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Mini review

Do Delta-9-tetrahydrocannabinol and Cannabidiol have opposed effects on male fertility?

Olivia L.M. Scandlan, Laura A. Favetta *

Reproductive Health and Biotechnology Laboratory, Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada

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ABSTRACT

Cannabis sativa is a complex plant, renowned for its diverse array of bioactive compounds, the most prominent of which are delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). These compounds exhibit markedly opposing pharmacological effects, with THC being primarily psychoactive and CBD known for its non-psychoactive properties. In recent years, there has been growing interest in the potential health implications of these compounds, particularly concerning male reproductive health. Accumulating evidence over the past decade has alluded to the potential negative effects of THC, including its association with reduced sperm quality, altered hormone levels, changes in genetic and epigenetic profiles, and potential impacts on fertility. Conversely, emerging studies suggest that CBD may exert protective and beneficial effects on male reproductive health, possibly through its anti-inflammatory and antioxidant properties. This review aims to provide a comprehensive analysis of the current scientific literature, delineating the mechanisms by which THC and CBD influence male reproductive health, highlighting the disparities in their effects, and discussing the clinical and therapeutic implications of these findings.

1. Introduction

On October 17th, 2018, the government of Canada enacted legislation to legalize, regulate, and restrict access to cannabis, allowing adults to possess up to 30 g of legally produced cannabis. This significant policy shift builds upon earlier efforts to regulate cannabis, as medical use and cultivation were legalized back in 2001 under the Marijuana for Medical Purposes Regulations by Health Canada. This program licenses cannabis producers to ensure a controlled and safe supply for medical purposes. It is indicated that over one-third of adults of reproductive age in Canada reported using cannabis recreationally, with approximately one in ten individuals using it daily or almost daily (Government of Canada, 2024). Similar trends in legalization and decriminalization are observed globally, with numerous countries adopting more permissive cannabis policies (Atakan, 2012; Capodice and Kaplan, 2021). Despite its increasing popularity, particularly among men of reproductive age, a significant gap in understanding the full impact of cannabis use on male reproductive health still exists (Capodice and Kaplan, 2021).

It is known that delta-9-tetrahydrocannabinol (THC) may influence hormone levels, sperm quality, and reproductive outcomes, however it is lesser known that cannabidiol (CBD), which is often prescribed as an antianxiety, may exert similar effects as those of THC, as they are structurally similar (Table 1). Comprehensive research can guide public health recommendations and inform users about possible risks. Additionally, such studies can help identify therapeutic benefits or adverse effects, leading to safer and more effective medical applications of cannabis.

1.1. Mechanisms of action in the endocannabinoid system

The endocannabinoid system (ECS) is a pro-homeostatic system conserved across many mammalian species and consists of endogenous ligands, regulatory enzymes, and receptors (Hua et al., 2016; Pařízek et al., 2023). The ECS is characterized by two cannabinoid receptors: cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2), both of which are G-protein coupled receptors (GPCR) (Bedse et al., 2017; Hua et al., 2016; Pařízek et al., 2023) The endogenous ligands (endocannabinoids) for these receptors are arachidonate-derived molecules, specifically N-arachidonylethanolamine (AEA) and 2-arachidonylglycerol (2AG), respectively (Marichal-Cancinoa et al., 2017; Pařízek et al.,

* Correspondence to: University of Guelph, 50 Stone Rd. East, Guelph, ON N1G 2W1, Canada.
E-mail address: lfavetta@uoguelph.ca (L.A. Favetta).

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2023). Both AEA and 2AG have distinct metabolic pathways. AEA is broken down by the fatty acid amide hydrolase (FAAH) pathway, whereas 2AG undergoes a more complex degradation process involving at least eight different enzymes, both presynaptic and postsynaptic (Sugiura et al., 2002). Monoacylglycerol lipase (MAGL) is the main enzyme responsible for 2AG degradation, but FAAH and α/β-hydrolase domain-containing 6 and 12 (ABHD6 and ABHD12) also play important roles (Ghosh et al., 2022; Matei et al., 2023; Sugiura et al., 2002). AEA synthesis occurs in two steps: initially, arachidonic acid (AA) is transferred to the amino group of the membrane lipid phosphatidylethanolamine, catalyzed by N-acyltransferase to form N-arachidonoylphosohatidylethanolamine (NAPE) (Pertwee et al., 2010). For 2AG, the process begins with the activation of phospholipase Cβ, which cleaves phosphatidylinositol bisphosphate (PIP2) containing arachidonic acid. This cleavage produces diacylglycerol (DAG) and inositol triphosphate (Maccarrone et al., 2021; Malheiro et al., 2023). Finally, 2AG is synthesized from DAG via one of two diacylglycerol lipases, DAGLα and DAGLβ. This intricate orchestration of enzymatic activity illustrates the finely tuned regulation of the ECS. Disruptions or alterations in this system, such as those potentially caused by cannabis use, can have profound effects on the overall metabolic health and the risk of developing complex metabolic diseases (Tomar et al., 2024).

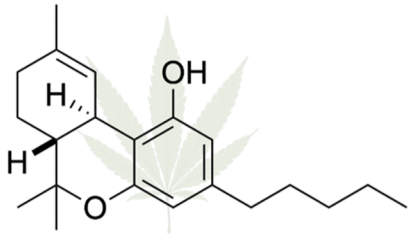
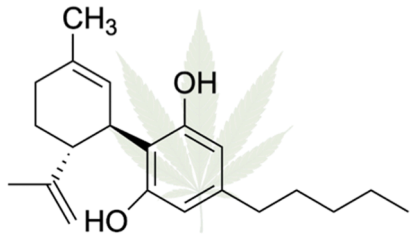
These endogenous lipids (AEA and 2AG) interact with the CB1 and CB2 receptors, influencing neurological activity and human behaviour in a manner similar to THC, CBD, and their associated metabolites. As outlined in Table 1, both THC and CBD elicit opposing pharmacological effects at the CB1, CB2 and GPR55 receptors. CBD has a high affinity for GPR55, where it functions as an antagonist. GPR55 is a GPCR that plays a role in cell proliferation, differentiation and cytoskeletal changes (Marichal-Cancinoa et al., 2017). Studies have shown that CBD has anti-inflammatory effects in experimental models of genetically induced Dravet syndrome (Kaplan et al., 2017) and Parkinson's disease (Celorrio et al., 2017). Another important receptor involved in the regulation of metabolism and energy homeostasis, cell differentiation and inflammation is the PPAR-γ receptor, part of the PPAR family (O'Sullivan, 2016). PPARs are nuclear transcription factors that bind to DNA sequences called PPAR response elements on various target genes, causing transcriptional changes.

