Cannabinoid signalling and effects of cannabis on the male reproductive system

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Abstract | Marijuana is the most widely consumed recreational drug worldwide, which raises concerns for its potential effects on fertility. Many aspects of human male reproduction can be modulated by cannabis-derived extracts (cannabinoids) and their endogenous counterparts, known as endocannabinoids (eCBs). These latter molecules act as critical signals in a variety of physiological processes through receptors, enzymes and transporters collectively termed the endocannabinoid system (ECS). Increasing evidence suggests a role for eCBs, as well as cannabinoids, in various aspects of male sexual and reproductive health. Although preclinical studies have clearly shown that ECS is involved in negative modulation of testosterone secretion by acting both at central and testicular levels in animal models, the effect of in vivo exposure to cannabinoids on spermatogenesis remains a matter of debate. Furthermore, inconclusive clinical evidence does not seem to support the notion that plant-derived cannabinoids have harmful effects on human sexual and reproductive health. An improved understanding of the complex crosstalk between cannabinoids and eCBs is required before targeting of ECS for modulation of human fertility becomes a reality.

Phytocannabinoids
Naturally occurring substances in the cannabis plant.

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Marijuana is the most widely used recreational drug in the Western world — it is consumed by an estimated 83 million individuals (~3% of the world population) and is easily accessible even to young adults (aged 15–24 years)[1]. In 2016, the Substance Abuse and Mental Health Services Administration's National Survey on Drug Use and Health established that 6.5% of adolescents aged 12–17 years and about one in five young people between 18 and 25 years of age were current consumers of marijuana[2]. In the USA alone, the legal cannabis-related financial turnover was ~$6.9 billion in 2016 and projections suggest that the market will grow to $21.6 billion by 2021 (REF.[3]). The legalization of cannabis in multiple regions of the USA — 36 and 15 states have now legalized medical marijuana and recreational marijuana, respectively — raises concerns about its potential hazard to health. Research into the therapeutic potential of cannabinoid-based drugs suggests that they are clinically useful in a wide range of pathological conditions, including neurological[4] and psychiatric disorders[5]. However, repeated recreational and medical cannabis use has been associated with short-term and long-term side effects, including respiratory and cardiovascular disorders, cognitive alterations, psychosis, schizophrenia and mood disorders[5]. In the past 5 years, an unfavourable effect on male reproductive health has also been claimed[6,7].

Cannabinoids and their endogenous counterpart (endocannabinoids; eCBs) bind to cannabinoid and non-cannabinoid receptors of the endocannabinoid system (ECS), thus regulating several aspects of male reproduction[6,8]. Smoking marijuana can negatively affect male reproductive potential by affecting the homeostasis of the ECS[6,9].

In this Review, we outline and discuss the available evidence for the involvement of eCBs and plant-derived cannabinoids in various aspects of male sexual and reproductive health.

Characteristics and pharmacokinetics of cannabis

The existence and characteristics of different species and cultivars of cannabis must be taken into account when evaluating their effects on health outcomes. *Cannabis sativa* and *Cannabis indica* are the most widespread and best-characterized species of cannabis; extracts of both plants contain phytocannabinoids of therapeutic interest, such as Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD)[10,11] (TABLE 1). THC and CBD have very different biological effects: THC is the psychoactive component, which produces euphoria and cognitive impairments and has also been linked to side effects such as anxiety and psychosis, whereas CBD has ~10% of the activity of THC and is considered to

Key points

- Marijuana has the highest consumption rate of any recreational drug in the Western world.
- Endocannabinoids and their receptors, enzymes and transporters, which together form the endocannabinoid system (ECS), are present in various components of the male reproductive tract, including male genital glands, testis and sperm.
- Preclinical studies have shown that the ECS is involved in negative modulation of testosterone secretion by acting at both central and testicular levels.
- As yet, clinical data are insufficient to conclude that cannabinoids have a harmful effect on human male sexual function and fertility.
- Although cannabinoid receptors are present in the testes and sperm, the effects of cannabinoid exposure on spermatogenesis largely remain to be clarified.
- The ECS has the potential to provide new drug targets in male reproductive disorders, and its components might be useful as biomarkers of male infertility.

be non-psychoactive. CBD reduces anxiety, psychosis, nausea and seizures and attenuates the psychotropic effects of THC[12].

The activity of cannabis depends on the amount of THC and CBD present, as well as on the presence and concentration of >110 additional phytocannabinoids and >440 non-phytocannabinoid compounds, including terpenoids, flavonoids and sterols[10]. In addition, different formulations and administration routes are used for cannabis extracts: inhalation by smoking or vaporization, oral, oromucosal, sublingual, topical or rectal administration[13]. The route of administration affects the rate of absorption; for example, smoking provides a rapid and efficient delivery from the lungs to the brain within minutes, contributing to its potential for abuse, whereas oral administration has a slower onset of action (within hours) with a bioavailability of 10–20%, and usually <15% of the bioavailability of the smoking route[13], which is reported as 2–56%, due in part to intrasubject and intersubject variability in smoking dynamics, which contribute to uncertainty in dose delivery[13]. Notably, plasma concentrations of THC decrease rapidly upon smoking cessation, owing to its rapid distribution into highly perfused tissues such as the lungs, heart, brain and liver[13]. However, chronic exposure to THC enables this lipophilic molecule to accumulate in the adipose tissue and to remain at low concentrations not exceeding 2–7 ng/g in plasma, brain and testis; however, the concentration of the tracer ^{14}C-Δ^8-THC (labelled at the C-11 position) has been found to be 40–80 times higher in the epididymal fat than in the brain[13]. The blood–testicular barrier limits storage of THC in the testis during acute exposure. However, during chronic THC exposure, pharmacokinetic mechanisms are insufficient to prevent accumulation of THC in tissues, with subsequent deregulation of cellular processes, including apoptosis of spermatogenic cells[13]. Differences in the THC content of different cannabis products (herb, resin and oil) are the result of the ratio of the different plant organs used for production: 10–12% in flowers, 1–2% in leaves, 0.1–0.3% in stalks and <0.03% in roots[14]. Cannabis oil usually contains the highest concentration of THC (>60%) as it is derived from a concentrated resin extract[11]. Thus, the physiological effects of cannabis can vary to a large extent depending on the form consumed and the qualitative and quantitative composition of magistral preparations, with a corresponding effect on the efficacy and safety of cannabis extracts as therapeutics[4].

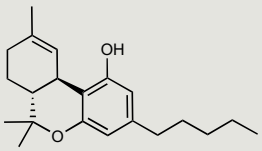
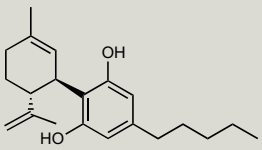
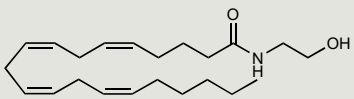
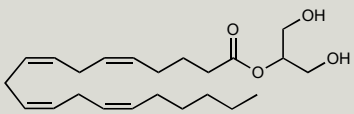
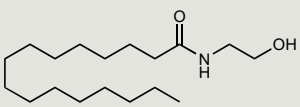
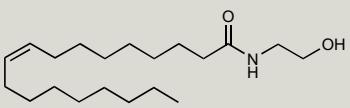
Sunflower oil
The non-volatile oil pressed from the seeds of sunflower.

Cannabinoids and endocannabinoids

Cannabinoids (or phytocannabinoids) are lipid components of cannabis that include THC and CBD (TABLE 1) as the most abundant and best-studied members[12]. These substances exert most of their biological activities of cannabinoids in humans, by interacting with the ECS[12]. In parallel, *N*-arachidonoylethanolamine (anandamide; AEA) and 2-arachidonoylglycerol (2-AG) (TABLE 1) are the most active eCBs discovered so far and are the main members of fatty acid amide and monoacylglycerol families, respectively[7]. Of the phytocannabinoids, THC has psychotropic effects, whereas CBD and many other cannabinoids do not; thus, CBD in particular is under investigation for therapeutic applications related to its anti-inflammatory, analgesic, anti-anxiety and anti-tumour properties[12]. For instance, in a mouse model of multiple sclerosis, treatment with CBD induced a significant decrease in pro-inflammatory cytokines and T cell infiltration in the central nervous system and alleviated experimental autoimmune encephalomyelitis in a myeloid-derived suppressor cell-mediated manner[15]. Nevertheless, functional impairment of the reproductive system has been reported after chronic CBD exposure in male mice[16]. In this study, sexual behaviour analysis revealed that the first mounting and intromission were delayed, and the number of mounts and ejaculations was significantly reduced in mice treated with CBD (15 mg/kg) compared with a control group treated with sunflower oil[16]. Furthermore, a group of mice treated with a double dose of CBD (30 mg/kg) showed a 30% reduction ($P < 0.001$) in fertility rate and a 23% reduction ($P < 0.05$) in the number of litters compared with a control group treated with sunflower oil[16]. Overall, the group treated with CBD (15 mg/kg) showed the poorest performance in terms of sexual behaviour, with no differences in fertility parameters compared with the control group, whereas the animals that received the highest dose of CBD had the poorest fertilization rate and the fewest litters[16].

