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Impact of maternal exposure to e-cigarette vapor on offspring health outcomes: A systematic review of animal studies

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Abstract

Since their introduction in 2007, electronic cigarettes have gained popularity, much of which come from the notion that it is a safe alternative to traditional cigarettes, even for women during pregnancy. These assertions are based in large part on the fact that e-cigarette vapors contain less toxicant compared with cigarette smoke. Little is known yet about the effects of prenatal exposure on offspring; hence, this paper aims to provide an overview of the current research on the impacts of prenatal e-cigarette exposure to offspring health outcomes. Since a clinical study in humans is scarce, this review refers only to animal studies published from 2018 to present. All relevant studies were systematically selected from online databases (Google Scholar, Web of Science, PubMed, NCBI, Research Gate, Science Direct). The search yielded 10 significant research papers. Most studies included made use of murine model for maternal e-cigarette exposure. Prenatal exposure to e-cigarettes of high nicotine levels resulted to reduced birth weight and litter size. Maternal vaping produced developmental dysfunction in cardiovascular, pulmonary, hepatic and renal systems of offspring. Moreover, afflicted offspring had short term memory deficits, and impaired learning and object recognition ability. Hyperactivity and decreased anxiety behavior are also exhibited by prenatally exposed offspring. It is also noted that e – cigarette vapor confers adverse health outcomes, independent of nicotine content. Based on these findings, it is then recommended that a more intense education and awareness program on the detrimental effects of e-cigarette smoking be given to pregnant women of child-bearing age, as well as prenatal care professionals.

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Introduction

Maternal smoking during pregnancy remains one of the most important modifiable risk factors for poor pregnancy outcomes (Li *et al*., 2020; Aboaziza *et al*., 2023). Maternal smoking not only adversely impacts maternal health but also results in poor fetal and postnatal outcomes including but not limited to tobacco-induced abortions and stillbirths, low birth weight (LBW) (Wetendorf *et al*., 2019; Wang *et al*., 2020; Archie *et al*., 2023), sudden infant death syndrome (SIDS), preterm birth, neurological and cognitive delays (Nguyen *et al*., 2018; Burrage *et al*., 2021; Archie *et al*., 2023), congenital disabilities, colic, asthma and atopic pregnancies.

Electronic cigarette or e-cigarette (e-cig), commonly known as vaping is a battery powered electronic nicotine delivery systems (ENDS), which has become extremely popular among all age groups and sexes since it entered the US market in 2007 (Li *et al*., 2020; Archie *et al*., 2023). Electronic cigarettes (commonly called "e-cigarettes" or “vapes”) are promoted as a smoking cessation aid with claims of safety over traditional cigarettes. Their popularity is based on perceptions of safety, current trends, and lower prices compared to traditional cigarette smoking. Even among those who have never smoked tobacco before, e-cigarette vaping has grown in popularity over the past ten years. Surprisingly, recent studies have reported that e-cigarette usage is also popular in women of child-bearing age and up to 15% of the pregnant women are now using e-cigs (Archie *et al*., 2023), a trend encouraged by the perception that vaping is safer than smoking for pregnant women. This may unintentionally increase the number of people exposed to e-vapor in utero given the prevalence of unplanned births and perceptions toward safety of e-cigarette smoking (Aboaziza *et al*., 2023). This is concerning since little is known about the overall health consequences of long-term e-cigarette usage, and even less in the context of pregnancy. E-cigarettes mainly contain a solution of nicotine along with several additives including propylene glycol, vegetable glycerin, acrolein, formaldehyde, favoring agents and other trace elements, some of which may be toxic for health including developing fetus and offspring. Although long-term toxic effects of prenatal tobacco smoke exposure on postnatal health are well-documented and well established, limited preclinical and clinical studies exist to evaluate the impact of maternal vaping on neonatal health outcomes.

As e-cigarettes are a relatively new product, there are only few researches conducted on how using them during pregnancy would affect the unborn child. Previous reviews emphasized the necessity for human research to provide findings that accurately reflect the outcome of prenatal e-cigarette use. The increasing number of vapers also shows that a better understanding of the effects of e-cigarettes on human fetal and postnatal development is essential and required in order to better inform pregnant women and assess the risks for the affected offspring. Hence, this current paper provides a review of the effects of maternal e-cigarette vapor exposure on offspring health outcomes based on animal models. This present the current state of research on the lack of human clinical studies.

Materials and Methods

Data Sources and Literature Search Strategy

This review utilized the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 (PRISMA) as its design and guideline in the selection and evaluation of published articles, as well as in reporting of summarized results.

