Marijuana, phytocannabinoids, the endocannabinoid system, and male fertility

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Abstract Marijuana has the highest consumption rate among all of the illicit drugs used in the USA, and its popularity as both a recreational and medicinal drug is increasing especially among men of reproductive age. Male factor infertility is on the increase, and the exposure to the cannabinoid compounds released by marijuana could be a contributing cause. The endocannabinoid system (ECS) is deeply involved in the complex regulation of male reproduction through the endogenous release of endocannabinoids and binding to cannabinoid receptors. Disturbing the delicate balance of the ECS due to marijuana use can negatively impact reproductive potential. Various in vivo and in vitro studies have reported on the empirical role that marijuana plays in disrupting the hypothalamus-pituitary-gonadal axis, spermatogenesis, and sperm function such as motility, capacitation, and the acrosome reaction. In this review, we highlight the latest evidence regarding the effect of marijuana use on male fertility and also provide a detailed insight into the ECS and its significance in the male reproductive system.

Capsule We highlight the latest evidence regarding the effect of marijuana use on male fertility and provide a detailed insight into its significance in the male reproductive system. Marijuana and its compounds can influence male fertility at multiple levels by acting through both the cannabinoid and vanilloid receptors.

Keywords Male infertility · Marijuana · Spermatozoa · Endocannabinoid system · Testosterone · LH · FSH · Estrogen · Sperm motility · Sperm viability

Introduction

Once a social taboo, medical, spiritual, and even recreational marijuana use is now increasingly accepted. Lobbying for the legalization of marijuana is at an unprecedented peak in the USA and becoming a global phenomenon. To date, medical marijuana use has been legalized in 23 states and the District of Columbia in the USA, while it has already been legalized for recreational use in four states. In Europe, and in specific the Netherlands, physicians have been able to prescribe cannabis preparations to patients for the last 10 years [1]. In Germany, medicinal use of cannabis are only granted for special cases while in Italy, cannabis are freely available to patients with a prescription since 2014. Proponents argue that it is an effective treatment for symptoms of patients with serious health issues, amongst other cancer-related pain and epilepsy. However, opponents maintain that it has several unwanted side effects that overshadow the beneficial effects and that too few valid scientific studies have been performed to support these claims. One specific area of concern is the effect of marijuana on the male reproductive system as epidemiological and experimental studies have shown that episodic marijuana use has long been associated with decreased testosterone release, reduced sperm counts, motility, viability, morphology, and inhibition of the acrosome reaction in humans. All of these factors can have drastic implications in the long term with regards to impairing male reproduction as well as negatively impacting the offspring [2–5].

Cannabis is undoubtedly the most widely cultivated, trafficked, and abused illicit drug in the world. Approximately 147 million people, or 2.5 % of the world population, consume

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cannabis [6]. According to the National Survey on Drug Use and Health, marijuana is the most commonly used among all illicit drugs in the USA. It is estimated that 80 % of the 24.6 million illicit drug users (i.e., 19.8 million) in the USA uses marijuana, with 64.7 % being marijuana-only users. Marijuana users are predominantly male. It is furthermore evident from the survey that marijuana use was more prevalent among men who are of reproductive age [7]. All of these facts combined are more than enough reason to raise awareness and debate about the effects and safety surrounding marijuana use.

Cannabis, commonly referred to as marijuana, is a product of the dried leaves and flowers from the plant *Cannabis sativa*. It is consumed for either its psychoactive (relaxation and mild euphoria) or physiological effects. Upon consumption, it acts via releasing of cannabinoid compounds that bind to cannabinoid receptors which form part of the endocannabinoid system (ECS). Numerous roles have been ascribed to the ECS, but it is known to also play a very important and specific role in the control of male reproduction [8]. An understanding of this system is therefore fundamental to be able to fully grasp the effect of exogenous cannabinoids (phytocannabinoids) on male reproductive function.

In the present paper, we will provide a comprehensive overview of the latest evidence regarding the effect of marijuana use on male infertility; however, this cannot be done in isolation as this drug exerts its effects via the ECS. We furthermore aim to also provide broad insight into the complicated ECS, its involvement, and its importance in the male reproductive system.

General pharmacobiology of marijuana

Marijuana consists of dried leaves and flowers from the plant *Cannabis sativa* and is also known under numerous street names, including weed, pot, grass, 420, hashish, joint, dope, and many more. It releases the psychoactive cannabinoid compound called tetrahydrocannabinol, with Δ^9 -tetrahydrocannabinol (THC) being much more abundant and active than Δ^8 -tetrahydrocannabinol [9]. It contains several other cannabinoids, such as cannabidiol (CBD) and cannabinol (CBN), but these are not as abundant and their psychoactive effects not as well-expressed as that of THC [10, 11]. Only through sufficient heating or dehydration the tetrahydrocannabinolic acid contained in marijuana can undergo decarboxylation and form the psychoactive THC [12, 13]. As previously mentioned, it exerts its effects via the ECS through binding to the cannabinoid receptors.

