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REVIEW

The Endocannabinoid System and Its Relationship to Human Reproduction

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Summary

The endocannabinoid system is among the most important regulators of human reproduction. It already applies at the level of the sperm and the egg, plays an important role in the fertilization of the egg, its implantation, regulates the function of the placenta and participates in childbirth. The aim of this work is to summarize the knowledge accumulated so far and to show that the endocannabinoid system must be perfectly regulated in order to maintain a physiological pregnancy from implantation to delivery. Only an exceptional interplay of enzymes such as NAPE-PDL or FAAH, endogenous cannabinoids and cannabinoid receptors CB1 and CB2 can ensure the proper functioning of the reproductive organs and thus lead to delivery on time. Changes in the endocannabinoid system can lead to a number of pathological conditions, e.g., during blastocyst implantation, retardation of embryo development, impaired placental function or miscarriage. Soon, we can expect not only an understanding of all the regulatory events associated with the endocannabinoid system and other regulatory systems that participate in reproduction, but also several possibilities for pharmacotherapeutic interventions that can modify the formation, degradation and effect of endocannabinoids. It cannot be ruled out that some components of the endocannabinoid system could become a marker for monitoring pregnancy and childbirth.

Keywords

Endocannabinoid system • Endocannabinoids • Anandamide • Pregnancy • Childbirth

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Introduction

The study of cannabis and its effects, the subsequent discovery of cannabinoid receptors, their endogenous ligands (endocannabinoids), detailed descriptions of their fate in organisms, and understanding their pathophysiological role have all provided robust and convincing evidence that the endocannabinoid system (ECS) is a phylogenetically very old and very important biological signaling mechanism. Involvement of the ECS in mammalian gametogenesis even invokes the idea that discovering further details of this system brings us closer to the very roots of the existence of living matter [1].

This paper provides a summary of basic information regarding involvement of the ECS in the physiology of human reproductive organs. Some components of the endocannabinoid system have shown great potential to be biomarkers for the future detection of pathophysiological changes in the human organism, and their modulation could also be a therapeutic target for treating some pathological conditions or various abnormalities, all of which could be facilitated by newly developed drugs affecting different components

of the ECS [2].

Mammalian reproduction is a complex process that is regulated by mechanisms of the hypothalamic-pituitary-gonadal axis (HPG). The regulation of both endocrine and cellular communication is critical for gamete formation and successful pregnancy. A number of factors are involved in the interplay of these processes. In the last two decades, key components of the ECS, i.e. endocannabinoids (eCBs), including the enzymatic apparatus necessary for their synthesis and degradation, and the high expression of cannabinoid receptors on the surface of gametes and in reproductive tissues have emerged as key modulators of male and female reproduction (Additional studies have shown that the ECS plays an essential role in human reproduction, from gametogenesis, fertilization, embryo implantation, through the course of pregnancy with prenatal fetal development to delivery and postnatal life of the newborn [1,2].

The complexity of interactions that are required in the body to ensure the maintenance of pregnancy led researchers at the University of Leicester 12 years ago to propose the term "endocannabinoid-hormone-cytokine network", pointing to the ECS as a key factor in the maternal-fetal compartment. Any significant deviation from balance in this network can result in a disruption of the course of pregnancy [2].

A brief history of the ECS

While the medicinal and psychotropic effects of cannabis (*Cannabis sativa* L.) have been known to mankind for millennia, the elucidation of these effects has occurred only recently. Palaeobotanical studies describe the geographical origin of cannabis as being in Central Asia, around the Altai Mountains. Hemp seeds were spread by the migration of nomadic peoples throughout the then-known world, including Egypt, Greece and Rome. In the 19th century, doctors who came into contact with Muslim and Indian cultures began to use cannabis medicinally in Europe. The second half of the 19th and the beginning of the 20th century is considered the first golden age of medicinal cannabis [3].

Over time, more than 100 cannabinoids (CBs) have been isolated from cannabis [4]. The most well-known CBs include (-)-trans-Δ-9-tetrahydrocannabinol, with the biologically active stereoisomer configuration 6aR,10aR (THC), and cannabidiol, with the stereoisomer configurations 1R,6R (CBD). Both THC and CBD were first isolated from hashish in the 1940s, but their chemical structure was not discovered until more than 20 years later [5]. Due to its psychotropic action, more attention has been focused on THC. Its chemical structure was also described by the Israeli chemist Mechoulam and co-workers in 1964 [6]. They were also the first to chemically synthesize first the racemate (±)-CBD and (±)-THC and subsequently the individual (+) and (-) enantiomers of CBD and THC [7].

Research on the pharmacology of cannabinoids expanded significantly in the mid 1960s and early 1970s, mainly due to the widespread use of cannabis as a recreational drug in Western countries. In contrast, there was less interest in the therapeutic potential of cannabinoids, despite the fact that cannabis tincture was still a registered medicine in the UK at the time [8]. Due to the marked lipophilicity of THC, the substance was thought to have psychotropic effects mediated by non-specific mechanisms. In the second half of the 1980s, two important discoveries were made, the application of which led to the discovery of cannabinoid receptors. The first was the Nobel Prize-winning understanding of cell signaling through G-protein coupled receptors. The second was the use of a technique to identify the localization of target structures using tritium-labeled synthetic cannabinoids. In 1988, the first cannabinoid receptor (CBR) was described in rat brain tissue [9] and later in humans [10].

In 1990, the cannabinoid receptor was cloned for the first time [11]. Today, this receptor is referred to as cannabinoid receptor type 1 (CBR1). Shortly after this discovery, another "peripheral" cannabinoid receptor was identified, which is now known as cannabinoid receptor type 2 (CBR2) [12]. However, even though the cannabinoid receptors were recognized, their endogenous ligands were still unknown. This was reminiscent of the history of the discovery of opioid receptors [13,14], where the discovery of endogenous opioids was made after the discovery of their receptors [15].

The same author who first described the cannabinoid receptor [16] isolated a lipophilic substance derived from arachidonic acid four years later, characterized by strong binding to cannabinoid receptors. This was arachidonoylethanolamide (AEA). He named it anandamide, referring to the Sanskrit word ānanda, meaning bliss, happiness or pleasure, which describes the experience of inhaling cannabis vapors, and at the same time introduced the term 'endocannabinoid'. The Czech chemist Lumír Ondřej Hanuš, who has spent his entire professional life dedicated to natural substances, especially cannabinoids and more importantly

endocannabinoids, collaborated on research into endocannabinoids [17,18].

The discovery of endocannabinoids significantly accelerated research activities in the field of the endocannabinoid system, particularly on pharmacodynamic aspects, the fate of endocannabinoids in the body, polymorphisms related to cannabinoid receptors, and enzymes involved in endocannabinoid degradation, the regulation of expression and links to other signaling pathways. Other research focused on the endocannabinoid system as a target for new potential drugs [19].

The endocannabinoid system

The endocannabinoid system (ECS) consists of the cannabinoid receptors CBR1 and CBR2, endogenous ligands (endocannabinoids), the most important of which are N-acetylethanolamides, represented mainly by anandamide (AEA), and monoacylglycerols, represented mainly by 2-arachidonoylglycerol (2-AG), as well as their synthesizing and degrading enzymes and transport systems ensuring their reuptake. As information on the ECS continues to expand, the term endocannabinoidome (eCBome) is beginning to gain prominence. The eCBome describes the environmental influences whose components interfere with the ECS. It encompasses all known components of the ECS, including proteins, enzymes and lipids, that are directly or indirectly involved in modulation of the ECS. It also includes issues surrounding the microbiome [20], dietary measures [21], and the whole plethora of digestive and metabolic diseases, as well as CNS diseases or malignancies. It can also not be excluded that proper modulation of the ECS can influence a healthy lifespan [22]. Interactions may not be only unidirectional, but rather a complex mechanism based on interplay between the ECS, the eCBome and the relevant pathophysiological state. The ECS is responsible for the energy homeostasis of the body and regulates food intake, metabolism and energy expenditure, thus contributing to the maintenance of a stable body weight. Moreover, proper function of the ECS can significantly contribute to preventing the development of metabolic syndrome and complex metabolic diseases such as diabetes mellitus, obesity and arterial hypertension [23].

