CIGARETTE SMOKE HINIBITS OOCYTE CUMULUS COMPLEX
PICK-UP BY THE OVITUD IN VITRO INDEPENDENT OF CILIARY
BEAT FREQUENCY

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Abstract — The purpose of this study was to quantify the effects of acute exposure to mainstream (MS) and sidestream (SS) smoke solutions on oocyte cumulus complex pick-up rate in explants of hamster oviducts using a newly developed in vitro assay. Experiments were performed in handmade perfusion chambers using infundibula from hamster oviducts and oocyte cumulus complexes harvested from mature ovarian follicles. Oocyte cumulus complex pick-up rate was measured by placing a stained oocyte cumulus complex at the base of the infundibulum and recording the length of time needed for the complex to traverse a defined path to the ostium. Addition of either whole MS or SS smoke solutions to the perfusion chamber caused a dose dependent decrease in oocyte cumulus complex pick-up rate. Unexpectedly, upon washout of smoke solutions with control medium, oocyte cumulus complex pick-up rate continued to decline. The gas phase of MS smoke is more inhibitory than the particulate phase, while SS gas and particulate phases inhibit oocyte cumulus complex pick-up rate at equivalent doses. Ciliary beat frequency and oocyte cumulus complex pick-up rate were measured using the same infundibular explants to determine if smoke solutions decrease oocyte cumulus complex pick-up rate by inhibiting ciliary beat frequency. Ciliary beat frequency decreased in MS smoke solutions and recovered either partially or completely after washout of the smoke solutions. SS smoke solutions either produced no change in ciliary beat frequency or stimulated ciliary beat frequency. Oocyte cumulus complex pick-up rate decreased in both MS and SS smoke solutions and further declined during washout when ciliary beat frequency was equivalent to or higher than controls. These data show that oocyte cumulus complex pick-up rate and ciliary beat frequency can be uncoupled and that smoke solutions inhibit oocyte cumulus complex pick-up rate by affecting factors in addition to ciliary beat frequency. Possible reasons for the smoke induced decrease in oocyte cumulus complex pick-up rate are discussed. These results may explain the increased incidence of tubal infertility and ectopic pregnancy observed in women who smoke. © 1998 Elsevier Science Inc.

Key Words: cigarette smoke; oviduct; oocyte; fertility; gamete transport; ectopic pregnancy; cilia.

INTRODUCTION

Women who smoke cigarettes have an increased incidence of tubal infertility (1,2) and ectopic pregnancy (3,4), although the causal mechanisms linking smoking with these reproductive problems are largely unknown. Using the hamster as a model, we have been addressing the possibility that smoke components carried by the circulatory system to the oviduct adversely affect oviductal functions.

The hamster oviduct is lined by ciliated and secretory cells, while the outer surface of the infundibulum is covered mainly by ciliated cells (5). After ovulation, the ciliated cells covering the infundibulum function in oocyte cumulus complex pick-up, which results in transfer of the oocyte cumulus complex over the surface of the infundibulum, through the ostium, and into the ampulla where fertilization occurs (6). Fertilized oocytes and preimplantation embryos are transported through the remainder of the oviduct by ciliary activity and perhaps by smooth muscle contraction (7,8). The secretory cells of the oviductal epithelium synthesize and secrete glycoproteins, which are thought to provide a suitable environment for fertilization and preimplantation development, although the detailed roles of these secretions are just beginning to be understood (9).

The oviduct is a target of cigarette smoke and its components (3). Nicotine alters tubal motility in Rhesus monkeys (10) and decreases tubal blood flow to the oviduct in rats (11). Hamster oviducts respond to inhaled doses of MS or SS smoke within the range received by humans who smoke actively or passively (12). Exposure of hamsters to realistic doses of cigarette smoke causes a small but significant increase in the ratio of secretory/ciliated cells lining the infundibulum of the hamster oviduct (12). Moreover, a single acute in vitro exposure of the infundibular cilia to either MS or SS smoke...
solutions causes a rapid decline in ciliary beat frequency which is generally reversible upon washout of the smoke solutions (13). While these experiments on ciliary beat frequency suggest that smoke can affect oocyte cumulus complex pick-up, they do not directly test the functioning of the cilia in oocyte cumulus complex pick-up during exposure to smoke solutions.