The pharmacological properties of THC are mediated mainly by its binding to the G protein-coupled CB_1 and CB_2 cannabinoid receptors[17]. However, THC has been reported to interact with other G protein-coupled receptors (GPCRs), such as the orphan receptors GPR55 and GPR18, and other well-known GPCRs, including the opioid or serotonin receptors[12]. By contrast, CBD has unclear actions on CB_1 and CB_2 and also acts via additional molecular targets including non-CB_1/non-CB_2 GPCRs such as serotonin receptors (5-HT_{1A}, 5-HT_{2A} and 5-HT_{3A}) and A_{1A} adenosine receptors[12], nuclear receptors such as the peroxisome proliferator-activated receptor-γ (PPARγ)[12], and ligand-gated ion channels including the glycine receptor, $GABA_A$ receptors and transient receptor potential (TRP) channels (TRPV1, TRPV2, TRPV3 and TRPA1)[12]. In parallel, eCBs have been shown to bind to and activate CB_1 and CB_2, which both have a central role in reproduction[18]. Additional eCB-like substances, such as *N*-palmitoylethanolamine (PEA) and *N*-oleoylethanolamine (OEA) (TABLE 1),

Table 1 | **Major cannabinoids and endocannabinoids, with their ECS targets and effects**

Name (abbreviation)	Chemical structure	ECS targets[a]	Effect
Cannabinoids			
Δ⁹-Tetrahydrocannabinol (THC)		CB_1	Activation
		CB_2	Activation
		GPR55	Activation
		PPARγ	Activation
Cannabidiol (CBD)		CB_1	Inhibition
		CB_2	Inhibition
		GPR55	Inhibition
		TRPV1	Activation
Endocannabinoids			
N-Arachidonoylethanolamine (anandamide, AEA)		CB_1	Activation
		CB_2	Activation
		GPR55	Activation
		TRPV1	Activation
		PPARα	Activation
		PPARγ	Activation
		PPARδ	Activation
2-Arachidonoylglycerol (2-AG)		CB_1	Activation
		CB_2	Activation
		GPR55	Activation
		TRPV1	Activation
		PPARγ	Activation
		PPARδ	Activation
Endocannabinoid-like compounds			
N-Palmitoylethanolamine (PEA)		GPR55	Activation
		PPARα	Activation
N-Oleoylethanolamine (OEA)		GPR55	Activation
		PPARα	Activation

CB_1, type 1 cannabinoid receptor; CB_2, type 2 cannabinoid receptor; ECS, endocannabinoid system; PPAR, peroxisome proliferator-activated receptor; TRPV1, transient receptor potential vanilloid 1. [a]Only targets activated at concentrations ≤1 μM are listed.

which are amides of long-chain polyunsaturated fatty acids, are structurally related to eCBs, are often found in much higher amounts than AEA, have no affinity for CB_1 or CB_2, and act via alternative intracellular targets; of note, these eCB-like compounds are metabolized by the same enzymes that synthesize and degrade authentic eCBs[19] and, indeed, might potentiate the activity of AEA and 2-AG at CB_1 and CB_2 receptors by inhibiting their degradation[18]. Both eCBs and eCB-like molecules have various biological effects, including those on the cardiovascular system, gastrointestinal tract and liver, immune system, muscles and bones, and skin[7]. Of particular interest, these molecules have roles in several physiopathological aspects of male reproduction[6,20,21], such as the control of testicular steroidogenesis and spermatogenesis[6], and sexual[6] and sperm functions[20], as well as of key processes for the acquisition of fertilizing ability[6].

The endocannabinoid system

The eCBs, their receptors, transporters and metabolic enzymes form the ECS[7] (FIG. 1). In particular, the endogenous level of eCBs is regulated by a metabolic equilibrium between biosynthetic and hydrolytic enzymes[22] in various cell types of the body, including the male gametes in humans[23,24] and other mammals[25,26] (FIG. 2). AEA is synthesized mainly by the sequential activity of *N*-acyltransferase and *N*-arachidonoylphosphatidylethanolamines-specific phospholipase D (NAPE-PLD)[27] and is degraded by

Intracellular trafficking
A general and tightly regulated process used by a variety of molecules to cross the membranes of, and move inside, living cells.

fatty acid amide hydrolase (FAAH)[28]. By contrast, 2-AG is mainly synthesized from membrane phosphatidylinositol by the sequential activity of phospholipase C (PLC) and diacylglycerol lipases (DAGLα and DAGLβ)[29] and is degraded by a specific monoacylglycerol lipase (MAGL)[30]. In 2016, another enzyme able to cleave 2-AG, α,β-hydrolase domain-containing protein 2 (ABHD2) (FIG. 2), was found to be strongly expressed in sperm, where it seems to have an unexpected role in sperm activation by mediating the non-genomic effects of progesterone[31]. In addition to metabolic enzymes, transport of eCBs across the plasma membrane is mediated by the putative endocannabinoid membrane transporter (EMT), which works bidirectionally to take up or release AEA and 2-AG. Of note, the molecular identity of EMT remains elusive, although its activity has been clearly documented in many cell types[32]. In addition, specific intracellular reservoirs (such as adiposomes)[33] cooperate in storing eCBs to guarantee their biological availability in various parts of the male reproductive tract, including sperm cells[24], prostate epithelial cells[34], urothelium[35] and testis[36] (FIG. 2). Moreover, intracellular trafficking drives eCBs to various receptor targets that include, in addition to CB_1 and CB_2, TRPV1 channels, which are strongly involved in regulating sperm functions[23] (FIG. 2). In fact, TRPV1 is a remarkable target within the ECS, with key roles in human sperm acquisition of fertilizing ability (TABLE 2). Moreover, GPR55, GPR119 and PPARs can also mediate many biological effects of eCBs, as well as of PEA and OEA[37]. However, our understanding of the involvement of these latter receptors in male reproduction remains limited to a study that showed a high expression of GPR55 in the human seminal vesicles[38].

Effects on male reproduction: preclinical studies

An effect of cannabinoid administration at the hypothalamus and pituitary level of the gonadal axis has been demonstrated in mice and rats. In rats, plasma levels of luteinizing hormone (LH) were significantly reduced after oral administration of THC compared with administration of a sesame oil vehicle[39]. In mice, intraperitoneal[40] or intracerebroventricular[41] microinjection of AEA produced a significant decrease in plasma LH levels compared with controls, which was accompanied by a fall in serum testosterone levels to one-third of its basal values[40]. The effect on LH secretion was shown to be mediated by CB_1, as it could be prevented by pretreatment with SR141716, a specific CB_1 antagonist[40], and was not observed in knockout mice in which the CB_1 gene *Cnr1* was deleted[40]. Although the expression of CB_1 was reported in rat adenohypophysis[42] and mouse hypothalamus[43], the inhibitory effect of cannabinoids on the release of gonadotrophins seems to occur upstream of the pituitary, because the addition of THC to cultured rat anterior pituitary cells had no effect on basal or gonadotropin-releasing hormone (GnRH)-stimulated release of LH[44]. Accordingly, Gammon and colleagues[45] demonstrated that immortalized mouse GnRH neurons express CB_1, which can be activated by selective agonists leading to the inhibition of pulsatile GnRH release.

