviductal Embryo Transport

To test the effect of smoke inhalation on embryo transport in the oviduct, female hamsters were exposed to MS or SS cigarette smoke on a smoking machine that was operated as previously described [11], then the distribution of preimplantation embryos in the oviduct and uterus was examined. For smoke exposure, females were restrained in vinyl-coated wire mesh tubes equipped with plastic nose cones that attach to the smoke exposure block for nose-only breathing. To operate the smoking machine, a cigarette was inserted into a cam-driven puffer box. Puffs of smoke were drawn from a cigarette at 1-min intervals. Each puff (35 ml) was diluted by 75% and distributed to females at a controlled concentration and rate. A peristaltic pump distributed 50% of the smoke collected from the burning end of the cigarette to the SS-exposed females. Unused MS and SS smoke were exhausted to a fume hood using a rotary pump. The smoking machine was thoroughly cleaned at the end of each day.

At the onset of the experiment, females were placed in one of four groups: cage controls, sham controls, MS smokers, or SS smokers. Each group had seven females. Cage controls were not handled except for cycle checking and cage cleaning, while sham smokers were placed on a sham smoking machine and exposed to ambient air but not smoke for 60 min, the maximum time smokers were on the smoking machine. Human smoking is not permitted in the building where smoke exposures are done, so ambient air is smoke free. MS and SS smokers were placed on a smoking machine and exposed to MS or SS smoke. Three doses of smoke were tested. These included exposure to the smoke from 2, 4, or 6 2R1 research cigarettes per day. The total exposure per day was delivered in a morning (1000–1100 h) and afternoon (1400–1500 h) session for seven days a week. For example, the females receiving two cigarettes per day were exposed to one cigarette in the morning and one in the afternoon. Dose is presented as cigarette equivalents per day (CEPD). One CEPD is the amount of smoke inhaled by one female hamster from one cigarette. This term is used to indicate that the smoke actually inhaled is only a small fraction of the total smoke produced by each cigarette.

Smoke exposure began 14 days before mating and continued through the third day of pregnancy. Females were killed using CO_2 within an hour of their last smoke exposure during the afternoon on Day 3 of pregnancy. Day 3 of pregnancy was chosen for analysis of preimplantation embryo transport since the embryos have not yet implanted and are still recoverable by flushing at this time. In addition, in control females, embryos are distributed in both the uterus and oviduct on Day 3 of pregnancy and this would enable us to determine if smoking increased or decreased transport to the uterus. Ligatures were placed between the oviduct and uterus, and at the ends of these organs, to prevent loss or redistribution of embryos. The oviducts and uteri were flushed with EBSS, and the number of preimplantation embryos recovered from each oviduct or uterine horn was determined with a dissecting microscope. Blood samples were collected by cardiac puncture at the end of the experiment. Serum was harvested and stored at −70°C until analyzed for cotinine concentration.

Cotinine Assays

Cotinine is a relatively stable metabolite of nicotine, and its concentration in serum is useful in evaluating actual inhalation of smoke during exposure. Cotinine levels in serum were measured in cage controls, sham smokers, MS smokers, and SS smokers (seven females per group) by Dr. Helen Van Vunakis of Brandeis University, using an RIA with a minimal detection limit of 1 ng/ml [16]. The inter- and intraassay coefficients of variation for this assay were 10% and 7%, respectively.

Measurement of Muscle Contraction Rate

A method was developed to make video recordings of oviductal muscle contractions before, during, and after MS or SS smoke exposure. On Day 1 of estrus, females were anesthetized with an i.p. injection of Nembutal (Abbott Labs., Abbott Park, IL; 0.1–0.3 ml at 50 mg/ml), and one oviduct was drawn through a small incision on the female's dorsal surface and placed in a handmade culture dish containing EBSS-HA at 37°C. While the oviduct remained attached to the female's circulatory and nervous system, it was observed with a Wild M5A dissecting microscope, and video recordings of muscle contraction were made using an Hitachi (Tokyo, Japan) color video camera attached to the dissecting microscope. Females were kept on a warm stage at 37°C, and body temperature and respiration rate were monitored throughout the procedure. Preliminary experiments showed that oviductal muscle contraction rates stabilized by 20 min of incubation in EBSS-HA, and all subsequent evaluations were made after the 20-min stabilization period.