Cannabis has varying psychoactive and physiological effects when consumed, depending on the strain, form (herb, resin, oil), and method (e.g., smoking, ingestion, tablets, tinctures, etc.) by which it is consumed [10]. The psychoactive effects of marijuana include that of stimulant, depressant, and hallucinogen leading to change of perception and mood. Physiologically, it lowers blood pressure and increases heart rate, while it also impairs memory (short-term and working), concentration, and

psychomotor coordination [6]. Other chronic health effects ascribe to marijuana use include airway injury, respiratory inflammation, bronchitis, and mental illnesses such as schizophrenia. The materia medica on marijuana as a therapeutic for nausea and glaucoma, a stimulant of appetite as well as an analgesic in advanced stages of disease has been well documented through several controlled trials and studies [10, 14]. However, the health consequences of marijuana warrants further investigation.

The endocannabinoid system—a brief overview

The ECS consists of the endogenous endocannabinoid ligands, their congeners, the biosynthetic and hydrolyzing enzymes involved in the metabolism of these ligands, their transporter proteins, and receptors [15, 16]. The ECS is present in both mammalian and non-mammalian vertebrates and appear to be an evolutionary conserved master system. Endocannabinoids are found to be widely dispersed in human tissues such as the central nervous system, peripheral nerves, leukocytes, spleen, uterus, and testicles [17]. It must therefore play a role in a number of physiological processes and appears to be deeply involved in the control of reproductive function [8, 18]. Please refer to Fasano et al. [8] for a comprehensive overview of the ECS.

The endocannabinoids

Endocannabinoids are endogenous lipids that mimic various actions of THC [4]. As of yet, four endocannabinoids have been characterized, i.e., arachidonoylglycerol ether, virodhamine, N-arachidonylethanolamine or anandamide (AEA), and 2-arachidonoylglycerol (2-AG). AEA and 2-AG are the best characterized members that belong to this family of biolipids, and both are regarded as the main endocannabinoids in the human body [19, 20]. They act on the cannabinoid receptors (CB1 and CB2) and therefore mimic some of the biological actions of cannabinoids (THC) originating from cannabis/marijuana. Interestingly, it is also believed that these endocannabinoids are not stored intracellularly but rather produced from membrane phospholipid precursors through the activation of specific phospholipases and are released on demand [21, 22]. Their extracellular bioavailability is subjected to an unsubstantiated endocannabinoid membrane transporter (EMT) [23]. These endocannabinoids can be synthesized and inactivated independently, while they also act promiscuously (i.e., do not only act on cannabinoid receptors). This allows for a high degree of differential flexibility of their actions, thereby making the ECS a highly complex system to understand [24].

AEA Phospholipase D catalyzes the release of AEA through the cleavage of a phospholipid precursor [25]. AEA acts as a

partial agonist for the cannabinoid receptors, being more selective for CB1 [25]. However, it also binds to the transient receptor potential cation channel subfamily V member 1 (TRPV1) or type-1 vanilloid receptor [26, 27]. It is metabolized, after cellular uptake by EMT, inside the cell to ethanolamine and arachidonic acid (AA) by fatty acid amide hydrolase (FAAH) which is membrane bound [28].

2-AG 2-AG belongs to the monoacylglycerol (MAG) family of endocannabinoids. It acts as a potent equal agonist for both CB1 and CB2 receptors; however, it does not act on the TRPV1 receptor [29, 30]. Various biosynthetic pathways (e.g., phospholipase C-dependent and independent) are responsible for the production of 2-AG [25]. The transport of 2-AG across the cell membrane may be mediated by EMT as well. Once inside the cell, 2-AG is a substrate for the cytosolic monoacylglycerol lipase (MAGL) and is mostly degraded to glycerol and AA [4].

The cannabinoid receptors

Two subtypes of cannabinoid receptors (CB1 and CB2) have been described as of yet, both of which belong to the family of transmembrane spanning G-protein coupled receptors (GPCRs) [31]. AEA and 2-AG bind to the extracellular site of these GPCRs [32]. Stimulation of these receptors can lead to either inhibition of adenylate cyclase and decreased c-AMP levels and/or inhibition of certain calcium channels, thereby reducing calcium influx [33–35]. Unlike most GPCRs, the cannabinoid receptors have more than one endogenous ligand. Other receptors that are stimulated by endocannabinoids have also been described.

