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The manifold actions of endocannabinoids on female and male reproductive events

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1. ABSTRACT

Epidemiological studies have highlighted the ever growing use of illegal drugs among teenagers. The negative effects of marijuana (a *Cannabis sativa* extract) on reproductive health are poorly known among young people, although chronic exposure to delta-9-tetrahydrocannabinol, the main psychoactive constituent of marijuana, impairs human reproductive potential by disrupting menstrual cycle, suppressing oogenesis and impairing embryo implantation and development, in women, and by increasing ejaculation problems, reducing sperm count and motility, and generating loss of libido and impotence, in men. Endocannabinoids, their metabolic enzymes and target receptors form the so called “endocannabinoid system” and they have been demonstrated to respond to fertility signals. In addition, they interfere with hormones, cytokines and other signalling molecules in both female and male reproductive events. In this review, we shall summarize the current knowledge on the endocannabinoid system, and on the multifaceted roles played by endocannabinoids in reproduction along the evolutionary axis from invertebrates to mammals. Furthermore, we shall discuss the potential use of distinct elements of the endocannabinoid system for the diagnosis and/or treatment of human infertility.

2. INTRODUCTION

Smoking marijuana during adolescence is not only a social problem that represents the starting point of potential health troubles at reproductive age. In fact, it is well-known that cannabis (*Cannabis sativa*), because of its euphoric effects, is the most used illegal drug among young adults in high-income countries, and more recently also in low-income and middle-income countries. Data published in the “2007 World Drug Report” (United Nations Office on Drugs and Crime) alert that, although there is a slight downward trend compared to the past, marijuana is used by over 160 million adults, is consumed already at age 12, and earlier by males than females. The main reason why most teenagers use cannabis is to experience a so-called “high”, that is mild euphoria, relaxation and perceptual alterations; among the latter are time distorsion and intensification of ordinary experiences with benefits in the social behaviours (1). One can suppose that the lack of addiction on chronic abuse and the federal approval for the use of marijuana in US and other Western countries (yet only for medical purposes), may encourage consumption in adolescents, who may neglect the long lasting effects on physical and mental performances like cognitive dysfunction and psychiatric illness, as well as cardiorespiratory, dental, immunological and reproductive deficits (2-8). Although the impact of

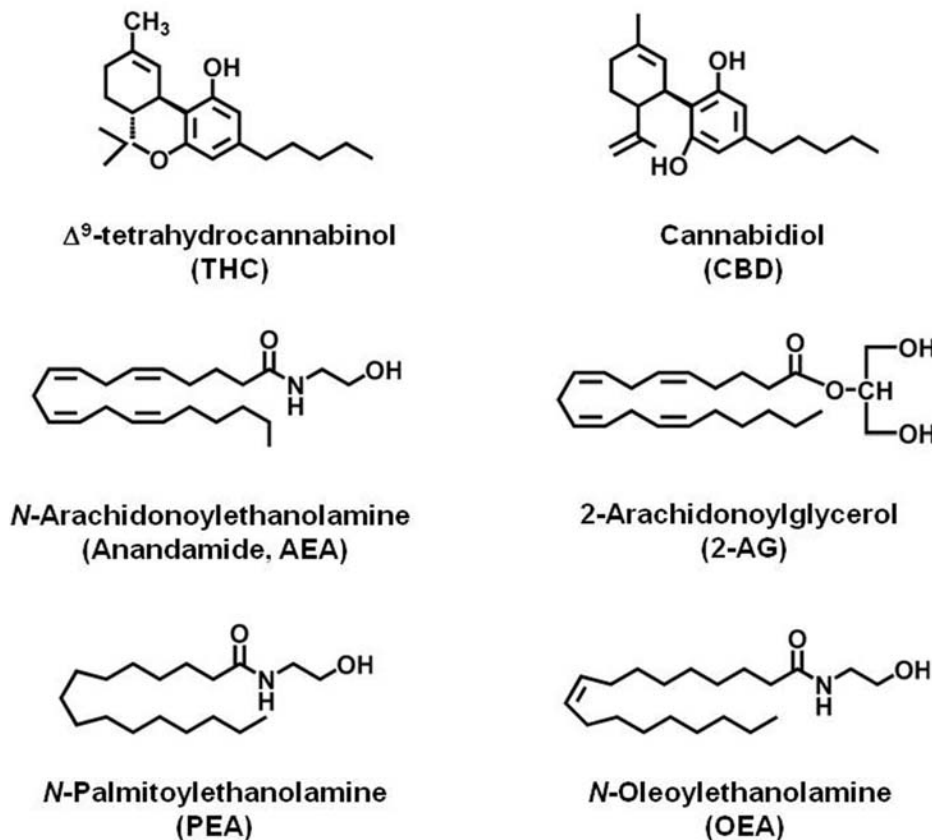

Figure 1. Chemical structures of the main plant-derived (THC and CBD), and endogenous (AEA, 2-AG, PEA, OEA) cannabinoids. See text for details.

cannabis smoke on female fertility is still controversial, tests carried out in women who regularly use marijuana have demonstrated in their reproductive organs trace amounts of delta-9-tetrahydrocannabinol (THC; Figure 1) and cannabidiol (CBD; Figure 1), two components of *Cannabis sativa* extracts (9). Small amounts of THC are even secreted in the vaginal fluid, where they over-stimulate spermatozoa (10). In addition, evidence obtained from animal models demonstrates that cannabinoids harm preimplantation events, and impact on pregnancy outcome (11). Cannabinoids are also able to cross the placental barrier during gestation (12), and can be transferred to the pups through maternal milk during lactation (13). Remarkably, clinical studies reported that children born to women who used cannabis during pregnancy have a reduced birthweight and show mild development abnormalities in both childhood (defective memory and poor verbal skills) and adulthood (impulsivity, delinquency and drugs addiction) (14, 15). Moreover, THC abuse during pregnancy influences gene expression of the neural adhesion molecule L1, a fundamental protein in synaptogenesis and brain development, and alters the development of nigrostriatal and mesolimbic dopaminergic neurons (16, 17). Overall, experimental and epidemiological studies demonstrate that THC negatively affects reproductive events both in females and males, and impairs the neurodevelopment and the social and psychological behaviour of the offspring born to cannabis users. On the other hand, the chronic and intense use of cannabis by men at reproductive age decreases testosterone release, as well as sperm production, motility and viability. In an invertebrate model, it has been demonstrated that THC is able to block the acrosome reaction, by affecting an event in the stimulus–secretion–mating mechanism of the sperm prior to the opening of ion channels; yet, it is unable to affect the physiological functions of sperm (18). Unfortunately, the lifestyle of chronic cannabis smokers is usually associated with abuse of alcohol and/or other drugs, and thus the potential hazard of cannabis to fertility and offspring's performance might be underestimated. Therefore, investigation of THC analogues like the endocannabinoids (eCBs), which act as endogenous ligands at cannabinoid receptors and are critically involved in the control of human reproduction, could help to better understand the molecular events occurring at the sex-addiction interface.

3. ENDOCANNABINOID SYSTEM

eCBs are endogenous lipids that mimic several actions of THC. Until now, the most bioactive eCBs are anandamide (*N*-arachidonoylethanolamine; AEA; Figure 1) and 2- arachidonoylglycerol (2-AG; Figure 1), that act principally through cannabinoid receptors; these are

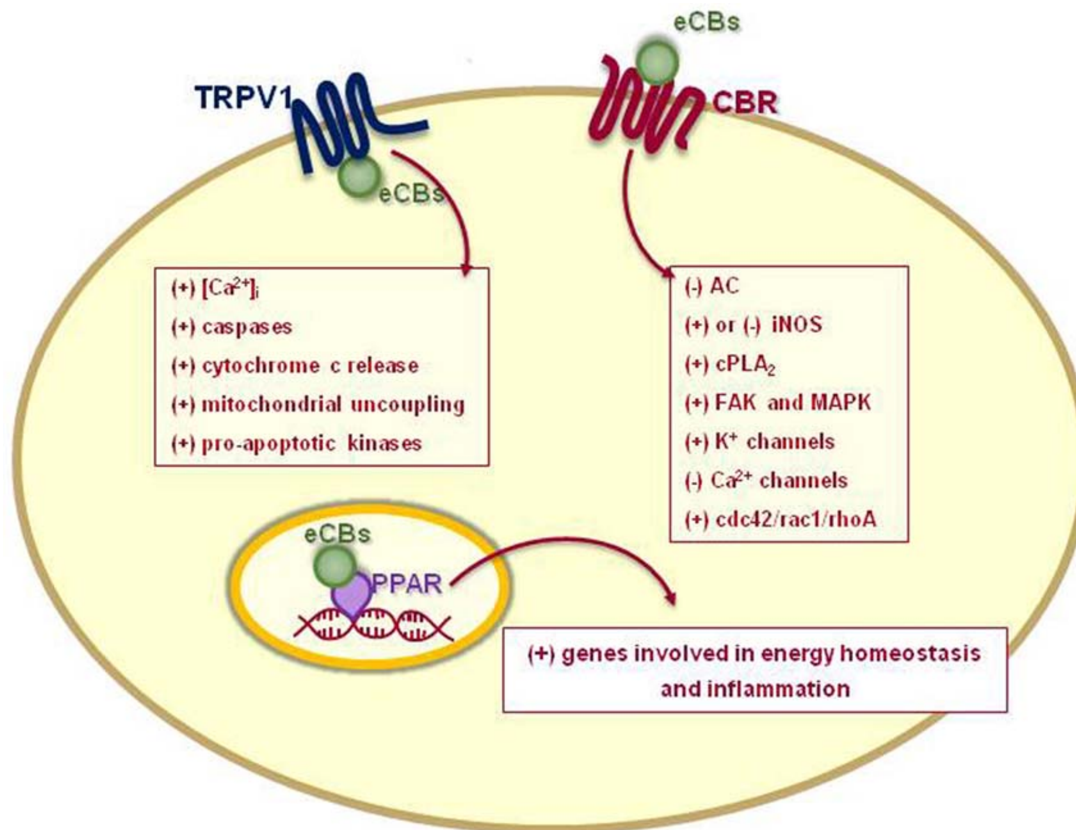

Figure 2. The binding of AEA and 2-AG to cannabinoid receptors (CBR) triggers different signalling cascades, such as reduction of adenylyl cyclase (AC), activation (by CB_1) or inhibition (by CB_2) of inducible nitric oxide synthase (iNOS), activation of cytosolic phospholipase A_2 ($cPLA_2$), focal adhesion kinase (FAK) and mitogen-activated protein kinase (MAPK), and regulation of ionic (K^+ and Ca^{2+}) currents. Binding to the purported CB_3 receptor (i.e., GPR55) triggers activation of the small GTPase proteins cdc42, rac and rhoA. eCBs might also affect lipid and glucose metabolism, as well as inflammation, through activation of PPAR nuclear receptors, whereas the interaction of AEA with the cytosolic binding site of TRPV1 channels triggers a cascade of intracellular responses that make of AEA a true "endovanilloid".

