Reproductive Toxicology 94 (2020) 22–30

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Effects of marijuana on human reproduction

Marina Dubovis[a,*], Ozgul Muneyyirci-Delale[b]

[a] Touro College of Osteopathic Medicine, New York, NY, USA
[b] Department of Obstetrics and Gynecology, SUNY Downstate Medical Center, Brooklyn, NY, USA

ARTICLE INFO

Keywords:
Cannabis
Marijuana
delta-9-tetrahydrocannabinol
Reproduction
Gametogenesis
Placenta
Pregnancy
Offspring

ABSTRACT

As U.S. states steadily legalize its distribution and the prevalence of its use in people of reproductive age continues to rise, the need to understand the effects of marijuana on human physiology is becoming increasingly urgent. While marijuana is well-known for its psychoactive effects and applications in controlling pain and nausea, little is known about its effects on reproduction. This review includes *in vitro* studies which consistently demonstrate associations between marijuana consumption and low sperm count, dysregulated menstruation, and abnormal placentation. While many *in vivo* studies associate maternal marijuana use with stillbirth, neonatal intensive care unit (NICU) admission, and offspring psychosis, significant literature negates these relationships by controlling for poly-substance use and socioeconomic status. Data limited by self-reporting and confounds precludes the drawing of definitive conclusions regarding the effects of marijuana on reproduction. This review serves as a call to action to elucidate these effects and discourage marijuana use in people of reproductive age.

1. Introduction

1.1. Prevalence and justification of use

As of this writing, marijuana use is legal to some extent in all but 12 U.S. states, with full legalization legislature having passed in 11 states. Unsurprisingly, widespread legalization is bringing with it widespread consumption. According to the most recent data available from the National Survey on Drug Use and Health (NSDUH), 24 million Americans over the age of 12 reported using marijuana in 2016. The 18-to-25-year age range saw the greatest prevalence of use, with 20.8 % of responders endorsing current use. Prevalence of use in this age group has been increasing at greater rates than any other age group [1]. As this study was conducted using self-report data only, it can be reasonably inferred that actual marijuana use is even more widespread. Given that so many Americans, especially those of reproductive age, endorse current marijuana use, the possibility and effects of marijuana use in pregnancy must be considered.

Analysis of 2007–2012 NSDUH data revealed that 7 % of pregnant women had used marijuana in the past year, with 3.9 % reporting use in the last month [2]. Co-use of marijuana and tobacco examined using 2005–2015 data from the National Health and Nutrition Examination Survey (NHANES) was found to be stable at 9.8 % of young adults aged 21–30 over the ten-year period. Interestingly, while tobacco-only and co-use of tobacco and marijuana were lower in pregnant women, there was no change in prevalence of marijuana-only use [3].

Several noteworthy trends related to socioeconomic factors, attitudes, and healthcare provider intervention with regard to marijuana use in women of reproductive age have recently come to light. The aforementioned NHANES study revealed that co-users of marijuana and tobacco were more likely to be unmarried, non-Hispanic black, to have used alcohol in the past month, and to have had other lifetime substance abuse. Prevalence of marijuana use was highest during the first trimester and lowest during the third. When asked if they worried marijuana use might negatively affect their pregnancy, 70 % of women who reported marijuana use in the 2007–2012 NSDUH felt the negative effects of using marijuana once or twice per week during pregnancy were negligible [2–4]. A qualitative study examining pregnant women's rationales for continued use during pregnancy revealed conflicting attitudes; while some women admitted to being somewhat concerned about the effects of marijuana on their pregnancies, many also expressed they felt marijuana was safer and more natural than pharmaceuticals [5]. A lack of healthcare provider intervention may also explain the prevalence of continued marijuana consumption during pregnancy. In an analysis of recording from 468 first-time prenatal visits, providers in 48 % of encounters responded with either generic statements regarding the risks of drug use in pregnancy or did not address the patient's admission of marijuana use at all [6]. Given these trends, the need for definitive data regarding the effects of marijuana use on reproductive success in order to more effectively counsel patients and address disparities, is glaringly obvious.

1.2. The endocannabinoid system in reproductive tissues

While marijuana use in humans is difficult to study given ethical considerations and confounding by socioeconomic factors and variability within the drug itself, strong efforts have been made to elucidate the effects of marijuana on reproduction in human and animal models.

The 1964 discovery of Δ^9-tetrahydrocannabinol (THC), the primary psychoactive compound in cannabis plants, ushered in an era of research whereby two transmembrane G-protein-coupled receptors which bind it, cannabinoid receptors type 1 and 2 (CB1 and CB2), were discovered. Further investigation revealed that these receptors have endogenous ligands, N-arachidonoylethanolamine (AEA), and 2-arachidonoylglycerol (2-AG), which came to be known as endocannabinoids. These receptors and their ligands have since been thoroughly characterized and dubbed the endocannabinoid system (ECS) [7].

Numerous *in vivo* and *in vitro* studies in humans and animals have demonstrated the presence and activity of the endocannabinoid system throughout the reproductive tract. Using cells cultured from human hysterectomy specimens, researchers were able to detect the presence of CB1 receptors, as well as enzymes such as *N*-acyl-phosphatidyl ethanolamine-specific phospholipase D (NAPE-PLD), endocannabinoid fatty acid amide hydrolase (FAAH), which synthesize and degrade endocannabinoids, respectively, throughout the myometrium [8]. The presence and distribution of cannabinoid receptors has also been elucidated in human oocytes. Activity of the ECS in male reproductive tissues is supported by repeated studies in sea urchin, bovine, murine, as well as human models [9].

Once the presence of the ECS was confirmed throughout reproductive systems, researchers set to work elucidating its role in reproduction. In an experiment using bovine luteal tissue, researchers found that progesterone secretion was decreased by CB1 and CB2 receptor agonists, and concluded that the ECS contributes to the regulation of luteal function [10]. A study in murine

* Corresponding author at: Touro College of Osteopathic Medicine, New York, 230 W. 125th Street, New York, NY 10027, USA.
E-mail address: mdubovis2@student.touro.edu (M. Dubovis).

https://doi.org/10.1016/j.reprotox.2020.04.071
Received 6 September 2019; Received in revised form 31 March 2020; Accepted 13 April 2020
Available online 18 April 2020
0890-6238/

oocytes demonstrated increased rate of meiotic entry, decreased rate of double stranded DNA break repair, and consequent oocyte apoptosis as a result of CB2 agonist administration [11]. A similar study in male mice also demonstrated an increase in rate of meiotic progression in spermatocytes *via* CB2 receptor signaling [12]. By deleting genes coding for CB1 in mice, researchers showed that dysregulation of CB1 signaling was associated with decreased sperm chromatin quality and subsequent DNA fragmentation [13].

Given the understanding that ECS dysregulation negatively impacts male and female reproductive tissues separately, researchers extrapolated their findings to show that such aberrant signaling also negatively impacts pregnancy. In a study on the coordination of murine embryo implantation, researchers demonstrated that elevated uterine endocannabinoid and CB1 receptor levels were associated with failed implantation [14]. A subsequent study posited a putative mechanism by which dysregulated ECS signaling leads to pregnancy failure. Antagonism of CB1 receptors in mice led to greater rates of embryo retention within the fallopian tube, an event analogous to ectopic pregnancy in humans [15].

These findings were confirmed to some extent in human studies of spontaneous abortion and failure to achieve ongoing pregnancy. Evaluation of placentas from women who suffered spontaneous abortion, when compared to controls who underwent elective abortion, demonstrated greater CB1 receptor expression, as well as lower concentrations of FAAH an enzyme which degrades endocannabinoids, in the placentas of women who had suffered miscarriage [16]. Elevated AEA and decreased FAAH levels were also associated with failure to achieve an ongoing pregnancy in women who had undergone *in vitro* fertilization (IVF) [17].

2. Methods

We performed a PubMed, Embase, JSTOR, EBSCOhost, and Google Scholar search for scientific papers describing *in vitro* and *in vivo* studies which explore the effects of marijuana on various aspects of human reproductive functioning. Another key resource was the 2017 National Academies of Sciences, Engineering, and Medicine (NASEM) expert report. This comprehensive review served as a powerful jumping-off point for article gathering and identifying gaps in the literature [18]. Animal studies were included for background information and to fill gaps in literature where human experimentation was not ethically tenable. We only included articles published in English in scholarly peer-reviewed journals within the last 20 years.

When searching for relevant *in vivo* studies in animals or *in vitro* studies in humans where control over dosing and administration was possible, we included only articles which examined the specific effects of THC on reproduction. As no randomized controlled trials of the effects of THC alone on human reproduction exist, we used the search terms "marijuana," and "cannabis" when searching for relevant *in vivo* studies on the subject. We excluded all studies lacking a defined control group such as case reports and case series, and thus our human studies were limited to cohort and case-control studies.

In order to evaluate the full scope of the effect of THC on human reproduction, we amassed articles pertaining to its effects on individual male and female reproductive functioning, the embryo, obstetrical and neonatal outcomes, as well as long-term neuropsychiatric and reproductive sequelae in offspring. Table 1 summarizes the specific endpoints studied in the primary literature examined for this review.

Overall, we included 61 human and 14 animal studies an in narrative literature review aimed at evaluating the contributions of various studies to knowledge on the subject and identifying gaps in the literature. Fig. 1 summarizes the types and number of studies included in this review.

3. Results

3.1. Animal studies

Male albino rats orally administered a daily weight-based THC bolus for 30 days exhibited overall decreased fertility as evidenced by less mounting behavior, lower sperm count, and fewer estrous females impregnated [19].

In a murine study demonstrating a pro-apoptotic relationship between CB2 receptor agonism and oocytes, researchers found that female offspring exhibited a decreased ovarian reserve and were less fertile than their non-exposed counterparts, but there was no effect on male offspring. While the mechanism of inheritance is unclear, this finding suggests that maternal cannabinoid dysregulation may lead to abnormal reproductive functioning in female offspring [11].