The fate of individual endocannabinoids in the body is crucial for understanding the regulation of ESCs and their mechanisms of action (Fig. 1). The metabolism

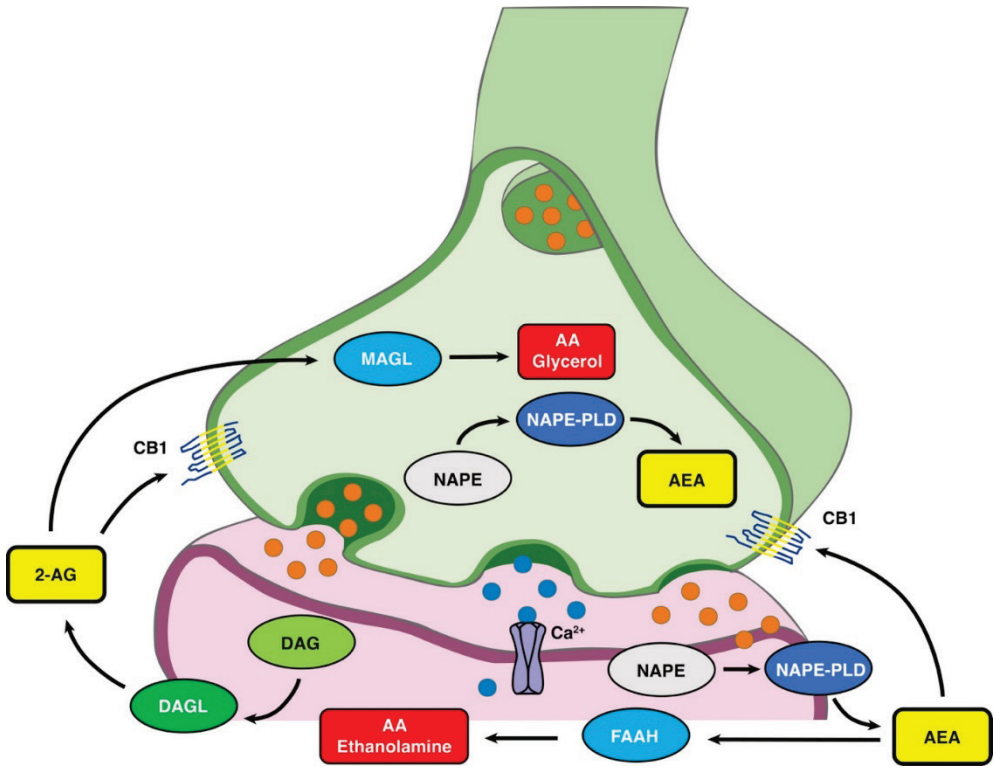

Fig. 1. A scheme of eCB synthesis and degradation (adapted and modified by Xiao *et al.* [25]).
Abbreviations: 2-AG, 2-arachidonoylglycerol; AEA, N-arachidonoylethanolamine; CB1, type 1 cannabinoid receptor; DAG, diacylglycerol; DAGL, diacylglycerol-lipase; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol-lipase; NAPE-PLD, N-acylphosphatydil ethanolamine-specific phospholipase D; NAPE, N-acylphosphatidyl ethanolamine; NPDL, N-arachidonoylphosphatidyl ethanolamine-specific phospholipase D.

of the two main groups of endocannabinoids – N-acetylethanolamides and monoacylglycerols – is fundamentally different. While AEA is degraded by the fatty acid amidohydrolase (FAAH) pathway, 2-AG is degraded in a rather complicated manner involving at least 8 enzymes localized both presynaptically and postsynaptically, with monoacylglycerol lipase (MAGL) being the main pathway for 2-AG degradation, but FAAH and α-β-hydrolase serine hydrolases (ABHD6 and ABHD12) also contributing. The synthesis of endocannabinoids is also different, except that it is calcium dependent for both eCBs. In the case of AEA, the synthesis involves two steps. The first consists of the transfer of arachidonic acid (AA) to the amino group of membrane phosphatidylethanolamine by the enzyme N-acyltransferase to form N-arachidonoylphosphatidyl-ethanolamine (NAPE). In the second step, by way of a specific NAPE-phospholipase D (NAPE-PLD) this "precursor" allows the formation of anandamide by phosphate cleavage [24]. Synthesis of 2-AG begins with the activation of phospholipase Cβ, which cleaves the membrane phosphatidylinositol bisphosphate (PIP2) containing arachidonic acid. PIP2 is further cleaved to diacylglycerol (DAG) and inositol triphosphate. From diacylglycerol of arachidonic acid, 2-AG is finally formed via one of the two diacylglycerol lipases (DAGL), DAGLα and DAGLβ. The interplay of all of these enzymes is a direct demonstration of the exquisite fine-tuning of the ECS [25].

ECS and the hypothalamo-pituitary system

The hypothalamo-pituitary system (HPS) is part of the mammalian neuroendocrine system, which links neural signals to endocrine signals in the central nervous system and controls other endocrine glands. It consists of the hypothalamus and pituitary gland and influences both male and female gonads, hence the hypothalamic-pituitary-gonadal axis (HPG). The first step in regulation of the HPG is the peptide gonadotropin releasing hormone (GnRH), which is produced by neurons in the preoptic region of the hypothalamus. GnRH secretion is pulsatile in nature and influences the release of two pituitary hormones via receptors in the adenohypophysis: low-frequency pulses of GnRH induce the release of follicle-stimulating hormone (FSH), while high-frequency pulses induce the release of luteinizing hormone (LH). In males, the frequency of GnRH release is constant, while in females the frequency increases significantly at the time of ovulation, leading to an increase in LH. FSH and LH regulate follicle growth, ovulation and maintenance of the corpus luteum in females and spermatogenesis in males. They are also involved in controlling the synthesis and release of sex steroid hormones. FSH and LH are thus key hormones in human reproduction.

The expression of individual components of the ECS has also been described in hypothalamic regions [10]. Here the ECS is involved in the control of female and male endocrine processes and is responsible for the regulation of central and peripheral gonadal tissues and their functions. The presence of the ECS has been demonstrated in many cell types that are subject to the influence of endogenous and exogenous cannabinoids and that are related to the regulation of female reproductive processes [1].

In women, chronic exposure to cannabinoids causes menstrual cycle disturbances, suppresses ovarian follicle maturation, and decreases serum concentrations of LH and other sex hormones [26]. Decreases in circulating GnRH, anovulatory cycles, and prolongation of the follicular phase, thereby delaying ovulation, have also been described. Most papers suggest that these effects are due to hypothalamic dysfunction [27]. However, the exact mechanisms and role of the ESC are still unclear whether the disorder is at the level of the pituitary and/or ovary. In an experimental model, CR1 density in the hypothalamus has been found to differ between male and female rodents [28]. This likely reflects important sex-related endocrine differences as well as differences in the effects of eCBs in male and female animals and humans. Experimental work has demonstrated an inhibitory effect of the ECS on GnRH synthesis and release and on some adenohypophysis hormones secretion [29], as well as on several neuronal systems that are actively involved in GnRH regulation, ultimately affecting gonadal function and steroidogenesis [30].

The activity of enzymes involved in the synthesis of eCBs and the density of CB1 in the brain fluctuate during a woman's menstrual cycle [31], in association with sex steroid metabolism. Estrogens especially estradiole (E2) modulate the ECS, with positive effects on fertility and other reproductive activities. In addition, E2 may also influence progesterone, which is closely associated with pregnancy, and their interaction with eCBs appears to be crucial for pregnancy [32]. These results have thus raised the hypothesis that ECS regulation could help to modify some fertility-related problems and even treat

reproductive disorders that are related to the ECS.

Endocannabinoid-mediated activation of cannabinoid receptors on GnRH neurons demonstrates that the ECS acts on gonadotropic hormone secretion directly. Animal studies have shown high levels of CR1 in the pituitary of males compared to females, whereas the pituitary content of anandamide (AEA) was also high in females. In terms of the ESC, there are sex-related differences in HPG [31]. GnRH-releasing hypothalamic neurons show an apparent sensitivity to the endocannabinoid signaling effects of AEA and 2-AG along with FAAH [26]. Endocannabinoids, especially AEA, inhibit GnRH release in the hypothalamus, thereby affecting reduced LH and FSH production via the pituitary gland, and thus reducing testosterone production. Reduced testosterone levels then feedback to reduce CR1 receptor expression, thereby inhibiting endocannabinoid signaling in both the hypothalamus and pituitary [1]. Endocannabinoids as well as cannabinoids also affect the functioning of GnRH neurons indirectly. They allow modulation of the activity of GABAergic nerve fibers. GABAergic nerve fibers express CB1 receptors, and activation of these receptors inhibits the release of γ-aminobutyric acid (GABA), which further influences the impaired activation of GABA receptors on GnRH neurons and ultimately inhibits GnRH release [33]. Furthermore, the ECS is present throughout the hypothalamic-pituitary-gonadal axis (HPG) and any disturbance in this tightly regulated endocrine system affects mammalian reproduction and reproductive performance [1]. Collectively, this information suggests that there could be evolutionarily conserved interactions between the ECS, hypothalamus and adenohypophysis.

Sperm

Long-term exposure to cannabinoids in males leads to reduced sperm count and motility [26], as well as serum testosterone [34] and luteinizing hormone (LH) levels [26]. The recognition that cannabinoids induce changes in reproductive processes has led to intensive study of the effect of endocannabinoids on the HPG axis, with effects seen in both sexes and in different species.

