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REVIEW

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Marijuana, the Endocannabinoid System and the Female Reproductive System

Lisa K. Brents

Department of Pharmacology and Toxicology and Department of Psychiatry, University of Arkansas for Medical Sciences

Marijuana use among women is highly prevalent, but the societal conversation on marijuana rarely focuses on how marijuana affects female reproduction and endocrinology. This article reviews the current scientific literature regarding marijuana use and hypothalamic-pituitary-ovarian (HPO†) axis regulation, ovarian hormone production, the menstrual cycle, and fertility. Evidence suggests that marijuana can reduce female fertility by disrupting hypothalamic release of gonadotropin releasing hormone (GnRH), leading to reduced estrogen and progesterone production and anovulatory menstrual cycles. Tolerance to these effects has been shown in rhesus monkeys, but the effects of chronic marijuana use on human female reproduction are largely unknown. Marijuana-induced analgesia, drug reinforcement properties, tolerance, and dependence are influenced by ovarian hormones, with estrogen generally increasing and progesterone decreasing sensitivity to marijuana. Carefully controlled regulation of the Endocannabinoid System (ECS) is required for successful reproduction, and the exogenous cannabinoids in marijuana may disrupt the delicate balance of the ECS in the female reproductive system.

INTRODUCTION

Marijuana is the most commonly abused illicit drug in the world [1]. In the United States (U.S.), about 40 to 50 percent of adults have used marijuana at least once [2,3]. Data from the 2014 National Survey on Drug Use and Health (NSDUH) indicate that 8.4 percent of Americans ages 12 and older are current marijuana users (i.e., have used marijuana in the past month) [4]. Out of all Americans who currently use *any* illicit drug, about 80 percent use marijuana [4]. The frequency and severity of marijuana use in the U.S. are also striking. Of current marijuana users, about 40 percent are daily or near daily users [5]. In 2014, approximately 4.2 million Americans endorsed cannabis dependence or abuse, as defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, or *DSM*-IV [4]. Cannabis use disorder, which is characterized by disruptions in daily functioning due to marijuana use, cravings for marijuana, and withdrawal upon marijuana abstinence, is endorsed by a far greater number of Americans *than any other illicit drug use disorder*, including prescription pain relievers (1.9 million), cocaine (0.9 million), heroin (0.6 million), or stimulants (0.5 million) [4].

Although men are more likely to use drugs [5] and have a substance use disorder [6], drug abuse seriously impacts women's health. After alcohol and heroin, marijuana is the most common primary drug of abuse for women entering treatment for substance abuse [7]. Females appear to be more sensitive to the behavioral and physiological effects of marijuana and marijuana-like substances [8], and treatment-seeking women endorse more severe marijuana withdrawal symptoms than treat-

*To whom all correspondence should be addressed: Lisa K. Brents, 4301 West Markham Street, Slot 554, Little Rock, AR, 72205, Phone: 501-526-8341, Fax: 501-526-8199.

†Abbreviations: 2-AG, 2-arachidonylglycerol; AEA, anandamide; CB1R, Cannabinoid Receptor type-1; CB2R, Cannabinoid Receptor type-2; CBs, cannabinoids; Δ^9-THC, Delta-9-tetrahydrocannabinol; eCBs, endocannabinoids; DAGL- α, diacylglycerol lipase α; DAGL- β, diacylglycerol lipase β; ECS, Endocannabinoid System; E2, estradiol; FAAH, fatty acid amide hydrolase; FSH, follicle stimulating hormone; GnRH, gonadotropin-releasing hormone; GPCR, G-protein coupled receptors; hCG, human chorionic gonadotropin; HPO axis, hypothalamic-pituitary-ovarian axis; inh, inhalation; IMm, intramuscular; IV, intravenous; IP, intraperitoneal; LH, luteinizing hormone; MAGL, monoacylglycerol lipase; mg/kg, milligrams (of drug) per kilogram (of body weight); NAPE-PLD, N-acyl phosphotidylethanolamine phospholipase D; NSDUH, National Survey on Drug Use and Health; n.d., not determined; PLC, phospholipase C; P, progesterone; PRL, prolactin; SC, subcutaneous; TRH, thyrotropin-releasing hormone.

Keywords: marijuana, endocannabinoid system, female reproduction, menstrual cycle, fertility, estrogen, progesterone, HPO axis

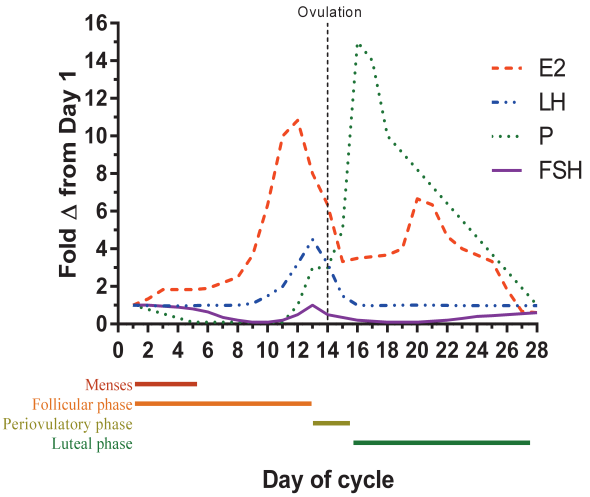

Figure 1. Relative changes in circulating gonadotropin and ovarian hormone levels throughout the phases of the menstrual cycle. Levels of each hormone are expressed relative to their Day 1 levels. E2 = estradiol; FSH = follicle stimulating hormone; LH = luteinizing hormone; P= progesterone.

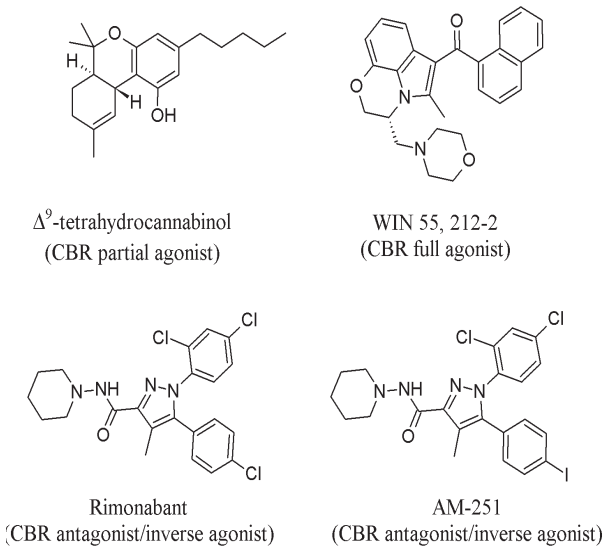

Figure 2. Chemical structures of cannabinoids, agonists and antagonists discussed in this review. Structures obtained from The PubChem Project [108-111].

ment-seeking men [9]. After tobacco and alcohol, marijuana is the most commonly abused substance by women of childbearing age [5]. According to the 2013 NSDUH, 5.4 percent of pregnant women and 11.4 percent of non-pregnant women ages 15 to 44 are current illicit drug users [5], with marijuana representing 64 to 79 percent of female drug use [5,10-12]. As marijuana becomes more widely legalized, marijuana use by women will likely increase. Although the impact of marijuana on women's reproductive health is rarely considered in the societal conversation on marijuana, reports that marijuana use disrupts female endocrinology and reproductive function should call greater attention to this issue. This review will focus on how marijuana and its biological target, the Endocannabinoid System (ECS), interface with the female reproductive system. This discussion will cover the impact of marijuana on the menstrual cycle, fertility and pregnancy, as well as the role of the ECS in each of these processes.