As research on the ECS expands, the concept of the endocannabinoidome (eCBome) is gaining recognition (Lian et al., 2022). The eCBome refers to the range of environmental factors that interact with the ECS (Di Marzo, 2020). It includes all known elements of the ECS, such as proteins, enzymes and lipids involved in its modulation, as well as aspects related to the microbiome, dietary influences, and various digestive, metabolic, and central nervous system diseases or cancers. There is also a possibility that proper regulation of the ECS may contribute to a healthy lifespan. The interactions within the ECS and the eCBome are not simply one-way processes, they involve a complex interplay that depends on the pathophysiological conditions present. The ECS plays a critical role in maintaining the body's energy balance by regulating food intake, metabolism, and energy expenditure, thus helping to sustain a stable body weight (Forteza et al., 2022). Proper ECS function is essential in preventing metabolic disorders such as metabolic syndrome, diabetes mellitus, cancer, obesity, and hypertension (Falasca and Falasca, 2022). Proper ECS function plays an important role in male fertility, as obesity, diabetes and metabolic syndromes can have negative impacts on various parameters of male fertility, from semen quality to sperm DNA integrity (Service et al., 2023).

1.2. *Epigenetics*

Epigenetics refers to the study of heritable changes in gene expression that do not involve alterations to the underlying DNA sequence (Reik, 2007). These changes are often influenced by environmental factors and can affect how genes are turned on or off, impacting various biological processes and development. The most common epigenetic mechanisms regarding infertility are histone post-transcriptional modifications, DNA methylation, and small non-coding RNA (sncRNA) interference (Joshi et al., 2023). During pregnancy, the maternal environment plays a crucial role in shaping the epigenetic landscape of the developing fetus. Important epigenetic biomarkers can also be derived from male factors as well. Sperm play a vital role in the epigenetic regulation of fetal development, and epigenetic disease markers can be transferred from men to offspring, such as in the case of overweight individuals (Tomar et al., 2024). Recent studies have also shown that men who consume cannabis have alterations in epigenetic markers which has potential to alter gene expression in the developing embryo (Kuzma-Hunt et al., 2024; Wanner et al., 2021). THC, the primary psychoactive component of cannabis, along with other cannabinoids, can cross the placental barrier and may alter the expression of genes involved in crucial developmental pathways. This disruption can lead to long-term consequences for the child, including neurobehavioral and

Table 1
Pharmacology of Delta-9-tetrahydrocannabinol and Cannabidiol. Overview of the mechanisms of action by which THC and CBD elicit various pharmacological effects.

Cannabinoid	ECS targets	Effect	Reference
Delta−9-tetrahydrocannabinol (THC) 	CB_1	Agonist	Laaris et al. (2010)
	CB_2	Agonist	Pertwee et al. (2010)
	GPR55	Agonist	Lauckner et al. (2008)
	PPAR-γ	Agonist	O'Sullivan (2016)
Cannabidiol (CBD) 	CB_1	Antagonist	Laprairie et al. (2015)
	CB_2	Antagonist	
	GPR55	Antagonist	
	PPAR-γ	Agonist	O'Sullivan (2016)
	TRPV1	Agonist	Anand et al. (2020)

cognitive impairments, as well as metabolic and reproductive issues (Kuzma-Hunt et al., 2024).

1.3. Histone acetylation

Histone acetylation is a major post-translational modification that contributes to the histone code (Lin et al., 2022). Acetylation of lysine residues on histone tails plays a direct role in gene transcription regulation. As early as 1964, it was demonstrated that histone binding to DNA inhibited gene transcription, and that chemical acetylation of histones could mitigate this inhibitory effect. The reduction in histone acetylation's inhibitory effect on gene transcription is attributed to a decrease in chromatin structure packing (Patel et al., 2024). This is due to the reduction in attractive forces between the positively charged histone tails and the negatively charged DNA, allowing transcription factors to access the DNA and promote transcription. Histone acetylation can also regulate gene transcription indirectly through interactions with bromodomains. Bromodomains are protein interaction domains that recognize ε-N-lysine acetylation and are often part of multidomain proteins (Fujisawa and Filippakopoulos, 2017). Proteins with bromodomains, mostly transcription factors and regulators (e.g., the BRD and BAZ families), may use acetylated lysine tails as anchors to approach the DNA. Additionally, several histone modulators, such as histone methyl- and acetyltransferases, contain bromodomains, indicating that acetylation of histone tail lysines can precede further modifications of the "histone code" (Filippakopoulos et al., 2012). Acetylation levels are controlled by a dynamic balance between acetylation and deacetylation reactions. Histone acetyltransferases (HATs) catalyze lysine acetylation, while histone deacetylases hydrolyze acetylated lysine residues (Filippakopoulos et al., 2012). Imbalance in the acetylation–deacetylation equilibrium is linked to diseases such as cancer and cardiac conditions. This chapter focuses on current knowledge about different families of HATs, their associations with specific diseases, and their respective small molecule inhibitors.

During spermatogenesis in mammals, germ cell chromatin experiences significant reorganization driven by histone acetylation, resulting in the eviction of 90–99 % of histones. Due to the important role that retained histones may play in fertility and embryonic development, understanding the genomic locations of these retained nucleosomes is highly valuable (Luense et al., 2019).

1.4. DNA methylation

DNA methylation is a heritable epigenetic modification that involves the addition of a methyl group to the C-5 position of the cytosine ring in DNA, facilitated by DNA methyltransferases (DNMTs) (Moore et al., 2013). In mammals, DNA methylation occurs at cytosines across the genome, although over 98 % of methylation in somatic cells occurs in a CpG dinucleotide context, whereas up to 25 % of methylation in embryonic stem cells (ESCs) occurs in non-CpG contexts (Jin et al., 2011). DNA methylation is generally removed during zygote formation and re-established in the embryo around implantation (Messerschmidt et al., 2014). This process is crucial for normal development and is involved in genomic imprinting, X-chromosome inactivation and the suppression of repetitive element transcription and transposition. Dysregulation of DNA methylation is associated with diseases like cancer (Messerschmidt et al., 2014).

