

Caenorhabditis elegans Under the Influence: Testing the Effects of Caffeine and Ethanol on C. elegans Reproduction

Lauren K. Welsh

Biology, The Bay School, San Francisco, California, United States, 94129.

KEYWORDS. Reproduction, endocrinology, *C. elegans*, infertility

BRIEF. Timed exposures to caffeine and ethanol in *Caenorhabditis elegans* reveal that both reduce reproductive success; chronic caffeine exposure nearly eliminated egg production and viable progeny, while brief peri-ovulatory ethanol exposure increased egg production but sharply reduced embryo viability.

ABSTRACT. Caffeine and ethanol are widespread exposures known to affect reproduction, the developmental timing of exposure and its effects on early embryogenesis remain poorly understood. This study used *Caenorhabditis elegans* (*C. elegans*), a rapid and genetically tractable model with conserved reproductive pathways, to examine how chronic and peri-ovulatory exposure to these substances impacts fecundity and embryo viability. Worms were reared under five conditions: control, chronic caffeine, chronic ethanol, caffeine during ovulation only, and ethanol during ovulation only. After correcting an initial ethanol over-concentration to 5%, single eggs or L4 hermaphrodites were transferred per plate, and adults were moved every three days to prevent generational overlap. Eggs and viable offspring were counted at 72 hours using microscopy. Control worms produced the most viable offspring, while all exposure groups showed impaired reproduction. Chronic caffeine nearly eliminated egg and offspring production, and chronic ethanol sharply reduced viable progeny. Ovulation-only ethanol produced the most eggs but very low viability, suggesting disruption after fertilization or during early embryogenesis. These results indicate that caffeine and ethanol reduce reproductive success in *C. elegans*, primarily through decreased embryo viability rather than reduced fertilization. The distinct effects of ovulation-only ethanol highlight the sensitivity of early developmental stages to transient exposures, offering insight into mechanisms relevant to assisted reproduction and embryo development outside the maternal environment.

INTRODUCTION.

Every complex organism begins as a single fertilized cell [1]. A human's journey from a single cell to a fully developed multicellular organism involves a series of steps in embryonic development. These steps include fertilization, cleavage, gastrulation, and organogenesis [2]. Each stage is sensitive to environmental conditions, such as exposure to substances like ethanol or caffeine.

Often in research, scientists will use model organisms to study biological processes. This experiment used *Caenorhabditis elegans* (*C. elegans*), tiny transparent roundworms that are widely used as model organisms in developmental biology. *C. elegans* is a commonly used organism for studying various biological processes, specifically reproduction, genetics, and toxicology. It was also the first multicellular organism to have its genome sequenced, which helped scientists determine the many genes and pathways *C. elegans* share with humans [3].

C. elegans has a limited life span of approximately 3.5 days from egg to adult. Even so, *C. elegans* produces around 300 offspring, which allows for fast observation of reproductive differences between *C. elegans* affected by different external factors. Hermaphrodites are the most prominent sex of *C. elegans* and can be self-fertilized, allowing for precise reproductive tracking [3]. On top of this, *C. elegans* can grow on a Petri dish and require easily accessible bacteria, such as *E. coli*, as food. They also do not require intense environmental changes, making them easy to maintain [3].

Humans and *C. elegans* share significant similarities in their reproductive systems, making *C. elegans* an ideal model for teaching humans about their own bodies. *C. elegans*' reproductive systems share several parts with humans'; both species have ovaries, sperm, eggs, and uteri. These parts are controlled by both organisms' vital processes, like egg production, sperm development, ovulation, and meiosis [4].

This experiment investigated the effect of caffeine and ethanol on *C. elegans* reproduction. Although previous studies have documented reproductive and developmental effects of these substances in mammals, fewer studies have examined how the timing of exposure influences early embryogenesis in invertebrate model systems. Caffeine is America's most popular drug [5], and over 60% of adults in America consume alcohol regularly [6]. Despite their widespread use, both caffeine and alcohol have documented negative effects on reproduction. Caffeine effects may involve delayed conception, increased risk of miscarriage, and fetal growth restrictions [7]. Alcohol has been proven to cause fetal alcohol spectrum disorders [8]. While prior studies have documented reproductive effects of caffeine and ethanol, few have examined how the timing of exposure influences early embryogenesis in invertebrate model systems. This study addresses that gap by comparing chronic and peri-ovulatory exposure in *C. elegans*, providing insight into how timing of exposure may influence fertilization success and embryo viability.

