Reproductive Sciences (2022) 29:2429–2439
https://doi.org/10.1007/s43032-021-00588-1

REVIEW

Cannabis and Cannabinoids in Reproduction and Fertility: Where We Stand

Bruno M. Fonseca [1] · Irene Rebelo [1]

Received: 8 February 2021 / Accepted: 15 April 2021 / Published online: 10 May 2021

Abstract

Although cannabis use is increasing in general population, their prevalence among young adults is remarkably high. In recent years, their medical use gained a renewed interest. However, it can underline the reputation of cannabis being a harmless drug. Between cannabinoids, uniquely found on the cannabis plant, Δ^9-tetrahydrocannabinol (THC) is the well-studied compound. It is responsible for the psychoactive effects via central cannabinoid receptors. Nevertheless, cannabinoids interact with other chemical signalling systems such as the hypothalamic–pituitary–gonadal axis. THC indirectly decreases gonadotropin-releasing hormone (GnRH) secretion by the hypothalamus. The consequences are diverse, and several key hormones are affected. THC disturbs important reproductive events like folliculogenesis, ovulation and sperm maturation and function. Although generally accepted that cannabinoid consumption impacts male and female fertility, prevailing evidence remains largely on pre-clinical studies. Here, we introduce cannabinoids and the endocannabinoid system, and we review the most prominent clinical evidence about cannabis consumption in reproductive potential and teratogenicity.

Keywords Cannabis · Cannabinoids · Male fertility · Female fertility · Pregnancy

Cannabis, Cannabinoid Receptors and Endocannabinoid System

Cannabis has been used worldwide for many years and is still one of the most consumed drugs. It derives from the *Cannabis sativa* L. plant that has a rich chemical composition. Besides primary metabolites (e.g., amino acids and fatty acids), cannabis secondary metabolites include flavonoids, terpenoids, alkaloids and cannabinoids. Nevertheless, the well-known class is cannabinoids of which Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the best-studied components.

The first cannabinoid binding site was cloned from the rat brain and denominated CB1 [1] that was followed by the characterization of a peripheral cannabinoid receptor (CB2) [2]. Despite such long history, the crystal structures of CB1 [3, 4] and CB2 [5] have been depicted more recently. It provided important insight into the activation mechanism of cannabinoid receptors and gave us some light about how cannabinoid agonists elicit biased signalling.

Besides cannabinoid receptors, the endocannabinoid system (ECS) includes the endocannabinoids (eCBs) and the enzymes responsible for their biosynthesis, degradation and transport. The ECS has emerged as an important modulatory system, namely for homeostatic synaptic plasticity mechanisms. In the periphery, the ECS has a regulatory role in energy balance, immune functions and reproductive physiology.

Endocannabinoids and Cannabinoid Receptors

The CB1 is mainly expressed in the central nervous system (CNS) [6], whereas CB2 is predominant in immune cells [7]. So far, CB1 is not restricted to CNS as well as CB2 is also present in the CNS, though at much lower levels [8, 9]. In that way, functionally relevant expression of CB1 is present in cardiovascular, endocrine and digestive tissues [10]. At the periphery, CB2 expression extends to the liver and digestive system [11] (Fig. 1). In addition, both receptors are also present in reproductive tissues [12].

✉ Bruno M. Fonseca
brunofonseca@ff.up.pt

[1] UCIBIO, REQUIMTE, Laboratório de Bioquímica, Departamento de Ciências Biológicas, Faculdade de Farmácia da Universidade do Porto, Rua de Jorge Viterbo Ferreira n° 228, 4050-313 Porto, Portugal

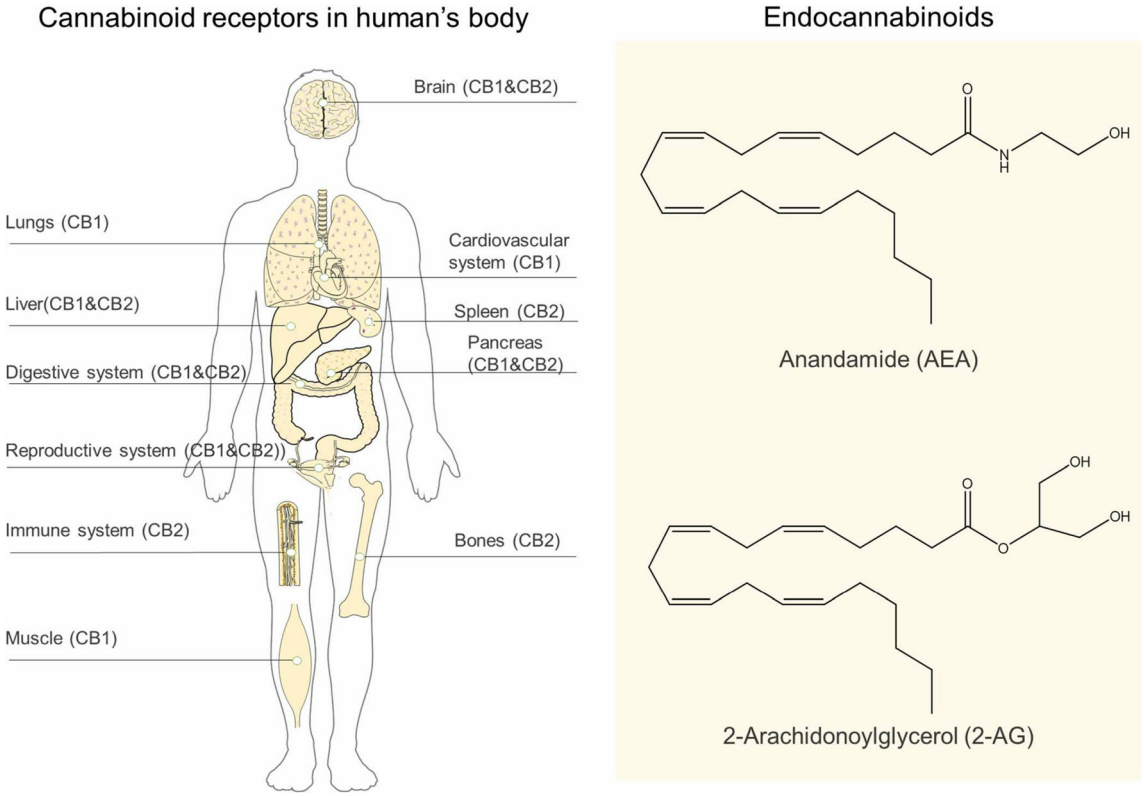

Fig. 1 The chemical structure of major endocannabinoids and cannabinoid receptors distribution. The two major endocannabinoids that have been discovered are anandamide (AEA) and 2-arachidonoyl glycerol (2-AG). Distribution of CB1 and CB2 in the body. CB1 is concentrated in the central and peripheral nervous systems and, to a lesser degree, in the lungs, pancreas, muscle, liver and cardiovascular, reproductive and digestive systems. CB2 is detected in a variety of tissues, like bones, spleen, liver and digestive and reproductive systems, but it is predominantly expressed in cells of the immune system

The identification and cloning of the CB1 receptor encouraged the discovery of its first endogenous agonist, called anandamide (*N*-arachidonoylethanolamine; AEA) [13]. Subsequently, 2-arachidonoylglycerol (2-AG), another important endocannabinoid, was the discovery [14, 15]. Despite the existence of other eCBs, most studies focus on both AEA and 2-AG. In addition, it was identified as eCB-interacting peptides and various arachidonic acid derivatives that can produce eCB-like effects [16]. Among those, the *N*-palmitoylethanolamide (PEA) and *N*-oleoylethanolamide (OEA) potentiate AEA responses (so-called entourage effects) and the nonapeptide hemopressin, which act as a negative allosteric modulator of CB1, is best documented [17].

