Progress in Lipid Research 77 (2020) 101019

Contents lists available at ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres

Review

The (endo)cannabinoid signaling in female reproduction: What are the latest advances?

Sandra Cecconi[a,1], Cinzia Rapino[b,1], Valentina Di Nisio[a], Gianna Rossi[a], Mauro Maccarrone[c,d,*]

[a] Department of Life, Health and Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy
[b] Faculty of Veterinary Medicine, University of Teramo, 64100 Teramo, Italy
[c] Department of Medicine, Campus Bio-Medico University of Rome, 00128 Rome, Italy
[d] European Center for Brain Research (CERC)/Santa Lucia Foundation, Via del Fosso di Fiorano, 64 - 00143 Rome, Italy

ARTICLE INFO

Keywords:
Cannabinoid receptors
Endocannabinoid signaling
Female reproduction
Mammals
Fertility biomarkers

ABSTRACT

Cannabis extracts like marijuana have the highest consumption rate worldwide. Yet, their societal acceptance as recreational and therapeutic drugs could represent a serious hazard to female human reproduction, because cannabis ingredients [termed (phyto)cannabinoids] can perturb an endogenous system of lipid signals known as endocannabinoids. Accumulated evidence on animal models and humans has demonstrated a crucial role of these endogenous signals on different aspects of female reproduction, where they act through an ensamble of proteins that synthesize, transport, degrade and traffic them. Several reports have recently evidenced the potential role of endocannabinoids as biomarkers of female infertility for disease treatment and prevention, as well as their possible epigenetic effects on pregnancy. The purpose of this review is to provide an update of data collected in the last decade on the effects of cannabinoids and endocannabinoids on female reproductive events, from development and maturation of follicles and oocytes, to fertilization, oviductal transport, implantation and labor. In this context, a particular attention has ben devoted to the ovary and the production of fertilizable oocytes, because recent studies have addressed this hot topic with conflicting results among species.

1. Introduction

1.1. State of the art on female fertility and infertility

The Western world is getting older because of increased life expectancy and reduced birth rates [1].

In recent years, lifestyle and socio-economic factors have led younger women to postpone motherhood, thereby increasing the number of reproductively aged subjects. Despite the chance of conceiving per month drops to low values by age 35 [2], women underestimate the risk of such decline and of increased obstetric complications [3].

Also the rapid increase of sexually transmitted diseases, obesity, alcohol, smoking and drug abuse negatively affect fecundity, independently of other factors [4–7]. In particular, recreational drug misuse is known to interphere with all reproductive events, ultimately leading to infertility [8].

1.2. Recreational use of cannabis

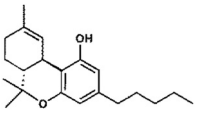
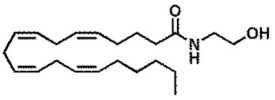
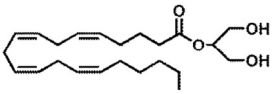
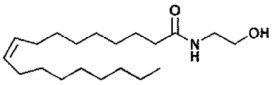
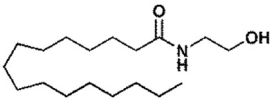
With the increasingly permissive legal and social environments in several countries (including Canada, New Zealand, Australia, The

Abbreviations: 2-AG, 2-arachidonoylglycerol; ABHD6, α/β-hydrolase domain protein 6; AEA, *N*-arachidonoylethanolamine; CB_1, type 1 cannabinoid receptor; CB_2, type 2 cannabinoid receptor; CC, cumulus cells; CL, corpus luteum; COX-2, cyclooxygenase-2; CYP, cytochrome P450; DAGL, *sn-1* diacylglycerol-lipase; E2, estrogen; eCBs, endocannabinoids; ECS, endocannabinoid system; EnCa, endometrial carcinoma; EMT, endocannabinoid membrane transporter; ER, endoplasmic reticulum; FAAH, fatty acid amide hydrolase; FSH, follicle stimulating hormone; GPR55, G protein-coupled receptor 55; GC, granulosa cells; GV, germinal vesicle; ICM, inner cells mass; LH, luteinizing hormone; LOXs, lipoxygenases; MAGL, monoacylglycerol lipase; MI, metaphase I; MII, metaphase II; NAEs, *N*-acylethanolamines; NAPE-PLD, N-acylphosphatidyl ethanolamines (NAPE)-specific phospholipase D; OC, ovarian cancer; OEA, *N*-oleoylethanolamine; P4, progesterone; PCOS, polycystic ovary syndrome; PEA, *N*-palmitoylethanolamine; pre-GC, pre-granulosa cells; PPARs, peroxisome proliferator-activated receptors; TC, theca cells; TRPV1, transient receptor potential vanilloid-1; THC, Δ^9-tetrahydrocannabinol.

* Corresponding author.
E-mail addresses: sandra.cecconi@univaq.it (S. Cecconi), crapino@unite.it (C. Rapino), valentina.dinisio@graduate.univaq.it (V. Di Nisio), gianna.rossi@univaq.it (G. Rossi), m.maccarrone@unicampus.it (M. Maccarrone).
[1] Equally first authors.

https://doi.org/10.1016/j.plipres.2019.101019
Received 16 September 2019; Received in revised form 11 October 2019; Accepted 4 November 2019
Available online 18 December 2019

Table 1
Major (endo)cannabinoids and endocannabinoid-like compounds, and main metabolic enzymes of the ECS involved in female reproductive events.

(Endo)cannabinoids	Chemical structure
Δ^9-Tetrahydrocannabinol (THC)	
N-Arachidonoylethanolamine (Anandamide, AEA)	
2-Arachidonoylglycerol (2-AG)	
Endocannabinoid-like compounds	**Chemical structure**
N-Oleoylethanolamine (OEA)	
N-Palmitoylethanolamine (PEA)	
Metabolic enzymes of AEA	**Intracellular localization**
N-acylphosphatidyl ethanolamines (NAPE)-specific phospholipase D (NAPE-PLD)	Membrane-associated
Fatty acid amide hydrolase (FAAH)	Membrane-associated (mainly ER)
Metabolic enzymes of 2-AG	Intracellular localization
Diacylglycerol lipase α (DAGLα)	Membrane-associated
Diacylglycerol lipase β (DAGLβ)	Membrane-associated
Monoacylglycerol lipase (MAGL)	Membrane-associated and cytosolic
α/β-Hydrolase domain protein 6 (ABHD6)	Membrane-associated enzyme

ECS, endocannabinoid system; ER, endoplasmic reticulum. See text for additional details.

Netherlands, Spain, United Kingdom, and more than 20 States in the USA), marijuana is the most common illicit recreational drug used before and during pregnancy [9]. In particular, ~9% of women are regular users before pregnancy and 2.5% during pregnancy [9], unfortunately with little (if any) understanding of the risk of its use even only once or twice a week [10].

Epidemiologic studies have delivered controversial results on the adverse effects of maternal use of cannabis for human pregnancy outcomes [11,12]. It is noteworthy that Δ^9-tetrahydrocannabinol (THC, shown in Table 1), the most important and abundant psychoactive component of cannabis preparations, enters maternal circulation and readily crosses the placental membrane [13]. Indeed, consumption during pregnancy of plant-derived and synthetic cannabinoids has been associated with gestational disorders such as abnormal embryo development, tubal pregnancy, implantation failure, preterm birth, intrauterine growth restriction, low birth weight and increased risk of miscarriage [14–16]. Recently, association of maternal use of marijuana with increased odds of adverse pregnancy outcomes and neonatal morbidity among liveborn controls has been assessed by the National Institute of Child Health and Human Development Stillbirth Collaborative Research Network [17]. Here, maternal marijuana use could not be associated with adverse neonatal or childhood outcomes, but with neonatal morbidity, probably because of long-term changes in the immunologic response upon *in utero* exposure [17,18]. Thus far, other human studies on marijuana consumption in pregnancy failed to demonstrate any association with fetal growth restriction [19], whereas a slightly increased risk for some congenital birth defects may be associated with early pregnancy following marijuana smoking [20]. In line with this, it should be recalled that many synthetic cannabinoids have higher affinity towards the same cellular receptors to which also THC binds, thus being more potent in adversely impacting pregnancy [21]. Discrepancies among studies may depend on different methodologies used to quantify the amount of marijuana ingested and to different types of biological samples available (plasma, urine and umbilical cord homogenates).

Depending on the amount ingested by the lactating mother, cannabis and its metabolites are excreted into breast milk in significant amounts. Indeed, human milk THC concentrations have been reported to be 8-fold higher than maternal serum concentrations, when cannabis is consumed regularly during breast feeding [22,23]. Overall, the limitation of many studies seems to be the paucity of long-term follow-up data on the exposed neonates, needed to assess childhood outcomes.

2. The modern view of the endocannabinoid system

2.1. Cannabinoids versus endocannabinoids

Cannabis (*Cannabis sativa* or *Cannabis indica*) extracts contain more than 550 known compounds, of which more than 110 have been identified as cannabinoids, including the potent psychoactive substance THC [24]. The latter compounds, also known as phytocannabinoids, are a family of lipophilic terpeno-phenolic compounds, that are structurally different (but pharmacologically similar) to their endogenous counterparts, called endocannabinoids (eCBs) [25].

Endocannabinoids (eCBs) are fatty acid-derived lipids produced by our body, where they interact with different proteins (receptors, transporters and enzymes) of a ubiquitous pro-homeostatic system known as endocannabinoid system (ECS), schematically depicted in Fig. 1. In particular, the major eCBs anandamide (*N*-arachidonoylethanolamine, AEA) and 2-arachidonoylglycerol (2-AG), both shown in Table 1, are neuromodulators and peripheral signals that control various metabolic, cardiovascular, immune and reproductive functions [26–28], as well as activities within the central nervous system [29,30].

2.2. Target receptors

Much alike eCBs, cannabinoids bind to type-1 (CB_1) and type-2 (CB_2) cannabinoid receptors, but also to transient receptor potential vanilloid-1 (TRPV1) channels, peroxisome proliferator-activated receptors (PPARs), and GPR55, with an impact on pregnancy [31]. The activation of CB receptors triggers different signaling pathways, including an inhibitory action on cyclic AMP/protein kinase A (cAMP/PKA) and mitogen-activated protein kinase (MAPK) p38 pathways, as well as extracellular signal-regulated kinases (ERK 1/2) phosphorylation, which are important for placental endocrine function [32].

2.3. Metabolic enzymes

Several enzymes are involved in the control of the biological activity of eCBs in female reproduction, as summarized in Table 1. An *N*-acylphosphatidyl ethanolamines (NAPE)-specific phospholipase D (NAPE-PLD) synthesizes AEA, that is cleaved by fatty acid amide hydrolase (FAAH or FAAH-1) and in higher placental mammals also by a second hydrolase, FAAH-2 [33]. 2-AG is mainly synthesized by two specific *sn*-1 diacylglycerol-lipases (DAGL) α and β, and is degraded by a specific monoacylglycerol lipase (MAGL). Recently, novel enzymes of the serine hydrolase family have been identified as regulators of the intracellular pool of 2-AG [34,35]. In particular, the expression of α/β-hydrolase domain protein 6 (ABHD6) seems to be regulated by hormones, such as estrogen (E2), suggesting sex-dependent differences in its expression [36]. In addition, to hydrolysis, AEA and 2-AG can be oxidized by cyclooxygenase-2 (COX-2), by different lipoxygenases (LOXs) or by cytochrome P450 (CYP) [37]. In particular, COX-2 turns AEA into prostaglandin-ethanolamides, while LOXs convert it into hydroxyanandamides or hydroxyeicosatetraenoyl-ethanolamides, and CYP into epoxyeicosatrienoyl-ethanolamides [27]. All eCBs can be inactivated

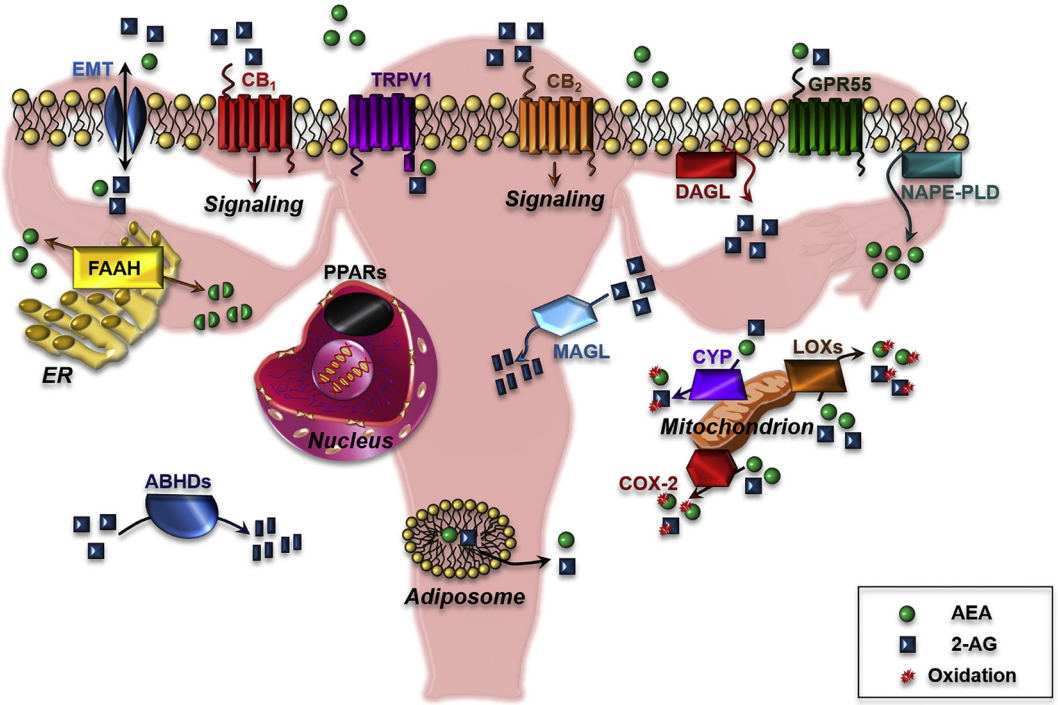


Fig. 1. Endocannabinoid system (ECS).
Cellular distribution of the different components of the ECS is shown.
Abbreviations: 2-AG, 2-arachidonoylglycerol; ABHDs, α/β-hydrolase domain protein; AEA, *N*-arachidonoylethanolamine; CB_1, type 1 cannabinoid receptor; CB_2, type 2 cannabinoid receptor; CYP, cytochrome P450; DAGL, *sn-1* diacylglycerol-lipase; EMT, endocannabinoid membrane transporter; FAAH, fatty acid amide hydrolase; GPR55, G protein-coupled receptor 55; LOXs, lipoxygenases; COX-2, cyclooxygenase-2; MAGL, monoacylglycerol lipase; NAPE-PLD, N-acylphosphatydil ethanolamines (NAPE)-specific phospholipase D; PPAR, peroxisome proliferator-activated receptor; TRPV1, transient receptor potential vanilloid-1.