To directly assess proper functioning of the oviductal cilia, an in vitro assay was recently developed to measure oocyte cumulus complex pick-up rate quantitatively (14). The assay is performed in a perfusion chamber that allows oocyte cumulus complex pick-up rate to be measured sequentially in control, experimental, and washout media. This assay has the advantage of allowing both oocyte cumulus complex pick-up rate and ciliary beat frequency to be compared in the same infundibulum. The pick-up rate assay has been used previously to evaluate the effects of temperature and viscosity of culture media on hamster oocyte cumulus complex pick-up rate (14).

The purpose of the present investigation was to use the oocyte cumulus complex pick-up rate assay to quantify the effects of acute exposure to MS and SS smoke solutions on pick-up rate of hamster infundibula and to determine if the effects of smoke components are reversible. Whole, gas, and particulate phase smoke solutions were tested at various concentrations. Experiments were designed to determine if the effects of smoke solutions on oocyte cumulus complex pick-up rate and ciliary beat frequency are correlated in the same preparation.

MATERIALS AND METHODS

Animals

Female golden hamsters (Mesocricetus auratus), purchased from Harlan Spague Dawley (San Diego, CA), were maintained on a 14:10 light:dark cycle (6 AM to 8 PM = light) in a room at 26°C and food was administered ad libitum as described previously (12). Hamsters were cycled daily by checking for a vaginal discharge which occurs on Day 1 of their estrous cycle. Day 3 hamsters were used for all experiments.

Media

Earle’s Balanced Salt Solution (EBSS) was made fresh daily from a 10× stock solution. To a 1.0× salt solution, 26.2 mm sodium bicarbonate and 25 mm HEPES were added, generating EBSS-H. EBSS-H was enriched with 0.5% BSA (EBSS-HA) and used for dissection and incubation. It was also used as the control medium in all experiments. The pH of all media was adjusted to 7.4 with NaOH and remained stable throughout each set of experiments. A 0.002% methylene blue solution was made in EBSS-HA and was used to stain oocyte cumulus complexes.

Preparation of smoke solutions

Smoke solutions were prepared as described previously (13) using an University of Kentucky analytical smoking machine (Figures 1 and 2). Smoke solutions were made in 10 mL of 1.0× EBSS-H, supplemented with 0.5% BSA, and then the pH was adjusted to 7.4.

Mainstream whole smoke solutions (MSW) were made from 60 puffs of MS smoke pushed through EBSS-H. The gas phase (MSG) and the particulate phase (MSP) were separated by placing a Cambridge filter in the line between the puffer box and the container with EBSS-H. MSG smoke solutions were made with 60 puffs of smoke that passed through the Cambridge filter. MSP smoke solutions were made by extracting the water soluble components from a used MS filter with 10 mL of EBSS-H. Sidestream whole smoke solutions (SSW) were made by collecting the smoke that was produced at the burning end of the cigarette during 30 MS puffs and pushing it through 10 mL of EBSS-H. SSG smoke solutions were produced by placing a Cambridge filter in the line carrying smoke to the container of EBSS-H. SSG smoke solutions were prepared by extracting water soluble components from a used SS filter with 10 mL of EBSS-H.
Fig. 2. Production of SS smoke solutions for use on infundibular explants. This set up requires both MS and SS peristaltic pumps and the puffer box. The puffer box and MS peristaltic pump generate MS smoke as described in Figure 1. While the MS puffs are made, the SS peristaltic pump continually collects the SS smoke generated from the burning end of a cigarette. The SS smoke is pushed by a peristaltic pump into a flask containing 10 mL of EBSS-H. Once the smoke is perfused through the EBSS-H, it is taken to an exhaust tube and then a fume hood.