Operation and calibration of the smoking machine have been described above and previously in detail [11]. Smoke was produced from 2R1 tobacco research cigarettes smoked at a rate of one 35-ml puff per minute. After a 20-min stabilization period, the oviducts were videotaped during 10 min of ambient air exposure (control period), and the animal was then exposed for the first time to 30 puffs of MS smoke or to 30 min of continual SS smoke (exposure period). The MS and SS group each contained seven females. Videotape monitoring continued for a 25-min recovery period following smoke exposure. Videotapes were replayed and the frequency of muscle contraction in the ampulla of the oviduct was determined at 5-min intervals. Control females ($n = 3$) were placed on the smoking machine but breathed ambient air for the entire 55-min observation period.

Statistical Analyses

For the preimplantation embryo transport experiment, the percentage of the total embryos retained in the oviduct was computed for each female, and these percentages were combined to give means and standard deviations for each group. Data were analyzed by a one-factor analysis of var-

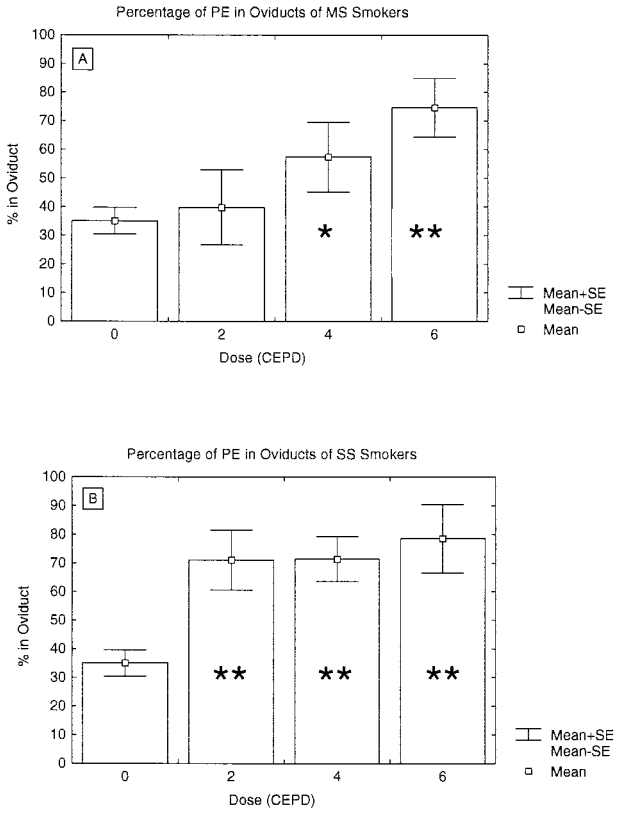

FIG. 1. Inhalation of MS and SS smoke inhibits preimplantation embryo (PE) transport. **A**) Percentage of embryos in oviducts of females exposed to 0, 2, 4, or 6 CEPD of MS smoke for 17 days total prior to evaluation. The zero dose group includes data from the cage and sham controls, which were not significantly different and are therefore combined. Significant inhibition of embryo transport occurred following exposure to 4 or 6 CEPD. **B**) Percentage of embryos in oviducts of females exposed to 0, 2, 4, or 6 CEPD of SS smoke for 17 days total before evaluation. The zero dose group includes cage and sham controls, which were not significantly different and are therefore combined. Data are plotted as means and SEM. n = 7. * $p < 0.05$, ** $p < 0.01$.

