Effect of nicotine-containing and nicotine-free e-cigarette vapor condensate on development of 4-day-cultured mouse embryos

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Background

The prevalence of vaping has risen precipitously since 2017, especially among young adults. Infertility currently affects 8-12% of couples worldwide[1]. From 2015 to 2019 the prevalence of vaping increased among high-schoolers from 24.0% to 32.7%[2]. From 2017 to 2020 e-cigarette use in the general population increased from 1.5% to 2.3% nationwide[3]. We investigated whether the fluids used in e-cigarettes exhibit a toxic effect on embryos.

Objective

1. Determine whether exposure to e-cigarette fluid distillate significantly disrupts mouse embryonic development.

2. Determine whether any observed disruption is correlated to nicotine content.

Methods

- Superovulated mice following published procedure[4].
- Distilled *Island Man Ice* flavored e-cigarette fluid following published procedure using atomizer set to 40 Watts.
- Repeated distillation process for fluids of 50mg/mL, 25mg/mL, or 0mg/mL nicotine.
- Prepared 96-well incubation plates with M-16 culture medium and concentrations of vaping fluid distillate ranging from 0.05% to 0.2%, with a row of 12 control wells of M-16 only on each plate.
- Dissected out embryos and added them to the 96-well incubation plates.
- Incubated embryos for 84 hours, recording developmental stage (figure 1) every 12 hours.
- Assigned values of -1 to 8 for developmental stages (-1=degenerated, 0-8= 2-cell to hatched blastula).
- Averaged the values of each row.
- Paired treatment row values to control row values from the same plate at the same time point and performed 1-tailed paired t-tests.
- Paired treatment rows of different nicotine concentrations at the same point in time and performed 1-tailed paired t-tests.
- Assessed for significance at $p < 0.05$

Meeting

Data presented by Student Doctor Thomas Hall at the annual American College of Osteopathic Internists (ACOI), October 11-14, 2023, Tampa, Florida, USA

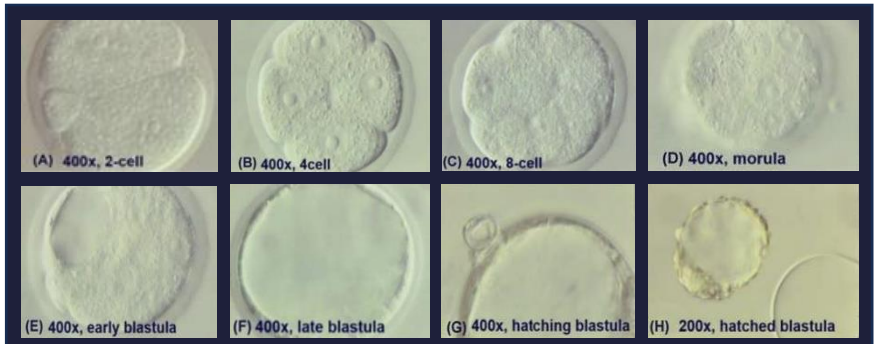

Figure 1 (A)-(H): Normal mouse embryo micrographs from 2-cell to hatched blastula. From the laboratory of *RJ Swanson*©

Results

P-values for treatment groups versus controls were as follows:

0.1% IMI (Island Man Ice flavored) fluid (50 mg/mL nicotine): 0.0048.

0.05% IMI fluid (50 mg/mL nicotine): 0.0096.

0.2% IMI fluid (25 mg/mL nicotine): 0.0018.

0.1% IMI fluid (25 mg/mL nicotine): 0.0006.

0.05% IMI fluid (25 mg/mL nicotine): 0.0127.

0.1% IMI fluid (0 mg/mL nicotine): 0.0114.

P-values of equal volumes of vaping fluid with different nicotine concentrations were as follows:

0.1% IMI fluid (25mg/mL nicotine) vs. 0.1% IMI fluid (50mg/mL nicotine): 0.1612

0.05% IMI fluid (25mg/mL nicotine) vs. 0.05% IMI fluid (50mg/mL nicotine): 0.3798

P-values of nicotine-free vs. nicotine-containing fluids were as follows:

0.1% IMI fluid (0mg/mL nicotine) vs. 0.1% IMI fluid (25mg/mL nicotine): 0.0300

0.1% IMI fluid (0mg/mL nicotine) vs. 0.1% IMI fluid (50mg/mL nicotine): 0.0709

Discussion

- All vaping fluid distillates tested, including those without nicotine, significantly disrupted embryo development.
- The significance of this disruption was lowest in the two groups with the lowest nicotine concentration.
- Compared to nicotine-free fluids, nicotine-containing fluids resulted in worse development, but the difference was not consistently significant.
- Doubling nicotine content of fluids did not significantly increase disruption of mouse embryo development.
- Therefore, it is likely that both nicotine and one or more other ingredients contribute to disruption of embryo development.

Conclusions

- Vaping fluid distillate appears to be toxic to mouse embryo development at concentrations of 0.05%-0.2%.
- This effect is not fully attributable to either the presence or concentration of nicotine.

Future Work

- Analysis of vaping fluids for component chemicals including carrier fluids, flavorings, flavor enhancers, and breakdown products.
- Testing of isolated compounds for toxicity.
- Pre-fertilization testing of vaping fluids on eggs and sperm.
- Testing using an in-vivo mouse model.

References and Acknowledgments

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