Both cannabinoids and eCBs can exert effects on GnRH neurons not only directly but also by modulating the activity of neighbouring neuronal fibres that control GnRH release, such as γ-aminobutyric acid (GABA)-ergic fibres (FIG. 3). In mice, Farkas and colleagues[46] demonstrated that presynaptic activation of CB_1 on GABA-ergic fibres inhibits spontaneous release of GABA and the lack of activation of GABA receptors

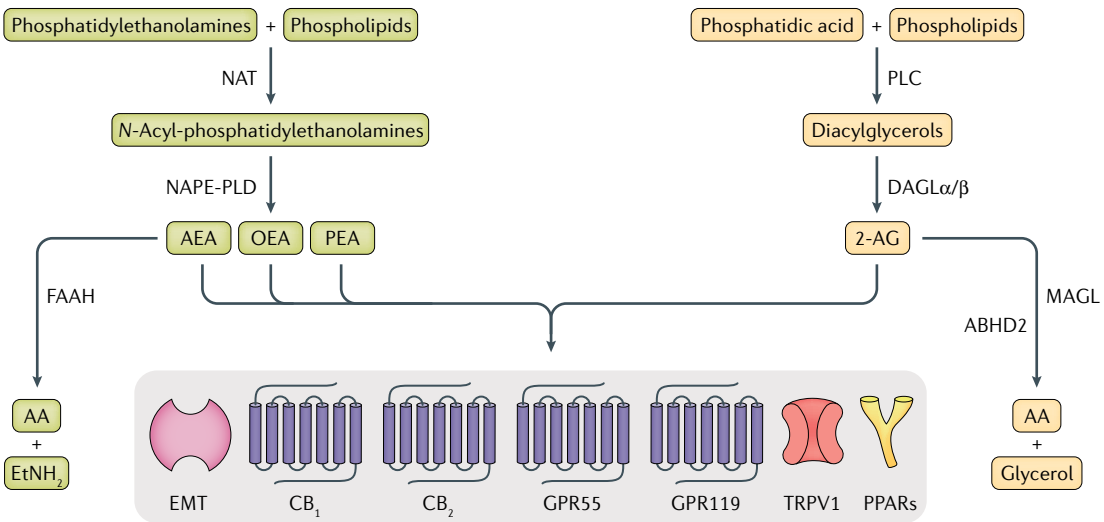


Fig. 1 | **The endocannabinoid system.** Schematic diagram showing synthesizing enzymes (*N*-acyltransferase (NAT), *N*-arachidonoylphosphatidylethanolamines-specific phospholipase D (NAPE-PLD), phospholipase C (PLC) and diacylglycerol lipase-α and diacylglycerol lipase-β (DAGLα/β)) and hydrolysing enzymes (fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL) and α,β-hydrolase domain-containing protein 2 (ABHD2)) of endocannabinoids (anandamide (AEA) and 2-arachidonoylglycerol (2-AG)) and related *N*-acylethanolamines (*N*-oleoylethanolamine (OEA) and *N*-palmitoylethanolamine (PEA)), as well as target receptors (type 1 cannabinoid receptor (CB_1), type 2 cannabinoid receptor (CB_2), GPR55, GPR119, transient receptor potential vanilloid 1 (TRPV1) and peroxisome proliferator-activated receptors (PPARs)). Also shown is the putative endocannabinoid membrane transporter (EMT). AA, arachidonic acid; $EtNH_2$, ethanolamine.

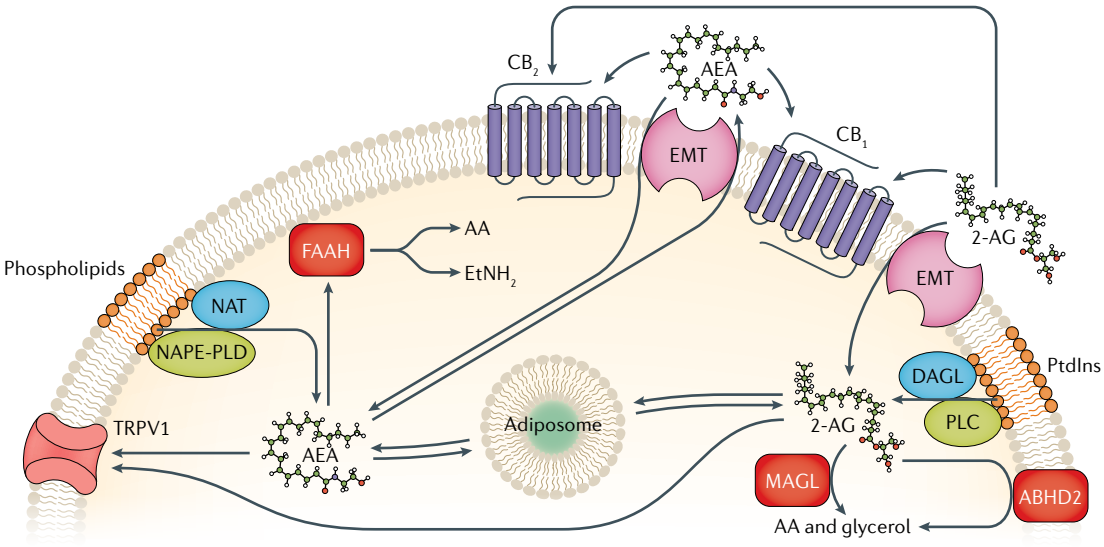


Fig. 2 | **Subcellular distribution of the main elements of the endocannabinoid system.** The synthesis of anandamide (AEA) from membrane phospholipid precursors is mainly catalysed by the sequential activity of *N*-acyltransferase (NAT) and *N*-arachidonoylphosphatidylethanolamines-specific phospholipase D (NAPE-PLD). AEA is carried through the cell membrane in both directions by a putative endocannabinoid membrane transporter (EMT) and, once inside the cell, AEA is hydrolysed by fatty acid amide hydrolase (FAAH) to ethanolamine ($EtNH_2$) and arachidonic acid (AA). The main targets of AEA are type 1 and type 2 cannabinoid receptors (CB_1 and CB_2), which have an extracellular binding site, and transient receptor potential vanilloid 1 (TRPV1) channels, which have an intracellular binding site. In addition, 2-arachidonoylglycerol (2-AG) is released from membrane phospholipids through the sequential activity of phospholipase C (PLC) and diacylglycerol lipases (DAGLα and DAGLβ). Subsequent hydrolysis of 2-AG is mainly via the action of monoacylglycerol lipase (MAGL) and α,β-hydrolase domain-containing protein 2 (ABHD2), which releases AA and glycerol. Much like AEA, 2-AG binds to and activates CB_1, CB_2 and TRPV1. Both AEA and 2-AG can be stored in adiposomes, specific intracellular reservoirs of lipids that can contribute to control their intracellular concentration and hence their biological activity. PtdIns, phosphatidylinositol.

on GnRH neurons prevents GnRH release. Incidentally, although GABA is typically an inhibitory neurotransmitter in the central nervous system, it exerts a paradoxical excitatory effect on mature GnRH neurons[47]. In addition to CB_1, CB_2 and TRPV1 are also expressed in mouse hypothalamus[48,49]. However, their involvement in the regulation of GnRH neuronal activity remains to be ascertained.

The decrease in testosterone levels in animals exposed to THC and AEA could reflect a combination of central (hypothalamic) and peripheral (testicular) effects (FIG. 3). Mouse[40] and rat[50] Leydig cells express CB_1, and the in vitro exposure of rat Leydig cells to THC inhibits steroidogenesis[51]. In rats, both acute (10 mg/kg) and chronic (2 mg/kg) in vivo administration of THC induced a significant decrease in the activity of testicular steroidogenic enzymes, resulting in depressed testosterone biosynthesis[52]. Cannabinoids and eCBs have been suggested to modulate the responsiveness of Leydig cells to LH: in vitro treatment of mouse testicular explants with low (1.5 mg/ml) and high (3 mg/ml) doses of bhang, an extract of the leaf and flower of *Cannabis sativa*, produced a significant dose-dependent decrease in testicular expression of the LH receptor[53]. Prolonged exposure of young adult rats to THC (1, 5 and 25 mg/kg/day) or crude marihuana extract (3, 15 and 75 mg/kg/day) reduced prostate, seminal vesicle and epididymal weight in a dose-related manner[54]. This change could be mediated by the concomitant decrease in serum androgen levels[47]. Furthermore, testes from *Cnr1*$^{-/-}$ mice exhibited a decreased basal secretion of testosterone in vitro[40], owing to the presence of a reduced number of Leydig cells compared with wild-type controls[50]. These findings suggest a role for CB_1 in stimulating the proliferation and differentiation of Leydig cells.