MARIJUANA AND THE FEMALE REPRODUCTIVE SYSTEM

Marijuana, the Menstrual Cycle, and the Hypothalamic-Pituitary-Ovarian Axis

The menstrual cycle is a multiphasic process of the female reproductive system that promotes readiness to conceive and carry out a pregnancy. The ovarian phases and hormonal changes associated with the menstrual cycle are illustrated in Figure 1 and are briefly described as follows [13]. The first half of the menstrual cycle is composed of the follicular phase. This phase is characterized by the maturation of a small subset of oocyte follicles in the ovaries, which is induced by the gonadotropin follicle-stimulating hormone (FSH). Estrogen production by the follicles sharply increases as the primary follicle matures and circulating estrogen levels peak as the phase ends. Soon afterwards, the release of an oocyte from the mature follicle is triggered by a surge of the gonadotropin luteinizing hormone (LH) in a process known as ovulation. During the luteal phase, which immediately follows ovulation, the empty ovarian follicle produces and secretes relatively large concentrations of progesterone. Progesterone promotes the maintenance of the endometrial lining in a state that can support implantation and the early stages of pregnancy. The absence of a conceptus leads to decreased progesterone production, which leads to the end of the luteal phase. Menses, which is Day 1 of the new cycle, signifies the end of the cycle.

Few studies have examined the effects of marijuana on the menstrual cycle, but these studies suggest that there exists an association between marijuana use and menstrual cycle disruptions. Women who use marijuana have a slightly elevated rate of menstrual cycles that lack ovulation (anovulatory cycles) [14]. Individuals in this population are also at higher risk for decreased fertility due to ovulatory abnormalities [15]. One study found an association between occasional marijuana use (self-reported 1 to 3 times in the three months preceding the study) and prolonged follicular phase (3.5 days), resulting in delayed ovulation [14]. The authors of this study remarked that in contrast to previous findings by other investigators, they found no difference with respect to marijuana use on the length of the luteal phase. In the study they referenced,

Table 1. Effects of marijuana or Δ^9-THC on circulating gonadotropins, prolactin and ovarian hormones in female humans and rhesus monkeys

			Treatment		Primary outcomes of treatment condition (marijuana or Δ^9-THC)					
Ref	**Subjects**	**Design**	**Dose**	**Route**	**Timing**	**FSH**	**LH**	**E2**	**P**	**PRL**
18	5 female rhesus monkeys	repeated measures	Δ^9-THC (2.5 mg/kg) or vehicle	IM	daily during the *follicular phase*	n.d.	Suppressed LH surge	Altered patterns of circulating total estrogens across the cycle (suppressed pre-ovulatory spike)	Suppressed luteal elevation of P	No effect
20	4 female rhesus monkeys	repeated measures	Δ^9-THC (2.5 mg/kg) or vehicle	IM	daily during the *luteal phase* (from the day after ovulation until menses)	n.d.	n.d.	n.d.	No effect	n.d.
21, 22	8 healthy human female marijuana users	repeated measures, double blind, placebo controlled	1-gram standardized marijuana joint (1.83% Δ^9-THC) or placebo joint	inh	single dose during *follicular phase*	n.d.	No effect	No effect	No effect	No effect
21, 22	8 healthy human female marijuana users	repeated measures, double blind, placebo controlled	1-gram standardized marijuana joint (1.83% Δ^9-THC) or placebo joint	inh	single dose during *luteal phase*	n.d.	Decreased 60-120 mins after dose	No effect	No effect	Decreased 60-120 mins and 150-180 mins after dose.
24	3 female rhesus monkeys	repeated measures	Δ^9-THC (2.5 mg/kg) or vehicle	IM	single dose during *mid-luteal phase* (day 20-22)	n.d.	n.d.	n.d.	Decreased 6-24 hours after dose; effect was prevented by hCG	n.d.
25	5 ovarectomized female rhesus monkeys	repeated measures performed at least 10 days apart	single dose of Δ^9-THC (0.3125, 0.625, 1.25, 2.5, 5.0 mg/kg) or vehicle.	IM	Non-cycling animals	Decreased 6-24 hours after 5.0 mg/kg dose, 12 hours after 2.5 mg/kg dose, and 6 hours after 0.625 and 1.25 mg/kg doses.	Decreased 6-12 hours after 2.5 and 5.0 mg/kg doses, and 6 hours after 0.625 and 1.25 mg/kg doses. No effect with 0.3 mg/kg.	n.d.	n.d.	n.d.
26	5 female rhesus monkeys	repeated measures, chronic study	Δ^9-THC (1.25 mg/kg in 2 monkeys or 2.5 mg/kg in 3 monkeys) or vehicle	IM	3X per week beginning on cycle day 1 and lasting for 230 days or until 2 consecutive ovulatory cycles occurred	n.d.	Decreased for ~100 days after treatment began in 2.5 mg/kg treated group	Decreased for ~100 days after treatment began in 2.5 mg/kg treated group	Decreased for ~100 days after treatment began in 2.5 mg/kg treated group	No effect

Note: Abbreviations: Δ^9-THC, delta-9-tetrahydrocannabinol; E2, estradiol; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; IM, intramuscular (injection); inh, inhalation; LH, luteinizing hormone; mg/kg, milligrams (of drug) per kilogram (of body weight); n.d., not determined; P, progesterone; PRL, prolactin

Table 2. Pregnenolone (PN) modulation of Δ^9-THC-induced effects [30]

Subjects	Treatment	Outcomes
Adult male Sprague Dawley rats	*In vitro* analysis of excitatory postsynaptic currents (EPSC) of NAc brain slices; pre-treatment of slices with 100 nM PN, followed by 20 μM bath of Δ^9-THC	PN decreased Δ^9-THC-induced inhibition of EPSCs; PN decreased Δ^9-THC-induced presynaptic suppression of glutamate release.
Adult male Sprague Dawley rats	Microdialysis probe and recording electrode placement in the right NAc and right ventral tegmental area (VTA), respectively; PN (2 mg/kg, SC) 30 minutes before Δ^9-THC (IV, 0.15-1.2 mg/kg). Recording began 15 minutes after Δ^9-THC administration	PN decreased Δ^9-THC-induced firing rate in the VTA; PN decreased Δ^9-THC-induced dopamine outflow to the NAc.
Adult male Wistar rats	*ad libitum* feeding; PN (0.5-2 mg/kg, SC), Δ^9-THC (0.5 mg/kg)	PN suppressed Δ^9-THC-induced hyperphagia.
C57BL/6N mice	Slightly food restricted; PN (2 mg/kg, SC), -THC (1 mg/kg, IP)	PN suppressed Δ^9-THC-induced hyperphagia.
	PN (6 mg/kg, SC, 30 minutes before Δ^9-THC, 10 mg/kg, IP)	PN decreased Δ^9-THC-induced locomotor suppression, hypothermia, catalepsy, analgesia, and memory impairment.
	PN-synthesis inhibitor aminogluthetimide (AMG, 50 mg/kg, IP, 30 minutes before Δ^9-THC, 10 mg/kg, IP)	PN increased Δ^9-THC-induced locomotor suppression, hypothermia, catalepsy and analgesia; Effect reversed by PN injection.
CD1 mice trained to self-administer WIN	PN (0, 2, 4 mg/kg, SC) 30 minutes before SA session	PN decreased responses on the active nosepoke hole; PN decreased breakpoint in progressive ratio schedule of reinforcement.

Note: Abbreviations: AMG, aminogluthetimide; Δ^9-THC, delta-9-tetrahydrocannabinol; EPSCs, excitatory postsynaptic currents; IP, intraperitoneal (injection); mg/kg, milligrams per kilogram; μM, micomolar; NAc, nucleus accumbens; PN, pregnenolone; SA, self-administration; SC, subcutaneous (injection); VTA, ventral tegmental area; WIN, WIN 55, 212-2.

self-reported chronic moderate-to-heavy marijuana use (at least 3 times per week over the six months preceding the study) was associated with greater frequency of menstrual cycles that were anovulatory and /or had a luteal phase shorter than 11 days (38.3 percent and 12.5 percent in marijuana users and control participants, respectively) [16]. No results pertaining to the follicular phase were reported in this study. The discrepancies regarding menstrual phase length reported in these studies may be due to differences in frequency of dosing (occasional versus moderate-to-heavy). These studies had relatively small sample sizes and did not control for the amount of marijuana used at each exposure or the use of other substances, such as alcohol or tobacco use, which could affect the menstrual cycle. Therefore, the results of these studies should be interpreted with caution and more studies that rigorously examine the relationship of marijuana use and menstrual cycle irregularities in humans are needed.