A study of bovine *in vitro* fertilization demonstrated that THC activates oocyte CB1 receptors, which leads to the phosphorylation of AKT and ERK1/2 proteins and the resumption of meiosis *via* the PI3/MAPK signaling pathway, ultimately resulting in faster oocyte maturation [26]. A study comparing the effects of endogenous and synthetic cannabinoids on frog oocytes examined another potential target for THC in reproductive tissues. Endogenous cannabinoids antagonized alpha7-nicotinic acetylcholine receptors in oocytes, while synthetic cannabinoids such as THC had no effect, which suggests these receptors are less likely to be involved in reproduction [27].

In the aforementioned study of the effects of THC exposure on bovine IVF, researchers also found that while THC exposure during the blastocyst stage did not speed up embryo maturation, it did increase the expression of interferon tau (IFNτ) and connexin 43 (GJA1), both of which are key to the establishment and maintenance of early pregnancy. The researchers go on to suggest that exocannabinoids such as THC may be useful in improving embryo quality in assisted reproduction, however, they did not examine whether this effect persists with chronic exposure or at larger doses [26].

Table 2 summarizes the routes of administration and THC concentrations used in the above animal experiments, as well as the associated study findings.

3.2. Human studies

3.2.1. Spermatozoa

Semen analyses of 1215 Danish men aged 18–28 demonstrated a 28 % decrease in sperm concentration as well as a 29 % decrease in sperm count in those who had used marijuana more than once per week in the 3 months leading up to the study [20]. A similar study in 229 Jamaican men aged 23–72 revealed a 2.7-fold increase in risk of abnormal sperm motility with recent marijuana use. Heavy use carried a 4.3-fold increase in risk for abnormal motility. Finally, moderate use carried a 3.4-fold increase in risk for abnormal morphology [21].

In an attempt to probe deeper into the effects of THC on sperm functioning, researchers treated neat human sperm with THC concentrations comparable to moderate use and found a dose-dependent decrease in cellular respiration, likely through direct mitochondrial damage. Of note, this effect was mitigated by the addition of seminal fluid, which suggests it may have cytoprotective properties [22]. Other studies supported these findings by also demonstrating a dose-dependent decrease in motility and ATP in sperm treated with THC [23–25].

3.2.2. Oocytes and ovulation

While the presence of cannabinoid receptors in human oocytes has been ascertained, to our knowledge no *in vivo* or *in vitro* studies exist on the effects of THC on human oocytes.

In a study comparing the menstrual cycles of marijuana users and non-users, researchers found an average 3.5-day increase in follicular phase length in women who reported occasional marijuana use than in non-users. The researchers thus posited that increased menstrual cycle length ultimately results in fewer ovulations per year and arguably decreased fertility [28]. Data from the National Survey of Family Growth, however, did not support this finding as time to pregnancy (TTP) in women actively trying to achieve pregnancy did not differ between marijuana users and non-users [29].

3.3. Embryo

A prospective study examining the dose-dependent relationship between marijuana consumption and success with assisted reproduction revealed that women who had more than 90 lifetime exposures to marijuana on average had 27 % fewer embryos harvested and 1 fewer viable embryo available for transfer through IVF [30].

Table 1
List of endpoints examined as measures of reproductive functioning in human an animal studies included in this review.

Reproductive Endpoints						
Male	Female	Embryo	Obstetrical Outcomes	Pregnancy Outcomes	Neonatal Outcomes	Long-Term Offspring Effects
• Sperm count • Sperm motility • Sperm morphology • Spermatogenesis	• oogenesis • ovulation • menstruation	• embryo quality • embryo quantity	• spontaneous abortion • placental abruption • emergency cesarean section • placenta previa • vasa previa • malpresentation • uterine rupture • gestational diabetes • protracted labor/arrest of labor/failure to progress • post-partum hemorrhage	• stillbirth • low birth weight • small for gestational age • intrauterine growth restriction • pre-term birth	• cerebral palsy • intraventricular hemorrhage • ARDS • Necrotizing enterocolitis • Cerebral palsy • NICU admission/ length of hospital stay • Neonatal abstinence syndrome • Congenital malformation • Sudden infant death syndrome (SIDS)	• Reproductive capacity (offspring gametogenesis) • Neurodevelopment • Neuropsychiatric disturbance • Substance use/abuse

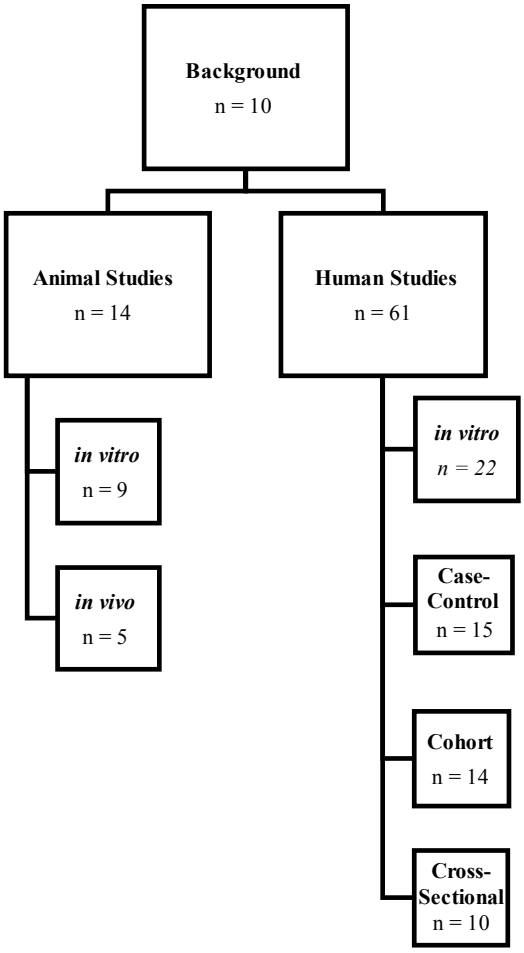

Fig. 1. Graphic representation of types of scholarly articles included in this review. A total of 10 meta-analyses were used for background and to identify gaps in literature. Animal studies included 9 *in vitro* and 5 *in vivo* experiments. Human studies included 14 cohort, 15 case-control, and 10 cross-sectional studies.

3.4. Placenta

In vitro studies of both post-partum placental cells and laboratory grown cell lines have demonstrated significant effects of THC on the expression of genes which regulate everything from cell morphology, migration, and apoptosis, to tissue microvascularization and endocannabinoid system homeostasis.

In a 2006 *in vitro* study, THC inhibited cell-cycle progression of BeWo trophobolast cells, through a purported downregulation of histone deacetylase as well as an upregulation of the pro-apoptotic genes Dip1 and CARP-1 [31]. A study using human placental trophoblastic cells, however, demonstrated a cytoprotective effect of THC as addition of the compound doubled reduced glutathione (GSH) concentration, decreased glutathione disulfide (GSSG) concentration by 50 %, and increased cellular ATP concentration by 55 %. This proved to be a dubious cytoprotective effect, however, as proper placentation relies on carefully coordinated apoptosis at certain points [32].

THC was also shown to decrease trophoblast motility and invasion of trophoblastic tissue by both BeWo and HTR-8-SVneo cells by decreasing phosphorylation of STAT3, a key protein in early embryonic development. By decreasing expression of the genes GCM-1, HERVW-1, HERVFRD-1, which code for the proteins syncytin-1 and syncytin-2, THC also decreased differentiation of cells from the cytotrophoblast to syncytiotrophoblast stage [33].

In a study using human primary amniotic epithelial cells (hAECs) and WISH cells, THC was again shown to inhibit migration and proliferation of trophoblastic cells, this time through decreased expression of MMP2 and MMP9 genes. Interestingly, both of these effects were more pronounced in cells obtained from pre-term placentas [34]. When applied to immortalized human endometrial stromal cells (THESCs) THC produced an overall decrease in the morphological changes which comprise decidualization, delayed migration of blastocyst trophoectoderm toward deciduum, and led to decreased secretion of prolactin from decidual tissues [35].

Studies in both post-partum placental cells and HUVE cells demonstrated abnormal microvascularization as a result of THC administration, likely *via* a downregulation of the RhoA/MLC signaling pathway [33]. Histological analysis of placentas obtained from mothers who self-reported smoking marijuana demonstrated narrowing of maternal sinusoids and increased diameters of trophoblastic septa within the labyrinth zone which would ultimately lead to a greater diffusion distance between maternal and fetal circulation and thus, poor fetal nutrition [36].

In a recent study, THC was noted to induce endoplasmic reticulum (ER) stress and to decrease mitochondrial respiration and ATP coupling by decreasing the expression of

Table 2
Doses and routes of administration reported in various *in vitro* and *in vivo* animal studies of the effects of THC on reproduction. (IV = intravenous injection, IP = intraperitoneal injection, PO = oral administration, GD = gestational day, PND = post-natal day).

Author	Model	Dose	Route	Endpoint	Findings
Lopez-Cardona et. al 2016	Heifer	100 nm in culture medium	*In vitro*	Blastocyst maturation rate	No change in maturation rate of THC exposed embryos, increased expression of genes associated with higher embryo quality
Lopez-Cardona et. al 2018	Mouse	10 mg/kg daily x30 days	PO	Reproductive organ size and histology	No change in size or histology of testes/epididymis
Oz et. al 2004	Xenopus frog	100 nm in culture medium	*In vitro*	α7nAch receptor activity	THC did not inhibit α7nACh receptor
Dhawan et. al 2003	Wistar strain rats	10 mg/kg daily x30 days	PO	Mounting behavior, sperm count, # females impregnated	Chronic THC exposure led to decreased all measured aspects of reproductive functioning
Morgan et. al 2012	CD1 mice	0.001/0.01.0.1/1/10 μM in culture medium	*In vitro*	Sperm motility, ATP production	Dose-dependent decrease in sperm motility and ATP production
DiNieri et. al 2011	Long-Evans rats	0.15 mg/kg daily GD5-PND2	IV	NAc functioning in offspring	Deresed dopamine D2 receptor mRNA, increased opiate reward sensitivity in NAc of exposed male offspring
Lombard et. al 2011	C57BL/6 mice	20-50 mg/kg (weight-based) on GD16	IP	Thymic development, T cell function	Acute prenatal THC exposure associated with thymic atrophy, fewer double-positive T cells, decreased response to HIV-1 p17/p24/gp120 antigens

mitochondrial complex proteins in trophoblasts. The authors posit that the effect of THC on these ubiquitous cellular mechanisms may underlie its negative effects on some of the aforementioned pathways. While these efforts in elucidating a common cytotoxic mechanism are noteworthy, it is important to remember that ER stress is a ubiquitous cytotoxic mechanism that is not specific to THC [37].