CB1 receptors These GPCRs are found primarily in the central nervous system [36–39]. It is also located in the ovary, uterine endometrium, testis, vas deferens, urinary bladder among others [19, 32, 40–42]. In the brain, CB1 receptors seem to be located in the preoptic area of the hypothalamus which is also the home of luteinizing hormone releasing hormone (LHRH) secreting neurons [43]. In the male reproductive system, they are located in the testis, prostate, and vas deferens [44, 45]. In humans, CB1 receptors are also expressed on the plasma membrane of the acrosomal region, midpiece, and on the tail of spermatozoa [46]. Rossato et al. [47] on the other hand showed the CB1 receptor to be only present in the sperm head and midpiece, but not the tail.

CB2 receptors CB2 receptors, which are also GPCRs, are mainly expressed in the immune system and peripheral cells as well as in neuronal cells [32, 48]. Initially, there was lack of clarity regarding the presence of CB2 receptors in spermatozoa, but a study conducted by Agirregoita et al. confirmed the presence of the CB2 receptor in human spermatozoa [46].

Here, these receptors are present in the postacrosomal region, midpiece, and tail of spermatozoa [46]. CB2 receptors were also found to be present on Sertoli cells [49].

Other receptors Some other receptors such as the purported CB3 receptor and non-CB1/non-CB2 receptors have also been described [50, 51]. The non-CB1/non-CB2 receptors include the TRPV1 receptor which is an intracellular target for AEA. However, 2-AG does not have an effect on the TRPV1 receptor [30]. In human spermatozoa, the TRPV1 receptor was restricted to the postacrosomal region of the sperm head [52]. These findings furthermore suggest an interaction between the cannabinoid and vanilloid systems [53]. Endocannabinoids and their congeners have also been implicated to activate peroxisome proliferator-activated receptors and thus play a role in energy homeostasis [54].

The endocannabinoid system and male reproduction

The presence of the ECS has been demonstrated in various cell types that are involved in male reproduction. As previously mentioned, endocannabinoids and cannabinoid receptors have been shown to be present in testicular tissue, including Sertoli and Leydig cells as well as spermatozoa in various species from invertebrates to mammals [4]. It was furthermore localized in areas of the hypothalamus responsible for the production of gonadotrophic releasing hormone (GnRH) and can thus also exert a role via the hypothalamus-pituitary-gonadal (HPG) axis. It is therefore clear that the ECS is deeply involved in the control of the male reproductive system and function of spermatozoa.

ECS and the hypothalamus-pituitary-gonadal axis

A fully functional HPG axis is needed to properly orchestrate and maintain the process of spermatogenesis [55]. GnRH is released from the hypothalamus which, in turn, stimulates specific nuclei in the pituitary to synthesize and release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These two gonadotropins act on their respective target tissues in the gonads. Basically, FSH stimulates the Sertoli cells to support developing spermatozoa, while LH leads to the release of testosterone from Leydig cells.

The ECS has been closely associated with the HPG pathway at multiple levels as CB1 receptors are expressed in the anterior pituitary, Leydig cells and Sertoli cells. CB2 was found in Sertoli cells while other components of the ECS, such as AEA, FAAH, and EMT have also been observed in testicular tissues [4, 19, 49, 56]. For example, administration of AEA, which usually binds to postsynaptic CB1 receptors, decreased serum LH. This action could be prevented by a specific CB1 antagonist (SR141716) [56, 57]. Farkas and

coworkers furthermore demonstrated that endocannabinoid activates CB1 which, in turn, inhibits spontaneous gamma aminobutyric acid (GABA) release [58]. Postsynaptic GABA receptors, located on GnRH neurons, are not activated, and as a consequence, GnRH is not released. Interestingly enough, the inhibitory effect of AEA was higher than that of 2-AG [58, 59]. Olah and coworkers postulated that the difference between the effects of AEA and 2-AG on the serum levels of LH is due to the difference in receptor activation as AEA can activate both CB1 and TRPV1 receptors while 2-AG acts only on the CB1 receptor [60]. Furthermore, CB1 receptor expression varies between males and females, thereby indicating that males are more sensitive to cannabinoid-induced changes and subsequently the secretion of pituitary hormones [61].

CB1 receptors have also been found to be present in the Leydig cells of mice and rats. LH and testosterone secretion were decreased in CB1 receptor-inactivated mice. However, in wild-type mice, AEA suppressed the levels of both of these hormones [56]. When they are activated through the endogenous cannabinoid AEA, it not only results in a drop in testosterone levels, but this alteration in sex steroid level can also disturb the spermatogenic process [19, 45, 56]. It was furthermore showed that these CB1 receptors are responsible for the actions of exogenous cannabinoids [56].

Sertoli cells play an important role during germ cell development as they nurture the developing spermatozoa. Sertoli cells not only have CB1 and CB2 receptors but also have TRPV1 receptors. AEA can act via these receptors to induce apoptosis of these cells [62] (see Fig. 1).

