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Effect of Vaping and Estrogen/Progesterone on Coagulation of Blood in Danio rerio: A Literature Review

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Effect of Vaping and Estrogen/Progesterone on Coagulation of Blood in *Danio rerio*: A Literature Review

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Honors Research Project

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Introduction:

With an increase in popularity among young adults using vaping devices (electronic cigarettes) instead of cigarettes, the effects of these vapors combined with other substances, such as oral contraceptives, is not widely studied. Today, many do not know the effects of vaping but view it as a safer alternative to smoking traditional cigarettes. Prior research has shown that toxicants in the vapor from electronic cigarettes (e-cigarettes) include formaldehyde, acetaldehyde, acrolein, volatile organic compounds, nitrosamines, nicotine, and metals (Williams, Villarreal, Bozhilov, Lin, & Talbot, 2013; Riley et al., 2016). These substances can also be found in higher concentrations in traditional cigarettes (Riley et al., 2016). A comparison of the levels of these toxicants in e-cigarettes and traditional cigarettes can be viewed in **Table 1**. For the past 50 years, a link between smoking traditional cigarettes and cardiovascular disease (CVD) has been formed, but an even higher risk of CVD, stroke, and venous thromboembolism exists for women who smoke and use combined hormonal contraceptives (Riley et al., 2016). Due to the health risks involved with smoking traditional cigarettes and taking combined hormonal contraceptives, it is important to understand the coagulation, or clotting, time of the blood after exposure to vapor from vaping devices, which include the same toxicants as traditional cigarettes, and oral contraceptives.

Table 1. This table created from a study by Rahman, Mohammed, & Jamshed (2015) compares the toxicants in the aerosol/smoke of traditional cigarettes and e-cigarettes. Traditional cigarettes contain much higher concentrations of toxicants.

Level of toxicants	Content in Aerosol from 12 E-cig samples/15 puffs	Content in tobacco cigarette mcg in mainstream smoke from 1 cigarette	Content in Nicotine inhaler mist Per 15 puffs
Formaldehyde,mcg	0.2-5.61	1.6-52	0.2
Acetaldehyde,mcg	0.11-1.36	52-140	0.11
Acrolein, mcg	0.07-4.19	2.4-62	ND
o-Methylbenzaldehyde, mcg	0.13-0.71	...	0.07
Toluene,mcg	ND-0.63	8.3-70	ND
p,m-xylene,mcg	ND-0.2	...	ND
NNN, ng	ND-0.00043	0.0005-0.19	ND
NNK, ng	ND-0.00283	0.012-0.11	ND
Cadmium, ng	ND-0.022	...	0.003
Nickel, ng	0.011-0.029	...	0.019
Lead, ng	0.003-0.057	...	0.004

The mechanism of coagulation starts off with an injured part of the body, such as a cut in the skin or an injured blood vessel, which then triggers a cascade of events to occur. Focusing on a caudal vein cut, a tear in the wall of this blood vessel would cause blood to flow out of the cut, activating tissue factor and collagen from the exposure of blood. Tissue factor would activate the conversion of fibrinogen to fibrin and the conversion of prothrombin to thrombin to activate platelets. Collagen would also activate platelets and its accumulation at the injured cell site to eventually form a clot (Barua & Ambrose, 2013). The process can be viewed in **Figure 1**. It is already known that traditional cigarette smoke effects the functions of platelets, endothelial cells, and coagulation factors in clot formation (Barua & Ambrose, 2013). For example, the body has nitric oxide (NO) molecules in the vascular tissue that can inhibit the accumulation of platelets, and vasodilate the vessel (Barua & Ambrose, 2013). However, NO

availability is limited in the presence of cigarette smoke in the blood due to free radicals (Barua & Ambrose, 2013). Therefore, clot formation is more likely to occur when NO availability is limited due to a decrease in vasodilation and regular accumulation of platelets. Due to similarities in the components of cigarette smoke and e-cigarette smoke, there would be the same effect on NO availability and coagulation in a vein in an individual who smokes e-cigarettes.

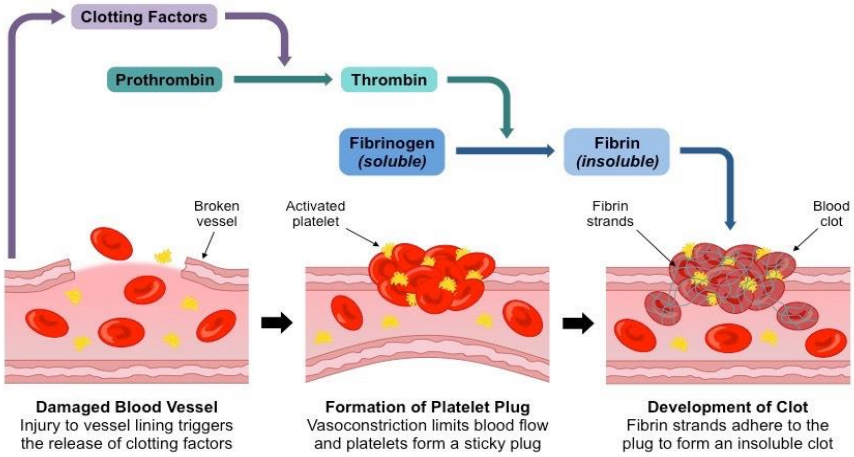

Figure 1. This figure from Cornell (2016) depicts the cascade of coagulation that occurs when a blood vessel is injured.