Independently of their inhibitory effect on gonadotrophins and testosterone secretion[39–41], cannabinoids could affect spermatogenesis also by acting directly on Sertoli cells (FIG. 3). Indeed, AEA has been shown to induce apoptosis of mouse Sertoli cells in vitro via TRPV1 (REF.[25]), an effect that is antagonized by follicle-stimulating hormone (FSH) through promotion of a cAMP–protein kinase A-dependent increase in FAAH expression[26]. Furthermore, FSH also induces oestrogen-dependent FAAH expression in mouse and rat Sertoli cells via increased aromatase activity[26,55,56]. However, the engagement of eCB signalling in spermatogenesis seems to be much more complex than just these inhibitory effects, and pro-apoptotic activation of TRPV1 (REF.[25]) is counterbalanced by anti-apoptotic activation of CB_2-dependent signalling pathways[25,26]. In fact, CB_2 inactivation by its selective antagonist SR144528 increased AEA-induced DNA fragmentation in isolated mouse Sertoli cells[25,26]. Furthermore, activation of CB_1 also has a role in the physiological spermatid DNA packaging in mouse, by promoting increase of transition proteins and histone displacement during spermatogenesis[57].

In animal models, the in vivo exposure to cannabis extracts has produced conflicting data on the effects of cannabinoids on spermatogenesis. Impairment of spermatogenesis after chronic exposure to THC has been reported in dogs[58], mice[23] and rats[59]. Histological changes — including widening of the intertubular spaces[53], high rates of apoptosis among spermatids[53] and Sertoli cells[59], and even complete arrest of spermatogenesis[58] — resulted in poor sperm output[53,59]. However, in a separate study, chronic oral treatment of mice with THC (10 mg/kg body weight every 2 days for 5 weeks) did not affect the frequency of males producing pregnancies, nor did it influence preimplantation loss or fetal mortality[60]. In keeping with these data, an additional independent investigation demonstrated that mice treated with a daily dose of 10 mg/kg body weight of THC for a period of 30 days had neither histological spermatogenesis abnormalities nor poorer sperm count than a vehicle-treated control group[61].

Overall, in the context of preclinical studies that have produced solid evidence for a role of the ECS in negative modulation of testosterone secretion by acting at both central and testicular levels, data regarding the effect of in vivo exposure to cannabinoids on spermatogenesis in animal models remain inconclusive, which is likely to reflect the complex role of eCB signalling in orchestrating key processes involved in spermatogenesis.

Effects on male reproduction: clinical studies

The lack of controlled clinical trials investigating the effects of exogenous cannabinoids on human sexual and reproductive health, for obvious ethical reasons, means that the scientific literature is largely based on observational findings from cannabis smokers (TABLE 3).

Effects on hypothalamic–pituitary–testicular axis hormones. CB_1 is expressed in human pituitary gonadotrophic cells[62]. This observation, coupled with data from preclinical models[39–42], suggests that impairment of gonadotropins and testosterone biosynthesis in cannabis users is biologically plausible. However, clinical evidence collected so far does not support definitive conclusions.

Reduced basal and GnRH-stimulated LH levels have been reported in a small group of chronic marijuana smokers[63], although in a separate study[64] chronic marijuana smokers did not exhibit significant differences in circulating LH levels, compared with age-matched controls and independently of the number of cannabis cigarettes (joints) smoked per week (TABLE 3).

As for testosterone levels, an early 1970s study found low total testosterone levels in chronic cannabis users, which increased, in some cases, after 2 weeks of discontinuation[64] (TABLE 3). However, subsequent studies failed to document differences between occasional and chronic marijuana smokers[9,65], between occasional smokers and controls[9,66], or between daily cannabis users and controls[65] (TABLE 3). In two large studies, a favourable effect on testosterone levels was suggested[67,68]. A cohort study enrolling 1,194 Danish men aged 18–28 years found increased testosterone levels in subjects smoking marijuana more than once per week, after adjustment for confounders[67]. In a separate sample of 1,577 US men from the National Health and Nutrition Examination Survey (NHANES), serum testosterone levels seemed to inversely correlate with time since last regular use of marijuana, although they were not significantly different between ever-users and never-users of marijuana after adjustment for demographic and lifestyle-related confounders[68]. Accordingly, a 2019 systematic review concluded that clinical studies assessing the association between chronic cannabis consumption and alterations in the hypothalamic–pituitary–testicular axis hormones overall produced inconclusive results[69]. A possible explanation for the heterogeneous findings in the literature is that cannabis could have only temporal and reversible effects on hormone secretions[69]. On this basis, a decline in circulating testosterone levels is more likely to be revealed in acute settings within a few hours of cannabis exposure than in chronic marijuana users[69]. At any rate, no study has definitively demonstrated to date that marijuana consumption, independently of dose or duration, is associated with statistically significant decreases in testosterone levels below the normal reference ranges.

Furthermore, differences in lifestyle and general health of the populations under study should also be taken into account. Indeed, residual confounding factors might remain even after adjustment for many variables linked to lifestyle habits; these uncontrolled variables might also have contributed to discrepancies in the results obtained from different studies.

Effects on male sexual function. To date, only a few studies have investigated the effects of cannabis on male sexual function in humans (TABLE 3).

In a case–control study of 434 Egyptian men with erectile dysfunction (ED) and 272 age-matched healthy controls, a multivariable model including common cardiovascular risk factors showed that the only two variables that exhibited an independent significant association with ED were cigarette smoking (OR 1.78, 95% CI 1.16–2.72) and the use of recreational drugs

Table 2 | **Roles of TrPV1 in human sperm function**

Proposed role	Experimental evidence
Prevention of spontaneous human sperm acrosome exocytosis, with preservation of responsiveness to progesterone at the fertilization site[23]	Exposure of sperm to a TRPV1 antagonist, capsazepine, during capacitation increases the incidence of spontaneous acrosome reactions, thus decreasing progesterone-induced acrosome reaction rate above the spontaneous rate
	The exposure of spermatozoa to capsazepine from the beginning of capacitation leads to a significant inhibition of the ability of the sperm to fuse with oocytes in response to progesterone
Facilitating human sperm thermotaxis[103]	Spermatozoa migrating along an increasing temperature gradient display a higher TRPV1 content
	Spermatozoa migrate along a gradient of capsaicin, a TRPV1 agonist, whereas the TRPV1 antagonist capsazepine blocks this effect
Involvement in capacitating effects exerted by fibronectin on human spermatozoa[104]	Capacitating effects of fibronectin are reversed by preincubation of spermatozoa with the TRPV1 antagonist capsazepine

TRPV1, transient receptor potential vanilloid 1.