also through their uptake by a purported "endocannabinoid membrane transporter" (EMT), whose molecular identity remains as yet unknown [38]. An additional layer of complexity is represented by new intracellular eCB reservoirs, such as adiposomes [39,40], that suggest the existence of potential platforms for eCB trafficking and accumulation. Finally, besides AEA other compounds of the family of long chain *N*-acylethanolamines (NAEs), involved in mammalian reproductive functions [41–43] have been proposed to belong to the ECS and named "eCB-like" compounds such as *N*-acylethanolamines (NAEs). Among them, *N*-palmitoylethanolamine (PEA) and *N*-oleoylethanolamine (OEA), shown in Table 1, are inactive at CB_1 or CB_2 receptors but inhibit AEA metabolism, thus prolonging its effects at cell receptors [44]. They increase the action of endogenous AEA through an increase in its affinity for receptors and/or a decrease in its enzymatic degradation. In particular, PEA and OEA could act as "entourage" compounds by interacting with TRPV1 receptors as well as with FAAH [44].

3. Cannabinoids, endocannabinoids and female reproduction

The presence of ECS elements in several reproductive fluids, cells and tissues like human serum and follicular fluid, ovary, uterus and placenta, have been documented by several studies in animal models and humans [45–50]. Cannabis extracts, THC and modulators of the ECS clearly have a major impact on virtually all steps of female reproduction [41,51,52].

3.1. The production of a fertilizable oocyte

In mammals, oocytes originate from actively proliferating primordial germ cells that enter meiosis and arrest at the germinal vesicle (GV) stage. Their very low number justifies the many ongoing attempts to increase their population *in vitro,* although good results in terms of live births have been obtained only for mouse oocytes [53]. In rodents, meiosis and follicle formation start during the first few days after birth, while in primates during fetal life. The formation of primordial follicles, which form the pool of resting follicles, occurs soon after the appearance of a pre-granulosa cell (GC) layer around the oocytes. After entering the growth phase, follicle development is under control of follicle stimulating hormone (FSH), E2 and luteinizing hormone (LH) that, by interacting with many other regulative molecules, activate proliferative and differentiative pathways [54–57]. FSH binds to GC, while LH binds to a layer of stroma-derived cells called theca cells (TC). Final follicle development (evidenced by the appearance of a fluid-filled cavity, the antrum) is completed by one or more follicles, depending on species. Preovulatory surge of LH triggers the activation of a complex series of molecular events that induce oocyte meiotic resumption, formation of the first meiotic spindle (metaphase I, MI), extrusion of the first polar body with the redundant genetic material and arrest at metaphase II (MII) [58–61]. Now oocytes, which are surrounded by an irregular ring of cumulus cells (CC), leave the ovary upon rupture of the follicular wall. Finally, LH induces changes that convert the follicle remnants into the corpus luteum (CL), an endocrine structure mainly responsible for progesterone production.

3.2. Localization of ECS components in the ovary of different mammalian species

Many reports have demonstrated that chronic exposure to cannabinoids negatively affects follicular development by reducing the release of hormones of the hypotalamic-pituitary-ovarian axis and of sex steroids [62–63]. More recently, it has been found that pharmacological activation of CB_2 in fetal female germ cells accelerates entrance of oocytes in meiosis and apoptosis, thereby reducing ovarian reserve [64]. Although the species-specific expression and distribution of ECS components makes it difficult to extrapolate a general model, recent data have pointed out new and interesting roles for ECS in the

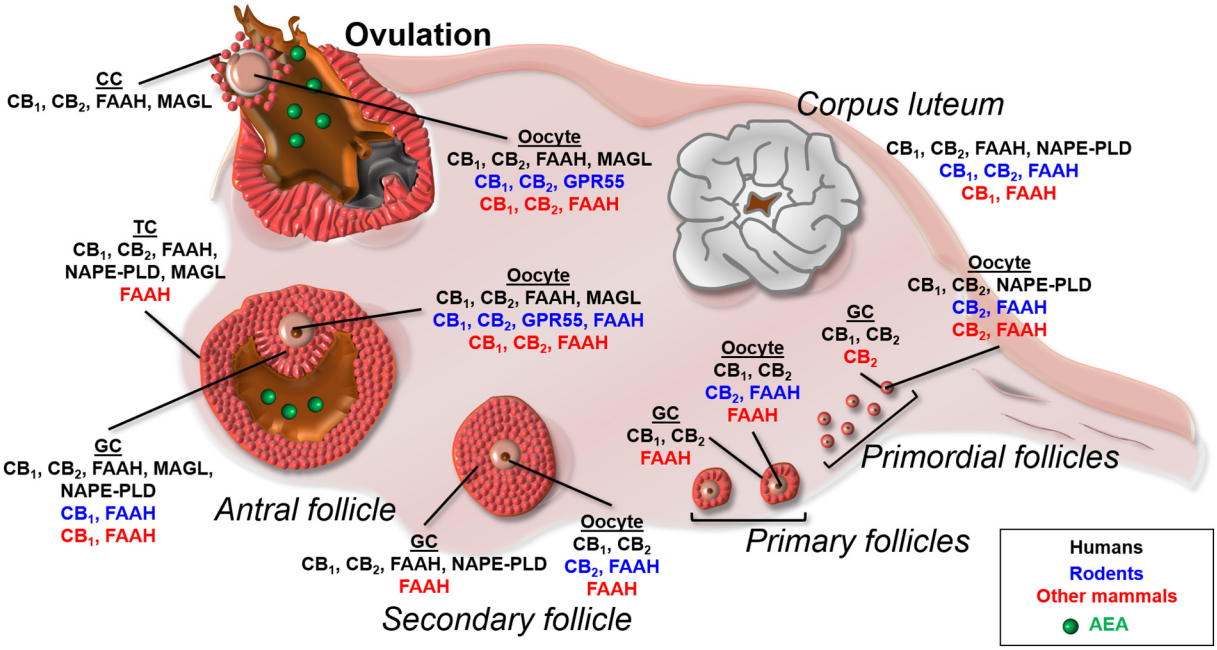


Fig. 2. Distribution of ECS components in the mammalian ovary.
Each follicle is formed by the oocyte and surrounding granulosa cells (GC); theca cells (TC) layers appear at the secondary stage. Gonadotropins (FSH, LH) are necessary to promote follicle development and ovulation, respectively. The expression of ECS components is differentially modulated during folliculogenesis, and among species (black: humans, blue: rodents, red: other mammals).
Abbreviations: AEA, *N*-arachidonoylethanolamine; CB_1, type 1 cannabinoid receptor; CB_2, type 2 cannabinoid receptor; CC, cumulus cells; FAAH, fatty acid amide hydrolase; GC, granulosa cells; GPR55, G protein-coupled receptor 55; MAGL, monoacylglycerol lipase; NAPE-PLD, *N*-acylphosphatydilethanolamines (NAPE)-specific phospholipase D; TC, theca cells.

production of healthy offspring in mammals, schematically depicted in Fig. 2 and summarized in Table 2.

3.2.1. Humans

In humans, the presence of ECS components was ascertained for the first time in sections of adult ovary by El-Talatini and collaborators [65]. Both CB_1 and CB_2 were present in oocytes enclosed into primordial, primary and secondary follicles, while only CB_2 was detectable after antrum formation. As for metabolic enzymes, NAPE-PLD was sporadically detectable only in primordial oocytes, while FAAH was never found. Although CB_1 and CB_2 have been immunolocalized in GC throughout folliculogenesis, FAAH and NAPE-PLD appeared in secondary follicles but decreased sharply in antral follicles. By contrast, TC expressed CB_1, CB_2, NAPE-PLD and FAAH in all follicles analyzed, apart from CL that was devoid of FAAH. It was concluded that AEA can act in an autocrine manner in TCs, while a FAAH-independent degradation of this eCB may be present in GC [65].

The expression levels and localization of CB_1, CB_2, MAGL and FAAH in human oocytes collected during assisted reproductive procedures have been assessed by Agirregoitia and collaborators [66–68]. They found that only CB_1 mRNA was present at very low levels during meiotic maturation, although both CB_1 and CB_2 proteins were detectable by Western blot and immunofluorescence. FAAH was expressed at mRNA and protein levels from GV to MII phase, while MAGL could be detected only as a protein. Fluorescence analysis also revealed that both CB_1 and FAAH were distributed at cell periphery, and that MAGL was cytoplasmic at GV and MI stages. At MII CB_1 localization remained the same, while both FAAH and MAGL appeared homogeneously distributed in the cytoplasm. The same analysis in human CCs detected CB_1, FAAH and MAGL either at mRNA or at protein level, whereas CB_2 could be detected only as a protein. It seems noteworthy that in these studies a mixture of GV, MI and MII-failed fertilized oocytes and CCs was used for molecular analysis, making it hard to quantify stage-specific expression of ECS proteins. For the same reason it is difficult to compare these studies with those by El-Talatini and colleagues [65], that yielded conflicting results about ECS distribution in human oocytes.

3.2.2. Rodents

In rat oocytes, Bagavandoss and Grimshaw [69] demonstrated the presence of CB_2 and FAAH from primordial to antral stage. Conversely, GC did not express CB_1, CB_2 nor FAAH, but both receptors were detected in CL. It was suggested that progressive increase of FSH during folliculogenesis stimulated the synthesis of CB_1, but not of FAAH and CB_2 that were both independent of gonadotropin action.

In mice, López-Cardona and collaborators [70] found that CB_1 and CB_2 were differently expressed depending on the stage of oocyte meiotic maturation and on the culture protocols. Although at GV only CB_1 mRNA was expressed, both CB receptors were immunolocalized in oocytes: CB_2 was always found in the cytoplasm, while CB_1 distribution changed from peripheral to cytoplasmic between *in vivo* and *in vitro* oocytes. Independently of maturation protocols, in MII oocytes CB_1 was localized at the periphery and CB_2 in the cytoplasm. The underlying molecular reasons for these differences have not been explained. Remarkably, experiments with agonists and antagonists demonstrated that only CB_1 triggers phosphorylation of protein kinase B (Akt) and ERK1/2 kinases, that stimulate or inhibit polar body 1 emission and spindle formation, respectively. Despite CB_2 did not seem to be involved in oocyte maturation, López-Cardona and coworkers [70] speculated that it could compensate for the possible lack of CB_1 signaling during embryo oviductal transport.

More recently, CB_1, CB_2 and GPR55 mRNAs were shown to be expressed to different extents during *in vivo* maturation of mouse oocytes, as their levels fell down from GV to MII stages [71]. Conversely, TRPV1 mRNA was never detected. Immunofluorescence showed that in GV oocytes CB_1 was almost exclusively localized at the plasma membrane, while a weak cytoplasmic distribution was observed for both CB_2 and GPR55. After meiotic resumption, formation of CB_1 clusters was

Table 2
Localization of ECS components in the ovary of humans, rodents and cats. All the data are extrapolated from IHC.

HUMANS					
Follicle stage	Protein	Oocyte	GC	TC	References
Primordial follicle	CB_1	+	+	×	[65]
	CB_2	++	++	×	
	FAAH	-	-	×	
	NAPE-PLD	+/-	-	×	
Primary follicle	CB_1	+	+	×	
	CB_2	++	+	×	
	FAAH	-	-	×	
	NAPE-PLD	-	-	×	
Secondary follicle	CB_1	+	+	+	
	CB_2	++	+	+	
	FAAH	-	+	++	
	NAPE-PLD	-	++	++	
Tertiary follicle	CB_1	-	++	+	
	CB_2	++	+	+	
	FAAH	-	+/-	++	
	NAPE-PLD	-	+/-	+	
Corpus luteum	CB_1	×	++		
	CB_2	×	+		
	FAAH	×	+		
	NAPE-PLD	×	++		
RODENTS					
Secondary follicle	CB_1	-	-	-	[69]
	CB_2	+	-	-	
	FAAH	+	-	-	
Tertiary follicle	CB_1	-	+	-	
	CB_2	+	-	-	
	FAAH	+	-	-	
Corpus luteum	CB_1	×	+		
	CB_2	×	+		
	FAAH	×	+		
CATS					
Primordial follicle	CB_1	-	-	×	[74]
	FAAH	+/-	+	×	
Primary follicle	CB_1	-	-	×	
	FAAH	+/-	+	×	
Secondary follicle	CB_1	-	-	-	
	FAAH	+	+	+/-	
Tertiary follicle	CB_1	+/-	+	-	
	FAAH	+	+/-	+/-	
Corpus luteum	CB_1	×	+		
	FAAH	×	++		

Notes: (-) absent; (+) present; (++) highly expressed; (+/-) occasionally observed. (x) indicates the absence of TC/oocyte. GC, granulosa cells; TC, theca cells. See text for additional abbreviations and details.

followed by disappearance from plasma membrane, with the exception of a residual low cytoplasmic signal at MI and MII. These observations could be a consequence of receptor endocytosis, as already documented for G protein-coupled receptor 3 [72]. Moreover, CB_2 and even more GPR55 protein contents increased significantly at MI and MII. A role for CB_1 and CB_2 in the control of meiotic resumption via G*αi* proteins was supported by quantitation of intracellular concentration of cAMP in oocytes cultured in the presence or absence of CB_1 and CB_2 antagonists SR141716 and SR144528, respectively. Both compounds were able to delay meiotic resumption documented by germinal vesicle breakdown, raising the possibililty that CB receptors could act as regulators of this process through adenylyl cyclase activity. Furthermore, an unexpected role for GPR55 in the formation of normal meiotic spindles arose from experiments where its ML193 antagonist caused a significant reduction of MI and MII spindle length, without affecting chromosome alignment at metaphase [71]. The consequences of these findings remain to be further investigated.