A family of DNMTs, including DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L, regulates DNA methylation (Moore et al., 2013). DNMT1 preferentially methylates hemimethylated DNA and is localized to replication foci during the S phase, acting as the maintenance methyltransferase responsible for copying methylation patterns to daughter strands during replication (Messerschmidt et al., 2014; Moore et al., 2013). DNMT2, in contrast, methylates cytosine-38 in the anticodon loop of aspartic acid tRNA instead of DNA. DNMT3A and DNMT3B prefer unmethylated CpG dinucleotides and are responsible for *de novo* methylation during development (Messerschmidt et al., 2014). Mice lacking DNMT3a die around four weeks of age (Messerschmidt et al., 2014), while DNMT3b knockout leads to embryonic lethality. DNMT3L, which is homologous to DNMT3A and DNMT3B, enhances the activity of *de novo* methyltransferases by increasing their binding affinity for the methyl group donor, S-adenosyl-L-methionine, despite having no catalytic activity itself (Messerschmidt et al., 2014). DNMT3L-null mice are viable, but heterozygous embryos from homozygous DNMT3L-null oocytes show lethality due to the lack of de novo methylation in the germline, which causes sterility in males These impaired maternal methylation imprints and abnormally expressed of imprinted genes typically derive from the paternal allele. Cooperation among different DNMTs is necessary for methylating certain genomic regions, particularly repetitive elements. While DNMT1 is primarily considered a maintenance methyltransferase and DNMT3A and DNMT3B are seen as *de novo* enzymes, evidence suggests that DNMT1 also participates in *de novo* methylation and that DNMT3A and DNMT3B contribute to maintenance methylation during replication (Messerschmidt et al., 2014). Cannabis use has been shown to alter sperm DNA methylation, but the potential reversibility of these changes is still unknown (Schrott et al., 2021).

1.5. small non-coding RNA interference

The control of gene expression by small, noncoding RNA was first observed in 1993 when researchers found a small, double-stranded RNA (dsRNA) in nematode larvae that bound to the 3′ untranslated region of a larger mRNA, inhibiting its translation (Lee et al., 1993). Since then, various mechanisms for translational control by small RNAs have been identified. mRNA can either be targeted for cleavage by a small-interfering RNA (siRNA) -protein complex or translation can be inhibited by microRNAs (miRNA), leading to the eventual destruction of the mRNA by the cell (Chatterjee et al., 2023).

siRNAs originate as small, double-stranded RNA molecules (approximately 20 base pairs) produced by the cleavage of dsRNA by the enzyme Dicer (Chatterjee et al., 2023). siRNAs have two-nucleotide overhangs at each 3′ end. miRNAs, however, start as a small, hairpin-shaped precursor that is similarly processed by Dicer (Huang et al., 2019). siRNAs and miRNAs inhibit translation through different mechanisms while forming a ribonucleoprotein complex called RNA-induced silencing complex (RISC). RISC proteins unwind siRNA and bind to a single antisense strand, which then attaches to mRNA in a sequence-specific manner (Chatterjee et al., 2023; Huang et al., 2019). A protein component of RISC, called Slicer (Huang et al., 2019), cuts the mRNA, leading to its recognition as abnormal and subsequent destruction by the cell. In miRNA, the microRNA-induced silencing complex (miRISC) associates with mature miRNA, binding to mRNA and blocking translation. miRNAs often form imperfectly complementary stem-loop structures on the target mRNA, while siRNAs require near-perfect matches (Niu et al., 2011). While the vast array of functions performed by siRNAs and miRNAs are still being categorized, they are known to elicit a wide array of effects on tissue systems and their functions include regulation of self-renewal, cellular differentiation, proliferation, and apoptosis (Niu et al., 2011). miRNA interference has been shown to affect fertility by down- regulating gene expression (Barbu et al., 2021; Muñoz et al., 2015). miRNAs have also shown a vast potential to be used as biomarkers for disease. Their resistance to nucleases has made them very attractive targets for diagnostic and therapeutic purposes. Both genetic and epigenetic markers provide a clear analysis of disruptions that may occur during protein synthesis.

In the male reproductive system, the androgen receptor (AR) has been considered an important genetic marker of interest. The AR transcript is nearly 11Kb long and contains a 3' untranslated region, indicating its biological role in post-transcriptional regulation. Additionally, AR expression in sperm has been linked to positive indicators of fertility (Dohle et al., 2003). Androgens are important steroid hormones that

play crucial developmental roles in the male reproductive system (Dohle et al., 2003; Zhou and Hylemon, 2014). They play an important role in the onset of puberty, male fertility, and male sexual function (Alexander et al., 2024; Pencina et al., 2024). Testosterone, or its metabolite dihydrotestosterone (DHT), is one of the most important male androgens and high levels of intratesticular testosterone secreted by Leydig cells are vital for spermatogenesis (Dohle et al., 2003; Grimes et al., 2007). Bound to AR, testosterone makes its way into the seminiferous tubules, where inside the Sertoli cells, AR activation results in the initiation and maintenance of the spermatogenic process (Bennett et al., 2010; Dohle et al., 2003). Full-length AR binds to the DHT in the cytoplasm, initiating the translocation of the AR-DHT complex into the nucleus (Gelmann, 2002; Miller et al., 2023). Once inside the nucleus, AR binds to androgen response elements (AREs) on DNA and drives the expression of cell survival and proliferation genes and target genes (Frati et al., 2015). AR has a ligand-binding domain which resembles that of other steroid hormone receptors (Gelmann, 2002). When a ligand binds to AR, the protein undergoes a conformational change, facilitating the binding of coactivator compounds which can enhance the hormonal signal and play a role in initiating transcription (Bennett et al., 2010; Frati et al., 2015; Gelmann, 2002). AR can also be influenced by intramolecular interactions that regulate its interactions with coactivators and influence its activity, such as in the case of small non-coding RNAs.

1.6. Pharmacokinetics and dosages

The effects of THC and CBD on male reproductive health can vary significantly depending on individual consumption patterns, dosages, and routes of administration. Therapeutic doses of cannabinoids prescribed for medical purposes are typically controlled and lower than the unregulated, often higher doses associated with recreational use (Kuzma-Hunt et al., 2023). Studies suggest that chronic, heavy cannabis use can reduce sperm quality, including motility, morphology, and concentration, thereby impacting fertility treatments like intrauterine insemination (IUI) and in vitro fertilization (IVF) that rely on high-quality sperm (Ismaeil et al. 2024; Kuzma-Hunt et al., 2023).