Either AEA or 2-AG is structurally distinct from phytocannabinoids (Fig. 1), which substantiates the distinct pharmacological activities. In fact, besides cannabinoid receptors, both AEA and 2-AG interact with other receptors. It includes the transient receptor potential vanilloid 1 (TRPV1) [18], the orphan G-protein-coupled receptor 55 (GPR55) [12] and the peroxisome proliferator-activated receptors (PPARs) family (De Petrocellis and Di Marzo), which are activated by AEA. The interaction of 2-AG with the K_{ATP} channel in pancreatic β-cells has been reported [19], as well as 2-AG ability to potentiate $GABA_A$ [20] or PPAR-γ receptors [21].

Endocannabinoid Metabolic Pathways and Related Enzymes

The synthesis, transport and degradation of the two major eCBs occur at target tissues. In brief, AEA synthesis is catalysed from *N*-acyl-phosphatidylethanolamine (NAPE) by NAPE-specific phospholipase D (NAPE-PLD) [22], while 2-AG is produced from diacylglycerol (DAG) by either DAG lipase (DAGL) α or β [23]. Both AEA and 2-AG syntheses are Ca^{2+} sensitive, though the rate-limiting step is, however, the formation of NAPE and DAG precursors [22]. Once synthesis of the eCBs is complete, they are able to diffuse into the extracellular space by simple diffusion [24] or by using specific transporters. However, conclusive evidence for the existence of an "anandamide membrane transporter" (AMT) capable of carrying eCBs is still lacking.

Outside the cells, eCBs may interact with local cannabinoid receptors and then rapidly be taken up by the cells and shuttled into intracellular targets. Fatty acid-binding proteins (FABP)-5 [25] and -7 [25], albumin [26], heat shock protein 70 [27] and the fatty acid amide hydrolase (FAAH)-like AEA transporter protein (FLAT) [28] have been identified as intracellular carrier proteins for endocannabinoids.

Prompt eCBs hydrolysis and/or oxidation terminates their action. The proteolytic degradation of AEA to

arachidonic acid and ethanolamine depends upon FAAH action [29], whereas 2-AG is mostly hydrolysed by monoacylglycerol lipase (MAGL) into arachidonic acid and glycerol [23]. Although less frequent, eCBs can be also oxidized by prostaglandin-endoperoxide synthase 2 (PTGS2) and/or cyclooxygenase 2 (COX-2) enzymes providing prostaglandin ethanolamides and prostaglandin-glycerol esters, respectively [30]. Alternatively, oxidative metabolism may involve the *N*-acylethanolamine acid amidase in AEA hydrolysis [31] and the ab-hydrolase domain-containing (ABDH) -6 and -12 enzymes in 2-AG hydrolysis [32]. Both ABDH-6 and ABDH-12 enzymes are expressed by the human placenta, though their physiological relevance remains uncertain [33].

Cannabinoid Signalling Pathways

Cannabinoid signalling is very complex. Besides many ligands (plant derived, synthetic or endogenous), cannabinoids bind and activate various targets (CB1, CB2, GRP55, TRPV1 and others) triggering different signalling pathways, including mitogen-activated protein kinase (MAPK) and protein kinase A (PKA). Despite such an array of targets, the best-studied signalling pathways involve cannabinoid receptors. They belong to the GPCR family, which are negatively coupled to adenylyl cyclase activity [34]. Through $G_{i/0}$ proteins, cannabinoids can inhibit N- or P/Q-type Ca^{2+} channels and activate inwardly rectifying K^+ channels [35]. In addition, CB1 receptor may be coupled to $G_{q/11}$ that increases intracellular Ca^{2+} concentrations [36] or to G_s that, under pertussis toxin treatment conditions, prevents coupling with $G_{i/o}$-coupled receptors [37]. In contrast, CB2 receptor activation does not modulate the activity of ion channels [38] or couple to G_s protein [37].

There are also evidences that CB1 activates the PI3K/Akt pathway [39] and modulates sphingolipid-metabolizing pathways by increasing the intracellular levels of ceramide [40]. The most pronounced cannabinoid-induced gene expression changes occur through the cAMP-responsive element-binding protein (CREB) and MAPK signalling. Figure 2 depicts the major cannabinoid signalling pathways.

Despite such evidence, cannabinoid pharmacology is still very intriguing. For example, cannabinoid receptors may present conformational heterogeneity, creating a complex energy landscape that may involve different intracellular effectors [4]. In addition, the CB1 may specifically interact with and is regulated by several other proteins, such as the interacting proteins, CRIP1a and CRIP1b [41], G protein receptor-associated sorting protein 1 (GASP1) [42] and β-arrestins [43]. Overall, these proteins play an extra role either in intracellular pathways or in receptor downregulation and desensitization of CB1 receptors.

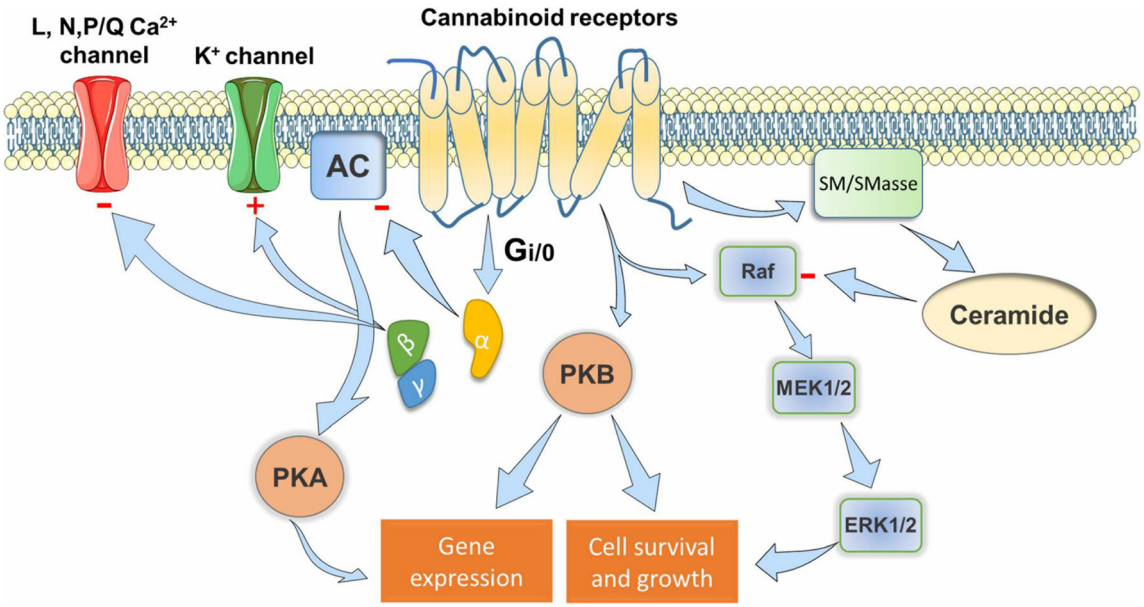

Fig. 2 Cannabinoid receptor signaling favours a range of receptor conformations that can variably affect different signalling pathways. Both CB1 and CB2 cannabinoid receptors are associated with Gi/o-dependent inhibition of adenylyl cyclase (AC) activity, decreased cAMP production and less activation of cAMP-dependent protein kinase (PKA,) culminating in the inhibition of some gene expression; cannabinoid receptors also activate Akt/protein kinase B, favouring cell survival and growth and modulating gene expression; activation of the MAPK cascade, favouring cell survival; through beta and gamma subunits, it may inhibit of specific calcium channels and enhanced opening of G protein-gated inwardly rectifying potassium (GIRK) channels; finally, the stimulation of de novo synthesis of ceramide and inhibition of the MAPK cascade, promoting apoptosis, is only associated to CB1 activation; + indicates activation of the pathway by cannabinoid agonists; − indicates inhibition/downregulation of pathway by cannabinoid receptor agonists

Physiological Effects of Cannabis and Cannabinoids: an Overview

Since 1964, when THC was isolated, it has been the primary focus of cannabis research [44]. In the last years, the non-psychoactive cannabinoid CBD, very common in certain strains of cannabis, has gained clinical relevance. Besides psychoactivity, cannabis have shown antinociceptive [45], anticonvulsant [46], immunosuppressive [47], antiemetic [48], appetite stimulation [49] and antimicrobial [50, 51] properties. These effects result from either cannabinoid receptor activation or eCBs-metabolic enzyme competition.