The literature search on impact of maternal exposure to offspring health outcomes was conducted in universally recognized searching online databases, namely Google Scholar, Web of Science, PubMed and National Center for Biotechnology Information (NCBI), Science Direct, and Research Gate. These meta-databases were chosen for their broad range of highly scientific publications.

Appropriate keywords and Boolean operators, such as “and” or “or” were used for a more efficient and effective search for publications of high relevance to the topic of this review.

The search used a combination of the following six keyword categories (A-F): [A] Maternal, mother, prenatal, pregnancy, pregnant woman; [B] Offspring, neonates, neonatal, fetus, litters, progeny; [C] impact, effect, outcomes, consequence; [D] E-cigarette, vape, vaping, ENDS, electronic nicotine delivery system, e-vapor, aerosol; [E] Health outcomes, birth outcomes, birth parameters, birth defects, congenital defects; and [F] Animal studies, animal models, rat, mouse, mice.

All relevant articles included in this review must belong to the following categories: [A] studies which involves exposure of pregnant mothers to e-cigarette vapor, and not the e-liquid; [B] studies where progeny are at postnatal stage, and not at embryonic stage [C] studies with findings related to the impact of maternal exposure to e-cigarette vapor on offspring; [D] studies that use animal models and not human subjects. Search results selection is restricted by year of publication, language, and study category. Only research articles published from 2018 to present, and written or translated to English language are considered. Review articles, case reports, case series, and narrative reviews are excluded, with that of articles with no available full text.

Following the literature search, and elimination of duplicates, the titles and abstracts of all papers found were read first. Final screening of research articles based on eligibility criteria came next to screen out irrelevant papers to the current topic. The full texts of the remaining papers were then reviewed.

Search Results and Data Extraction

A total of 62 articles were initially identified and downloaded from the combination of search terms from the six online databases. Initial screening resulted from exclusion of 28 studies that were published beyond the range of specified year of publication (2018-2023), and/or are written in non-English language with no available translation.

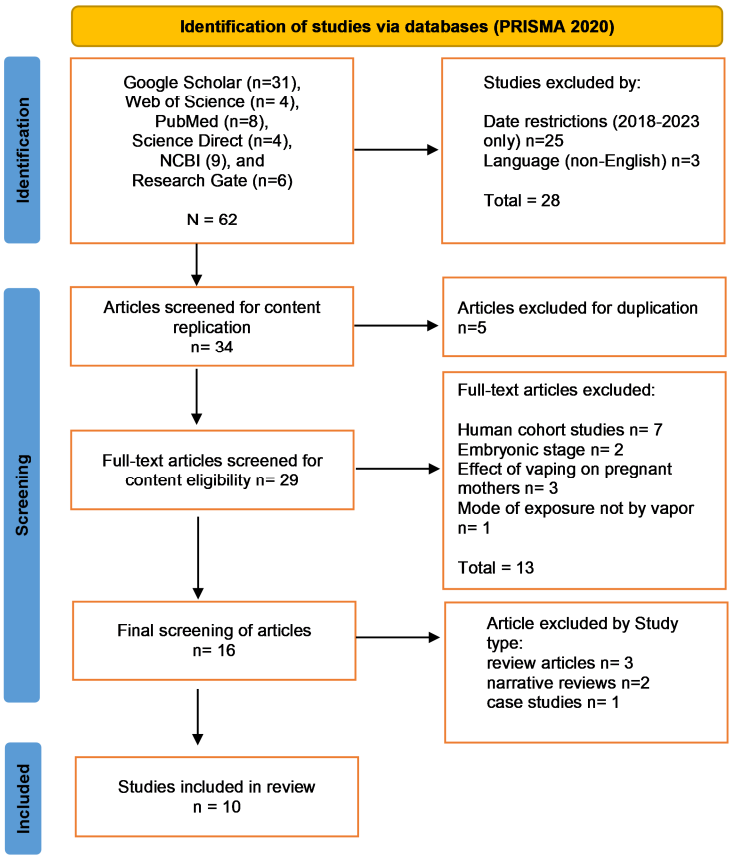

Fig. 1. PRISMA 2020 flow diagram of the stages of study identification and screening results.

After removal of duplicated (6) studies, only 29 studies remained and subjected to further screening based on inclusion and other exclusion criteria. Sixteen articles remained after evaluating the content based on the abstracts. Removed articles were those that used human cohort studies (7), where findings were that on the effect of vaping on the pregnant mothers and not on offspring (2), involved subjects were at embryonic stage and not of full-term or postnatal offspring (3), and where mode of e-cigarette exposure is by e-liquid and not by e-vapor or aerosol (1). The last stage of screening finally resulted to 10 eligible articles for full text review. The six excluded articles were that of review articles, narrative reviews or case studies. The stages of study selection and results are presented in the PRISMA 2020 flow diagram (Fig. 1).