The method of administration—such as oral ingestion, inhalation, or topical application—significantly impacts the bioavailability, absorption, distribution, metabolism, and excretion of THC and CBD. Inhaled THC, for example, is rapidly absorbed, reaching peak plasma concentrations within minutes, while orally ingested THC undergoes first-pass metabolism in the liver, which reduces its bioavailability and delays its effects (Ismaeil et al. 2024). This difference in pharmacokinetics can lead to varying impacts on reproductive health; inhaled THC may contribute to oxidative stress in sperm cells, potentially resulting in DNA damage that could affect embryo quality in assisted reproductive technologies (ART) like IVF or intracytoplasmic sperm injection (ICSI) (Ismaeil et al. 2024; Kuzma-Hunt et al., 2023; Wehrli et al. 2023). Similarly, CBD has reduced bioavailability with oral ingestion compared to inhalation. Both cannabinoids are lipophilic, accumulating in fatty tissues, including the brain, leading to prolonged presence and gradual release within the body. Metabolism of THC primarily involves the liver enzymes CYP2C9 and CYP3A4, which convert it into the psychoactive metabolite 11-hydroxy-THC, followed by the inactive THC-COOH (Ismaeil et al. 2024; Jiang et al., 2011). CBD is metabolized by a broader range of CYP enzymes, including CYP2C19, CYP3A4, and CYP2C9. Both THC and CBD can inhibit CYP enzymes, potentially causing drug-drug interactions (DDIs) when used with other medications (Jiang et al., 2011). Excretion occurs mainly through feces, with minor amounts in urine, and their fat accumulation contributes to prolonged detection times (Jiang et al., 2011).

Toxicokinetically, high doses of THC, particularly with chronic use, may negatively affect male fertility by increasing oxidative stress and metabolite accumulation in reproductive tissues. CBD, while generally less toxic, can still interact with medications and, at high doses, may affect liver enzymes (Jiang et al., 2011). Concurrent use of THC and CBD, or their combination with other substances, further complicates their impact on fertility.

1.7. Overview of sperm viability and fertilization capability

Spermatogenesis is the intricate process by which sperm cells, or spermatozoa, are produced within the testes (Nishimura and L'Hernault, 2017). Spermatogonial stem cells (SSCs) differentiate into haploid male gametes within the seminiferous tubules (STs) of the testes, and once formed, these sperm cells migrate from the STs to the epididymis, where they undergo maturation and preparation for ejaculation (Nishimura and L'Hernault, 2017; Suede et al., 2023). The process of spermatogenesis begins with diploid stem cells called spermatogonia, in the basal compartment of the seminiferous tubules, which undergo several mitotic divisions to form primary spermatocytes. These primary spermatocytes then undergo the first meiotic division to produce two secondary spermatocytes, which subsequently undergo the second meiotic division to yield four round spermatids. The spermatids then undergo spermiogenesis, a process of morphological and physiological transformation into mature spermatozoa, involving nuclear condensation, acrosome development, flagellum formation, and cytoplasm reduction (Suede et al., 2023).

The successful production of sperm relies on a complex interaction of endocrine and paracrine factors from Leydig, Sertoli, and mesenchymal cells (Neto et al., 2016). Spermatocytogenesis starts in early embryonic development with the differentiation of primordial germ cells (PGCs) into gonocytes, regulated by factors like estrogen, SDF-1, and estradiol (Neto et al., 2016). Gonocytes remain in the G0 phase during fetal development until early infancy, when they differentiate into spermatogonia (SPG) (Neto et al., 2016). Male gametes, unique for their self-renewing SPGs, can be produced throughout life (Lin et al., 2022). Research into SPG self-renewal involves genetic and epigenetic factors such as GDNF-stimulated transcription factors and H3K79 methylation via DOT1L (Lin et al., 2022; Oatley and Brinster, 2008). The regulation of spermatogenesis is tightly controlled by the hypothalamic-pituitary-gonadal axis (Xie et al., 2022). Gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates the pituitary gland to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH activates Sertoli cells within the seminiferous tubules to support developing sperm, while LH prompts Leydig cells in the testes to produce testosterone, which is crucial for sperm maturation (Neto et al., 2016; Xie et al., 2022). Sertoli cells provide nutritional and structural support, form the blood-testis barrier, and secrete fluid for sperm transport, while Leydig cells produce testosterone in response to LH stimulation.

At puberty, three SPG subsets are identified: Type A Dark (Ad), Type A Pale (Ap), and Type B-SPG (Neto et al., 2016; Rooij and de, 2001). Ad-SPGs are the most undifferentiated, located near the basement membrane, while Ap-SPGs, derived from Ad-SPGs, are lighter and also near the basement membrane (Neto et al., 2016; Rooij and de, 2001; Suede et al., 2023). Ap-SPGs undergo mitosis to maintain their population and differentiate into Type B SPGs, larger cells located near the lumen of seminiferous tubules (STs) (Neto et al., 2016; Oatley and Brinster, 2008; Rooij and de, 2001; Suede et al., 2023; Xie et al., 2022). Type B SPGs divide mitotically to form primary spermatocytes, the last diploid cells before the mitotic phase of spermatogenesis (Nishimura and L'Hernault, 2017). The differentiation of Ad-SPGs into Type B SPGs and the start of meiosis are regulated by factors like retinoic acid (RA), influenced by FSH in the testes (Khanehzad et al., 2021). At puberty, increased levels of testosterone, FSH, and LH are crucial for primary spermatocytes to cross the blood-testes barrier (BTB) and begin meiotic division (Khanehzad et al., 2021; Neto et al., 2016; Nishimura and L'Hernault, 2017). The BTB, composed of tight junctions, gap junctions, and desmosomes, connects Sertoli cells and peritubular tissues, protecting developing sperm during meiosis, spermiogenesis, and spermiation (Dym, 1973; Staub and Johnson, 2018). During the first meiotic

division, primary spermatocytes produce secondary spermatocytes, which undergo meiosis II to yield four haploid spermatids. These spermatids, located at the ST lumen's border, differentiate into sperm cells during spermiogenesis (Neto et al., 2016; Nishimura and L'Hernault, 2017; Suede et al., 2023).

Spermiogenesis encompasses several distinct stages: initially, proacrosomal vesicles are formed from the Golgi apparatus, which is followed by the development of the acrosomal cap. Subsequently, the acrosome is fully formed, and nuclear condensation occurs along with the removal of excess cytoplasm. The centrioles then migrate to establish the sperm tail. This is followed by the formation of the axoneme and the organization of mitochondria. The process continues with the maturation of the sperm and the formation of residual bodies, which are eventually discarded to produce mature spermatozoa (Dam et al., 2007; Greenbaum et al., 2011; Neto et al., 2016; Nishimura and L'Hernault, 2017; Suede et al., 2023; Weber and Russell, 1987). Molecular changes during spermiogenesis include DNA condensation, histone replacement with transition proteins, transition proteins replaced with protamines for DNA supercoiling and DNA methylation changes crucial for gene regulation and genomic stability in mature sperm (Cannarella et al., 2020). Mature spermatozoa are released from Sertoli cells into the ST lumen and undergo further biochemical and molecular changes in the epididymis to become capable of fertilization (Gervasi and Visconti, 2017). Epididymal maturation involves nuclear compaction, non-coding RNA and protein accumulation, motility acquisition, and cytoskeletal restructuring (Vallet-Buisan et al., 2023). The entire spermatogenesis process spans approximately 64–72 days in humans, characterized by continuous production and yielding millions of sperm daily from puberty onwards. This process contrasts with female oogenesis, which is cyclical and finite. Human spermatogenesis takes approximately 42–76 days, while in bulls it takes about 61 days (Cannarella et al., 2020; Staub and Johnson, 2018). Bull and murine spermatogenesis is comparable to humans in terms of efficiency and product variability (Fang et al., 2019; Staub and Johnson, 2018; Yao et al., 2022). These similarities make bovine and murine sperm efficient as translational models of study and can be used to assess male factor infertility.