Spermatogenesis is a continuous process of the production of a large number of spermatozoa, which in humans occurs throughout the reproductive life of the individual. A highly coordinated process is essential for the continuous production and maintenance of sperm, in which the ECS is significantly involved [35], and the male reproductive tract possesses a complete enzymatic apparatus for the synthesis and metabolism of endocannabinoids [36].

In human semen, the actions of AEA and 2-AG on their target receptors CB1, CB2 and transient receptor potential vanilloid-1 (TRPV1) play an important role. The major metabolic enzymes of eCBs (NAPE-PLD and FAAH for AEA; DAGL and MAGL for 2-AG) also influence sperm control and function, and this has a distinct impact on male reproductive potential. Compared to fertile men, a significant decrease in the content of AEA and 2-AG has been described in the semen of infertile men, together with an increased degradation : biosynthesis ratio of both substances. In addition, in one study binding to TRPV1 was detected in fertile sperm, but was undetectable in infertile sperm, while binding to CB1 and CB2 receptors was not statistically different in the two groups [36]. The ESC affects the sperm capacitation and acrosome response, and thus fertilization outcomes [37].

It has been shown that seminal plasma contains significant amounts of anandamide, the amount of which gradually decreases in the uterus, fallopian tube and follicular fluid [38]. As sperm leave the seminal plasma and approach the egg in the female reproductive tract, they are exposed to progressively decreasing concentrations of AEA. It is speculated that the high concentrations of anandamide observed in seminal plasma may contribute to maintaining spermatozoa in a resting metabolic state to make them fully activatable in the female reproductive tract [39].

In this regard, decreasing concentrations of AEA in secretions of the female reproductive tract could reduce its inhibitory effect on spermatozoa, thereby making them suitable for gaining capacitation and fertilization capacity [1]. ECS components are expressed differently in different regions of spermatozoa. The CB1 receptor is present throughout the sperm head membrane, midpiece and tail region, while CB2 is localized in the sperm head membrane. TRPV1 is localized in the midpiece and tail region. The enzymes NAPE-PLD and FAAH are present in the head and midpiece membranes. CB1 activation is associated with immobile spermatozoa, while CB2 activation is associated with the production of progressively slow spermatozoa [1].

Spermatozoa reach fertilization capacity only after a complex series of physicochemical modifications have been completed, called capacitation. The interaction of eCBs with CB1 and TRPV1 plays a key role in this process. In particular, CB1, via Gi protein/cAMP/PKA, maintains low levels of cAMP in the early stages of the

post-ejaculatory life of male gametes [39]. In this way, it promotes membrane stability, preventing premature fusion of the plasma membrane (PM) and the outer acrosomal membrane (OAM), which is essential for exocytosis of the acrosome contents. TRPV1, in contrast, becomes active only during the final stages of capacitation, allowing a rapid increase in intracellular calcium concentrations, allowing the removal of the F-actin network between the PM and OAM so they can be fused and ultimately leading to the acrosome reaction [40].

The ESC and female reproductive organs

The presence of ECS elements in the fluids, cells and tissues of a woman's reproductive organs, such as the follicular fluid, ovary, uterus and placenta, has been documented by several studies on animal models and the human ECS, and as such clearly has a major impact on all stages of female reproduction [41].

Oocytes

In humans, the presence of ECS components was first detected in sections of adult human ovaries by El-Talatini and co-workers [42].

Both CB1 and CB2 have been described in primordial, primary and secondary follicles, whereas only CB2 was detectable after follicular cavity formation. Regarding metabolic enzymes, NAPE-PLD was sporadically detectable only in primordial oocytes, whereas FAAH was never found. Although CB1 and CB2 were immunolocalized in granulosa cells throughout folliculogenesis, FAAH and NAPE-PLD were found in secondary follicles but declined sharply in antral follicles [42]. In contrast, theca cells expressed CB1, CB2, NAPE-PLD and FAAH in all follicles analyzed, except CL, which lacked FAAH. This led to the conclusion that AEA may act in an autocrine manner in theca cells, whereas in granulosa cells, degradation of this eCB may occur independently of FAAH [2,41,42] (Fig. 2).

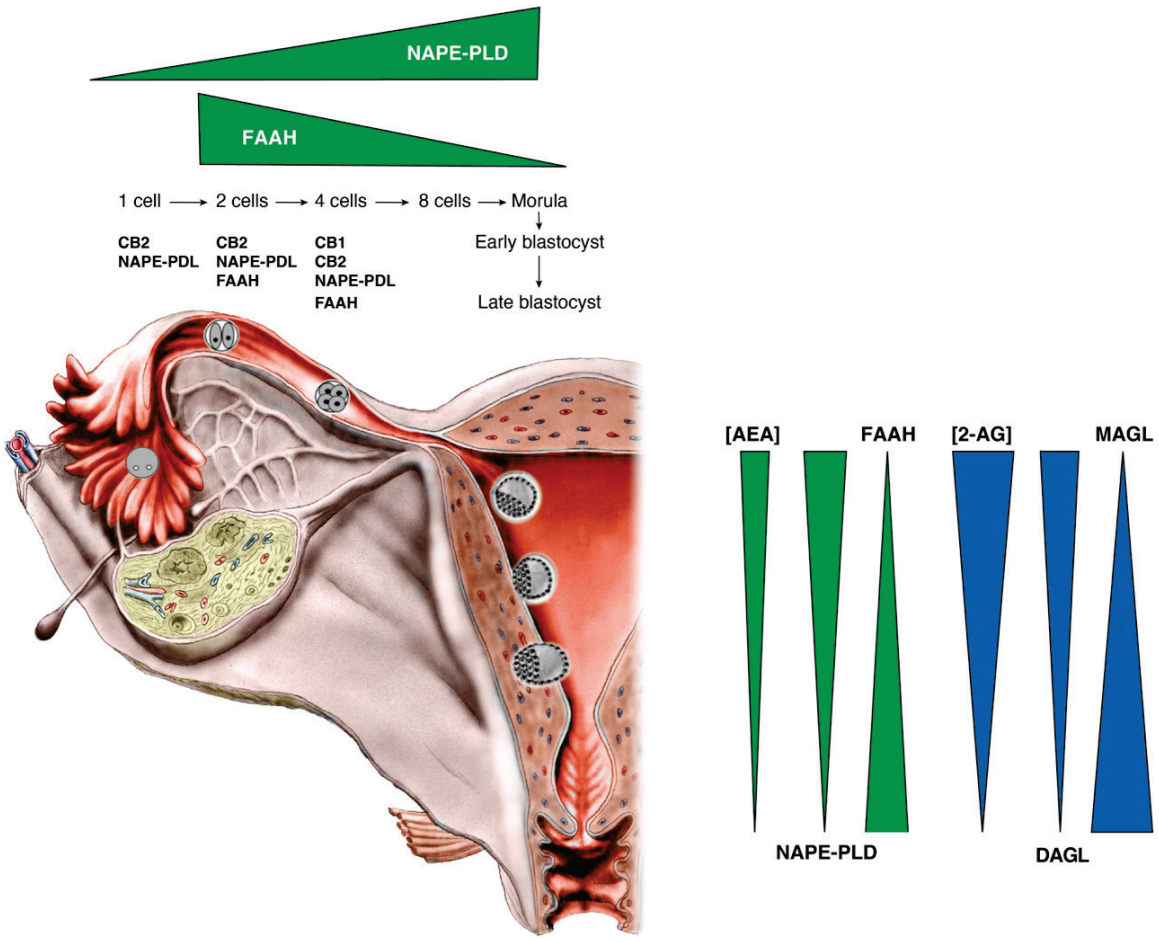

Fig. 2. Oogenesis and the ECS (adapted and modified by Karasu *et al.* [2] and Cecconi *et al.* [41]).
Abbreviations: 2-AG, 2-arachidonoylglycerol; AEA, N-arachidonoylethanolamine; CB1, type 1 cannabinoid receptor; CB2, type 2 cannabinoid receptor; DAGL, sn-1 diacylglycerol-lipase; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAPE-PLD, N-acylphosphatydil ethanolamine-specific phospholipase D.

Preimplantation embryonic development

In the case of natural reproduction, the fusion of the sperm and egg occurs in the fallopian tube. and the fertilized egg is subsequently called an embryo. This then begins to go through several rounds of successive mitotic cell divisions from the two-cell to the sixteen-cell stage, then a ball of compacted cells called a morula is formed. The morula migrates towards the uterine cavity. As the morula enters the uterus, a small cavity (blastocoel) begins to appear within the morula, and this is the beginning of the blastocyst stage. The blastocyst consists of an outer layer of trophectoderm (TE) cells and an inner cell mass (ICM).