Given that appropriate AEA "tone" is pivotal to proper placentation and pregnancy maintenance, a study was conducted to examine the effects of THC on placental AEA concentrations. Though total AEA concentrations were virtually unchanged throughout the experiment, researchers noted a temporal pattern in the expression of enzymes responsible for the synthesis of AEA, NAPE-PLD as well as enzymes involved in its breakdown, such as FAAH [38,39]. Taken together, studies on this topic suggest THC hinders important cellular processes involved in placentation and pregnancy maintenance in animals and humans, and can thus threaten or even prevent pregnancy. The above *in vitro* human studies are summarized in Table 3.

3.5. Obstetrical outcomes

The U.S., the Centers for Disease Control and Prevention estimate that 700 women die from pregnancy-related complications annually, with 60 % of those considered to result from preventable causes [40]. Given that substance use is a modifiable risk factor for health complications, is it necessary to examine the role of marijuana consumption and negative obstetrical outcomes such as miscarriage, placental abruption, and hypertensive disorders.

3.5.1. Spontaneous abortion

In a study of 500 urban women, Coleman-Cowager and colleagues found a 12-fold increase in the risk of non-live birth in mothers who reported marijuana use during pregnancy. It is important to note however, that the statistical analysis made no distinction between stillbirth and spontaneous abortion, examining only the likelihood of live *vs*. non-live birth as a function of marijuana use. Furthermore, data regarding pregnancy outcomes and substance use was obtained *via* self-report, which leaves outcomes vulnerable to misclassification. Finally, the fact that data reported in this study were gathered from a primarily African American population, the generalizability of this study is tenuous [41].

In a study using 1994–2017 data from the U.S. Women's Interagency HIV Study, researchers found a statistically significant increase in the risk of spontaneous abortion in women who used marijuana at least once per day throughout pregnancy. While this study makes a compelling argument regarding the dose-dependent relationship between marijuana consumption and miscarriage, researchers emphasize that high HIV viral load, medication non-compliance, and tobacco use predicted spontaneous abortion more reliably than marijuana use [42]. A study examining the effects of pre-conception marijuana consumption association with spontaneous abortion, suggesting the preconception period may be less susceptible to insult by THC than the pregnancy period itself [43].

3.5.2. Placental abruption

Though data on timing and degree of use was not collected, a retrospective cohort study of over 650,000 women in Canada demonstrated an increased likelihood of placental abruption in mothers who used marijuana during pregnancy [43]. In a case-control study in a low socioeconomic area in Australia, researchers failed to demonstrate a correlation between marijuana use during pregnancy and placental abruption. It should be noted, however, this study also failed to demonstrate a correlation between cigarette smoking and abruption, a well-established relationship in obstetrical literature, and thus the validity of these results may be questionable [44,45].

3.5.3. Hypertensive disorders

In another retrospective cohort study researchers failed to demonstrate an association between marijuana use and hypertensive disorders such as pre-eclampsia and eclampsia, when controlling for concurrent tobacco use. The researchers took care to collect self-report data *via* trained research personnel rather than healthcare providers, which may strengthen validity of self-report data [46].

To our knowledge, no studies exist on the relationship between THC and other obstetrical complications such as postpartum hemorrhage, amniotic fluid embolism, emergency cesarean section, placenta/vasa previa, gestational diabetes, and protracted labor. While several studies have linked marijuana consumption during pregnancy with potentially catastrophic complications such as stillbirth and placental abruption, the above data come from studies subjected to a lack of standardization and participants not representative of the general population, and must thus be interpreted with caution.

3.5.4. Stillbirth

In 2014 a seminal case-control study using data from the Stillbirth Collaborative Research Network demonstrated an increased risk of stillbirth in mothers exposed to marijuana [48]. While the researchers worked to control for self-reporting bias by verifying data through urine toxicology screening, it should be noted that verification occurred primarily in subjects who were white, had commercial insurance, and delivered at a later gestational age. A later study examining birth records of over 200,000 women again demonstrated equivocal results regarding marijuana consumption and increased risk of stillbirth, however, exposure was associated with increased risk for small for gestational age (SGA) and spontaneous preterm birth [49].

3.6. Neonatal outcomes

According to Harrison and Goodman, between 2007–2012 NICU admission rates across the U.S. rose from 64.0 to 77.9 neonates per 1000 births [47]. As healthcare costs continue to rise it becomes increasingly important to identify and intervene upon modifiable risk factors to such increased healthcare usage.

3.6.1. Low birth weight, IUGR, small for gestational age

Several recent studies have examined the relationship between marijuana use and low birth weight. A 2016 meta-analysis revealed an increased likelihood of low birth weight in women exposed to marijuana during pregnancy [50]. However, in the same year, another meta-analysis noted no association between exposure and low birth weight when controlling for maternal tobacco use [51]. In an attempt to broaden the generalizability of this line of inquiry, researchers examined the association between marijuana use and low birth weight in Aboriginal women in South Australia. When controlling for education level, researchers were able to demonstrate an association between marijuana use in pregnancy and low birth weight and SGA [52].

In a retrospective cohort study of women who initiated prenatal care at a university based hospital, no association existed between marijuana use and low birth weight when controlling for adequacy of prenatal care [53]. Interestingly, given current trends in marijuana legalization, a cross-sectional study of over 3000 pregnancies in post-legalization Colorado demonstrated an increase in incidence of low birth weight in prenatal marijuana users [54]. The same year, a population-based study of over 9000 women elsewhere failed to demonstrate an association between marijuana use and low birth weight when controlling for tobacco use [55]. When ascertained by urine toxicology screening, prenatal marijuana use was found to be associated with low birth weight, however, it should be noted that due to variability in drug half-life and excretion, urine toxicology may only detect exposures occurring within 3–7 days [56].

A recent study of the relationship between prenatal marijuana exposure and low birth weight suggests the effects of prenatal exposure may be mediated by the sex of the offspring. In a study of over 1000 mother-baby dyads, male infants exposed to marijuana during the prenatal period exhibited an average 153.09 g decrease in birth weight when compared to un-exposed male infants. This association persisted even when controlling for gestational age at birth. Conversely, exposed female infants did not exhibit any change in birth weight [57].

In a study of 7452 mothers, marijuana use in pregnancy was associated with intrauterine growth restriction (IUGR) and small head circumference. Importantly, however, this association existed only in mothers who used marijuana during pregnancy and was not shown in those who only endorsed pre-pregnancy use, mirroring the 2019 findings of Corsi and colleagues regarding the relationship between marijuana and spontaneous abortion. Here the researchers posited that prenatal marijuana exposure may hinder intrauterine growth *via* the same mechanism of carboxyhemoglobin-induced fetal hypoxia understood to underline IUGR associated with maternal cigarette smoking [58].

3.6.2. Pre-term birth

Inquiries have also been made into the association between maternal marijuana use and preterm birth. From data collected from over 20,000 women, a statistically significant association was found between prenatal marijuana consumption and preterm birth [59]. In one of the few studies examining the temporal relationship between marijuana use and pregnancy outcomes, continuation of use past 20 weeks of pregnancy was associated with greater risk of preterm birth in a large prospective cohort study of nulliparous women [60].

3.6.3. Complications of prematurity

Research into neonatal outcomes such as complications of prematurity and NICU admission rates has again produced mixed results. Researchers conducted a secondary analysis of data obtained from a randomized controlled trial evaluating the benefits of magnesium sulfate in the prevention of cerebral palsy in babies born prior to 35 weeks' gestation. Using self-report data regarding maternal marijuana use during pregnancy, researchers evaluated whether *in utero* exposure was associated with increased risk for known complications of prematurity such as intraventricular hemorrhage, necrotizing enterocolitis, bronchopulmonary dysplasia, periventricular leukomalacia, and cerebral palsy and were unable to demonstrate a correlation [61].

A 2015 retrospective cohort study demonstrated an increased likelihood of NICU admission in mothers exposed to marijuana during pregnancy [62]. A study comparing incidence of the same outcome in pre-and-post-legalization Colorado demonstrated an increased incidence of NICU admission, however, researchers did not examine specific reasons for admission [63]. Given that NICU admission is often the result of prematurity, infection, or signs of neonatal distress, Gray and colleagues examined whether prenatal marijuana exposure was associated with lower Apgar scores. In a study of 86 mothers who admitted to marijuana use during pregnancy, the researchers did not find any association between prenatal marijuana exposure and lower Apgar scores at 1 and 5 min after birth [64].

3.6.4. Neonatal abstinence syndrome

Neonatal abstinence syndrome (NAS) is a well-known consequence of maternal substance use in pregnancy, however, most research on the topic focuses on mothers who use opioids during pregnancy. A step toward evaluating for the presence of NAS in neonates exposed to marijuana in utero was made through a retrospective cohort study of 191 infants of opioid dependent-buprenorphine treated mothers who admitted to marijuana consumption during the third trimester of pregnancy. Researchers found no increased risk of low Apgar scores, NAS symptoms, or longer NICU stay in infants exposed to marijuana during the third trimester of pregnancy. As with the majority of the human studies discussed in this review, the above data must be considered in the context of polysubstance use [65]. Neonates born to mothers who used marijuana during pregnancy also exhibited more tremors, a more pronounced startle response, and greater nighttime arousal compared to their non-exposed peers [66].