Infertility is a medical condition affecting the male or female reproductive system, characterized by the inability to conceive after engaging in regular unprotected sexual intercourse for a period of 12 months or longer (WHO, 2023). Estimates suggest that one in every six people of reproductive age will experience infertility at some point in their lifetime (WHO, 2023). About 50 % of global infertility cases are attributed to male factors infertility, with roughly 20 % of cases solely resulting from male factors (Leslie et al., 2023). Sperm count, movement, and morphology are all important factors contributing to male fertility and alarmingly, the average global sperm counts have dropped by 51.6 % from 1973 to 2018 (Levine et al., 2023). Many biological factors can predispose men to infertility including; genetics, congenital malformations, hormones, behaviours, iatrogenic, obesity and lifestyle factors (Kumar and Singh, 2022). Sperm are also sensitive to a variety of environmental elements that can have an effect on individual fertility such as, recreational drug use, alcohol, cannabis, heavy metals, and endocrine disrupting compounds (EDCs) (Davis et al., 2024; Kumar and Singh, 2022; Lockington and Favetta, 2024; Nguyen et al., 2022; Sabry et al., 2024). There is much debate surrounding the effects of cannabis, as there are many links attributed to male infertility and potential benefits associated with CBD.

2. Methods

A literature search was conducted using PubMed, Web of Science, and Google Scholar. We chose these databases to locate accurate and relevant publications. We searched the databases using the following keywords; THC, CBD, fertility, endocannabinoid system, sperm, embryo, reproduction alone and in combination. Our primary search terms used were 'CBD effects on male gametes' and 'THC effects on male gametes'. We searched for publications up to August 2024 and prioritized more recent publications to ensure that our review has an accurate representation of current data. We applied filters for [2012-present] as well as [English] and examined those selections first. Primarily, we preferred studies performed on human subjects, but since this topic is niche, we accepted any appropriate and significant results. Methods and data were extracted from studies to compare and compile a current assembly of results.

2.1. Effects of delta-9-tetrahydrocannabinol on male reproduction

Despite the widespread use of cannabis among adults of reproductive age, there is limited understanding of its effects on fertility (Jordan et al., 2020). Clinical studies examining the impact of cannabis on male fertility have produced highly inconsistent results (Srinivasan et al., 2021). Studies indicate that recreational cannabis use, especially at moderate (5–15 mg) to high doses (> 30 mg), can adversely affect sperm parameters, including sperm counts, morphology, motility, capacitation, and the acrosome reaction (Banerjee et al., 2011; Carroll et al., 2020; Gundersen et al., 2015; Hehemann et al., 2021; Payne et al., 2019; Srinivasan et al., 2021; Truong et al., 2023; Whan et al., 2006). Conversely, recreational cannabis use has been linked to protection against abnormal sperm motility (Hehemann et al., 2021) and increased sperm counts (Nassan et al., 2019).

Components of the ECS are involved in several crucial reproductive functions. ECS signaling through CB receptors regulates the hypothalamic-pituitary-gonadal (HPG) axis' endocrine function (Nielsen et al., 2019). Within the hypothalamus, CB receptors influence GnRH, which affects pituitary secretion of LH and FSH, testosterone production, and spermatogenesis in the testis (Nielsen et al., 2019). Both CB1 and CB2 receptors are also found on spermatozoa. CB1 receptors are located on the plasma membrane of the acrosomal region, midpiece, and tail of the sperm cell, whereas CB2 receptors are primarily found in the postacrosomal region, midpiece, and tail (Agirregoitia et al., 2010; Aquila et al., 2010; Rossato et al., 2005). In mice, AEA injection suppressed GnRH, and CB1 knockout reduced LH and testosterone, indicating the ECS's role in sex hormone production (Battista et al., 2012; Nielsen et al., 2019; Scorticati et al., 2004; Wenger et al., 1988). In the male reproductive system, ECS components are found in Sertoli cells, Leydig cells, and spermatozoa (Agarwal et al., 2015; du Plessis et al., 2015; Maccarrone et al., 2021; Nielsen et al., 2019; Scorticati et al., 2004; Wenger et al., 1988). ECS tone is precisely regulated during spermatogenesis, with 2AG levels being highest in spermatogonia and decreasing as diacylglycerol lipase expression increases and monoacylglycerol lipase decreases (Grimaldi et al., 2009, 2013). Human spermatozoa contain N-acyl phosphatidylethanolamine phospholipase D and FAAH to regulate AEA activity, along with diacylglycerol lipase and monoacylglycerol lipase and/or abhydrolase domain-containing 2 (ABHD2), to control 2-AG activity. Mature human sperm expresses CB1 in the head, midpiece, and tail, CB2 in the post-acrosomal region, and TRPV1 in the head and tail (Aquila et al., 2010; Sayed et al., 2021). Overall, sperm cell CB1 receptor density is higher than CB2 (Aquila et al., 2010; Sayed et al., 2021). AEA synthesized in sperm by NAPE-PLD is transported outside via the endocannabinoid membrane transporter (EMT), suggesting that ECBs activate both CB1 and CB2 receptors in different sperm locations, resulting in varied functional outcomes.

Cannabis consumption in men may impact the steroidogenic function of the testes, particularly through its influence on androgen metabolism and estrogen receptor (ER) signaling. Cannabinoids like THC and CBD can interfere with the hypothalamic-pituitary-gonadal axis, potentially reducing testosterone production. Lower testosterone levels may, in turn, alter the levels of its androgen metabolites, including those that are converted into estrogens (Scorticati et al., 2004).

Additionally, THC has been shown to interact with estrogen receptors (ERα and ERβ) in various tissues, and this interaction might extend to the testes. Since estrogen and ER signaling play a role in

spermatogenesis and the health of testicular cells, disruptions in ER signaling from cannabis exposure could impair these processes, potentially affecting fertility and overall reproductive health in men (Scorticati et al., 2004). There are many contradictory findings pertaining to decreased testosterone levels, sperm production, sperm motility, and elevated sperm abnormalities with the use of cannabis. These studies have since been challenged by a more extensive and methodically rigorous study encompassing chronic-heavy users. The most recent literature states no discernable differences in plasma testosterone either at the beginning of the study periods or following three weeks of substantial daily Cannabis consumption (Chai et al., 2020). The full extent of these effects is still under investigation, but the interaction between cannabis, androgens, estrogens, and ER signaling represents a complex area of study with significant implications for male reproductive health. These varied outcomes are highlighted in Fig. 1, demonstrating the differences in hormonal regulation across the hypothalamic-pituitary-testicular axis.