**Oocyte cumulus complex collection and infundibulum preparation**

To collect oocyte cumulus complexes, hamsters were injected with 25 I.U. of hCG on the evening of Day 3 of their estrous cycle. On the day of the experiment, approximately 12 h after injection of hCG, hamsters were sacrificed using CO₂, and oviducts and ovaries were removed. During dissection, the ovary was removed from the oviduct, and the dissecting medium (EBSS-HA) was changed frequently to minimize contamination from blood. Once the ovary was separated from other tissues, expanded follicles were poked with a dissecting needle, and oocyte cumulus complexes were transferred to a fresh Petri dish.

The infundibulum was cut from the oviduct leaving part of the ampulla attached to function as a handle. The infundibulum/ampulla preparation was then placed into a perfusion chamber (15) containing EBSS-HA (control medium), and the infundibulum was kept stationary by inserting the ampullary portion of the preparation into a holding pipette. Paraffin oil was added to the surface of the medium to prevent evaporation.

**Oocyte cumulus complex pick-up rate and ciliary beat frequency measurements**

Perfusion chambers containing infundibular preparations were viewed with either a Nikon SMZ-10 or Wild M5A stereoscopic microscope containing ocular mi-

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measurements were made. For each phase of an experiment (control, exposure, or washout), six pick-up rate measurements were made along the same path using a different oocyte cumulus complex for each phase. The six replicate measurements were used to compute means for each phase of a particular experiment. The data in Figures 4 through 7 are based on 4 or 5 separate experiments, each done on different day using different infundibula.

For dose experiments, only oocyte cumulus complex pick-up rate data were collected. Stock smoke solutions were prepared daily. The stock (1.0×) smoke solutions were then serially diluted to produce 0.5× and 0.1× concentrations of smoke solution. Experiments began by taking control oocyte cumulus complex pick-up rate measurements as described previously. Infundibula were first exposed to the lowest concentration of diluted smoke solution, and oocyte pick-up rate was measured. Progressively higher doses of smoke solution were perfused into the chamber, and oocyte cumulus complex pick-up rate was measured after the addition of each dose.

Statistics

Statistical analyses on dose response data were performed using the DOS Instat (GraphPad) program. For each type of smoke solution, means for oocyte cumulus complex pick-up rate obtained at each dose and the initial control readings were compared by one factor ANOVA. When significant (P < 0.05), the experimental means were compared to the initial control mean (EBSS-HA) by Dunnett’s post hoc test. For the remaining experiments, mean exposure and washout pick-up rates and ciliary beat frequencies were compared to initial control values using Students t-test (STATISTICA for Windows (StatSoft)). Differences were considered significant for P < 0.05 (*) or P < 0.01 (**)..

RESULTS

Representative oocyte cumulus complex pick-up rate experiments

To determine if 1.0× MS or SS smoke solutions can alter the rate of oocyte cumulus complex pick-up, infundibula were placed in perfusion chambers and exposed sequentially to control medium (EBSS-HA), a smoke solution (exposure phase), and control medium (washout phase). Oocyte cumulus complex pick-up rate was measured over a 10 min interval in each solution (Figure 3).

Single representative experiments (from 10 experiments total) showing the effects of exposure to MSW and SSW smoke solutions are presented in Figure 3.

Some variation exists among control oocyte cumulus complex pick-up rate measurements on different infundibula (range for all infundibula including those not shown was 40 to 80 mm/s). However, for a particular infundibulum, oocyte cumulus complex pick-up rate was relatively constant in control medium over the 10 min period. In both MSW and SSW smoke solutions, oocyte cumulus complex pick-up rate decreased immediately after addition of smoke solution and continued to decrease over the 10-min exposure period. Unexpectedly, following washout of smoke solutions with control medium, oocyte cumulus complex pick-up rate remained depressed. Similar data were obtained with the particulate and gas phase of both MS and SS smoke (not shown).
Fig. 4. Effects of exposure of various dilutions of MSW, MSG, and MSP smoke solution on oocyte cumulus complex pick-up rate (OPR). Pick-up rate is plotted in μm/s as a function of MS smoke solution concentration. Solid bars represent the means of all oocyte cumulus complex pick-up rate measurements taken over a 10 min treatment period. Hatched bars represent the means of oocyte cumulus complex pick-up rate measurements taken at just the end of the 10 min period for control, exposure, and washout intervals. Standard deviation is indicated on all bars. Each bar is based is the mean of 4 or 5 separate experiments. ANOVA and Dunnett's post-hoc test were performed for each type of MS smoke solution. *p < 0.05, **p < 0.01.