This review includes a systematic overview of the impact of maternal exposure to e-cigarette vapor to offspring health outcomes. All the retrieved publications were evaluated, and data pertinent to the specific objectives of the review was manually recorded in MS Word. The data collected from each study include: animal model used, study population, active substance in e-cigarette liquid, period of maternal exposure to e-cigarette vapor, and health outcomes of offspring. Details of the study such as name of the author/s and publication date of article are also recorded.

Results and discussions

Neurobiology, Cognition and Behavior

The following section describes the effects of prenatal e-cigarette exposure on neurobiology, cognition, and behavior in affected offspring. Of the 10 research articles reviewed, only three studies provided information on this, which is listed on Table 1.

Table 1. Animal studies on maternal e-cigarette vapor exposure and its neurobiological, cognitive and behavioral effects on offspring.

Author and Year of Publication	Animal Model Used	Study Population	Active Substance	Period of Exposure	Findings
Burrage *et al.*, 2021 [3]	Sprague Dawley Rats	15 mothers	E-liquid with and without nicotine (18mg/mL) + French vanilla flavor additive	GD 2 until PD 21 5x a week to e- cig vapor 1 hour per day with 1 puff (5 sec) every after 3 minutes	Maternal vaping during pregnancy confers significant cerebrovascular health risk and dysfunction to offspring which persist till adulthood.
Nguyen *et al.*, 2018 [8]	BALB/c mice	24 females Male offspring n=126	E-liquid with and without nicotine (18mg/mL) + tobacco flavor additives	6 weeks PrGD up to PD20 2x daily for 30 min (2x 15 min)	Offspring from mothers exposed to e-cigarette aerosols showed short-term memory deficits, reduced anxiety and hyperactivity in adulthood. Global DNA methylation and alterations in chromatin modification enzymes in the brain were found in all stages of the developing offspring
Archie *et al.*, 2023 [10]	CD1 mice	16 mothers 160 offspring	E-liquid with 24mg/mL nicotine	GD 5 up to PD 7 6x a day, 32 puffs per session. 2 puffs per 60sec	Prenatally e-cig exposed adolescent and adult offspring showed impaired locomotor, learning, and memory function. Prenatal e-cig exposure also induces long-term neurovascular changes of neonates by disrupting postnatal blood – brain barrier (BBB) integrity and worsening behavioral outcomes.

Legend: GD- Gestational Day (During Pregnancy) PD- Postnatal Day (After parturition)
PrGD- Pregestational Day (Pre-conception)

The middle cerebral arteries (MCAs), is of special importance as they are the biggest cranial vessels and supply 80% of the blood flow to the brain. Therefore, in terms of cerebrovascular reactivity, these vessels are the ones that have been examined the most. A simple constriction or blockage to MCA prevents oxygenated blood from reaching the brain parenchyma will result to ischemic stroke and cause cerebral edema and tissue necrosis.

The study of Burrage *et al.* (2021) on the effects of maternal vaping on the cerebrovascular reactivity in mice progeny, demonstrated that prenatally e-vapor exposed pups had more than 50% reduction in maximal MCA EDD than control group at 1,3, and 7 months of age, suggesting that maternal vaping, with or without nicotine, confers significant cerebrovascular health risk/dysfunction to offspring that persists into adult life. Examination of MCA microvessels of e-cigarette exposed pups revealed presence of oxidative stress higher than that of control group. Oxidative stress impedes availability of nitric oxide (NO), which serves as a vasodilator of MCA, thus play a pivotal role in brain blood flow regulation. The study therefore implies that maternal vaping contributes to cerebrovascular dysfunction by reducing availability of NO, which in turn cause constriction of MCA and impairs brain blood flow.

Changes in short-term memory in mice can be measured by the novel object recognition (NOR) test, whereby test objects are placed in a box together with the test animal. After a familiarization phase, the animal is removed and one of the blocks is replaced by a block of different shape and color. For the test phase, the animal is returned back to the box, and time spent by the animal on exploring the new block is measured. The idea behind this experiment is that a mouse with cognitive impairment won't be able to recall the old item during the test phase and will, as a result, spend about the same amount of time investigating each object. When tested, both adolescent male and female mice prenatally exposed to e-cigarette vapor exhibited significant deterioration in object recognition (Nguyen *et al.*, 2018; Archie *et al.*, 2023).