The fallopian tube and associated tissues play a key role in sperm capacitation, fertilization and transport of the embryo into the uterine cavity to the site of implantation. In the isthmus, at the junction of the fallopian tube with the uterus, sperm cells not yet capacitated first bind to the cells of the epithelium. Capacitation of the spermatozoa occurs, and they are then released and can move to the distal part of the fallopian tube, the ampulla. After ovulation, the oocyte is moved from the ovary to the ampulla by the fimbriae of the fallopian tube, and fertilization occurs in the ampullary part of the tube [43]. In the fallopian tube, the production of not only AEA but also other ECS components such as NAPE-PLD, CB1, CB2 and FAAH have been demonstrated [44].

There is longitudinal gradient of AEA, which is required to prolong sperm fertility as they progress through the fallopian tube to the oocyte. The maximum AEA concentration is in the isthmus, where it affects sperm capitation. Sperm are then released from the fallopian tube epithelial cells by the stimulation of Ca^{2+} influx via CB1 and TRPV1, but not CB2 [40]. After capitation, sperm are able to secrete AEA and 2-AG, which activate CB1 and TRPV1 [45]. In humans, AEA binds to TRPV1 of spermatozoa, promoting their fusion with the egg by inhibiting premature acrosome reactions and membrane fusion in non-fertilizing spermatozoa [46,47]. FAAH is strongly expressed in the ampullary region and to a lesser extent in the isthmus of the fallopian tube [48]. In the case of NAPE-PLD, the opposite is the case. FAAH activity and AEA levels could serve to monitor early pregnancies, with high levels of AEA in the isthmic part of the fallopian tube being necessary for implantation [2].

The mouse embryo expresses CB1 and CB2 from the zygote to the blastocyst stage. In the blastocyst, CB2 is found in the inner cell mass (ICM) and CB1 in the trophectoderm cells [49]. Both FAAH and NAPE-PLD are present from the two-cell embryo stage to the blastocyst. FAAH was first identified in outer morula cells and then in trophectoderm cells [50]. Recently, the involvement of CB1 in the regulation of embryonic transport rates has been proposed [51], as it had previously been described that embryo development and trophoblast differentiation are impaired by high levels of AEA and 2-AG [43].

Implantation

Successful implantation and subsequent pregnancy are dependent on optimal communication between the decidua, trophoblast and maternal immune cells, specifically NK cells (natural killer cells, a subpopulation of lymphocytes). Their main role is to create an ideal environment at the decidua-trophoblast boundary. NK cells play an indispensable role in trophoblast invasion, spiral artery remodeling and modulation of the local inflammatory response. They are a major producer of many growth factors (PLGF - placental growth factor and others), proteases, cytokines including tumor necrosis factor a (TNF-α), interferon γ (IFN-γ), interleukin 10 (IL-10) and many other effectors, including the ability to produce endocannabinoids [52].

For successful implantation, AEA levels in the uterine microenvironment must be low and this requires an increase in FAAH and a decrease in NAPE-PLD. Otherwise, high AEA levels can lead to miscarriage [53]. AEA acts on NK cells both directly and indirectly *via* trophoblast cells [54]. In the case of direct action, there is an association between higher levels of AEA and higher levels of proangiogenic factors involved in spiral artery remodeling. However, the complex effects of eCBs on NK cells, especially on the modulation of the inflammatory response, deserve great attention. AEA has been shown to play a role in the modulation of IFN-γ, TNF-α and IL-10 secretion by NK cells, the balance of which is essential for successful embryo implantation and the maintenance of pregnancy [55].

Administration of AEA *in vitro* arrests embryonic development at the morula stage, whereas 2-AG administration arrests development already at the two-cell stage [56]. The mechanism of action is mediated primarily through the CB1 receptor. When a CB1 receptor antagonist is administered, the effects of

both AEA and 2-AG are reversible. AEA itself also blocks blastocyst development, and high concentrations of AEA inhibit trophoblast cell differentiation. AEA binding to the blastocyst decreases at the time of implantation into the decidua. The decrease in binding may be explained by decreased expression of the CB1 receptor at the time of implantation; however, the exact mechanism controlling the expression has not yet been satisfactorily elucidated. Many-fold lower concentrations of both AEA and 2-AG have been demonstrated for the uterine mucosal epithelium in the nidation region compared to the surrounding uterine mucosa. In addition, the low concentration of AEA accelerates the attachment of the blastocyst to the uterine mucosa [53,57] (Fig. 3).

AEA may further modulate the expression of COX-2 [58], which is essential for the decidualization process and thus for the initiation and progression of pregnancy.

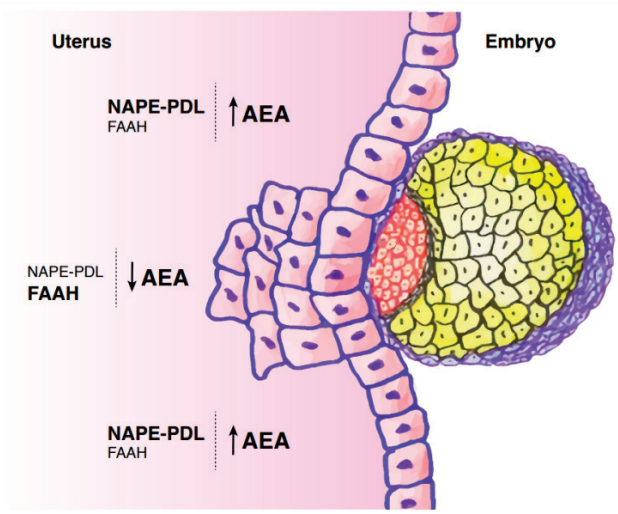

Fig. 3. Schematic representation of endocannabinoid signaling during implantation based on rodent and human studies (adapted and modified by Correa *et al.* [57] from Fonseca *et al.* [53]).
Abbreviations: AEA, N-arachidonoylethanolamine; FAAH, fatty acid amide hydrolase; NAPE-PLD, N-acylphosphatydil ethanolamine-specific phospholipase D.

The fetus

Dynamic expression of the ECS also occurs in the fetus during pregnancy, particularly during brain development [59]. In the human fetal brain, CB1 has been detected as early as 14 weeks of gestation, with preferential expression in the cerebral cortex, hippocampus, caudate, putamen and cerebellar cortex [60], which corresponds to the distribution of CB1 in adulthood. At week 20, intense expression was found to be particularly evident in the hippocampus and basal nuclei of the amygdala [61].

The two major ligands of the ECS, AEA and 2-AG, show different ontogenic bioavailability and different developmental patterns. While increasing levels of 2-AG during embryonic development correlate with cell differentiation and axon elongation in the brain, AEA has been shown to be essential in the early stages of pregnancy for embryonic implantation in utero. Moreover, 2-AG levels peak on the first day after birth and then remain constant until puberty, when they begin to fluctuate. High levels appear during early and late adolescence, returning to their original levels in adulthood. In contrast, in most of the brain regions studied, AEA concentrations gradually increase from gestational day 21 and reach a maximum during adolescence.

Notably, 2-AG concentrations (2-8 nmol/g tissue) are approximately 1000 times higher than AEA concentrations (3-6 pmol/g tissue) during brain development [25].

The ESC and the placenta

The human placenta has several essential functions during pregnancy and fetal development, including nutrition, gas exchange, protein biosynthesis, support and protection. This transient but highly specialized pregnancy organ is made up of many different cell types. The most characteristic placental cells are the trophoblasts, the epithelial cells of the placenta that are divided into four subtypes with different morphology and function: cytotrophoblasts (CTs), syncytiotrophoblasts (STs), extravillous trophoblasts (EVTs) and giant trophoblast cells.

Cytotrophoblasts are cells capable of proliferation, differentiation into other trophoblast types and death by apoptosis. They can differentiate by both villous and extravillous pathways. In the villous pathway, CTs undergo syncytialization, where they fuse and biochemically differentiate to form the syncytiotrophoblast (ST). These ST cells are responsible for endocrine function and form a physical barrier between the maternal and fetal body through the blood, where nutrients and gases are exchanged. The ST forms a multinucleated layer that is already devoid of proliferative capacity, and is in contact with the maternal blood, which is essential for maternal-fetal interaction. In

the extravillous space, the ST develops an invasive capacity and an extravillous trophoblasts are formed. EVTs invade and remodel uterine tissues, vessels and glands to form interstitial, endovascular and endoglandular trophoblasts. The former aggregate and fuse to form giant trophoblastic cells. The proliferation, differentiation, invasive properties, apoptosis, and function of the trophoblast are highly regulated by several cytokines, growth factors, lipids, and other molecules that collectively contribute to the normal formation and development of the placenta.