In addition, THC possesses several effects beyond those directly mediated by CB1 and/or CB2 receptors. For example, the anti-inflammatory properties of THC involve diverse mechanisms. It results from THC ability to inhibit prostaglandin E2 (PGE2) production [52], reduce platelet aggregation [53], decrease the synthesis and secretion of pro-inflammatory cytokines and, in some models, upregulate T-regulatory cells response [54]. At physiological concentrations, cannabinoids can also promote proper recycling of damaged macromolecules and organelles, thus helping to maintain the structural integrity and functional balance of lysosomes. In neurons, these abilities contribute to neuroprotection [55, 56].

Like THC, CBD presents neuroprotective effects and displays potent antioxidant [57] and antimicrobial [51] properties. CBD, in contrast to THC, may activate the TRPV1 receptor [58] and inhibit both FAAH activity and AEA reuptake [59]. Cannabinoids can affect tumour growth directly by inducing cancer cell death and/or by inhibiting cancer cell proliferation [60] and, in part, indirectly via reduction in vascular endothelial growth factor (VEGF) expression [61]. Nevertheless, cannabinoids are capable of inhibiting cancer cell migration and invasion without triggering apoptosis [62].

The psychoactive properties of cannabis, mainly as a result of THC activity, are well documented (Fig. 3). The most common short-term effects include an altered sense of time and other senses (e.g. seeing brighter colours), memory and other thinking problems, changes in body movements and cognitive impairment [63]. In high doses, it may cause hallucinations, delusions or psychosis [64]. In the long term, cannabis use impairs memory and learning functions and affects the communication between neurons associated with these functions [65]. As eCBs are critical for normal brain development, these effects seem to result from cannabinoid disruption of eCBs homeostasis. Therefore, children and adolescents can be especially vulnerable to cannabis misuse.

Besides mental effects, cannabis use can cause fatigue, dry mouth, blurred vision and increase heart and breathing rates [66, 67] (Fig. 3). Cannabis smoke, like tobacco, produces various carcinogens and irritates the lungs [68]. Although controversial, some studies show that continuous use of cannabis is associated with reduced fertility in both male [69] and female [70]. During pregnancy and breastfeeding, cannabis is also contraindicated.

Cannabis in Reproduction and Fertility

Despite the perceptions of fertility and pregnancy complications, cannabis use is increasing among the general population [71]. Although there is a significant variation between studies, the prevalence among young adults is growing in either women or men [72, 73]. In addition, their use is also rising among

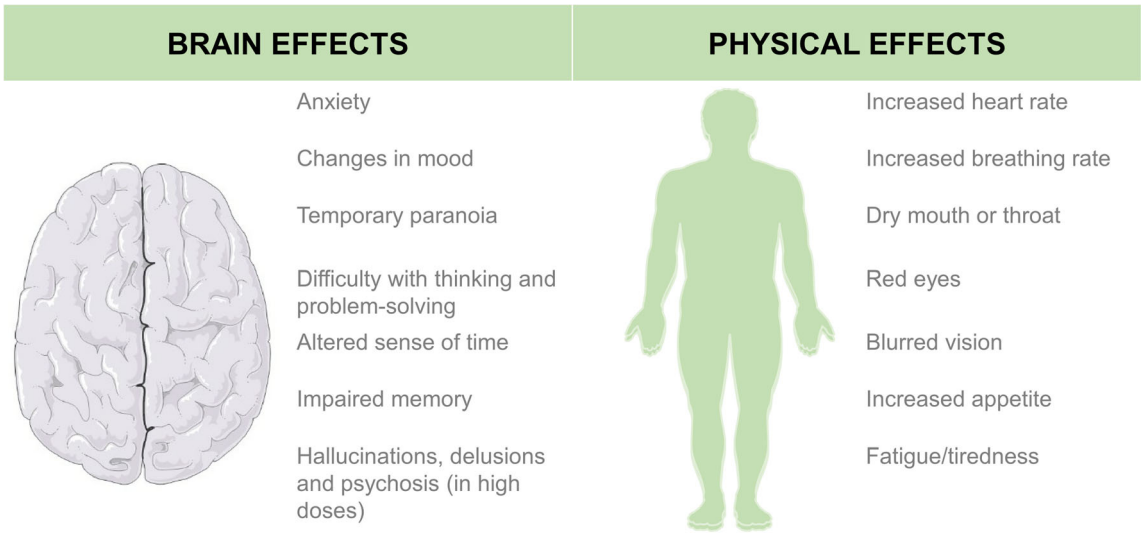

Fig. 3 Major central and peripheral effects of *Cannabis sativa* consumption. Short-term effects on the brain include anxiety, changes in mood, paranoia, difficulty with thinking and problem-solving, altered sense of time and memory patterns and, when ingested in high doses, hallucinations, delusions and psychosis. The peripheral effects are more diverse, but the major cannabis-associated physical effects are increased breathing and heart rate, dry mouth or throat, red eyes and blurred vision, increased appetite and fatigue

pregnant patients. A recent study found an increase, from 2.2 to 3.3%, in the proportion of pregnant women who self-reported cannabis use [74]. The referred proportion may be even higher as self-reporting surveys commonly underestimate the prevalence when compared with that found via toxicology studies [75]. Cannabinoids exert potential negative effects on human reproduction, though there still exist some controversies. Moreover, their use is frequently associated with alcohol, illicit drugs and tobacco, which may increase the risk of detrimental effects [76].

Cannabis and Female Fertility

Cannabinoids are associated with the hypothalamic–pituitary–gonadal (HPG) axis control of reproductive function. The CB1 receptor is present in the hypothalamus and anterior pituitary as well as in reproductive tissues, including the ovary [77] and endometrium [78]. Chronic exposure to cannabis decreases the pulsatile gonadotropin-releasing hormone (GnRH) secretion indirectly by inhibiting glutamate-releasing (glutamatergic) neurons [79]. In humans, THC decreases GnRH concentrations in a dose-dependent manner [80].

Besides plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels [81], THC also decreases prolactin levels [82]. Consequently, cannabis users are more likely to cause menstrual cycle disruption [83]. In addition, animal studies demonstrated that chronic cannabis use delays sexual maturation [84] and endometrial stromal cells exposed to THC failed to differentiate [85].

Although the exact mechanism is not clear, it involves disruption of multiple pathways, namely deregulation of the pituitary and ovarian hormone levels [86]. Nevertheless, accumulating evidence demonstrates that cannabis effects in reproductive function results, at least partially, from disturbances in the ECS [87]. Sex steroid hormones and eCBs are closely associated [88] and in uterine tissues, estradiol and ECS may regulate each other's activities [89]. During the menstrual cycle, there is also a dynamic change of AEA plasmatic levels [90]. The activity of eCB metabolic enzymes is crucial to regulate eCBs levels, playing a part in reproductive success. Thus, when perturbed by exogenous cannabinoids, enhanced eCBs signalling may lead to reproductive failure. In addition, AEA can inhibit the mouse blastocyst attachment and outgrowth [91]. Overall, there is evidence that an abnormal synthesis of AEA in the ovary and/or uterus may be a determining factor for female infertility or early pregnancy failure.