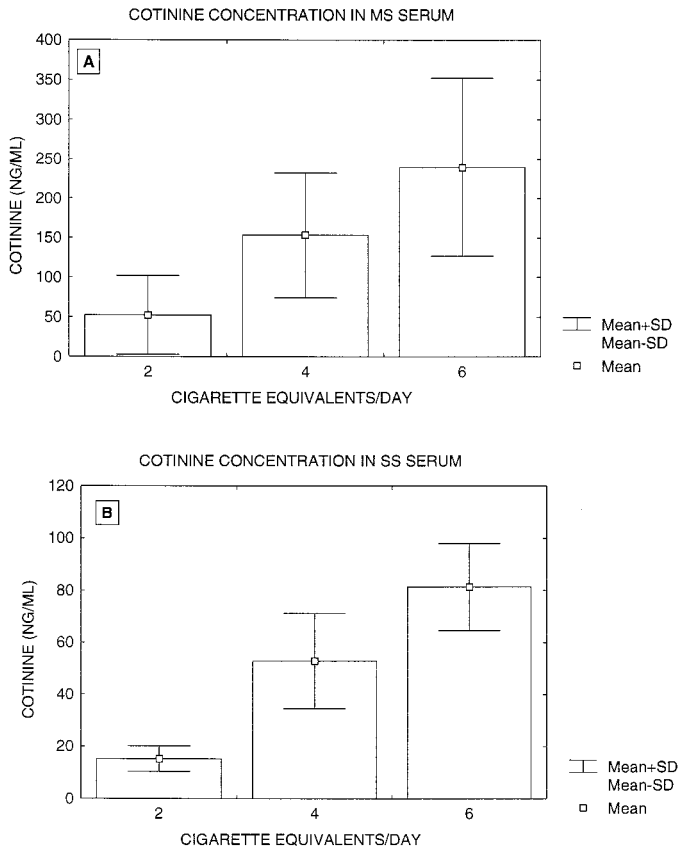

FIG. 2. Cotinine levels in serum of females exposed to 2, 4, or 6 CEPD for 17 days total. **A**) MS-Exposed females. **B**) SS-Exposed females. Cotinine was not detected in the serum of control females. Data are plotted as the mean and SEM for n = 7.

iance (ANOVA) followed by Dunnett's (embryo transport and muscle contraction) or Neuman-Keuls (cotinine assays) post hoc test. All data were examined to verify that they satisfied the assumptions of ANOVA. Analyses were done using either Statistica (StatSoft, Tulsa, OK) or Instat (GraphPad, San Diego, CA). Means were considered significantly different when $p < 0.05$.

RESULTS

Effect of Smoke Inhalation on Embryo Transport (Fig. 1)

To determine if the rate of oviductal preimplantation embryo transport is affected by smoke exposure, females were exposed to one of three doses of MS or SS cigarette smoke for 14 days prior to mating and for the first three days of pregnancy. The total number of embryos recovered from the oviducts and uteri was determined for each female and used to compute the mean total recovery of embryos for each group of females in the experiment. The groups (smokers, controls, and shams) with the lowest and highest means had 13.1 and 16.8 total embryos recovered, respectively. In a previous study, the mean number of corpora lutea on ovaries in our colony was computed for eight groups of females [11]. The lowest and highest means for corpora lutea/ovary were 14.7 and 16.4, respectively. Comparison of the means from both studies indicates that we recovered most or all embryos in the current study.

The percentage of embryos recovered from the oviducts of cage and sham controls were not significantly different from each other ($p > 0.05$). This indicates there was not a handling effect, and these data are shown combined in Figure 1 (dose = 0 CEPD). At the time of recovery, about 35% of the embryos were in the oviducts and 65% were in the uterine horns of the controls. In contrast, females exposed to MS smoke showed a dose-dependent inhibition of oviductal preimplantation embryo transport (Fig. 1A). At both 4 and 6 CEPD, MS smokers retained significantly more embryos in their oviducts than the control groups. At the highest MS dose used (6 CEPD), about 75% of the embryos were retained in the oviducts. SS smoke caused significant retention of embryos in oviducts at all doses tested (Fig. 1B). Even at 2 CEPD, 70% of the embryo were recovered from the oviducts as compared to 35% in the combined controls.