On an elevated plus maze test that assess anxiety and exploratory activity in mice, Nguyen *et al.* (2018) observed that both the E-cig (+nic) group and E-cig (-nic) group spent a higher percentage of time in the open arms, and more head dipping compared to the sham group. These actions are an indication of reduced anxiety levels. In addition, offspring exposed to e-cigarette aerosols, regardless of nicotine content, showed increase in the number of center crosses compared to the sham group, which suggests that exposed mice have higher exploratory and locomotor activity, which further implies increased curiosity and hyperactivity. A similar finding is reported by Archie *et al.* (2023), prenatally e-cig exposed pups showed significantly higher locomotor activity in the open field test, but only for female offspring. No significant difference was observed in prenatally e-cig exposed male offspring with that of the control group.

Furthermore, Morris water maze test (MWMT) was performed to assess spatial acquisition and reference memory function in offspring at adolescent and adult age (Archie *et al.*, 2023). No significant difference was observed in control and treated group in reference memory however, prenatally e-cig exposed female and male group had slow learning process (learning the location of hidden platform) compared to control group. Moreover, only adult male offspring had impaired learning compared to control group.

An effect of prenatal e-cigarette exposure to brain development and cognition is also investigated at a molecular level (Nguyen *et al.*, 2018; Archie *et al.*, 2023). Epigenetic modification, such as DNA methylation and histone modifications provide mechanisms by which early events can influence development, cognition, and health outcomes. In the study of Nguyen *et al.* (2018) on the result of maternal e-cigarette exposure to cognitive and epigenetic alteration, it was found that mice whose intrauterine exposure was switched from cigarette smoke to +Nic e-cigarettes, the level of DNA methylation was noticeably lower than when they were exposed to regular cigarette smoke. However, when compared with control group exposed with

ambient air, e-cigarette smoke exposed offspring showed significantly higher global DNA methylation. It appears that exposure to cigarette and vape smoke has a significant correlation with DNA methylation.

The blood-brain barrier (BBB) is a crucial part of the central nervous system (CNS), and it is responsible for regulating immune cell trafficking between the blood and the brain as well as preventing drugs from passing through and maintaining the homeostasis of the brain microenvironment [10]. The studies of Archie et al. (2023) have also shown that prenatal e-cigarette exposure alters expressions of blood-brain barrier (BBB) component markers. The study has shown that maternal e-cig exposure can decrease the expression of tight junction proteins, astrocyte marker and AQP4 at PD7, PD23, PD45 and PD90. Many a study has shown that BBB disruption has been associated with the onset and/ or progression of major neurological disorders.

Organ Systems

Table 2 lists studies that show effect of maternal vaping to organ systems of offspring. Two studies provided information on effects on lungs, and another two studies on the impact to the liver. Other effects include cardiovascular and renal outcomes.

Table 2. Animal Studies on Maternal E-cigarette Vapor Exposure and Effect on Organ Systems of Offspring.

Author and Year of Publication	Animal Model Used	Study Population	Active Substance	Period of Exposure	Findings
Aboaziza, E. A., 2022 [1]					Significant alteration in arterial structure and function is observed in adolescent and adult offspring.
	Sprague Dawley Rats	15 dams	E-liquid with and without nicotine (18mg/mL) + French vanilla flavor additive	GD 2 to PD 21 5x a week to e- cig vapor 1 hour per day with 1 puff every after 3 minutes	Impaired aortic relation in prenatally e-cig exposed mice is observed, regardless of nicotine content. Carotid artery stiffness and aortic reactivity deficit is observed in prenatally e-cig exposed offspring
Li *et al.*, 2020 a [2]	BALB/c mice	24 dams	E-liquid with and without nicotine (18mg/mL) + Tobacco flavor additive	6 weeks PrGD, throughout gestation and lactation (2x a day)	Maternal e-vapor exposure, irrespective of nicotine content, caused impaired glucose tolerance, increased liver damage and liver triglyceride accumulation in offspring.
Wetendorf, M., 2019 [4]	C57BL/6J mice	Not specified	E-liquid with 24mg/mL nicotine	GD 0 to 4 months (for fertility trial) 4 weeks PrGD until GD 5 (for implantation studies) 5x a week to e-cig vapor 3 hours per day with 2 puffs (2 sec) per minute	Exposures during early pregnancy significantly impair embryo implantation.
Wang *et al.* 2020 [5]	CD1 mice	1 male, 30 females	E-liquid with and without nicotine (16%) 3 hours	GD 0 to 3 weeks 5x a week to e-cig vapor 3 hours per day	Maternal exposure during pregnancy to e-cig aerosols, with or without nicotine, induces myogenesis with dysregulated ECM remodeling in a sex-dependent manner.