In addition, activation and overexpression of cannabinoid receptors, especially CB1, may be involved in the pathophysiology of reproductive disorders. CB1 activation appears to be associated with polycystic ovary syndrome (PCOS) through dysregulation of energy metabolism [87]. In turn, endometriotic lesions [92] and ectopic endometrium from patients diagnosed with adenomyosis express less CB1 and CB2 [93]. Although the involvement of the ECS in these pathologies requires further elucidation, it clearly demonstrates that eCBs importance for the reproductive function is far beyond the HPG axis. In the same line of evidence, AEA inhibits aromatase activity and affects estradiol signalling [85], indicating that dysfunctions of the ECS may be implicated in endometrial disease and infertility [94]; however, the evidence is still limited in humans.

Despite substantial evidence that cannabis use can disrupt ovulation, tubal transportation and embryo implantation, large-scale cohort studies have failed to demonstrate reduced fertility in women who use the drug. The American National Survey for Family Growth, which retrospectively followed 1076 female participants, reported that neither cannabis use nor frequency was associated with time to pregnancy [95]. Moreover, cannabis use was independently associated with increased sexual frequency [96]. Another large observational study, the Pregnancy Study Online (PRESTO), which followed 4194 women (1125 couples) prospectively from 2013 to 2017, reported a poor association between cannabis use and fecundability [73]. Nonetheless, an prospective cohort study involving patients undergoing in vitro fertilization found that current cannabis users had more than double the adjusted probability of pregnancy loss after fertility treatment (54% versus 26%) when compared with those who were past cannabis users or had never smoked cannabis [97]. In addition, the study also demonstrated that treatment outcomes of past cannabis smokers did not differ significantly from those women who had never smoked marijuana [97]. However, only nine women were included in the cannabis users' group making it difficult to generalize to couples from the general population.

Cannabis and Pregnancy

Several studies suggest that, during pregnancy, cannabis is the most used illicit substance, despite it is associated with adverse neonatal outcomes. THC, and its metabolites, readily crosses the placenta [98] and are detectable in breast milk samples up to 6 days after maternal cannabis use [99]. Recent data suggests an expansion in their use [74], which is greater in the first trimester, and it is frequently associated with alcohol, illicit drugs and tobacco [76]. In addition, some women use cannabis to treat pregnancy-related nausea because they believe it is safe as it is natural.

The Screening for Pregnancy Endpoints (SCOPE) study recruited a cohort of 5610 pregnant nulliparous women at low risk of complications, from 2004 to 2011. The authors showed that frequent cannabis users during pregnancy presented a higher risk of spontaneous pre-term birth [100]. However, the risk of pre-eclampsia, gestational hypertension or gestational diabetes was not increased [100]. The authors have then assessed adverse neonatal outcomes [101]. They reported lower

birth weight, length and head circumference for gestational age patterns in babies of a mother who still used cannabis compared to babies of mothers who stopped using cannabis. Heavy users revealed even greater differences, except for gestational age at birth [101]. Overall, it suggests a dose-dependent effect for cannabis detrimental effects on neonatal outcomes. Another study, from J.E. Ehiri and collaborators, further demonstrated that infants exposed to cannabis during pregnancy had low birth weight and more frequently required neonatal intensive care [102]. However, a systematic review and meta-analysis of 31 studies assessed the effects of maternal cannabis use on adverse neonatal outcomes. The authors have concluded that the association between maternal cannabis use and adverse neonatal outcomes, namely increased risk of low birth weight and premature birth, appears attributable to confounding factors, particularly tobacco use [103].

Few studies have addressed children's long-term neurobehavioural effects. Daniel J Corsi and collaborators performed a retrospective analysis using medical records. The offspring of mothers who reported using marijuana during pregnancy were at increased risk of autism spectrum disorder. The incidence of intellectual disability and learning disorders was also higher, though less statistically robust [104]. Other potential long-term health consequences of prenatal exposure include sleep disorders [105] and affective symptoms [106].

The risk of prenatal exposure to cannabis or maternal cannabis use during lactation is unclear; however, data also suggest an association of cannabis exposure with development and behaviour [107]. Human infants exposed to cannabis in utero exhibit decreased dopamine receptor 2 mRNA in nucleus accumbens, through regulation of histone lysine methylation [108]. These epigenetic modifications in individuals prenatally exposed to cannabis may contribute to addiction vulnerability later in life. Moreover, as far as we know, there are no studies about the potential effect on infants exposed to secondary cannabis smoke. Although some findings associate maternal cannabis use and adverse outcomes, the whole range of short- and long-term health effects requires further studies.

Cannabis and Sperm Function

Cannabis consumers are predominantly male, particularly at reproductive age. A recent prospective cohort of North American couples reports that 14.2% of males use cannabis compared to 11.6% of females [73]. Cannabis prevalence can even be higher among men. For example, according to the Canadian Tobacco, Alcohol and Drugs Survey, the prevalence of cannabis use is nearly double among males (15%) compared to females (7.4%) [109].

THC crosses the blood-testis barrier and its penetration is correlated with serum THC [110]. Like females, cannabinoids affect the HPG axis, which are crucial to produce spermatozoa and sex steroids. Chronic exposure to cannabinoids results in reduced serum LH; however, FSH levels remained unchanged and testosterone levels are inconclusive. Primary studies conducted by Kolodny et al. suggest that THC cause a dose-dependent reduction in testosterone levels and sperm concentration [111]. However, in a recent study, sperm samples from 5146 men, of which 3027 using THC, show that, despite the fact that testosterone levels decline as THC use increases, testosterone is higher in THC users [112]. Nevertheless, most evidence of alterations in male fertility resulting from cannabis exposure is associated with semen parameters. Heavy users present reduced sperm count and concentrations [111]. These effects are accompanied by changes in sperm morphology and motility [113].

Studies analysing the impact of cannabis on male fecundity have raised controversy. In couples using assisted reproductive technologies to conceive, male partners with positive cannabis status at the time of enrollment were associated with a significantly higher probability of live birth compared with former consumers or non-consumers independently of women's cannabis using status [97]. While cannabis may increase libido in short term, in long term, regular use induces gynecomastia, erectile disorders and reduce erectile function [114]. Nevertheless, the impact of cannabis consumption on male fertility is still unclear. Among the 758 male participants of the American National Survey, neither cannabis use nor frequency was associated with men's fertility [95].

The ECS also plays a role in male reproductive function. Cannabinoid receptors and AEA have been shown to be present and operate in testicular tissue, including Sertoli and Leydig cells, as well as spermatozoa [115, 116]. Interestingly, within spermatozoa, CB1 and CB2 have different locations. Although midpiece and tail express both cannabinoid receptors, the plasma membrane of the acrosomal region expresses predominantly CB1, whereas the postacrosomal region is CB2 [117]. Besides differently located, cannabinoid receptors also participate in distinct manners in regulating sperm potential. The activation of CB1 decreases motility and viability of spermatozoa [118] as well as inhibits the capacitation-induced acrosomal reaction [119]. Activation of CB2 increases in the slow/sluggish progressive sperm population [120]. Besides CB1 and CB2 receptors, Sertoli cells express TRPV1 receptors, which when activated triggers apoptosis of these cells [121]. Accordingly, seminal endocannabinoid levels and sperm motility correlate inversely [122]. Thus, alteration in the balance of endocannabinoids within the seminal plasma may affect sperm production and reduce fertility in men. DNA methylation differs in the sperm of human users from non-users [123]. Moreover, paternal activation of CB2 can alter the epigenome of spermatozoa [124]. Although largely unknown, these heritable changes in gene expression highlight the potential risks induced by the father's pre-mating exposure to cannabinoids.