FSH acts on its receptor on the Sertoli cell to activate two separate pathways. It activates adenylate cyclase which, in turn, causes PKA activation via cAMP, thereby causing increased expression of FAAH [63]. The other pathway triggered by FSH is by the activation of PI-3 which stimulates the expression of aromatases (at a transcriptional level) and thus increase estrogen levels in the cell (see Fig. 1). This subsequently causes an increase in FAAH expression by activation of the FAAH promoter through the estrogen response element. FAAH helps to hydrolyze AEA and thereby decrease the intracellular level of AEA. Thus, FAAH has a protective role in preventing AEA-induced apoptosis [62]. Interestingly, studies showed that the CB2 receptor can also play a protective role by decreasing programmed cell death [62]. Activation of CB2 receptors protects Sertoli cells against AEA-induced/mediated apoptosis [49, 62].

ECS and sperm function

Both CB1 and CB2 receptors are present on spermatozoa. CB1 has been localized to the plasma membrane of the acrosomal region, midpiece, and tail of the spermatozoon, while CB2 receptors are mostly localized in the postacrosomal region as well as midpiece and tail [46, 47, 64]. The transporters

as well as enzymes responsible for synthesis and hydrolysis of endocannabinoids have also been identified in the male gametes of various species, including humans. Francavilla et al. therefore concluded that human spermatozoa exhibit a completely functional ECS [52]. As AEA is present in human seminal plasma [65, 66], spermatozoa are therefore also exposed to this compound in the epididymis [67], and it is inevitable that the ECS thus play a potential modulatory role in sperm function [27] (see Fig. 2).

AEA, as mentioned earlier, is a primary endocannabinoid. As shown in Fig. 2, it is synthesized from the membrane phospholipid N-archidonyl-phosphatidyl ethanolamine (NAPE) by the enzyme NAPE-PLD [68] inside the spermatozoa from where it is transported to the outside via the EMT. [23] AEA can also move back into the cell via the EMT. Once outside, it can act on both CB1 and CB2 receptors [46]. Activation of these receptors modulates the motility of spermatozoa. CB1 receptor activation was found to not only decrease motility and viability of spermatozoa [69] but also inhibit the capacitation-induced acrosomal reaction [47]. Similarly, the CB1 antagonist, rimonabant (SR141716), increased sperm motility and viability, while it also induced capacitation and the acrosome reaction. It had an overall lipolytic action on the spermatozoa, and it also induced energy expenditure possibly through induction of the pAkt and pBc12 proteins that control pro-survival pathways and regulate metabolism [70]. Studies also showed that CB2 modulated the motility of spermatozoa. It was shown that CB2 activation caused an increase in the slow/sluggish progressive sperm population and CB1 activation increased the immobile spermatozoa [46]. In humans, the endogenous agonists activate both CB1 and CB2 receptors. Therefore, motility will depend on the dose of the agonist. This is particularly important as the exogenous cannabinoids might cause an inappropriate decrease in motility of spermatozoa. If these substances cause poor motility, it will result in inappropriate completion of capacitation in an area of the female reproductive tract prior to meeting the oocyte [46]. Spermatozoa also express the vanilloid TRPV1 receptor. Along with CB1 receptors, the TRPV1 receptor has been found to play a role in spermatozoa capacitation [71]. The activation of the TRPV1 receptor through AEA binding helps to prevent the spontaneous acrosome reaction to occur in an untimely manner before reaching the oocyte. Unlike the CB1 and CB2 receptors, binding to the TRPV1 receptor occurs intracellularly [71, 72].

Supporting the physiological observations mentioned previously, a study of 86 men presenting at an infertility clinic showed that the levels of AEA in the seminal plasma of both asthenozoospermic and oligoasthenozoospermic men were significantly lower compared to normozoospermic men [69].

Fig. 1 The involvement of cannabinoids, vanilloid receptors, and FSH in Sertoli cell function. (AA arachidonic acid, EtNH₂ ethylamine, FSH follicle-stimulating hormone)

Similarly, the levels of CB1 mRNA were also decreased significantly in the spermatozoa from these men [69].

The endocannabinoid AEA was found to decrease the mitochondrial activity of spermatozoa, likely through CB1-

mediated inhibition, which, in turn, will hamper sperm viability and functions such as motility in a dose-dependent manner [47, 69]. AEA also affected motility, capacitation, and acrosome reaction in human spermatozoa in a similar dose-

Fig. 2 The influence of the endocannabinoid system on sperm function. (AA arachidonic acid, AEA N-arachidonylethanolamine or anandamide, CB1R cannabinoid receptor 1, CB2R cannabinoid receptor 2, EMT endocannabinoid membrane transporter, EtNH₂ ethylamine, FAAH fatty acid amide hydrolase, PA phosphatidic acid, PL phospholipid, PLD phospholipase D, TRPV1 transient receptor potential cation channel subfamily V member 1)

dependent manner [9, 47]. These findings suggest a possible role for the cannabinoid system in the pathogenesis of some forms of male infertility.