3.6.5. Sudden infant death syndrome (SIDS)

Sudden infant death syndrome remains a tragic and perplexing affliction of the newborn. In an effort to examine whether prenatal marijuana exposure may increase the risk of SIDS, Klonoff-Cohen and Lam-Kruglic conducted a study of 239 SIDS cases and controls matched by age, sex, date of birth, and even birth hospital. The researchers found no association between self-reported maternal marijuana use during pregnancy and sudden infant death syndrome. Upon expanding the study to examine the fathers, the researchers found a fascinating two-fold increase in SIDS risk associated with paternal marijuana consumption. Despite this strong

Table 3
The models, THC doses, endpoints, and findings of *in vitro* studies using human tissues are summarized above.

Author	Model	THC Dose	Endpoint	Findings
Badawy, Z et. al 2009 Whan et. al 2004	Washed sperm, semen Washed sperm	20 mg/mL in 95% EtOH solution Therapeutic (0.032 μM) or recreational (0.32 μM) plasma concentration	Cellular respiration Motility	THC inhibited respiration of neat sperm, seminal fluid mitigated this effect Dose-dependent ↓ in motility
Lojpur, T. et. al 2019	BeWo trophoblast cells	3-30 μM	Endoplasmic reticulum stress	Dose-dependent ↑ in markers of ER stress, upregulation of ER stress-sensitive genes ERRγ, VEGFA, FLT-1
Maia, J et. al 2019	Villous explants from term placentas	1-40 μM	ECS functioning	All concentrations disrupt ratio of enzymes which synthesize and degrade AEA, respectively, but only 40 μM led to increase in AEA level itself
Neradugomma, N. K. et. al 2019	THESCs	0.2-20 μM	Decidualization	Inhibition of decidualization at dose of 0.5 μM, cytotoxicity at 20 μM
Chang, X et. al 2018	HUVE cells	20 μM	Endothelial cell migration	Inhibition of endotheial cell migration via Rho/MLC pathway
Chang, X. et. al 2017	BeWo and HTR-8/SVneo cells	0.3-30 μM	Cell migration	Concentrations ≥ 15 μM inhibited migration via STAT3 pathway dysregulation
Costa, M. A. et. al 2015	Cytotrophoblasts, syncytiotrophoblasts from term placentas	10-75 μM	Reactive oxygen species	1-25 μM ↓ ROS while 75 μM ↑. 10 μM ↑GSH/GSSG ratio
Keating, E. et. al 2009	Cytotrophoblasts from term placentas	Acute: 0.001-1 μM for 26 min Chronic: 0.001-0.1 μM for 48 hrs	Folic acid uptake	Folic acid uptake decreased with chronic, but not acute, exposure
Khare, M. et. al 2006	BeWo cells	0.3-30 μM	Cellular proliferation	Concentrations ≥ 20 μM led to ↓ expression of transcription factors and ↓ cellular proliferation

correlation, the authors were unable to propose a convincing biological mechanism which may underlie this phenomenon [86].

To our knowledge, no data exists regarding a relationship between in utero THC exposure and other specific neonatal outcomes such as jaundice, acute respiratory distress syndrome (ARDS), and retinopathy of prematurity. While studies offering quantitative evidence of the effects of prenatal marijuana exposure on the incidence of stillbirth, low birth weight, and preterm birth lend support the proposed negative effects of marijuana on neonates, in many studies these effects disappear when controlling for major confounds such as concurrent tobacco use or adequacy of prenatal care. Furthermore, while studies demonstrating higher rates of NICU admission raise concerns over marijuana consumption leading to greater healthcare usage, such increases in the absence of evidence of neonatal distress may be more indicative of changing hospital policies, rather than negative effects of marijuana on newborn health.

3.7. Long-term effects

While it is difficult to parse the effects of prenatal events from outside influences as a child ages, much research has been conducted in order to elucidate the long-term effects of parental marijuana use on offspring wellbeing.

3.7.1. Congenital defects

Several animal and human studies have examined the relationship between THC and congenital birth defects in order to elucidate whether parental marijuana use leads to long-term sequelae in exposed offspring. An analysis of birth records from 1986 to 2002 in Hawaii demonstrated a positive correlation between maternal marijuana use in pregnancy and increased risk for congenital defects such as phocomelia and cleft lip/palate [67]. Data from the Colorado Responds to Children with Special Needs (CRCSN) study on birth defects, along with data regarding the prevalence of marijuana use from the SDUH and Drug Enforcement Administration demonstrates an increase in birth defects such as spina bifida, trisomy 21, and various cardiovascular malformations out of proportion to the birth rate between 2000–2014, a period where the prevalence of only marijuana use rose as compared to other illicit substances [68].

A case control analysis of data from the National Birth Defects Prevention study demonstrated an association between periconception marijuana consumption and an increased risk for anencephaly. Researchers were unable, however, to demonstrate the same relationship between marijuana and the over 20 other congenital defects studied [69]. In a secondary analysis of the data five years later, the researchers employed a strategy to better account for maternal substance use under-reporting and misclassification, and ultimately demonstrated an association between marijuana use in pregnancy and the risk of gastroschisis, esophageal atresia, and diaphragmatic hernia in offspring [70].

A case-control study of 122 neonates found a two-fold increase in risk of isolated simple ventricular septal defect in children whose mothers admitted to using marijuana during pregnancy when compared with over 3000 controls [71]. Finally, data from the Canadian Pediatric Surgery Network (CAPSNET) database was analyzed regarding the progress of 114 infants born with gastroschisis as a function of maternal substance use in pregnancy. In general, maternal substance use was associated with an increased risk of varying degrees of bowel necrosis, with marijuana specifically associated with an increased risk for matting of the bowel [72].

3.7.2. Neuropsychiatric disturbance

In a study of human cord blood induced pluripotent stem cell (hCBiPSCs)-derived neural precursor cells exposed to the endocannabinoid AEA or THC at high and low concentrations, high concentrations of both types of cannabinoids led to decreased ion channel currents and synaptic function. Interestingly, a similar, though diminished effect was noted at low levels of AEA, but not THC, suggesting a critical dose at which THC begins to cause harm to this particular cell type [73].

Researchers studying the brains of fetuses collected from elective abortions at 18–22 weeks' gestation from mothers who endorsed marijuana use during pregnancy decreased expression of SCG10/stathmin-2, a microtubule-binding axonal protein involved in cytoskeletal development, in the brains of fetuses exposed to marijuana in utero. They went on to posit that CB1 activation by THC in the fetal brain likely recruits c-Jun to phosphorylate and thus degrade SCG10, leading to abnormal axonal development [74].

Marijuana's history as a psychogenic drug of abuse raises serious concerns for long-term neuropsychiatric functioning of offspring exposed to it *in utero*. In a longitudinal study of 6356 adolescents as part of the Avon Longitudinal Study of Parents and Children (ALSPAC), researchers found maternal tobacco use during pregnancy was associated with psychotic symptoms in offspring, however, no association existed between marijuana use in pregnancy and psychosis [75]. A more recent study analyzing data from the Adolescent Brain Cognitive Development (ABCD) study demonstrated a small increase likelihood of psychosis in offspring exposed to marijuana in utero, specifically if maternal marijuana use continued beyond 7.7 weeks' gestation [76]. In a prospective cohort study of 596 mothers followed from 4 months' gestation until offspring age 22, Day and colleagues demonstrated an increased risk of psychotic symptoms in offspring whose mothers used marijuana during pregnancy. Given the difficulty of parsing the effects of prenatal events from a child's surroundings as he ages, the researchers took care to control for early onset marijuana consumption in the offspring themselves, and the aforementioned association persisted [77].

3.7.3. Cognitive impairment

Given the purported association between marijuana use and psychiatric disturbance, researchers set to work exploring the effects of prenatal exposure to THC-containing products on cognitive functioning. Noland and colleagues measured the performance of 330 four-year-olds whose mothers endorsed marijuana use during pregnancy on several attention tasks. When compared with non-exposed controls, children whose mothers used marijuana during pregnancy committed significantly more errors on each measure of attention [78]. In a prospective cohort study of 524 mothers recruited during pregnancy and offspring followed up at age 14, researchers found that first trimester prenatal marijuana exposure was associated with impaired reading ability as measured by the Wechsler Individual Achievement Test (WIAT) Screener. This effect was not observed for any other skills measured by the WIAT. The researcher also did not