Rhesus monkeys provide one of the best animal models for the human female reproductive system because they have a 28-day menstrual cycle that is regulated by mechanisms similar to those in the human [17]; therefore, most primate studies on the effects of marijuana on the female reproductive system have been conducted in the rhesus monkey. In many of the studies cited in this review, the menstrual cycle phase-specific effects of marijuana use are determined by restricting acute or sub-chronic exposure to one phase and measuring the effects of exposure on hormonal profile, phase length and markers of phase hallmarks (e.g., menses, ovulation). Furthermore, most studies in non-human species administer the isolated psychoactive component of marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC, Figure 2), via injection for ease and consistency in dosing (compared to inhalation). These injections are usually intramuscular (IM) in non-human primates and intraperitoneal (IP,) or sometimes subcutaneous (SC,) in mice and rats. Almost all rhesus monkey studies cited in this review administered 2.5 mg/kg (milligrams per kilogram) Δ^9-THC, IM, because this dose produces Δ^9-THC blood levels comparable to those of human moderate-to-heavy marijuana users [18]. In addition to Δ^9-THC, other similar drugs (called "cannabinoids") are administered to rodent models. One such drug is WIN 55,212-2 (WIN), which is used instead of Δ^9-THC for intravenous (IV) cannabinoid self-administration (SA) studies because rodents acquire and maintain WIN SA, but not Δ^9-THC SA [19].

Table 3. Combined estrogen/progesterone modulation of Δ^9-THC-induced effects

Subjects	Treatment	Outcomes	Ref
28 adult female humans	1-gram standardized marijuana joint (1.83% Δ^9-THC) or placebo joint during the follicular, luteal, and ovulatory phases	Menstrual cycle phase had no effect on marijuana-induced changes to pulse rate or subjective ratings of intoxication and confusion.	[38]
30 adult female human with moderate-to-heavy marijuana use	No treatment; participants completed marijuana use diaries and the Moos Menstrual Distress Questionnaire (MDQ) daily for 3 consecutive menstrual cycles	No covariance of marijuana use and menstrual cycle phase.	[39]
Adult intact and ovariectomized female and intact male Lister Hooded and Long Evans rats	Ovarian hormone depletion (ovariectomy at 9-10 weeks of age and male rats) or presence (intact female rats); WIN self-administration acquisition, maintenance, and extinction	Intact females of both strains acquired WIN SA faster, administered more drug per session, and resisted extinction of WIN SA more robustly than did males and ovariectomized females.	[40]
Adult intact and ovariectomized female and intact male Lister Hooded rats	WIN self-administration acquisition and extinction; drug- and cue-induced reinstatement by priming with WIN (0.15 or 0.03 mg/kg, IP) with and without visual and/or auditory cues	Intact female rats reinstated WIN SA more robustly than did intact male or ovariectomized female rats across all drug- and cue-priming conditions.	[41]
Adult intact male and female Sprague-Dawley rats	Intracerebroventricular (ICV) administration of Δ^9-THC (100 µg) five minutes before testing session	Females had shorter latencies to withdraw in nociceptive tests than males; females in estrus had shorter latency to withdraw than those in proestrus-estrus; Females in proestrus-estrus showed greater Δ^9-THC-antinocieption than females in other phases and males.	[44]
Four-month old intact male and female Sprague-Dawley rats	Quantification of Δ^9-THC and 11-OH- Δ^9-THC in brain and serum 15, 30, 60, 120, and 240 minutes after Δ^9-THC (10 mg/kg, IP); SKF525A (cytochrome P450 inhibitor, 25 mg/kg, IP) thirty minutes before Δ^9-THC (10 mg/kg, IP) fifteen minutes before testing session; using HPLC	Females exhibited greater brain concentrations of 11-OH- Δ^9-THC than males at 120 minutes post-injection; SKF525A decreased Δ^9-THC-induced antinociception in females, but not males.	[45]
Adult gonadectomized or sham-gonadectomized female and male Sprague-Dawley rats	E2 (females) or testosterone (males) replacement or blank capsule controls (SC implants) immediately after gonadectomy; P (500 µg, SC, females only) or vehicle every 3 days, beginning 4 days after gonadectomy; Δ^9-THC (30 mg/kg, IP) or vehicle twice daily for 6.5 days, with the final dose administered 30 minutes before tolerance testing session; Rimonabant (10.0 mg/kg, IP) or vehicle 4 hours after final Δ^9-THC treatment, 5 minutes before dependence testing session.	Sham-gonadectomized females developed greater tolerance to Δ^9-THC-induced hypothermia than sham males; E2 and P increased rimonabant-induced chewing in chronic Δ^9-THC-treated female rats.	[46]
Adult intact male and female Sprague-Dawley rats	ED80 dose of Δ^9-THC (IP) or vehicle twice daily for 9 days; cumulative dosing of Δ^9-THC (IP) on pre-chronic (1.8-32.0 mg/kg) and post-chronic (18.0-180.0 mg/kg) test days	Females developed greater tolerance to Δ^9-THC-induced antinociception than males.	[47]
Adult gonadectomized or sham-gonadectomized female and male Sprague-Dawley rats	E2 (females) or testosterone (males) replacement or blank capsule controls (SC implants) immediately after gonadectomy; daily P (500 µg, SC, females only) or vehicle, beginning 4 days after gonadectomy; ED80 dose of Δ^9-THC (IP) or vehicle twice daily for 9 days; cumulative dosing of Δ^9-THC (IP) on pre-chronic (1.8-32.0 mg/kg) and post-chronic (18.0-180.0 mg/kg) test days	Females developed greater tolerance to Δ^9-THC-induced antinociception than males in a non-ovarian-hormone- dependent manner.	[48]

Note: Abbreviations: Δ^9-THC, delta-9-tetrahydrocannabinol; E2, estradiol; HPLC, high performance liquid chromatography; ICV, intracerebroventricular (injection); IP, intraperitoneal (injection); µg, micrograms; MDQ, Moos Menstrual Distress Questionnaire; mg/kg, milligrams per kilogram; P, progesterone; SC, subcutaneous (injection).

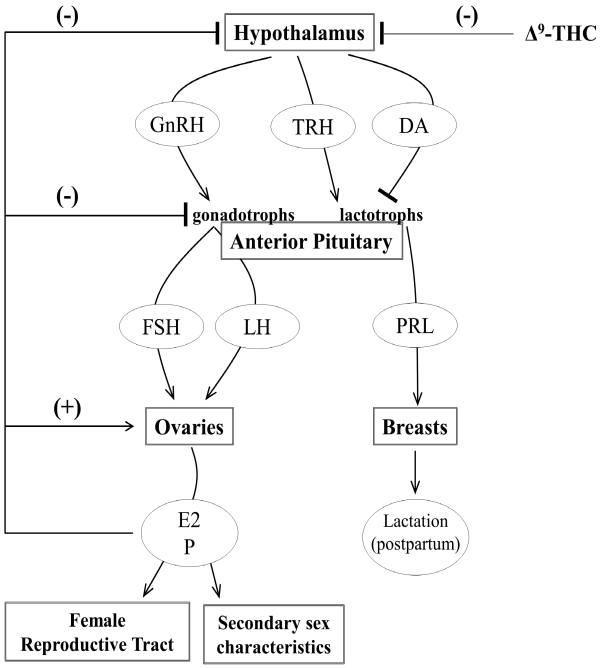

Figure 3. **A simplified representation of the hypothalamic-pituitary-ovarian (HPO) axis.** Hypothalamic stimulation elicits the release of gonadotropin-releasing hormone (GnRH), thyrotropin-releasing hormone (TRH), and dopamine (DA) onto the anterior pituitary, which contains specialized neurons that are sensitive to these hormones. TRH stimulates and dopamine inhibits the release of prolactin (PRL) from the lactotrophs of the anterior pituitary. Prolactin promotes milk production during the postpartum period. GnRH stimulates the release follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from gonadotrophs in the anterior pituitary. FSH and LH promote the ovarian production of estrogen (primarily estradiol, E2), development of mature ovarian follicles, the release of oocytes from the mature ovarian follicles during ovulation and the production of progesterone (P) from the post-ovulatory follicle. The ovarian hormones, particularly E2, signal at the ovaries to promote follicle maturation. The ovarian hormones also exert negative feedback on the pituitary and hypothalamus to decrease release of FSH, LH and GnRH.