The first evidence of the expression of ECS components in the human placenta dates back to 1999, when Kenney et al. reported the presence of CB1 and CB2 mRNA in placental tissues at the end of the third trimester [62]. Later, immunohistochemical studies confirmed the presence of CB1 in all human placental tissues at the end of pregnancy. CB1 expression is stronger in amnion cells than in decidua cells [63]. Moreover, CB1 expression has been shown to be higher in placentas at term of women who had undergone caesarean section than in placentas of women after spontaneous delivery [64]. This finding suggests that CB1-mediated signaling may be involved in maintaining pregnancy stability when the uterine smooth muscle is at rest, without contractile activity.

CB1 and CB2 are also expressed in first trimester placentas [65]. Initially, it was thought that CB1 was not present early in pregnancy and that CB2 expression was mainly linked to placental macrophages. However, a later study [66] showed that CB1 transcripts are already upregulated during early pregnancy (weeks 7-12), with a peak at week 10 of gestation and the lowest levels recorded at week 12. In addition, CB1 immunolabeling showed that this receptor is present in all trophoblast and endothelial cell types, although its expression in the ST decreased by week 10. CB2 transcript levels were expressed throughout early pregnancy and, similar to CB1, all trophoblast types and placental vascular endothelial cells showed immunoreactivity for this receptor, indicating constant expression during this period [66].

The human placenta also expresses the eCB metabolic enzymes. FAAH has been shown to be present already during early pregnancy. All trophoblast phenotypes expressed FAAH, but the signal in the ST peaked at 10 weeks of gestation and decreased significantly after 11 weeks, when the signal was barely detectable. FAAH was also expressed from EVT cells that had migrated from the villi but had not yet reached the decidua, while it was absent in EVT cells that had already come into contact with this maternal tissue [67]. FAAH is also immunoreactively detected in the placenta at the end of pregnancy. Here, FAAH expression was higher in decidua cells and the amniotic epithelium, whereas lower expression was identified in vascular tissue and trophoblasts [63]. Detection was more evident in the ST than in the cytotrophoblast (CT). On the other hand, other authors have reported that FAAH was not detected in placentas that were collected from women without uterine contractions in placentas obtained at caesarean section [64]. One possible explanation is that this very low expression of FAAH at term delivery may be related to the increased plasma levels of AEA, decreased levels of arachidonic acid, and decreased prostaglandin production demonstrated in spontaneously laboring women with myometrial contraction [68]. Transcripts of the major AEA biosynthetic enzyme, NAPE-PLD, are also present in first trimester placental tissues [69]. Although AEA has been a major focus of ECS research, the human placenta also expresses enzymes of 2-AG metabolism. DAGL-α and MAGL have been identified in the human placenta, both in primary cultures of the CT and ST from term placentas, and in BeWo cells [70,71].

In immunohistochemical studies, COX-2 has been shown to be further expressed in human placental tissue at the end of pregnancy, particularly in the ST layer, EVTs, villous stroma and capillary endothelium [72]. In addition, however, COX-2 was also expressed in first trimester placentas, particularly in the ST, villous stroma and EVTs [73].

In addition to CB1/CB2, the human placenta expresses other types of eCB-triggered receptors. Indeed, the nuclear receptor PPAR- γ was shown to be expressed in the placenta as early as the first trimester, in the CT and EVTs [74], whereas expression in the ST and endothelial cells was negligible. In the placenta at the end of pregnancy, PPAR-γ was predominantly expressed in the ST, although the CT and EVTs were also immunopositive for this receptor. These data suggest that PPAR-γ expression in human trophoblasts is modulated during pregnancy, which may be related to the role of this receptor in trophoblast differentiation and invasion [75].

TRPV1 and a putative endocannabinoid receptor (GPR55) have also been described in the placenta, when all indications are that TRPV1 expression does not change with trophoblast differentiation [76]. Tissues from

term placentas had higher levels of GPR55 transcripts compared to first trimester placentas [77]. Furthermore, immunological studies revealed that GPR55 expression is restricted to the fetal endothelium in the CT of placentas from both stages of pregnancy. Indeed, no immunoreactivity was detected in the ST or in the villous stroma.

The results of these studies support a role for eCBs, particularly in trophoblast cells, reinforcing the importance of cannabinoid signaling in fundamental cellular processes of the human placenta [78]. There is thus increasing evidence that the ECS and associated mechanisms help to regulate the complex and as yet unsatisfactorily explained functions of the human placenta.

The uterus and the course of pregnancy

The uterus expresses all elements of the ECS, and its microenvironment is critical to the outcome of reproductive physiological events of pregnancy and subsequent birth. Steroidogenesis is also essential for these events. The steroid metabolome demonstrates a direct regulatory role in modulation of the ECS in female reproductive organs [78], including stabilization of the smooth muscle of the uterus, the myometrium.

In addition to placental development and function, the myometrium plays a critical role in the course of pregnancy. The resting stage of the myometrium during pregnancy physiologically lasts in humans for about 267 days after the last ovulation. After about this time, the contraction of the myometrium, a key event for spontaneous birth, begins, though the precise mechanism of the onset of labor has not yet been satisfactorily explained [79, 80].

Anandamide suppresses oxytocin-induced contractions in the human myometrium, and this inhibition is concentration dependent and mediated by the CB1 receptor in the placenta. In experimental rodent models, administration of CB1 receptor antagonists early in the third trimester resulted in a higher incidence of preterm birth. Furthermore, in an experimental rodent model, the absence of the CB1 receptor results in changes in serum concentrations of progesterone, estrogen, corticosterone, and corticotropin-releasing hormone, all hormones involved in the course of pregnancy [81].

There is a significant increase in human plasma AEA concentrations from the beginning of the third trimester to term delivery. However, after the onset of uterine contractions, the role of AEA is unclear. Habayeb et al observed a moderate but positive correlation between plasma AEA concentrations, the duration of contractions and cervical dilation at the time of sampling at delivery [66].

Although AEA prevents the onset of myometrial contractions, its plasma concentration have been shown to be elevated during labor. These results are supported by another study which reported a significant, up to threefold increase in plasma AEA concentrations in women giving birth spontaneously compared to women not giving birth at term. AEA concentrations were also significantly increased after artificially induced uterine contractions compared to the pre-induction state [82].

CB1 receptor expression is significantly reduced in placental villi after spontaneous delivery compared to placental villi obtained from pregnancies terminated by elective caesarean section at term. However, this does not suggest that the amount of AEA during labor activates the placental CB1 receptor. Overall, AEA has an inhibitory effect on myometrial contractions and prolongs pregnancy, but does not appear to have an effect on the onset of myometrial contractions leading to human birth [83].

Birth

The described effects of AEA at the time of the onset of labor can be explained by its dual action. AEA is transformed into arachidonic acid and subsequently into prostaglandins (PGs), which directly induce myometrial contractions when FAAH is sufficiently active. However, with reduced FAAH activity, AEA is not sufficiently converted to arachidonic acid and is converted to prostamides (PMs) by an alternative metabolic pathway. The poor myometrial contractility induced by PMs may be due to their low affinity for prostaglandin receptors. If AEA is hydrolyzed to PMs, their elevated concentrations inhibit myometrial contractility [84]. Therefore, the paradox of AEA activity can be explained by the fact that it will depend on the metabolic pathway and the concomitant activity of FAAH. If FAAH activity is high, increased AEA would lead to increased PG production. Alternatively, if FAAH activity is low, less AEA would be converted to arachidonic acid, which limits the rate of PG production but increases the rate of PM production.

The question thus arises as to whether increased plasma AEA concentrations may predict the onset of labor. Endocannabinoids stimulate the CB1 receptor and

induce the production of PGs, which causes uterine contractions and thus labor [85]. As mentioned above, however, when FAAH activity is low or absent, AEA is not readily converted to arachidonic acid and PGs but is instead metabolized to PM. A certain amount of AEA can be converted to PGs by binding to the CB1 receptor despite the lack of or inefficient FAAH activity. Both scenarios result in markedly opposite actions. To what extent the conversion of AEA via the CB1 receptor pathway is utilized is not yet fully known. Establishing a normal range of the PG to PM ratio for a specific week of pregnancy and accurately measuring the ratio in clinical samples could be a useful tool in assessing the risk of preterm birth. The effects of 2-AG have not yet been sufficiently investigated in this respect. For AEA, measurements of serum concentrations in relation to miscarriage or preterm birth are already gaining experimental interest, and studies are emerging that show associations between serum AEA levels and the risk of preterm birth [2,41].

Our team focus long times on research in the perinatal medicines [79,80, 86-89], and now ECS and preterm birth, it is in this direction that our research activity will be heading in the near future, and elsewhere we present a proposed study protocol to determine whether it will be possible in the future to use information on specific parameters of a woman's ECS to predict preterm birth. We are fully aware that unraveling the very complex ECS relationships has been and will continue to be dependent on discoveries in other disciplines and the ever-improving capabilities of analytical methods.