Dose experiments

To determine what doses of MS and SS smoke solutions affect oocyte cumulus complex pick-up rate, dilutions (0.1×, 0.5×, and 1.0×) of smoke solutions were tested on infundibula (Figures 4 and 5). Control pick-up rate readings were taken for 10 min, then the lowest concentration of smoke solution was added to the chamber, and after a 5 min incubation period, oocyte cumulus complex pick-up rate readings were taken for 10 min. The next two higher concentrations of smoke solution were then sequentially perfused into the chamber, and oocyte cumulus complex pick-up rate measurements were taken after each.

The whole and the gas and particulate phases of MS and SS smoke solutions inhibited oocyte cumulus complex pick-up rate in a dose dependent manner (Figures 4 and 5). Both the overall means of all pick-up rates collected during the 10 min incubation interval and the means of rates at just the 10 min point (final oocyte cumulus complex pick-up rate) were inhibited signifi-
Fig. 5. Effects of exposure of various dilutions of SSW, SSG, and SSP smoke solution on oocyte cumulus complex pick-up rate (OPR). Pick-up rate is plotted as a function of SS smoke solution concentration. Solid bars represent the means of all pick-up rate measurements taken over a 10 min treatment period. Hatched bars represent the means of the oocyte cumulus complex pick-up rate measurements taken just at the end of the 10 min period for control, exposure, and washout intervals. Standard deviations are indicated on all bars. Each bar is based on 4 or 5 separate experiments. ANOVA and Dunnett's post-hoc test were performed for infundibula in each type of MS smoke solution. *P < 0.05, **P < 0.01.

Significantly at some doses when compared to control rates. The mean oocyte cumulus complex pick-up rate at the 10 min time point was always less than the overall mean pick-up rate taken from the entire 10 min time period for MS smoke, indicating that pick-up rate continued to decrease after initial addition of MS smoke solution. The gas phase of MS smoke inhibited pick-up rate at higher dilutions than MSP smoke. MSW and MSG smoke solutions were more inhibitory at high dilutions than any of the SS smoke solutions.

Oocyte cumulus complex pick-up rate and ciliary beat frequency experiments

The beating of infundibular cilia was also inhibited by exposure to smoke solutions (13). Experiments were performed to determine if the decrease in oocyte cumulus complex pick-up rate can be attributed to the inhibition of ciliary beat frequency when infundibula are exposed to smoke solutions (Figures 6 and 7). Infundibula were placed in perfusion chambers, and oocyte cumulus complex pick-up rate then ciliary beat fre-
frequency were measured on the same preparation. The chambers were perfused with a 1.0× concentration of smoke solution, and pick-up rate and ciliary beat frequency were again measured. The chambers were then flushed with control medium to determine if recovery of oocyte cumulus complex pick-up rate and/or ciliary beat frequency occurred.

Each panel in Figure 6 shows the overall mean oocyte cumulus complex pick-up rate (mm/s) and ciliary beat frequency (Hz) during control incubation (C), exposure to MS smoke solution (E), and washout with control medium (W). Oocyte cumulus complex pick-up rate decreased during exposure to MS smoke solutions with MSW smoke solution producing the greatest effect. Pick-up rate decreased further during the washout phase and was significantly less than the control in all cases. Ciliary beat frequency was inhibited during exposure to the MSW and MSP smoke solutions, but was not affected by exposure to MSG solutions. During washout, ciliary beat frequency recovered in the MSW and MSP groups, returning to initial control values for MSW. The decrease in oocyte cumulus complex pick-up rate observed during exposure to MSW and MSP smoke solutions could have been due to a decrease in ciliary beat frequency. However, the additional decrease in pick-up rate observed during washout occurred while ciliary beat frequency was increasing.