members of the rhodopsin family of G protein-coupled seven-transmembrane spanning receptors (19), and include type-1 and type-2 (CB_1 and CB_2) receptors as the best characterized targets of eCBs. CB_1 has been found mainly in the central nervous system (20), and is present also in ovary (21), uterine endometrium (22), testis (23), vas deferens (24), urinary bladder (25), and other peripheral endocrine and neurological tissues (26). CB_2 has been found mainly in peripheral and immune cells (22), and is also present in neuronal cells (27, 28). CB_1 and CB_2 transcripts have been also found in mouse pre-implantation embryos (29) and in human placenta (30). Recently, other CB receptors, like a purported CB_3 (GPR55) receptor (31, 32), and non-CB_1/non-CB_2 receptors have been identified. Among the latter entities, transient receptor potential vanilloid-1 (TRPV1) channel has been shown to be activated by AEA, but not by 2-AG, suggesting an interplay between the cannabinoid and vanilloid systems (33, 34). In addition, AEA, 2-AG and congeners like *N*-palmitoylethanolamine (PEA; Figure 1) and *N*-oleoylethanolamine (OEA; Figure 1) have been shown to activate peroxisome proliferator-activated receptors (PPARs) under physiological and pathological conditions, thus contributing to the control of energy homeostasis and inflammation (see ref. 35 for a recent review). Signal transduction pathways triggered by eCBs binding to CB, TRPV1 and PPAR receptors (36-38) are schematically depicted in Figure 2.

eCBs are released from membrane phospholipid precursors through the activation of specific phospholipases (39), that are activated "on demand", i.e. when and where needed. Although there are multiple biosynthetic pathways involved in the formation of AEA (40-43; reviewed in ref. 44), the most prominent route engages a transacylation by a Ca^{2+}-dependent *N*-acyltransferase (NAT) to produce *N*-arachidonoyl-phosphatidylethanolamine (NArPE) (45), with the subsequent conversion of this lipid into AEA through the activity of a specific phospholipase D (NAPE-PLD) (46). Much alike AEA, 2-AG is synthesized in a two-step pathway, which includes a rapid hydrolysis of inositol phospholipids by a specific phospholipase C (PLC) to generate diacylglycerol (DAG), sequentially converted into 2-AG by a *sn*-1-DAG lipase (DAGL) (47). After re-uptake

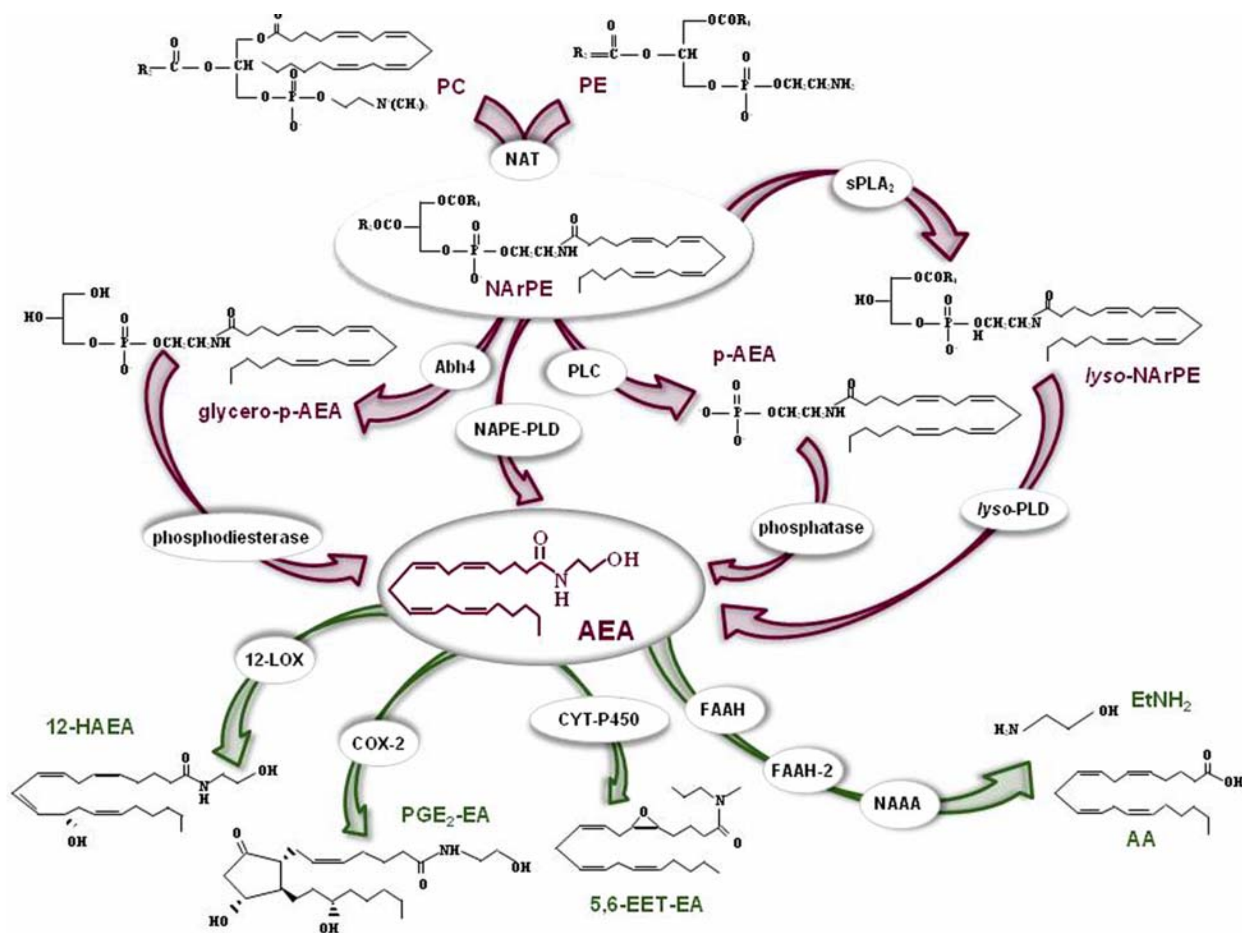

Figure 3. Biosynthetic and catabolic pathways of AEA. *N*-Acyltransferase (NAT) transfers arachidonic acid from the *sn*-1 position of 1,2-*sn*-di-arachidonoylphosphatidylcholine (PC) to phosphatidylethanolamine (PE), thus generating *N*-arachidonoylphosphatidylethanolamine (NArPE). Then, *N*-acyl-phosphatidylethanolamines (NAPE)-specific phospholipase D (NAPE-PLD) cleaves NArPE, releasing AEA and phosphatidic acid (not shown). Alternatively, cleavage of NArPE by phospholipase C (PLC) produces phospho-AEA (p-AEA), which is then dephosphorylated by a phosphatase. Also secretory phospholipase A_2 ($sPLA_2$) can hydrolyze NArPE to *N*-arachidonoyl-*lyso*-phosphatidylethanolamine (*lyso*-NArPE), that is further hydrolyzed to AEA by a *lyso*-phospholipase D (*lyso*-PLD). Finally, NArPE can be deacylated by a *lyso*-phospholipase/ phospholipase B named Abh4 (alpha-/beta-hydrolase), thus generating glycero-phospho-AEA (glycero-p-AEA) that is cleaved to AEA by a phosphodiesterase. AEA is hydrolyzed to arachidonic acid (AA) and ethanolamine ($EtNH_2$) by FAAH, FAAH-2 or the lysosomal *N*-acylethanolamine-hydrolyzing acid amidase (NAAA). Alternatively, AEA is metabolized to 12-hydroxy-AEA (12-HAEA) by 12-lipoxygenase (12-LOX), to PGE_2-ethanolamide (PGE_2-EA) by cyclooxygenase-2 (COX-2), or to 5,6-epoxyeicosatrienoic acid ethanolamide (5,6-EET-EA) by members of the cytochrome P450 (CYT-P450) family. Also LOX isozymes different from 12-LOX can oxygenate AEA, but were omitted for the sake of clarity.

through a purported specific transporter, called endocannabinoid membrane transporter (EMT) (48, 49), eCBs signalling is terminated by hydrolysis via enzymes showing “amidase signature”: two fatty acid amide hydrolases (FAAH and FAAH-2) (50, 51), and the *N*-acylethanolamine-hydrolyzing acid amidase (NAAA) (52). These hydrolases break the amide bond of AEA to release arachidonic acid and ethanolamine. On the other hand, FAAH and more importantly a specific monoacylglycerol lipase (MAGL) transform 2-AG into arachidonic acid and glycerol (53, 54). Also cyclooxygenase-2 (COX-2), different lipoxygenase (LOX) isozymes and cytochrome P450 are able to accept AEA as a substrate, to generate prostaglandin-ethanolamides (55), hydroxy-anandamides (56), and epoxy-eicosatrienoyl-glycerols (57), respectively. In addition, COX-2 and LOXs can dioxygenate 2-AG to produce prostaglandin-glyceryl esters (58) and hydroxyleicosatetraenoyl-glycerols (56). Figures 3 and 4 summarize the metabolic pathways that regulate the endogenous tone of AEA and 2-AG, respectively, in several organs of the body, including reproductive cells and tissues. Taken together eCBs, their receptors, biosynthetic and catabolic enzymes, as well as putative transporter(s), are collectively termed endocannabinoid system **(**ECS). This system is depicted in Figure 5, and has been detected in female and male germ cells at various stages, and