A final note

The late Professor Luboslav Stárka continued to be mentally alert and capable until the last moments of his life, and we had taken the liberty of asking him to be a member of our new team for studying the endocannabinoid system. Professor Stárka immediately accepted the offer without a second thought, with the plan that he would initially perform literature research and organize his own memories. However, fate willed otherwise, and he is unfortunately no longer with us.

Our interdisciplinary team has set up a project to search for new predictors of preterm birth. Together with ECS units, we will look for associations with steroidogenesis in women at high risk of preterm birth. In the context of the professional life of Professor Stárka [90], we see this continuation of more detailed studies of the steroid metabolome along with the ECS as more than symbolic.

Conflict of Interest

There is no conflict of interest.

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Abbreviations

2-AG, 2-arachidonoylglycerol; AEA, N-arachidonoyl-ethanolamine; CB1, type 1 cannabinoid receptor; CB2, type 2 cannabinoid receptor; DAG, diacylglycerol; DAGL, diacylglycerol-lipase; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAPE-PLD, N-acylphosphatydil ethanolamine-specific phospholipase D. NAPE, N-acylphosphatidyl ethanolamine; NPDL, N-arachidonoylphosphatidyl ethanolamine-specific phospholipase D.

References

1. Sayed TS, Balasinor NH, Nishi K. Diverse role of endocannabinoid system in mammalian male reproduction. Life Sci 2021;286:120035. https://doi.org/10.1016/j.lfs.2021.120035
2. Karasu T, Marczylo TH, Maccarrone M, Konje JC. The role of sex steroid hormones, cytokines and the endocannabinoid system in female fertility. Hum Reprod Update 2011;17:347-361. https://doi.org/10.1093/humupd/dmq058
3. Crocq MA. History of cannabis and the endocannabinoid system. Dialogues Clin Neurosci 2020;22:223-228. https://doi.org/10.31887/DCNS.2020.22.3/mcrocq

4. Siracusa L, Ruberto G, Cristino L. Recent Research on Cannabis sativa L.: Phytochemistry, New Matrices, Cultivation Techniques, and Recent Updates on Its Brain-Related Effects (2018–2023). Molecules 2023;28:3387. https://doi.org/10.3390/molecules28083387
5. Mechoulam R, Shvo Y. Hashish. I. The structure of cannabidiol. Tetrahedron 1963;19:2073-2078. https://doi.org/10.1016/0040-4020(63)85022-X
6. Gaoni Y, Mechoulam R. Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. Journal of the American Chemical Society 1964;86:1646-1647. https://doi.org/10.1021/ja01062a046
7. Mechoulam R, Hanus L. A historical overview of chemical research on cannabinoids. Chem Phys Lipids 2000;108:1-13. https://doi.org/10.1016/S0009-3084(00)00184-5
8. Pertwee RG. The pharmacology of cannabinoid receptors and their ligands: an overview. Int J Obes (Lond) 2006;30 Suppl 1:S13-18. https://doi.org/10.1038/sj.ijo.0803272
9. Devane WA, Dysarz FA, 3rd, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 1988;34:605-613.
10. Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC. Cannabinoid receptor localization in brain. Proc Natl Acad Sci U S A 1990;87:1932-1936. https://doi.org/10.1073/pnas.87.5.1932
11. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 1990;346:561-564. https://doi.org/10.1038/346561a0
12. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61-65. https://doi.org/10.1038/365061a0
13. Snyder SH, Pert CB, Pasternak GW. The opiate receptor. Ann Intern Med 1974;81:534-540. https://doi.org/10.7326/0003-4819-81-4-534
14. Terenius L, Wahlström A. Search for an endogenous ligand for the opiate receptor. Acta Physiol Scand 1975;94:74-81. https://doi.org/10.1111/j.1748-1716.1975.tb05863.x
15. Hughes J. Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. Brain Res 1975;88:295-308. https://doi.org/10.1016/0006-8993(75)90391-1
16. Devane W, Hanus L, Breuer A, Pertwee R, Stevenson L, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 1992;258:1946-1949. https://doi.org/10.1126/science.1470919
17. Hanus L, Gopher A, Almog S, Mechoulam R. Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. J Med Chem 1993;36:3032-3034. https://doi.org/10.1021/jm00072a026
18. Mechoulam R, Hanus L, Martin BR. Search for endogenous ligands of the cannabinoid receptor. Biochem Pharmacol 1994;48:1537-1544. https://doi.org/10.1016/0006-2952(94)90197-X
19. Stasiulewicz A, Znajdek K, Grudzień M, Pawiński T, Sulkowska AJI. A Guide to Targeting the Endocannabinoid System in Drug Design. Int J Mol Sci 2020;21. https://doi.org/10.3390/ijms21082778
20. Silvestri C, Di Marzo V. The Gut Microbiome-Endocannabinoidome Axis: A New Way of Controlling Metabolism, Inflammation, and Behavior. Function (Oxf) 2023;4:zqad003. https://doi.org/10.1093/function/zqad003
21. Forteza F, Bourdeau-Julien I, Nguyen GQ, Guevara Agudelo FA, Rochefort G, Parent L, Rakotoarivelo V, Feutry P, Martin C, Perron J, Lamarche B, Flamand N, Veilleux A, Billaut F, Di Marzo V, Raymond F. Influence of diet on acute endocannabinoidome mediator levels post exercise in active women, a crossover randomized study. Sci Rep 2022;12:8568. https://doi.org/10.1038/s41598-022-10757-0
22. Manca C, Carta G, Murru E, Abolghasemi A, Ansar H, Errigo A, Cani PD, Banni S, Pes GM. Circulating fatty acids and endocannabinoidome-related mediator profiles associated to human longevity. Geroscience 2021;43:1783-1798. https://doi.org/10.1007/s11357-021-00342-0
23. Dörnyei G, Vass Z, Juhász CB, Nádasy GL, Hunyady L, Szekeres M. Role of the Endocannabinoid System in Metabolic Control Processes and in the Pathogenesis of Metabolic Syndrome: An Update. Biomedicines 2023;11. https://doi.org/10.3390/biomedicines11020306
24. Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N. Molecular characterization of a phospholipase D generating anandamide and its congeners. J Biol Chem 2004;279:5298-5305. https://doi.org/10.1074/jbc.M306642200