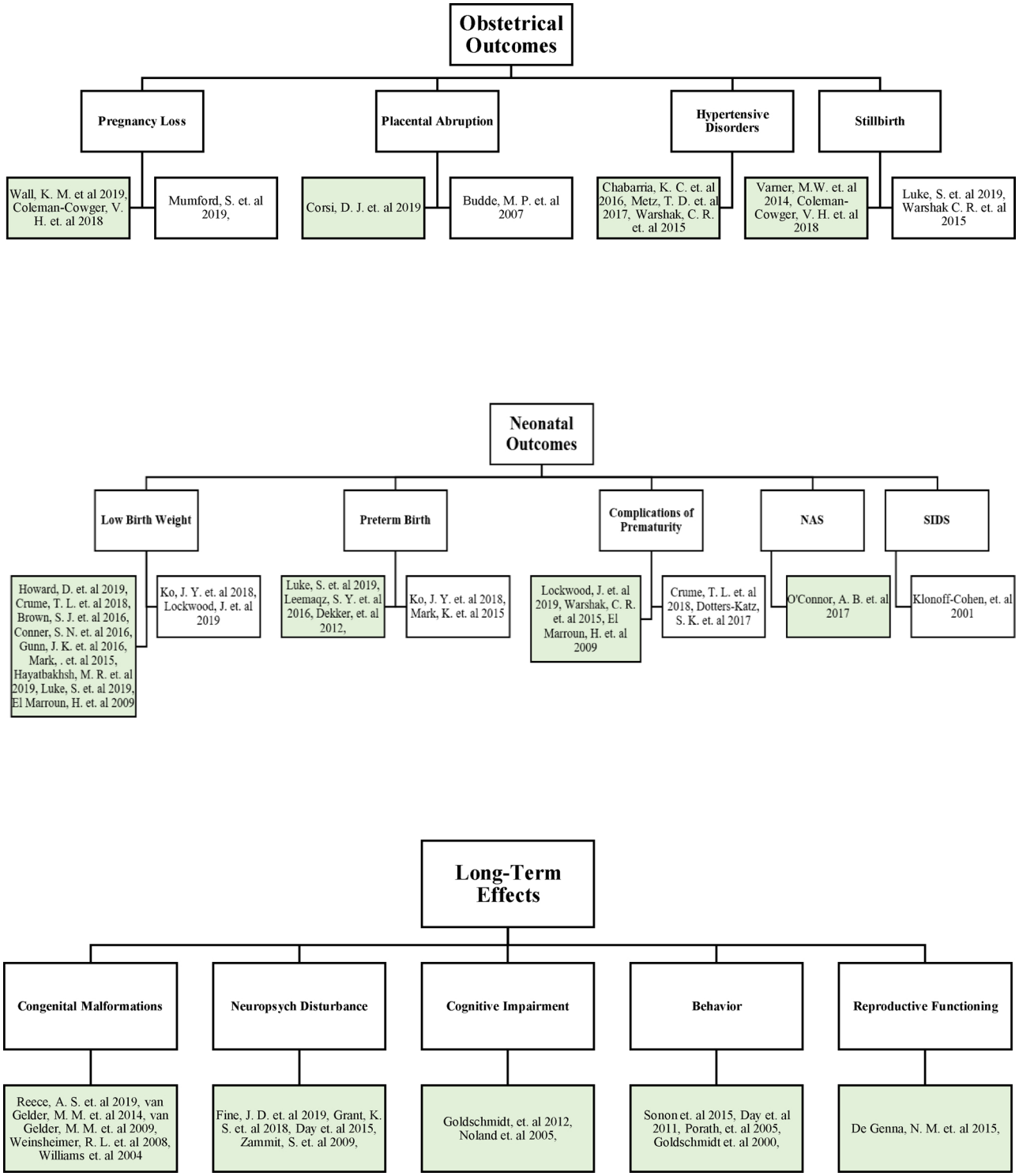

Fig. 2. Graphic representation of the human *in vivo* studies discussed in this review. Under each endpoint, the item on the left (in green) lists all studies which found a positive correlation between marijuana consumption and the given endpoint. The item on the right lists studies which did not demonstrate any correlation (this is omitted for endpoints where no studies demonstrating a lack of correlation exist.) Some studies appear more than once as they address multiple endpoints.

find any association between marijuana exposure during the second or third trimesters of pregnancy, suggesting the first trimester may be particularly susceptible to disturbances affecting later cognitive ability [79].

3.7.4. Behavior

Numerous human and animal studies provide evidence for increased risk of substance use in offspring exposed to marijuana during the prenatal period. In a study using aborted 18–22 week human fetuses exposed to marijuana in utero, researchers noted decreased expression of the D2 dopamine receptor in the nucleus accumbens (NA), a brain area central to reward processing. In order to evaluate the long-term significance of this finding in offspring, researchers exposed pregnant rats to THC and noted that offspring exhibited fewer D2 receptor sites in the NA, and greater reward sensitivity in opiate self-administration trials. While this data suggests in utero THC exposure may predispose offspring to addiction later in life, it should be noted that in this study researchers only evaluated the brains of male offspring [80].

In a prospective cohort study of 152 offspring aged 16–21, Porath and Fried found a dose-dependent association between degree of maternal marijuana use during pregnancy and likelihood of cigarette and marijuana use in offspring. Interestingly, this effect was even more pronounced in male offspring [81]. Sonon and colleagues followed 763 offspring until the age of 22 in a prospective cohort study and found that prenatal exposure to marijuana was associated with an increased risk of marijuana use in offspring regardless of

age, race, gender, and even first trimester alcohol exposure [82].

The discussion of the use of an (at least somewhat) illegal substance would not be complete without consideration of its relationship with delinquent behavior. In a prospective cohort study of 565 families, Goldschmidt and colleagues evaluated offspring of mothers who admitted to marijuana use during pregnancy using the Child Behavior Checklist, Teachers Report Form, and Swanson, Noland, and Pelham (SNAP) checklist and found prenatal marijuana exposure to be associated with increased risk of impulsivity, hyperactivity, and delinquent behavior at age 10 [83]. A similar study of 580 mother-child dyads demonstrated that children exposed to one or more joints per day during the prenatal period were more likely to exhibit delinquent behavior at age 14 [84].

3.7.5. Reproductive functioning

A study of sexual behavior in offspring exposed to marijuana in utero revealed early onset of sexual activity, defined as oral or vaginal sex prior to age 14, than that found in non-exposed peers. It should be noted, however, that the mothers in question were themselves teenagers, age 12–18, when they entered the study, so early sexual activity in this cohort may simply represent cultural or social differences [85]. The cross-sectional and cohort studies described in section 3.3 are summarized in Fig. 2. Taken together, the findings discussed above raise concerns regarding the long-term effects of parental marijuana consumption on offspring physical and emotional wellbeing.

The above literature paints a concerning picture regarding the relationship between parental marijuana use and the risk of congenital malformation, psychosis, cognitive impairment, dangerous or delinquent behavior, and reproductive dysfunction in prenatally exposed offspring. The data is perhaps most convincing when examining the relationship between prenatal marijuana exposure and congenital defects as these defects are often apparent at birth and their diagnoses are not subject to provider interpretation. Although efforts were made to control for substance use in the offspring themselves, as well as a variety of socioeconomic factors, the above data must still be considered within the context of a given child's environment. Specifically, aberrations in cognitive ability and behavior may have more to do with neighborhood influences and priorities than with *in utero* marijuana exposure.

4. Discussion

4.1. Main points

Here we review the most up to date evidence regarding the effects of marijuana on human reproductive functioning. Given the sensitivity of the prenatal period as well as nationwide legalization trends, several notable reviews have already been published on this topic. A 2018 review by Grant and colleagues summarized available data regarding the effects of prenatal marijuana exposure on cognitive development in offspring [87]. In 2019, Guille and Aujla described a body of work wherein maternal cannabis use was associated with impaired executive and intellectual functioning in children whose mothers consumed marijuana during pregnancy [88]. Thomas and colleagues examined the prenatal and neonatal periods more closely in their 2019 review of the association between maternal marijuana use and negative obstetrical outcomes such as miscarriage and preterm birth [89]. Our aim is to build upon this work with a review that is, to our knowledge, the first to encompass virtually all aspects of human reproduction from gametogenesis, embryo quality, implantation, placentation, and obstetric course, to neonatal and long-term neuropsychiatric and reproductive outcomes in offspring. The literature presented here for the first time makes clear that exposure at any stage of life may carry previously undescribed consequences for human reproduction in parents and offspring.

Semen analyses of men who reported varying degrees of marijuana use have shown abnormalities in sperm count, concentration, motility, cellular respiration, and morphology. Marijuana consumption was also associated with longer follicular phase length in the female reproductive cycle, however, this finding was not supported by increased TTP in women actively attempting to become pregnant. Although to our knowledge no *in vitro* studies of the effects of THC on human oocytes exist, research into the relationship between marijuana use and assisted reproduction demonstrated lower numbers of harvested oocytes and viable embryos fertilized in women who used marijuana.

Given the sensitive nature of the prenatal period, the effects of marijuana exposure on pregnancy and neonatal outcomes continue to be of great interest. Numerous studies support an association between marijuana use either prior to or during pregnancy with low birth weight, preterm birth, and even pregnancy loss. However, when controlling for concurrent tobacco use and a variety of socioeconomic factors, many studies failed to replicate this relationship. Studies examining the effects of maternal marijuana use on neonatal outcomes show increased rates of NICU admission following delivery, however, the lack of consensus regarding the medical need for such admissions may be more suggestive of changing hospital policies rather than actual neonatal health outcomes.

Although many animal studies clearly demonstrate negative effects of THC on reproductive functioning, human lives are complicated by disparities in socioeconomic status as well as variability in the makeup of the marijuana people consume. Thus, while it is reasonable to conclude that THC, one of myriad compounds found in marijuana, may have negative effects on numerous aspects of human reproduction, it is not possible to definitively describe those effects or generalize them to all marijuana products at this time.

4.2. Strengths and limitations

While literature reviews do not provide the strongest level of scientific evidence, the high caliber of research included in this review makes it a strong contribution to the study of reproductive toxicology. First, while no randomized controlled trials of the effects of marijuana on human reproduction exist, this review features 15 case-control, 14 cohort studies, and 10 cross-sectional studies, the strongest study designs possible given the ethical limitations of human research. Furthermore, we include 22 human *in vitro* experiments demonstrating the effects of THC specifically on various human tissues. We also describe 9 *in vitro* and 5 *in vivo* animal studies in support of the effects of THC exposure on the animal as a whole as well as its offspring.

Despite significant efforts to maximize generalizability, the scope of this work is limited in several important ways. First, we focused only on papers written in English and published within the last twenty years. Whenever possible, we selected studies on the effects of THC which is only one of the myriad bioactive compounds found within marijuana. Due to ethical limitations, *in vivo* human studies described here were exclusively cohort or cross-sectional in nature. While the animal studies we described to address gaps in understanding used models with similar reproductive biology to that of humans, data obtained from such studies is not completely generalizable to humans as these studies used rigorously controlled doses and routes of administration which are not possible to ensure in humans. Specifically, many studies exposed reproductive tissues to THC *in vitro*, or administered THC intravenously or *via* the intraperitoneal route, while humans typically consume marijuana through smoking. While researchers made efforts in some cases to administer THC to animals orally in order to mimic THC-containing edibles, the plasma concentration of THC resulting from the various weight-based dosing protocols and how these compare to plasma concentrations found in humans who consume marijuana are unknown.

The human studies included in this search were complicated by many confounding factors. First, all studies relied heavily on self-report data which is subject to recall bias and underreporting due to social pressures, especially when data is collected by a participant's own healthcare provider. While verification of self-report data by toxicology screening improves validity to some degree, it is important to note that variability in drug excretion and half-life may affect detection in screening tests. Furthermore, toxicology screening methods may identify only one or two compounds found within marijuana, while the actual negative endpoints in this study may be the result of contaminants in THC-containing products or simply the act of smoking itself. Much of the available research on this topic was also conducted on samples not immediately generalizable to the general population. For example, some studies used primarily African American populations, while others gathered data only from mothers who were HIV-positive or undergoing substance abuse rehabilitation.