Cotinine Concentration in Smokers Serum (Fig. 2)

Cotinine, a metabolite of nicotine, is a widely used marker for cigarette smoke exposure [3]. To compare our doses in the preceding embryo transport experiment with exposures received by active and passive human smokers, serum cotinine levels were measured in animals at the completion of the transport study. In both the MS and SS groups, serum cotinine concentrations increased as the CEPD increased (Fig. 2, A and B). In MS smokers, the

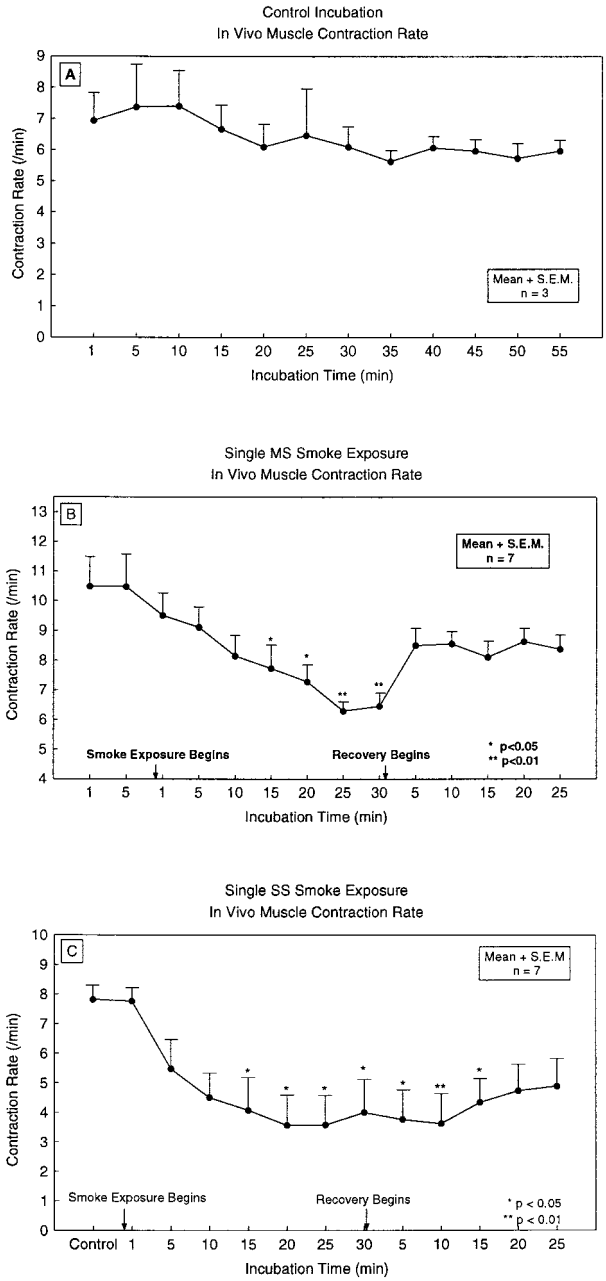

FIG. 3. A one-time exposure to MS or SS smoke inhibited the in vivo contraction rate of hamster oviductal smooth muscle. **A**) Contraction rate (contractions/min) for control females not exposed to smoke. Rate does not change significantly over 55 min of observation (n = 3). **B**) Contraction rate for females exposed to fresh air for 10 min, MS smoke for 30 min, then fresh air (recovery) for 25 min. Rate decreased significantly by 15 min of exposure and partially recovered after exposure (n = 7). **C**) Contraction rate for females exposed to fresh air for 10 min, SS smoke for 30 min, then fresh air (recovery) for 25 min. Rate decreased significantly by 15 min of exposure and remained depressed during most of the recovery period (n = 7). Data were obtained after a 20-min stabilization period. Data are plotted as means and SEM. * $p < 0.05$, ** $p < 0.01$.

mean serum cotinine concentration ranged from 50 to 240 ng/ml, while in SS smokers, the mean ranged from 14.8–82 ng/ml (Fig. 2, A and B). Significant differences in cotinine concentration were found between 2 and 4 CEPD in both the MS ($p = 0.016$) and SS ($p = 0.002$) group and between the 4 CEPD and 6 CEPD in the MS ($p = 0.049$) and SS ($p = 0.002$) group.

Effect of a Single Exposure to Cigarette Smoke on Oviductal Muscle Contraction (Fig. 3)

To determine if the observed effect of smoke on embryo transport rate could be due to an alteration in oviductal muscle contraction, we developed a technique to video tape contraction of oviducts that were drawn into a medium-filled dish (*Materials and Methods*). In all experiments, oviducts were allowed to stabilize for 20 min prior to measuring muscle contraction. To determine if muscle contraction rates remained constant while oviducts were exteriorized in culture medium, a control experiment was first performed in which oviductal contractions were observed over a 55-min incubation interval in EBSS-HA without any smoke exposure (Fig. 3A). Muscle contraction rates in these control females that breathed only ambient air for 55 min during video recording were measured at 5-min intervals and did not change significantly during the observation period (Fig. 3A). This result indicated that any changes observed during smoke exposure were not due to instability in contractions caused by the technique.