25. Xiao J, Zhou Y, Sun L, Wang H. Role of integrating cannabinoids and the endocannabinoid system in neonatal hypoxic-ischaemic encephalopathy. Front Mol Neurosci 2023;16:1152167. https://doi.org/10.3389/fnmol.2023.1152167
26. Gammon CM, Freeman GM, Jr., Xie W, Petersen SL, Wetsel WC. Regulation of gonadotropin-releasing hormone secretion by cannabinoids. Endocrinology 2005;146:4491-4499. https://doi.org/10.1210/en.2004-1672
27. Brents LK. Marijuana, the Endocannabinoid System and the Female Reproductive System. Yale J Biol Med 2016;89:175-191.
28. Craft RM, Marusich JA, Wiley JL. Sex differences in cannabinoid pharmacology: a reflection of differences in the endocannabinoid system? Life Sci 2013;92:476-481. https://doi.org/10.1016/j.lfs.2012.06.009
29. Murphy LL, Muñoz RM, Adrian BA, Villanúa MA. Function of cannabinoid receptors in the neuroendocrine regulation of hormone secretion. Neurobiol Dis 1998;5:432-446. https://doi.org/10.1006/nbdi.1998.0224
30. Scorticati C, Fernández-Solari J, De Laurentiis A, Mohn C, Prestifilippo JP, Lasaga M, Seilicovich A, Billi S, Franchi A, McCann SM, Rettori V. The inhibitory effect of anandamide on luteinizing hormone-releasing hormone secretion is reversed by estrogen. Proc Natl Acad Sci U S A 2004;101:11891-11896. https://doi.org/10.1073/pnas.0404366101
31. González S, Bisogno T, Wenger T, Manzanares J, Milone A, Berrendero F, Di Marzo V, Ramos JA, Fernández-Ruiz JJ. Sex steroid influence on cannabinoid CB(1) receptor mRNA and endocannabinoid levels in the anterior pituitary gland. Biochem Biophys Res Commun 2000;270:260-266. https://doi.org/10.1006/bbrc.2000.2406
32. Maia J, Almada M, Silva A, Correia-da-Silva G, Teixeira N, Sa SI, Fonseca BM. The endocannabinoid system expression in the female reproductive tract is modulated by estrogen. J Steroid Biochem Mol Biol 2017;174:40-47. https://doi.org/10.1016/j.jsbmb.2017.07.023
33. Farkas I, Kalló I, Deli L, Vida B, Hrabovszky E, Fekete C, Moenter SM, Watanabe M, Liposits Z. Retrograde endocannabinoid signaling reduces GABAergic synaptic transmission to gonadotropin-releasing hormone neurons. Endocrinology 2010;151:5818-5829. https://doi.org/10.1210/en.2010-0638
34. Kolodny RC, Masters WH, Kolodner RM, Toro G. Depression of plasma testosterone levels after chronic intensive marihuana use. N Engl J Med 1974;290:872-874. https://doi.org/10.1056/NEJM197404182901602
35. 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;90:984-991. https://doi.org/10.1210/jc.2004-1287
36. Lewis SE, Rapino C, Di Tommaso M, Pucci M, Battista N, Paro R, Simon L, Lutton D, Maccarrone M. Differences in the endocannabinoid system of sperm from fertile and infertile men. PLoS One 2012;7:e47704. https://doi.org/10.1371/journal.pone.0047704
37. Francou MM, Girela JL, De Juan A, Ten J, Bernabeu R, De Juan J. Human sperm motility, capacitation and acrosome reaction are impaired by 2-arachidonoylglycerol endocannabinoid. Histol Histopathol 2017;32:1351-1358.
38. Schuel H, Burkman LJ, Lippes J, Crickard K, Mahony MC, Giuffrida A, Picone RP, Makriyannis A. Evidence that anandamide-signaling regulates human sperm functions required for fertilization. Mol Reprod Dev 2002;63:376-387. https://doi.org/10.1002/mrd.90021
39. Truong VB, Davis OS, Gracey J, Neal MS, Khokhar JY, Favetta LA. Sperm capacitation and transcripts levels are altered by in vitro THC exposure. BMC Mol Cell Biol 2023;24:6. https://doi.org/10.1186/s12860-023-00468-3
40. Gervasi MG, Osycka-Salut C, Sanchez T, Alonso CA, Llados C, Castellano L, Franchi AM, Villalon M, Perez-Martinez S. Sperm Release From the Oviductal Epithelium Depends on Ca(2+) Influx Upon Activation of CB1 and TRPV1 by Anandamide. J Cell Biochem 2016;117:320-333. https://doi.org/10.1002/jcb.25273
41. Cecconi S, Rapino C, Di Nisio V, Rossi G, Maccarrone M. The (endo)cannabinoid signaling in female reproduction: What are the latest advances? Prog Lipid Res 2020;77:101019. https://doi.org/10.1016/j.plipres.2019.101019
42. El-Talatini MR, Taylor AH, Elson JC, Brown L, Davidson AC, Konje JC. Localisation and function of the endocannabinoid system in the human ovary. PLoS One 2009;4:e4579. https://doi.org/10.1371/journal.pone.0004579

43. Wang H, Xie H, Guo Y, Zhang H, Takahashi T, Kingsley PJ, Marnett LJ, Das SK, Cravatt BF, Dey SK. Fatty acid amide hydrolase deficiency limits early pregnancy events. J Clin Invest 2006;116:2122-2131. https://doi.org/10.1172/JCI28621
44. Wang H, Xie H, Dey SK. Endocannabinoid signaling directs periimplantation events. Aaps j 2006;8:E425-432. https://doi.org/10.1007/BF02854916
45. Catanzaro G, Battista N, Rossi G, Di Tommaso M, Pucci M, Pirazzi V, Cecconi S, Maccarrone M. Effect of capacitation on the endocannabinoid system of mouse sperm. Mol Cell Endocrinol 2011;343:88-92. https://doi.org/10.1016/j.mce.2011.01.022
46. Maccarrone M, Barboni B, Paradisi A, Bernabò N, Gasperi V, Pistilli MG, Fezza F, Lucidi P, Mattioli M. Characterization of the endocannabinoid system in boar spermatozoa and implications for sperm capacitation and acrosome reaction. J Cell Sci 2005;118:4393-4404. https://doi.org/10.1242/jcs.02536
47. Francavilla F, Battista N, Barbonetti A, Vassallo MR, Rapino C, Antonangelo C, Pasquariello N, Catanzaro G, Barboni B, Maccarrone M. Characterization of the endocannabinoid system in human spermatozoa and involvement of transient receptor potential vanilloid 1 receptor in their fertilizing ability. Endocrinology 2009;150:4692-4700. https://doi.org/10.1210/en.2009-0057
48. Scotchie JG, Savaris RF, Martin CE, Young SL. Endocannabinoid regulation in human endometrium across the menstrual cycle. Reprod Sci 2015;22:113-123. https://doi.org/10.1177/1933719114533730
49. Paria BC, Das SK, Dey SK. The preimplantation mouse embryo is a target for cannabinoid ligand-receptor signaling. Proc Natl Acad Sci U S A 1995;92:9460-9464. https://doi.org/10.1073/pnas.92.21.9460
50. Paria BC, Song H, Wang X, Schmid PC, Krebsbach RJ, Schmid HH, Bonner TI, Zimmer A, Dey SK. Dysregulated cannabinoid signaling disrupts uterine receptivity for embryo implantation. J Biol Chem 2001;276:20523-20528. https://doi.org/10.1074/jbc.M100679200
51. López-Cardona AP, Pérez-Cerezales S, Fernández-González R, Laguna-Barraza R, Pericuesta E, Agirregoitia N, Gutiérrez-Adán A, Agirregoitia E. CB(1) cannabinoid receptor drives oocyte maturation and embryo development via PI3K/Akt and MAPK pathways. Faseb J 2017;31:3372-3382. https://doi.org/10.1096/fj.201601382RR
52. Gellersen B, Brosens JJ. Cyclic decidualization of the human endometrium in reproductive health and failure. Endocr Rev 2014;35:851-905. https://doi.org/10.1210/er.2014-1045
53. 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 2013;2013:510540. https://doi.org/10.1155/2013/510540
54. Fonseca BM, Cunha SC, Goncalves D, Mendes A, Braga J, Correia-da-Silva G, Teixeira NA. Decidual NK cell-derived conditioned medium from miscarriages affects endometrial stromal cell decidualisation: endocannabinoid anandamide and tumour necrosis factor-alpha crosstalk. Hum Reprod 2020;35:265-274. https://doi.org/10.1093/humrep/dez260
55. Torella M, Bellini G, Punzo F, Argenziano M, Schiattarella A, Labriola D, Schettino MT, Ambrosio D, Ammaturo FP, De Franciscis P. TNF-alpha effect on human delivery onset by CB1/TRPV1 crosstalk: new insights into endocannabinoid molecular signaling in preterm vs. term labor. Analysis of the EC/EV pathway and predictive biomarkers for early diagnosis of preterm delivery. Minerva Ginecol 2019;71:359-364. https://doi.org/10.23736/S0026-4784.19.04405-8
56. Cui N, Wang C, Zhao Z, Zhang J, Xu Y, Yang Y, Hao G. The Roles of Anandamide, Fatty Acid Amide Hydrolase, and Leukemia Inhibitory Factor on the Endometrium during the Implantation Window. Front Endocrinol (Lausanne) 2017;8:268. https://doi.org/10.3389/fendo.2017.00268
57. Correa F, Wolfson ML, Valchi P, Aisemberg J, Franchi AM. Endocannabinoid system and pregnancy. Reproduction 2016;152:R191-r200. https://doi.org/10.1530/REP-16-0167
58. Almada M, Piscitelli F, Fonseca BM, Di Marzo V, Correia-da-Silva G, Teixeira N. Anandamide and decidual remodelling: COX-2 oxidative metabolism as a key regulator. Biochim Biophys Acta 2015;1851:1473-1481. https://doi.org/10.1016/j.bbalip.2015.08.011
59. Fernández-Ruiz J, Berrendero F, Hernández ML, Ramos JA. The endogenous cannabinoid system and brain development. Trends Neurosci 2000;23:14-20. -https://doi.org/10.1016/S0166-2236(99)01491-5