Two studies in monkeys reported the effects of Δ^9-THC exposure on menstrual cycle phase length and ovulation. Daily intramuscular injections of Δ^9-THC during the follicular phase of the menstrual cycle induced longer, anovulatory cycles [18]. Luteal phase length was not affected by daily Δ^9-THC administration during the luteal phase at doses up to 5.0 mg/kg [20].

The mechanisms by which marijuana disrupts the menstrual cycle involve the hypothalamic-pituitary-ovarian (HPO) axis, which regulates female reproduction [13] (Figure 3). Overall findings from human and animal studies suggest that acute Δ^9-THC suppresses the release of gonadotropin-releasing hormone (GnRH) and thyrotropin-releasing hormone (TRH) from the hypothalamus, preventing these hormones from stimulating the release of prolactin and the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), from the anterior pituitary. The gonadotropins maintain the menstrual cycle by promoting ovarian follicle maturation, stimulating production of the ovarian steroids estradiol and progesterone, and inducing ovulation, and alterations in circulating gonadotropin can disrupt these processes. Studies examining the effects of marijuana and Δ^9-THC on plasma gonadotropin, prolactin and ovarian hormone levels are summarize in Table 1. An acute inhaled dose totaling 1 gram of marijuana that was smoked in a single session was sufficient to suppress plasma LH during the luteal, but not follicular, phase of the menstrual cycle in humans [21,22]. The suppression of LH during the early luteal phase may terminate early pregnancies by reducing ovarian production of progesterone [23], a hormone that is necessary to maintain and support pregnancy. Consistent with this finding in humans, a study in rhesus monkeys found that a single, clinically relevant intramuscular dose of Δ^9-THC (2.5 mg/kg) during the mid-luteal phase of the menstrual cycle decreased circulating progesterone levels in rhesus monkeys [24]. This effect was reversed by human chorionic gonadotropin (hCG) [24], suggesting that Δ^9-THC-induced inhibition of progesterone was caused by suppression of gonadotropin release from the anterior pituitary gland. Studies in ovariectomized monkeys indicate that like LH and prolactin, FSH levels decrease in response to acute Δ^9-THC administration [25]. Consequences of FSH suppression in cycling, intact females include reductions in ovarian follicle development, oocyte maturation, and gonadal steroid production, potentially leading to anovulatory menstrual cycles and infertility.

Like acute dosing, chronic and sub-chronic (i.e., isolated to a single phase of the menstrual cycle) dosing can alter HPO axis function, but sub-chronic administration of Δ^9-THC during the luteal phase appears to affect HPO axis function less than acute administration with the same dose. For example, daily administration of 2.5 mg/kg (IM) of Δ^9-THC throughout the entire luteal phase (day after ovulation until menses) failed to alter serum progesterone levels in rhesus monkeys [20]. This discrepancy between acute and sub-chronic studies of Δ^9-THC administration during the luteal phase may be caused by development of rapid tolerance to the luteum-disrupting effects of Δ^9-THC, but further study is needed to confirm this possibility. In contrast to this possible rapid tolerance during the luteal phase, daily administration of Δ^9-THC (2.5 mg/kg IM) throughout the follicular phase disrupted follicle development, decreased estrogen and progesterone produc-

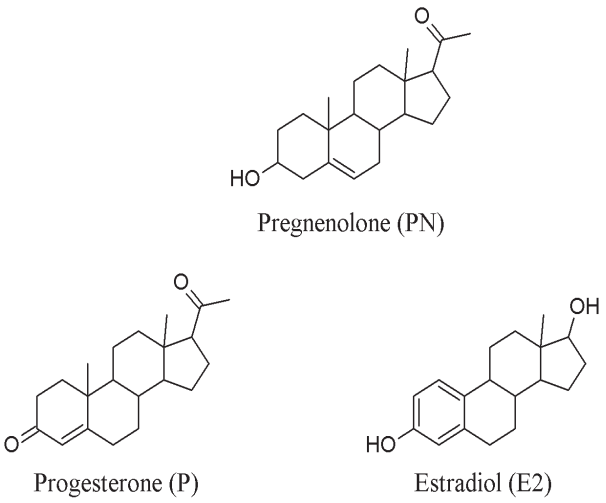

Figure 4. Chemical structures of pregnenolone, progesterone and estrogen. Structures obtained from The PubChem Project [112-114].

tion, blocked LH surge and prevented ovulation, which was rescued by mid-cycle gonadotropin administration [18]. This finding suggests that centrally mediated functions of the HPO axis are susceptible to disruption by sub-chronic Δ^9-THC exposure during the follicular phase. Chronic Δ^9-THC administration across several consecutive menstrual cycles transiently disrupts HPO axis function in the rhesus monkey. Thrice-weekly administration of Δ^9-THC (2.5 mg/kg) robustly suppressed serum estradiol, progesterone, LH, and prolactin, and inhibited ovulation and menses, but the monkeys developed complete tolerance to these effects 103 to 135 days after the initial administration of Δ^9-THC [26]. I could identify no comparable studies of chronic or sub-chronic marijuana or Δ^9-THC administration that have been conducted in humans.

The possibility that Δ^9-THC disrupts the HPO axis at the hypothalamus or pituitary gland instead of the ovary is supported by several previous findings, including Δ^9-THC-induced suppression of circulating prolactin and gonadotropins, the reversal of Δ^9-THC-induced ovarian steroid suppression by exogenous gonadotropins [18, 24], and the lack of Δ^9-THC affinity for estrogen and progesterone receptors [27]. Furthermore, suppression of gonadotropins and prolactin by Δ^9-THC can be reversed by exogenous gonadotropin-releasing hormone (GnRH) and thyrotropin-releasing hormone (TRH), respectively [25,28]. This finding indicates that the anterior pituitary can be stimulated by hypothalamic hormones in the presence of Δ^9-THC, providing strong evidence that the hypothalamus, not pituitary gland, is suppressed by Δ^9-THC (Figure 3).

As described above, the suppression of the HPO axis and dysregulation of female reproduction by marijuana and its psychoactive component Δ^9-THC is well-established in non-human primates, but evidence suggests that ovarian hormones and their neuroactive precursors (neurosteroids) also influence the subjective, behavioral and physiological effects of marijuana. The influence of ovarian hormones and neurosteroids on the effects of marijuana has been investigated in preclinical studies by directly manipulating ovarian hormones. Such manipulations include treating ovariectomized rodents with estradiol and/or progesterone replacement, or modifying the hormonal systems of intact rodents using pharmacological agents or genetic modification (e.g., knock-out rodents). Other studies have also observed how estrous cycle phase affects the behavior of intact rodents. This approach is useful because each phase (diestrus, proestrus, estrus, and metestrus) has its own distinct ovarian hormone profile, with progesterone rapidly peaking during the second half of proestrous, and estrogen gradually climbing and peaking during proestrus as well [29]. Table 2, Table 3, Table 4, and 5 summarize the findings of these studies by hormone. One notable recent study found that pregnenolone, the precursor for progesterone, estrogen, and all other steroid hormones, was induced in the brain by Δ^9-THC administration [30]. Pregnenolone, in turn, suppressed Δ^9-THC-induced effects in rodents, including hypothermia, locomotor suppression, analgesia, catalepsy, hyperphagia, and intravenous self-administration of the Δ^9-THC-like drug WIN (Table 2). Pregnenolone also suppressed Δ^9-THC-induced neurobiological effects in areas of the brain important in reward processing, including increased firing rates of neurons in the ventral tegmental area (VTA), increased dopamine outflow from the VTA onto the nucleus accumbens (NAc), and inhibition of excitatory post synaptic currents in nucleus accumbal neurons [30]. This study indicates that a hormone that is structurally almost identical to progesterone (Figure 4) can "dampen" a wide range of marijuana's effects, raising the possibility that progesterone may possess similar activity. While the interaction of progesterone with marijuana has not yet been extensively investigated, preclinical and human studies of other drugs of abuse suggest that progesterone may antagonize the abuse-related effects of other drugs. For example, a state of suppressed progesterone and elevated estrogen (i.e. the follicular phase, Figure 1) was associated with enhanced ratings of drug liking, euphoria, and "high" for cocaine [31,32] and amphetamines [33,34]. Because reward processing of drugs from different classes share the common mechanism of stimulating dopamine transmission in the NAc shell and extended amygdala complex [35], the finding that ovarian hormone profile can modulate cocaine and amphetamine reward signifies that the effects of ovarian hormones on marijuana-induced reward processing should also be considered and investigated. Ovarian hormone profiles may also affect the risk of drug use relapse. In human studies of males and females, exogenous progesterone attenuated the urge to smoke in abstinent nicotine-dependent smokers [36] and reduced cue-induced drug cravings in cocaine-dependent individuals. Cocaine-dependent women with higher levels