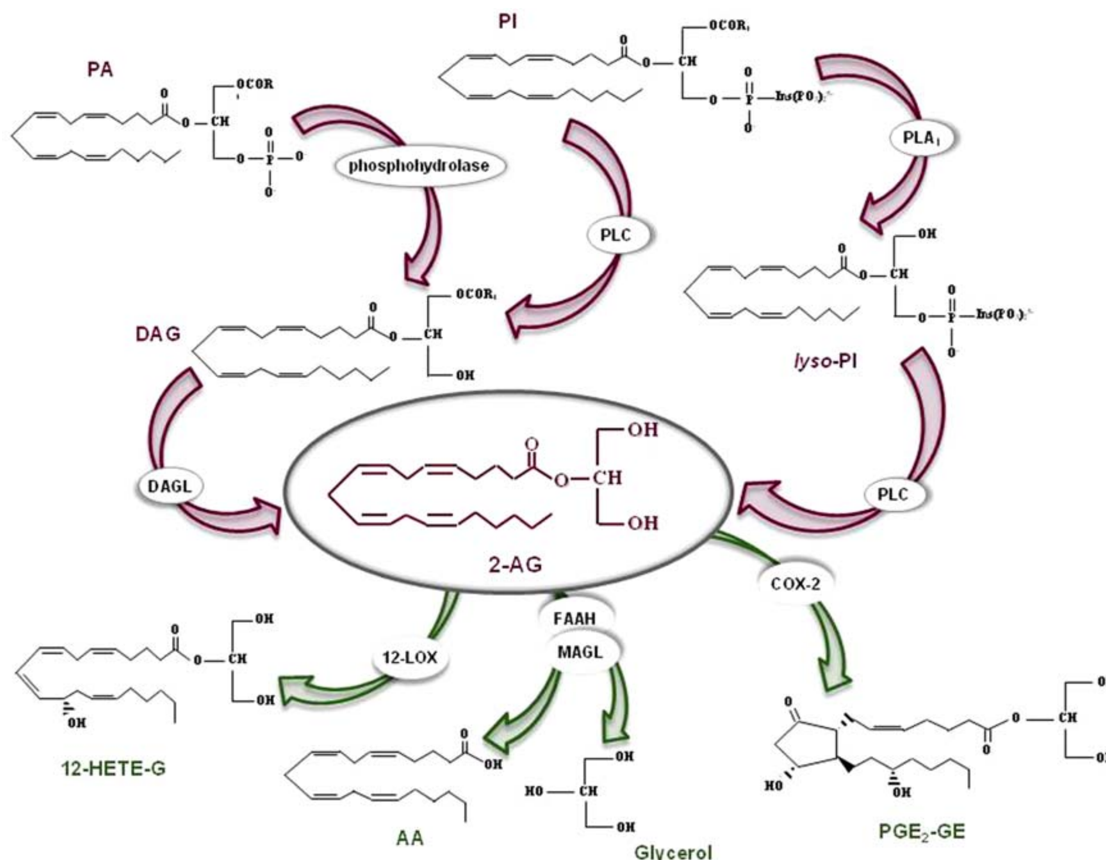

Figure 4. Biosynthetic and catabolic pathways of 2-AG. Precursors of 2-AG are inositol phospholipids (PI) or phosphatidic acid (PA), which are hydrolyzed by a specific phospholipase C (PLC) or by a PA-phosphohydrolase, respectively, to generate diacylglycerol (DAG), which in turn is converted to 2-AG by a *sn*-1-DAG lipase (DAGL). On the other hand, 2-AG can be degraded by FAAH, although the main 2-AG hydrolase is the cytosolic monoacylglycerol lipase (MAGL), which releases arachidonic acid (AA) and glycerol. In addition, both 12-LOX and COX-2 efficiently catalyze the oxygenation of 2-AG, and produce 12-hydroxy-eicosatetraenoic-glycerol (12-HETE-G) and PGE_2 glyceryl-ester (PGE_2-GE), respectively. Also LOX isozymes different from 12-LOX can oxygenate 2-AG, but were omitted for the sake of clarity.

throughout the pre- and postnatal embryonic development (59). It should be stressed that the existence of a close link between ECS, sex hormones and other fertility signals in male and female reproductive organs (29) could be useful to exploit distinct members of ECS as novel targets for therapeutic intervention against fertility problems. On the other hand, the presence of CB_1 receptors in reproductive cells raises concerns against the use of CB_1 agonists (like marijuana) during the reproductive age. Not surprisingly, the manifold roles of ECS in mammalian reproduction have been recently the subject of comprehensive reviews (59-66). Here, we shall briefly discuss the state of the art and the most recent advances on the physiological distribution, functional role, dysregulation and therapeutic exploitation of ECS in male and female reproductive organs.

4. ECS IN FEMALE FERTILITY

4.1. Physiological distribution

In order to understand the role of ECS elements in female fertility, and the mechanisms whereby they influence the outcome of implantation and other aspects of reproduction, it is essential to know their localization under normal conditions. In the last few years, many studies have been performed in different animal models, with the aim of characterizing the distribution and function of ECS elements in reproductive organs. AEA was found in female reproductive fluids (10), as well as in the human ovary, where it may play a role in folliculogenesis, preovulatory follicle maturation, oocyte maturity and ovulation (67). In the same context, it was suggested that high plasma levels of AEA are required at ovulation (67), whereas low levels are necessary to achieve successful pregnancy (68). Conversely, high plasma AEA, paralleled by low FAAH activity in peripheral lymphocytes (69), is almost 100% predictive of spontaneous miscarriage (70). From these data it appears that the positive or negative effect of AEA on reproductive events critically depends on a balance between synthesis and degradation (by NAPE-PLD and FAAH, respectively). In line with this, a recent study has shown that in mouse embryos and oviductal epithelium, a locally appropriate “AEA tone” is created to permit a normal embryo development and oviductal transport (71). Accordingly, the AEA metabolizing enzymes were found

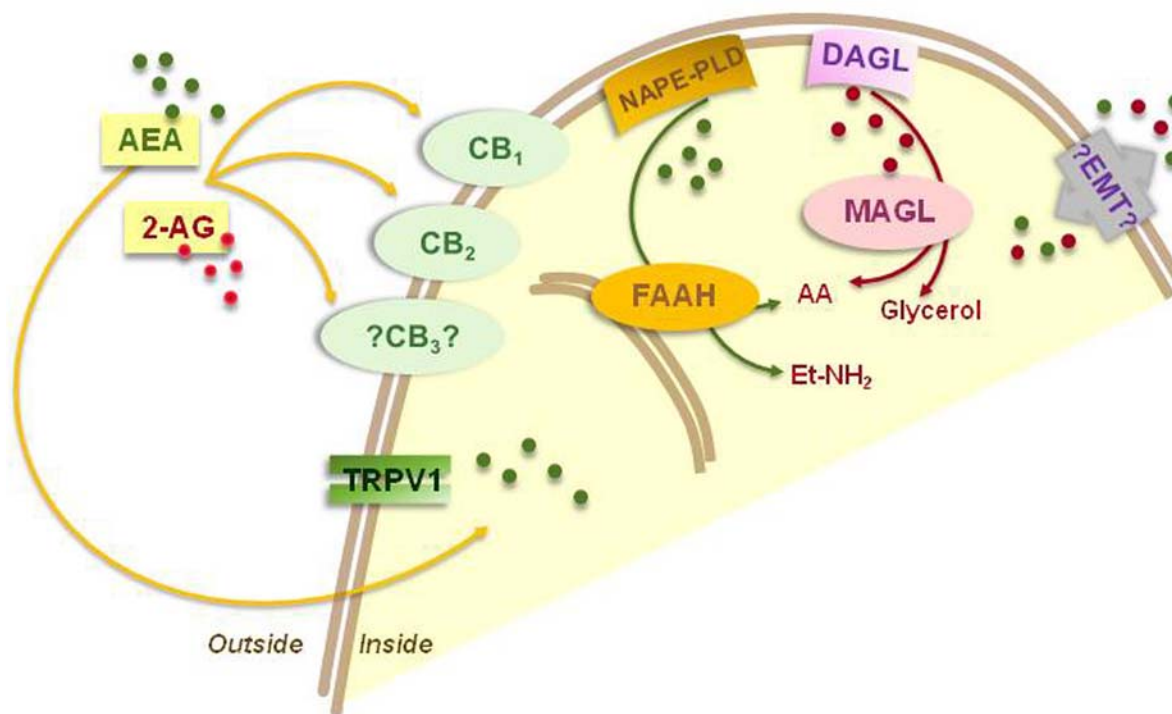

Figure 5. Schematic representation of the best characterized members of the endocannabinoid system. *N*-arachidonoyl-ethanolamine (AEA) is produced by the sequential activity of *N*-acyltransferase (NAT, not shown) and NAPE-specific phospholipase D (NAPE-PLD), which releases AEA and phosphatidic acid. The biological actions of AEA are terminated by a two-step mechanism, consisting of cellular uptake through a purported endocannabinoid membrane transporter (EMT), followed by intracellular degradation by fatty acid amide hydrolase (FAAH) to ethanolamine ($EtNH_2$) and arachidonic acid (AA).2-Arachidonoylglycerol (2-AG) is also released from membrane lipids through the activity of diacylglycerol lipase (DAGL), and can be hydrolyzed by a cytosolic monoacylglycerol lipase (MAGL) that releases glycerol and AA. The transport of 2-AG across the cell membrane may be mediated by the same EMT that takes up AEA, or by other membrane carriers. Both AEA and 2-AG trigger several signal transduction pathways by acting at their targets, CB_1, CB_2 and purported CB_3 receptors. Other targets of AEA and 2-AG are the nuclear PPARs, that were omitted for the sake of clarity. AEA, but not 2-AG, binds intracellularly to TRPV1, which is the natural target of capsaicin, the pungent ingredient of hot peppers. See also Figures 3-4 for further details on metabolic enzymes of AEA and 2-AG, and Figure 2 for receptor-dependent signal transduction. Also note that most proteins of the endocannabinoid system are bound to the plasma membrane, whereas FAAH is bound to endomembranes (especially those of the endoplasmic reticulum) and MAGL is cytosolic.

in growing secondary and tertiary follicles, and in corpus luteum and corpus albicans (21). High levels of FAAH were also found in the human villous cytothrophoblast (68) and syncytiotrophoblast (72), suggesting that FAAH has a protective role by cleaving AEA and preventing its passage at the materno-fetal interface. In mouse, FAAH is expressed at the 2-cell stage of the early pre-implantation embryo (73-75), while NAPE-PLD, CB_1 and CB_2 are expressed at later stages. Also human placenta synthesizes AEA through NAPE-PLD and expresses CB_1, CB_2 and FAAH at the mRNA and protein levels, with a cellular localization confined to the syncytiotrophoblast layer (21). The entire ECS has been detected also in the human ovary, where CB receptors were found in oocytes (76) and ovary itself, with higher expression of CB_2 than CB_1 in the latest phases of follicle maturation (21). By using CB_1 knockout (KO) mice, it has been shown that CB_1 regulates the oviductal transport of embryos, while CB_2 is not involved in this process (74). Recently, CB_1 mRNA has been shown to undergo a temporal variation in human Fallopian tubes, where a low expression has been detected in women with ectopic pregnancy, paralleled by a lower expression in the endometrium (77). CB_1 and CB_2 proteins were found in medulla and cortex of the ovary, in particular in the granulosa cells of primordial, primary, secondary and tertiary follicles, as well as in the theca cells of secondary and tertiary follicles (21). Both CB receptors were also observed in the corpus luteum and corpus albicans (21), and CB_2 was also expressed in blastocyst trophoblast with an as-yet-unclear function (78, 79). In rat deciduas it has been demonstrated that CBs and TRPV1 expression fluctuates during pregnancy, showing high levels in the early phases of gestation and low levels at later stages (80). In the uterus 2-AG showed a similar distribution as AEA during early pregnancy, suggesting that also this compound could take part in the regulation of implantation (75). Consistently, DAGL is highly expressed in both circular and longitudinal muscle layers of the uterus, particularly in the myometrium, and MAGL is primarily localized in the luminal and glandular epithelia (75). Taken together, these

findings denote a coordinated activity of NAPE-PLD and FAAH, as well as of DAGL and MAGL, in the regulation of uterine levels of eCBs during normal pregnancy, although details of the regulation of reproductive events by 2-AG remain elusive.