Looking Ahead

The growing movement towards the legalization of medical cannabis has brought a renewed interest in the effects of cannabinoids on reproductive function. The use of cannabis among individuals who are on reproductive age, as well as pregnant women is becoming more frequent, particularly in countries that legalized medical cannabis use. Moreover, cannabis consumers are more likely to use tobacco, alcohol and other addictive drugs and to have a psychiatric disorder. In addition, the use of more potent cannabis products will certainly increase in the future.

Despite such importance, the short- and long-term effects of cannabis use on reproductive function require further elucidation. In women, cannabis users revealed reduced hormonal levels, which may disrupt ovulation, tubal transportation and embryo implantation. Nevertheless, large-scale prospective cohort studies have failed to demonstrate reduced fertility in women who use cannabis. Cannabis use during pregnancy is associated with a higher risk of spontaneous pre-term birth and adverse neonatal outcomes. It includes lower birth weight, length and head circumference. In addition, current research suggests that babies with in utero exposure to cannabis more frequently required neonatal intensive care and, in long term, revealed intellectual disability and learning disorders.

In men, THC, as well as its major metabolites, is present in the semen of men who are heavy or long-term cannabis users. Although contradictory data persists, regarding hormone plasma levels in cannabis users, numerous in vitro and animal studies demonstrate reduced sperm quality with cannabis use. These effects, expected in human spermatozoa, are varying; cohort studies fail to provide robust conclusions that cannabis harms male fertility. Prospective, long-term studies, with appropriate control of confounders, are essential for further elucidation of these effects.

Overall, these effects need further studies to validate the robust findings in pre-clinical models about cannabis impact upon human fertility. Although the impact of cannabis use on female fertility remains inconclusive, current evidence of harm recommends patients avoid cannabis when trying to conceive; however, there is sufficient data indicating a negative impact during pregnancy. Thus, women when pregnant or breastfeeding may be advised about potential risks. In men, data from pre-clinical studies suggest that sperm parameters become altered in cannabis users; however, this data is conflicting and larger clinical studies are required. Nevertheless, men should be aware of its dangers and be counselled to stop when trying to conceive.

In addition, far more research is needed on the implications of cannabis consumption for reproductive potential. For example, few studies addressed the impact of CBD, which is increasingly used in place of the psychoactive THC, on fertility and pregnancy outcomes. Likewise, pre-clinical evidence supports a negative effect of CBD on the reproductive system, but it is currently limited to acute and subacute dosing in rodents and to in vitro models. Therefore, until high-quality scientific evidence is available to show that cannabis is safe, clinicians should be aware of the potential benefits and risks when prescribing medical cannabis. In addition, cannabis use should be considered in a comprehensive infertility evaluation. It is also relevant to promote the development and dissemination of tools to raise public awareness and knowledge of the health risks associated with cannabis use for individuals at reproductive age and for pregnant women.

Author's Contributions B.M.F. conceived the idea and wrote the manuscript in consultation with I.R. All authors read and approved the final manuscript.

Funding This work was supported by the Applied Molecular Biosciences Unit - UCIBIO which is financed by national funds from Fundação para a Ciência e a Tecnologia (FCT) (UIDB/04378/2020); FEDER - Fundo Europeu de Desenvolvimento Regional funds through the COMPETE 2020 – Operacional Programme for Competitiveness and Internationalisation (POCI) and FCT in the framework of the project PTDC/MEC-OUT/28931/2017.

Declarations

Conflict of Interest The authors declare no competing interests.

References

1. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature. 1990;346:561–4.
2. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature. 1993;365:61–5.
3. Hua T, Vemuri K, Pu MC, Qu L, Han GW, Wu YR, Zhao SW, Shui WQ, Li SS, Korde A, Laprairie RB, Stahl EL, Ho JH, Zvonok N, Zhou H, Kufareva I, Wu BL, Zhao Q, Hanson MA, Bohn LM, Makriyannis A, Stevens RC, Liu ZJ. 2016. Crystal structure of the human cannabinoid receptor CB1. Cell, 167, 750-+.
4. Hua T, Vemuri K, Nikas SP, Laprairie RB, Wu Y, Qu L, Pu M, Korde A, Jiang S, Ho JH, Han G. W, Ding K, Li X, Liu H, Hanson MA, Zhao S, Bohn LM, Makriyannis A, Stevens RC. Liu ZJ. 2017. Crystal structures of agonist-bound human cannabinoid receptor CB1. Nature, 547, 468-+.
5. Li XT, Hua T, Vemuri K, Ho JH, Wu YR, Wu LJ, Popov P, Benchama O, Zvonok N, Locke K, Qu L, Han GW, Iyer MR, Cinar R, Coffey NJ., Wang JJ, Wu M, Katritch V, Zhao SW, Kunos G, Bohn LM, Makriyannis A, Stevens RC. Liu ZJ. 2019. Crystal structure of the human cannabinoid receptor CB2. Cell, 176, 459-+.
6. Mackie K. 2005. Distribution of cannabinoid receptors in the central and peripheral nervous system. Handb Exp Pharmacol, 299-325.
7. Chen DJ, Gao M, Gao FF, SU, Q. X. & Wu, J. Brain cannabinoid receptor 2: expression, function and modulation. Acta Pharmacol Sin. 2017;38:312–6.