Second, since the marijuana in question was not provided by the researchers, there was no standardization in routes of administration or doses of various cannabinoids to which participants were exposed. Available studies for the most part examined marijuana use only in a qualitative, rather than quantitative manner. Efforts to quantify marijuana consumption invariably fell short of precise classification as researchers used arbitrary amounts such as "less than or equal to 3 joints per day" to establish a dichotomy between heavy and light use. This lack of standardization creates an unclear picture of the effects of timing and degree of use on various measures of reproductive health.

While we examined the effects of parental marijuana use on offspring in the neonatal period and beyond, it is important to note that long-term neuropsychiatric development is subject to innumerable influences such as quality of parenting and socioeconomic status and thus it is impossible to attribute the development of psychosis, cognitive disability, and delinquency entirely to parental marijuana exposure.

Finally, while studies such as the NSDUH make strides towards quantifying the prevalence of marijuana use in the U.S., the lack of standardized definitions for marijuana use, variability in THC content of various cannabis strains, advent of non-combustible THC-containing products, and changing attitudes in the setting of widespread legalization, make it difficult to reliably predict the scope of this problem at the population level.

4.3. Future directions

Given the inconclusive data presented above, further research is needed to parse the effects of marijuana use on reproductive health from confounds and to identify critical time points and degrees of exposure. Care should be taken to minimize confounds such as concurrent tobacco or other substance use, adequacy of prenatal care, gravity/parity, and socioeconomic factors such as race, age, income, health insurance, education, desired pregnancy, and local legal status of marijuana. More detailed substance use histories should be obtained to delineate duration and degree of use, as well as route of administration, specific strain consumed, and timing of use as it relates to a given pregnancy.

Assisted reproduction technologies such as IVF provide a unique opportunity to study the effects of marijuana on human gametocytes and embryos. Fetal tissue from elective abortions should also continue to be studied in order to elucidate the effects of THC on later stages of fetal development. Finally, offspring of marijuana users should be followed and evaluated for long-term developmental and reproductive sequelae of marijuana exposure. Given some limited evidence of sex differences in the effects of prenatal marijuana exposure on birth weight and offspring reproductive functioning, these differences in risk should continue to be explored.

Inquiries should also be made into protective factors. The aforementioned study by Dhawan and colleagues demonstrated a protective effect of benzoflorane moiety extracted from *Passiflora incarnate*, a type of passionflower, when co-administered to sperm with THC [19]. Such information may be particularly helpful for patients who prefer to use THC-containing products over traditional pharmaceuticals for cultural or other reasons.

The above data regarding the effects of ECS dysregulation on the quality of gametocytes, as well as associations between marijuana use and negative obstetric and offspring outcomes present a strong cause for concern. Given that THC is only one of the over 70 known compounds in cannabis, future exploration should also be aimed at elucidating the effects of other, more poorly-understood marijuana constituents, as well as the effects of other THC-containing preparations such as edibles and vape cartridges. While this data does not provide conclusive evidence of a ubiquitously negative impact of either THC specifically or marijuana generally on human reproduction, healthcare providers should continue to counsel patients against its use, especially during pregnancy, until convincing evidence of its safety comes to light.

Despite some disquieting findings, this data also suggests that aspects of the ECS may be targeted therapeutically in order to optimize reproductive functioning. Our hope is that this review will point out gaps in the literature and help direct future researchers in answering these questions.

Funding

The researchers did not receive special funding in support of this work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to acknowledge their respective academic institutions, Touro College of Osteopathic Medicine – New York and SUNY Downstate Medical Center Department of Obstetrics and Gynecology, for their support in completing this work.