Figure 7 shows the overall mean pick-up rate (μm/s) and ciliary beat frequency (Hz) for control (C), SS smoke exposure (E), and washout (W) incubations. Both SSW and SSG smoke solutions caused a significant inhibition of oocyte cumulus complex pick-up rate, which continued to decrease during washout. Ciliary beat frequency was not inhibited by any SS smoke solution, and in SSW and SSP, a significant increase in ciliary beat frequency was observed during the exposure phase. In the washout phase, ciliary beat frequency was equivalent to controls, except for SSP which remained elevated. These experiments demonstrate that while oocyte cumulus complex pick-up rate decreases significantly during SSW and SSG exposure and during the corresponding washout phases, ciliary beat frequency either remains equivalent to control values (SSG) or is stimulated by SS smoke solutions (SSW).

**DISCUSSION**

Oocyte cumulus complex pick-up by the oviduct is essential for reproduction in mammals (6,16). In this study, a newly developed functional assay was used experimentally to show that both MS and SS smoke solutions inhibit oocyte cumulus complex pick-up rate in a dose dependent manner. Inhibition occurs independently of the effect of smoke solutions on ciliary beat frequency. Moreover, decreases in oocyte cumulus complex pick-up rate became more pronounced during washout, indicating that inhibition of oocyte cumulus complex pick-up rate by smoke solutions is not rapidly reversible.

Oocyte cumulus complex pick-up rate is more sensitive to the gas than the particulate phase of MS and SS smoke solutions. Inhibition of pick-up rate by gas phase components could be important in vivo. The gas phase of both MS and SS smoke may gain access to the circulatory system and be carried to organs such as the oviduct. The gas phase of MS smoke is the major form of smoke inhaled by most active smokers who now, in general, use filtered cigarettes. The gas phase of SS smoke, the major component of environmental tobacco smoke (ETS) (17,18), may likewise enter the circulatory system and potentially affect oocyte cumulus complex pick-up rate in passive smokers.

Nicotine is a commonly used biomarker in smoke related studies (17). Serum concentrations of nicotine in human smokers are about 40 ng/mL at steady state but may be as high as 72 ng/mL in chronic smokers (19–22). Tissue levels of nicotine are generally higher (23–25), and in some organs, including the ovary and uterus, nicotine concentrations are 10 times the levels found in blood (26,27). Based on previously obtained data (13), 1.0× MSG phase smoke solutions contain nicotine (700 ng/mL) at the high end of the range found in tissues of various species, while 0.1× solutions of MSG phase smoke have nicotine (70 ng/mL) well within tissue levels. Both the 1.0× and 0.1× concentrations of MSG smoke solutions inhibited oocyte cumulus complex pick-up rate. This does not indicate that nicotine per se affected oocyte cumulus complex pick-up rate, but that its concentration in the MSG phase smoke solutions was within the range found in human smokers. Thus, whatever component(s) in MSG smoke inhibits oocyte cumulus complex pick-up rate, it is probably present in smokers at a level high enough to cause similar effects in vivo. Nicotine levels in the serum of passive smokers are about 5% the levels in MS smokers (17). Although tissue levels of nicotine in passive smokers are not well documented, if they follow the trend for active smokers, tissue levels could be as high as 35 ng/mL. All SS smoke solutions had nicotine levels higher than 35 ng/mL. Our data address a single acute exposure to SS smoke constituents; chronic exposure to SSG phase smoke could reveal additional effects at lower concentrations.