3.2.3. Other mammals

CB_1 and CB_2 mRNA levels and protein localization have been assessed also in bovine oocytes undergoing *in vitro* maturation [73]. Here, mRNAs of both CB receptors were detected at GV and MI, but not at MII stage where CB_1 was absent. Also receptor localization by immunofluorescence was different at MI and MII: CB_2 was always distributed homogeneously in the cytoplasm, while CB_1 had a peripheral localization from MI onward. In the same study, the authors showed that both CB_1 agonists and epidermal growth factor modulated positively Akt and ERK1/2 kinases in GC and oocytes, likely via CB_1 activation. At the same time, CB_1 agonists did not impair embryo development, and positively regulated embryo quality-related genes.

More recently, Pirone and coworkers [74] described the distribution of CB_1 and FAAH in sections of cat ovaries. CB_1 was not expressed in early follicles, but was detected in GC of the antral follicles and CL; instead, FAAH was strongly detectable in GC and TC up to CL formation. In addition, CB_1 agonists decreased progesterone (P4) levels, suggesting that injection of these CB_1 modulators could be used to regulate cat fertility. These observations are in line with data on ewes treated with CB_1/CB_2 agonists, which affect luteal P4 secretion by downregulating LH receptor expression [75].

3.3. The role of AEA in the mammalian ovary

In the context of eCB signaling in the mammalian ovary, a key question is the source of AEA and other eCBs. It has been shown that systemic and ovarian AEA concentrations are positively related to each other [45]. In fertile women, AEA is produced by follicle cells and its concentration correlates with follicle growth, due to an AEA-dependent autocrine mechanism acting on GC [76]. The maintenance of low AEA concentration during the first steps of folliculogenesis is required to avoid inappropriate oocyte maturation [52]. Consistently, the maximum level of AEA in the follicular fluid is reached in the peri-ovulatory stage, and ~1.0 nM AEA can even predict follicle/oocytes maturity, but not embryo quality [45]. Also in cattle, AEA varies similarly during estrus cycle reaching the maximum value in the peri-ovulatory phase [77].

Fluctuations of plasma FAAH and AEA levels occur physiologically also during human ovulatory cycle [78]. In the periovulatory phase, AEA and E2 levels are high and those of FAAH low, while the opposite occurs in the post-ovulatory phase [76]. Moreover, the increase of gonadotropin and AEA concentrations in serum are positively related to each other and to E2 levels, but not to P4 concentration [45]. The link between E2 and AEA is supported by the finding that E2 treatment is able to restore normal plasmatic LH and gonadotropin-releasing hormone (GnRH) levels, as well as AEA synthesis in the hypothalamus of ovariectomyzed rats, while no E2-dependent variation of 2-AG concentration could be observed [79].

3.4. Into the oviduct: the place of fertilization and early embryogenesis

The oviduct plays key roles in sperm capacitation, fertilization and embryo transport towards the implantation site. In its lower region, called isthmus or oviduct-uterine junction, uncapacitated sperm firstly bind to epithelial cells, from which they are released after capacitation and move to the upper region (ampulla), where fertilization occurs; indeed, the ovulated oocyte is driven by the fimbriae to the same ampullary region.

Several studies have clearly demonstrated that AEA and other components of ECS like NAPE-PLD, CB_1, CB_2 and FAAH are detectable in the oviduct (Fig. 3) [80,81]. In mice, CB_1 colocalization with α1 and α2 adrenergic receptors allows normal transfer to uterus [80]. The existence of a longitudinal gradient of AEA in the oviduct is required to prolong sperm fertile life and progression towards the oocyte. AEA is mostly concentrated in the isthmus, where it regulates sperm capacitation and release from oviductal cells by stimulating Ca^{2+} influx via CB_1 and TRPV1, but not CB_2 [82]. Afterwards, capacitated sperm are able to secrete AEA and 2-AG, both activating CB_1 and TRPV1 [83].

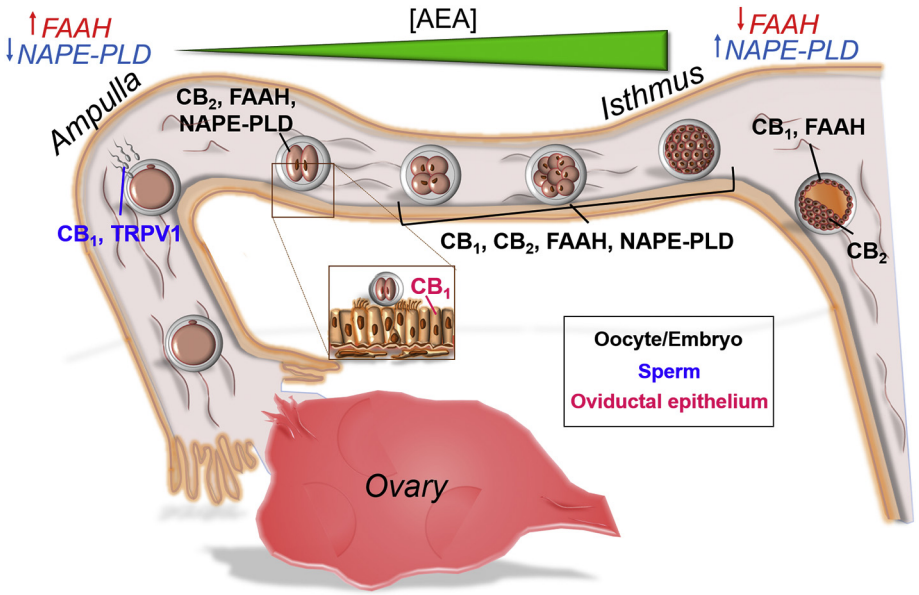


Fig. 3. ECS, embryo development and oviductal transport.
The expression of ECS components is modulated during fertilization, embryo development and oviductal transport (embryo: black; oviduct: green; sperm: blue). An increasing gradient of AEA is present in the oviduct from ampulla region to isthmus, along with the increase/decrease of AEA synthesis/degrading enzymes.
Abbreviations: AEA, *N*-arachidonoylethanolamine; CB_1, cannabinoid receptor type 1; CB_2, cannabinoid receptor type 2; FAAH, fatty acid amide hydrolase; NAPE-PLD, N-acylphosphatydil ethanolamines (NAPE)-specific phospholipase D; TRPV1, transient receptor potential vanilloid-1.

Accumulated evidence supports the hypothesis that aberrant CB_1 signaling negatively affects sperm-oocyte interaction. In women, reduced CB_1 expression in both oviduct and decidua could predispose to ectopic pregnancy [84]. Elevated levels of AEA, as those obtained in *faah-/-* mice, compromise sperm fertilizing capacity [85]. In humans, AEA binds to sperm TRPV1 and promotes sperm-egg fusion by inhibiting premature acrosome reaction and membrane fusion of non-fertilizing sperm [86,87]. In cattle, AEA can increase nitric oxide concentration in sperm, but not in oviductal cells, likely via activation of nitric oxide synthesis during capacitation [88]. Consistently, FAAH is strongly expressed in the ampullary region, and to a lesser extent in isthmus, while the opposite occurs for NAPE-PLD [89]. Maternal FAAH deficiency causes embryo retention, due to high AEA levels in the oviduct [90], that increase the number of embryos with retarted development [45].

Mouse embryo expresses CB_1 and CB_2 from 1-cell and from 4-cells up to blastocyst stage, respectively; in the blastocyst CB_2 appear localized in the inner cell mass (ICM) and CB_1 in trophectoderm cells [91]. FAAH and NAPE-PLD are both present from 2-cells to blastocyst; FAAH, in particular, has been recognized in the outer cells of morula and then in trophectoderm cells. Embryo development and transport are modulated by CB_1, because altered receptor signaling impairs dramatically embryonic development, and the use of specific receptor agonists or antagonists causes a dose-dependent increase or reduction of blastocysts number, respectively [92]. Conversely, embryonic CB_2 is unresponsive to agonist stimulation, and its role remains elusive [8]. Recently, involvement of CB_1 in regulation of embryo transport speed has been proposed [70]. Finally, embryo development and trophoblast differentiation are also impaired by high levels of AEA and 2-AG [81].

3.5. Implantation

Implantation represents the phase where the blastocyst successfully adheres to the receptive maternal endometrium. In humans, the implantation of a blastocyst is estimated to take place ~9 days after ovulation and is marked by the secretion of human chorionic gonadotropin.

eCB signaling modulates endometrial plasticity and uterine receptivity [8]. Indeed, regulation of AEA tone by FAAH and NAPE-PLD appears fundamental for implantation, and any alteration of this fine tuning can induce pregnancy failure [93], also acting on endometrial stromal cell migration via CB_1, but not CB_2 [94]. These observations are summarized in Fig. 4.

In the murine uterus, the levels of AEA and 2-AG, and the expression of the enzymes involved in their synthesis and degradation are modulated by P4 and E2 [95,96]. Timing of NAPE-PLD and FAAH expression in early pregnant mice is regulated as follows: before implantation (days 1-4), NAPE-PLD is more expressed in luminal and glandular epithelium than in stroma; on days 5-7, NAPE-PLD expression is restricted to interimplantation sites and is very low at sites of implantation; consistently, FAAH expression is the opposite. Fluctuation of AEA concentration in the uterus on day 4 of pregnancy (showing high levels in the morning and low levels in the evening) is concomitant with the expression of embryonic CB_1. Xie and collaborators [97] found that ~100 genes are irreversibly changed by an AEA-dependent CB_1 activation in day 4 embryos. Maintenance of the appropriate AEA tone requires that the adjacent inter-implantation sites have high levels of AEA and NAPE-PLD and low levels of FAAH, as indeed observed. On day 5, in coincidence with the implantation window, FAAH expression is consistently enhanced [98].

In the human uterus, while CB_2 is distributed in both glandular and stromal tissues and is maximally expressed during late proliferative phase, CB_1 endometrial distribution and regulation still remains a matter of debate. Taylor and colleagues [99] found that CB_1 was mainly expressed in the glandular epithelium and was not regulated by steroids, while Resuehr and coworkers [100] reported a CB_1 expression restricted to the stromal tissue, with a strong dependence on P4. Moreover, FAAH and NAPE-PLD were detected in both glandular and stromal cells, but their expression was orchestrated to maintain low AEA contents during the mid-luteal phase (Fig. 3). Notably, low P4/high AEA levels in serum have been considered indicative of miscarriage [101,102]. In line with these findings, El-Talatini and coworkers [45] found that women undergoing *in vitro* fertilization have very low serum AEA levels, and that LH and E2 regulate these levels from ovulation to early pregnancy.

3.6. Decidualization, placentation and labor

As soon as the blastocyst contacts the stroma, the primary decidualization reaction occurs, consisting of a rapid and sustained stimulus to increase endometrial vascular permeability at the site of implantation. The morphological and functional changes of the stromal cells surrounding the implanting blastocyst determine a localized endometrial decidualization, with luminal epithelial apoptosis and subsequent invasion of the trophoblast through the basement membrane

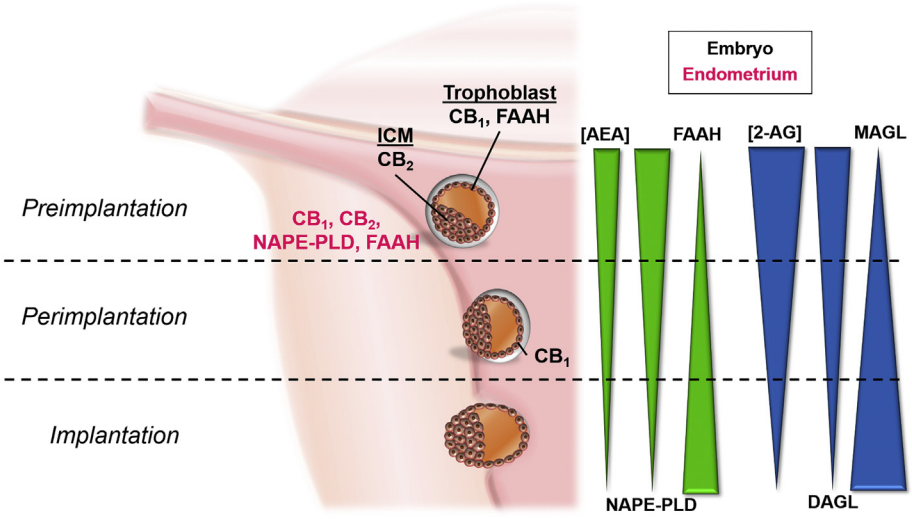


Fig. 4. ECS and embryo implantation.
AEA (green) and 2-AG (blue) fluctuations and relative biosynthetic/degradative enzymes concentrations during the process of implantation in mammals.
Abbreviations: 2-AG, 2-arachidonoylglycerol; AEA, *N*-arachidonoylethanolamine; CB_1, type 1 cannabinoid receptor; CB_2, type 2 cannabinoid receptor; DAGL, *sn-1* diacylglycerol-lipase; FAAH, fatty acid amide hydrolase; ICM, inner cell mass; MAGL, monoacylglycerol lipase; NAPE-PLD, *N*-acylphosphatydil ethanolamines (NAPE)-specific phospholipase D.

into the stroma (Fig. 4) [8]. Also trophoblast differentiation is tightly controlled by AEA, that stimulates embryonic fibronectin binding and MAPK activity [103].