Mice spermatozoa are exposed to decreasing concentrations of 2-AG, from caput to cauda, during epididymal transit. This gradient is probably necessary to counteract CB1-dependant inhibition of motility and to keep spermatozoa quiescent until release [73]. Ricci et al. [74] also concluded that CB1 receptors play a central role in preventing the acquisition of motility at too early a stage in the epididymis.

Endocannabinoids inhibit the biochemical and physiological changes needed for sperm to undergo capacitation through a CB1-mediated mechanism [74–78] and subsequently reduces the ability to AR in various species [76]. In addition, capacitated spermatozoa show a general downregulation of the expression of ECS elements compared to non-capacitated sperm [67, 75].

The distinct compartmentalization of CB1/CB2 receptors and of TRPV1 in spermatozoa as well as their levels of expression may critically regulate sperm function. Additionally, the presence of an endocannabinoid gradient in both the male and female reproductive tract can lead to differential spatiotemporal activation of these receptors, thereby affecting sperm function and the various fertilization steps [27, 79].

Marijuana, phytocannabinoids, and male reproduction

It is to be expected that exogenous cannabinoids, such as those present in marijuana, compete with endocannabinoids for binding on the cannabinoid receptors. This can disturb the ECS, and the resultant imbalance can impact fertility [69]. It is not surprising then that studies consistently conclude that marijuana negatively affects male fertility.

Effect on the HPG axis

As previously mentioned, cannabinoid receptors are closely related to neurons in the hypothalamus and GnRH release has been shown to be inhibited in males by AEA and THC through interaction with GABA and other systems [58, 80–82]. This reduction in gonadolibersins can cascade to the rest of the HPG axis as to be discussed subsequently (see Table 1). Similarly to the effects on the HPG axis, the hypothalamus-pituitary-adrenal axis activity has also been showed to be affected by marijuana use in adolescents [88].

FSH Many studies showed that FSH levels were not significantly affected by THC as it presumably acts through LHRH [83, 84, 89]. Thus far, only a single study has reported that chronic marijuana use decreased FSH levels, but this was exclusively found in high consumption users [85]. However,

FSH has an important influence on the ECS as it increases FAAH (enzyme which degrades AEA) expression through different pathways in Sertoli cells. Thereby, FSH regulates AEA-mediated apoptosis in Sertoli cells [62].

LH Similar to the effects of marijuana on FSH levels, inconclusive findings are also reported in the literature with regards to its effects on LH. In general, it is believed that marijuana consumption decreases LH levels [83–85]. These findings are supported by a study conducted by Wenger and colleagues who injected THC into the third cerebral ventricle of male rats. It showed that THC indirectly decreased the level of LH by inhibiting the release of LHRH from the hypothalamus [89]. These results are similar to those observed in Rhesus monkeys [90]. In a later study, the Wenger group showed that CB1 receptors are actually present in the anterior pituitary and cannabinoids can therefore exert their action at both pituitary and hypothalamic levels [91]. In short, LH levels can be decreased by THC mediated through CB1 receptors.

Testosterone There have been contradictory results as far as the effect of marijuana on testosterone levels is concerned. In a case control study conducted on males (18 to 26 years) who used marijuana for a minimum of 4 days a week for at least a period of 6 months without the use of other drugs, it was reported that there was a statistically significant drop in testosterone levels. The findings were similar after chronic and acute exposure [85]. However, another study conducted on 66 males showed that neither chronic nor acute intake of marijuana had a significant effect on plasma testosterone levels [92]. The main difference between the two studies is the fact that the latter study also included subjects who drank cannabis as a tea. Some other studies also showed that testosterone levels did not vary much after marijuana use [83, 86]. These observations are interesting in spite of the fact that CB1 receptor activation by AEA caused a drop in testosterone levels [56] and that animal models (rats and monkeys) showed a marked reduction in testosterone in response to THC and CBD treatment [93, 94].

Estrogen To investigate the possible estrogenic effects of marijuana smoke condensate (MSC) and cannabinoids, a study was conducted on human breast cancer cells. It was reported that THC, CBD, and CBN had no effect, but MSC stimulated cell proliferation [95]. Some studies propose that marijuana use can even lead to gynecomastia. Moreover, the estrogenic effects of MSC were also observed during the immature rat uterotrophic assay as evidenced by an increase in uterus to body weight ratio [95]. As THC, CBD, and CBN did not have any estrogenic actions on their own, either the combined effects of these must be responsible for the changes observed or perhaps the phenolic compounds contained in MSC may play a role.