MS and SS smoke solutions inhibited hamster oviductal ciliary beat frequency in vitro and beat frequency recovered during washout (13). In the present study, similar effects were found for MS smoke solutions; however, SS smoke solutions produced either no effect (SSG) on ciliary beat frequency or actually stimulated ciliary beat frequency (SSW, SSP). It is probable...
Fig. 6. Effect of the 1× concentration of MSW, MSG, and MSP smoke solutions on oocyte cumulus complex pick-up rate (OPR) and ciliary beat frequency (CBF) of infundibular preparations. Pick-up rate and ciliary beat frequency are plotted as a function of incubation medium: C = control medium (EBSS-HA), E = MS smoke solution (MSW, MSG, or MSP), and W = washout medium (EBSS-HA). Solid bars show the mean pick-up rate and hatched bars show the mean ciliary beat frequency. Standard deviations are given above each bar. Each bar is based on 4 or 5 separate experiments. Student's t-test was used to determine if a significant difference exits between control and exposure groups and between control and washout groups. *P < 0.05, **P < 0.01.
Fig. 7. Effect of the 1.0× concentration of SSW, SSG, and SSP smoke solutions on oocyte cumulus complex pick-up rate (OPR) and ciliary beat frequency (CBF) of infundibular preparations. Pick-up rate and ciliary beat frequency are plotted as a function of incubation medium: C = control medium (EBSS-HA), E = SS smoke solution (SSW, SSG, or SSP), and W = washout medium (EBSS-HA). Solid bars show the mean pick-up rate and hatched bars show the mean ciliary beat frequency measurements. Standard deviation of the mean is given above each bar. Each bar is based on 4 or 5 separate experiments. Student's t-test was used to determine if a significant difference exits between control and exposure groups and between control and washout groups. *P < 0.05, **P < 0.01.
that the difference between this result and our previous study is due to the inclusion of 0.5% BSA in the smoke solutions used in the current study. BSA, which has protective functions in both serum and in vitro culture media (27), can scavenge oxygen and carbon free radicals (28,29) and binds various types of metals, including cadmium, mercury, and copper (30). The BSA in smoke solutions in the current study may have bound a toxicant(s) that is inhibitory to ciliary beat frequency and thereby unmasked the stimulatory effect of other factors in the SSW smoke solutions. Although we have not yet characterized the stimulatory factor(s), nicotine enhances ciliary beat frequency in the respiratory epithelium (31). The observation that ciliary beat frequency can be manipulated by the presence or absence of BSA provides a useful means to control the direction of the effect of smoke solutions on ciliary beat frequency.

Because ciliary beating is the main mechanism for oocyte cumulus complex pick-up by the infundibulum (8,32) and because ciliary beat frequency is inhibited in vitro by smoke solutions (13), we originally hypothesized that pick-up rate would be inhibited by smoke solutions and return to normal during washout. While oocyte cumulus complex pick-up rate did decrease upon exposure to smoke solution, it unexpectedly did not recover to control values during washout, even though ciliary beat frequency did recover. Moreover, in SSW smoke solutions, ciliary beat frequency was significantly stimulated during exposure, while pick-up rate simultaneously decreased significantly. These two observations show that oocyte cumulus complex pick-up rate can be uncoupled from ciliary beat frequency.

The uncoupling of oocyte cumulus complex pick-up rate and ciliary beat frequency by smoke solutions could be explained by several factors. First, metachrony may have been disturbed by the smoke solutions; if ciliary power strokes are not in unison in one direction, the oocyte cumulus complex would not move toward the ostium. This explanation is unlikely since we did not observe any evidence for loss in metachrony when performing the ciliary beat frequency assay, and our ciliary beat frequency data would have been difficult to subject to Fourier transformation and to interpret had metachrony been lost. It is also possible that ciliostasis occurred in regions of the infundibulum and precluded efficient movement of the oocyte cumulus complex toward the ostium. However, during measurement of ciliary beat frequency, four different regions of each infundibulum were analyzed simultaneously, and since no region ever showed ciliostasis, it is unlikely that this possibly explains the uncoupling of oocyte cumulus complex pick-up rate and ciliary beat frequency.