2.2. *Effects of delta-9-tetrahydrocannabinol and cannabidiol on sperm in vitro*

Many studies have examined the effects of THC on human sperm function and shown a dose-dependent negative effect. Higher level THC exposure impacts sperm progressive motility percentage, becoming more highlighted as semen quality diminishes overall. Additionally, THC reduces spontaneous acrosome reaction and can inhibit acrosome reaction even when induced artificially at both therapeutic (0.032 μM) and recreational (0.32 μM) plasma concentrations (Kuzma-Hunt et al., 2023; Whan et al., 2006). Ismaeil et al. (2024) compared sperm from men between the ages of 20–35, regardless of their fertility status, and analyzed sperm cell quality before and after incubation with cannabis extracts (THC, CBD, and THC-COOH). They found that after treatment with 1 mg/mL of either THC, CBD or THC-COOH, total sperm motility was significantly reduced when exposed to THC and CBD ($P \leq 0.001$ and $P \leq 0.003$ respectively). Progressive motility was decreased significantly ($P \leq 0.001$) and the mean number of immotile sperm was significantly increased ($P \leq 0.003$) after incubation with THC and CBD. However, slight inhibition of total sperm's motility was observed after incubation with THC-COOH (Ismaeil et al., 2024).

A similar study conducted by Wehrli et al. (2023) analyzed the effects of THC and CBD as well as their major metabolites on Ca^{2+} influx via CatSper channels in human sperm. In mammalian sperm cells, regulation of spatiotemporal Ca^{2+} signaling relies on the quadrilinear Ca^{2+} signaling nanodomains in the flagellar membrane. The sperm-specific, multi-subunit CatSper Ca^{2+} channel, which is crucial for sperm hyperactivated motility and male fertility, organizes the nanodomains (Hwang et al., 2022; Wehrli et al., 2023). This group further studied the effects of THC and CBD action on progesterone-, prostaglandin-, and pH-induced activation of human CatSper. Both THC and CBD suppress natural ligand-induced calcium influx via CatSper. In particular, THC inhibited progesterone-induced Ca^{2+} influx via CatSper at pharmacologically relevant concentrations in a non-competitive manner and reduces the pH-induced activation of CatSper. In addition, THC impaired sperm hyperactivation and penetration into viscous media and induced spontaneous acrosomal exocytosis *in vitro* (Ismaeil et al., 2024). The action of THC on CatSper in human sperm might impair the fertilization process, stressing the negative association with THC and, to a lesser extent, CBD.

An *in vitro* translational study conducted by Kuzma-Hunt et al. (2023) used bovine sperm and cumulus oocyte complexes (COCs),

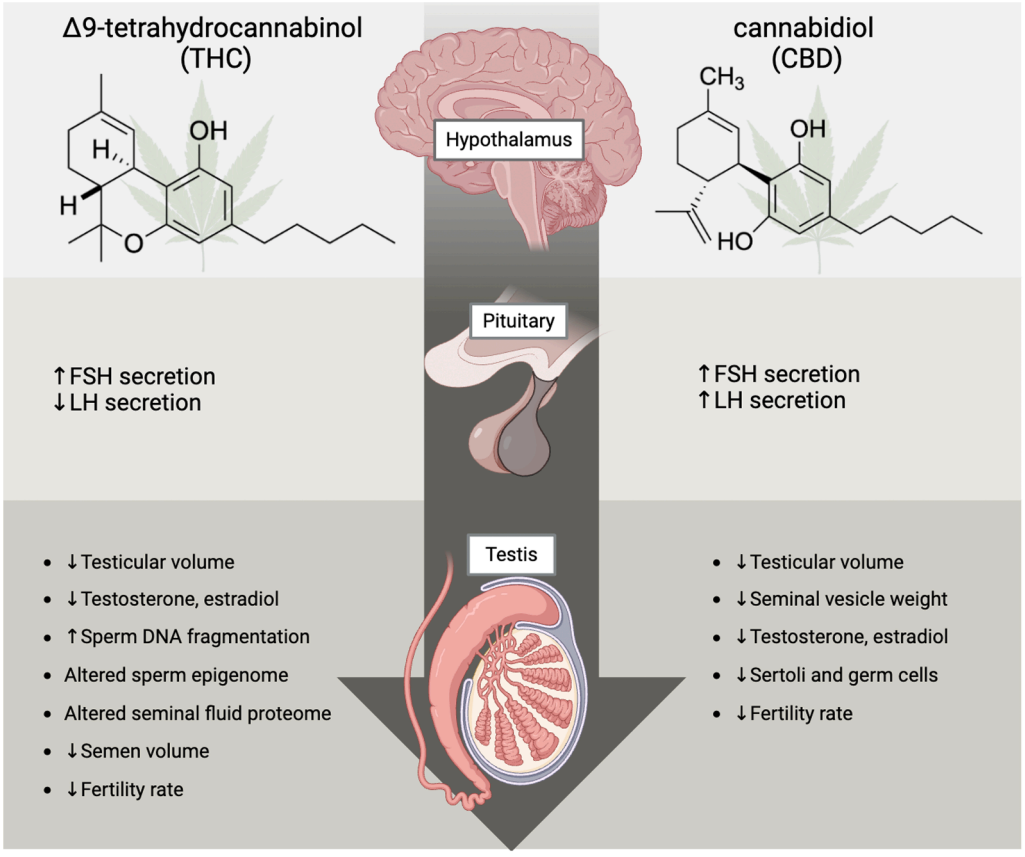


Fig. 1. Compared effects of delta-9-tetrahydrocannabinol and cannabidiol on the hypothalamic-pituitary-gonadal axis in males. Drug exposure causes FSH and LH fluctuations, and ultimately affects the production of sex hormones. Decreases in testicular volume and decreases in fertility factors are observed in both CBD and THC exposed men and male gametes. [Created in biorender.com].

analysing a minimum of 30,000 sperm and 60 COCs per group. Sperm were treated with THC at low-THC (0.032 μM), mid-THC (0.32 μM), and high-THC (4.8 μM) – concentrations equivalent to THC plasma levels following therapeutic and mid-high recreational use, respectively. Results indicate a significant reduction in mitochondrial membrane potential (38 %) in the high THC-exposed sperm group (4.8 μM) compared to control (51 %) ($p = 0.0417$, $n = 6$) (Kuzma-Hunt et al., 2023). Similarly, a study analyzing the effects of THC on bovine sperm found that 0.32 μM of THC caused an increased proportion of capacitating sperm ($p < 0.05$) compared to controls (Truong et al., 2023). Transcriptome analysis revealed that 39 genes were found to be differentially expressed at a concentration of 0.032 μM of THC, 196 genes at 0.32 μM of THC, and 33 genes at 3.2 μM of THC. Secondary analysis revealed pathways involving development, nucleosomes, ribosomes and translation, and cellular metabolism to be significantly enriched.