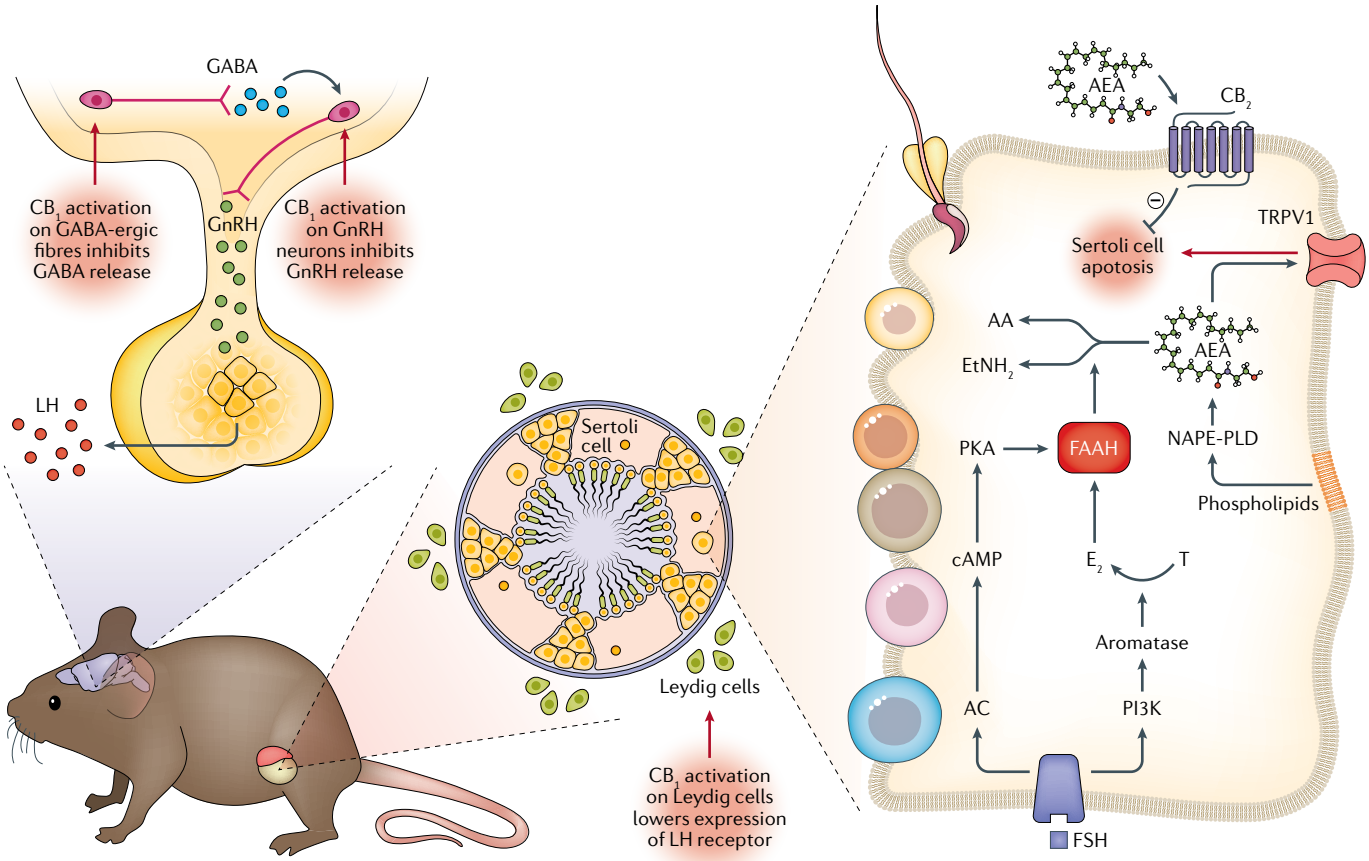


Fig. 3 | **Involvement of the endocannabinoid system in controlling rodent testicular steroidogenesis and spermatogenesis.** At the hypothalamic level, γ-aminobutyric acid (GABA)-ergic fibres express type 1 cannabinoid receptor (CB_1), activation of which decreases release of gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH). GABA also exerts excitatory effects on GnRH neurons; thus, GnRH release can be inhibited directly by the activation of CB_1 in GnRH neurons. At the testicular level, activation of CB_1 in Leydig cells negatively affects testosterone biosynthesis by decreasing Leydig cell responsiveness to LH. In Sertoli cells, anandamide (AEA) generated by *N*-arachidonoylphosphatidylethanolamines-specific phospholipase D (NAPE-PLD) can induce apoptosis via transient receptor potential vanilloid 1 (TRPV1) channels. This effect is antagonized by follicle-stimulating hormone (FSH), which activates adenylyl cyclase (AC) and, therefore, promotes a cAMP/protein kinase A (PKA)-dependent increase in the expression of fatty acid amide hydrolase (FAAH). FAAH cleaves AEA into ethanolamine ($EtNH_2$) and arachidonic acid (AA). FSH also triggers the phosphatidylinositol-3-kinase (PI3K) pathway, which in turn induces the expression of aromatase and leads to increased production of oestradiol (E_2) from testosterone (T). Increased oestrogen levels result in higher expression of FAAH, due to an oestrogen-responsive element in its gene promoter. Finally, activation of TRPV1 promotes apoptosis, whereas type 2 cannabinoid receptor (CB_2) exerts an anti-apoptotic effect. Together these effects maintain a balance between survival and death of Sertoli cells and ultimately control spermatogenic output.

(OR 3.18, 95% CI 1.15–8.82)[70]. However, reliable conclusions cannot be drawn from the same study on the specific effect of cannabis, because the specific use of marijuana was not separated from the use of other recreational substances, such as a hybrid strain of marijuana, codeine and narcotic analgesics. Another study in men with ED aged 30.0 ± 3.6 years[71] showed that a significantly higher proportion of patients with arteriogenic ED (as assessed by dynamic penile Doppler ultrasonography) reported habitual consumption of cannabis than the group with non-arteriogenic ED. Based on the fact that the incidence of cigarette smoking was similar in both groups, the authors suggested a possible relationship between chronic cannabis use and vascular damage.

A large epidemiological study that included >3,000 adults in the St. Louis area in the USA[72] found that lifetime use of marijuana was associated with higher odds of reporting inhibited orgasm (as defined in the 3rd edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III)[73]) in a multiple logistic regression analysis, after adjustment for sociodemographic and health status-related confounders. By contrast, no association was found between lifetime use of marijuana and reduced sexual excitement or desire (TABLE 3). Unfortunately, this study did not assess the temporal association between sexual dysfunction and marijuana use; furthermore, data from men and women were not analysed separately, meaning that conclusions cannot be drawn regarding effects specifically in men. However, a survey of 8,650 Australians[74] did show an effect of daily cannabis use in orgasm disorders when the analysis was restricted to the men ($n = 4,350$) and after adjustment for confounding factors (TABLE 3). In this study, daily cannabis use was associated with a significantly increased likelihood of men reporting orgasm disorders, not only in terms of inability to reach orgasm or reaching orgasm too slowly but also reaching orgasm too quickly, compared with no cannabis use. However,

Table 3 | **Clinical data regarding cannabis and male sexual and reproductive health**

Ref.	Study design	Study population	Outcomes	Results
Effects on LH and testosterone levels				
Kolodny et al.[64]	Case–control study	40 men aged 18–28 years: 20 chronic cannabis users (at least 4 days/week for ≥6 months) and 20 controls (never-users)	LH and TT	No differences in LH levels between chronic users and controls Significantly lower TT levels in chronic users than in controls (416 ± 34 ng/dl vs 742 ± 29 ng/dl[a], $P < 0.001$) Significantly lower TT levels in chronic users smoking ≥10 joints than in those smoking <10 joints per week (309 ± 34 ng/dl vs 503 ± 40 ng/dl[a], $P < 0.005$) TT levels increased from 57% to 141% in three chronic users after 2 weeks discontinuation
Mendelson et al.[65]	Longitudinal study including three consecutive phases: a 5-day baseline period, a 21-day smoking period (during which men smoked joints at will) and a 5-day post-smoking period	27 men aged 21–26 years: 15 'heavy' cannabis users (42.0 ± 24.5 joints per month), and 12 'casual' cannabis users (11.5 ± 6.5 joints per year)	TT	No differences in TT were noted between casual and heavy cannabis users during any phase of the study (the mean number of joints smoked during the 21-day smoking period by casual and heavy users was 54.3 and 119.5, respectively)
Cushman[66]	Case–control study	38 male university students: 25 cannabis users (≥1 joint per week) and 13 controls (never-users)	TT	No differences in TT between cannabis users and controls
Vescovi et al.[63]	Case–control study	20 men aged 19–20 years: 10 cannabis users (~1 g once every day) and 10 controls (never-users)	Basal and GnRH-stimulated LH levels	Significantly lower basal LH levels in cannabis users (5.8 ± 1.5 mIU/ml) than in controls (10.5 ± 1.3 mIU/ml, $P < 0.05$) Significantly lower AUC of GnRH-stimulated LH levels in cannabis users (37.8 ± 4.4 mIU/3 h) than in controls (50.4 ± 4.9 mIU/3 h, $P < 0.01$)
Gundersen et al.[67]	Cohort study	1,194 Danish men aged 18–28 years: 131 (11%) cannabis users more than once per week; 405 (34%) cannabis users once per week or less; 658 (55%) never-users	LH, TT and cFT levels	TT levels in more than once per week users were 7% higher than in non-users (95% CI 0–14), after adjustment for lifestyle-related variables
Thistle et al.[68]	Population cross-sectional survey	Nationally representative sample of 1,577 US men from the NHANES: 1,044 (66.2%) cannabis ever-users, and 533 (33.8%) cannabis never-users	TT levels	No differences between ever-users and never-users, after adjustment for demographic and lifestyle-related variables Inverse association between TT levels and time since last regular use ($P_{trend} = 0.02$)
Nassan et al.[9]	Longitudinal study	317 men enrolled at the Massachusetts General Hospital Fertility Center: 37 (11.7%) current cannabis users, 131 (41.3%) past cannabis users, and 149 (47%) never-users	LH and TT levels	No differences between past and current users, past users and never-users, or current users and never-users, after adjustment for demographic and lifestyle-related variables
Effects on male sexual function				
Johnson et al.[72]	Retrospective study	Community sample of 3,004 subjects (1,203 men and 1,801 women) from the Epidemiological Catchment Area Project	Sexual excitement, sexual desire, and orgasm	Significant association between cannabis use and 'inhibited orgasm' (OR 1.76, 95% CI 1.12–2.74), after adjustment for sociodemographic and health status-related variables No association between cannabis use and poorer sexual excitement (OR 1.14, 95% CI 0.55–2.34) or desire (OR 1.02, 95% CI 0.56–1.88)
Aversa et al.[71]	Retrospective study	64 young men with ED: 30 (47%) with arteriogenic ED, and 34 (53%) with non-arteriogenic ED	Erectile function	More frequent cannabis use in arteriogenic group than in non-arteriogenic ED group (78% vs 3%, $P < 0.001$)
Elbendary et al.[70]	Case–control study	434 Egyptian men <40 years of age with ED and 272 age-matched controls	Erectile function	Significant association between ED and use of recreational drugs (including cannabis), after adjustment for cardiovascular risk factors (OR 3.18, 95% CI 1.15–8.82)