In humans, ECS contributes to placenta formation. It has been found that 2-AG, MAGL and DAGL are all present in human cytotrophoblast [101,104], and that high levels of CB_1, CB_2 and FAAH mRNAs are found within the syncytiotrophoblast during the first trimester [101]. Moreover, CB_1 and FAAH are detectable in amniotic epithelial cells, chorionic cytotrophoblast and especially in syncytiotrophoblast [101]. Appropriate eCB signaling is necessary to drive normal proliferation rate of trophectodermal stem cells, because trophoblast invasiveness towards maternal decidual zona stimulates maternal blood vessel growth to support embryo development [103]. A high abortion rate in the first trimester is marked by low FAAH expression and (consistently) high AEA levels in placenta [46], FAAH being localized in the nucleus of trophoblastic cells [102,105]. Also reduced NAPE-PLD and elevated CB_1 expressions in placenta are linked to miscarriage [46].

It is generally accepted that normal labor is controlled by P and corticotrophin hormone, but recent findings point to ECS as a key regulator also of the final event of pregnancy.

Experiments in mice showed that *cnr1*, but not *cnr2*, deficiency determines a high incidence of preterm birth, likely due to decreased P/E2 ratio [90,106]. The concomitant alteration of corticotrophin hormone/corticosterone axis in these mice strongly supports the existence of a regulative axis between ECS and these hormones. Also in women AEA controls the onset and duration of labor [106,107]. Indeed, plasma AEA content rises significantly (1.5-fold, from ~1.2 to ~1.8 nM) only in women at term. Such an increase is controlled by P via FAAH, which in turns regulates AEA-dependent activation of CB receptors [108]. Indeed, CB_1 and FAAH are highly expressed by placental cells at term [76,78]. More recently, Sun and collaborators [109] showed that *faah-/-* mice exposed to inflammatory stimuli were more prone to preterm birth, because decidual cells underwent premature senescence with increased p38 MAPK signaling. Thus, high AEA tone activates CB_1-dependent senescence-associated enzymes, leading to preterm birth. When these *faah-/-* mice were treated with metformin, decidual senescence was significantly attenuated without adverse effects on pregnancy outcome [110]. In addition, also a high plasma level of PEA has been recently recognized as a biomarker of preterm birth [111].

4. Endocannabinoids as biomarkers of reproductive diseases

The National Institutes of Health define a serum biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological and pathogenic processes, or pharmacological responses to a therapeutic intervention" [112]. Accordingly, in reproductive female medicine biomarkers are useful to follow physiological reproductive functions or to diagnose reproductive defects. In

Table 3
eCBs, NAEs and other ECS elements as biomarkers of female human reproduction.

eCBs and NAEs	Fluids, cells and tissues	Reproductive Conditions	Potential biomarker role	References
AEA	Plasma	Menstrual cycle	Uterine Receptivity	[45,107,113]
		Early Pregnancy	Progression of Pregnancy and/or Embryo Transfer	[45,155]
		Labor	Parturition	[107,108,114]
AEA, OEA and PEA	Plasma	Early pregnancy complications	Ectopic Pregnancy	[126]
AEA	Decidual cells/Uterine tissue	Endometrium preparation	Natural or Assisted Pregnancy	[115,116]
2-AG			Implantation and Uterine Remodelling	[95,117]
2-AG	Cytotrophoblasts/choriocarcinoma cells	Altered placental development	Placenta Development	[118]
AEA	Serum	Anovulation (Infertility)	PCOS	[120–122]
AEA	Placentas	Pregnancy disorder	Preeclampsia	[123]
2-AG	Endometrial Carcinoma	Gynecological cancer	Growth of Endometrial Carcinoma	[129]
AEA and PEA	Plasma and Endometrial tissues		Early Diagnosis of Endometrial Carcinoma	[42,129,130]
Other ECS components				
CB_1 and CB_2	Endometrial tissues	Gynecological cancer	Endometrial Carcinoma	[130]
MAGL, GPR55 and CB_1	Ovarian cancer cells/ epithelial ovarian tumors	Gynecological cancer	Ovarian Cancer	[132–134]
FAAH, PEA, CB_1 and CB_2	Uterine leiomyomas	Non-cancerous pelvic tumors	Leiomyomata	[43]
FAAH-2	Blastocoel fluid	Infertility diagnosis	Embryo quality	[119]

eCBs, endocannabinoids; NAEs, *N*-acylethanolamines; ECS, endocannabinoid system. See text for additional abbreviations and details.

line with this, eCBs and other ECS elements have been proposed as suitable biomarkers of fertility alterations (Table 3). AEA and 2-AG as well as their congeners PEA and OEA have been quantified in several fluids like blood, plasma, follicular fluid, amniotic fluid, breast milk and oviductal fluid from women at different stages of the menstrual cycle, or undergoing *in vitro* fertilization and embryo transfer programs [41]. It was found that spatiotemporal distribution of AEA undergoes specific changes, in order to support pregnancy onset: during the early stage, at the implantation site low levels are needed to promote uterine receptivity and maintenance of pregnancy, while at the time of labor high levels may be useful for parturition, probably because AEA hydrolysis releases arachidonic acid, which in turn increases the concentration of prostaglandins [45]. In particular, AEA levels fluctuate during the menstrual cycle from the follicular phase (~1.5 nM) to ovulation, where they remain high until the luteal phase (~0.8 nM) [107,113]. Furthermore, AEA levels in the active labor are high to ensure the final stage of pregnancy [107,108,114].

In addition, low AEA levels may be essential for the onset of decidualization, where the endometrium is remodeled in preparation for pregnancy through a dramatic morphological and functional differentiation of human endometrial stromal cells [115]. Any perturbation of this phase is associated with pregnancy alterations, such as infertility, recurrent miscarriages, and utero-placental disorders. Consistently, AEA levels in decidualizing cells were found to be lower than those in non-differentiated cells, whereas prostaglandin E2 levels and COX-2 expression were up-regulated [116]. Thus, AEA levels in plasma of pregnant women might become a diagnostic biomarker for natural or assisted pregnancy outcome. On the other hand, since murine decidual cells and uterine tissues show higher levels of 2-AG than of AEA [95,117], it is likely that also 2-AG plays a role in the implantation and uterine remodelling. In line with this, an involvement of 2-AG signaling in cytotrophoblast cell turnover has been described, whereby a deregulation may be responsible for altered placental development and poor pregnancy outcome [118]. In particular, investigation of 2-AG metabolic enzymes in primary human cytotrophoblasts and in human choriocarcinoma BeWo cells allowed to propose 2-AG as a potentially important biomarker of placenta development [118].

Interestingly, a reliable and sensitive microextraction-chemical derivatization method coupled with liquid chromatography-tandem mass spectrometry (LC-MS) has been used for the quantification of eCBs and NAEs in peripheral serum and follicle fluids from infertile women [49]. In this study, significant differences were found in serum AEA and PEA levels, as well as in follicular fluid OEA content, between infertile and fertile women [49], opening the avenue to their further exploitation as potential biomarkers of female infertility.

Of note, maternal aging is a common cause of female infertility, that increases the rate of implantation failure probably due to old blastocysts. In this context, a recent proteomic study showed that levels of FAAH-2, highly expressed in higher placental mammals [33], are lower in the blastocoel fluid of aged woman with respect to young ones [119]. Hence, this eCB hydrolase may serve as potential biomarker of embryo quality [119].

Altered eCB levels can be found under some pathological conditions, as well as in pregnancy complications where they could become candidate hallmarks [26,52]. For instance, serum AEA could serve as a potential biomarker for the diagnosis of polycystic ovary syndrome (PCOS), a metabolic and endocrine disorder that commonly causes infertility [120,121]. Indeed, a significant increase in plasma AEA has been documented in women with PCOS compared with infertile women without PCOS, along with lower endometrial expression of FAAH in the PCOS group [122]. Moreover, a recent study has shown low serum levels of AEA in women with preeclampsia [123], and a markedly higher expression of CB_1 protein in preeclamptic placental tissues [124]. In placentas from women with preeclampsia also higher and lower expression of NAPE-PLD and FAAH, respectively, was observed [125]. Collectively, these data suggest a potential role of AEA-dependent signaling in preeclampsia, and support serum AEA as a putative biomarker of this disorder. Finally, ectopic pregnancy has been associated with elevated levels of eCBs and reduced FAAH activity, and notably OEA seems to drive embryo-tubal transport by decreasing cilia beat frequency in Fallopian tube epithelial cells [126].

Deregulation of distinct ECS components has been highlighted also in gynecological cancers [52,107,127,128]. For instance, 2-AG levels are elevated as a result of reduced MAGL expression, and CB_2 is significantly up-regulated in biopsies from women affected by endometrial carcinoma (EnCa) [129]. Also AEA levels are elevated in patients with EnCa, in both plasma and endometrial tissue [129], and indeed AEA induces a decrease in EnCa (Ishikawa) cell number, probably due to inhibition of cell proliferation [130]. These data suggest that increased plasma and tissue AEA concentrations in EnCa patients may be a countermeasure against further cancer growth, and point to AEA-related ECS elements as potentially new therapeutic targets [130]. In line with this, transcript levels of CB_1 and CB_2 have been found to be significantly decreased in endometrial biopsies from women with EnCa, and their expression could serve as a new histological marker to treat and/or prevent EnCa [131]. More recently, positive correlations between plasma concentrations and tissue levels of AEA and PEA have been found in patients with EnCa, and were linked to an imbalanced E2/P4 ratio, known to be involved in EnCa development [42]. In addition, a possible role of ECS has been recognized in uterine leiomyomas (fibroids), that are the most common non-cancerous pelvic tumors found in women [43]. A significantly lower FAAH expression was shown in fibroids, thus favoring higher AEA levels in pre-menopausal tissues, along with significantly lower PEA levels particularly in post-menopausal women, who may be protected against fibroid pathogenesis. In addition, the CB_1/CB_2 ratio was lower in fibroids, suggesting that loss of CB_1 expression may affect the fibroid cell phenotype [43]. Significant correlations between reduced FAAH, CB_1, GPR55 expression and PEA levels in fibroids indicated that loss of these selected ECS components could mark leiomyomata [43].

As far as other gynecoalogical pathologies are concerned, MAGL and its fatty acid products were found to be elevated in aggressive ovarian cancer (OC) cells, as well as in high-grade primary human ovarian tumours [132]. Also GPR55 expression increased in OC cells (like OVCAR3 and A2780), and its downregulation by means of siRNAs or pharmacological blockade led to strong inhibition of cell proliferation [133]. These findings point to GPR55 as a potential therapeutic target of OC. In line with this, CB_1 expression has been found to increase in human epithelial ovarian tumors, from benign up to malignant stadium [134]. Thus, CB_1 might be used in clinical practice, alone or in combination with other markers, to identify or better characterize ovarian tumors [134].

Based on available evidence, the possible exploitation of selected ECS elements as potential biomarkers for monitoring physiological conditions (i.e., pregnancy), as well as pathological events like infertility, pregnancy complications and gynecological cancers, summarized in Table 3, may open new arenas for prediagnosis and care in reproductive clinical practice.