4.2. Functional role

The ability of ECS elements to critically regulate embryo implantation and development has been investigated in details. In fact, many studies were designed to localize the ECS in the ovary and to dissect processes that depend on eCBs in reproductive organs (29, 66, 81-86). The earliest event in mammalian reproduction at the female side is follicologenesis. As described above, many elements of ECS were found in oocytes at various stages of maturation, and in particular it appeared that AEA levels found in follicular fluid may be strongly correlated to oocyte quality and maturation (67). In this context, recent human studies have shown that plasma levels of AEA fluctuate during menstrual cycle, and that FAAH activity is upregulated in the period that coincides with the putative window of implantation (87). Interestingly, FAAH and progesterone showed the same fluctuations during the menstrual cycle (87), and it was suggested that AEA may be controlled by gonadotrophins, estrogen, or a combination of both, during this period (67). After fertilization and before implantation the oocyte travels through the oviduct to reach the uterus. For mammals embryo transport and attachment to the luminal epithelium of the uterus are crucial events. In fact, during oviductal transport many events concur to ensure that i) blastocyst is ready for implantation when it arrives in the uterine cavity, and ii) the latter organ is ready to receive the blastocyst. A normal oviductal transport is a prerequisite for implantation, and indeed any dysfunction of this process causes oviductal embryo retention with increased incidence of pregnancy failure due to tubal pregnancy and abortion. Many observations demonstrate that aberrant cannabinoid signalling, either silenced or enhanced, impairs embryo transport (74, 88). In fact, eCBs regulate normal embryo transport via oviductal CB_1 receptors. Then, embryos at the late morula or early blastocyst stage enter in the uterus, where they differentiate to become implantation-competent. In the uterine luminal epithelium low levels of AEA are beneficial, because implantation competency requires down-regulation of AEA binding to blastocyst CB_1 (75, 83). The mechanism by which uterine AEA levels is kept at different concentrations requires a differential expression of NAPE-PLD and FAAH: high levels of NAPE-PLD activity and expression have been found at inter-implantation sites of non-receptive uterus, whereas lower NAPE-PLD levels were found at implantation sites of receptive uterus (89). Conversely, FAAH activity and expression showed an opposite distribution, being high at implantation sites of receptive uterus and low at inter-implantation sites of non-receptive uterus (89). As a consequence, uterine receptivity strongly depends on AEA concentration, so that a receptive area is marked by low AEA content, whereas a non-receptive area has high AEA levels. Recent evidence also points to the possibility that the implanting blastocyst enhances uterine FAAH by releasing a “FAAH activator” (90), and concomitantly inhibits uterine NAPE-PLD expression (91). A recent study on cow infertility has lent support to the role of FAAH in regulating AEA levels during implantation (92). In fact, it was demonstrated that CB_1 or CB_2 receptor agonists decrease progesterone levels in luteal tissues and, since progesterone upregulates FAAH (93), they increase eCBs levels. Evidence that local AEA concentrations are critical for implantation was reported also in studies on embryos exposed to high levels of AEA, showing embryotoxicity, reduced trophoblast proliferation and implantation failure (78, 79, 94). Similarly, women undergoing an *in vitro* fertilization (IVF) programme and achieving successful implantation, were found to have low levels of plasma AEA, associated with elevated levels of FAAH in their peripheral lymphocytes (95). Overall, decreased activity and expression of FAAH in maternal lymphocytes could be used as an early marker for first trimester miscarriage, both in normal gestation (69) and assisted reproduction (95). This concept has found support in recent studies, that showed low levels of AEA in plasma of healthy women in the first trimester of gestation (68), but high levels in blood (68) and placental tissues (96) of women who had spontaneous miscarriage.

Taken together, available data demonstrate that under physiological conditions, eCBs signalling through CB_1 is crucial to various female reproductive events, that span from oogenesis to oviductal transport, homing and implantation, and development of embryos. Any aberration of eCBs signalling can severely derange these processes.

Further evidence has shown that plasma AEA levels, low in the first trimester of normal gestation, increase at the onset of labour (68), whereas CB_1 expression has been found to be higher in non-labouring than in labouring villi of human placenta (97). It was proposed that the latter switch may contribute to differentially regulate placental actions of AEA. These data were further extended by demonstrating that placental tissue from women with spontaneous miscarriage had very low levels of FAAH and increased expression of CB_1 (96), whereas in human placenta the levels of FAAH increase in the first trimester of pregnancy and decline by the early second trimester (67). Moreover, CB_1, CB_2 and FAAH mRNAs and proteins have been observed in the human first trimester placenta, with cellular localization for all three ECS elements confined within the syncytiotrophoblast layer (96). These data confirm that high FAAH levels in normal placenta, during the first trimester of pregnancy, represent a pro-fertility factor at the materno-fetal interface. On the other hand, human placenta is able to synthesize AEA through NAPE-PLD, whose mRNA expression is higher in women undergoing normal termination than in those who miscarried (96). It is noteworthy that peripheral AEA levels remained relatively low during implantation, whereas they increased before and during parturition in humans. It remains to be established which advantage can be conferred by high NAPE-PLD under these conditions. At any rate, these observations add a new dimension to the concern that the adverse effects of maternal use of cannabis on offspring may be seeded very early in pregnancy. A summary of the ECS elements detected in female reproductive cells and tissues is reported in Table 1.

Table 1. ECS elements detected in female reproductive cells and tissues. See text for abbreviations and details

Cell/Tissue	ECS elements	References
Oocyte	CB_1; CB_2	76
Blastocyst	CB_2	78, 79
Ovary	AEA; CB_1; CB_2; NAPE-PLD; FAAH	21, 156
Uterus	CB_1 FAAH; NAPE-PLD DAGL; MAGL	91 89 75
Embryo	CB_1; CB_2; NAPE-PLD (at late stages) FAAH (at early stages)	21 73-75
Placenta	CB_1; CB_2; NAPE-PLD; FAAH TRPV1 (deciduas)	21 80
Peripheral lymphocytes	FAAH; NAPE-PLD; AEA	21, 69

4.3. Dysregulation and therapeutic exploitation

The involvement of ECS in embryo oviductal transport, blastocyst development and implantation in females has been deeply discussed, and the reduced level of the eCBs-hydrolase FAAH in peripheral lymphocytes has been recognized as a predictor of spontaneous miscarriage in healthy women (69), as well as of failure to achieve an ongoing pregnancy after *in vitro* fertilization (IVF)-embryo transfer in women undergoing assisted reproduction cycles (95). It seems that eCBs have the potential to direct human fertility towards a positive or negative outcome, and that assays of eCBs levels and/or of the activity of their metabolic enzymes in human blood hold the promise to become reliable markers of natural and assisted reproduction. Therefore, defective FAAH in peripheral blood has been proposed as a diagnostic marker of human infertility, and in line with this a recent study has demonstrated that the content of AEA in blood is predictive of miscarriage in women at risk (70). In the same context, detection of AEA in follicular fluid might be a predictor of oocyte maturity (67). On the other hand, the drop of plasma AEA level that occurs at ovulation, and less during implantation, could be used as a biomarker for the appropriate timing of embryo transfer in IVF protocols, and/or in intracytoplasmatic sperm injection (ICSI) procedures. Furthermore, CB_1 holds promise as a diagnostic marker of reproduction, because high CB_1 expression in first trimester placenta corresponds to spontaneous miscarriage and low CB_1 expression in Fallopian tubes and endometrium seems to be associated with genetic predisposition to ectopic pregnancy. Incidentally, the latter defect is the most common cause of pregnancy-related first trimester death in the United States. Overall, these data demonstrate that eCBs signalling is at least one of the pathways determining the fate of implantation in all mammals.

5. ECS IN MALE FERTILITY

5.1. Physiological distribution

The investigation of ECS in male reproductive organs has been performed from invertebrate to mammals, and the presence of CB receptors in spermatozoa isolated from sea urchin (98), sea squirt (99), frog (100), mouse (100), rat (100), boar (101), bovine (102) and human (103) supported the "evolutionary" theory that ECS (and in particular CB_1 and FAAH) are check points in reproduction (29, 62, 85, 104). The starting point in the investigation of ECS in male reproductive organs was the demonstration that rat testis is able to synthesize endocannabinoids (105).

Endogenous *N*-acylethanolamines were quantified in the same tissue shortly after (106). Later on, the supposedly ancient association between ECS and male reproductive events was confirmed by mRNA and protein expression of CB_1 and FAAH in the gonads of teleosts (107) and in the testis of the frog *Rana esculenta*, collected during the annual reproductive cycle (100). Interestingly, quantitative and qualitative immunohistochemical analysis showed a peak of signals in September-October, when the entire process of sperm maturation was completed (100).

The internal structure of the male gland is a net of primary cell types within the seminiferous tubule (germ cells and Sertoli cells) and interstitial cells, such as Leydig cells, macrophages and epithelial cells, supplied by blood vessels and lymphatic drainage. Sertoli cells, considered a "nurse" of testis, are part of the seminiferous tubule and sustain the developing sperm during spermatogenesis (108). The presence of several elements of ECS has been demonstrated by our group at different developmental stages of these cells in mouse (109, 110). In fact, we found that immature Sertoli cells, obtained from 4 to 16 day-old mice, express CB_2 receptors (109). Moreover, these cells are equipped with all metabolic enzymes to synthesize and degrade AEA and 2-AG, as demonstrated by functional assays (109), quantitative polymerase chain reaction, and Western blot analysis (111). Incidentally, also a faint signal for CB_1 receptor has been detected in rat Sertoli cells, particularly at stages VI–X of spermatogenesis, thus it cannot be ruled out that this receptor subtype is expressed also at other stages of sperm development (23).