The most probable explanation for uncoupling of oocyte cumulus complex pick-up rate and ciliary beat frequency is that smoke solutions disrupt adhesion between the tips of the cilia and the oocyte cumulus complex. Oviductal cilia have a negatively charged glycoprotein crown (33–35) that binds polycations, such as cationic ferritin and poly-L-lysine (34,36). In rabbits, polycationic ions inhibit pick-up of cumulus masses in vitro and in vivo, while cilia beat normally (36,37). Removal of the cumulus cells and matrix of hamster oocyte cumulus complexes results in inefficient pick-up of oocytes by infundibula (38). In addition, humans with endometriosis have a factor in their peritoneal fluid that blocks oocyte cumulus complex pick-up in an in vitro hamster assay, probably by binding to the cilia (39,40).

Adhesion can be directly visualized with a dissecting microscope because the matrix between cumulus cells is stretched and released during oocyte cumulus complex pick-up (41,42). In our experiments, when oocyte cumulus complex pick-up rate decreased, the cilia had little or no ability to stretch the matrix of the oocyte cumulus complex, suggesting that normal adhesion between the cilia and matrix of the oocyte cumulus complex had been disturbed by smoke solutions. As in the experiments using polycationic ferritin and poly-L-lysine (36), cilia exposed to smoke solutions did not recover the ability to pick up oocyte cumulus complexes even after washout, indicating a tight, perhaps, permanent binding of a smoke component(s).

In the respiratory tract, cilia are responsible for mucociliary clearance and are also targets of cigarette smoke; however, the precise effect of smoke on respiratory tract cilia is unclear (43,44). Some studies have provided evidence that mucociliary transport is reduced in smokers (44). Respiratory cilia possess a crown-shaped glycolcalyx similar to the glyccalcalyx of oviductal cilia (45), and only the tips of the cilia interact with the layer of mucus that coats the respiratory epithelia (46). Similar adhesive defects may occur in airway cilia that are directly exposed to smoke components in both active and passive smokers. Our results show that monitoring only ciliary beat frequency does not provide accurate functional data with respect to pick-up of the oocyte cumulus complex. If a similar situation exists in the respiratory system (i.e., if mucociliary transport rate and ciliary beat frequency do not necessarily correlate), some of the discrepancies in the literature dealing with respiratory cilia may be understood. For example, in vivo stimulation of ciliary beat frequency by nicotine (31) would not necessarily be in conflict with the reported decrease in mucociliary transport in human smokers (47–49) if adhesion of the mucus to the ciliary crown is impaired by other particulate or gaseous factors in smoke.

In conclusion, the effects of cigarette smoke on the oviductal cilia are more complex than previously real-
Cigarette smoke inhibits oocyte cumulus complex pick-up rate • M. Knoll and P. Talbot

ized. Both MS and SS smoke solutions inhibit oocyte cumulus complex pick-up rate in a dose dependent manner, and this inhibition is not rapidly reversible. In some cases, pick-up rate inhibition occurs in smoke solutions thought to contain doses similar to those found in vivo in human smokers. Inhibition of oocyte cumulus complex pick-up rate by smoke solutions is not due to a sustained depression of ciliary beat frequency, and in some cases oocyte cumulus complex pick-up rate decreased while ciliary beat frequency was stimulated. These observations are consistent with the idea that cigarette smoke solutions inhibit oocyte cumulus complex pick-up rate by blocking proper adhesion between oocyte cumulus complexes and the ciliary crown. Inhibition of oocyte cumulus complex pick-up by the infundibulum could be a factor contributing to tubal infertility and ectopic pregnancy in women who smoke.

Acknowledgments — We are grateful to Dr. Leah Haimo who provided access to image processing equipment in her laboratory, and to the Department of Botany and Plant Sciences which provided the inverted microscope used to measure ciliary beat frequency. We also thank Dr. Leah Haimo and Dr. Manuela Martins-Green for their helpful suggestions on the manuscript.

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