5. Current issues and future challenges for clinical practice

Over the last few years, technical improvements of LC-MS methods (such as nanoLC/nanoESI-MS system and various sample pretreatment strategies, i.e. chemical derivatization) have led to better analytical performances in terms of improved linearity, limit of detection, limit of quantitation, precision, recovery, and matrix effect. As a consequence, quantitative determination of eCBs was made possible also in tiny biological samples like female reproductive fluids [41,49,135]. Unfortunately, additional factors such as low chemical stability of eCBs (i.e., due to acyl transmigration from position 2 to position 1 in arachidonoyl-glycerol) during and after sampling may increase variability and inaccuracy of the measurements, and so do delays in processing

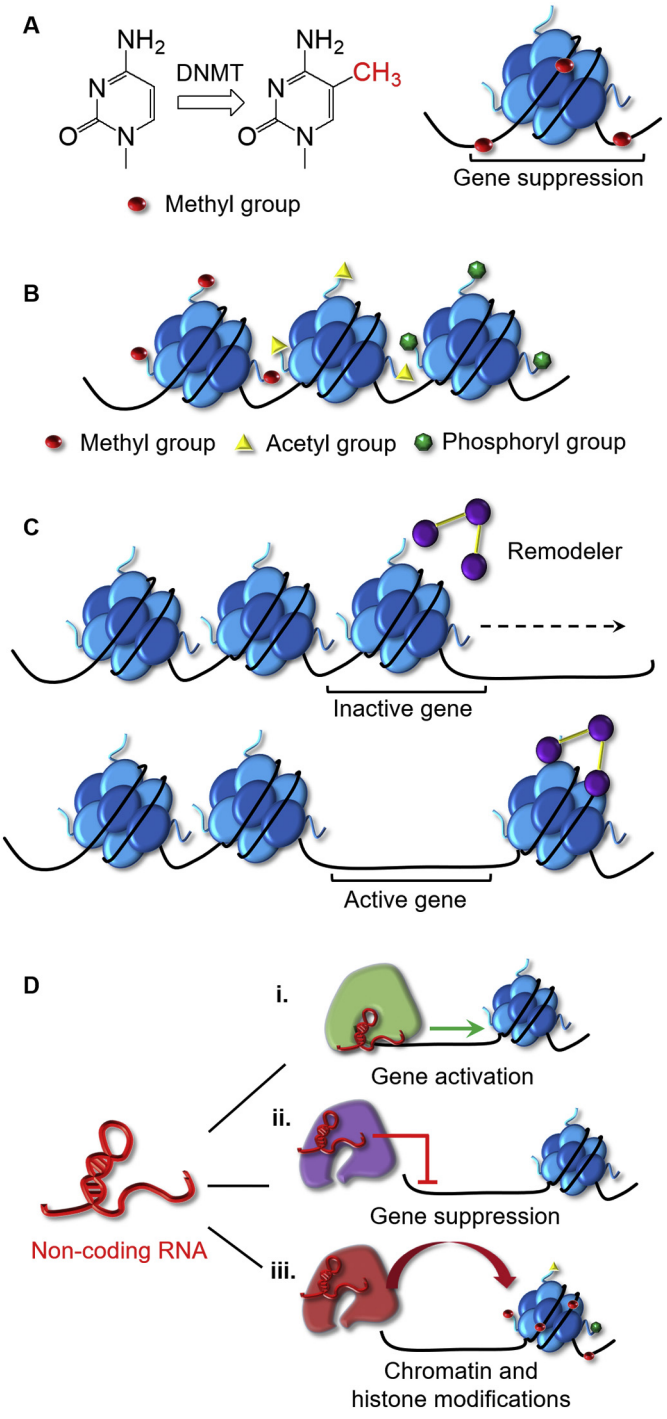


Fig. 5. Epigenetic modifications.
Schematic representation of the major epigenetic modifications: DNA methylation (A), histone modifications (B), histone positioning (C), and non-coding RNA (D).
Abbreviations: DNMT, DNA methyltransferase.

and freeze–thaw cycles. Furthermore, it seems of note that low concentrations of eCBs can be found in organic solvents, plastics and glassware [136], thus calling for caution when measuring these compounds. Recently, an innovative liquid biosensor (BIONOTE) was developed, able to quantify eCBs content by means of a screen-printed electrode probe and a dedicated electronic interface [137]. This BIONOTE sensor was shown to measure both AEA and 2-AG concentrations with a threshold of ~7 nM and ~24 nM, respectively [137]. Though its use as an easy and cheap tool to screen eCBs content at large needs to be further validated, BIONOTE seems to hold promise for the detection and quantification of eCBs in female reproductive matrices (e.g., cells, tissues and fluids), eventually to be used as biomarkers of infertility or gynecological disorders.

6. (Endo)cannabinoids and epigenetic modulation

Epigenetic modifications include a series of biochemical processes, such as DNA methylation, histone modification, histone positioning and non-coding RNAs (Fig. 5). These modifications produce heritable changes in gene expression during chromatin development, without changes in DNA sequences, and might affect the early and late stages of gametogenesis, ultimately leading to reproductive infertility [138].

Epigenetic alterations brought about by environmental insults, including cannabis exposure during pregnancy, may have transgenerational and immunological consequences for offspring [18]. It is possible that during THC exposure *in utero*, epigenetic alterations might occur in the brain, which would be propagated throughout development stably enough to cause enduring phenotypical abnormalities [139]. For instance, maternal cannabis use alters developmental regulation of mesolimbic dopamine receptor subtype 2 in offspring through histone lysine methylation, and the ensuing reduction of this receptor may contribute to increased vulnerability to drug abuse later in life [140]. It has been demonstrated that THC at the concentration detected in patients using cannabis for recreational use [141] inhibits cytotrophoblast BeWo cell proliferation in a dose-dependent manner and increases the transcription of histone deacetylase 3 [142]. The increased expression of the latter enzyme upon THC exposure may suggest a link between inhibition of cytotrophoblast cell cycle progression and subsequent placental development [142].

Among the different ECS components, growing attention has been paid to the epigenetic regulation of both cannabinoid receptors, that in different cellular models occurs by both histone modification and DNA methylation [143]. Indeed, CB_1 and CB_2 are engaged in several immune responses [144] that are activated by plant-derived cannabinoids through epigenetic marks [145]. For instance, THC exposure of pregnant mice results in markedly defective T cell differentiation and impaired T cell function in offspring, that are reversed by CB_1 and CB_2 antagonists [146]. Hence, during pregnancy a profound T cell dysfunction could lead to increased susceptibility of offspring to certain infections and cancers. More recently, histone modifications have been associated with THC-mediated alterations in antigen-specific T cell response [147]. In particular, THC was proposed as a driver of a switch from T helper 1 genes to T helper 2 genes [147]. Keeping in mind that the balance between these two genes is a key in reproductive immunology [8], the potential impact of this epigenetic activity of THC (and potentially also of other cannabinoids) on female fertility seems apparent.

More recently, one of the circular RNA isoforms of NAPE-PLD transcript (circNAPEPLD) was found to be largely expressed in mammalian sperm, possibly leading to the expression of a short form of NAPE-PLD protein [148]. Of note, such a circNAPEPLD may be transferred from sperm to oocytes during fertilization, and it may function as a decoy to inhibit the anti-proliferative activity of some oocyte-derived miRNAs, thus allowing cell proliferation during the first stages of embryo development [148].

Additional lifestyle factors, including behavior, nutrition, and exposure to toxins and pollutants, are known to be associated with epigenetic modifications. In this context, maternal high fat diet can promote obesity and hypothalamic leptin resistance in male rat offspring at weaning and adulthood [149]. It should be recalled that obesity is a condition closely associated with an overactive ECS [150]. A recent study hypothesized that maternal high fat diet down-regulates leptin signaling and up-regulates CB_1 mRNA levels in the hypothalamus of the offspring at birth, associated with sex-specific changes in epigenetic markers and sex steroid signaling [151]. Besides, maternal high fat diet

increased histone acetylation of *cnr1* gene promoter (encoding for CB_1) in male offspring, and increased androgen receptor binding to the same promoter, thus contributing to higher expression of CB_1 in newborn offspring [151].

Future investigations are warranted, to better interrogate the impact of epigenetic regulation of ECS genes on female reproductive events, in both humans and animal studies.

7. Conclusions and future directions

The overall picture derived from available data suggests that a fine tuning of the ECS in female fertility contributes to wellness and success of reproductive events, from gametogenesis to fertilization, embryo implantation, fetal development and parturition. Instead, dysregulation of the eCB tone impairs reproductive performances by altering either hormonal homeostasis (e.g., endometriosis, gynecological cancers) or various reproductive steps, as those causing miscarriages and pregnancy complications (e.g., ectopic pregnancies and preeclampsia). In this context, eCB-based drugs should be developed as treatment for female infertility. To date, creams containing the endogenous cannabimimetic compound PEA appeared useful for the treatment of chronic pelvic and vaginal pains [152,153]. In addition, an association between micronized PEA and the polyphenolic compound transpolydatin proved to be effective in the management of pelvic pain related to endometriosis after laparoscopy [154]. Lastly, a novel approach to the development of effective cannabinoid-based treatments for endometriosis, dysmenorrhoea and menopause symptoms is being investigated in Israel, and medical cannabis vaginal suppositories are currently available in the United States for women suffering from pelvic conditions.

Also the involvement of ECS in ovarian and uterine cancers has allowed the identification of new targets able to arrest disease progression, and the discovery of novel mechanisms linking ECS to sex hormone signaling, immune system and epigenetic modifications.

Excitation for this challenging arena is dampened by the prevalence of use of illicit drugs by women of reproductive age before and during pregnancy. Cannabis is the most common illicit drug used, and a recent study reported that more than 1 in 10 pregnant and nonpregnant women used marijuana in the past 12 months [10], also because they understimated the associated risks [10]. Therefore, it seems now urgent to define the true impact of cannabis on reproduction, in particular during pregnancy [155], and its long-term outcomes on future children.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

None.

Acknowledgements

The authors wish to thank all colleagues who contributed over the last 25 years to their studies on endocannabinoid signaling in female reproduction.

References

[1] MC Inhorn, P Patrizio, Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century, Hum Reprod Update 21 (4) (2015) 411–426, https://doi.org/10.1093/humupd/dmv016.
[2] FJ Broekmans, EA Knauff, ER te Velde, NS Macklon, BC Fauser, Female reproductive ageing: current knowledge and future trends, Trends Endocrinol Metab 18 (2) (2007) 58–65, https://doi.org/10.1016/j.tem.2007.01.004.
[3] GM Lockwood, Social egg freezing: the prospect of reproductive "immortality" or a dangerous delusion? Reprod Biomed Online 23 (3) (2011) 334–340, https://doi.org/10.1016/j.rbmo.2011.05.010.
[4] National Institute on Alcohol Abuse and Alcoholism, Alcohol facts and statistics, National Institute on Alcohol Abuse and Alcoholism (NIAAA), 2017, https://www.niaaa.nih.gov/alcohol-health/overview-alcoholconsumption/alcohol-facts-and-statistics Accessed on May 2019.
[5] BV Rossi, KF Berry, MD Hornstein, DW Cramer, S Ehrlich, SA Missmer, Effect of alcohol consumption on in vitro fertilization, Obstet Gynecol 117 (1) (2011) 136–142, https://doi.org/10.1097/AOG.0b013e31820090e1.
[6] K Van Heertum, B Rossi, Alcohol and fertility: how much is too much? Fertil Res Pract 3 (2017) 10, https://doi.org/10.1186/s40738-017-0037-x.
[7] The Practice Committee of the American Society for Reproductive Medicine (PCASRM), Smoking and infertility, Fertil Steril 81 (4) (2004) 1181–1186, https://doi.org/10.1016/j.fertnstert.2003.11.024.
[8] M Maccarrone, Endocannabinoids: friends and foes of reproduction, Prog Lipid Res 48 (6) (2009) 344–354, https://doi.org/10.1016/j.plipres.2009.07.001.
[9] MR Hayatbakhsh, AM Kingsbury, V Flenady, KS Gilshenan, DM Hutchinson, JM Najman, Illicit drug use before and during pregnancy at a tertiary maternity hospital 2000-2006, Drug Alcohol Rev 30 (2) (2011) 181–187, https://doi.org/10.1111/j.1465-3362.2010.00214.x.
[10] JY Ko, SL Farr, VT Tong, AA Creanga, WM Callaghan, Prevalence and patterns of marijuana use among pregnant and nonpregnant women of reproductive age, Am J Obstet Gynecol 213 (2) (2015) 201.e1–201.e10, https://doi.org/10.1016/j.ajog.2015.03.021.
[11] PH Shiono, MA Klebanoff, RP Nugent, MF Cotch, DG Wilkins, DE Rollins, et al., The impact of cocaine and marijuana use on low birth weight and preterm birth: a multicenter study, Am J Obstet Gynecol 172 (1 Pt 1) (1995) 19–27, https://doi.org/10.1016/0002-9378(95)90078-0.
[12] DM Fergusson, LJ Horwood, K Northstone, ALSPAC Study Team. Avon longitudinal study of pregnancy and childhood. maternal use of cannabis and pregnancy outcome, BJOG 109 (1) (2002) 21–27, https://doi.org/10.1111/j.1471-0528.2002.01020.x.
[13] D Marchetti, G Di Masi, F Cittadini, G La Monaca, N De Giovanni, Placenta as alternative specimen to detect in utero cannabis exposure: a systematic review of the literature, Reprod Toxicol 73 (2017) 250–258, https://doi.org/10.1016/j.reprotox.2017.06.049.
[14] X Sun, SK Dey, Synthetic cannabinoids and potential reproductive consequences, Life Sci 97 (1) (2014) 72–77, https://doi.org/10.1016/j.lfs.2013.06.018.
[15] MW Varner, RM Silver, CJ Rowland Hogue, M Willinger, CB Parker, VR Thorsten, et al., Eunice Kennedy Shriver National Institute of Child Health and Human Development Stillbirth Collaborative Research Network. Association between stillbirth and illicit drug use and smoking during pregnancy, Obstet Gynecol 123 (1) (2014) 113–125, https://doi.org/10.1097/AOG.0000000000000052.
[16] KA Richardson, AK Hester, GL McLemore, Prenatal cannabis exposure - The "first hit" to the endocannabinoid system, Neurotoxicol Teratol 58 (2016) 5–14, https://doi.org/10.1016/j.ntt.2016.08.003.
[17] TD Metz, AA Allshouse, CJ Hogue, RL Goldenberg, DJ Dudley, MW Varner, et al., Maternal marijuana use, adverse pregnancy outcomes, and neonatal morbidity, Am J Obstet Gynecol 217 (4) (2017) 478.e1–478.e8, https://doi.org/10.1016/j.ajog.2017.05.050.
[18] EE Zumbrun, JM Sido, PS Nagarkatti, M Nagarkatti, Epigenetic regulation of immunological alterations following prenatal exposure to marijuana cannabinoids and its long term consequences in offspring, J Neuroimmune Pharmacol 10 (2) (2015) 245–254, https://doi.org/10.1007/s11481-015-9586-0.
[19] TD Metz, EH Stickrath, Marijuana use in pregnancy and lactation: a review of the evidence, Am J Obstet Gynecol 213 (6) (2015) 761–778, https://doi.org/10.1016/j.ajog.2015.05.025.
[20] P Merlob, B Stahl, G Klinger, For Debate: does cannabis use by the pregnant mother affect the fetus and newborn? Pediatr Endocrinol Rev 15 (1) (2017) 4–7, https://doi.org/10.17458/per.vol15.2017.msk.fd.cannabispregnantmother.
[21] JW Huffman, LW Padgett, Recent developments in the medicinal chemistry of cannabimimetic indoles, pyrroles and indenes, Curr Med Chem 12 (2005) 1395–1411, https://doi.org/10.2174/0929867054020864.
[22] SC Jaques, A Kingsbury, P Henshcke, C Chomchai, S Clews, J Falconer, et al., Cannabis, the pregnant woman and her child: weeding out the myths, J Perinatol 34 (6) (2004) 417–424, https://doi.org/10.1038/jp.2013.180.
[23] KA Belendiuk, LL Baldini, MO Bonn-Miller, Narrative review of the safety and efficacy of marijuana for the treatment of commonly state-approved medical and psychiatric disorders, Addict Sci Clin Pract 10 (2015) 10, https://doi.org/10.1186/s13722-015-0032-7.
[24] M El Sohly, W Gul, Constituents of Cannabis sativa, in: RG Pertwee (Ed.), Handbook of Cannabis, Oxford University Press, 2014, pp. 3–22.
[25] RG Pertwee, RA Ross, Cannabinoid receptors and their ligands, Prostaglandins Leukot Essent Fatty Acids 66 (2–3) (2002) 101–121, https://doi.org/10.1054/plef.2001.0341.
[26] M Maccarrone, I Bab, T Bíró, GA Cabral, SK Dey, V Di Marzo, et al., Endocannabinoid signaling at the periphery: 50 years after THC, Trends Pharmacol Sci 36 (5) (2015) 277–296, https://doi.org/10.1016/j.tips.2015.02.008.
[27] M Maccarrone, Metabolism of the endocannabinoid anandamide: Open questions after 25 years, Front Mol Neurosci 10 (2017) 166, https://doi.org/10.3389/fnmol.2017.00166.
[28] C Rapino, D Tortolani, L Scipioni, M Maccarrone, Neuroprotection by (endo)Cannabinoids in glaucoma and retinal neurodegenerative diseases, Curr Neuropharmacol 16 (7) (2018) 959–970, https://doi.org/10.2174/1570159X15666170724104305.