Furthermore, to understand the coagulation of blood in a smoker, reviewing nicotine's role in the body is significant, as well as the other materials/chemicals in the vaping liquid. Nicotine and other cigarette constituents from traditional cigarettes or e-cigarettes enters the lungs, gets transferred into the arterial system, and eventually gets circulated up to the brain (Armitage et al., 1975). In the brain, nicotine acts as dopamine to stimulate the pleasure pathways of the brain (Balfour, 2009). Repetitively activating the pleasure pathway with nicotine can eventually form the addiction to nicotine (Balfour, 2009). In the blood, nicotine and the other substances from cigarettes/e-

cigarettes increase blood pressure in the vascular system (Armitage et al., 1975). An increase in heart rate is also observed (Armitage et al., 1975). Heart rate is increased because of the increased number of molecules in the blood the heart must circulate until they're excreted from the body or metabolized. Overall, a sustained increase in heart rate and blood pressure over a long period of time, such as in a habitual smoker, can be a predisposition of heart failure or CVD.

Equally important, reviewing what estradiol and progesterone do in the body is necessary for researchers to understand when studying women who use oral contraceptives. Women take oral contraceptives for a variety of reasons including to prevent pregnancy, reduce acne, and control their menstrual cycles. There can be different concentrations or doses of oral contraceptives. The dosage refers to the two hormones found in oral contraceptives: estradiol and progesterone. These two hormones are produced by the ovaries in the female body. (Fleischman, Navarrete, & Fessler, 2010). The release of these hormones is first stimulated by the hypothalamus, which communicates to the pituitary gland through blood circulation via the gonadotropin releasing hormone (GnRH). The pituitary then releases follicle stimulating hormone (FSH) and luteinizing hormone (LH) into circulation in order to cause the ovaries to release estrogen and progesterone. As estrogen rises during the follicular phase, it has negative feedback on FSH release. When estrogen is at a high concentration, a large amount of LH is released to stimulate a large release of progesterone during the luteal phase (Shukla, Jamwal, & Bala, 2016). Together, these hormones maintain the menstrual cycle. Unfortunately, synthetic estrogen and progesterone in oral contraceptives have been known to cause venous

thromboembolism; a condition where a clot forms and obstructs a smaller vein, often times leading to stroke, CVD or death (Shukla, Jamwal, & Bala, 2016). Additionally, taking an oral contraceptive may lead to irregular bleeding during menstrual cycles (Shukla, Jamwal, & Bala, 2016). Another study by Olatunji et al. (2016) has shown an increase in hypertension and endothelial cell dysfunction when taking oral contraceptives.

Altogether, the interaction between vaping e-cigarettes and oral contraceptive components, estradiol and progesterone, equate to possible effects in blood coagulation time. As described earlier, smoke inhalation from traditional or e-cigarettes can decrease the amount of NO present in the blood, leading to decreased vasodilation and accumulation of platelets (Barua & Ambrose, 2013). However, smoke inhalation can also increase heart rate which in turn, increases blood pressure because there is lack of vasodilation occurring at the blood vessels. Lastly, progesterone and estradiol in the blood from oral contraceptives increases chances of throwing a clot, while also maintaining a higher than normal blood pressure (Olatunji et al., 2016). With these effects in mind, there is reason to believe that young women using e-cigarettes while taking an oral contraceptive are in danger of blood coagulation deficiencies.

Hypotheses:

Two hypotheses can be made from this literature review. One hypothesis is that the combined use of vaping devices while taking oral contraceptives increases the coagulation time of the blood. In other words, the blood will take longer to form a clot when injured. This hypothesis is reasonable because the oral contraceptives women take evoke an innate fibrinolytic mechanism in the body that works to break up possible

clots (Fruzzetti, 1999). In conjunction with oral contraceptives and e-cigarettes ability to induce higher blood pressure in women, it is likely a woman may bleed more easily if injured (Olatunji et al., 2016). The effects of vaping e-cigarettes and oral contraceptives combined are likely to induce hypertension, increased heart rate, and clotting abnormalities that could lead to the longer clotting time proposed by this hypothesis.

Another hypothesis that can be proposed from this literature review is the greater the amount of vapor ingested, the greater the clotting time of blood will be in people who utilize vaping devices and oral contraceptives. In this way, the effects of being a heavy smoker vs. a lighter smoker would be tested with varying amounts of puffs of vapor. A larger amount of puffs would be in relation to a heavier smoker and a smaller amount of puffs would correlate to a lighter smoker. More puffs for heavier smokers will cause an increase in particle and nicotine levels from e-cigarettes in the blood. An increase in nicotine and constituents in the blood may have an increase in heart rate and blood pressure (Armitage et al., 1975). Together with oral contraceptives and their known individual effects in the body, there may be a longer blood clotting time observed when injured.