Table 4. Estrogen modulation of Δ^9-THC-induced effects

Subjects	Treatment	Outcomes	References
Ovariectomized female Long-Evans rats	The dose-effect of Δ^9-THC (0.56-3.2 mg/kg, IP) on repeated acquisition and performance of a 4-response sequence memory and learning operant task in the presence of either E2 replacement or cholesterol control via SC implanted capsule	E2 alone improved response accuracy. E2 attenuated the Δ^9-THC-induced reduction in response accuracy during acquisition and performance and reduction in response rate during acquisition.	[42]
Intact or ovariectomized female Sprague-Dawley rats	E2 replacement or blank capsule controls (SC implants) immediately after ovariectomy; determination of estrous cycle phase of intact females by daily vaginal lavage; acute Δ^9-THC (5 or 10 mg/kg, IP) 15 minutes before testing session	E2 enhanced Δ^9-THC-induced antinociception; Δ^9-THC-induced antinociception was greatest during the estrus phase in intact cycling females; E2 had no effect on Δ^9-THC-induced locomotor supression or catalepsy; Testosterone attenuated Δ^9-THC-induced locomotor suppression.	[43]
Adult ovariectomized or sham- ovariectomized female Sprague-Dawley rats	E2 replacement or blank capsule controls (SC implants) immediately after ovariectomy; P (500 µg, SC) or vehicle every 3 days, beginning 4 days after ovariectomy ; Δ^9-THC (30 mg/kg, IP) or vehicle twice daily for 6.5 days, with the final dose administered 30 minutes before tolerance testing session. Rimonabant (10 mg/kg, IP) or vehicle were administered 4 hours later, 5 minutes before dependence testing session	E2 increased locomotor activity in Rimonabant-treated ovariectomized female rats.	[46]

Note: Abbreviations: Δ^9-THC, delta-9-tetrahydrocannabinol; E2, estradiol; IP, intraperitoneal (injection); µg, micrograms; mg/kg, milligrams per kilogram; P, progesterone; SC, subcutaneous (injection).

of circulating progesterone reported lower cue- and stress-induced drug craving after individualized guided script imagery compared to cocaine-dependent women with lower levels of progesterone [37], suggesting that progesterone may have protective effects against relapse for some drugs.

The two studies that have examined the effects of marijuana across the menstrual cycle in humans produced null results [38,39], but several animal studies provide evidence that gonadal hormone regulation affects the abuse potential of cannabinoids (Table 3). One showed that intact female rats acquired self-administration of WIN much quicker, more often, and more robustly than male and ovariectomized female rats [40], suggesting that ovarian hormones (likely estrogen) contribute to the reinforcing effects of cannabinoid agonists like Δ^9-THC. As demonstrated in another study that also compared intact females and males to ovariectomized female rats, ovarian hormones also promote cue- and drug-induced reinstatement of WIN self-administration and increase cannabinoid-seeking behavior [41].

In addition to possibly augmenting the abuse-related effects of cannabinoids, ovarian hormones may alter other Δ^9-THC-related effects. Estradiol replacement treatment in ovariectomized adult rats attenuated Δ^9-THC-induced decrements in learning and memory, as measured by response rate and accuracy in a nonspatial repeated acquisition and performance task [42] (Table 4). Overall, estradiol increases the sensitivity of female rats to Δ^9-THC-induced antinociception, but not locomotor suppression or catalepsy [43]. Estrogen-modulated Δ^9-THC-induced antinociception is largely, but not completely, due to supraspinal (central) mechanisms [44]. Peripheral mechanisms related to the metabolism of Δ^9-THC likely also contribute to this effect. The induction of the CYP450 enzyme system by estrogen leads to greater metabolism of Δ^9-THC to its active metabolite 11-OH-THC, which enhances antinociception in females versus males [45]. In a study examining the role of gonadal hormones on Δ^9-THC tolerance and dependence, progesterone enhanced tolerance to the locomotor-suppressing effects of Δ^9-THC (Table 5), and estrogen and progesterone in-

Table 5. Progesterone modulation of Δ^9-THC-induced effects

Subjects	Treatment	Outcomes	References
Adult ovariectomized or sham-ovariectomized female Sprague-Dawley rats	E2 replacement or blank capsule controls (SC implants) immediately after ovariectomy; P (500 µg, SC) or vehicle every 3 days, beginning 4 days after ovariectomy; Δ^9-THC (30 mg/kg, IP) or vehicle twice daily for 6.5 days, with the final dose administered 30 minutes before tolerance testing session. Rimonabant (10 mg/kg, IP) or vehicle were administered 4 hours later, 5 minutes before dependence testing session	P enhanced tolerance to Δ^9-THC-induced locomotor suppression in ovariectomized female rats; P enhanced Rimonabant-induced sniffing in chronic Δ^9-THC-treated female rats.	[46]
Adult ovariectomized or sham-ovariectomized female Sprague-Dawley rats	E2 replacement or blank capsule control (SC implants) immediately after ovariectomy; daily P (500 µg, SC) or vehicle, beginning 4 days after ovariectomy; acute Δ^9cumulative dosing of Δ^9-THC (1.8-32.0 mg/kg, IP) 15 mins before testing session	P enhanced Δ^9-THC-induced nociception in ovariectomized female rats.	[48]

Note: Abbreviations: Δ^9-THC, delta-9-tetrahydrocannabinol; E2, estradiol; IP, intraperitoneal (injection); µg, micrograms; mg/kg, milligrams per kilogram; P, progesterone; SC, subcutaneous (injection).

creased precipitated withdrawal symptoms in rats chronically treated with Δ^9-THC [46]. Another study found that intact female rats exhibited enhanced tolerance to Δ^9-THC-induced antinociception compared to males [47]. Interestingly, this effect was not due to estrogen or progesterone [48]. Altogether, these findings indicate that there exists a bidirectional relationship between marijuana and the HPO axis, meaning that use of marijuana can alter HPO axis functionality, while ovarian hormones produced by the HPO axis impact the ultimate physiological, behavioral and subjective effects of marijuana.

Marijuana in Pregnancy Maintenance, Parturition and Lactation

Marijuana use during pregnancy is highly prevalent. Approximately 3.9 percent of pregnant women are current (past-month) users of marijuana [49], but few studies have examined the effects of marijuana on pregnancy, delivery, and lactation. Furthermore, experimental studies in which marijuana is administered to pregnant women are unethical, and observational studies in humans can be confounded by inaccurate self-reporting of marijuana use and of behavioral and sociodemographic variables that correlate with prenatal marijuana use and may impact pregnancy outcomes (e.g., age, socioeconomic status, prenatal care access, and use of tobacco, alcohol and other illicit drugs). For these reasons, *in vitro* and animal studies are vital for understanding how marijuana affects pregnancy. *In vitro* studies of early pregnancy indicate that Δ^9-THC interferes with trophoblast proliferation [50] and turnover [51], which may negatively impact placentation. Marijuana can also affect hormonal regulation during pregnancy. One study showed that large doses of Δ^9-THC-like drugs increased LH and estrogen in pregnant rats [52]. In contrast, a study in humans found no difference between pregnant marijuana users and gestational age-matched controls in serum concentrations of several pregnancy hormones and proteins, including human chorionic gonadotropin, pregnancy-specific beta-1-glycoprotein, placental lactogen, progesterone, 17-hydroxyprogesterone, estradiol, and estriol [53]. More studies on the effects of marijuana use on sex hormones in both pregnant and non-pregnant female humans are needed to confirm the relevance of these previous findings to human health.