[29] MP Baggelaar, M Maccarrone, M van der Stelt, 2-Arachidonoylglycerol: a signaling lipid with manifold actions in the brain, Prog Lipid Res 71 (2018) 1–17, https://doi.org/10.1016/j.plipres.2018.05.002.

[30] V Chiurchiù, M van der Stelt, D Centonze, M Maccarrone, The endocannabinoid system and its therapeutic exploitation in multiple sclerosis: clues for other neuroinflammatory diseases, Prog Neurobiol 160 (2018 Jan) 82–100.

[31] BM Fonseca, NA Teixeira, M Almada, AH Taylor, JC Konje, G Correia-da-Silva, Modulation of the novel cannabinoid receptor - GPR55 - during rat fetoplacental development, Placenta 32 (6) (2011) 462–469, https://doi.org/10.1016/j.placenta.2011.03.007.

[32] MA Costa, BM Fonseca, A Mendes, J Braga, NA Teixeira, G Correia-da-Silva, The endocannabinoid 2-arachidonoylglycerol dysregulates the synthesis of proteins by the human syncytiotrophoblast, Biochim Biophys Acta 1861 (3) (2016) 205–212, https://doi.org/10.1016/j.bbalip.2015.12.008.

[33] BQ Wei, TS Mikkelsen, MK McKinney, ES Lander, BF Cravatt, A second fatty acid amide hydrolase with variable distribution among placental mammals, J Biol Chem 281 (48) (2006) 36569–36578, https://doi.org/10.1074/jbc.M606646200.

[34] M van Tienhoven, J Atkins, Y Li, P Glynn, Human neuropathy target esterase catalyzes hydrolysis of membrane lipids, J Biol Chem 277 (23) (2002) 20942–20948, https://doi.org/10.1042/0264-6021:3610119.

[35] JL Blankman, GM Simon, BF Cravatt, A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol, Chem Biol 14 (12) (2007) 1347–1356, https://doi.org/10.1016/j.chembiol.2007.11.006.

[36] MN Drehmer, YCN Muniz, AR Marrero, SE Löfgren, Gene expression of ABHD6, a key factor in the Endocannabinoid System, Can Be modulated by female hormones in human immune cells, Biochem Genet 57 (1) (2019) 35–45, https://doi.org/10.1007/s10528-018-9871-8.

[37] S Nakane, S Oka, S Arai, K Waku, Y Ishima, A Tokumura, et al., 2-Arachidonoyl-sn-glycero-3-phosphate, an arachidonic acid-containing lysophosphatidic acid: occurrence and rapid enzymatic conversion to 2-arachidonoyl-sn-glycerol, a cannabinoid receptor ligand, in rat brain, Arch Biochem Biophys 402 (1) (2002) 51–58, https://doi.org/10.1016/S0003-9861(02)00038-3.

[38] A Chicca, S Nicolussi, R Bartholomäus, M Blunder, A Aparisi Rey, V Petrucci, et al., Chemical probes to potently and selectively inhibit endocannabinoid cellular reuptake, Proc Natl Acad Sci U S A 114 (25) (2017) E5006–E5015, https://doi.org/10.1073/pnas.1704065114.

[39] S Oddi, F Fezza, N Pasquariello, C De Simone, C Rapino, E Dainese, et al., Evidence for the intracellular accumulation of anandamide in adiposomes, Cell Mol Life Sci 65 (5) (2008) 840–850, https://doi.org/10.1007/s00018-008-7494-7.

[40] M Maccarrone, V Gasperi, MV Catani, TA Diep, E Dainese, HS Hansen, et al., The endocannabinoid system and its relevance for nutrition, Annu Rev Nutr 30 (2010) 423–440, https://doi.org/10.1146/annurev.nutr.012809.104701.

[41] C Rapino, N Battista, M Bari, M Maccarrone, Endocannabinoids as biomarkers of human reproduction, Hum Reprod Update 20 (4) (2014) 501–516, https://doi.org/10.1093/humupd/dmu004.

[42] T Ayakannu, AH Taylor, TH Marczylo, M Maccarrone, JC Konje, Identification of novel predictive biomarkers for endometrial malignancies: N-Acylethanolamines, Front Oncol 11 (9) (2019) 430, https://doi.org/10.3389/fonc.2019.00430.

[43] T Ayakannu, AH Taylor, TH Marczylo, JC Konje, New insights of Uterine Leiomyoma pathogenesis: Endocannabinoid system, Med Sci Monit Basic Res 25 (2019) 76–87, https://doi.org/10.12659/MSMBR.914019.

[44] WS Ho, DA Barrett, MD Randall, "Entourage" effects of N-palmitoylethanolamide and N-oleoylethanolamide on vasorelaxation to anandamide occur through TRPV1 receptors, Br J Pharmacol 155 (6) (2008) 837–846, https://doi.org/10.1038/bjp.2008.324.

[45] MR El-Talatini, AH Taylor, JC Konje, Fluctuation in anandamide levels from ovulation to early pregnancy in in-vitro fertilization-embryo transfer women, and its hormonal regulation, Hum Reprod 24 (8) (2009) 1989–1998, https://doi.org/10.1093/humrep/dep065.

[46] E Trabucco, G Acone, A Marenna, R Pierantoni, G Cacciola, T Chioccarelli, et al., Endocannabinoid system in first trimester placenta: low FAAH and high CB1 expression characterize spontaneous miscarriage, Placenta 30 (6) (2009) 516–522, https://doi.org/10.1016/j.placenta.2009.03.015.

[47] AH Taylor, AA Amoako, K Bambang, T Karasu, A Gebeh, PM Lam, et al., Endocannabinoids and pregnancy, Clin Chim Acta 411 (13–14) (2010) 921–930, https://doi.org/10.1016/j.cca.2010.03.012.

[48] BM Fonseca, G Correia-da-Silva, AH Taylor, PM Lam, TH Marczylo, JC Konje, et al., Characterisation of the endocannabinoid system in rat haemochorial placenta, Reprod Toxicol 34 (3) (2012) 347–356, https://doi.org/10.1016/j.reprotox.2012.05.036.

[49] J Ding, XT Luo, YR Yao, HM Xiao, MQ Guo, Investigation of changes in endocannabinoids and N-acylethanolamides in biofluids, and their correlations with female infertility, J Chromatogr A 1509 (2017) 16–25, https://doi.org/10.1016/j.chroma.2017.06.029.

[50] J Maia, M Almada, A Silva, G Correia-da-Silva, N Teixeira, SI Sá, et al., The endocannabinoid system expression in the female reproductive tract is modulated by estrogen, J Steroid Biochem Mol Biol 174 (2017) 40–47, https://doi.org/10.1016/j.jsbmb.2017.07.023.

[51] S Cecconi, G Rossi, A Castellucci, G D'Andrea, M Maccarrone, Endocannabinoid signaling in mammalian ovary, Eur J Obstet Gynecol Reprod Biol 178 (2014) 6–11, https://doi.org/10.1016/j.ejogrb.2014.04.011.

[52] OS Walker, AC Holloway, S Raha, The role of the endocannabinoid system in female reproductive tissues, J Ovarian Res 12 (1) (2019) 3, https://doi.org/10.1186/s13048-018-0478-9.

[53] G Rossi, V Di Nisio, G Macchiarelli, SA Nottola, I Halvaei, L De Santis, et al., Technologies for the production of fertilizable mammalian oocytes, Appl Sci 9 (8) (2019) 1536, https://doi.org/10.3390/app9081536.

[54] PA Hunt, TJ Hassold, Human female meiosis: what makes a good egg go bad? Trends Genet 24 (2) (2008) 86–93, https://doi.org/10.1016/j.tig.2007.11.010.

[55] S Cecconi, C Ciccarelli, M Barberi, G Macchiarelli, R Canipari, Granulosa cell-oocyte interactions, Eur J Obstet Gynecol Reprod Biol 115 (Suppl. 1) (2004) S19–S22, https://doi.org/10.1016/j.ejogrb.2004.01.010.

[56] M Zuccotti, V Merico, S Cecconi, CA Redi, S Garagna, What does it take to make a developmentally competent mammalian egg? Hum Reprod Update 17 (4) (2011) 525–540, https://doi.org/10.1093/humupd/dmr009.

[57] R Canipari, V Cellini, S Cecconi, The ovary feels fine when paracrine and autocrine networks cooperate with gonadotropins in the regulation of folliculogenesis, Curr Pharm Des 18 (3) (2012) 245–255, https://doi.org/10.2174/138161212799040411.

[58] G Coticchio, G Rossi, A Borini, C Grøndahl, G Macchiarelli, C Flamigni, et al., Mouse oocyte meiotic resumption and polar body extrusion in vitro are differentially influenced by FSH, epidermal growth factor and meiosis-activating sterol, Hum Reprod 19 (12) (2004) 2913–2918, https://doi.org/10.1093/humrep/deh514.

[59] S Cecconi, A Mauro, G Capacchietti, P Berardinelli, N Bernabò, AR Di Vincenzo, et al., Meiotic maturation of incompetent prepubertal sheep oocytes is induced by paracrine factor(s) released by gonadotropin-stimulated oocyte-cumulus cell complexes and involves mitogen-activated protein kinase activation, Endocrinology 149 (1) (2008) 100–107, https://doi.org/10.1210/en.2007-0874.

[60] S Cecconi, A Mauro, V Cellini, F Patacchiola, The role of Akt signalling in the mammalian ovary, Int J Dev Biol 56 (10–12) (2012) 809–817, https://doi.org/10.1387/ijdb.120146sc.

[61] MG Palmerini, SA Nottola, WA Tunjung, A Kadowaki, S Bianchi, S Cecconi, et al., EGF-FSH supplementation reduces apoptosis of pig granulosa cells in co-culture with cumulus-oocyte complexes, Biochem Biophys Res Commun 481 (1–2) (2016) 159–164, https://doi.org/10.1016/j.bbrc.2016.10.151.

[62] CM Gammon, GM Freeman Jr, W Xie, SL Petersen, WC Wetsel, Regulation of gonadotropin-releasing hormone secretion by cannabinoids, Endocrinology 146 (10) (2005) 4491–4499, https://doi.org/10.1210/en.2004-1672.

[63] AM Jukic, CR Weinberg, DD Baird, AJ Wilcox, Lifestyle and reproductive factors associated with follicular phase length, J Womens Health (Larchmt) 16 (9) (2007) 1340–1347, https://doi.org/10.1089/jwh.2007.0354.

[64] E De Domenico, F Todaro, G Rossi, S Dolci, R Geremia, P Rossi, et al., Overactive type 2 cannabinoid receptor induces meiosis in fetal gonads and impairs ovarian reserve, Cell Death Dis 8 (10) (2017) e3085, https://doi.org/10.1038/cddis.2017.496.

[65] MR El-Talatini, AH Taylor, JC Elson, L Brown, AC Davidson, JC Konje, Localisation and function of the endocannabinoid system in the human ovary, PLoS One 4 (2) (2009) e4579, , https://doi.org/10.1371/journal.pone.0004579.

[66] L Peralta, E Agirregoitia, R Mendoza, A Expósito, L Casis, R Matorras, et al., Expression and localization of cannabinoid receptors in human immature oocytes and unfertilized metaphase-II oocytes, Reprod Biomed (2011) 372–379, https://doi.org/10.1016/j.rbmo.2011.05.011.