2.3. Effects of delta-9-tetrahydrocannabinol and cannabidiol on sperm in vivo

A study conducted by Schrott et al. (2021) analyzed semen samples from 18 cannabis users and 24 non-user controls at baseline and again following a 77-day period of cannabis abstinence (one spermatogenic cycle) (Schrott et al., 2020). Users and controls did not significantly differ by demographics or semen analysis, however a whole-genome bisulfide sequencing identified 163 CpG sites with significantly different DNA methylation in sperm groups ($P < 2.94 \times 10^{-9}$) and 127 significantly different methylated CpG sites after cannabis abstinence, with only three CpG sites being common between the two data sets. Bisulfide sequencing allows users to detect methylated cytosines in genomic DNA: after treatment with sodium bisulfide the DNA is sequenced, providing single-base resolution of methylated cytosines in the genome. Genes associated with altered CpG sites were enriched with those involved in development, including cardiogenesis and neurodevelopment. DNA methylation resulting from cannabis-associated gene alterations were often diminished, as these sites were often located on genes important for early embryonic development (Schrott et al., 2020).

Carvalho et al. (2022) examined the impact of CBD on spermatogenesis and sperm quality. Twenty-one-day-old Swiss mice were given CBD for 34 days at doses of either 15 or 30 mg/kg. Chronic CBD exposure led to a decrease in the frequency of stages VII-VIII and XII of spermatogenesis, and an increase in stage IX (Carvalho et al., 2022). Additionally, the seminiferous epithelium height decreased at stage IX and increased at stage XII in both CBD-treated groups. There was a notable increase in sperm DNA damage, although no genotoxic effects were found in leukocytes. The activities of superoxide dismutase and catalase decreased, while malondialdehyde levels rose in the sperm of mice treated with a higher CBD dose. Mice exposed to 30 mg/kg of CBD experienced a reduction in mobile spermatozoa percentage and curvilinear velocity, with straight line and average path velocity also decreasing in both treated groups. The number of acrosome-intact spermatozoa declined in the 30 mg/kg CBD group, and the number of abnormal acrosomes increased in both CBD groups. Conversely, the weight of reproductive organs, sperm count, and hormone levels were unaffected by CBD treatment. These results indicate that CBD can impair sperm quality through endocannabinoid system dysregulation, likely involving disruptions in spermatogenesis, particularly during the final stages of nuclear remodeling and acrosome assembly, as well as alterations in mitochondrial function and decreased antioxidant enzyme activities during epididymal transit (Carvalho et al., 2022).

In the study by Zufferey et al. (2024), a Swiss cohort was classified based on cannabis consumption using both self-reports and THC-COOH levels in urine. Cannabis users were divided into "recent consumers" (those who used cannabis within 24 h) and "non-recent consumers" (those who last used it more than 24 h prior), with timing estimated from blood THC concentrations. Users were also categorized by frequency, with "chronic" users having THC-COOH blood levels over 40 ng/mL and "occasional" users below 3 ng/mL. Blood phytocannabinoids, endocannabinoids, male steroids, and hormones related to the hypothalamus-pituitary-gonadal axis were analyzed through liquid chromatography–mass spectrometry and immunoassays. Semen parameters such as sperm concentration and motility were recorded using computer-assisted sperm analysis (CASA). Anandamide, N-palmitoyl ethanolamide, androgens, estradiol and sex hormone binding globulin levels were all higher in cannabis users, particularly in chronic, recent and cannabidiol-positive consumers. Gonadotropin levels were not significantly different in these user subpopulations, whereas prolactin and albumin concentrations were lower. In addition, cannabis users had a more basic semen pH and a higher percentage of spermatozoa with progressive motility. However, the two latter observations seem to be related to a shorter period of sexual abstinence in this group rather than to the use of cannabis. Since both CBD and THC are frequently used by men of reproductive age, it is highly relevant to elucidate the potential effects they may have on human reproductive health. Table 2. breaks down the various effects of THC and CBD exposure both in vivo and in vitro, as they pertain to male reproductive health. This study demonstrates that the mode of cannabis consumption must be considered when evaluating the effect of cannabis on semen quality (Zufferey et al., 2024).

2.4. Effects of cannabis use prior to mating and developmental effects in offspring

A study conducted by Slotkin et al. (2021) used a male rat model to demonstrate the effects of cannabis exposure. Cannabis extract, specifically THC, was given to male rats prior to mating, delivered at 4 mg/kg/day. This group used two different 28-day exposures, with one having a 56-day interval between the end of exposure and mating; with the end of exposure being “Early Cannabis”, and the other just prior to mating being “Late Cannabis.” They found that paternal cannabis

Table 2
Delta-9-tetrahydrocannabinol and Cannabidiol exposure *in vivo* and *in vitro*. Summary of observed effects *in vitro* and *in vivo* following THC and CBD exposure in human, bovine, and murine sperm.

In Vitro	References
Effect of CBD	
↓ sperm motility at 1 mg/mL CBD	Ismaeil et al. (2024)
↓ ligand-induced calcium influx (CatSper)	Wehrli et al. (2023)
Effect of THC	
↑ progressive motility at 1 mg/mL THC	Ismaeil et al. (2024)
↓ acrosomal reaction at 1 mg/mL THC	
↓ ligand-induced calcium influx (CatSper)	Wehrli et al. (2023)
Impaired:	
- sperm hyperactivation	
- penetration	
Induced:	
- acrosomal exocytosis	
↑ capacitating sperm at 0.32 μM THC	Truong et al. (2023)
Transcriptome analysis:	
0.032 μM, 39 genes altered	
0.32 μM, 196 genes altered	
3.2 μM, 33 genes altered	
↓ sperm mitochondrial membrane potential at 4.8 μM THC	Kuzma-Hunt et al. (2023)
In Vivo	
Effect of CBD	
↑ sperm DNA damage at 30 mg/kg	Carvalho et al. (2022)
↓ spermatogenesis at 30 mg/kg	
↓ mobile sperm at 30 mg/kg	
Effect of THC	
Altered sperm DNA methylation at 4 mg/kg	Schrott et al. (2021)
* 163 CpG sites	
↓ prolactin and albumin in self-reported cannabis consumption	Zufferey et al. (2024)
↑ semen pH (↓ motility) in self-reported cannabis consumption	

exposure prior to mating evokes deficits in dopamine utilization in male offspring throughout maturation and adolescence through middle age, indicative of reduced overall presynaptic activity. They noted that this adverse effect was not regionally selective, pointing to an underlying impact on the development of the dopamine systems in general, rather than an alteration in the regulation of specific dopamine synaptic circuits. There were two additional, notable features of the effect. First, the reduction in turnover in the offspring was seen with both paternal cannabis regimens, even though, for the Early Cannabis treatment group, mating occurred after a prolonged, drug-free hiatus. Worrisomely, this indicates a persistent effect on developing sperm even with abstinence for more than a full spermatogenerational cycle (Slotkin et al., 2021).