References

[1] U.S. Department of Health and Human Services, Substance Abuse and Mental Health Services Administration, Center for Behavioral Health Statistics and Quality. (2018), National Survey on Drug Use and Health, 2016 NSDUH-2016-DS0001.
[2] Q.L. Brown, A.L. Sarvet, D. Shmulewitz, S.S. Martins, M.M. Wall, D.S. Hasin, Trends in marijuana use among pregnant and nonpregnant reproductive-aged women, 2002-2014, JAMA. 317 (2) (2017) 207–209, https://doi.org/10.1001/jama.2016.17383.
[3] E.L. Seaman, K.M. Green, M.Q. Wang, S.C. Quinn, C.S. Fryer, Examining prevalence and correlates of cigarette and marijuana co-use among young adults using ten years of NHANES data, Addict. Behav. 96 (2019) 140–147, https://doi.org/10.1016/j.addbeh.2019.04.014.
[4] K.C. Young-Wolff, V. Sarovar, L.-Y. Tucker, et al., Trends in marijuana use among pregnant women with and without nausea and vomiting in pregnancy, 2009–2016, Drug Alcohol Depend. 196 (2019) 66–70, https://doi.org/10.1016/j.drugalcdep.2018.12.009.
[5] H. Bayrampour, M. Zahradnik, S. Lisonkova, P. Janssen, Women's perspectives about cannabis use during pregnancy and the postpartum period: an integrative review, Prev. Med. 119 (2019) 17–23, https://doi.org/10.1016/j.ypmed.2018.12.002.
[6] Cl L. Holland, D. Rubio, Kl L. Rodriguez, et al., Obstetric health care providers' counseling responses to pregnant patient disclosures of marijuana use, Obstet. Gynecol. 127 (4) (2016) 681–687, https://doi.org/10.1097/aog.0000000000001343.
[7] F. Fezza, M. Bari, R. Florio, E. Talamonti, M. Feole, M. Maccarrone, Endocannabinoids, Related Compounds and Their Metabolic Routes, Molecules. 19 (11) (2014) 17078–17106, https://doi.org/10.3390/molecules191117078.
[8] P.J. Brighton, T.H. Marczylo, S. Rana, J.C. Konje, J.M. Willets, Characterization of the endocannabinoid system, CB1 receptor signalling and desensitization in human myometrium, Br. J. Pharmacol. 164 (5) (2011) 1479–1494, https://doi.org/10.1111/j.1476-5381.2011.01425.x.
[9] L. Peralta, E. Agirregoitia, R. Mendoza, A. Expósito, L. Casis, R. Matorras, et al., Expression and localization of cannabinoid receptors in human immature oocytes and unfertilized metaphase-II oocytes, Reprod. Biomed. Online 23 (2011) 372–379.
[10] Y.S. Weems, A.W. Lewis, D.A. Neuendorff, R.D. Randel, C.W. Weems, Endocannabinoid 1 and 2 (CB1; CB2) receptor agonists affect negatively cow luteal function in vitro, Prostaglandins Other Lipid Mediat. 90 (3-4) (2009) 89–93, https://doi.org/10.1016/j.prostaglandins.2009.09.003.
[11] E. De Domenico, F. Todaro, G. Rossi, et al., Overactive type 2 cannabinoid receptor induces meiosis in fetal gonads and impairs ovarian reserve, Cell Death Dis. 8 (10) (2017) e3085, https://doi.org/10.1038/cddis.2017.496.
[12] T. Chioccarelli, Cannabinoid receptor 1 influences chromatin remodeling in mouse spermatids by affecting content of transition protein 2 mRNA and histone displacement, Endocrinology (Philadelphia). 151 (10) (2010) 5017–5029, https://doi.org/10.1210/en.2010-0133 10/.
[13] P. Grimaldi, The endocannabinoid system and pivotal role of the CB2 receptor in mouse spermatogenesis, Proceedings of the National Academy of Sciences - PNAS 106 (27) (2009) 11131–11136, https://doi.org/10.1073/pnas.0812789106 07/.
[14] H. Wang, H. Xie, X. Sun, et al., Differential regulation of endocannabinoid synthesis and degradation in the uterus during embryo implantation, Prostaglandins Other Lipid Mediat. 83 (1-2) (2007) 62–74, https://doi.org/10.1016/j.prostaglandins.2006.09.009.
[15] A.W. Horne, J.A. Phillips, N. Kane, et al., CB1 expression is attenuated in fallopian tube and decidua of women with ectopic pregnancy. Aziz SA, ed, PLoS One 3 (12) (2008) e3969, https://doi.org/10.1371/journal.pone.0003969.
[16] E. Trabucco, G. Acone, A. Marenna, et al., Endocannabinoid system in first trimester placenta: low FAAH and high CB1 expression characterize spontaneous miscarriage, Placenta 30 (6) (2009) 516–522, https://doi.org/10.1016/j.placenta.2009.03.015.
[17] M. Maccarrone, Low fatty acid amide hydrolase and high anandamide levels are associated with failure to achieve an ongoing pregnancy after IVF and embryo transfer, Mol. Hum. Reprod. 8 (2) (2002) 188–195, https://doi.org/10.1093/molehr/8.2.188.
[18] National Academics of Sciences, Engineering, and Medicine, The health effects of Cannabis and cannabinoids, The Current State of Evidence and Recommendations for Research. Washing, The National Academies Press, DC, 2017, https://doi.org/10.17226/24625.
[19] K. Dhawan, A. Sharma, Restoration of chronic- Δ9-THC-induced decline in sexuality in male rats by a novel benzoflavone moiety from Passiflora incarnata Linn, Br. J. Pharmacol. 138 (2003) 117–120, https://doi.org/10.1038/sj.bjp.0705015.
[20] T.D. Gundersen, N. Jørgensen, A.-M. Andersson, et al., Association between use of marijuana and male reproductive hormones and semen quality: a study among 1,215 healthy young men, Am. J. Epidemiol. 182 (6) (2015) 473–481, https://doi.org/10.1093/aje/kwv135.
[21] K. Carroll, A.M. Pottinger, S. Wynter, V. DaCosta, Marijuana Use and Its Influence on Sperm Morphology and Motility: Identified Risk for Fertility Among Jamaican Men, Andrologia (2019), https://doi.org/10.1111/andr.12670.
[22] L.B. Whan, Effects of delta-9-tetrahydrocannabinol, the primary psychoactive cannabinoid in marijuana, on human sperm function in vitro, Fertil. Steril. 85 (3) (2006) 653–660, https://doi.org/10.1016/j.fertnstert.2005.08.027 03/.
[23] Z.S. Badawy, Cannabinoids inhibit the respiration of human sperm, Fertil. Steril. 91 (6) (2009) 2471–2476, https://doi.org/10.1016/j.fertnstert.2008.03.075 06/.
[24] D.J. Morgan, C.H. Muller, N.A. Murataeva, B.J. Davis, K. Mackie, Δ9-Tetrahydrocannabinol (Δ9-THC) attenuates mouse sperm motility and male fecundity, Br. J. Pharmacol. 165 (2012) 2575–2583, https://doi.org/10.1111/j.1476-5381.2011.01506.x.
[25] A.P. López-Cardona, Effect of chronic THC administration in the reproductive organs of male mice, spermatozoa and in vitro fertilization, Biochem. Pharmacol. 157 (2018) 294–303, https://doi.org/10.1016/j.bcp.2018.07.045 11/.
[26] A.P. López-Cardona, M.J. Sánchez-Calabuig, P. Beltran-Breña, N. Agirregoitia, D. Rizos, E. Agirregoitia, A. Gutierrez-Adán, "Exocannabinoids effect on in vitro bovine oocyte maturation via activation of AKT and ERK1/2", Reproduction 152.6 (2019) 603–612, https://doi.org/10.1530/REP-16-0199 Web. 2 Jul..
[27] M. Oz, Differential effects of endogenous and synthetic cannabinoids on 7-Nicotinic acetylcholine receptor-mediated responses in Xenopus oocytes, J. Pharmacol. Exp. Ther. 310 (3) (2004) 1152–1160, https://doi.org/10.1124/jpet.104.067751.
[28] A.M.Z. Jukic, C.R. Weinberg, D.D. Baird, A.J. Wilcox, Lifestyle and reproductive factors associated with follicular phase length, J. Womens Health 16 (9) (2007) 1340–1347, https://doi.org/10.1089/jwh.2007.0354.
[29] A.M. Kasman, Association between use of marijuana and time to pregnancy in men and women: findings from the national survey of family growth, Fertil. Steril. 109 (5) (2018) 866–871, https://doi.org/10.1016/j.fertnstert.2018.01.015 05/.
[30] H.S. Klonoff-Cohen, L. Natarajan, R. Victoria Chen, A prospective study of the effects of female and male marijuana use on in vitro fertilization (IVF) and gamete intrafallopian transfer (GIFT) outcomes, Am. J. Obstet. Gynecol. 194 (2) (2006) 369–376, https://doi.org/10.1016/j.ajog.2005.08.020.
[31] M. Khare, 9-Tetrahydrocannabinol inhibits cytotrophoblast cell proliferation and modulates gene transcription, Mol. Hum. Reprod. 12 (5) (2006) 321–333, https://doi.org/10.1093/molehr/gal036.
[32] M.A. Costa, B.M. Fonseca, F. Marques, N.A. Teixeira, G. Correia-da-Silva, The psychoactive compound of Cannabis sativa, Δ9-tetrahydrocannabinol (THC) inhibits the human trophoblast cell turnover, Toxicology. 334 (2015) 94–103, https://doi.org/10.1016/j.tox.2015.06.005.
[33] X. Chang, Y. Bian, Q. He, et al., Suppression of STAT3 signaling by Δ9-Tetrahydrocannabinol (THC) induces trophoblast dysfunction, Cell. Physiol. Biochem. 42 (2) (2017) 537–550, https://doi.org/10.1159/000477603.
[34] J.L. Yao, Q.Z. He, M. Liu, et al., Effects of Δ(9)-tetrahydrocannabinol (THC) on human amniotic epithelial cell proliferation and migration, Toxicology. 394 (2018) 19–26, https://doi.org/10.1016/j.tox.2017.11.016.
[35] N.K. Neradugomma, K. Drafton, D.R. O'Day, et al., Marijuana use differentially affects cannabinoid receptor expression in early gestational human endometrium and placenta, Placenta. 66 (2018) 36–39, https://doi.org/10.1016/j.placenta.2018.05.002.
[36] X. Chang, H. Li, Y. Li, et al., RhoA/MLC signaling pathway is involved in Δ^9-tetrahydrocannabinol-impaired placental angiogenesis, Toxicol. Lett. 285 (2018) 148–155.
[37] T. Lojpur, Z. Easton, S. Raez-Villanueva, S. Laviolette, A.C. Holloway, D.B. Hardy, Δ9-Tetrahydrocannabinol leads to endoplasmic reticulum stress and mitochondrial dysfunction in human BeWo trophoblasts, Reprod. Toxicol. 87 (2019) 21–31, https://doi.org/10.1016/j.reprotox.2019.04.008.
[38] J. Maia, L. Midão, S.C. Cunha, et al., Effects of cannabis tetrahydrocannabinol on endocannabinoid homeostasis in human placenta, Arch. Toxicol. 93 (3) (2019) 649–658, https://doi.org/10.1007/s00204-019-02389-7.
[39] L.W. Chamley, A. Bhalla, P.R. Stone, et al., Nuclear localisation of the endocannabinoid metabolizing enzyme fatty acid amide hydrolase (FAAH) in invasive trophoblasts and an association with recurrent miscarriage, Placenta 29 (11) (2008) 970–975.
[40] E.E. Petersen, N.L. Davis, D. Goodman, et al., Vital signs: pregnancy-related deaths, United States, 2011–2015, and strategies for prevention, 13 states, 2013–2017, MMWR Morb. Mortal. Wkly. Rep. 68 (2019) 423–429.
[41] V.H. Coleman-Cowger, G.L. Schauer, E.N. Peters, Marijuana and tobacco co-use among a nationally representative sample of US pregnant and non-pregnant women: 2005–2014 national survey on drug use and health findings, Drug Alcohol Depend. 177 (2017) 130–135, https://doi.org/10.1016/j.drugalcdep.2017.03.025.
[42] K.M. Wall, L.B. Haddad, C.C. Mehta, et al., Miscarriage among women in the United States women's interagency HIV study, 1994-2017, Am. J. Obstet. Gynecol. 221 (2019), https://doi.org/10.1016/j.ajog.2019.05.034 347.e1-13.
[43] D.J. Corsi, L. Walsh, D. Weiss, et al., Association between self-reported prenatal Cannabis use and maternal, perinatal, and neonatal outcomes, JAMA. 322 (2) (2019) 145, https://doi.org/10.1001/jama.2019.8734.
[44] K. Mark, A. Desai, M. Terplan, Marijuana use and pregnancy: prevalence, associated characteristics, and birth outcomes, Arch. Womens Ment. Health 19 (1) (2015) 105–111, https://doi.org/10.1007/s00737-015-0529-9.