Table 3 (cont.) | **Clinical data regarding cannabis and male sexual and reproductive health**

Ref.	Study design	Study population	Outcomes	Results
Effects on male sexual function (cont.)				
Smith et al.[74]	Computer-assisted telephone survey	4,350 Australian men aged 16–64 years: 96 (2.2%) daily cannabis users, 86 (2.0%) weekly cannabis users, 307 (7.0%) less than weekly cannabis users, and 3,861 (88.8%) never-users	Interest in sex, erectile function and orgasm	Association between daily cannabis use and increased likelihood of reporting inability to reach orgasm (OR 3.94, 95% CI 1.71–9.07), reaching orgasm too slowly (OR 2.05, 95% CI 1.02–4.12) and reaching orgasm too quickly (OR 2.68, 95% CI 1.41–5.08), after adjustment for sexual identity, number of partners, demographic and lifestyle-related variables No association between cannabis use and ED
Sun and Eisenberg[80]	Cross-sectional survey	Nationally representative sample of 22,943 US men from the NSFG	Sexual frequency	Significant association between daily (IRR 1.36, 95% CI 1.21–1.53) or weekly (IRR 1.22, 95% CI 1.06–1.41) use of cannabis and increased coital frequency, after adjustment for demographic, socioeconomic and anthropometric variables
Effects on male fertility				
Kolodny et al.[64]	Case–control study	17 chronic cannabis users (at least 4 days a week for ≥6 months)	Sperm count	Significantly lower sperm count in men smoking ≥10 than in those smoking <10 joints/weeks (26.6 ± 7.3 vs $67.9 \pm 6.3 \times 10^6$/ml, $P < 0.01$)
Close et al.[82]	Case–control study	169 men from infertile couples: 10 (6%) regular cannabis users, and 159 (94%) non-users (controls)	Standard semen parameters	Significantly higher seminal leukocyte concentration ($P = 0.007$) and percentage of motile spermatozoa ($P = 0.006$) in samples from regular cannabis users No differences in sperm count or sperm morphology
Pacey et al.[83]	Unmatched case–referent study	1,970 male partners from infertile couples: 318 cases (men with <4% morphologically normal spermatozoa, as assessed by CASMA) and 1,652 referents (with at least 4% morphologically normal spermatozoa)	Sperm morphology	Independent association between poor sperm morphology and use of cannabis in the 3 months prior to sample collection in men aged ≤30 years (OR 1.94, 95% CI 1.05–3.60)
Gundersen et al.[67]	Cohort study	1,172 Danish men aged 18–28 years: 399 (34%) cannabis users once per week or less, 130 (11%) cannabis users more than once per week, and 643 (55%) never-users	Standard semen parameters	Association between regular cannabis use and a 28% (95% CI −48 to −1) lower sperm concentration and a 29% (95% CI −46 to −1) lower total sperm count, after adjustment for abstinence period, sexually transmitted diseases and lifestyle-related variables No differences in sperm motility and morphology
Kasman et al.[87]	Retrospective analysis of cross-sectional data	758 men from the NSFG who were actively trying to conceive	Time to pregnancy	No difference in time to pregnancy between never-users (83.4% of the study population) and daily cannabis users (5%) (time ratio 1.08, 95% CI 0.79–1.47, $P = 0.65$), after adjustment for age, marital status, previous children, partner age, previous fertility evaluation or treatment, year of survey, income, ethnicity and education
Murphy et al.[81]	Case–control study	12 cannabis users (at least weekly cannabis use for the past 6 months) and 12 non-users	Standard semen parameters and sperm DNA methylation	Significantly lower sperm concentration in cannabis users ($58.1 \pm 26.5 \times 10^6$/ml[b]) vs non-users ($96.3 \pm 49.7 \times 10^6$/ml[b], $P < 0.05$) No differences in semen volume, sperm motility, or percentage of normal sperm morphology Association between cannabis use and altered sperm DNA methylation status
Nassan et al.[9]	Longitudinal study	1,143 ejaculates from 662 men undergoing semen analysis at the Massachusetts General Hospital Fertility Center: 365 (55%) cannabis ever-users, and 297 (45%) cannabis never-users	Standard semen parameters and sperm DNA integrity (comet assay)	Significantly higher sperm concentration in ever-users (62.7×10^6/ml, 95% CI 56.0–70.3×10^6/ml[c]) vs never-users (45.4×10^6/ml, 95% CI 38.6–53.3×10^6/ml[c]; $P < 0.05$), after adjusting for abstinence period, demographic and lifestyle-related variables No significant differences in sperm motility, morphology or DNA integrity

AUC, area under the curve; CASMA, computer-aided sperm morphometric assessment; cFT, calculated free testosterone; ED, erectile dysfunction; GnRH, gonadotropin-releasing hormone; IRR, incidence rate ratio; LH, luteinizing hormone; NHANES, National Health and Nutrition Examination Survey; NSFG, National Survey of Family Growth; TT, total testosterone. [a]Values are means ± standard error. [b]Values are means ± standard deviation. [c]Values are means (95% CI).

in the same study, cannabis use was not associated with ED[74]. Accordingly, a meta-analysis of population-based research[75], despite being limited by the dearth of studies in the area (only three studies were included in the quantitative synthesis[70,72,76]), suggested that cannabis consumption could indeed be a risk factor for orgasm dysfunctions (pooled OR 2.09, 95% CI 1.36–3.20), but not other sexual dysfunctions in men.

Despite data suggesting that cannabis use can affect human sexual function, its effects remain under debate, as favourable effects on sexual behaviour and motivational, hedonic and/or perceptual aspects of sexual intercourse have been also reported. Some studies[77] have suggested that 70–85% of marijuana consumers experience increased sexual pleasure and satisfaction, 25–40% prolonged duration of intercourse, and 55–70% heightened orgasmic sensation[78,79]. Furthermore, the National Survey of Family Growth (NSFG), a representative cross-sectional survey on a well-controlled cohort of almost 23,000 men in the USA, showed that increased frequency of marijuana use was associated with increased coital frequency, even after adjustment for demographic, socio-economic and anthropometric variables[80] (TABLE 3). Notably, these data are all self-reported and, therefore, are at risk of both recall bias and exaggeration by participants; thus, they should be interpreted with caution. The authors hypothesized that individuals who engage in marijuana use might be more psychologically disinhibited than those who do not, which could be reflected in their sex life as high coital frequency[80]. Furthermore, although sexual pleasure could be at least partially attributed to an actual enhancement of sensory experience, especially in terms of an increased sensitivity to touch, which has been reported in cannabis users[78], a placebo effect cannot be ruled out, given the anecdotal reputation of cannabis as an aphrodisiac[77]. These aspects should be taken into account when interpreting the increased sexual pleasure and satisfaction among marijuana consumers reported in previous studies[77].