Despite this null finding, marijuana use during pregnancy is associated with decreased gestational length (0.8 to 2.2 weeks earlier than non-marijuana-using sample) [10,54], and preterm birth (before 37 weeks gestation, OR = 1.5) [55]. One study suggests that cannabinoid use during pregnancy is associated with elevated risk of stillbirth (OR = 2.34), but tobacco use appears to partially confound this results, and the authors were unable to control the results for potential sociodemographic confounders [56]. A study in rhesus monkeys similarly found that administration of Δ^9-THC early in pregnancy (starting on the day of pregnancy diagnosis) led to a high rate of spontaneous abortion 14 to 18 days after dosing initiation [57]. These spontaneous abortions were associated with a rapid drop in maternal chorionic gonadotropin and progesterone levels [57], an effect that is expected to terminate pregnancy. In the few pregnancies that continued to term, animals who received Δ^9-THC had significantly elevated estrogen levels compared with vehicle controls [57], which is consistent with findings from a similar rat study [52]. The ef-

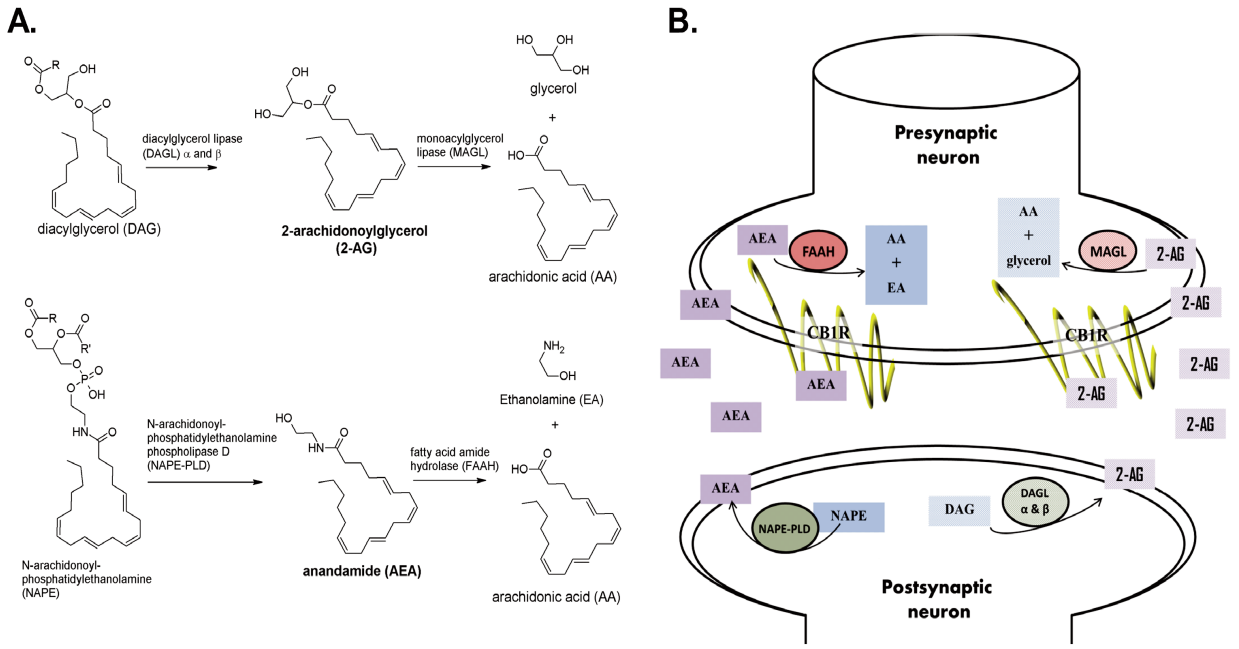

Figure 5. **Formation, degradation and retrograde signaling of endocannabinoids**. A. The eCB 2-AG is formed primarily from DAG by DAGL-α and DAGL-β and is degraded by MAGL into AA and glycerol. The eCB AEA is formed primarily from NAPE by NAPE-PLD and degraded to AA and EA by FAAH. B. Neuronal eCB signaling occurs by retrograde processes; that is, eCBs are made in the postsynaptic neuron, passively diffuse out of the neuron, through the synaptic cleft and bind to cannabinoid receptors on pre-synaptic axonal terminals. eCBs activate CB1R, which tends to have an inhibitory, hyperpolarizing effect on the presynaptic neuron. The formation of eCBs is stimulated by increased signaling from the presynaptic neuron. Retrograde eCB signaling acts to suppress presynaptic neuronal activity by depolarization-induced suppression of inhibition (DSI, in GABAergic neurons) or depolarization-induced suppression of excitation (DSE, in glutamatergic neurons). Structures obtained from [65-67].

fect of in utero marijuana exposure on offspring outcomes is an important topic, but is outside the scope of the current discussion and is reviewed in greater depth elsewhere [58,59].

The effects of marijuana use during late pregnancy on parturition are largely unknown, but *in vitro* and rodent studies suggest that marijuana interferes with the production of several signaling molecules that are important in orchestrating labor and delivery, including prostaglandins [60], oxytocin [61], estrogen and progesterone.

Marijuana appears to have a suppressive effect on lactation. Acute Δ^9-THC administered to lactating rats transiently suspended milk ejections and decreased ejection frequency compared to vehicle-treated controls [62]. Delta-1-THC administered to mice throughout pregnancy and lactation suppressed mammary gland lipoprotein lipase activity, delayed peak prolactin, and depressed mammary gland growth and development [63]. Marijuana use consistently suppresses prolactin levels in non-pregnant female humans [21], as does Δ^9-THC administration in rhesus monkeys [18,26], but cannabinoid effects on prolactin levels, milk production and milk release in lactating primates are largely unknown. Oxytocin is a peptide hormone that is important for milk release during lactation, stimulating uterine contractions during parturition, and social bonding. In rats, chronic Δ^9-THC exposure downregulates oxytocin in the nucleus accumbens and ventral tegmental area, which are two brain regions that are important for reward processing [61]. I could not identify research addressing the effects of marijuana or Δ^9-THC on circulating oxytocin levels in humans or animal models.

To explore how marijuana exerts its effects on the female reproductive system, the discussion will now focus on the basic biological mechanisms of marijuana and how these mechanisms may interact with female reproduction.

FEMALE REPRODUCTION AND THE ENDOCANNABINOID SYSTEM (ECS)

The Endocannabinoid System

The Endocannabinoid System (ECS) is the biological system that mediates the effects of cannabinoids, including Δ^9-THC, WIN, Rimonabant, AM-251 (Figure 2) and many others. The ECS is composed of the cannabinoid receptors (CB1R and CB2R), which are rhodopsin-like G-protein coupled receptors (GPCR), their endogenous ligands, and the enzymes that synthesize and degrade these endogenous ligands. Cannabinoids alter cannabinoid receptor activity by binding to the receptor and increasing its activity (i.e., acting as an agonist), decreasing its constitutive activity (i.e., acting as an inverse agonist) or blocking other ligands from accessing it (i.e., acting as an antagonist). Delta-9-THC, which is a CB1R and CB2R agonist, is one of hundreds of CBs expressed by the cannabis plant. Endogenous CBs, or endocannabinoids (eCBs), are lipophilic chemical messengers of the ECS that are produced "on demand" instead of being stored and transported in vesicles. The best-characterized eCBs are 2-arachidonylglycerol (2-AG), a full CBR agonist, and anandamide (AEA), a partial CB receptor agonist [64]. Figure 5 illustrates the formation, degradation, and retrograde signaling of these eCBs at the synapse [65-67]. In short, 2-AG is primarily synthesized by diacylglycerol lipase α and β (DAGL- α and DAGL- β) from diacylgylercerol (DAG) and is primarily broken down by monoacylglycerol lipase (MAGL). AEA is primarily synthesized by phospholipase C (PLC) and N-acyl phosphotidylethanolamine phospholipase D (NAPE-PLD) from phosphotidylethanolamine (NAPE) and is primarily degraded by fatty acid amide hydrolase (FAAH) (for more in-depth reviews of the ECS, please see [65-67]).