60. Mato S, Del Olmo E, Pazos A. Ontogenetic development of cannabinoid receptor expression and signal transduction functionality in the human brain. Eur J Neurosci 2003;17:1747-1754. https://doi.org/10.1046/j.1460-9568.2003.02599.x
61. Wang X, Dow-Edwards D, Keller E, Hurd YL. Preferential limbic expression of the cannabinoid receptor mRNA in the human fetal brain. Neuroscience 2003;118:681-694. https://doi.org/10.1016/S0306-4522(03)00020-4
62. Kenney SP, Kekuda R, Prasad PD, Leibach FH, Devoe LD, Ganapathy V. Cannabinoid receptors and their role in the regulation of the serotonin transporter in human placenta. Am J Obstet Gynecol 1999;181:491-497. https://doi.org/10.1016/S0002-9378(99)70583-1
63. Park B, Gibbons HM, Mitchell MD, Glass M. Identification of the CB1 cannabinoid receptor and fatty acid amide hydrolase (FAAH) in the human placenta. Placenta 2003;24:990-995. https://doi.org/10.1016/S0143-4004(03)00165-6, https://doi.org/10.1053/plac.2002.0926
64. Acone G, Trabucco E, Colacurci N, Cobellis L, Mackie K, Meccariello R, Cacciola G, Chioccarelli T, Fasano S, Pierantoni R, Cobellis G. Low type I cannabinoid receptor levels characterize placental villous in labouring delivery. Placenta 2009;30:203-205. https://doi.org/10.1016/j.placenta.2008.11.018
65. Helliwell RJ, Chamley LW, Blake-Palmer K, Mitchell MD, Wu J, Kearn CS, Glass M. Characterization of the endocannabinoid system in early human pregnancy. J Clin Endocrinol Metab 2004;89:5168-5174. https://doi.org/10.1210/jc.2004-0388
66. Habayeb OM, Taylor AH, Bell SC, Taylor DJ, Konje JC. Expression of the endocannabinoid system in human first trimester placenta and its role in trophoblast proliferation. Endocrinology 2008;149:5052-5060. https://doi.org/10.1210/en.2007-1799
67. Chamley LW, Bhalla A, Stone PR, Liddell H, O'Carroll S, Kearn C, Glass M. Nuclear localisation of the endocannabinoid metabolizing enzyme fatty acid amide hydrolase (FAAH) in invasive trophoblasts and an association with recurrent miscarriage. Placenta 2008;29:970-975. https://doi.org/10.1016/j.placenta.2008.08.003
68. Habayeb OM, Taylor AH, Evans MD, Cooke MS, Taylor DJ, Bell SC, Konje JC. Plasma levels of the endocannabinoid anandamide in women--a potential role in pregnancy maintenance and labor? J Clin Endocrinol Metab 2004;89:5482-5487. https://doi.org/10.1210/jc.2004-0681
69. Trabucco E, Acone G, Marenna A, Pierantoni R, Cacciola G, Chioccarelli T, Mackie K, Fasano S, Colacurci N, Meccariello R, Cobellis G, Cobellis L. Endocannabinoid system in first trimester placenta: low FAAH and high CB1 expression characterize spontaneous miscarriage. Placenta 2009;30:516-522. https://doi.org/10.1016/j.placenta.2009.03.015
70. Costa MA, Fonseca BM, Keating E, Teixeira NA, Correia-da-Silva G. 2-arachidonoylglycerol effects in cytotrophoblasts: metabolic enzymes expression and apoptosis in BeWo cells. Reproduction 2014;147:301-311. https://doi.org/10.1530/REP-13-0563
71. Costa MA, Keating E, Fonseca BM, Teixeira NA, Correia-da-Silva G. 2-Arachidonoylglycerol impairs human cytotrophoblast cells syncytialization: influence of endocannabinoid signalling in placental development. Mol Cell Endocrinol 2015;399:386-394. https://doi.org/10.1016/j.mce.2014.09.005
72. Xu Y, Knipp GT, Cook TJ. Expression of cyclooxygenase isoforms in developing rat placenta, human term placenta, and BeWo human trophoblast model. Mol Pharm 2005;2:481-490. https://doi.org/10.1021/mp0500519
73. Meadows JW, Pitzer B, Brockman DE, Myatt L. Differential localization of prostaglandin E synthase isoforms in human placental cell types. Placenta 2004;25:259-265. https://doi.org/10.1016/j.placenta.2003.09.004
74. Capparuccia L, Marzioni D, Giordano A, Fazioli F, De Nictolis M, Busso N, Todros T, Castellucci M. PPARgamma expression in normal human placenta, hydatidiform mole and choriocarcinoma. Mol Hum Reprod 2002;8:574-579. https://doi.org/10.1093/molehr/8.6.574
75. Schaiff WT, Carlson MG, Smith SD, Levy R, Nelson DM, Sadovsky Y. Peroxisome proliferator-activated receptor-gamma modulates differentiation of human trophoblast in a ligand-specific manner. J Clin Endocrinol Metab 2000;85:3874-3881. https://doi.org/10.1210/jcem.85.10.6885, https://doi.org/10.1210/jc.85.10.3874
76. Costa MA, Fonseca BM, Keating E, Teixeira NA, Correia-da-Silva G. Transient receptor potential vanilloid 1 is expressed in human cytotrophoblasts: induction of cell apoptosis and impairment of syncytialization. Int J Biochem Cell Biol 2014;57:177-185. https://doi.org/10.1016/j.biocel.2014.10.008

77. Kremshofer J, Siwetz M, Berghold VM, Lang I, Huppertz B, Gauster M. A role for GPR55 in human placental venous endothelial cells. Histochem Cell Biol 2015;144:49-58. https://doi.org/10.1007/s00418-015-1321-7
78. Maia J, Fonseca BM, Teixeira N, Correia-da-Silva G. The fundamental role of the endocannabinoid system in endometrium and placenta: implications in pathophysiological aspects of uterine and pregnancy disorders. Hum Reprod Update 2020;26:586-602. https://doi.org/10.1093/humupd/dmaa005
79. Hill M, Pařízek A, Jirásek J E, Jirkovská M, Velíková M, Dušková M, Klímková M, Pašková A, Žižka Z, Germanová A, Koucký M, Kalousová M, Stárka L. Is maternal progesterone actually independent of the fetal sterois? Physiol Res 2010;59:211-224. https://doi.org/10.33549/physiolres.931807
80. Adamcová K, Kolátorová L, Škodová T, Šimková M, Pařízek A, Stárka L, Dušková M. Steroid hormone levels in the peripartum period - differences caused by fetal sex and delivery type. Physiol Res 2018; 67 (Suppl. 3); S489-S497. https://doi.org/10.33549/physiolres.934019
81. Almada M, Oliveira A, Amaral C, Fernandes PA, Ramos MJ, Fonseca B, Correia-da-Silva G, Teixeira N. Anandamide targets aromatase: A breakthrough on human decidualization. Biochim Biophys Acta Mol Cell Biol Lipids 2019;1864:158512. https://doi.org/10.1016/j.bbalip.2019.08.008
82. Nallendran V, Lam PM, Marczylo TH, Bankart MJ, Taylor AH, Taylor DJ, Konje JC. The plasma levels of the endocannabinoid, anandamide, increase with the induction of labour. Bjog 2010;117:863-869. https://doi.org/10.1111/j.1471-0528.2010.02555.x
83. Laštůvka Z, Suchopár J, Mašková S, Alblová M, Pařízek A. The role of endocannabinoids in pregnancy. Ceska Gynekol 2021;86:349-354. https://doi.org/10.48095/cccg2021349
84. Suchopár J, Laštůvka Z, Mašková S, Alblová M, Pařízek A. Endocannabinoids. Ceska Gynekol 2021;86:414-420. https://doi.org/10.48095/cccg2021414
85. Bachkangi P, Taylor AH, Bari M, Maccarrone M, Konje JC. Prediction of preterm labour from a single blood test: The role of the endocannabinoid system in predicting preterm birth in high-risk women. Eur J Obstet Gynecol Reprod Biol 2019;243:1-6. https://doi.org/10.1016/j.ejogrb.2019.09.029
86. Pařízek A, Mikešová M, Jirák R, Hill M, Koucký M, Pašková A, Velíková M, Adamcová K, Šrámková M, Jandíková H, Dušková M, Stárka L. Steroid hormones in the development of postpartum depression. Physiol Res 2014;63 Suppl 2:S277-S282. https://doi.org/10.33549/physiolres.932788
87. Adamcová K, Kolátorová L, Jandíková H, Chlupáčová T, Šmídová M, Pařízek A, Stárka L, Dušková M. Changes to fetal steroidogenesis caused by maternal smoking. Physiol Res 2017; 66(Suppl. 3);S375-S386. https://doi.org/10.33549/physiolres.933728
88. Kolatorova L, Vitku J, Vavrous A, Hampl R, Adamcova K, Simkova M, Parizek A, Starka L, Duskova M. Phthalate metabolites in maternal and cord plasma and their relations to other selected endocrine disruptors and steroids. Physiol Res. 2018; 67 (Suppl. 3); S473-S487. https://doi.org/10.33549/physiolres.933962
89. Šimják P, Hill M, Pařízek A, Vítek L, Velíková M, Dušková M, Kancheva R, Bulant J, Koucký M, Kokrdová Z, Adamcová K, Černý A, Hájek Z, Stárka L. May circulating steroids reveal a predisposition to intrahepatic cholestasis of pregnancy in non-pregnant women? Physiol Res 2018;67(Suppl. 3);S499-S510. https://doi.org/10.33549/physiolres.934028
90. Stárka L. My life studying steroids. Physiol. Res. 2020; 69 (Suppl. 2): S181-S182.