In the MS exposure experiment (Fig. 3B), females were first exposed to ambient air for 10 min (control period). The two consecutive readings taken at 1 and 5 min during the control period were not significantly different from each other (Fig. 3B), as would be expected from the preceding experiment in which rates did not change over 55 min of incubation in control medium. However, once smoke exposure began, there was a rapid decrease in contraction rate that became significantly different than the initial control values by 15 min of exposure (equivalent to 15 puffs of MS smoke). Contraction rate continued to decrease during the remainder of the MS exposure period. When smoke exposure stopped, contraction rate showed a rapid partial recovery, but rates did not return to initial control values during the 25-min recovery period.

In the SS exposure experiment, the two readings taken at 1 and 5 min during the control period were not significantly different from each other (Fig. 3C). However, once females began to inhale SS smoke, there was an immediate decrease in contraction rate, and by 15 min of smoke exposure, the rate was significantly less than the initial control values. The rate remained depressed throughout the smoke exposure period. Recovery from SS smoke exposure was slower than recovery from MS smoke, and the contraction rate remained significantly less than control rates during most of the recovery period. At the end of the recovery period, rates were still depressed when compared to initial control values.

DISCUSSION

Our study has shown that exposure to either MS or SS smoke prior to and during early pregnancy retards the rate of embryo transport through the oviduct. The doses of MS and SS smoke that retard embryo transport produced serum cotinine levels equivalent to those present in humans who smoke actively and passively, respectively. In addition, a one-time exposure to either MS or SS cigarette smoke decreased the contraction rate of the oviductal smooth muscle. In both the embryo transport and muscle contraction experiments, SS smoke appeared to exhibit greater toxicity than MS smoke.

Embryos are normally transported through the oviduct at a precisely regulated rate so that they arrive in the uterus at the proper time for implantation [15, 17]. In humans, embryos that travel through the oviduct too fast (for ex-

ample due to exposure to ergonovine maleate, which stimulates oviductal muscle contraction) pass through the uterus prematurely and fail to implant [15, 18]. A similar outcome occurs in rats given the weak estrogenic pesticide methoxychlor, which produces preimplantation embryonic loss due to acceleration of embryo transport through the reproductive tract [19]. Conversely, if preimplantation embryo transport is retarded in humans, embryos fail to arrive in the uterus at the blastocyst stage and may implant ectopically in the oviduct, as reviewed by Harper [15]. Numerous studies have shown that women who smoke have an increased incidence of ectopic pregnancy [4, 20–22], which adds $86 million dollars to American health care costs annually [23]. In one recent study, the risk factor for ectopic pregnancy was shown to increase significantly with increases in the number of cigarettes smoked per day [24]. Our results show that embryo transport through the oviduct is retarded in smoke-exposed females, an observation which could explain the increase in ectopic pregnancy in human smokers.

The dose of smoke received by females in this study was determined by measuring serum cotinine at the end of the transport experiment so that comparisons could be made to human smokers. Cotinine levels in humans have been measured in serum, urine, and saliva in several studies [3, 25–28]. In a recent study, serum cotinine in pregnant women who were active smokers (exposed to MS smoke) ranged from 0–569 ng/ml [25]. The authors of this study broke this range into tertiles of 0–78, 79–165, and 166–569 ng/ml, with each tertile indicating an increase in the number of cigarettes smoked per day. Although the exact number of cigarettes smoked per tertile was not given, the tertile ranges probably reflect levels of cotinine in light, moderate, and heavy smokers. In our study, all MS-exposed females had serum cotinine levels within the above range (0–569 ng/ml) reported for active human smokers. Our lowest MS dose (2 CEPD), which did not significantly retard embryo transport, produced a mean serum cotinine level of 52.5 ng/ml which is in the light smoker tertile of Eskenaszi et al. [25]. At the two higher doses, transport rate was retarded, and mean serum cotinine levels were in the moderate smoker tertile (150 ng/ml for 4 CEPD) and heavy smoker tertile (240 ng/ml for 6 CEPD) [25]. Since hamsters were only exposed to smoke for a total of 17 days, longer exposures may have produced significantly more retardation at lower doses.