Leydig cells, also known as interstitial cells, are adjacent to the seminiferous tubules in the testicle and are in charge of producing androgens, in particular testosterone in the presence of luteinizining hormone (LH). The immunohistological demonstration of CB_1 receptors in mouse Leydig cells has been reported in 2001 by Wenger and coworkers (112), and a spatio-temporal expression of CB_1 in interstitial cells during postnatal development of testis has been proposed more recently (113). The simultaneous analysis of testosterone and LH levels in wild-type mice pretreated with the CB_1 antagonist SR141716, or in CB_1 knockout animals, has shown a CB_1-mediated effect of AEA on the regulation of steroidogenesis, through promotion or suppression of testicular hormone secretion (112). In fact, the trend of CB_1 immunoreactivity in Leydig cells, isolated from testis at 1, 2, 4 and 8-week post partum, showed a moderate expression in the early stage of cell development, followed

Table 2. ECS elements detected in male reproductive cells and tissues. See text for abbreviations and details

Cells/Tissue	ECS elements	Animal model	Reference
Testis	AEA and other *N*-acylethanolamines	Rat	106
	FAAH; CB_1	Frog, Rat	100, 105, 107
Sertoli cells	FAAH; EMT; CB_2	Mouse	109
	CB_1	Mouse	111
Leydig cells	CB_1	Mouse, Rat	23, 112, 113
Spermatozoa	AEA; NAPE-PLD; EMT; FAAH; CB_1; TRPV1	Boar, Bovine, Frog, Human, Invertebrate, Mouse, Rat,	100-103, 117, 146, 156, 157
	2-AG; DAGL; MAGL	Mouse	111

by a decrease during the passage from undifferentiated cells to adult Leydig cells up to the detection of a strong immunochemical signal at the beginning of puberty and during adulthood (113). The normal development of these soma cells was corroborated by the identification of a conventional marker for adult type Leydig cells, 17-beta-hydroxysteroid dehydrogenase (17-beta-HSD-3), whose expression pattern was closely overlapping on that of CB_1 (113, 114).

Similarly, temporal expression of CB_1 in rat Leydig cells showed that CB_1-dependent signalling negatively regulates adult Leydig cells proliferation between postnatal days 28 and 56. In fact, at this stage immature Leydig cells undergo mitotic division before their final differentiation into adult cells, and it seems that only immature mitotic, but not non-mitotic, Leydig cells express CB_1 (23). These data support the hypothesis that CB_1 signalling is a positive effector in the regulation of cell differentiation, whereas it plays a negative role on cell growth (23). This concept was further supported by the observation that the number of adult Leydig cells in CB_1 KO mice was lower than in wild-type mice (23). Spermatozoa are continuously produced throughout the life of adult male primates, a time-span that encompasses a few years in some non-human primates, but 50 or more years in man (115). This process is called spermatogenesis and begins with the division of cells, usually identifiable morphologically as primary spermatogonia (116), which in turn proliferate and differentiate into type A1 spermatogonia. The latter cells undergo a series of synchronized mitotic divisions, giving rise to type B spermatogonia, that develop into spermatocytes and then undergo meiotic division to produce spermatids. Spermiogenesis represents the morphological transformation from spermatids to elongated and highly motile cells, spermatozoa, the latter cells are differentially released from Sertoli cells (spermiation), depending on the species (115). As reported above, a fully active ECS, or at least some of its components, has been identified in spermatozoa obtained from human (103, 117), bovine (102), boar (101), mouse (111, 113), frog (100) and sea urchin (118). Recently, it has been demonstrated that ECS might be modulated in the phases of spermatogenesis (111). The same authors showed different translational and transcriptional levels of both CB receptors and TRPV1 at the postmeiotic stage of sperm maturation. In particular, CB_2 mRNA and protein expression was detectable in spermatogonia, spermatocytes and spermatids, while at the end of spermatogenesis its immunofluorescence staining was negative in mature spermatozoa and was localized in the residual bodies of cytoplasm only (111). Interestingly, functional experiments in the presence of the potent selective CB_2 agonist JWH133, and biochemical analysis of AEA and 2-AG metabolism and content, performed at all stages of spermatogenesis, unveiled a key-role for CB_2 in promoting meiotic progression of male germ cells, through a 2-AG-dependent pathway (111). The metabolic control of 2-AG tone was demonstrated by showing that the highest levels of 2-AG in spermatogonia (compared to spermatocytes and spermatids) were due to increased transcriptional and translational levels of DAGL, and decreased levels of MAGL (111). On the other hand, AEA levels remained unaltered during spermatogenesis, and its biosynthetic and catabolic enzymes (NAPE-PLD and FAAH, respectively) were both increased during germ cell meiosis (111). It was suggested that a steady AEA tone in testes might allow TRPV1 activation, that protects germ cells against the effects of heat stress (119). In fact, experiments performed on mice lacking TRPV1 demonstrated that this ion channel interferes with spermatogenesis, by promoting apoptosis of spermatogonia and blocking meiosis of germ cells as a defensive mechanism in response to abnormally elevated testicular temperatures (119). The ECS elements detected in male reproductive cells and tissues from different animal models are reported in Table 2.

5.2. Functional role

The spermatogenic output of mammals is strictly controlled by estrogens, which are produced mainly by Sertoli cells (120), and by the follicle stimulating hormone (FSH), that is a member of the glycoprotein hormone family essential for normal reproduction, both in males and females. FSH is required during puberty, in order to assure the initiation of spermatogenesis, and in adulthood, in order to guarantee sperm production. Additionally, FSH can contribute to estrogen production (121), and controls normal Sertoli cell functions, by binding to FSH receptors in testis (122). Activation of FSH receptors on Sertoli cells triggers several signal transduction pathways that regulate gene expression and cell differentiation and proliferation (65). For instance, phosphorylation of protein kinase B by FSH enhances gene promoter activity of the androgen-converting enzyme aromatase (123), thus affecting the balance between estrogen and testosterone along male reproduction. Recently, an elegant ECS-mediated mechanism has been proposed for the modulation of Sertoli cell number by FSH, that is based on the control of the pro-apoptotic activity of AEA. The latter is triggered by activation of TRPV1 and is prevented by activation of CB_2 (109). FSH dose-dependently enhances FAAH activity and expression, thus reducing the endogenous content of AEA, and hence TRPV1 binding and subsequent apoptosis (109). Remarkably, none of the other eCBs-related metabolic

enzymes of Sertoli cells (e.g., NAT, NAPE-PLD, DAGL and MAGL) was under control of FSH (110), identifying FAAH as one of the few specific targets of this gonadotropin as yet known (65). Also protein kinase A (PKA) and aromatase are engaged by FSH to modulate FAAH in Sertoli cells: the PKA-dependent pathway acts directly on FAAH activity, possibly by phosphorylating transcription factors and accessory proteins; the aromatase-dependent pathway increases FAAH expression at the transcription and translation levels (110). Incidentally, these data are in keeping with the presence of an estrogen response element in the mouse *faah* promoter (124), and corroborate the hypothesis of a tight link between ECS and sex hormones also in male reproductive events. After ejaculation, and during sperm transit through the female reproductive tract, sperm motility is initiated and then hyperactivated to allow the spermatozoa to penetrate through the viscous oviductal mucus and the egg's protective vestments, thus completing a successful fertilization. The acquisition of fertilizing ability, termed capacitation (125), includes reorganisation of membrane proteins, metabolism of membrane phospholipids, reduction of membrane cholesterol (126), and changes in sperm motility, which altogether are thought to aid sperm progression to the oviduct (127) and to improve its ability to break the egg's extracellular coat (the so-called zona pellucida; ZP) (128). As reported above, it is well-documented that the phytocannabinoid THC decreases testicular weight (129), has adverse actions on the release of testosterone (7), disrupts spermatogenesis when administrated for a long time (130), induces anomalies in sperm morphology, and affects sperm motility and viability (118). Overall, THC negatively impacts the fertilizing potential of man (131). In mammals, acquisition of sperm motility is a two step mechanism, that starts in the epididymus and continues in the female reproductive tract. In fact, after spermiation immotile spermatozoa reach the epididymus by passive transport, and on their way from caput to cauda they become motile (132). Remarkably, eCBs are present in reproductive fluids along evolution, from cloacal fluid of amphibians (100) to seminal plasma of humans (10); across species eCBs inhibit the biochemical and physiological changes needed for sperm capacitation, through a CB_1-mediated mechanism (101, 117, 133, 134). In this context, preliminary data were obtained by comparing the percentage of motile spermatozoa in the caput and cauda of wild-type and CB_1 KO mice (133). The count of motile sperm in wild-type mice was significantly lower in the caput than in the cauda, whereas a dramatic increase of motile sperm, comparable to that observed in the cauda, was detected in the caput of transgenic mice; therefore, it was suggested that acquisition of progressive motility by sperm is under control of eCBs through CB_1 signalling in the epididymus (133, 135).

More recently, a regulatory role of 2-AG has been identified in mouse epididymial sperm start up, showing that throughout their transit from caput to cauda spermatozoa are in close contact with decreasing concentrations of 2-AG itself (136). The resulting gradient is maintained by a putative EMT, and is controlled by a tight equilibrium between DAGL and MAGL activity in the epididymal tissue and cauda; this gradient is likely to be necessary to counteract the CB_1-dependent inhibition of motility, and to keep the spermatozoa quiescent until their final release (136). Although some aspects of eCBs signalling in male reproduction are overlapping in different species along the evolutionary axis, the 2-AG gradient seems to be regulated in different manners: mammals use an autocrine mechanism, whereas amphibians take advantage of an "acquatic dilution" of eCBs, that makes the spermatozoa motile (100). The effects of AEA on human sperm motility have been ascertained both at pharmacological (117) and physiological (134) doses, and in all cases it has been demonstrated a dose-dependent reduction, mediated by CB_1, of sperm viability and mitochondrial activity, paralleled by modulation of lipid and glucose metabolism (134). Altered levels of intracellular tryglycerides, increased activity of lipase and glucose-6-phosphate dehydrogenase (G6PDH), and modulation of insulin release after treatment with the non-hydrolyzable AEA analogue methanandamide (Met-AEA), might indeed contribute to the control of sperm energy homeostasis by eCBs, which in turn affects critical sperm functions like motility, capacitation and acrosome reaction (61). On the other hand, capacitated sperm show a general down-regulation of the expression of ECS elements compared to non-capacitated sperm (101). Additionally, under capacitating conditions Met-AEA reduces tyrosine phosphorylation of sperm proteins, an event which marks the capacitation status in mouse (134, 137). These inhibitory effects of Met-AEA are also attributable to its ability to reduce, in a CB_1-dependent manner, the intracellular levels of cyclic adenosine monophosphate (cAMP), that are necessary for the capacitation process (61, 101, 138). Another well-known effect of AEA is the inhibition of ZP-induced acrosome reaction (AR) (101).