The ECS is distributed extensively throughout the human body and exerts influence on a multitude of biological processes. CB1R is densely expressed in the brain and modulates many CNS functions, including mood [68], appetite [69], and pain signaling [70]. Peripherally, the ECS is involved in bone remodeling [71], heavily modulates the immune system via CB2R [72], and promotes "thrifty" energy homeostasis by its CB1R-mediated actions in the liver, pancreas, gastrointestinal system, skeletal muscle and adipose tissue [73]. As described in the following sections, the ECS is also intricately involved in the female reproductive system [74], where a delicate balance of endocannabinoid production and degradation and well-regulated CBR activity are required for the reproductive tract and HPO axis to function optimally.

INTERACTIONS BETWEEN THE ECS ANDTHE FEMALE REPRODUCTIVE SYSTEM

ECS Expression in the Female Reproductive Tract

Components of the ECS are located throughout the reproductive tract, including in the ovary [75,76], Fallopian tubes [77], myometrium [78], and endometrium [79].

The ECS likely plays a role in the cycle of endometrial development by promoting endometrial plasticity. Methanandamide, a hydrolysis-resistant form of the eCB AEA, stimulates migration of endometrial stromal cells via ERK1/2 and PI3K/Akt activation downstream of CB1R [80]. Estradiol, but not progesterone, induces endometrial stromal cell migration by similar mechanisms [81]. Several components of the ECS, including CB1R, CB2R, the synthetic enzyme NAPE-PLD and the degradative enzyme FAAH, are highly expressed in the human endometrium, and expression levels of each protein changes throughout the menstrual cycle [79]. For example, CB1R protein expression in endometrial stroma is significantly lower during the mid-follicular phase, while CB2R in endometrial glands and stroma are robustly elevated from the mid-follicular to the late luteal phases [79]. FAAH expression in the endometrial glands is highest during menses and NAPE-PLD is lowest from the late follicular to late luteal phases [79].

The myometrium expresses several components of the ECS, including NAPE-PLD, FAAH, CB1R, but not CB2R [78]. The myometrium is also responsive to AEA stimulation of the CB1R, leading to signaling via Gαi/o-dependent inhibition of adenylate cyclase and activation of PI3K and ERK activation [78].

The ECS is active in the ovary during folliculogenesis, the process that produces mature oocytes and ovarian hormones. In both rat and human ovary, CB1R and CB2R are present in granulosa cells of follicles in several stages of maturity, from primordial tertiary, and in theca cells of secondary and tertiary follicles and corpus luteum and corpus albicans throughout the ovary [75,76]. FAAH and NAPE-PLD are detectable in theca cells and NAPE-PLD in granulosa cells [76] and AEA concentrations in follicular fluid increase as the ovarian follicle matures [76]. The functions of the ECS in the female reproductive tract remain unclear, but, as discussed below, their disruption can negatively impact reproduction and thus warrant further investigation.

ECS Activity in the Menstrual Cycle and HPO Axis

Multiple studies have determined that AEA concentrations in circulating lymphocytes are significantly elevated during the periovulatory phase of the menstrual cycle [82,83]. One study examined the relationships between AEA and HPO axis hormones and found that AEA was significantly correlated with serum LH, FSH, and estradiol, but not progesterone, in pre-menopausal women throughout the menstrual cycle [83]. Another study deter-

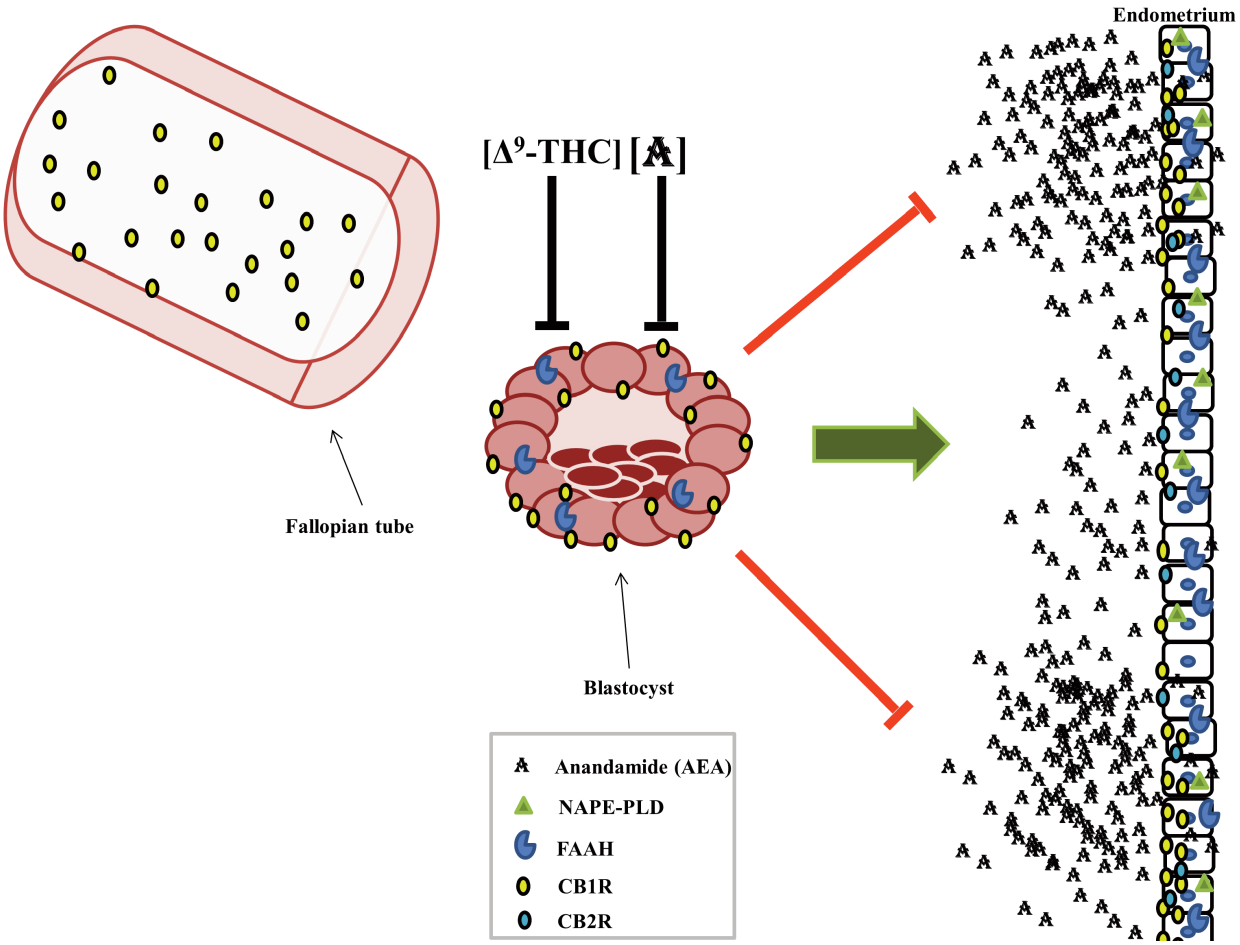

Figure 6. The ECS promotes embryonic migration through the oviducts and uterine implantation. Adequate levels of CB1R expression are required in the Fallopian tube to allow passage of the embryo into the uterus and prevent ectopic implantation. Exposure to high concentrations of Δ^9-THC or anandamide will arrest the development of the blastocyst, but relatively low levels of AEA are required to activate the blastocyst and promote implantation. The endometrium is spatially varied with respect to CB1R and AEA expression; implantation can only occur in areas that express low CB1R and AEA.

mined that expression of FAAH, the enzyme that degrades AEA, is the highest and AEA is the lowest during the postovulatory phase, and that FAAH is expressed at its lowest when AEA is at its highest during the periovulatory phase [82]. These findings suggest that regulation of the ECS and HPO axis are linked. More studies are needed to further uncover the mechanisms underlying this link.