The guiding question of this experiment was: How does exposure to caffeine or ethanol affect reproduction in *C. elegans*? The experimental research examines the effects of continuous (chronic) exposure to substances throughout life and limited exposure during ovulation. The findings of this project may help shed light on the effects of caffeine and ethanol during early stages of embryonic development and advance public understanding of the risks associated with caffeine and alcohol exposure during pregnancy.

IVF involves fertilizing an egg outside the body and nurturing it through the earliest phases of embryonic development before implantation [9]. For a pregnancy to progress successfully, fertilization, cleavage, gastrulation, and organogenesis must all occur properly. Because IVF removes the protective maternal environment during early development, embryos may be especially vulnerable to environmental exposures. This experiment allows for a more hands-on way to explore the environment required for successful fertilization and embryo development. It is hypothesized that *C. elegans* exposed to caffeine or ethanol during their lifespan or ovulation will show reduced reproductive success.

MATERIALS AND METHODS.

To collect the data, *C. elegans* were kept at room temperature in a semi-controlled laboratory environment. The experiment aimed to assess reproductive rates of *C. elegans* under five different conditions: control (no exposure), chronic caffeine exposure, chronic ethanol exposure, caffeine exposure during ovulation only, and ethanol exposure during ovulation only.

Nematode growth media (NGM) agar plates were prepared using standard laboratory materials. NGM media (RPI CAS 9002-18-0) was used at 23 g/L and dissolved in deionized water and autoclaved. For each 100 mL of NGM media, 80 μ L 1M calcium chloride, 80 μ L 1M magnesium sulfate, and 2 mL 1M potassium phosphate buffer was added. For the substance treatments, different solutions of caffeine and ethanol were prepared to be mixed into separate agar solutions. For the NGM solution with caffeine, 0.194 g of caffeine powder was dissolved into 100 mL of NGM agar to form a 10% solution. For the ethanol solution, 5 mL of pure ethanol was mixed into 95 mL of the NGM media, creating a 5% ethanol solution.

The NGM agar solutions were poured into Petri dishes, and after solidifying, the plates were labeled according to their treatment group. Group A was labeled the control and had no substance exposure. Group B was labeled the chronic caffeine plate and had chronic exposure. Group C was labeled chronic ethanol, as it had chronic ethanol exposure. Group D was labeled caffeine exposure during ovulation, as *C. elegans* were only exposed to the substance during ovulation. Group E was labeled ethanol ovulation as it only had exposure to ethanol during ovulation. After labeling, approximately 100 μ L of *E. coli* was pipetted on each agar plate solution and let sit for 24 hours before transferring *C. elegans* onto each plate.

To transfer *C. elegans*, a 32g kanthal A-1 wire pick was used, which was sterilized with a 95% ethanol solution between uses. The process of transferring worms involved gently tapping the kanthal wire onto the *E. coli* lawn of the NGM agar to make the worm stick before moving the worm onto an agar plate. For groups A, B, and C, one *C. elegans* egg was placed onto each plate; for groups D and E, one L4-stage worm was transferred onto each plate. Adults in groups A, B, and C were transferred to fresh NGM agar plates without substance exposure every three days to prevent generational overlap.

Data Collection Seventy-two hours after the *C. elegans* were transferred onto the NGM agar plates, data were recorded. This data included the number of eggs present and the number of viable offspring. To collect this information, grids were drawn on the back of the plate to ensure accurate data collection and no repetition of counting. Counts were done visually under a microscope.

The instruments used in this experiment include a dissecting light microscope for worm identification, worm transfer, and counting eggs and offspring. Micropipettes were used for precise application of substances and bacterial lawns. A kanthal wire pick, sterilized by ethanol, was used for worm transfer.

After data collection, all plates were disposed of in a biohazard waste container. Tools like kanthal wire picks were sterilized, and pipette tips were disposed of following safety protocols.