In mammals AR is the fusion, at multiple points, of the outer acrosomal membrane with the overlying sperm plasma membrane. This fusion causes the release of acrosomal contents and the exposure of the inner acrosomal membrane (139), as well as of other membrane domains known to be essential for fertilization (140). The recognition and binding of capacitated spermatozoa to ZP in a receptor-ligand manner is the starting point of this process (126, 141, 142), and in humans it engages at least two different sperm receptors : a G_i-coupled receptor, that activates phospholipase C (PLC) and is able to regulate adenylyl cyclase activity to produce cAMP and to activate protein kinase A (PKA); and a tyrosin-kinase receptor, that is coupled to PLC and activates, through an inositol triphosphate (IP_3)-dependent cascade, the opening of a voltage-dependent Ca^{2+} channel in the outer acrosomal membrane. The latter event allows the release of Ca^{2+} from the interior of the acrosome to the cytosol (140), where the subsequent phosphorylation of proteins leads to AR (143). Although ZP provides the primary ligand site(s) for the receptor(s) present on the surface of the anterior head of capacitated spermatozoa (126, 141, 142), a number of physiological and non-physiological substances are also known to induce AR in the cauda epididymal or ejaculated spermatozoa. The physiological inducers are substances that sperm will encounter during *in vivo* fertilization (144),

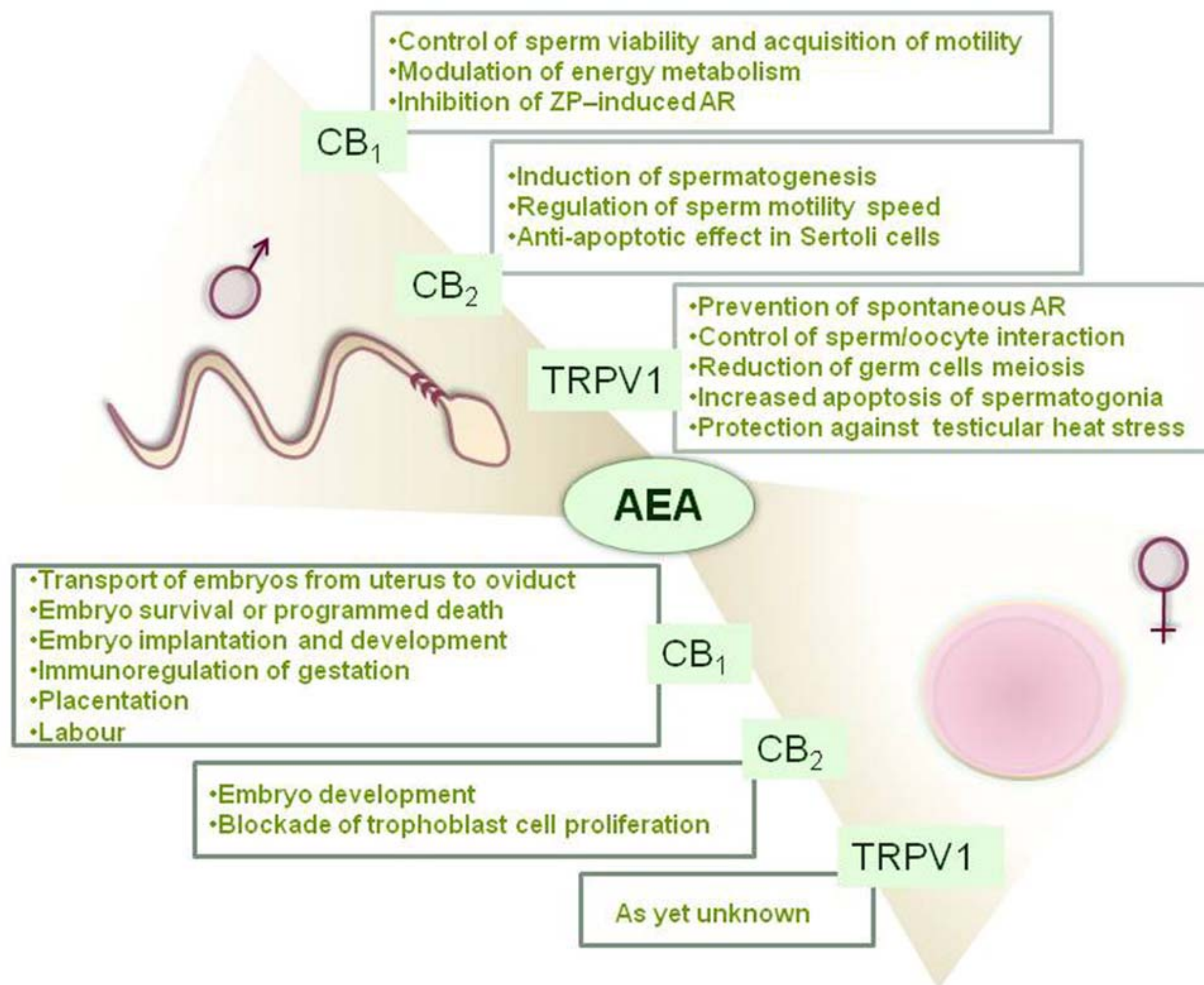

Figure 6. Main effects of AEA on female and male fertility, mediated by CB_1, CB_2 or TRPV1 receptors. See text for details.

such as progesterone that enhances both sperm binding to ZP and ZP-mediated induction of AR (145). Substantially homogeneous data exist indicating that CB_1 activation reduces the ability of sperm to undergo AR, from invertebrates to vertebrates (101, 104, 117, 138, 146). In particular, data on boar sperm, whose reproductive biology resembles several aspects of that of humans, have shown that Met-AEA reduces sperm capacitation, and hence the ability of sperm to react with ZP proteins and lead to acrosome exocytosis (101). It is noteworthy that CB_1 controls the effect of Met-AEA on ZP-induced AR, while the effect of this AEA anologue on spontaneous AR was under control of TRPV1 receptor (101). This ion channel is expressed also by human sperm (103), where it prevents spontaneous AR and favours the sperm/oocyte interaction stimulated by physiological inducers of AR (103). In addition, TRPV1 has been detected in murine sperm, where it contributes to control the choice between survival and death during spermatogenesis (111). On the other hand, CB_2 has been shown to be involved in shifting the number of motile cells to slow motile/sluggish spermatozoa (147), suggesting that the two cannabinoid receptor subtypes may regulate different aspects of sperm physiology *in vivo*: motility *versus* immotility (CB_1), and fast motility *versus* slow motility (CB_2). In addition, activation of CB_2 by 2-AG has been recently demonstrated to represent the true key-point in the regulation of spermatogenesis in mammals (111). It can be speculated that the two CB receptors may be engaged differently in response to eCBs gradients along the male and female tracts, because they differ significantly for their affinity towards AEA and 2-AG (19).

5.3. Dysregulation and therapeutic exploitation

The reasons for male infertility remain, most often, unknown and it cannot be ruled out that dysregulation of the ECS may perturb the finely tuned events associated with male fertilizing ability (81). We can speculate that the presence of an eCBs gradient through the male tract, and the need for a precise spatio–temporal activation of cannabinoid and vanilloid receptors in sperm, might control the quality of semen, and might affect negatively the interaction between sperm and oocyte, ultimately impairing male reproductive potential (64). Here it seems noteworthy that, in male idiopathic infertility, the

AEA congener PEA modulates some kinematic parameters of spermatozoa, and makes them more susceptible to develop hyperactivated motility (148). These effects could be due to perturbations in the physico-chemical properties of the lipid bilayer of membranes (149, 150), or to a direct interaction between PEA and membrane-bound enzymes. All together, these findings seem to point to PEA as a potential therapeutic target for idiopathic infertility. On the other hand, elevated AEA levels may compromise sperm viability, motility and capacitation, thus a tight control of AEA tone in male reproductive tissues could have therapeutic relevance to treat infertility in man. To date, most studies have been focused on CB_1 as the main responsible for the activity of eCBs (in particular of AEA) on male reproduction; however, very recently it has been demonstrated that mice lacking FAAH have reduced fertilizing ability and slow spermatozoa, that are unable to adhere to and penetrate the ZP of the egg (151). Interestingly, these defects associated with FAAH deficiency could be relapsed by the simultaneous genetic loss of CB_1, lending support to the critical role of the metabolic control of AEA content (and, hence, of its signalling through CB_1) for the design of eCBs-oriented drugs as novel anti-infertility therapeutics. Taken together, it should be recalled that the risk of altered semen parameters and infertility is associated, mainly in obese men, to an imbalance in hormone profile and to a deregulation of the hypothalamus-pituitary-gonadal axis (152). The presence of ECS elements along this axis (59, 81), and their involvement in hormonal control, adds a new dimension to the intricate network of autocrine, endocrine and paracrine signals that regulate reproductive events, and open new perspectives for the treatment of male fertility problems.

6. CONCLUSIONS

Mammalian reproduction is a complex process, involving germ cell development (spermatogenesis and oogenesis), sperm activation, oocyte fertilization, preimplantation embryo development, timely passage of embryos through the oviduct, their implantation in the uterus, and further development aided by a functional placenta. Each step in the reproductive process is spatio-temporally regulated by various networks of hormones, endocrine, paracrine, autocrine modulators, as well as by other critical signals like cytokines. A further level of complexity in these networks is represented by endocannabinoids, and the proteins that bind, synthesize, transport, transform or degrade them. Molecular, genetic, functional and pharmacological approaches have demonstrated that apparently all steps of reproduction, both at the female and male side, engage to some extent endocannabinoids, either to enhance or to reduce a certain signalling pathway. Remarkably, some alterations of the endocannabinoid system in blood cells mirror defects in the reproductive organs, and thus promise to have a potential diagnostic value. For instance, from the data reviewed above, it appears that assays of AEA levels in blood (153, 154), and/or of the activity and expression of the AEA-hydrolyzing enzyme FAAH (e.g., through a simple immunochemical test like ELISA), can be predictive of spontaneous miscarriage in healthy women, both under physiological conditions and during assisted reproduction techniques. On the other hand, ECS-oriented drugs may become useful therapeutics to combat female and male infertility, for instance by enhancing FAAH activity in those pathological conditions where it is too low. On a final note, it should be stressed that research efforts should be supported by funding agencies, in order to further our understanding of fertility defects, so that the ever-declining human reproductive potential may be restored, and then preserved against exogenous insults. Once the value of "natural" reproduction is re-established, research efforts should be focused on a better understanding of the mechanisms that underlie assisted reproduction technologies, thus improving their safety. Finally, the manifold roles of endogenous cannabinoids on reproduction raise caution on the potential thread represented by marijuana (155), especially for female consumers bearing a child, and for their offspring during childhood and adulthood.