Li *et al.*, 2020 b [6]	BALB/c mice	24 mothers	E-liquid with 18mg/mL nicotine + tobacco flavor additives	6 weeks PrGD until lactation 2x daily	E-cigarette replacement during pregnancy in a low dose setting seems to ameliorate the adverse impact of cigarette smoke exposure on maternal and offspring liver metabolic profile in mice.
Li *et al.*, 2019 c [7]	BALB/c mice	24 mothers	E-liquid with and without nicotine (18mg/mL) + tobacco flavor additives	6 weeks PrGD up to lactation 2x daily for 30 min (2x 15 min)	Kidney weights is reduced in prenatal smoke-exposed offspring (+nic < -nic < sham) at birth Continuous e-vapor exposure adversely affects renal development, by increasing oxidative stress and related injury in offspring's kidney.
Chen *et al.*, 2018 [9]	BALB/c mice	Not specified	E-liquid with and without nicotine (18mg/mL) + tobacco flavor additives	6 weeks PrGD to PD20 2x daily for 30 min (2x 15 min)	E-cigarette exposure during pregnancy adversely affected maternal and offspring lung health. In utero e-cigarette exposure leads to abnormalities in DNA methylation, with or without nicotine,

Legend: GD- Gestational Day (During Pregnancy) PD- Postnatal Day (After parturition)
PrGD- Pregestational Day (Pre-conception)

Pulmonary system

The outcomes of the study of Chen *et al.* (2018) show that both +Nic and –Nic e-cigarette exposure elevates certain growth factors in the lung tissue of affected offspring and alters DNA methylation processes, which confers adverse effects to both offspring and maternal lung health. A noteworthy finding in this study is the reported increase in PDGFα. PDGF signaling is important to lung development, and elevated PDGF signaling has been linked to lung fibrosis. The study suggests that elevated PDGFα is caused by the e-cigarette humectant or flavor additives, rather than the nicotine content of e-liquid.

The study of Wang *et al.* (2020) reports on the effects of maternal e-cig aerosol exposure throughout gestation on lung ECM remodeling using in vivo mouse model. The pulmonary extracellular matrix (ECM) establishes the structure of the lung's tissue and offers elastic recoil and mechanical stability, both of which are necessary for healthy lung function. Findings suggest that prenatal e-cigarette aerosol exposure, even with humectant alone, results in myogenic lung profile and sex-dependent pulmonary ECM buildup. Moreover, lungs from adult male and female offspring are particularly sensitive to increased ECM deposition. It appears that maternal vaping, with or without nicotine, induces myogenesis in offspring with dysregulated ECM remodeling in a sex-dependent manner. The study concludes that e-cigarette use during pregnancy is not a safe substitute for tobacco or cigarette use and raises the risk of lung problems in later life.

Hepatic organ system

Li and associates (2019, 2020) made an extensive study on the effects of maternal smoking and vaping on offspring liver and its metabolic outcomes. In their 2019 study, where they look into the impact of replacing tobacco smoke with e-cigarette during pregnancy in the metabolic health of mice offspring, it was found that Replacement offspring had no changes in body and liver weights, compared to cigarette smoke exposed pups that had significantly reduced body mass and liver weight. Moreover, e-vapor replacement restored lipid homeostasis.

It seems that in mice, switching to e-cigarettes during pregnancy appears to mitigate the negative effects of cigarette smoke exposure on maternal and offspring liver metabolic profile. E-cigarette vapor contains lesser quantity of toxic chemicals than cigarette smoke, which likely results in fewer inflammatory reactions. Therefore, it is not much of a surprise that using an e-cigarette instead of tobacco smoke during pregnancy had less of an adverse effect (Li *et al.*, 2020).

However, on a subsequent study, comparing e-cigarette exposure, with and without nicotine, with that of a control group exposed to ambient air, offspring of dams exposed to nicotine-containing e-vapor developed glucose intolerance associated with impaired insulin signaling element, and had increased hepatic gluconeogenesis, as well as increased hepatic triglyceride accumulation associated with increased de-novo lipogenesis. On the other hand, prenatal exposure to nicotine free e-vapor had led to increased oxidative stress and inflammation in the offspring's liver, impaired liver mitochondrial health, and impaired glucose metabolism. Overall, this suggest that maternal e-vapor exposure, irrespective of nicotine content, caused impaired glucose tolerance, increased liver damage and liver triglyceride accumulation in offspring (Li *et al.*, 2020).

Cardiovascular organ system

The study of Aboaziza *et al.* (2022) describes the consequence of maternal vaping on central arterial stiffness and aortic reactivity in adolescent and adult mice offspring. Perinatal e-cigarette exposed offspring were found to have two to three-fold increase in carotid artery stiffness and 20 – 30% reduction in aortic reactivity compared to control groups. Increasing maternal exposure and nicotine levels in the e-liquid did not create a dose-dependent effect on the offspring's vascular outcomes. This finding implies that maternal vaping during pregnancy, regardless of nicotine content, leads to maladaptation in vascular development that persist into adult life of offspring.