8. Chung YC, Shin WH, Baek JY, Cho EJ, Baik HH, Kim SR, et al. CB2 receptor activation prevents glial-derived neurotoxic mediator production, BBB leakage and peripheral immune cell infiltration and rescues dopamine neurons in the MPTP model of Parkinson's disease. Exp Mol Med. 2016;48:e205.
9. Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, et al. Identification and functional characterization of brainstem cannabinoid CB2 receptors. Science. 2005;310:329–32.
10. Dai E, Zhang L, Ye L, Wan S, Feng L, Qi Q, et al. Hepatic expression of cannabinoid receptors CB1 and CB2 correlate with fibrogenesis in patients with chronic hepatitis B. Int J Infect Dis. 2017;59:124–30.
11. Galiazzo G, Giancola F, Stanzani A, Fracassi F, Bernardini C, Forni M, et al. Localization of cannabinoid receptors CB1, CB2, GPR55, and PPARalpha in the canine gastrointestinal tract. Histochem Cell Biol. 2018;150:187–205.
12. Fonseca BM, Teixeira NA, Almada M, Taylor AH, Konje JC, Correia-Da-Silva, G. Modulation of the novel cannabinoid receptor-GPR55-during rat fetoplacental development. Placenta. 2011;32:462–9.
13. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science. 1992;258:1946–9.
14. Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol. 1995;50:83–90.
15. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, et al. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun. 1995;215:89–97.
16. Di Marzo V, DE Petrocellis L. Why do cannabinoid receptors have more than one endogenous ligand? Philos Trans R Soc Lond Ser B Biol Sci. 2012;367:3216–28.
17. Bomar MG, Galande AK. Modulation of the cannabinoid receptors by hemopressin peptides. Life Sci. 2013;92:520–4.
18. Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V, et al. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature. 1999;400:452–7.
19. Spivak CE, Kim W, Liu QR, Lupica CR, Doyle ME. Blockade of beta-cell K(ATP) channels by the endocannabinoid, 2-arachidonoylglycerol. Biochem Biophys Res Commun. 2012;423:13–8.
20. Sigel E, Baur R, Racz I, Marazzi J, Smart TG, Zimmer A, et al. The major central endocannabinoid directly acts at Gaba(A) receptors. Proc Natl Acad Sci U S A. 2011;108:18150–5.
21. Matias I, Gonthier MP, Orlando P, Martiadis V, De Petrocellis L, Cervino C, et al. Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglycemia. J Clin Endocrinol Metab. 2006;91:3171–80.
22. Fonseca BM, Costa MA, Almada M, Correia-Da-Silva G, Teixeira NA. Endogenous cannabinoids revisited: a biochemistry perspective. Prostaglandins Other Lipid Mediat. 2013b;102-103:13–30.
23. Murataeva N, Straiker A, Mackie K. Parsing the players: 2-arachidonoylglycerol synthesis and degradation in the CNS. Br J Pharmacol. 2014;171:1379–91.
24. Hillard CJ, Jarrahian A. The movement of N-arachidonoylethanolamine (anandamide) across cellular membranes. Chem Phys Lipids. 2000;108:123–34.
25. Kaczocha M, Glaser ST, Deutsch DG. Identification of intracellular carriers for the endocannabinoid anandamide. Proc Natl Acad Sci U S A. 2009;106:6375–80.
26. Ranganarayanan P, Thanigesan N, Ananth V, Jayaraman VK, Ramakrishnan V. Identification of glucose-binding pockets in human serum albumin using support vector machine and molecular dynamics simulations. IEEE/ACM Trans Comput Biol Bioinform. 2016;13:148–57.
27. Kopczynska B, Sulejczak D, Welniak-Kaminska M, Gietka A, Grieb P. Anandamide enhances expression of heat shock proteins Hsp70 and Hsp25 in rat lungs. Eur J Pharmacol. 2011;668:257–63.
28. Kaczocha M, Vivieca S, Sun J, Glaser ST, Deutsch DG. Fatty acid-binding proteins transport N-acylethanolamines to nuclear receptors and are targets of endocannabinoid transport inhibitors. J Biol Chem. 2012;287:3415–24.
29. Muccioli GG. Endocannabinoid biosynthesis and inactivation, from simple to complex. Drug Discov Today. 2010;15:474–83.
30. Kozak KR, Prusakiewicz JJ, Marnett LJ. Oxidative metabolism of endocannabinoids by COX-2. Curr Pharm Des. 2004;10:659–67.
31. Ueda N, Tsuboi K, Uyama T. N-acylethanolamine metabolism with special reference to N-acylethanolamine-hydrolyzing acid amidase (NAAA). Prog Lipid Res. 2010;49:299–315.
32. Blankman JL, Simon GM, Cravatt BF. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol. 2007;14:1347–56.
33. Maia J, Fonseca BM, Cunha SC, Braga J, Goncalves D, Teixeira N, Correia-Da-Silva G 2020. Impact of tetrahydrocannabinol on the endocannabinoid 2-arachidonoylglycerol metabolism: ABHD6 and ABHD12 as novel players in human placenta. Biochim Biophys Acta Mol Cell Biol Lipids, 1865, 158807.
34. Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, et al. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev. 2002;54:161–202.
35. Pertwee RG. Cannabinoid receptor ligands: clinical and neuropharmacological considerations, relevant to future drug discovery and development. Expert Opin Investig Drugs. 2000;9:1553–71.
36. Navarrete M, Araque A. Endocannabinoids mediate neuron-astrocyte communication. Neuron. 2008;57:883–93.
37. Glass M, Felder CC. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. J Neurosci. 1997;17:5327–33.
38. Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O, et al. Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Mol Pharmacol. 1995;48:443–50.
39. Gomez Del Pulgar T, Velasco G, Guzman M. The CB1 cannabinoid receptor is coupled to the activation of protein kinase B/Akt. Biochem J. 2000;347:369–73.
40. Velasco G, Galve-Roperh I, Sanchez C, Blazquez C, Haro A, Guzman M. Cannabinoids and ceramide: two lipids acting hand-by-hand. Life Sci. 2005;77:1723–31.
41. Niehaus JL, Liu Y, Wallis KT, Egertova M, Bhartur SG, Mukhopadhyay S, et al. CB1 cannabinoid receptor activity is modulated by the cannabinoid receptor interacting protein CRIP 1a. Mol Pharmacol. 2007;72:1557–66.
42. Martini L, Waldhoer M, Pusch M, Kharazia V, Fong J, Lee JH, et al. Ligand-induced down-regulation of the cannabinoid 1 receptor is mediated by the G-protein-coupled receptor-associated sorting protein GASP1. FASEB J. 2007;21:802–11.
43. Jin WZ, Brown S, Roche JP, Hsieh C, Celver JP, Kovoor A, et al. Distinct domains of the CB1 cannabinoid receptor mediate desensitization and internalization. J Neurosci. 1999;19:3773–80.
44. Gaoni Y, Mechoulam R. Isolation, structure, and partial synthesis of an active constituent of Hashish. J Am Chem Soc. 1964;86:1646–7.