RESULTS.

The number of eggs produced varied substantially across treatment conditions (Figure 1). Control plates produced an average of 35 eggs across replicates. In contrast, chronic caffeine exposure resulted in extremely low egg production, with an average of 9 eggs per animal. Chronic ethanol exposure produced a large number of eggs (~140) across replicates, suggesting that ethanol exposure did not suppress egg laying.

The ovulation-only caffeine treatment also resulted in little to no egg production (0 eggs). While ovulation-only ethanol exposure produced the highest egg counts observed in the experiment, the number of *C. elegans* eggs in this treatment ranged from 230 to 59, showing strong variability.

These results suggest that caffeine exposure suppresses egg production, whereas ethanol exposure does not prevent egg laying and may be associated with increased egg deposition.

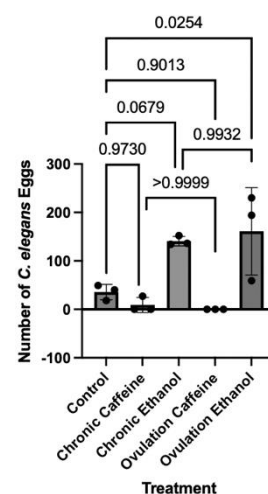


Figure 1. Egg production per treatment group. Individual points represent biological replicates (plates), bars represent the mean, and error bars represent standard deviation.

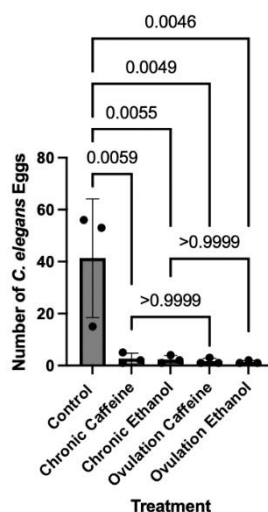


Figure 2. Number of viable *C. elegans* offspring produced per treatment group. Individual points represent plates, bars represent the mean, and error bars represent standard deviation.

Despite high egg counts in some treatment groups, the number of viable offspring differed dramatically among treatments (Figure 2). Control plates produced the highest number of offspring overall, with multiple plates producing more than 50 viable worms.

In contrast, all treatment groups exposed to caffeine or ethanol showed dramatically reduced offspring production, with average living offspring between 1-3 animals. Chronic caffeine exposure resulted in almost no viable offspring. Chronic ethanol exposure also produced very few offspring despite high egg counts. Similarly, both ovulation-only exposure groups produced extremely low offspring numbers, even when egg production was high in the ovulation-ethanol treatment.

These results suggest that exposure to caffeine or ethanol substantially reduces reproductive success in *C. elegans*.

Embryo viability, defined as the successful transformation from egg to viable offspring, varied between treatment groups (Figure 3). Embryo viability was calculated by dividing the number of viable offspring by the number of eggs. Control plates exhibited the highest embryo viability, with an average of 152%, which suggests uncounted eggs in figure 1. In contrast, all caffeine and ethanol exposure groups

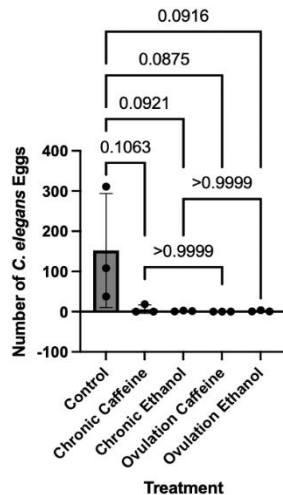


Figure 3. Embryo viability across treatment groups. Individual points represent plates, bars represent the mean, and error bars represent standard deviation.

showed extremely low embryo viability, with values of 0-2% viability; many plates produced eggs that failed to develop into viable offspring. This pattern was especially pronounced in ethanol-treated groups, where egg production remained high, but successful embryo development remained extremely low.

This pattern suggests that ethanol exposure may allow egg production but disrupts embryonic development, leading to reduced reproductive success.

DISCUSSION.