Table 1 Effect of marijuana use on reproductive hormones in males

Parameter	Effect	Intervention	Author
FSH	↔	Frequent marijuana smoking	Cone et al. [83]
	↔	Chronic marijuana users	Vescovi et al. [84]
	↔	Chronic marijuana users (<10 joints per week)	Kolodny et al. [85]
LH	↓	Chronic marijuana users (>10 joints per week)	Kolodny et al. [85]
	↓	Frequent marijuana smoking	Cone et al. [83]
	↓	Chronic marijuana users	Vescovi et al. [84]
Testosterone	↔	Chronic marijuana users (>10 joints per week)	Kolodny et al. [85]
	↔	Frequent marijuana smoking	Cone et al. [83]
	↓	Chronic marijuana use	Kolodny et al. [85]
	↓	Acute marijuana use	Kolodny et al. [85]
	↔	Chronic marijuana use	Mendelson et al. [86]
	↔	Chronic and acute marijuana use	Friedrich et al. [86]
	↔	Daily marijuana use	Hembree et al. [87]

Effect on reproductive organs

Not many reports are available on the direct and physical effects of marijuana use on the reproductive organs of men. Kolodny et al. [85] reported no change in testicular size and texture in chronic marijuana users. However, a number of animal studies have reported direct effects on various reproductive organs. Prolonged cannabis exposure reduced the ventral prostate, seminal vesicle, and epididymal weights in both rats and mice [96–100]. These findings were accompanied by histological evidence showing disruption of the basement membrane, significant shrinkage of the seminiferous tubules marked by appearance of giant cells in their lumen, reduction in the number of spermatogonia, and furthermore spermatogenic cells showing degeneration, vacuolated/scanty cytoplasm, and small dense nuclei. It was also reported that testicular degeneration and necrosis was induced in dogs after only 30 days of cannabis administration [101]. Results from various experiments of a very eloquent study not only showed a significant decrease in weight and increase in apoptosis of mice testes (in vivo) after cannabis treatment, but it also reports on significantly decreased testicular LH receptor (LHR) and FAAH expression, thus suggesting that cannabis has a direct action on testicular activity [102]. Hypogonadism was also reported by Harclerode et al. [98]. A number of other animal studies correspondingly reported that THC reduces the activities of the enzymes, beta-glucuronidase, alpha-glucosidase, acid phosphatase, and fructose-6-phosphatase in a dose-related manner in the testis, prostate as well as in the epididymis [103]. From these findings, it can be concluded that THC interfere with the normal physiology and functioning of the male reproductive organs.

Interestingly, in a recent population-based case–control study, a specific association was observed between marijuana use and the risk of testicular tumors (non-seminoma and mixed histology). The authors went on to caution that recreational and therapeutic use of cannabinoids by young men may confer malignant potential to testicular germ cells [97].

Effect on sperm parameters and function

As the ECS is so deeply involved in the regulation of the male reproductive system, a number of studies have investigated the effect of cannabis on various sperm parameters. Just as the blood–brain barrier protects the brain, the blood–testis barrier provides protection to the testis against harmful substances. However, cannabinoids are lipophilic, and they accumulate in membranes and testicular/epididymal fat from where it can be released slowly, and this exposure can affect spermatozoa and their function [104] (see Table 2).

A decrease in sperm concentration has been reported in both humans [85, 87, 105] and animals [102] after regular exposure to cannabis. It also appears that sperm counts are inversely proportional to the amount of drug taken [85]. There is limited evidence for marijuana use to be associated with morphological abnormalities in human spermatozoa [87]. However, in a recently performed unmatched case-referent study with 1700 participants, it was clearly reported that cannabis exposure is a risk factor for poor sperm morphology (OR $\frac{1}{4}$ 1.94, 95 % CI 1.05–3.60) [106]. Morphological abnormalities due to cannabis have been well documented in animal studies. Interestingly, it appears as if THC and CBN, but not CBD, leads to more morphological abnormalities [109].

Table 2 Effect of marijuana and cannabinoids on human sperm parameters and function