Serum cotinine levels in pregnant women who are passive smokers (exposed to sidestream smoke) are considerably lower than those of active smokers, and in humans ranged from 0–15 ng/ml [26]. Interestingly, embryo transport rate in our study was inhibited significantly in all SS exposure groups suggesting that the effects of SS smoke exposure are more detrimental to embryo transport than MS smoke exposure. The cotinine levels in the females exposed to 2 CEPD of SS smoke were 14.8 ng/ml, which is at the high end of the range (0–15 ng/ml) reported for passive human smokers [3, 26]. Further increases in SS dose did not increase retardation of embryo transport, indicating that maximal inhibition is achieved at a dose that falls within the range received by passive human smokers.

An important point revealed by our inhalation study is that SS smoke appeared more toxic than MS smoke with respect to both embryo transport and decreased frequency of oviductal muscle contraction. This suggests that SS smoke either contains a toxicant(s) not present in MS smoke or that the toxicant(s) that produces these effects is present in higher concentration in SS than in MS smoke. The latter idea is more probable since MS and SS smoke have similar overall compositions, but many toxicants are present in 2–100 times higher concentrations in SS than in MS smoke [3]. Our experiments tested the effects of MS and SS smoke separately. Active human smokers are exposed to both MS and SS smoke simultaneously, and therefore our results with MS smoke probably underestimate the effects of active smoking on the oviduct.

Cigarette smoke could act on the oviduct at several levels to delay movement of the gamete from the ovary to the ampulla or embryo from the ampulla to the uterus. Movement of the oocyte and preimplantation embryo into and through the oviduct is accomplished by ciliary beating and muscle contraction [15]. Previous studies have shown that ciliary beat frequency is inhibited in vitro by smoke solutions [7] and that cyanide is present in smoke solutions in sufficiently high concentration to account for this inhibition [9]. The rate at which the oocyte cumulus complex is picked up by the infundibulum of the oviduct is irreversibly inhibited in vitro by smoke solutions [8], and this inhibition can occur independently of the effect on ciliary beat frequency [8]. Others have shown that in addition to ciliary beating, proper adhesion between the matrix and cilia is required for successful pick-up [29, 30]. Our preliminary observations suggest that inhibition of oocyte pickup by smoke solutions is due to faulty adhesion between the oocyte cumulus complex matrix and the tips of the cilia during smoke treatment. The current study furthers our earlier findings in showing that inhalation of smoke affects contraction of the oviductal smooth muscle cells in a manner that could retard embryo transport rate. The rate of muscle contraction in the oviduct is inhibited by a one-time exposure to either MS or SS smoke. Approximately 15 puffs of smoke were required to inhibit contraction rates significantly, and rates did not fully recover to control values following exposure. The effect of SS smoke appeared to be longer-lived than that of MS smoke. Taken together, our previous studies support the idea that cigarette smoke exposure alters oocyte pick-up rate by decreasing ciliary beat frequency and possibly by altering adhesion of the oocyte cumulus complex to the cilia. The present in vivo study extends our earlier work by showing that smoke inhalation retards preimplantation embryo transport through the oviduct and inhibits contraction of oviductal smooth muscle. Thus cigarette smoke appears to act on both the ciliary and muscular transport mechanisms of the oviduct in a manner that could affect pregnancy.

No previous studies have directly evaluated the effect of smoke inhalation on oviductal contractions. The frequency of human oviductal muscle contraction appeared not to change significantly during smoking when monitored using the Rubin test [12]. However, the Rubin test, which monitors rhythmic pressure changes in insufflated oviducts, provides indirect evidence on contraction and is invasive, which may affect muscle contractions [12]. Moreover, the source of contractions (oviduct, uterus, or both) made with the Rubin test have been debated [12]. An advantage of our video system is that it allows the oviductal muscle to be directly observed during an experiment without subjecting the oviduct to implanted balloons or electrodes. Our direct observations on hamster oviducts showed a significant decrease in contraction frequency during and after inhalation of both MS and SS smoke. However, our method does not permit quantitative measurements of strength of contraction, so while contraction rates decreased, we can not determine quantitatively from our tapes whether strength of

contraction and tone were affected by smoke exposure. In rabbits, there is a short burst of electrical activity in oviductal muscle following smoke inhalation [13], which we did not observe. However, the sustained decrease in peristaltic contractions that we did observe during smoking is more likely to influence embryo transport rate.