The results of this experiment support the original hypothesis that exposure to both caffeine and ethanol negatively impacts the reproductive success of *C. elegans*. Across all groups, the control worms produced the highest number of viable offspring. In contrast, worms exposed to caffeine or ethanol, especially under chronic conditions, produced significantly fewer eggs and almost no viable offspring. Although egg counts were markedly higher in ethanol-exposed groups, particularly in ovulation-only ethanol plates (Figure 1), offspring production remained extremely low (Figure 2). This indicates that ethanol exposure disrupted embryonic development and did not enhance reproductive success. Egg counts for the control group may be higher than originally counted; there were often more viable offspring than original egg counts on control plates. Due to this discrepancy, the error in embryo viability is much higher, leading to reduced statistical significance. We are not concerned with this discrepancy because even if some eggs were missed in the treatment groups, the embryo viability would still be low, if not lower.

Ethanol is recognized as a teratogen, and its detrimental effects on embryonic development have been well documented in both human and animal studies¹⁰. The extreme reduction in viable offspring observed in ethanol-exposed worms is consistent with findings on fetal alcohol spectrum disorders and ethanol's disruption of gastrulation and organogenesis¹¹. Similarly, caffeine has been associated with delayed conception and increased miscarriage risk⁷. Caffeine and ethanol may impair reproduction through distinct biological mechanisms. Caffeine is

known to interfere with adenosine signaling and cellular energy balance, which can disrupt oocyte maturation and early embryonic cell division⁷. Ethanol induces oxidative stress and disrupts membrane integrity and cell signaling pathways critical for gastrulation and organogenesis¹⁰. These mechanisms may explain the observed reduction in embryo viability despite continued egg production in some exposure groups.

Future studies could investigate dose-dependent effects, recovery after exposure, and molecular markers of oxidative stress or apoptosis to better understand the mechanisms underlying reduced embryo viability.

ACKNOWLEDGMENTS.

I acknowledge Dr. Molly Matty for her wisdom and guidance through the process of this research. I would also like to acknowledge The Bay School for providing lab space and funding.

REFERENCES

1. B. Alberts et al., *Molecular Biology of the Cell*, 6th ed. (Garland Science, New York, 2015).
2. L. A. Urry, M. L. Cain, S. A. Wasserman, P. V. Minorsky, J. B. Reece, *Campbell Biology*, 11th ed. (Pearson, New York, 2017).
3. P. M. Meneely, C. L. Dahlberg, J. K. Rose, Working with worms: *Caenorhabditis elegans* as a model organism. *Curr. Protoc. Essent. Lab. Tech.* 19, e35 (2019).
4. Y. Kim, Y. Park, J. Hwang, K. Kwack, Comparative genomic analysis of the human and nematode *Caenorhabditis elegans* uncovers potential reproductive genes and disease associations in humans. *Physiol. Genomics* 50, 1002–1014 (2018).
5. Kuakini Health System, Caffeine: America's most popular drug (2018); <https://www.kuakini.org/wps/portal/public/Health-Wellness/Health-Info-Tips/Miscellaneous/Caffeine--America-s-Most-Popular-Drug>.
6. Gallup, What percentage of Americans drink alcohol? (2022); <https://news.gallup.com/poll/467507/percentage-americans-drink-alcohol.aspx>
7. H. Lakin, P. Sheehan, V. Soti, Maternal caffeine consumption and its impact on the fetus: A review. *Cureus* 15, e48266 (2023).
8. Centers for Disease Control and Prevention, About alcohol use during pregnancy (2024); <https://www.cdc.gov/alcohol-pregnancy/about/index.html>
9. Cleveland Clinic, IVF (In Vitro Fertilization): Procedure & how it works (2022); <https://my.clevelandclinic.org/health/treatments/22457-ivf>
10. D. D. Chung et al., Toxic and teratogenic effects of prenatal alcohol exposure on fetal development, adolescence, and adulthood. *Int. J. Mol. Sci.* 22, 8785 (2021).
11. A. Alsakran, T. Kudoh, Zebrafish as a model for fetal alcohol spectrum disorders. *Front. Pharmacol.* 12, 721924 (2021).



Lauren Welsh is a student at The Bay School in San Francisco, California. She participated in a research program through University of Cincinnati and independently conducted a bench study.