Renal organ system

A mice study conducted by Li *et al.* (2019) on the effects of maternal e-cigarette vapor exposure on renal health in the offspring showed that replacement of tobacco cigarettes with e-cigarettes resulted in increased total ROS and mitochondrial specific ROS, and improvement of overall renal development and function compared to continuous cigarette smoke exposure during pregnancy. However, continuous e-vapor exposure during pregnancy was still found to be detrimental to fetal development, with increased kidney markers of oxidative stress, renal inflammation and fibrosis in the adult offspring, independent of nicotine.

Birth Parameters

The impact of maternal e-cigarette exposure on birth parameters such as birth weight and litter size of offspring is given in Table 3. Eight of the total 10 publications examined provided information on these two parameters.

In all 10 studies analyzed, neither increased infantile mortality nor stillbirths were noted. This is valid for both parental exposure to e-cigarettes in the weeks preceding pregnancy (Chen *et al.*, 2017; Wetendorf *et al.*, 2019; Li *et al.*, 2019 & 2020) as well as for maternal prenatal exposure beginning at conception (Wang *et al.*, 2020; Burrage *et al.*, 2021; Aboaziza *et al.*, 2023; Archie *et al.*, 2023). Most of the studies reported that prenatal exposure to e-cigarette vapor, with or without nicotine, had no effect on litter size (Chen *et al.*, 2017; Wang *et al.*, 2020; Burrage *et al.*, 2021; Aboaziza *et al.*, 2023; Archie *et al.*, 2023) and birth weight (Chen *et al.*, 2017; Li *et al.*, 2019; Wetendorf *et al.*, 2019; Wang *et al.*, 2020; Burrage *et al.*, 2021; Aboaziza *et al.*, 2023) when compared to the control group exposed with ambient air. Some studies contradict these findings as they have observed reduction in birth weight (Chen *et al.*, 2017; Wetendorf *et al.*, 2019; Li *et al.*, 2020; Archie *et al.*, 2023) and litter size (Wetendorf *et al.*, 2019) in pups of e-cigarette exposed dams. It is also noted that offspring with reduced weight at birth gradually catch up with that of the sham group later in adulthood.

This finding is in consonance with other studies (tobacco exposed vs sham) which reported that any weight differences among offspring, regardless of exposure group, will level out later in life. However, a deviation from this observation is reported in the study of Wetendorf (2019), where weight reduction in adulthood is observed but only in female offspring, while Chen, *et al.* (2018) also reported the same trend of weight reduction, but only for male offspring.

Table 3. Animal Studies on Maternal E-cigarette Vapor Exposure and Effect on Birth Parameters.

Author and Year of Publication	Animal Model Used	Study Population	Active Substance	Period of Exposure	Findings
Aboaziza, E. A., 2022 [1]	Sprague Dawley Rats	15 dams	E-liquid with and without nicotine (18mg/mL) + French vanilla flavor additive	GD 2 to PD 21 5x a week to e- cig vapor 1 hour per day with 1 puff every after 3 minutes	Litter size and body mass at birth were not different between groups. Higher body fat (BF) mass and lower lean body mass is observed in e-cig exposed offspring (Ecig0)
Burrage *et al.*, 2021[3]	Sprague Dawley Rats	15 mothers	E-liquid with and without nicotine (18mg/mL) + French vanilla flavor additive	GD 2 until PD 21 5x a week to e- cig vapor 1 hour per day with 1 puff (5 sec) every after 3 minutes	Body mass at birth and at weaning were not different between groups. No difference in litter size within exposed and control dams. Anthropometric measures are not significantly different within any age or by exposure group
Wetendorf, M., 2019 [4]	C57BL/6J mice	Not specified	E-liquid with 24mg/mL nicotine	GD 0 to 4 months (for fertility trial) 4 weeks PrGD until GD 5 (for implantation studies) 5x a week to e-cig vapor 3 hours per day with 2 puffs (2 sec) per minute	Delay onset of first litter, and cause slight reduction on litter size. No effect on overall birth weight, but caused reduced weight in female offspring
Wang *et al.* 2020 [5]	CD-1 mice	1 male, 30 females	E-liquid with and without nicotine (16%)	GD 0 to 3 weeks 5x a week to e-cig vapor 3 hours per day	E-cig aerosol exposure had no effect on pregnancy incidence, litter size, or male-to-female offspring sex ratio.
Li *et al.*, 2019 c [6]	BALB/c mice	24 mothers	E-liquid with nicotine (18mg/mL) + tobacco flavor additives	6 weeks PrGD until lactation 2x daily for 30 min (2x 15 min)	Birth weight is reduced for prenatally e-cig exposed offspring, regardless of nicotine concentration.
Nguyen *et al.*, 2018 [7]	BALB/c mice	24 females Male offspring n=126	E-liquid with and without nicotine (18mg/mL) + tobacco flavor additives	6 weeks PrGD up to PD20 2x daily for 30 min (2x 15 min)	There was no difference in birth weights or morbidity between the exposure status of the dams.
Chen *et al.*, 2018 [9]	BALB/c mice	Not specified	E-liquid with and without nicotine (18mg/mL) + tobacco flavor additives	6 weeks PrGD to PD20 2x daily for 30 min (2x 15 min)	No difference in body weight or organ weight at birth among the offspring groups, but weight reduction is observed in e-cig (+nic) pups in adulthood.
Archie *et al.*, 2023 [10]	CD1 mice	16 mothers 160 offspring	E-liquid with 24mg/mL nicotine	GD 5 up to PD 7 6x a day, 32 puffs per session. 2 puffs per 60sec	No difference was observed in litter size between control and exposed offspring. Birth weight and brain-body weight ratio is significantly reduced in e-cig exposed offspring.