Parameter	Effect	Intervention	Study	Author
Sperm concentration	↓	Marijuana (smoking)	In vivo	Kolodny et al. [85]
	↓	Marijuana (smoking)	In vivo	Hembree et al. [105]
Morphology	↓	Marijuana (smoking)	In vivo	Pacey et al. [106]
	—	Marijuana (smoking)	In vivo	Hembree et al. [87]
Viability	↓	Anandamide	Invitro	Rossato et al. [47]
	↓	Anandamide	Invitro	Schuel et al. [67]
	↑	Rimonabant (CB1 receptor antagonist)	Invitro	Aquila et al. [70]
	↓	MF-AEA, URB597 (CB1 receptor agonist)	Invitro	Aquila et al. [70]
	↓	Met-AEA (non-hydrolyzable analog of AEA)	Invitro	Barbonetti et al. [107]
	↓	MF-AEA (anandamide analog)	Invitro	Aquila et al. [64]
	↓	Meth-AEA	Invitro	Amoaka et al. [69]
Sperm motility	↓	Anandamide	Invitro	Rossato et al. [47]
	↓	THC (therapeutic and recreational levels)	Invitro	Whan et al. [108]
	↑	Rimonabant (CB1 receptor antagonist)	Invitro	Aquila et al. [70]
	↓	MF-AEA, URB597 (CB1 receptor agonist)	Invitro	Aquila et al. [70]
	↓	Meth-AEA	Invitro	Amoaka et al. [69]
	↓	ACEA (CB1 selective agonist)	Invitro	Agirregoitia et al. [46]
	↓	JWH-015 (CB2 selective agonist)	Invitro	Agirregoitia et al. [46]
	↓	Met-AEA (non-hydrolyzable AEA analog)	Invitro	Barbonetti et al. [107]
Hyperactivated motility	↓ (Biphasic)	Anandamide (high concentration—2.5 nM)	Invitro	Schuel et al. [67]
	↑ (Biphasic)	Anandamide (low concentration—0.25 nM)	Invitro	Schuel et al. [67]
Capacitation/acrosome reaction	↓	Anandamide	Invitro	Rossato et al. [47]
	↓	Anandamide	Invitro	Schuel et al. [67]
		THC		
Acrosome reaction (spontaneous/induced)	↓	THC (therapeutic and recreational levels)	Invitro	Whan et al. [108]
Acrosin activity	↑	MF-AEA (physiological levels)	Invitro	Aquila et al. [64]
	↔	MF-AEA (supra physiological levels)	Invitro	Aquila et al. [64]
Hemizona binding	↓	Anandamide	Invitro	Schuel et al. [67]

ACEA Arachidonyl-2'-chloroethylamide, *JWH-015* (2-methyl-1-propyl-1H-indol-3-yl)-1-naphthalenyl-methanone, *Met-AEA* R(+)-methanandamide, *Meth-AEA* methanandamide, *MF-AEA* 2-methylarachidonyl-2'-fluoroethylamide, *THC* Δ^9 -tetrahydrocannabinol, *URB597* 3'-carbamoyl-biphenyl-3-yl-cyclohexylcarbamate

Human seminal plasma, mid-cycle fallopian tubal fluid as well as follicular fluid contains AEA which suggests that human spermatozoa are sequentially exposed to AEA, indicating a potential modulatory role for the ECS on sperm function [40, 65, 66]. Even more profound is the fact that small amounts of THC have been shown to be secreted by the vagina into the vaginal fluid in women who regularly use marijuana, leading to stimulation of spermatozoa and possibly affecting sperm function [4, 110].

From the literature, it is evident that sperm motility and viability is mediated via endocannabinoids and CB receptors. Met AEA (stable form of AEA) was shown to decrease human sperm motility and viability via its action through CB1 [69]. Several other *in vitro* studies on human spermatozoa are in agreement with these findings [46, 47, 64, 107]. Whan et al. [108] exposed human spermatozoa to both therapeutic and recreational levels of THC and showed clearly that it reduced the percentage of motile and progressively motile spermatozoa, while the kinematic parameters such as straight line velocity and average path velocity were also decreased. These observations are supported by both *in vitro* [111] and *in vivo* animal studies [102] where it is clearly shown that THC attenuates sperm motility and viability. The fact that THC impairs sperm motility and viability can be explained partially by the fact that it inhibits mitochondrial respiration and activity; therefore, the exposed spermatozoa are starved from energy [112]. These findings are supported by the marked reduction in sperm ATP levels due to THC [111]. It was also shown that THC inhibits fructose metabolism. With fructose being a major energy source for spermatozoa, this could further hamper sperm motility [113]. Glycolysis combined with oxidative phosphorylation also provides fuel for many other energy-dependent processes including capacitation and the acrosome reaction [114]. Disturbing the ECS homeostasis will subsequently adversely affect these energy-dependent processes with implications for gaining fertilizing potential.

The ECS is important in keeping the spermatozoa from undergoing capacitation before reaching of the oocyte [47]. This is essential in preventing the spermatozoa from undergoing untimely capacitation in an unusual location. The fact that the process of capacitation is inhibited by cannabinoids means that this effect can be extrapolated to marijuana. It was shown that Met AEA, which is the stable analogue of AEA, inhibits capacitation via the activation of CB1 receptor [75].

Cannabinoids (AEA, THC) has an effect on the acrosome reaction too. CB1 receptor activation prevents the acrosome reaction from occurring [47, 67, 75]. Similar inhibitory findings were observed for both the spontaneous and induced AR after *in vitro* treatment of spermatozoa with either therapeutic or recreational concentrations of THC [108].