Tissue concentrations of AEA in the anterior pituitary, but not hypothalamus, fluctuated significantly with estrous cycle phase in the intact female rat, with the highest concentrations expressed during estrus and the lowest during proestrous [84]. Estrogen replacement in ovariectomized rats was found to stimulate synthesis of AEA in medial basal hypothalamus neurons [85]. There were no phase-dependent changes in 2-AG tissue concentrations in the hypothalamus and pituitary. CB1R is also expressed in the anterior pituitary [84,86]. One study of intact female rabbits found that CB1R is expressed in the cytoplasm of anterior pituitary neurons where it co-localizes with estradiol-17β receptor type 1 [86]. A rat study was used in determining that gene expression of CB1R in the anterior pituitary was greatest during day 2 of diestrous and was more than double that of diestrous day 1 [84], indicating that robust changes to CB1R expression in the HPO axis can occur very rapidly. CB1R is also expressed in the hypothalamus and downregulated by estrogen [87].

In ovariectomized rats, centrally administered AEA significantly inhibited plasma LH levels relative to vehicle controls [85]. This observation is consistent with Δ^9-THC-induced suppression of gonadotropins in rhesus monkeys. The AEA-induced inhibition of circulating LH was reversed by treating ovariectomized rats with estrogen, such that AEA stimulated plasma LH levels in the presence of estrogen. AEA also stimulated hypothalamic release of GnRH, but only in the presence of estrogen [85]. While this effect may signify that estrogen antagonizes the suppressive effect of AEA on LH release, these results may also be due, in part, to the difference in LH

tone measured in an estrogen-deficient vs. estrogen-replacement state; ovariectomized rats had much higher plasma LH levels than did ovariectomized rats on estrogen replacement [85]. Interestingly, central administration of AEA produced equivalent levels of LH, regardless of basal LH tone; that is, AEA decreased elevated LH levels (in ovariectomized rats) and increased suppressed LH levels (in ovariectomized rats receiving estrogen replacement). This suggests that AEA is able to buffer estrogen-dependent fluctuations in circulating LH levels via mechanisms that are not well-understood, but may be induced by the downregulation of hypothalamic CB1R expression by estrogen [87]. Central administration of the CB1R antagonist/inverse agonist AM-251 in this study likewise yielded unexpected results. In ovarectomized rats, AM-251 reduced LH release even more than did AEA, and this effect appeared to be reversed by estrogen replacement treatment [85]. This paradoxical finding was also observed in intact female rabbits who were administered Rimonabant, another CB1R inverse agonist [86]. The mechanism identified in this study was Rimonabant-induced inhibition of LH secretory capacity by the pituitary, suggesting that the endocannabinoid system can signal at both the hypothalamus and pituitary to control LH release. These multiple sites of action, along with the sensitivity of hypothalamic CB1R expression to estrogen may contribute to the dependence of the LH-releasing actions of AEA and AM-251 on estrogen state.

The ECS in Conception, Implantation and Placentation

The appropriate expression of the ECS during conception and the peri-implantation period is imperative to successful implantation [88]. The CB1R is expressed extensively in the non-pregnant mouse and human uterus [79,89], and the ECS is tightly regulated in the decidua of the mouse and human endometrium [90]. Both cannabinoid receptors are expressed by the embryonic 2-cell stage [91], but activation of the embryonic CB1R by exogenous cannabinoids, such as those from marijuana, at the peri-implantation stage can result in arrested development of the embryo [92]. Studies with CB1R and CB2R knockout mice show that oviduct transport of a fertilized egg from the Fallopian tube to the uterus for implantation is CB1R-dependent, and oviducts that lack maternal CB1R retain zygotes, resulting in failed pregnancies [93]. Reduced CB1R expression in the Fallopian tube and decidua is also associated with ectopic pregnancies in humans [77]. Blastocysts that do not express CB1R or FAAH (i.e., Cnr-/- and Faah-/-) have compromised migration, attachment, and spreading capacities, which can interfere with their ability to successfully implant and invade the uterine wall [93]. Implantation can occur only in endometrial areas that express reduced levels of CB1R and the endocannabinoid AEA; the endometrial surface of the pre-receptive uterus and areas between perspective implantation sites express much more CB1R and AEA than implantation sites [88] (Figure 6). The blastocyst is rendered competent for implantation by low, but not high, concentrations of AEA in the endometrial environment, which activate blastocysts via embryonic CB1R [88]. The development of the placenta also appears to be regulated by the ECS. The metabolic and degradative enzymes of 2-AG, DAGL and MAGL, respectively, are expressed in human cytotrophoblasts [94]. AEA and 2-AG each induce apoptosis in cytotrophoblasts by CBR-mediated mechanisms [94-96]. Altogether, these reports indicate that tightly synchronized regulation of the maternal and embryonic ECS is required for successful oviduct transport, embryonic implantation, and placentation.

Pregnancy Maintenance, Parturition, Lactation and the ECS

Postimplantation, the ECS also plays a dynamic role in maintaining pregnancy, from the early 1st trimester [97] to parturition [98,99]. Deviations in the expression of one or more ECS components are associated with miscarriage. For example, in a large proportion of women with recurrent miscarriage, FAAH is aberrantly localized in the nuclei of trophoblastic cells [100], possibly reducing FAAH activity. Reduced FAAH activity, especially in circulating lymphocytes, can lead to elevated plasma AEA, which is also associated with increased risk of miscarriage [101]. Spontaneous miscarriage during the first trimester is also associated with a reduction or absence of placental FAAH with reduced placental NAPE-PLD expression and elevated placental CB1R [102]. Throughout pregnancy, placental endocannabinoid levels increase [103] and CB1R and FAAH are highly expressed in the placenta at term [104,105], suggesting that the ECS plays a role in pregnancy maintenance and preparation for parturition [105]. Prostaglandins induce cervical ripening and parturition late in pregnancy. The synthetic cannabinoid CP55,940 and endocannabinoids 2-AG and AEA increased prostaglandin PGE2 production in term amniotic and chorionic tissues in a CB1R- and COX-2 dependent manner [99]. Furthermore, plasma AEA levels increase during labor [98], but whether this effect contributes to labor, is a consequence of labor or is a correlate of labor has not yet been determined.

CONCLUSIONS AND OUTLOOK

The female reproductive system and ECS are intricately linked. Hormonal fluctuations throughout the menstrual cycle and pregnancy lead to changes in the expression of the cannabinoid receptors, endocannabinoids, and their associated synthetic and metabolic enzymes in the brain, ovaries, oviducts, uterus and in circulation. Altered ECS expression is associated with reduced fertility, ectopic pregnancy and spontaneous abortion. In addition to altering the ECS, cannabinoids found

in marijuana, particularly Δ^9-THC, exert inhibitory effects on hypothalamic release of GnRH and alter HPO axis regulation, potentially leading to disruption of the reproductive system. There remains much work to be done to understand how the ECS interacts with female reproduction. One intriguing consistency that should be explored further is the paradoxical effect of CB1R inverse agonists exhibiting agonist-like activity, particularly on LH release [85] and sexual motivation [106,107] in female rats. Understanding the basic mechanisms underlying these unexpected findings will provide greater insight into the functioning of the ECS in female reproduction. Of great importance, more human studies are needed to more fully understand the effects of marijuana dosing and chronicity on gonadotropin and ovarian hormone regulation, fertility, pregnancy maintenance, parturition and lactation. The changing legal status of marijuana in several US states simultaneously makes these types of studies more practical in more places and prompts their necessity in order to accurately inform the public on how marijuana affects women's reproductive health.

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