The components in smoke that produce alterations in embryo transport and muscle contraction rates are not known. Cotinine is unlikely to be an active factor as relatively low levels of cotinine (14.8 ng/ml at CEPD) were present in SS-exposed females that exhibited maximal inhibition of embryo transport, while much higher levels of cotinine (52.5 ng/ml at 2 CEPD) were present in MS-exposed females that showed no effect on transport. Yoshinaga et al. [31] have reported that pharmacological doses of nicotine retard embryo transport rates in rats, but it was not determined in their study if normal doses of nicotine produce similar effects. Neri and Eckerling [32] have shown that nicotine affects oviductal muscle contraction during the first but not later half of the Rhesus monkey menstrual cycle. They found that nicotine injections produced a short-lived increase in tone and amplitude followed by a prolonged period of complete inhibition of tubal activity. While this latter response could be interpreted to be similar to our observations, our data suggest that nicotine was either not involved or was not the only agent affecting muscle contractions. In our experiments, the SS smokers were affected maximally at all three doses, but the nicotine exposure in the SS group, as estimated from cotinine values, was much less than in the MS groups, including the 2 CEPD MS group which showed no effect. Thus, SS smoke and probably MS smoke must contain component(s) other than nicotine that affected muscle contraction rate in our experiments.

In conclusion, we have found that exposure of female hamsters to doses of MS and SS smoke that are similar to those experienced by active and passive human smokers retarded embryo transport within the oviduct and decreased the contraction rate of oviductal smooth muscle. These data support the idea that the increased incidence of ectopic pregnancy seen in women smokers is due to a direct effect of smoke on the oviduct, including the musculature, and that both active and passive smoking could be causative. The effects of SS smoke on reproduction are not well characterized. Our data clearly link exposure to SS smoke, at doses within the range of human exposure, to oviductal complications that could affect human reproduction and fertility.

ACKNOWLEDGMENTS

We are grateful to Ray Talbot, Maria Rubio, Karen Riveles, and Christine Gieseke for their helpful suggestions on the manuscript.