Legend: GD- Gestational Day (During Pregnancy) PD- Postnatal Day (After parturition)
PrGD- Pregestational Day (Pre-conception)

Large bodies of literature have shown that dose-dependent maternal exposure to cigarette smoke caused decrease in birth weight and litter size. However as reported in this review, there are more studies suggesting that prenatal exposure of e-cigarette, regardless of exposure (+nic, -nic, sham), bears no effect in birth weight and litter size. This discrepancy, as explained by Burrage *et al.* (2021), is most likely due to relatively low exposure (only 20 puffs within 1 hr/day exposure window from GD 0 to PD21) of dams to e-cigarette vapor (Wang *et al.*, 2020; Burrage *et al.*, 2021; Aboaziza *et al.*, 2023; Archie *et al.*, 2023) and low concentration of nicotine in the e-liquid (18 mg/mL) that were used in these studies (Chen *et al.*, 2017; Li *et al.*, 2019 & 2020; Burrage *et al.*, 2021; Aboaziza *et al.*, 2023; Archie *et al.*, 2023). Whereas, studies whose findings reported a decline on birth weight and/or litter size used a more intense vaping paradigm (Chen *et al.*, 2017; Wetendorf *et al.*, 2019; Li *et al.*, 2020) (3hr exposure/day exposure window from 6 weeks before mating until weaning), and with higher concentration of nicotine (24 mg/mL) in e-liquid (Wetendorf *et al.*, 2019; Wang *et al.*, 2020; Archie *et al.*, 2023).

Fat distribution is also one of the parameters examined in animal studies as it provides information on signs of obesity. The study of Aboaziza (2022) revealed that higher body fat (BF) mass and lower lean body mass is observed in e-cigarette exposed offspring. This result is in agreement with research literatures that report on the association of prenatal e-cigarette exposure on increased fat mass in the abdominal cavity.

Summary

This review analyzed 10 research papers for the last five years on the effects of prenatal exposure of offspring to e-cigarettes. These papers included research on maternal e-cigarette vapor exposure and neurobiological, cognitive and behavioral effects (n=3), and on the effects on body organs (n=7). Some of the studies also provided information on effects of prenatal e-cigarette exposure on birth parameters (n=8). This current review only includes animal studies on the impact of maternal exposure to e-cigarette vapor on offspring's health outcomes.

It is noted that all studies included in this review made use of murine model for the perinatal e-cigarette exposure studies. Mice species utilized were the following: Sprague Dawley (n=2), BALB/c mice (n=5), C57BL/6J mice (n=1), CD1 mice (n=2). When evaluating the impacts of e-cigarette exposure, human subject research may prove to be challenging, invasive, and impractical, particularly when it comes to prenatal studies. This is partly because gestational period in humans is longer than that of rodents. Furthermore, perinatal studies in humans would require an extensive timeline to study effects during pregnancy, and even longer to study offspring effects into adolescence and adulthood. For this reason, using animal models is common. Due to their genetic and physiological similarities to humans, rodents have traditionally been the preferred animal model for biomedical research. The abundance of data gathered over the years has led to a greater understanding of the physiological reactions and pathways in rats than in other species (Aboaziza *et al.*, 2023). Additionally, rats have a clear advantage, being relatively small which makes them easier to handle and manage.