Bolhuis et al. (2018) investigated the relationship between parental cannabis use daily (n = 124, 42.6 %), weekly (n = 87, 29.9 %) or monthly (n = 80, 27.5 %) during pregnancy and the occurrence of psychotic-like experiences in their offspring. Conducted within the Generation R birth cohort with 3692 participants, they used self-reported data regarding cannabis consumption. This study suggests that paternal cannabis use, even if infrequent, correlates with an increased risk of psychotic-like experiences (OR= 1.44, 95 % CI 1.14 – 1.82) (Bolhuis et al., 2018). Paternal cannabis use has also been linked to adverse neurodevelopmental outcomes in offspring, though the transmission mechanisms are still unclear. Using reduced representation bisulphite sequencing (RRBS), it was found that cannabis use leads to widespread DNA methylation changes in human and rat sperm (Schrott et al., 2020). The gene Discs-Large Associated Protein 2 (DLGAP2), which plays a role in synapse organization and neuronal signaling, and is also implicated in autism, showed significant hypomethylation at 17 CpG sites in human sperm ($p < 0.05$). This differential methylation at nine CpG sites in intron seven of DLGAP2 was validated using quantitative bisulphite pyrosequencing ($p < 0.05$). There was an inverse correlation between intron 7 DNA methylation and DLGAP2 expression in human embryonic brain tissue ($p < 0.01$). In adult male rats exposed to THC, differential DNA methylation at DLGAP2 was observed in sperm ($p < 0.03$), and similar changes were found in the nucleus accumbens of rats whose fathers were exposed to THC before conception ($p < 0.05$). Fig. 2 illustrates the effects of paternal cannabis use and the associated epigenetic changes observed in offspring. A metanalysis explored the impact of male marijuana use on the risk of spontaneous abortion (Harlow et al., 2021). Using data from the PRESTO study, which tracked North American pregnancy planners between 2013 and 2019, researchers found that 19 % of pregnancies with sperm from cannabis users ended in spontaneous abortion. They observed that male partners who used marijuana at least once per week before conception had a twofold higher risk of their partners experiencing spontaneous abortion compared to non-users. The risk was even more pronounced for losses occurring before 8 weeks of gestation and for men aged 35 and older (Harlow et al., 2021). The findings suggest that frequent male marijuana use during the preconception period may increase the risk of spontaneous abortion, however there is a need for further research on the effects of preconception cannabis use in males and its potential impact on future generations (Bolhuis et al., 2018).

3. Conclusion

In conclusion, this review highlights that paternal cannabis consumption, particularly involving THC, adversely affects male fertility parameters and is associated with epigenetic changes in offspring. Evidence suggests that higher frequency and chronic use, rather than occasional consumption, have a greater impact on reproductive health, although further studies are needed to establish safe thresholds. Both THC and CBD—used recreationally and medicinally for chronic pain and anxiety—can influence the hypothalamic-pituitary-testicular axis, alter hormone production, decrease testicular volume, reduce fertility, increase DNA methylation, and potentially elevate the risk of miscarriage. Given that men of reproductive age are among the most frequent cannabis users, it is crucial to inform and educate them about the potential reproductive health impacts. Notably, most research has focused on THC, leaving a significant gap in understanding CBD's specific effects on sperm. Future research should further examine the genetic and epigenetic effects of both THC and CBD on sperm, with a focus on understanding the role of small non-coding RNAs in these epigenetic changes and their implications for embryonic development. Future studies should also investigate whether occasional cannabis consumption is safe or if more frequent use, such as weekly or daily consumption, poses greater risks to male fertility.

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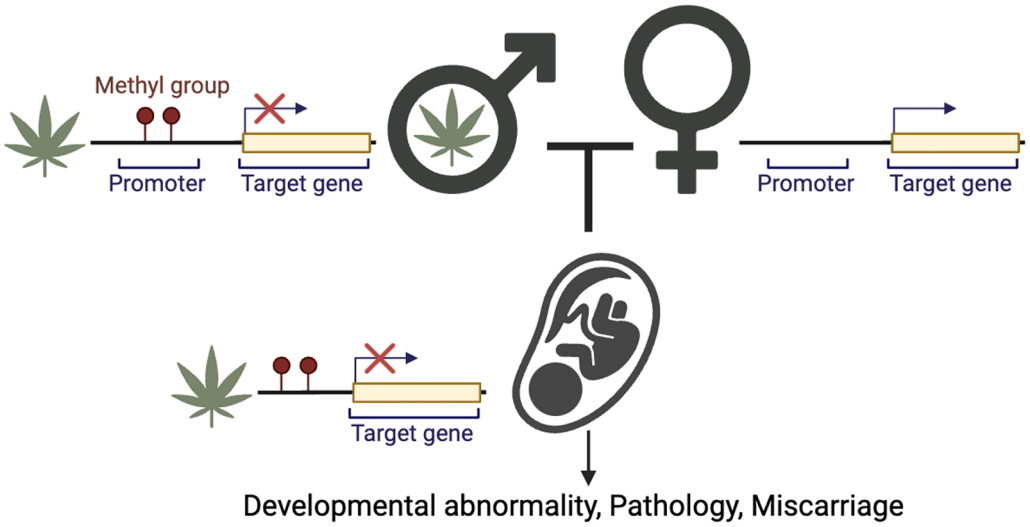

Fig. 2. Paternal cannabis use and the transfer of epigenetic modifications to offspring. Cannabis use is associated with DNA methylation at various sites known to play important roles in fetal and placental development. These methylation changes have been correlated with developmental abnormalities and fetal pathology, as well as miscarriage. [Created in Biorender.com].

CRediT authorship contribution statement

Olivia L. M. Scandlan: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Laura A Favetta**: Writing – review & editing, Supervision, Conceptualization.

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.

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Data Availability

No data was used for the research described in the article.

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