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8. REFERENCES

1. W. Hall, L. Degenhardt: Adverse health effects of non-medical cannabis use. *Lancet* 374, 1383-13891 (2009)

2. M. Schneider: Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. *Addiction Biology* 13, 253-263 (2008)

3. T.H. Moore, S. Zammit, A. Lingford-Hughes, T.R. Barnes, P.B. Jones, M. Burke, G. Lewis: Cannabis use and risk of psychotic or affective mental health outcomes: A systematic review. *Lancet* 370, 319-328 (2007)

4. J. M. Tetrault, K. Crothers, B. A. Moore: Effects of marijuana smoking on pulmonary function and respiratory complications: A systemic review. *Arch Intern Med*167, 221-228 (2007)

5. W. M. Thomson, R.. PoultonJ. M. Broadbent: Cannabis smoking and periodontal disease among young adults. *JAMA* 299, 525-531(2008)

6. T.W. Klein, H. Friedman, C. Specter: Marijuana, immunity and infection. *J Neuroimmunol* 83, 102-115 (1998)

7. R.C. Kolodny, W.H. Masters, R.M. Kolodner, G. Toro: Depression of plasma testosterone levels after chronic

intensive marihuana use. *N Engl J Med* 290, 872-874 (1974)

8. A. J. Gruber, H.G. Jr.Pope: Marijuana use among adolescents. *Pediatric Clinics of North America* 49, 389-341(2002)

9. A.C. Huizink: Moderate use of alcohol, tobacco and cannabis during pregnancy: new approaches and update on research findings. *Reprod Toxicol* 28, 143-151 (2009)

10. H. Schuel, L.J, Burkman, J. Lippes, K. Crickard, E. Forester,D. Piomelli, A. Giuffrida: N-Acylethanolamines in human reproductive fluids. *Chem Phys Lipids* 121, 211–227 (2002)

11. M. Schneider: Cannabis use in pregnancy and early life and its consequences: animal models. *Eur Arch Psychiatry Clin Neurosci* 259, 383-393 (2009)

12. D.E. Hutchings, S.C. Brake, B. Morgan: Animal studies of prenatal delta-9-tetrahydrocannabinol: female embryolethality and effects on somatic and brain growth. *Ann N Y Acad Sci* 562, 133-144 (1989)

13. A. Jakubovic, T. Hattori, P.L. McGeer: Radioactivity in suckled rats after giving 14 C-tetrahydrocannabinol to the mother. *Eu J Pharmacol* 22, 221-223 (1973)

14. L. Goldschmidt, N.L. Day, G.A. Richardson: Effects of prenatal marijuana exposure on child behavior problems at age 10. Neurotoxicol Teratol 22, 325-336 (2000)

15. A.C. Huizink, E.J. Mulder: Maternal smoking, drinking or cannabis use during pregnancy and neurobehavioral and cognitive functioning in human offspring. *Neurosci Biobehav Rev* 30, 24-41 (2006)

16. M. Gómez, M. Hernández, B. Johansson, R. de Miguel, J.A. Ramos, J. Fernández-Ruiz: Prenatal cannabinoid and gene expression for neural adhesion molecule L1 in the fetal rat brain. *Brain Res Dev Brain Res* 147, 201-207 (2003)

17. F. Rodríguez de Fonseca, M. Cebeira, J.J. Fernández-Ruiz, M. Navarro, J.A.Ramos: Effects of pre- and perinatal exposure to hashish extracts on the ontogeny of brain dopaminergic neurons. *Neuroscience* 43, 713-23 (1991)

18. H. Schuel, R.Schuel, A.M. Zimmerman, S. Zimmerman: Cannabinoids reduce fertility of sea urchin sperm. *Biochem Cell Biol* 65, 130–136 (1987)

19. A.C. Howlett, F. Barth, T.I. Bonner, G. Cabral, P. Casellas, W.A. Devane, C.C. Felder, M. Herkenham, K. Mackie, B.R. Martin, R. Mechoulam, R.G: Pertwee International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* 54, 161-202 (2002)

20. M. Herkenham, A.B. Lynn, M.R. Johnson, L.S.Melvin, B.R. de Costa, K.C. Rice: Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J Neurosci* 11, 563–583 (1991)

21. M.R. El-Talatini, A.H. Taylor, J.C. Elson, L. Brown, A.C. Davidson, J.C. Konje: Localisation and function of the endocannabinoid system in the human ovary. *PLoS ONE* 4, e4579 (2009)

22. S. Munro, S. Munro, K.L. Thomas, M. Abu-Shaar: Molecular characterization of a peripheral Receptor for cannabinoids. *Nature* 365, 61–65 (1993)

23. G. Cacciola, T. Chioccarelli, G. Ricci, R. Meccariello, S. Fasano, R. Pierantoni, G. Cobellis: The endocannabinoid system in vertebrate male reproduction: a comparative overview. *Mol Cell Endocrinol* 286, S24-30 (2008)

24. S. Tambaro, R. Mongeau, C. Dessi, L. Pani, S. Ruiu: Modulation of ATP-mediated contractions of the rat vas deferens through presynaptic cannabinoid receptors. *Eur J Pharmacol* 525, 150-153 (2005)

25. J.S. Walczak, T.J. Price, F. Cervero: Cannabinoid CB1 receptors are expressed in the mouse urinary bladder and their activation modulates afferent bladder activity. *Neuroscience* 159, 1154-1163 (2009)

26. I. Matias, T. Bisogno, V. Di Marzo: Endogenous cannabinoids in the brain and peripheral tissues: regulation of their levels and control of food intake. *Int J Obes* 30, S7–S12 (2006)

27. M.D. Van Sickle, M. Duncan, P.J. Kingsley, A. Mouihate, P. Urbani, K. Mackie, N. Stella, A. Makriyannis, D. Piomelli, J.S. Davison, L.J. Marnett, V. Di Marzo, Q.J. Pittman, K.D. Patel, K.A. Sharkey: Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* 310, 329-332 (2005)

28. M.T. Viscomi, S. Oddi, L. Latini, N. Pasquariello, F. Florenzano, G. Bernardi, M. Molinari, M. Maccarrone: Selective CB2 receptor agonism protects central neurons from remote axotomy-induced apoptosis through the PI3K/Akt pathway. *J Neurosci* 29:4564-4570 (2009)

29. N. Battista, N. Pasquariello, M. Di Tommaso, M. Maccarrone: Interplay between endocannabinoids, steroids and cytokines in the control of human reproduction. *J Neuroendocrinol* 20, 82–89 (2008).

30. A. Park, H.M. Gibbons, M.D. Mitchell, M. Glass: Identification of the CB1 cannabinoid receptor and fatty acid amide hydrolase (FAAH) in the human placenta. *Placenta*. 24, 990-995 (2003)

31. J.E. Lauckner, J.B. Jensen, H.Y. Chen, H.C. Lu, B. Hille, K. Mackie: GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc Natl Acad Sci U S A* 105, 2699-2704 (2008)

32. A. Moriconi, I. Cerbara, M. Maccarrone, A. Topai: GPR55: Current Knowledge and Future Perspectives of a

Purported "Type-3" Cannabinoid Receptor. *Curr Med Chem* In press (2010)

33. A. Tóth, P.M. Blumberg, J. Boczán: Anandamide and the vanilloid receptor (TRPV1). *Vitam Horm* 81, 389-419 (2009)

34. V. Di Marzo, L. De Petrocellis: Endocannabinoids as regulators of transient receptor potential (TRP) channels: a further opportunity to develop new endocannabinoid-based therapeutic drugs. *Curr Med Chem* In press (2010)

35. M. Pistis, M. Melis: From Surface to Nuclear Receptors: The Endocannabinoid Family Extends its Assets. *Curr Med Chem* In press (2010)

36. A.C. Howlett: Efficacy in CB1 receptor-mediated signal transduction. *Br J Pharmacol*. 142, 1209-1218 (2004)

37. K. Starowicz ,S. Nigam, V. Di Marzo: Biochemistry and pharmacology of endovanilloids. *Pharmacol Ther* 114, 13–33 (2007)

38. S.E. O'Sullivan: Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. *Br J Pharmacol* 152, 576–582 (2007)

39. K. Ahn, MK. BF. Cravatt: Enzymatic pathways that regulate endocannabinoid signaling in the nervous system. *Chem Rev* 108, 1687–1707 (2008)

40. D. Leung , A. Saghatelian, GM. Simon, BF. Cravatt: Inactivation of N-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. *Biochemistry* 45, 4720-4726 (2006)

41. J. Liu, L. Wang, J. Harvey-White, D. Osei-Hyiaman, R. Razdan, Q. Gong, AC Chan, Z. Zhou, B.X. Huang, H.Y. Kim, G. Kunos: A biosynthetic pathway for anandamide. *Proc Natl Acad Sci USA* 103, 13345-13350 (2006)

42. G.M. Simon, B.F. Cravatt: Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/beta-hydrolase 4 in this pathway. *J Biol Chem* 28, 26465-26472 (2006)

43. G.M. Simon, B.F. Cravatt: Anandamide biosynthesis catalyzed by the phosphodiesterase GDE1 and detection of glycerophospho-N-acyl ethanolamine precursors in mouse brain. *J Biol Chem* 283, 9341-9349 (2008)

44. G.M. Simon, B.F. Cravatt: Characterization of mice lacking candidate N-acyl ethanolamine biosynthetic enzymes provides evidence for multiple pathways that contribute to endocannabinoid production *in vivo*. *Mol Biosyst* In press (2010)

45. X-H. Jin, Y. Okamoto, J. Morishita , K. Tsuboi, T. Tonai, N. Ueda: Discovery and characterization of a Ca2+-independent phosphatidylethanolamine N-acyltransferase generating the anandamide precursor and its congeners. *J Biol Chem* 282, 3614–3623 (2007)

46. Y. Okamoto, J. Morishita, K. Tsuboi, T. Tonai, N. Ueda: Molecular characterization of a phospholipase D generating anandamide and its congeners. *J Biol Chem* 279, 5298–5305 (2004)

47. T. Bisogno, F. Howell, G. Williams, A. Minassi, M.G. Cascio, A. Ligresti, I. Matias, A. Schiano-Moriello, P. Paul, E.J.Williams, U. Gangadharan, C Hobbs, V. Di Marzo, P. Doherty: Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J Cell Biol* 63, 463-468 (2003)