[67] E Agirregoitia, I Ibarra-Lecue, L Totorikaguena, R Mendoza, A Expósito, R Matorras, et al., Dynamics of expression and localization of the cannabinoid system in granulosa cells during oocyte nuclear maturation, Fertil Steril 104 (3) (2015) 753–760, https://doi.org/10.1016/j.fertnstert.2015.06.013.

[68] E Agirregoitia, L Totorikaguena, A Expósito, R Mendoza, R Matorras, N Agirregoitia, Dynamic of expression and localization of cannabinoid-degrading enzymes FAAH and MGLL in relation to CB1 during meiotic maturation of human oocytes, Cell Tissue Res 365 (2) (2016) 393–401, https://doi.org/10.1007/s00441-016-2381-2.

[69] P Bagavandoss, S Grimshaw, Temporal and spatial distribution of the cannabinoid receptors (CB1, CB2) and fatty acid amide hydroxylase in the rat ovary, Anat Rec (Hoboken) 293 (8) (2010) 1425–1432, https://doi.org/10.1002/ar.21181.

[70] AP López-Cardona, S Pérez-Cerezales, R Fernández-González, R Laguna-Barraza, E Pericuesta, N Agirregoitia, et al., CB1 cannabinoid receptor drives oocyte maturation and embryo development via PI3K/Akt and MAPK pathways, FASEB J 31 (8) (2017) 3372–3382, https://doi.org/10.1096/fj.201601382RR.

[71] S Cecconi, G Rossi, S Oddi, V Di Nisio, M Maccarrone, Role of major endocannabinoid-binding receptors during mouse oocyte maturation, Int J Mol Sci 20 (12) (2019) 2866, https://doi.org/10.3390/ijms20122866.

[72] KM Lowther, TF Uliasz, KR Götz, VO Nikolaev, LM Mehlmann, Regulation of constitutive GPR3 signaling and surface localization by GRK2 and β-arrestin-2 overexpression in HEK293 cells, PLoS One 8 (6) (2013) e65365, , https://doi.org/10.1371/journal.pone.0065365.

[73] AP López-Cardona, MJ Sánchez-Calabuig, P Beltran-Breña, N Agirregoitia, D Rizos, E Agirregoitia, et al., Exocannabinoids effect on in vitro bovine oocyte maturation via activation of AKT and ERK1/2, Reproduction 152 (6) (2016) 603–612, https://doi.org/10.1530/REP-16-0199.

[74] A Pirone, C Lenzi, A Briganti, F Abbate, M Levanti, F Abramo, et al., Spatial distribution of cannabinoid receptor 1 and fatty acid amide hydrolase in the cat ovary and oviduct, Acta Histochem (2017) 417–422, https://doi.org/10.1016/j.acthis.2017.04.007.

[75] NM Tsutahara, YS Weems, JA Arreguin-Arevalo, TM Nett, ME LaPorte, J Uchida, et al., Effects of endocannabinoid 1 and 2 (CB1; CB2) receptor agonists on luteal weight, circulating progesterone, luteal mRNA for luteinizing hormone (LH) receptors, and luteal unoccupied and occupied receptors for LH in vivo in ewes, Prostaglandins Other Lipid Mediat 94 (1–2) (2011) 17–24, https://doi.org/10.1016/j.prostaglandins.2010.11.002.

[76] LK Brents, Marijuana, the Endocannabinoid System and the Female Reproductive System, Yale J Biol Med 89 (2) (2016) 175–191.

[77] MG Gervasi, TH Marczylo, PM Lam, S Rana, AM Franchi, JC Konje, et al., Anandamide levels fluctuate in the bovine oviduct during the oestrous cycle, PLoS One 8 (8) (2013) e72521, , https://doi.org/10.1371/journal.pone.0072521.
[78] F Correa, ML Wolfson, P Valchi, J Aisemberg, AM Franchi, Endocannabinoid system and pregnancy, Reproduction 152 (6) (2016) R191–R200, https://doi.org/10.1530/REP-16-0167.
[79] C Scorticati, J Fernández-Solari, A De Laurentiis, C Mohn, JP Prestifilippo, M Lasaga, et al., The inhibitory effect of anandamide on luteinizing hormone-releasing hormone secretion is reversed by estrogen, Proc Natl Acad Sci U S A 101 (32) (2004) 11891–11896, https://doi.org/10.1073/pnas.0404366101.
[80] H Wang, H Xie, SK Dey, Endocannabinoid signaling directs periimplantation events, AAPS J8 (2006) E425–E432, https://doi.org/10.1007/BF02854916.
[81] H Wang, H Xie, Y Guo, H Zhang, T Takahashi, PJ Kingsley, et al., Fatty acid amide hydrolase deficiency limits early pregnancy events, J Clin Invest 116 (8) (2006) 2122–2131, https://doi.org/10.1172/JCI28621.
[82] MG Gervasi, C Osycka-Salut, T Sanchez, CA Alonso, C Llados, L Castellano, et al., Sperm Release From the Oviductal Epithelium Depends on Ca(2+) Influx Upon Activation of CB1 and TRPV1 by Anandamide, J Cell Biochem 117 (2) (2016) 320–333, https://doi.org/10.1002/jcb.25273.
[83] G Catanzaro, N Battista, G Rossi, M Di Tommaso, M Pucci, V Pirazzi, et al., Effect of capacitation on the endocannabinoid system of mouse sperm, Mol Cell Endocrinol 343 (1–2) (2011) 88–92, https://doi.org/10.1016/j.mce.2011.01.022.
[84] AW Horne, JA Phillips 3rd, N Kane, PC Lourenco, SE McDonald, AR Williams, et al., Critchley HO. CB1 expression is attenuated in Fallopian tube and decidua of women with ectopic pregnancy, PLoS One 3 (12) (2008) e3969, , https://doi.org/10.1371/journal.pone.0003969.
[85] X Sun, H Wang, M Okabe, K Mackie, PJ Kingsley, LJ Marnett, et al., Genetic loss of Faah compromises male fertility in mice, Biol Reprod 80 (2) (2009) 235–242, https://doi.org/10.1095/biolreprod.108.072736.
[86] M Maccarrone, B Barboni, A Paradisi, N Bernabò, V Gasperi, MG Pistilli, et al., Characterization of the endocannabinoid system in boar spermatozoa and implications for sperm capacitation and acrosome reaction, J Cell Sci 118 (Pt 19) (2005) 4393–4404, https://doi.org/10.1242/jcs.02536.
[87] F Francavilla, N Battista, A Barbonetti, MR Vassallo, C Rapino, C Antonangelo, et al., Characterization of the endocannabinoid system in human spermatozoa and involvement of transient receptor potential vanilloid 1 receptor in their fertilizing ability, Endocrinology 150 (10) (2009) 4692–4700, https://doi.org/10.1210/en.2009-0057.
[88] C Osycka-Salut, MG Gervasi, E Pereyra, M Cella, ML Ribeiro, AM Franchi, et al., Anandamide induces sperm release from oviductal epithelia through nitric oxide pathway in bovines, PLoS One 7 (2) (2012) e30671, , https://doi.org/10.1371/journal.pone.0030671.
[89] JG Scotchie, RF Savaris, CE Martin, SL Young, Endocannabinoid regulation in human endometrium across the menstrual cycle, Reprod Sci 22 (1) (2015) 113–123, https://doi.org/10.1177/1933719114533730.
[90] X Sun, SK Dey, Endocannabinoid signaling in female reproduction, ACS Chem Nerosci 3 (5) (2012) 349–355, https://doi.org/10.1021/cn300014e.
[91] BC Paria, SK Das, SK Dey, The preimplantation mouse embryo is a target for cannabinoid ligand-receptor signaling, Proc Natl Acad Sci U S A 92 (21) (1995) 9460–9464 doi:10.1073%2Fpnas.92.21.9460.
[92] BC Paria, H Song, X Wang, PC Schmid, RJ Krebsbach, HH Schmid, et al., Dysregulated cannabinoid signaling disrupts uterine receptivity for embryo implantation, J Biol Chem 276 (23) (2001) 20523–20528, https://doi.org/10.1074/jbc.M100679200.
[93] X Sun, SK Dey, Cannabinoid/Endocannabinoid signaling impact on early pregnancy events, Curr Top Behav Neurosci 1 (2009) 255–273, https://doi.org/10.1007/978-3-540-88955-7_10.
[94] D Gentilini, A Besana, P Vigano, P Dalino, M Vignali, M Melandri, et al., Endocannabinoid system regulates migration of endometrial stromal cells via cannabinoid receptor 1 through the activation of Pi3K and ERK1/2 pathways, Fertil Steril 93 (8) (2010) 2588–2593, https://doi.org/10.1016/j.fertnstert.2010.02.006.
[95] BM Fonseca, G Correia-da-Silva, AH Taylor, PM Lam, TH Marczylo, SC Bell, et al., The endocannabinoid 2-arachidonoylglycerol (2-AG) and metabolizing enzymes during rat fetoplacental development: a role in uterine remodelling, Int J Biochem Cell Biol 42 (11) (2010) 1884–1892, https://doi.org/10.1016/j.biocel.2010.08.006.
[96] BM Fonseca, G Correia-da-Silva, AH Taylor, PM Lam, TH Marczylo, JC Konje, et al., N-acylethanolamine levels and expression of their metabolizing enzymes during pregnancy, Endocrinology 151 (8) (2010) 3965–3974, https://doi.org/10.1210/en.2009-1424.
[97] H Xie, X Sun, Y Piao, AG Jegga, S Handwerger, MS Ko, et al., Silencing or amplification of endocannabinoid signaling in blastocysts via CB1 compromises trophoblast cell migration, J Biol Chem 287 (38) (2012) 32288–32297, https://doi.org/10.1074/jbc.M112.381145.
[98] MS Sordelli, JS Beltrame, M Cella, MG Gervasi, S Perez Martinez, J Burdet, et al., Interaction between lysophosphatidic acid, prostaglandins and the endocannabinoid system during the window of implantation in the rat uterus, PLoS One 7 (9) (2012) e46059, , https://doi.org/10.1371/journal.pone.0046059.
[99] AH Taylor, MS Abbas, MA Habiba, JC Konje, Histomorphometric evaluation of cannabinoid receptor and anandamide modulating enzyme expression in the human uterus through the menstrual cycle, Histochem Cell Biol 133 (2010) 557–565, https://doi.org/10.1007/s00418-010-0695-9.
[100] D Resuehr, DR Glore, HS Taylor, KL Bruner-Tran, KG Osteen, Progesterone-dependent regulation of endometrial cannabinoid receptor type 1 (CB1-R) expression is disrupted in women with endometriosis and in isolated stromal cells exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), Fertil Steril 98 (4) (2012), https://doi.org/10.1016/j.fertnstert.2012.06.009 948-56.e1.
[101] OM Habayeb, AH Taylor, SC Bell, DJ Taylor, JC Konje, Expression of the endocannabinoid system in human first trimester placenta and its role in trophoblast proliferation, Endocrinology 149 (10) (2008) 5052–5060, https://doi.org/10.1210/en.2007-1799.
[102] AH Taylor, M Finney, PM Lam, JC Konje, Modulation of the endocannabinoid system in viable and non-viable first trimester pregnancies by pregnancy-related hormones, Reprod Biol Endocrinol 9 (2011) 152, https://doi.org/10.1186/1477-7827-9-152.
[103] X Sun, H Xie, J Yang, H Wang, HB Bradshaw, SK Dey, Endocannabinoid signaling directs differentiation of trophoblast cell lineages and placentation, Proc Natl Acad Sci U S A 107 (39) (2010) 16887–16892, https://doi.org/10.1073/pnas.1010892107.
[104] BM Fonseca, G Correia-da-Silva, M Almada, MA Costa, NA Teixeira, 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.
[105] LW Chamley, A Bhalla, PR Stone, H Liddell, S O'Carroll, C Kearn, et al., Nuclear localisation of the endocannabinoid metabolizing enzyme fatty acid amide hydrolase (FAAH) in invasive trophoblasts and an association with recurrent miscarriage, Placenta 29 (11) (2008) 970–975, https://doi.org/10.1016/j.placenta.2008.08.003.
[106] H Wang, H Xie, SK Dey, Loss of cannabinoid receptor CB1 induces preterm birth, PLoS One 3 (10) (2008) e3320, , https://doi.org/10.1371/journal.pone.0003320.
[107] OM Habayeb, AH Taylor, MD Evans, MS Cooke, DJ Taylor, SC Bell, et al., Plasma levels of the endocannabinoid anandamide in women–a potential role in pregnancy maintenance and labor? J Clin Endocrinol Metab 89 (11) (2004) 5482–5487, https://doi.org/10.1210/jc.2004-0681.
[108] V Nallendran, PM Lam, TH Marczylo, MJ Bankart, AH Taylor, DJ Taylor, et al., The plasma levels of the endocannabinoid, anandamide, increase with the induction of labour, BJOG 117 (7) (2010) 863–869, https://doi.org/10.1111/j.1471-0528.2010.02555.x.
[109] X Sun, W Deng, Y Li, S Tang, E Leishman, HB Bradshaw, et al., Sustained Endocannabinoid Signaling Compromises Decidual Function and Promotes Inflammation-induced Preterm Birth, J Biol Chem 291 (15) (2016) 8231–8240, https://doi.org/10.1074/jbc.M115.707836.
[110] X Sun, A Tavenier, W Deng, E Leishman, HB Bradshaw, SK Dey, Metformin attenuates susceptibility to inflammation-induced preterm birth in mice with higher endocannabinoid levels, Biol Reprod 98 (2) (2018) 208–217, https://doi.org/10.1093/biolre/iox164.
[111] P Bachkangi, AH Taylor, M Bari, M Maccarrone, JC Konje, 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), https://doi.org/10.1016/j.ejogrb.2019.09.029 in press.
[112] Biomarkers Definitions Working Group, Biomarkers and surrogate endpoints: preferred definitions and conceptual framework, Clin Pharmacol Ther 69 (3) (2001) 89–95, https://doi.org/10.1067/mcp.2001.113989.
[113] M Maccarrone, T Bisogno, H Valensise, N Lazzarin, F Fezza, C Manna, et al., Low fatty acid amide hydrolase and high anandamide levels are associated with failure to achieve an ongoing pregnancy after IVF and embryo transfer, Mol Hum Reprod 8 (2) (2002) 188–195, https://doi.org/10.1093/molehr/8.2.188.
[114] PM Lam, TH Marczylo, M El-Talatini, M Finney, V Nallendran, AH Taylor, et al., Ultra performance liquid chromatography tandem mass spectrometry method for the measurement of anandamide in human plasma, Anal Biochem 380 (2) (2008) 195–201, https://doi.org/10.1016/j.ab.2008.05.033.
[115] H Okada, T Tsuzuki, H Murata, Decidualization of the human endometrium, Reprod Med Biol 17 (3) (2018) 220–227, https://doi.org/10.1055/s-2007-991042.
[116] M Almada, S Cunha, BM Fonseca, C Amaral, F Piscitelli, V Di Marzo, et al., Anandamide interferes with human endometrial stromal-derived cell differentiation: An effect dependent on inhibition of cyclooxygenase-2 expression and prostaglandin E2 release, Biofactors 42 (3) (2016) 277–286, https://doi.org/10.1002/biof.1270.
[117] H Wang, H Xie, X Sun, PJ Kingsley, LJ Marnett, BF Cravatt, et al., Differential regulation of endocannabinoid synthesis and degradation in the uterus during embryo implantation, Prostaglandins Other Lipid Mediat 83 (1–2) (2007) 62–74, https://doi.org/10.1016/j.prostaglandins.2006.09.009.
[118] MA Costa, BM Fonseca, E Keating, NA Teixeira, G Correia-da-Silva, 2-arachidonoylglycerol effects in cytotrophoblasts: metabolic enzymes expression and apoptosis in BeWo cells, Reproduction 147 (3) (2014) 301–311, https://doi.org/10.1530/REP-13-0563.
[119] G Tedeschi, E Albani, EM Borroni, V Parini, AM Brucculeri, E Maffioli, et al., Proteomic profile of maternal-aged blastocoel fluid suggests a novel role for ubiquitin system in blastocyst quality, J Assist Reprod Genet (2017) 34225–34238, https://doi.org/10.1007/s10815-016-0842-x.
[120] A Gambineri, C Pelusi, S Genghini, AM Morselli-Labate, M Cacciari, U Pagotto, et al., Effect of flutamide and metformin administered alone or in combination in dieting obese women with polycystic ovary syndrome, Clin Endocrinol (Oxf) 60 (2) (2004) 241–249, https://doi.org/10.1111/j.1365-2265.2004.01973.x.
[121] R Pasquali, A Gambineri, U Pagotto, The impact of obesity on reproduction in women with polycystic ovary syndrome, BJOG 113 (10) (2006), https://doi.org/10.1111/j.1471-0528.2006.00990.x 1148–5.
[122] N Cui, X Feng, Z Zhao, J Zhang, Y Xu, L Wang, et al., Restored plasma anandamide and endometrial expression of fatty acid amide hydrolase in women with polycystic ovary syndrome by the combination use of diane-35 and metformin, Clin