45. Smith FL, Fujimori K, Lowe J, Welch SP. Characterization of delta9-tetrahydrocannabinol and anandamide antinociception in nonarthritic and arthritic rats. Pharmacol Biochem Behav. 1998;60:183–91.
46. Sofia RD, Solomon TA, Barry H, 3RD. Anticonvulsant activity of delta9-tetrahydrocannabinol compared with three other drugs. Eur J Pharmacol. 1976;35:7–16.
47. Croxford JL, Yamamura T. Cannabinoids and the immune system: potential for the treatment of inflammatory diseases? J Neuroimmunol. 2005;166:3–18.
48. Darmani NA. Delta(9)-tetrahydrocannabinol and synthetic cannabinoids prevent emesis produced by the cannabinoid CB(1) receptor antagonist/inverse agonist SR 141716A. Neuropsychopharmacology. 2001;24:198–203.
49. Mattes RD, Engelman K, Shaw LM, Elsohly MA. Cannabinoids and appetite stimulation. Pharmacol Biochem Behav. 1994;49:187–95.
50. Appendino G, Gibbons S, Giana A, Pagani A, Grassi G, Stavri M, et al. Antibacterial cannabinoids from Cannabis sativa: a structure-activity study. J Nat Prod. 2008;71:1427–30.
51. Van Klingeren B, Ten Ham M. Antibacterial activity of delta9-tetrahydrocannabinol and cannabidiol. Antonie Van Leeuwenhoek. 1976;42:9–12.
52. Burstein S, Levin E, Varanelli C. Prostaglandins and cannabis. II. Inhibition of biosynthesis by the naturally occurring cannabinoids. Biochem Pharmacol. 1973;22:2905–10.
53. Schaefer CF, Brackett DJ, Gunn CG, Dubowski KM. Decreased platelet aggregation following marihuana smoking in man. J Okla State Med Assoc. 1979;72:435–6.
54. Kozela E, Juknat A, Kaushansky N, Rimmerman N, Ben-Nun A, Vogel Z. Cannabinoids decrease the th17 inflammatory autoimmune phenotype. J NeuroImmune Pharmacol. 2013;8:1265–76.
55. Garcia C, Palomo-Garo C, Garcia-Arencibia M, Ramos J, Pertwee R, Fernandez-Ruiz J. Symptom-relieving and neuroprotective effects of the phytocannabinoid delta(9)-THCV in animal models of Parkinson's disease. Br J Pharmacol. 2011;163:1495–506.
56. Noonan J, Tanveer R, Klompas A, Gowran A, McKiernan J, Campbell VA. Endocannabinoids prevent beta-amyloid-mediated lysosomal destabilization in cultured neurons. J Biol Chem. 2010;285:38543–54.
57. Hacke ACM, Lima D, De Costa F, Deshmukh K, Li N, Chow AM, et al. Probing the antioxidant activity of delta(9)-tetrahydrocannabinol and cannabidiol in Cannabis sativa extracts. Analyst. 2019;144:4952–61.
58. Giudice ED, Rinaldi L, Passarotto M, Facchinetti F, D'arrigo A, Guiotto A, et al. Cannabidiol, unlike synthetic cannabinoids, triggers activation of RBL-2H3 mast cells. J Leukoc Biol. 2007;81:1512–22.
59. De Petrocellis L, Bisogno T, Maccarrone M, Davis JB, Finazzi-Agro A, Di Marzo V. The activity of anandamide at vanilloid VR1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism. J Biol Chem. 2001;276:12856–63.
60. Fonseca BM, Correia-Da-Silva G, Teixeira NA. Cannabinoid-induced cell death in endometrial cancer cells: involvement of TRPV1 receptors in apoptosis. J Physiol Biochem. 2018;74:261–72.
61. Blazquez C, Gonzalez-Feria L, Alvarez L, Haro A, Casanova ML, Guzman M. Cannabinoids inhibit the vascular endothelial growth factor pathway in gliomas. Cancer Res. 2004;64:5617–23.
62. Solinas M, Massi P, Cantelmo AR, Cattaneo MG, Cammarota R, Bartolini D, et al. Cannabidiol inhibits angiogenesis by multiple mechanisms. Br J Pharmacol. 2012;167:1218–31.
63. Volkow ND, Baler RD, Compton WM, Weiss SR. Adverse health effects of marijuana use. N Engl J Med. 2014;370:2219–27.
64. Rabin RA, George TP. Cannabis and psychosis: understanding the smoke signals. Lancet Psychiatry. 2016;3:909–10.
65. Cohen K, Weizman A, Weinstein A. Modulatory effects of cannabinoids on brain neurotransmission. Eur J Neurosci. 2019;50:2322–45.
66. Ribeiro LI, Ind PW. Effect of cannabis smoking on lung function and respiratory symptoms: a structured literature review. NPJ Prim Care Respir Med. 2016;26:16071.
67. Schmid K, Schonlebe J, Drexler H, Mueck-Weymann M. The effects of cannabis on heart rate variability and well-being in young men. Pharmacopsychiatry. 2010;43:147–50.
68. Tashkin Dp, Roth MD 2019. Pulmonary effects of inhaled cannabis smoke. Am J Drug Alcohol Abuse**,** 1-14.
69. Payne KS, Mazur DJ, Hotaling JM, Pastuszak AW 2019. Cannabis and male fertility: a systematic review. J Urol**,** 101097JU0000000000000248.
70. Fonseca BM, Correia-Da-Silva G, Almada M, Costa MA, Teixeira NA. The endocannabinoid system in the postimplantation period: a role during decidualization and placentation. Int J Endocrinol. 2013a;2013:510540.
71. Jordan T, Ngo B, Jones CA 2020. The use of cannabis and perceptions of its effect on fertility among infertility patients. Hum Reprod Open*,* 2020**,** hoz041.
72. Metz TD, Silver RM, Mcmillin GA, Allshouse AA, Jensen TL, Mansfield C, et al. Prenatal marijuana use by self-report and umbilical cord sampling in a state with marijuana legalization. Obstet Gynecol. 2019;133:98–104.
73. Wise LA, Wesselink AK, Hatch EE, Rothman KJ, Mikkelsen EM, Sorensen HT, et al. Marijuana use and fecundability in a North American preconception cohort study. J Epidemiol Community Health. 2018;72:208–15.
74. Luke S, Hutcheon J, Kendall T. Cannabis use in pregnancy in British Columbia and selected birth outcomes. J Obstet Gynaecol Can. 2019;41:1311–7.
75. Young-Wolff KC, Tucker LY, Alexeeff S, Armstrong MA, Conway A, Weisner C, et al. Trends in self-reported and biochemically tested marijuana use among pregnant females in California from 2009-2016. Jama-Journal of the American Medical Association. 2017;318:2490–1.
76. Singh S, Filion KB, Abenhaim HA, Eisenberg MJ. Prevalence and outcomes of prenatal recreational cannabis use in high-income countries: a scoping review. Bjog-an International Journal of Obstetrics and Gynaecology. 2020;127:8–16.
77. Cecconi S, Rossi G, Castellucci A, D'andrea G, Maccarrone M. Endocannabinoid signaling in mammalian ovary. Eur J Obstet Gynecol Reprod Biol. 2014;178:6–11.
78. Resuehr D, Glore DR, Taylor HS, Bruner-Tran KL, Osteen KG. Progesterone-dependent regulation of endometrial cannabinoid receptor type 1 (CB1-R) expression is disrupted in women with endometriosis and in isolated stromal cells exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Fertil Steril. 2012;98:948–56 e1.
79. Liu X, Herbison AE. Dopamine regulation of gonadotropin-releasing hormone neuron excitability in male and female mice. Endocrinology. 2013;154:340–50.
80. Chakravarty I, Shah PG, Sheth AR, Ghosh JJ. Mode of action of delta-9-tetrahydrocannabinol on hypothalamo-pituitary function in adult female rats. J Reprod Fertil. 1979;57:113–5.
81. Mendelson JH, Mello NK, Ellingboe J, Skupny AS, Lex BW, Griffin M. Marihuana smoking suppresses luteinizing hormone in women. J Pharmacol Exp Ther. 1986;237:862–6.
82. Mendelson JH, Mello NK, Ellingboe J. Acute effects of marihuana smoking on prolactin levels in human females. J Pharmacol Exp Ther. 1985;232:220–2.
83. Brents LK. Marijuana, the endocannabinoid system and the female reproductive system. Yale J Biol Med. 2016;89:175–91.