Effects on male fertility. Clinical studies on the effects of cannabis on male fertility have mostly used semen quality as a surrogate end point, leading to mixed results (TABLE 3). An adverse effect on sperm count has been found in some studies[64,67,81]. However, such adverse effects were not confirmed in other studies[9,82]. In accordance with a potential reduction in sperm count, a negative effect on sperm morphology was found by Pacey and colleagues[83]. However, this effect on morphology was not observed in other studies[9,67,82]. In one early study by Close and co-workers[82], marijuana smokers exhibited higher concentrations of semen leukocytes. However, whether and to what extent an increased seminal leukocyte count could affect male fertility remains a matter of debate. In fact, although it has been reported that semen leukocytes could promote sperm damage by producing reactive oxygen species[84], in a meta-analysis of 28 studies[85], leukocytospermia ($\geq 1 \times 10^6$ leukocytes/ml of semen, according to the WHO[86]) was not associated with altered semen quality or poorer outcomes of assisted reproduction technology. Finally, in a study of 24 men aged 18–40 years, the group reporting at least weekly cannabis use for the past 6 months ($n = 12$) exhibited lower sperm concentrations than non-users ($58.1 \pm 26.5 \times 10^6$/ml versus $96.3 \pm 49.7 \times 10^6$/ml, $P < 0.05$), whereas no significant differences were found in other standard semen parameters, including semen volume, sperm motility and percentage of normal sperm morphology[81]. In that study, the authors also found that cannabis use was associated with altered methylation status of genes involved in controlling organ size by modulating cell proliferation and apoptosis. Although this latter finding points to possible heritable epigenetic changes following pre-conception cannabis exposure[81], the researchers did not adjust analyses for several potential confounders that could have influenced sperm DNA methylation (lifestyle habits, physical condition, diet and nutrition, sleep and alcohol use). This major limitation means that this study could not establish a clear cause–effect relationship between cannabis use and altered sperm methylation status[81].

Two 2019 systematic reviews concluded that cannabis use has an adverse effect on male fertility: Rajanahally and colleagues[69] reviewed seven clinical studies suggesting overall that cannabis consumption has a negative impact on fertility when using semen parameters as a surrogate; and Payne and colleagues[8] reviewed a total of 48 studies, including both clinical and preclinical (in vitro and animal) studies, and reached the same conclusion. However, these systematic reviews did not include two subsequently published large studies[9,87], the results of which somewhat undermined concerns about a possible clinical impact of marijuana use (TABLE 3). In the first of these subsequent studies, Nassan and colleagues[9] assessed 1,143 ejaculates from 662 men undergoing semen analysis at a fertility centre and failed to find any association between marijuana smoking and poorer sperm motility or poorer sperm morphology. Surprisingly, in the same study, men who had ever smoked marijuana exhibited significantly higher sperm concentration than those who had never smoked marijuana, after adjusting data for abstinence period and demographic and lifestyle-related confounders[9]. Even more reassuring were data from the population based NSFG, assessing time to pregnancy in partners of men who were actively trying to conceive[87]. In this study, no significant associations were found between daily use of marijuana in men and longer duration of pregnancy attempts, after controlling for possible confounders that included partner age.

Conclusions of clinical studies. Taken together, a combination of anecdotal reports on erectile function, scanty evidence regarding ejaculatory and orgasmic function and inconclusive findings on testosterone levels and male fertility potential do not seem to substantiate the notion that plant-derived cannabinoids exert certain harmful effects on human sexual and reproductive health. Indeed, clinical data regarding adverse effects on male fertility are based on results from conventional semen analysis, which is undermined by considerable spontaneous variability between patients and even between samples from the same patient[88]. The strong

Thermotaxis
Movement towards or away from a thermal stimulus.

Orphan enzyme
An enzyme for which activity has been experimentally characterized but amino acid sequences are lacking.

evidence from the large NSFG study[87], in which no significant associations were found between marijuana use in men and longer duration of pregnancy attempts, seems to support the safety of marijuana with respect to time to pregnancy, which is a more clinically relevant end point than semen parameters. Nevertheless, further large longitudinal analyses are warranted to definitively prove the safety of cannabis on clinically relevant reproductive end points, including natural and medically assisted pregnancies.

Endocannabinoids and human sperm function

Human spermatozoa contain the necessary ECS biochemical machinery to enable the synthesis (by NAPE-PLD) and degradation (by FAAH)[23] of AEA, as well as the synthesis (by DAGL)[24] and degradation (by MAGL[24] and/or ABHD2 (REF.[31])) of 2-AG. In addition, mature spermatozoa express the major eCB-binding receptors CB_1 (REFS[23,89]), CB_2 (REFS[23,90]) and TRPV1 (REF.[23]).

Evidence indicates that the highly organized ECS exerts a complex (and not yet fully understood) role in modulating human sperm functions, including motility and key processes involved in the acquisition of sperm-fertilizing ability, as well as in mediating relevant non-genomic responses to progesterone (FIG. 4). In vitro studies have suggested that cannabinoids and eCBs negatively affect human sperm motility[18,89,91–95], via CB_1 activation[89,92–94]. In particular, Rossato and colleagues[89] found that exposure of human spermatozoa to AEA produced a significant decrease in sperm motility, down to 10% of the baseline value, and that this effect was prevented by preincubation with the CB_1 antagonist SR141716. Notably, cannabinoids and eCBs not only affect the physiology of isolated mitochondria, by disrupting their O_2 consumption[96] and membrane integrity[97], but in human spermatozoa they also reduce mitochondrial membrane potential ($\Delta\Psi m$)[89] via CB_1 activation[98,99]. Indeed, both in mice[100] and humans[101], under normal physiological conditions in standard glucose-containing incubation media, glycolysis can compensate for loss of ATP produced by mitochondria in maintaining sperm motility. Thus, the effect of CB_1-mediated $\Delta\Psi m$ loss on sperm motility could be more clearly detected under experimental conditions of glycolysis blockade. Accordingly, our group have found that, in the presence of glucose in the extracellular environment, the stable AEA analogue methanandamide (Met-AEA) inhibited human sperm $\Delta\Psi m$ without affecting motility[98]. Instead, when spermatozoa were incubated in a glucose-free medium and in the presence of the glycolysis inhibitor, 2-deoxy-D-glucose, the addition of Met-AEA (1 μM) led to complete sperm immobilization, an effect that was prevented by preincubation with the CB_1 antagonist SR141716. In addition to exogenous cannabinoids, CB_1 activation leading to loss of sperm $\Delta\Psi m$ can also be triggered by eCBs generated by sperm cells themselves in response to in vitro treatment with lipopolysaccharide (LPS), an endotoxin sourced from Gram-negative bacteria[102]. In fact, exposure of human spermatozoa to *Escherichia coli* LPS produced a significant decrease in sperm $\Delta\Psi m$, similar to that induced by Met-AEA and prevented by SR141716 (REF.[102]). This finding could be clinically relevant in a context of genital tract infections, where LPS-induced mitochondrial dysfunction could make spermatozoa more vulnerable to the effects of other inflammatory mediators[102].

In terms of sperm fertilizing ability, exposure to cannabinoids or eCBs has also been reported to reduce both spontaneous and calcium ionophore-induced acrosome reactions[18], as well as zona pellucida binding[91] in human sperm samples under capacitation conditions.

From a physiological point of view, the inhibition of spontaneous acrosome reactions is not supportive of harmful effects of eCBs on sperm fertilizing ability, owing to the role of TRPV1 in controlling acrosomal integrity[23]. In fact, in human sperm, the activation of TRPV1 by intracellular AEA during capacitation maintains acrosomal integrity by preventing spontaneous acrosome reactions[23]; this effect preserves the sperm responsiveness to progesterone, which induces a functional acrosome reaction (followed by sperm–oocyte fusion) at the fertilization site (TABLE 2). It has been also suggested that TRPV1 might have a role in mediating both human sperm thermotaxis[103] and fibronectin-induced capacitating effects on human spermatozoa[104] (TABLE 2). In a study by De Toni and colleagues[103], spermatozoa that migrated along an increasing temperature gradient exhibited a higher TRPV1 content. Furthermore, the TRPV1 agonist, capsaicin, promoted sperm migration, an effect that was prevented by the TRPV1 antagonist capsazepine. In another study[104], the capacitating effects of fibronectin were inhibited by TRPV1 blockade with capsazepine. Taken together, these findings indicate a physiological role of TRPV1 in the acquisition of sperm fertilizing ability during capacitation.

Progesterone is a key hormone in promoting the ability of spermatozoa to fertilize oocytes. This hormone, released in the oviduct from the cumulus cells that surround the oocyte, is known to stimulate an increase in intracellular calcium concentration in human sperm (which are transcriptionally silent cells) via a non-genomic mechanism, leading to the acrosome reaction and hyperactivated motility[105,106]. A 2016 study revealed a role for eCB signalling in the non-genomic activity of progesterone in spermatozoa, demonstrating that the orphan enzyme ABHD2, which is highly expressed in sperm, acts as a progesterone-dependent hydrolase that depletes endogenous 2-AG content[31]. Thus, in the absence of progesterone, 2-AG inhibits the sperm-specific calcium channel CatSper[31] and the removal of 2-AG, induced by ABHD2 activation via progesterone, leads to calcium influx via CatSper, causing sperm activation[31] (FIG. 4). Interestingly, intracellular levels of 2-AG seem to be controlled by its degradation. This suggests that the widely accepted dogma of 'on-demand' synthesis of eCBs (that is, that eCBs including 2-AG are produced only upon stimulus-dependent synthesis from phospholipid precursors) needs to be reconsidered[107]. In sperm, on-demand synthesis does not seem to be the mechanism controlling levels of 2-AG, which instead seems to be degraded on demand from a pre-existing pool in the presence of progesterone within the female reproductive tract[31].