REFERENCES

1. DHHS US. The Health Consequences of Smoking: Cancer. A Report of the Surgeon General. Washingtion, DC: Public Health Service; 1982.
2. DHHS US. Public Health Service Report: The Health Consequences of Smoking: Cardiovascular Disease. A Report of the Sugeon General. Washington, DC: Public Health Service; 1983.
3. EPA. EPA Report/600/6–90/006F: Respiratory Health Effects of Passive Smoking: Lung Cancer and Other Disorders. Washington, DC: Public Health Service; 1992.
4. Stillman RJ, Rosenberg MJ, Sachs BP. Smoking and reproduction. Fertil Steril 1986; 46:545–566.
5. Hunter RHF. Modulation of gamete and embryonic microenvironments by oviduct glycoproteins. Mol Reprod Dev 1994; 39:176–81.
6. Puikkinen MO. Oviductal function is critical for very early human life. Ann Med 1995; 27:307–310.
7. Knoll M, Shaoulian R, Magers T, Talbot P. Ciliary beat frequency of hamster oviducts is decreased in vitro by exposure to solutions of mainstream and sidestream cigarette smoke. Biol Reprod 1995; 53: 29–37.
8. Knoll M, Talbot P. Cigarette smoke inhibits oocyte cumulus complex pick-up by the oviduct independent of ciliary beat frequency. Reprod Toxicol 1998; 12:57–68.
9. Talbot P, DiCarlantonio G, Knoll M. Identification of cigarette smoke components that alter functioning of hamster (*Mesocricetus auratus*) oviducts in vitro. Biol Reprod 1998; 58:1047–1053.
10. Talbot P, Gieseke C, Knoll M. Oocyte pick-up by the mammalian oviduct. Mol Biol Cell 1999; 10:5–8.
11. Magers T, Talbot P, DiCarlantonio G. Cigarette smoke inhalation affects the reproductive system of female hamsters. Reprod Toxicol 1995; 9:513–525.
12. Neri A, Eckerling B. Influence of smoking and adrenaline (epinephrine) on the uterotubal insufflation test (Rubin test). Fertil Steril 1969; 20:818–828.
13. Ruckebusch Y. Relationship between the electrical activity of the oviduct and the uterus of the rabbit in vivo. J Reprod Fertil 1975; 45: 73–82.
14. Blandau R, Verdugo P. An overview of gamete transport—comparative aspects. In: Harper MJK, Pauerstein CJ, Adams CE, Coutinho EM, Paton DM (eds.), Ovum Transport and Fertility Regulation. Copenhagen: Scriptor; 1976: 138–146.
15. Harper JK. Gamete and zygote transport. In: Knobil E, Neill JD (eds.), The Physiology of Reproduction. Second ed. New York: Raven Press; 1994: 123–185.
16. Langone JJ, Gjika B, Van Vunakis H. Nicotine and its metabolites. Radioimmunoassay for nicotine and cotinine. Biochemistry 1973; 12: 5025–5030.
17. Adams CE. Consequences of accelerated ovum transport, including a re-evaluation of Estes operation. J Reprod Fertil 1979; 55:239–246.
18. Coutinho AM, Maia H, Nascimento L. The response of the human fallopian tube to ergonovine and methyl-ergonivine in vivo. Am J Obstet Gyncecol 1976; 126:48–54.
19. Cummins AM, Perreault SD. Methoxychlor accelerates embryo transport through the rat reproductive tract. Toxicol Appl Pharmacol 1990; 102:110–116.
20. Phipps W, Cramer D, Schiff I, Belisle S, Stillman R. The association between smoking and female infertility as influenced by cause of infertility. Steril Fertil 1987; 48:377–382.
21. Chow W-H, Daling J, Weiss N. Maternal cigarette smoking and tubal pregnancy. Obstet Gynecol 1988; 71:167–170.
22. Tuomivaara L, Ronnberg L. Ectopic pregnancy and infertility following treatment of infertile couples: a follow-up of 929 cases. Eur J Obstet Gynecol Reprod Biol 1991; 42:33–38.
23. Adams KE. Costs of maternal conditions attributable to smoking during pregnancy. Am J Prev Med 1998; 15:212–219.
24. Saraiya M, Berg CJ, Kendrick JS, Strauss LT, Atrach HK, Ahn YW. Cigarette smoking as a risk factor for ectopic pregnancy. Am J Obstet Gynecol 1998; 178:493–498.
25. Eskenaszi B, Prehn AW, Christianson RE. Passive and active maternal smoking as measured by serum cotinine: the effect on birthweight. Am J Public Health 1995; 85:395–398.
26. Ford RPK, Schuter PJ, Tappini DM. Changes in cotinine levels during pregnancy. Aust N Z J Obstet Gynaecol 1998; 38:50–55.
27. Rebagliato M, Bolumar F, Florey CV. Variations in cotinine levels in smokers during and after pregnancy. Am J Obstet Gynecol 1998; 178: 568–571.
28. Foundas M, Hawkrigg NC, Smith S, Devadason SG, Le Souef P. Urinary cotinine levels in early pregnancy. Aust N Z J Obstet Gynaecol 1997; 37:383–386.
29. Mahi-Brown CA, Yanagimachi R. Parameters influencing ovum pick-up by oviductal fimbria in the golden hamster. Gamete Res 1983; 8: 1–10.
30. Norwood JT, Anderson RGW. Evidence that adhesive sites on the tips of oviduct cilia membranes are required for ovum pickup in situ. Biol Reprod 1980; 23:788–791.
31. Yoshinaga K, Rice C, Krenn J, Pilot CL. Effects of nicotine on early pregnancy in the rat. Biol Reprod 1979; 20:294–303.
32. Neri A, Marcus SL. Effect of nicotine on the motility of the oviducts in the Rhesus monkey: a preliminary report. J Reprod Fertil 1972; 31: 91–95.