[45] M.P. Budde, T.E. De Lange, G.A. Dekker, A. Chan, Nguyen A-MT, Risk factors for placental abruption in a socio-economically disadvantaged region, J. Matern. Neonatal Med. 20 (9) (2007) 687–693, https://doi.org/10.1080/14767050701482738.
[46] K.C. Chabarria, D.A. Racusin, K.M. Antony, et al., Marijuana use and its effects in pregnancy, Am. J. Obstet. Gynecol. 215 (4) (2016) 506, https://doi.org/10.1016/j.ajog.2016.05.044 e1-506.e7.
[47] W. Harrison, D. Goodman, Epidemiologic Trends in Neonatal Intensive Care, 2007-2012, JAMA Pediatr. 169 (9) (2015) 855–862, https://doi.org/10.1001/jamapediatrics.2015.1305.
[48] T.D. Metz, A.A. Allshouse, C.J. Hogue, et al., Maternal marijuana use, adverse pregnancy outcomes, and neonatal morbidity, Am. J. Obstet. Gynecol. 217 (4) (2017) 478, https://doi.org/10.1016/j.ajog.2017.05.050 e1-478.e8.
[49] S. Luke, J. Hutcheon, T. Kendall, Cannabis use in pregnancy in British Columbia and selected birth outcomes, J. Obstet. Gynaecol. Canada 41 (9) (2019) 1311–1317, https://doi.org/10.1016/j.jogc.2018.11.014.
[50] J.K.L. Gunn, C.B. Rosales, K.E. Center, et al., Prenatal exposure to cannabis and maternal and child health outcomes: a systematic review and meta-analysis, BMJ Open 6 (4) (2016) e009986, , https://doi.org/10.1136/bmjopen-2015-009986.
[51] S.N. Conner, V. Bedell, K. Lipsey, G.A. Macones, A.G. Cahill, M.G. Tuuli, Maternal marijuana use and adverse neonatal outcomes, Obstet. Gynecol. 128 (4) (2016) 713–723, https://doi.org/10.1097/aog.0000000000001649.
[52] S.J. Brown, F.K. Mensah, J. Ah Kit, et al., Use of cannabis during pregnancy and birth outcomes in an Aboriginal birth cohort: a cross-sectional, population-based study, BMJ Open 6 (2) (2016) e010286, , https://doi.org/10.1136/bmjopen-2015-010286.
[53] K. Mark, A. Desai, M. Terplan, Marijuana use and pregnancy: prevalence, associated characteristics, and birth outcomes, Arch. Womens Ment. Health 19 (1) (2015) 105–111, https://doi.org/10.1007/s00737-015-0529-9.
[54] T.L. Crume, A.L. Juhl, A. Brooks-Russell, K.E. Hall, E. Wymore, L.M. Borgelt, Cannabis use during the perinatal period in a state with legalized recreational and medical marijuana: the association between maternal characteristics, breastfeeding patterns, and neonatal outcomes, J. Pediatr. 197 (2018) 90–96, https://doi.org/10.1016/j.jpeds.2018.02.005.
[55] J.Y. Ko, V.T. Tong, J.M. Bombard, D.K. Hayes, J. Davy, K.A. Perham-Hester, Marijuana use during and after pregnancy and association of prenatal use on birth outcomes: a population-based study, Drug Alcohol Depend. 187 (2018) 72–78, https://doi.org/10.1016/j.drugalcdep.2018.02.017.
[56] D.S. Howard, D.N. Dhanraj, C.G. Devaiah, D.S. Lambers, Cannabis use based on urine drug screens in pregnancy and its association with infant birth weight, Journal of Addiction Medicine. March (2019) 1, https://doi.org/10.1097/adm.0000000000000516.
[57] S.H. Massey, D.K. Mroczek, D. Reiss, et al., Additive drug-specific and sex-specific risks associated with co-use of marijuana and tobacco during pregnancy: evidence from 3 recent developmental cohorts (2003-2015), Neurotoxicol. Teratol. 68 (2018) 97–106, https://doi.org/10.1016/j.ntt.2018.06.002.
[58] Marroun El, H. Tiemeier, H. Steegers EAP, et al., Intrauterine Cannabis exposure affects fetal growth trajectories: the generation r study, J. Am. Acad. Child Adolesc. Psychiatry 48 (12) (2009) 1173–1181, https://doi.org/10.1097/chi.0b013e3181bfa8ee.
[59] M.R. Hayatbakhsh, Birth outcomes associated with cannabis use before and during pregnancy, Pediatr. Res. 71 (2) (2012) 215–219, https://doi.org/10.1038/pr.2011.25 02/.
[60] S.Y. Leemaqz, G.A. Dekker, L.M. McCowan, et al., Maternal marijuana use has independent effects on risk for spontaneous preterm birth but not other common late pregnancy complications, Reprod. Toxicol. 62 (1) (2016) 77–86.
[61] S.K. Dotters-Katz, M.C. Smid, T.A. Manuck, T.D. Metz, Risk of neonatal and childhood morbidity among preterm infants exposed to marijuana, J. Matern. Neonatal Med. 30 (24) (2017) 2933–2939, https://doi.org/10.1080/14767058.2016.1269165.
[62] C.R. Warshak, J. Regan, B. Moore, K. Magner, S. Kritzer, J. Van Hook, Association between marijuana use and adverse obstetrical and neonatal outcomes, J. Perinatol. 35 (12) (2015) 991–995, https://doi.org/10.1038/jp.2015.120.
[63] J. Lockwood, A. Moss, A. Beck, I. Francis, E. Schmoll, E. Wymore, The association between the legalization of recreational marijuana and both small for gestational age births and NICU admissions in Colorado, J. Perinatol. 39 (2019) 1165–1174, https://doi.org/10.1038/s41372-019-0416-8.
[64] T.R. Gray, R.D. Eiden, K.E. Leonard, G.J. Connors, S. Shisler, M.A. Huestis, Identifying prenatal Cannabis exposure and effects of concurrent tobacco exposure on neonatal growth, Clin. Chem. 56 (9) (2010) 1442–1450, https://doi.org/10.1373/clinchem.2010.147876.
[65] A.B. O'Connor, B.K. Kelly, L.M. O'Brien, Maternal and infant outcomes following third trimester exposure to marijuana in opioid dependent pregnant women maintained on buprenorphine, Drug Alcohol Depend. 180 (2017) 200–203, https://doi.org/10.1016/j.drugalcdep.2017.08.012.
[66] P.A. Fried, B. Watkinson, R. Gray, Growth from birth to early adolescence in offspring prenatally exposed to cigarettes and marijuana, Neurotoxicol. Teratol. 21 (5) (1999) 513–525, https://doi.org/10.1016/S0892-0362(99)00009-4.
[67] M.B. Forrester, R.D. Merz, Risk of selected birth defects with prenatal illicit drug use, Hawaii, 1986–2002, J. Toxicol. Environ. Health Part A 70 (1) (2006) 7–18, https://doi.org/10.1080/15287390600748799.
[68] A.S. Reece, G.K. Hulse, Cannabis teratology explains current patterns of coloradan congenital defects: the contribution of increased cannabinoid exposure to rising teratological trends, Clin. Pediatr (2019) 992281986128, , https://doi.org/10.1177/0009922819861281 July.
[69] M.M.H.J. van Gelder, J. Reefhuis, A.R. Caton, M.M. Werler, C.M. Druschel, N. Roeleveld, Maternal periconceptional illicit drug use and the risk of congenital malformations, Epidemiology. 20 (1) (2009) 60–66, https://doi.org/10.1097/ede.0b013e31818e5930.
[70] M.M.H.J. van Gelder, A.R.T. Donders, O. Devine, N. Roeleveld, J. Reefhuis, Using bayesian models to assess the effects of under-reporting of Cannabis use on the association with birth defects, national birth defects prevention study, 1997-2005, Paediatr. Perinat. Epidemiol. 28 (5) (2014) 424–433, https://doi.org/10.1111/ppe.12140.
[71] L.J. Williams, A. Correa, S. Rasmussen, Maternal lifestyle factors and risk for ventricular septal defects, Birth Defects Res. Part A Clin. Mol. Teratol. 70 (2) (2004) 59–64, https://doi.org/10.1002/bdra.10145.
[72] R.L. Weinsheimer, N.L. Yanchar, Impact of maternal substance abuse and smoking on children with gastroschisis, J. Pediatr. Surg. 43 (5) (2008) 879–883, https://doi.org/10.1016/j.jpedsurg.2007.12.032.
[73] N. Stanslowsky, Functional effects of cannabinoids during dopaminergic specification of human neural precursors derived from induced pluripotent stem cells, Addict. Biol. 22 (5) (2017) 1329–1342, https://doi.org/10.1111/adb.12394 09/.
[74] G. Tortoriello, Miswiring the brain: Δ9-tetrahydrocannabinol disrupts cortical development by inducing an SCG10/stathmin-2 degradation pathway, EMBO J. 33 (7) (2014) 668–685, https://doi.org/10.1002/embj.201386035 04/.
[75] S. Zammit, K. Thomas, A. Thompson, et al., Maternal tobacco, cannabis and alcohol use during pregnancy and risk of adolescent psychotic symptoms in offspring, Br. J. Psychiatry 195 (4) (2009) 294–300, https://doi.org/10.1192/bjp.bp.108.062471.
[76] J.D. Fine, A.L. Moreau, N.R. Karcher, et al., Association of prenatal Cannabis exposure with psychosis proneness among children in the adolescent brain cognitive development (ABCD) study, JAMA Psychiatry 76 (7) (2019) 762, https://doi.org/10.1001/jamapsychiatry.2019.0076.
[77] N.L. Day, L. Goldschmidt, R. Day, C. Larkby, G.A. Richardson, Prenatal marijuana exposure, age of marijuana initiation, and the development of psychotic symptoms in young adults, Psychol. Med. 45 (8) (2014) 1779–1787, https://doi.org/10.1017/s0033291714002906.
[78] J.S. Noland, L.T. Singer, E.J. Short, et al., Prenatal drug exposure and selective attention in preschoolers, Neurotoxicol. Teratol. 27 (3) (2005) 429–438, https://doi.org/10.1016/j.ntt.2005.02.001.
[79] L. Goldschmidt, G.A. Richardson, J.A. Willford, S.G. Severtson, N.L. Day, School achievement in 14-year-old youths prenatally exposed to marijuana, Neurotoxicol. Teratol. 34 (1) (2012) 161–167, https://doi.org/10.1016/j.ntt.2011.08.009.
[80] J.A. DiNieri, X. Wang, H. Szutorisz, et al., Maternal Cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring, Biol. Psychiatry 70 (8) (2011) 763–769, https://doi.org/10.1016/j.biopsych.2011.06.027.
[81] A. Porath, P. Fried, Effects of prenatal cigarette and marijuana exposure on drug use among offspring, Neurotoxicol. Teratol. 27 (2) (2005) 267–277, https://doi.org/10.1016/j.ntt.2004.12.003.
[82] K.E. Sonon, G.A. Richardson, J.R. Cornelius, K.H. Kim, N.L. Day, Prenatal marijuana exposure predicts marijuana use in young adulthood, Neurotoxicol. Teratol. 47 (2015) 10–15, https://doi.org/10.1016/j.ntt.2014.11.003.
[83] L. Goldschmidt, N.L. Day, G.A. Richardson, Effects of prenatal marijuana exposure on child behavior problems at age 10, Neurotoxicol. Teratol. 22 (3) (2000) 325–336, https://doi.org/10.1016/s0892-0362(00)00066-0.
[84] N.L. Day, S.L. Leech, L. Goldschmidt, The effects of prenatal marijuana exposure on delinquent behaviors are mediated by measures of neurocognitive functioning, Neurotoxicol. Teratol. 33 (1) (2011) 129–136, https://doi.org/10.1016/j.ntt.2010.07.006.
[85] N.M. De Genna, L. Goldschmidt, M.D. Cornelius, Maternal patterns of marijuana use and early sexual behavior in offspring of teenage mothers, Matern. Child Health J. 19 (3) (2014) 626–634, https://doi.org/10.1007/s10995-014-1550-8.
[86] H. Klonoff-Cohen, P. Lam-Kruglick, Maternal and paternal recreational drug use and sudden infant death syndrome, Arch. Pediatr. Adolesc. Med. 155 (7) (2001) 765, https://doi.org/10.1001/archpedi.155.7.765.
[87] K.S. Grant, R. Petroff, N. Isoherranen, N. Stella, T.M. Burbacher, Cannabis use during pregnancy: pharmacokinetics and effects on child development, Pharmacol. Ther. 182 (2018) 133–151, https://doi.org/10.1016/j.pharmthera.2017.08.014.
[88] R. Thompson, K. DeJong, J. Lo, Marijuana use in pregnancy: a review, Obstet. Gynecol. Surv. 74 (7) (2019) 415–428, https://doi.org/10.1097/OGX.0000000000000685.
[89] C. Guille, R. Aujla, Developmental consequences of prenatal substance use in children and adolescents, J. Child Adolesc. Psychopharmacol. 29 (7) (2019) 479–486, https://doi.org/10.1089/cap.2018.0177.