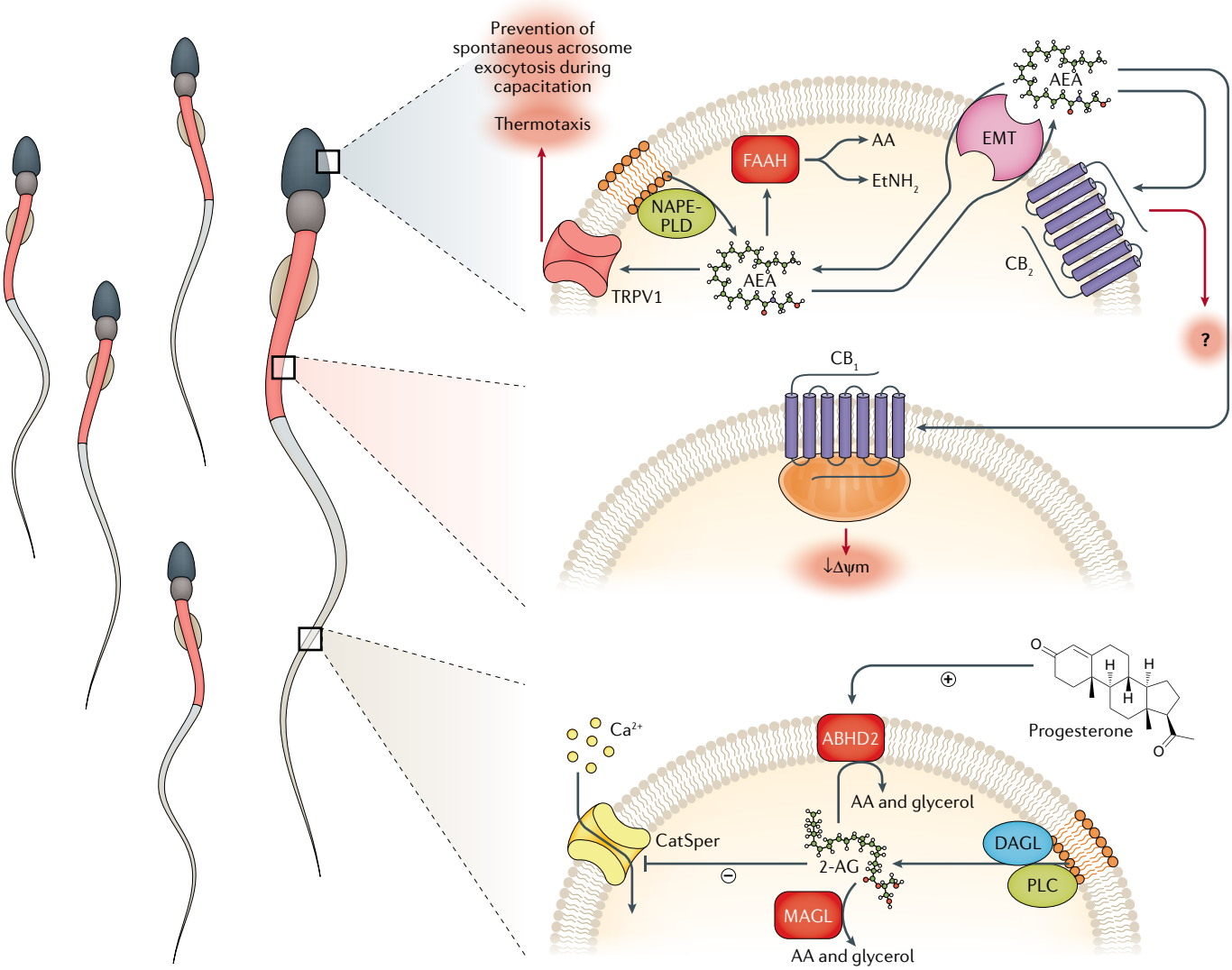


Fig. 4 | **The endocannabinoid system in human sperm.** Anandamide (AEA), generated during capacitation by *N*-arachidonoylphosphatidylethanolamines-specific phospholipase D (NAPE-PLD), binds to the intracellular site of transient receptor potential vanilloid 1 (TRPV1) channels, activation of which prevents spontaneous (unfunctional) acrosome exocytosis. A role for TRPV1 in sperm thermotaxis has also been proposed. Intracellular AEA content is dependent on its transport across the plasma membrane, which is mediated by a putative endocannabinoid membrane transporter (EMT), and is followed by hydrolysis to ethanolamine ($EtNH_2$) and arachidonic acid (AA) by fatty acid amide hydrolase (FAAH). Type 1 and type 2 cannabinoid receptors (CB_1 and CB_2) can be activated by extracellular cannabinoids and endocannabinoids. Although CB_2 does not have any overt function in human sperm, activation of CB_1 inhibits mitochondrial membrane potential ($\Delta\Psi m$), with a possible effect on sperm motility when glycolysis substrates are lacking. 2-Arachidonoylglycerol (2-AG), generated by the sequential activity of phospholipase C (PLC) and diacylglycerol lipase (DAGL), inhibits CatSper, a sperm-specific calcium channel expressed in the principal piece of the sperm flagellum. Of note, upon progesterone binding the α,β-hydrolase domain-containing protein 2 (ABHD2) degrades 2-AG into AA and glycerol, thus relieving CatSper from inhibition. 2-AG catabolism can also occur via monoacylglycerol lipase (MAGL).

ECS elements as biomarkers in reproduction

The possible exploitation of ECS components as biomarkers of male reproductive disorders[20] is probably somewhat premature, owing to the inconclusive data produced thus far. Indeed, studies by Amoako and colleagues[94,108] showed that seminal AEA, PEA and OEA levels are significantly lower in men with asthenozoospermia or oligoasthenoteratozoospermia than in normozoospermic men, but a separate study by Lewis and co-workers[24] showed that AEA (and 2-AG) are significantly higher in fertile men than in men with infertility, but sperm parameters did not differ between the two groups.

This controversial relationship between seminal eCBs and sperm parameters and/or fertility potential might be attributable to the different sources of eCBs in the ejaculate. The levels of 2-AG have been shown to be significantly higher in ejaculates from patients with leukocytospermia than in those from healthy controls, and 2-AG levels are significantly correlated with concentrations of macrophages and activated macrophages in semen[21]. As mammalian macrophages can produce

2-AG in response to inflammatory stimuli[109–111], they might be an important source of this eCB in ejaculates from patients with leukocytospermia. In this way, 2-AG could represent a biochemical marker of macrophage activation in the context of an inflammatory response.

Conclusions

Preclinical and clinical studies have investigated the role of eCB signalling in male reproductive events. Overall, preclinical studies have clearly shown that the ECS is involved in negative modulation of testosterone secretion, by acting both at central[39–41,44–46] and testicular[51–53] levels. However, in animal models, the effect of in vivo exposure to cannabinoids on spermatogenesis remains a matter of debate[53,58–61]. Furthermore, anecdotal reports on erectile function[70,71], limited evidence on ejaculatory and orgasmic function[72,74,75] and inconclusive findings of studies in humans regarding testosterone levels[64–69] and male fertility potential[9,64,67,81–83,87] do not seem to support the notion that plant-derived cannabinoids have definite harmful effects on human sexual and reproductive health.

The major ECS components have been identified in human sperm cells[23,24,31,89,90] and eCBs have also been detected in seminal plasma[21,24,94,108], pinpointing eCBs as potential new biomarkers that could be used to evaluate male reproductive defects[20].

A challenge for future research is to improve understanding of the crosstalk between plant-derived cannabinoids and eCBs, which could open new avenues for exploiting distinct ECS components as possible targets of innovative diagnostic and therapeutic approaches.

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Author contributions

All authors researched data for the article, made substantial contributions to discussions of content and wrote the manuscript. M.M. reviewed and edited the manuscript before submission.

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