The impact of a 10% Ethanol Diet on Fertility of Aged Drosophila

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Introduction

Drosophila encounter ethanol in their natural environment because they prefer to lay their eggs on ethanol-containing food. Females knew certain amounts will be detrimental to them and their progeny, and when given the option of 0%, 5%, and 10% ethanol food, the females chose the 5% which was beneficial to them (Azanchi et al., 2013). A link between embryonic ethanol exposure and slowed development has already been established in *Drosophila*. When *Drosophila* embryos were exposed to greater than 5% ethanol, they showed a decrease in survival and development (Devineni and Heberlein, 2013). Pre-pregnancy ethanol exposure has been studied in mice and has shown similar effects. When exposed to ethanol prior to pregnancy, there was a decrease in liter size, embryo number, and pregnancy rate (Lee et al., 2020). However, maternal pre-exposure in *Drosophila* has not been studied. Here, we hypothesize that maternal pre-mating exposure to a 10% ethanol diet will decrease egg production and percentage of hatched eggs.

Methods

All parts of the experiment were conducted between 12:00 to 13:00 and occurred over a period of 4 weeks and 4 days.

Collection

30 male and 30 female virgin flies were collected under a Leica S7 E Stereo Microscope. The males were stored across 3 vials with 10 flies in each vial. The females were stored across 6 vials with 5 flies in each vial.

Food preparation

A 500 mL corn meal-agar base bagged food was adjusted to contain 10% ethanol in the final product. The contents were split into two beakers, 225 mL each. 22.5 mL of 100% ethanol was added to one beaker, being the experimental, and 22.5 mL of deionized water was added to the other, being the control food. 5 mL of food was dispensed into each vial.

Ethanol Exposure

1 week prior to mating, half of the virgin females were placed on the 10% food. The females remained on the food for an entire week and then removed once mating began.

Mating cages and egg lay plates

2 males and 2 females were set into a mating cage with a grape agar plate. There were 7 pairs per group. The pairs remained in the cages for 48 hours and the plate was changed after 24 hours. The second 24-hour plate was counted for eggs.

Larvae Quantification

Each plate was extensively examined for larvae using a thin paint brush one at a time. The number of larvae was recorded, and the plates were placed in a plastic bag for proper disposal.

Statistical analysis

Microsoft Excel was used to generate graphs and run statistical analysis. The percentage of eggs hatched was a ratio of the number of larvae to the original number of eggs. The averages were represented by a bar graph. Then, each group was subject to a one-tailed t-Test.

Results

Overall, the results of the experiment showed a statistically significant decrease in the number of eggs laid by females that had been exposed to a 10% ethanol diet the week before mating. On average, the control group laid 53 eggs per egg-lay plate, while the experimental group laid 33 (Figure 1 & Table 1). This demonstrates a negative effect on fertility. The number of eggs hatched, which served as a measure of development, was also negatively impacted by maternal exposure to ethanol. 66% of the eggs in the control group hatched, whereas only 55% of those in the experimental group hatched (Figure 2 & Table 2). The one-tailed p-value was less than 0.05, confirming statistical significance (Table 2).

Discusssion

Results indicate that both fertility and embryo development were negatively impacted from maternal pre-exposure to a 10% ethanol diet. One explanation for these results is that ethanol affects motor function (Scholz et al., 2000), which may have affected mating behavior. However, it's possible that the observed results were exaggerated due to sex depravation the females experienced while on the ethanol diet, which has been linked to increased ethanol consumption (Shohat-Ophir et al., 2012). In their natural habitat, this would not be a variable and could lead to less significant effects on fertility and development.

Effects on development and fertility after maternal pre-exposure were observed as opposed to examining the direct effects of ethanol on embryo development. There has been limited research done looking at the maternal pre-exposure in Drosophila, however a previous mouse model had observed detrimental effects (Lee et al., 2020). Drosophila has been used as a model for maternal ethanol exposure effects and has shown similarities to humans (McClure et al., 2011). By studying Drosophila, mechanisms, and effects of fetal alcohol syndrome in humans can be further understood.

References

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Appendix

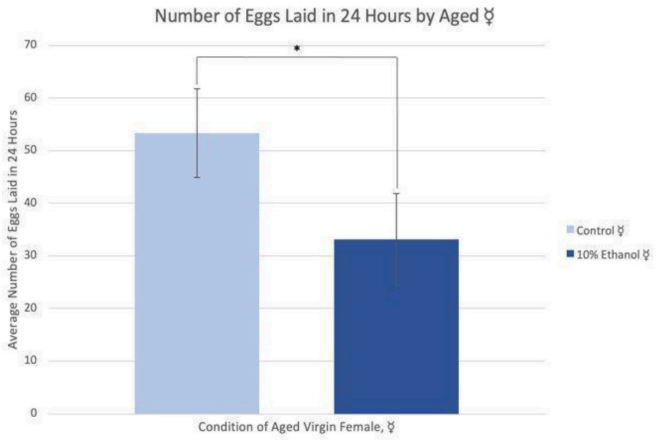

Figure 1. The number of eggs decreased after exposure to a 10% ethanol diet. The experimental group produced a significantly lower number of eggs. The number of eggs was determined by counting each plate. The groups were compared by the average number of eggs laid in 24-hours.

	Control ☿	10% Ethanol ☿
Mean	53.29	33.14
P(T<=t) one-tail	0.000440	

Table 1. The number of eggs between the control and 10% ethanol virgin females was statistically significant. The average number of eggs for the control group was 53.29 and the experimental group was 33.14. The p-value from the one-tail T-test was 0.000440.

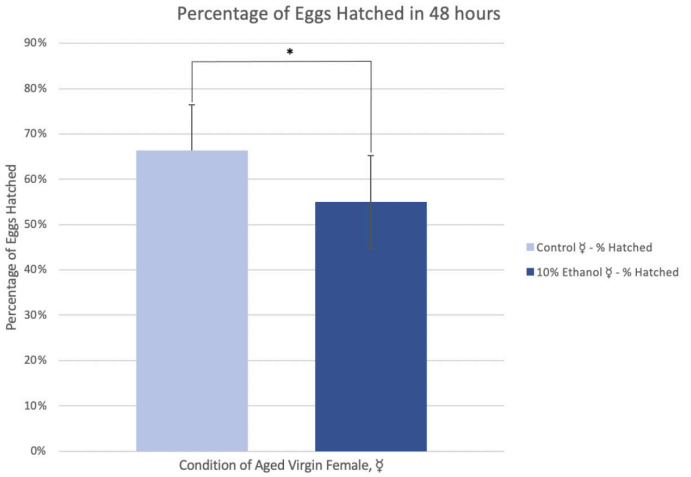

Figure 2. The percentage of eggs hatched in 48 hours was lower in the 10% ethanol group. rhe control group had a higher average compared to the experimental group. The percentage was determined by a ratio of the number of larvae by the original number of eggs.

	Control ☿ - % Hatched	10% Ethanol ☿ - % Hatched
Mean	66.30%	55.01%
P(T<=t) one-tail	0.0302	

Table 2. The percentage of eggs hatched between the control and experimental group was statistically significant. The average eggs hatched for the control group was 66.30% and the experimental group was 55.01%. The difference was significant as confirmed by the p-value from the one-tail T-test, p = 0.0302.