Based on the review, e-vapor exposure during critical developmental periods, especially in utero can impair organ development and lead to organ defect and dysfunction. Organ systems greatly impaired due to maternal vaping includes cerebrovascular, pulmonary, hepatic and renal systems. It is found that e-cigarette exposure, even without nicotine, confers equal, if not greater, alteration in organ structure, and impairment of organ and metabolic functions.

Prenatal e-cigarette exposure also confers significant neurological, cognitive and behavioral risks and dysfunctions to offspring which persist up to adulthood. Exposed offspring were found to have short term memory deficits, hyperactivity and impaired locomotors and learning functions. A number of reviewed articles have findings that show maternal exposure to e-vapor

had no effect on litter size and birth weight, but some studies noted that with increased exposure to e-liquid aerosol of higher nicotine levels significantly reduce birth weight and litter size.

Effects of e-cigarette exposure on offspring birth parameters appear to be dose-dependent, whereas the organ systems, cognition, and behavior of prenatally exposed offspring are equally compromised regardless of nicotine content, which suggests that other than nicotine, the e-liquid component and flavoring additives may contain toxicants that may cause adverse effect and health risk.

Limitations in Methodology and Evidence

With regard to findings, there are instances that a result of one study is in contradiction with another. There is always an outlier to an identified pattern or similarities among the findings. Upon close examination, it is presumed that the difference in results lies within the heterogeneity of methods of exposing animal subjects to e-cigarette vapor. An example is the protocol on the period of exposure to e-cigarette vapors of pregnant dams, the amount, intensity and length of exposures vary. Additionally, a nicotine level in e-liquid is not uniform. E-liquid used in some of the studies contains flavorings, while others do not. These variations may have corresponding effect on the degree of impact or scale of effects on the parameters observed in the offspring.

There is also an issue on the representation of offspring used in some studies. All of the studies used randomization in assigning dams to control and treatment groups, and most randomly select offspring, taking into account equal or nearly equal representation of male and female offspring. However, three studies intentionally focused on offspring of male sex only. As sex is one of the determinants of physical and physiological outcomes, it would also be appropriate to include both sexes in the observation of morphological, physiological and behavioral effects of maternal exposure to smoking in offspring.

Most of the findings show that maternal exposure to nicotine containing and nicotine free e-cigarette vapor produce similar adverse effect on offspring health outcomes. While some of the studies presume that nicotine is not the only substance that brings health risk, but could also be by the humectant and the flavoring additives in e-liquid, nothing can be made conclusive as the elucidation of chemical components of e-liquid and their toxicity is beyond the scope of the studies.

Though this review presents findings on the clear association between maternal smoking and adverse health outcomes in offspring mice, the translation of the outcomes from this murine model to humans is not yet definitive.

Implications for Future Research, Clinical Practice, and Policy

For future research, with respect to methodology, the procedures can be standardized. The protocol on maternal exposure like amount, intensity and length of exposure to e-vapor can be applied uniformly, same is true with the nicotine levels and ratio and kinds of chemical constituents and additives in the e-liquid. Offspring sex ratio must also be equally represented as much as possible to prevent one-sided result.

The current review primarily depicts negative effects of nicotine as a component of e-cigarettes. Due to the additional substrates and flavors, investigating into their effects and toxicity would also be essential. This is particularly important for adequate risk assessment of e-cigarette vaping during pregnancy.

In clinical practice, preventive information should be provided on the basis of current research findings about the numerous associated health consequences of maternal vaping to offspring. This reinforces the need for ongoing public health interventions regarding smoking and e-vaping cessation in all countries, particularly focusing on pregnant and women of child bearing age. It must be made clear that e-cigarettes can no longer be regarded as a safe alternative as originally claimed, but rather it has been disproved by current research outcomes.

Health-related institutions in the municipality and national level, including the World Health Organization should promote its support and recommendation against e-cigarette consumption for wider public awareness.

Conclusion

Despite the marketing as healthier alternatives to tobacco cigarette smoking and subsequent widespread public perception of e-cigarette harmlessness, findings of this review of animal studies on the effect of maternal exposure to e-cigarette vapor on offspring health outcomes suggest otherwise. Maternal vaping, with or without nicotine, produces profound cardiovascular, pulmonary, renal and hepatic systems dysfunction, and cognitive and behavioral impairment to offspring that could persist until adulthood. These data add to the emerging body of literature that concludes that e-cigarette vaping during pregnancy is not a safe alternative, nor a harmless and risk-free cessation tool to tobacco cigarette smoking.

It is crucial to inform healthcare professionals, such as midwives, psychologists, and doctors, who support pregnant patients, about these current results. This information should also be shared so the public can make an informed decision, especially pregnant and women of child-bearing age. This can guarantee that pregnant women receive thorough information about using electronic cigarettes and the risks involved.

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