Fertilizing ability of spermatozoa also appears to be affected as hyperactivated motility, necessary for penetration of zona pellucida, as well as hemizona binding were negatively

affected in AEA-treated spermatozoa. Interestingly, low/physiological concentrations of AEA stimulated hyperactivated motility while it was attenuated at higher dosages. This biphasic effect was shown between 1 to 6 h of incubation in AEA [67].

Spermatozoa can also be cytogenetically affected by marijuana as Zimmermann et al. demonstrated that as little as five consecutive days of treatment with THC, CBN, or CBD, respectively, caused increased ring and chain translocations but showed no difference in chromosome breaks, deletions, and aneuploidy in mice spermatozoa [2].

Effect on libido and sexual function

In both males and females, arousability and sexual behavior appear to be modulated by ECBs. It is well established that a group of oxytocinergic neurons containing CB1 receptors in the paraventricular nucleus of the hypothalamus (PVN) regulate erectile function and copulatory behavior of males [115]. The use and effect of cannabis on sexual function are extremely controversial and more than likely subject-specific. Anecdotal aphrodisiac-like properties of cannabis as described by some users are likely the result of altered perceptual processing of the sexual encounter.

Currently limited evidence from human clinical trials is available to suggest any beneficial and/or detrimental effects of cannabis on male libido and sexual function [10]. In one study, acute use of marijuana has been shown to increase sexual drive, but chronic use of marijuana was reported to decrease libido in males [116]. These sentiments were echoed by Abel who stated that a lesser amount of cannabis can enhance sexual activity, but larger quantities may impede sexual motivation [117]. Besides, similar dose effects were reported by American Indian men who were chronic cannabis users [118].

A study conducted in mice exposed to chronic administration of THC for 30 days showed that there was significant loss of libido in these rodents [119]. It was also shown in a rat model that marijuana use was associated with impotence [120]. Results from studies on non-human primates suggest that cannabinoids have a predominant detrimental effect on male sexual motivation and erectile function [121]. Various other studies however report that cannabis intensified arousal and enhanced sexual pleasure in men [122, 123]. Di Marzo and coworkers reported that THC weakens sexual drive by interfering with the production of testosterone [124].

In a large Swiss study ($n > 9000$), cannabis was indirectly associated to both premature ejaculation and erectile dysfunction (ED) [125]. Similarly, it was also showed in another recent study that chronic cannabis consumption can cause vascular ED in young habitual cannabis users through its effect on endothelial function [126]. However, no link between frequency of cannabis use and trouble keeping an erection was

reported in a study where 4350 men were screened for the use of cannabis and its sexual effects [127].

Despite marijuana use being implicated to cause reduced libido, gynecomastia, and erectile disorders [128], no properly controlled study has been performed in humans to substantiate these speculations.

Conclusion

It is beyond doubt that recreational and medicinal marijuana usage will increase and become even more prevalent. Given the deep involvement of the ECS in the regulation of male reproduction and the direct impact of exogenous cannabinoids on the homeostasis of the ECS, the potential thread represented by marijuana on the finely tuned events associated with male fertilizing ability must definitely be considered [4, 82]. Surprisingly, very few studies have explored the direct effect of marijuana on male fertility. This can mainly be ascribed to legislation and ethical considerations making it virtually impossible to pursue in vivo human studies. The current body of knowledge pertaining to this topic mainly consists of a number of earlier human studies and more recently animal, in vitro, and retrospective studies. Despite these limitations, it is clear that marijuana and its compounds can influence male fertility at multiple levels. A number of studies have attributed dysregulation of the HPG axis, and in specific reduction in a key hormone such as LH, which, in turn, can affect testosterone and spermatogenesis to marijuana. It appears as if marijuana can actually affect semen parameters and sperm function by acting through both the cannabinoid and vanilloid receptors. Furthermore, sexual health has also been linked to marijuana as it seems to have an effect on erectile function.

With the change in legislation and decriminalization of marijuana use, as well as the fact that some studies report conflicting and contradictory findings, it is paramount that more clinical studies should be undertaken to examine the effects of marijuana use in greater detail. Despite that human studies are currently few and limited by their observational nature, the existing proof substantiates the claim that marijuana use has a detrimental effect on male reproductive potential [129]. Of interest would also be to explore the confounding effects of marijuana use on tobacco smokers as a recent study revealed that cigarette smokers are greater abusers of cannabis, whilst cigarette smoking males of infertile couples showed lower ejaculate volumes despite higher testosterone levels [130]. All the above findings underline the fact that clinicians should include questions on marijuana usage while evaluating infertility in males. Health professionals should definitely also keep the association and potential impact of marijuana on male fertility in mind when prescribing medical marijuana.

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Author contributions S.S.D.P. conceived the idea, researched data, and wrote the article. All authors made substantial contributions to the discussion of content and reviewed/edited the manuscript before submission.

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