84. Field E, Tyrey L. Delayed sexual-maturation during prepubertal cannabinoid treatment - importance of the timing of treatment. J Pharmacol Exp Ther. 1990;254:171–5.
85. Neradugomma NK, Drafton K, Mor GG, Mao QC. Marijuana-derived cannabinoids inhibit uterine endometrial stromal cell decidualization and compromise trophoblast-endometrium cross-talk. Reprod Toxicol. 2019;87:100–7.
86. Gammon CM, Freeman GM Jr, Xie W, Petersen SL, Wetsel WC. Regulation of gonadotropin-releasing hormone secretion by cannabinoids. Endocrinology. 2005;146:4491–9.
87. Juan CC, Chen KH, Wang PH, Hwang JL, Seow KM. Endocannabinoid system activation may be associated with insulin resistance in women with polycystic ovary syndrome. Fertil Steril. 2015;104:200–6.
88. Cui N, Wang L, Wang W, Zhang J, Xu Y, Jiang L, et al. The correlation of anandamide with gonadotrophin and sex steroid hormones during the menstrual cycle. Iran J Basic Med Sci. 2017;20:1268–74.
89. Maia J, Almada M, Silva A, Correia-Da-Silva G, Teixeira N, Sa SI, et al. The endocannabinoid system expression in the female reproductive tract is modulated by estrogen. J Steroid Biochem Mol Biol. 2017;174:40–7.
90. Scotchie JG, Savaris RF, Martin CE, Young SL. Endocannabinoid regulation in human endometrium across the menstrual cycle. Reprod Sci. 2015;22:113–23.
91. Liu WM, Duan EK, Cao YJ. Effects of anandamide on embryo implantation in the mouse. Life Sci. 2002;71:1623–32.
92. Tanaka K, Mayne L, Khalil A, Baartz D, Eriksson L, Mortlock SA, et al. The role of the endocannabinoid system in aetiopathogenesis of endometriosis: a potential therapeutic target. Eur J Obstet Gynecol Reprod Biol. 2020;244:87–94.
93. Shen X, Duan H, Wang S, Gan L, Xu Q, Li JJ. 2019. Decreased expression of cannabinoid receptors in the eutopic and ectopic endometrium of patients with adenomyosis. Biomed Res Int, 2019, 5468954.
94. almada m, Oliveira A, Amaral C., Fernandes Pa, Ramos MJ, Fonseca B, Correia-Da-Silva G, Teixeira N. 2019. Anandamide targets aromatase: a breakthrough on human decidualization. Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids, 1864.
95. Kasman AM, Thoma ME, McLain AC, Eisenberg ML. Association between use of marijuana and time to pregnancy in men and women: findings from the National Survey of Family Growth. Fertil Steril. 2018;109:866–71.
96. Sun AJ, Eisenberg ML. Association between marijuana use and sexual frequency in the United States: a population-based study. J Sex Med. 2017;14:1342–7.
97. Nassan FL, Arvizu M, Minguez-Alarcon L, Gaskins AJ, Williams PL, Petrozza JC, et al. Marijuana smoking and outcomes of infertility treatment with assisted reproductive technologies. Hum Reprod. 2019;34:1818–29.
98. Blackard C, Tennes K 1984. Human Placental-Transfer of Cannabinoids. N Engl J Med, 311, 797
99. Bertrand KA, Hanan NJ, Honerkamp-Smith G, Best BM, Chambers CD. Marijuana use by breastfeeding mothers and cannabinoid concentrations in breast milk. Pediatrics. 2018;142: e20181076.
100. Leemaqz SY, Dekker GA, Mccowan LM, Kenny LC, Myers JE, Simpson NAB, et al. Maternal marijuana use has independent effects on risk for spontaneous preterm birth but not other common late pregnancy complications. Reprod Toxicol. 2016;62:77–86.
101. Grzeskowiale LE, Grieger JA, Andraweera P, Knight E, Leemaqz S, Poston L, et al. The deleterious effects of cannabis during pregnancy on neonatal outcomes. Med J Aust. 2020;212:519–24.
102. Gunn JKL, Rosales CB, Center KE, Nunez A, Gibson SJ, Christ C, et al. Prenatal exposure to cannabis and maternal and child health outcomes: a systematic review and meta-analysis. BMJ Open. 2016;6:e009986.
103. Conner SN, Bedell V, Lipsey K, Macones GA, Cahill AG, Tuuli MG. Maternal marijuana use and adverse neonatal outcomes: a systematic review and meta-analysis. Obstet Gynecol. 2016;128: 713–23.
104. Corsi DJ, Donelle J, Sucha E, Hawken S, Hsu H, El-Chaar D, et al. Maternal cannabis use in pregnancy and child neurodevelopmental outcomes. Nat Med. 2020;26:1536–40.
105. Winiger Ea, Hewitt JK 2020. Prenatal cannabis exposure and sleep outcomes in children 9-10 years of age in the adolescent brain cognitive development (SM) study. Sleep Health.
106. Lisdahl KM, TAMM L, Epstein JN, Jernigan T, Molina BSG, Hinshaw SP, et al. The impact of ADHD persistence, recent cannabis use, and age of regular cannabis use onset on subcortical volume and cortical thickness in young adults. Drug Alcohol Depend. 2016;161:135–46.
107. Eidelman AI. Public health implications of recent breastfeeding research regarding cannabis use and risk of childhood obesity. Breastfeed Med. 2018;13:549–51.
108. Dinieri JA, Wang X, Szutorisz H, Spano SM, Kaur J, Casaccia P, et al. Maternal cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring. Biol Psychiatry. 2011;70:763–9.
109. CANADIAN TOBACCO, A. A. D. S. C. 2017. Available at: https://www.canada.ca/en/health-canada/services/canadian-tobacco-alcohol-drugs-survey/2017-summary.html (Accessed february 1, 2021).
110. Lee MS, Lanes A, Ginsburg ES, Fox JH. Delta-9 THC can be detected and quantified in the semen of men who are chronic users of inhaled cannabis. J Assist Reprod Genet. 2020;37:1497–504.
111. Kolodny RC, Masters WH, Kolodner RM, Toro G. Depression of plasma testosterone levels after chronic intensive marihuana use. N Engl J Med. 1974;290:872–4.
112. Fantus RJ, Lokeshwar SD, Kohn TP, Ramasamy R. The effect of tetrahydrocannabinol on testosterone among men in the United States: results from the National Health and Nutrition Examination Survey. World J Urol. 2020;38:3275–82.
113. Carroll K, Pottinger AM, Wynter S, Dacosta V. Marijuana use and its influence on sperm morphology and motility: identified risk for fertility among Jamaican men. Andrology. 2020;8:136–42.
114. Pizzol D, Demurtas J, Stubbs B, Soysal P, Mason C, Isik AT, et al. Relationship between cannabis use and erectile dysfunction: a systematic review and meta-analysis. American Journal of Mens Health. 2019;13:155798831989246.
115. Amoako AA, Marczylo TH, Lam PM, Willets JM, Derry A, Elson J, et al. Quantitative analysis of anandamide and related acylethanolamides in human seminal plasma by ultra performance liquid chromatography tandem mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci. 2010;878:3231–7.
116. Bari M, Battista N, Pirazzi V, Maccarrone M. The manifold actions of endocannabinoids on female and male reproductive events. Front Biosci (Landmark Ed). 2011;16:498–516.
117. Aquila S, Guido C, Santoro A, Perrotta I, Laezza C, Bifulco M, et al. Human sperm anatomy: ultrastructural localization of the cannabinoid1 receptor and a potential role of anandamide in sperm survival and acrosome reaction. Anat Rec (Hoboken). 2010;293: 298–309.
118. Amoako AA, Marczylo TH, Marczylo EL, Elson J, Willets JM, Taylor AH, et al. Anandamide modulates human sperm motility: implications for men with asthenozoospermia and oligoasthenoteratozoospermia. Hum Reprod. 2013;28:2058–66.
119. Rossato M, Ion Popa F, Ferigo M, Clari G, Foresta C. Human sperm express cannabinoid receptor Cb1, the activation of which

inhibits motility, acrosome reaction, and mitochondrial function. J Clin Endocrinol Metab. 2005;90:984–91.
120. Agirregoitia E, Carracedo A, Subiran N, Valdivia A, Agirregoitia N, Peralta L, et al. The CB(2) cannabinoid receptor regulates human sperm cell motility. Fertil Steril. 2010;93:1378–87.
121. Rossi G, Gasperi V, Paro R, Barsacchi D, Cecconi S, Maccarrone M. Follicle-stimulating hormone activates fatty acid amide hydrolase by protein kinase A and aromatase-dependent pathways in mouse primary Sertoli cells. Endocrinology. 2007;148:1431–9.
122. Zufferey F, Donze N, Rahban R, Senn A, Stettler E, Rudaz S, Nef S, Rossier MF 2020. Semen endocannabinoids are correlated to sperm quality in a cohort of 200 young Swiss men. Andrology.
123. Murphy SK, Itchon-Ramos N, Visco Z, Huang Z, Grenier C, Schrott R, et al. Cannabinoid exposure and altered DNA methylation in rat and human sperm. Epigenetics. 2018;13:1208–21.
124. Innocenzi E, De Domenico E, Ciccarone F, Zampieri M, Rossi G, Cicconi R, Bernardini R, Mattei M., Grimaldi P 2019. Paternal activation of CB2 cannabinoid receptor impairs placental and embryonic growth via an epigenetic mechanism. Scientific Reports, 9.