Ther 39 (4) (2017) 751–758, https://doi.org/10.1016/j.clinthera.2017.02.007.
[123] A Molvarec, G Fügedi, E Szabó, B Stenczer, S Walentin, J Rigó Jr, Decreased circulating anandamide levels in preeclampsia, Hypertens Res 38 (6) (2015) 413–418, https://doi.org/10.1038/hr.2015.20.
[124] G Fügedi, M Molnár, J Rigó Jr, J Schönléber, I Kovalszky, A Molvarec, Increased placental expression of cannabinoid receptor 1 in preeclampsia: an observational study, BMC Pregnancy Childbirth 14 (2014) 395, https://doi.org/10.1186/s12884-014-0395-x.
[125] C Abán, GF Leguizamón, M Cella, A Damiano, AM Franchi, MG Farina, Differential expression of endocannabinoid system in normal and preeclamptic placentas: effects on nitric oxide synthesis, Placenta 34 (1) (2013) 67–74, https://doi.org/10.1016/j.placenta.2012.10.009.
[126] AK Gebeh, JM Willets, M Bari, RA Hirst, TH Marczylo, AH Taylor, et al., Elevated anandamide and related N-acylethanolamine levels occur in the peripheralblood of women with ectopic pregnancy and are mirrored by changes in peripheralfatty acid amide hydrolase activity, J Clin Endocrinol Metab 98 (3) (2013) 1226–1234, https://doi.org/10.1210/jc.2012-3390.
[127] E Contassot, M Tenan, V Schnüriger, MF Pelte, PY Dietrich, Arachidonyl ethanolamide induces apoptosis of uterine cervix cancer cells via aberrantly expressed vanilloid receptor-1, Gynecol Oncol 93 (1) (2004) 182–188, https://doi.org/10.1016/j.ygyno.2003.12.040.
[128] T Ayakannu, AH Taylor, JM Willets, JC Konje, The evolving role of the endocannabinoid system in gynaecological cancer, Hum Reprod Update 21 (4) (2015) 517–535, https://doi.org/10.1093/humupd/dmv022.
[129] M Guida, A Ligresti, D De Filippis, A D'Amico, S Petrosino, M Cipriano, et al., The levels of the endocannabinoid receptor CB2 and its ligand 2-arachidonoylglycerol are elevated in endometrial carcinoma, Endocrinology 151 (3) (2010) 921–928, https://doi.org/10.1210/en.2009-0883.
[130] T Ayakannu, A Taylor, J Willets, T Marczylo, L Brown, Q Davies, et al., Effect of anandamide on endometrial adenocarcinoma (Ishikawa) cell numbers: implications for endometrial cancer therapy, Lancet 385 (1) (2015) S20, https://doi.org/10.1016/S0140-6736(15)60335-X.
[131] T Ayakannu, AH Taylor, JC Konje, Cannabinoid receptor expression in estrogen-dependent and estrogen-independent endometrial cancer, J Recept Signal Transduct Res 38 (5–6) (2018) 385–392, https://doi.org/10.1080/10799893.2018.1531890.
[132] DK Nomura, JZ Long, S Niessen, HS Hoover, SW Ng, BF Cravatt, Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis, Cell 140 (1) (2010) 49–61 doi:10.1016%2Fj.cell.2009.11.027.
[133] R Piñeiro, T Maffucci, M Falasca, The putative cannabinoid receptor GPR55 defines a novel autocrine loop in cancer cell proliferation, Oncogene 30 (2) (2011) 142–152, https://doi.org/10.1038/onc.2010.417.
[134] EM Messalli, F Grauso, R Luise, A Angelini, R Rossiello, Cannabinoid receptor type 1 immunoreactivity and disease severity in human epithelial ovarian tumors, Am J Obstet Gynecol 211 (3) (2014), https://doi.org/10.1016/j.ajog.2014.04.004 234.e1-6.
[135] V Kantae, S Ogino, M Noga, AC Harms, RM van Dongen, GL Onderwater, et al., Quantitative profiling of endocannabinoids and related N-acylethanolamines in human CSF using nano LC-MS/MS, J Lipid Res 58 (3) (2017) 615–624, https://doi.org/10.1194/jlr.D070433.
[136] C Skonberg, A Artmann, C Cornett, SH Hansen, HS Hansen, Pitfalls in the sample preparation and analysis of N-acylethanolamines, J Lipid Res 51 (10) (2010) 3062–3073, https://doi.org/10.1194/jlr.D004606.
[137] S Grasso, M Santonico, T Bisogno, G Pennazza, A Zompanti, A Sabatini, et al., An innovative liquid biosensor for the detection of lipid molecules involved in diseases of the nervous system, Proceedings 2 (760) (2018) 1–4 https://www.mdpi.com/2504-3900/2/13/760.
[138] L Das, S Parbin, N Pradhan, C Kausar, SK Patra, Epigenetics of reproductive infertility, Front Biosci (Schol Ed) 9 (2017) 509–535.
[139] CV Morris, JA DiNieri, H Szutorisz, YL Hurd, Molecular mechanisms of maternal cannabis and cigarette use on human neurodevelopment, Eur J Neurosci 34 (10) (2011) 1574–1583 doi:10.1111%2Fj.1460-9568.2011.07884.x.
[140] JA DiNieri, X Wang, H Szutorisz, SM Spano, J Kaur, P Casaccia, et al., Maternal cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring, Biol Psychiatry 70 (8) (2011) 763–769, https://doi.org/10.1016/j.biopsych.2011.06.027.
[141] T Cherlet, JE Scott, Tetrahydrocannabinol (THC) alters synthesis and release of surfactant-related material in isolated fetal rabbit type II cells, Drug Chem Toxicol 25 (2) (2002) 171–190, https://doi.org/10.1081/DCT-120003258.
[142] M Khare, AH Taylor, JC Konje, SC Bell, Delta9-tetrahydrocannabinol inhibits cytotrophoblast cell proliferation and modulates gene transcription, Mol Hum Reprod 12 (5) (2006) 321–333, https://doi.org/10.1093/molehr/gal036.
[143] C D'Addario, A Di Francesco, M Pucci, A Finazzi Agrò, M Maccarrone, Epigenetic mechanisms and endocannabinoid signalling, FEBS J 280 (9) (2013) 1905–1917, https://doi.org/10.1111/febs.12125.
[144] DR Fitzpatrick, CB Wilson, Methylation and demethylation in the regulation of genes, cells, and responses in the immune system, Clin Immunol 109 (1) (2003) 37–45, https://doi.org/10.1016/S1521-6616(03)00205-5.
[145] GA Cabral, TJ Rogers, AH Lichtman, Turning over a new leaf: cannabinoid and endocannabinoid modulation of immune function, J Neuroimmune Pharmacol 10 (2) (2015) 193–203, https://doi.org/10.1007/s11481-015-9615-z.
[146] C Lombard, VL Hegde, M Nagarkatti, PS Nagarkatti, Perinatal exposure to Δ9-tetrahydrocannabinol triggers profound defects in T cell differentiation and function in fetal and postnatal stages of life, including decreased responsiveness to HIV antigens, J Pharmacol Exp Ther 339 (2) (2011) 607–617, https://doi.org/10.1124/jpet.111.181206.
[147] X Yang, VL Hegde, R Rao, J Zhang, PS Nagarkatti, M Nagarkatti, Histone modifications are associated with Δ9-tetrahydrocannabinol-mediated alterations in antigen-specific T cell responses, J Biol Chem 289 (27) (2014) 18707–18718, https://doi.org/10.1074/jbc.M113.545210.
[148] M Ragusa, D Barbagallo, T Chioccarelli, F Manfrevola, G Cobellis, C Di Pietro, et al., CircNAPEPLD is expressed in human and murine spermatozoa and physically interacts with oocyte miRNAs, RNA Biol 16 (9) (2019) 1237–1248, https://doi.org/10.1080/15476286.2019.1624469.
[149] CP Dias-Rocha, MM Almeida, EM Santana, JCB Costa, JG Franco, CC Pazos-Moura, et al., Maternal high-fat diet induces sex-specific endocannabinoid system changes in newborn rats and programs adiposity, energy expenditure and food preference in adulthood, J Nutr Biochem 51 (2018 Jan) 56–68, https://doi.org/10.1016/j.jnutbio.2017.09.019.
[150] C D'Addario, MV Micioni Di Bonaventura, M Pucci, A Romano, S Gaetani, R Ciccocioppo, et al., Endocannabinoid signaling and food addiction, Neurosci Biobehav Rev 47 (2014) 203–224, https://doi.org/10.1016/j.neubiorev.2014.08.008.
[151] MM Almeida, CP Dias-Rocha, CF Reis-Gomes, H Wang, GC Atella, A Cordeiro, et al., Maternal high-fat diet impairs leptin signaling and up-regulates type-1 cannabinoid receptor with sex-specific epigenetic changes in the hypothalamus of newborn rats, Psychoneuroendocrinology 103 (2019) 306–315, https://doi.org/10.1016/j.psyneuen.2019.02.004.
[152] JM Keppel Hesselink, TAM Hekker, Therapeutic utility of palmitoylethanolamide in the treatment of neuropathic pain associated with various pathological conditions: a case series, J Pain Res 5 (2012) 437–442, https://doi.org/10.2147/JPR.S32143.
[153] L Gabrielsson, S Mattsson, CJ Fowler, Palmitoylethanolamide for the treatment of pain: pharmacokinetics, safety and efficacy, Br J Clin Pharmacol 82 (4) (2016) 932–942, https://doi.org/10.1111/bcp.13020.
[154] L Cobellis, MA Castaldi, V Giordano, E Trabucco, P De Franciscis, M Torella, et al., Effectiveness of the association micronized N-Palmitoylethanolamine (PEA)-transpolydatin in the treatment of chronic pelvic pain related to endometriosis after laparoscopic assessment: a pilot study, Eur J Obstet Gynecol Reprod Biol 158 (1) (2011) 82–86, https://doi.org/10.1016/j.ejogrb.2011.04.011.
[155] M Maccarrone, H Valensise, M Bari, N Lazzarin, C Romanini, A Finazzi-Agrò, Relation between decreased anandamide hydrolase concentrations in human lymphocytes and miscarriage, Lancet 355 (9212) (2000) 1326–1329, https://doi.org/10.1016/S0140-6736(00)2115-2.