Methodology:

The proposed hypotheses would have been tested using *Danio rerio*, a combination of estrogen (or estradiol) and progesterone, and vapor from a vape pen. Utilizing these resources, there would be three treatment groups and a control group. The control group would consist of zebrafish exposed to their normal living conditions for up to one week. The test groups would be a vape treated group, a progesterone/estrogen treated group, and a vape + progesterone/estrogen treated

group. After exposures for up to one week, the clotting time of the zebrafish would be observed. The zebrafish would be anesthetized with 0.05% MS-222 buffered to pH 7 with sodium bicarbonate and put on a petri dish with a small amount of dechlorinated water. An incision no longer than 5 mm would be made with dissecting scissors or a sterilized blade in the region of the caudal vein, and the clotting time would be determined by recording a video with either high speed video camera attached to an inverted microscope or a standard digital video camera. Videos would then be analyzed to determine clotting time. The fish would be euthanized with an overdose of 0.05% MS-222, buffered to pH 7 with sodium bicarbonate after observation and placed in the freezer to confirm death.

Zebrafish would be chosen due to their status as a model organism for coagulation studies. Zebrafish have coagulation factors that are similar to mammalian factors on several levels (Weyend & Shavit, 2014). Additionally, the hemostatic system in zebrafish has a significant homology with mammals compared to other experimental species, and is therefore the best species to use for this experiment (Weyend & Shavit, 2014). In regards to the vapor, toxicity of the substances in the vapor is not well studied in zebrafish, however, there is a positive correlation between the concentration of nicotine and heart defect in developing zebrafish (Palpant et al., 2015). With this in mind, the well-being of the zebrafish is not expected to significantly change with the use of vape pens containing nicotine. In relation to women, nicotine in vape pens may have an effect on cardiovascular disease in females who smoke e-cigarettes and use hormonal contraceptives (Riley et al., 2016). For this reason, a vape pen that contains nicotine will be used. Furthermore, the oral contraceptive in this experiment will be a

combination of progesterone and estrogen. Only these two components will be used because they are the main components of combined hormonal contraceptives (Riley et al., 2016).

The proposed experimentation builds on existing research and efforts. For example, a study by Liang et al. (2019) performed an analysis on the effects of estradiol and progesterone on aquatic organisms by measuring the transcriptional changes on 42 genes related to the circadian rhythm and hypothalamic-pituitary-gonadal axis. Another study by Liu et al. (2017) performed an analysis on the risk of acetochlor to the cardiovascular system by observing thrombosis in the caudal vein of a zebrafish using the intensity of red blood cells to measure the thrombosis degree. Altogether, this research project will observe the effects of progesterone and estrogen which was also utilized in Liang et al. (2019) experiment, and thrombosis observation will be performed using a similar method like the one used in Liu et al. (2017).

Future Direction:

If the first hypothesis is supported about the combined effects of oral contraceptive and e-cigarettes producing a longer clotting time, further research may exploit the type of vaping devices used for vaping or the flavor of vape liquid used. For example, there are more than 8000 different flavors for e-cigarette users to choose from including, vanilla, cinnamon, cotton candy, café Cubano, mountain du voltage, and grape vape (Gerloff et al., 2017). In addition, there are over 466 brands of e-cigarette devices such as e-hookah, e-pipes, vape pens, and e-cigars produced by different manufacturers (Gerloff et al., 2017). Each one of these systems has the ability to deliver unequal amounts of vaping liquid, chemicals, and metals from the vaping device to the

person inhaling the vapor. Research by Cheng (2014) determined that there is a 'uniqueness' to each liquid of each brand, as well as the performance of the vaping device used. With this in mind, it would be beneficial to test different brands of vaping devices along with different flavors to see if there is an additional effect on the type of vape or device used along with the oral contraceptive on clot time.

Regarding the second hypothesis, one could research the different concentrations of estradiol and progesterone used in oral contraceptives, as well as different amounts of nicotine concentrations in the liquid cartridges used in vaping devices. E-cigarette liquid cartridges have nicotine concentrations that range between 0 mg/mL and 48 mg/mL (Lopez et al., 2015). Prior research has determined the concentration of nicotine in the liquid of e-cigarette cartridges have effects on plasma nicotine concentrations (Lopez et al., 2015). Different nicotine concentrations in the blood may lead to increased effects in heart rate or blood pressure which are already associated with nicotine use. In addition, there are different concentrations of estrogen and progesterone in oral contraceptives women have the option to take. A range of 0-50 micrograms of estradiol can be in an oral contraceptive (Fruzzetti, 1999). Oral contraceptives containing a higher concentration of estradiol are more likely to form blood clots (Fruzzetti, 1999). Interestingly, the body has a natural fibrinolytic mechanism that counters the clotting of low dose estradiol oral contraceptives, but the activity is suppressed when higher dosages of estradiol are used in combination with smoking (Fruzzetti, 1999). Therefore, it is imperative to research more than one type of dosage of oral contraceptive and one type of dosage of nicotine level in e-cigarette vaping liquid in order to determine possible clotting effects.

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