48. M. Beltramo, D. Piomelli: Carrier-mediated transport and enzymatic hydrolysis of the endogenous cannabinoid 2-arachidonylglycerol. *Neuroreport* 11, 1231-1235 (2000)

49. M. Kaczocha, S.T. Glaser, D.G. Deutsch: Identification of intracellular carriers for the endocannabinoid anandamide. *Proc Natl Acad Sci USA* 106, 6375–80 (2009)

50. B.F. Cravatt, D.K. Giang, S.P. Mayfield, D.L. Boger, R.A. Lerner, N.B. Gilula: Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* 384, 83-87 (1996)

51. B.Q. Wei, T.S. Mikkelsen, M.K. McKinney, E.S. Lander, B.F. Cravatt: A second fatty acid amide hydrolase with variable distribution among placental mammals. *J Biol Chem* 281,36569-36578 (2006)

52. K. Tsuboi, Y.X. Sun, Y. Okamoto, N. Araki, T. Tonai, N. Ueda: Molecular characterization of N-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. *J Biol Chem* 280, 11082-11092 (2005)

53. T.P. Dinh, T.F. Freund, D.B. Piomelli: A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. *Chem Phys Lipids* 121, 149-158 (2002)

54. S.Y. Ho, L. Delgado, J. Storch J: Monoacylglycerol metabolism in human intestinal Caco-2 cells: evidence for metabolic compartmentation and hydrolysis. *J Biol Chem* 277, 1816-1823 (2002)

55. K.R. Kozak, B.C. Crews, J.D. Morrow, L.H. Wang, H.Y. Ma, R. Weinander , P.J. Jakobsson, L.J. Marnett: Metabolism of the endocannabinoids, 2-arachidonoyglkycerol and anandamide, into prostaglandin, tromboxane, and prostacyclin glycerol esters and ethanolamides. *J Biol Chem* 277, 44877-44885 (2002)

56. M. Van der Stelt, J.A. van Kuik, M. Bari, G. van Zadelhoff, B.R. Leeflang, G.A. Veldink, A. Finazzi Agro', J.F. Vliegenthart, M. Maccarrone: Oxygenated metabolites of anandamide and 2-arachidonoyl-glycerol: conformational analysis and interaction with cannabinoid

receptors, membrane transporter and fatty acid amide hydrolase. *J Med Chem* 4, 3709-3720 (2002)

57. J.K. Chen, J. Chen, J.D. Imig, S. Wei, D.L. Hachey, J.S. Guthi, J.R. Falck, J.H. Capdevila, R.C. Harris: Identification of novel endogenous cytochrome p450 arachidonate metabolites with high affinity for cannabinoid receptors. *J Biol Chem* 283, 24514-24524 (2008)

58. K.R. Kozak, B.C, Crews, J.L. Ray, H.H. Tai, J.D. Morrow, L.J. Marnett: Metabolism of prostaglandin glycerol esters and prostaglandin ethanolamides *in vitro* and *in vivo*. *J Biol Chem* 276, 36993-36998 (2001)

59. M. Maccarrone: Endocannabinoids: friends and foes of reproduction. *Prog Lipid Res* 48, 344-354 (2009)

60. N. Battista, C. Rapino, M. Di Tommaso, M. Bari, N. Pasquariello, M. Maccarrone: Regulation of male fertility by the endocannabinoid system. *Mol Cell Endocrinol* 286 , S17-S23 (2008)

61. M. Rossato, F. Ion Papa, M. Ferigo, G. Clari, C. Foresta: Human sperm express cannabinoid receptor Cb1, the activation of which inhibits motility, acrosome reaction, and mitochondrial function. *J Clin Endocrinol Metab* 90, 984-991 (2005)

62. S. Fasano, R. Meccariello, G. Cobellis, R. Chianese, G. Cacciola, T. Chioccarelli, R. Pierantoni: The endocannabinoid system: an ancient signaling involved in the control of male fertility. *Ann NY Acad Sci* 1163, 112–124 (2009)

63. E. Fride, N. Gobshtis, H. Dahan, A. Weller, A. Giuffrida, S. Ben-Shabat: The endocannabinoid system during development: emphasis on perinatal events and delayed effects. *Vitam Horm* 81, 139-158 (2009)

64. S.E. Lewis, M. Maccarrone: Endocannabinoids, sperm biology and human fertility. *Pharmacol Res* 60, 126-131 (2009)

65. P. Grimaldi, G. Rossi, G. Catanzaro, M. Maccarrone: Modulation of the endocannabinoid-degrading enzyme fatty acid amide hydrolase by follicle-stimulating hormone. *Vitam Horm* 81, 231-261 (2009)

66. A.H. Taylor, A.A. Amoako, K. Bambang, T. Karasu, A. Gebeh, P.M. Lam, T.H. Marzcylo, J.C. Konje: Endocannabinoids and pregnancy. *Clin Chim Acta* In press 2010

67. M.R. El-Talatini, A.H. Taylor, J.C. Konje: Fluctuation in anandamide levels from ovulation to early pregnancy in in-vitro fertilization-embryo transfer women and its hormonal regulation. *Hum Reprod* 24, 1989–1998 (2009)

68. O.M. Habayeb, A.H. Taylor, M.D. Evans, M.S. Cooke, D.J. Taylor, S.C. Bell, J.C. Konje: Plasma levels of the endocannabinoid anandamide in women — a potential role in pregnancy maintenance and labor? *J Clin Endocrinol Metab* 89, 5482–5487 (2004)

69. M. Maccarrone, H. Valensise, M. Bari, N. Lazzarin, C. Romanini, A. Finazzi Agro': Relation between decreased anandamide hydrolase concentrations in human lymphocytes and miscarriage. *Lancet* 355, 1326–1329 (2000)

70. O.M. Habayeb, A.H. Taylor, M. Finney, M.D. Evans, J.C. Konje: Plasma anandamide concentration and pregnancy outcome in women with threatened miscarriage. *JAMA* 299, 1135–1136 (2008)

71. H. Schuel: Tuning the oviduct to the anandamide tone. *J Clin Invest* 116, 2087–2090 (2006)

72. R.J. Helliwell, L.W. Chamley, K. Blake-Palmer, M.D. Mitchell, J. Wu, C.S. Kearn, M. Glass: Characterization of the endocannabinoid system in early human pregnancy. *J Clin Endocrinol Metab* 89, 5168-5174 (2004)

73. B.C. Paria, S.K. Das, S.K. Dey:The preimplantation mouse embryo is a target for cannabinoid ligand-receptor signaling. *Proc Natl Acad Sci USA* 92, 9460–9464 (1995)

74. H. Wang, Y. Guo, D. Wang, P.J. Kingsley, L.J. Marnett, S.K. Das, R.N. DuBois, S.K. Dey: Aberrant cannabinoid signaling impairs oviductal transport of embryos. *Nat Med* 10, 1074–1080 (2004)

75. H. Wang, H. Xie, Y. Guo, H. Zhang, T. Takahashi, P.J. Kingsley, L.J. Marnett, S.K. Das, B.F. Cravatt, S.K.Dey: Fatty acid amide hydrolase deficiency limits early pregnancy events. *J Clin Invest* 116, 2122–2131 (2006)

76. L. Peralta, E. Agirregoitia, R. Mendoza, A. Exposito, R. Matrorras, N. Agirregoitia: Human oocytes express CB1 and CB2 cannabinoid receptors and their localization changes in the different oocyte maturation stages. In: IV European workshop on cannabinoid research (7–10 May 2009, S. Lorenzo de El Escorial, Madrid, Spain). p. 146.

77. A.W. Horne, J.A Phillips, N. Kane, P.C. Lourenco, S.E. McDonald, A.R. Williams, C. Simon, S.K. Dey, H.O. Critchley: CB1 expression is attenuated in Fallopian tube and decidua of women with ectopic pregnancy. *PLoS One* 3, e3969 (2008)

78. B.C. Paria, S.K. Dey: Ligand-receptor signaling with endocannabinoids in preimplantation embryo development and implantation. *Chem Phys Lipids* 108, 211-220 (2000)

79. Z.M. Yang, B.C. Paria, S.K. Dey: Activation of brain-type cannabinoid receptors interferes with preimplantation mouse embryo development. *Biol Reprod* 55, 756-761 (1996)

80. B.M. Fonseca, G. Correia-da-Silva, A.H. Taylor, J.C. Konje, S.C. Bell, N.A. Teixeira: Spatio-temporal expression patterns of anandamide-binding receptors in rat implantation sites: evidence for a role of the endocannabinoid system during the period of placental development. *Reprod Biol Endocrinol* 7, 121 (2009)

81. H. Wang, S.K. Dey, M. Maccarrone: Jekyll and hyde: two faces of cannabinoid signaling in male and female fertility. *Endocr Rev* 2, 427–448 (2006)

82. AH.Taylor, C. Ang, SC. Bell, JC Konje: The role of the endocannabinoid system in gametogenesis, implantation and early pregnancy. *Hum Reprod Update* 1, 501–513 (2007)

83. X.Sun, S.K. Dey: Aspects of endocannabinoid signaling in periimplantation biology. *Mol Cell Endocrinol* 286, 3–11 (2008)

84. M. Maccarrone: CB2 receptors in reproduction. *Br J Pharmacol* 153, 189–198 (2008)

85. M. Maccarrone: Endocannabinoids and reproductive endocrinology. *Curr Opin Investig Drugs* 10, 305–310 (2009)

86. M. Maccarrone, V. Gasperi, M. Bari, L. Avigliano, A. Finazzi Agro': Sistema nervoso e organi periferici. In. Gli endocannabinoidi: nuovi mediatori lipidici di rilevanza biomedica. Eds. Piccin, Padua, Italy (2009)

87. N. Lazzarin, H. Valensise, M. Bari, F. Ubaldi, N. Battista, A. Finazzi Agro', M. Maccarrone: Fluctuations of fatty acid amide hydrolase and anandamide levels during the human ovulatory cycle. *Gynecol Endocrinol* 18, 212-218 (2004)

88. B.C. Paria, H. Song, X. Wang, P.C. Schmid, RJ. Krebsbach, H.H. Schmid, T.I. Bonner, A. Zimmer, S.K. Dey: Dysregulated cannabinoid signaling disrupts uterine receptivity for embryo implantation. *J Biol Chem* 276, 20523–20528 (2001)

89. H. Wang, H. Xie, X Sun, P.J. Kingsley, L.J. Marnett, B.F.Cravatt, S.K. ,Dey: Differential regulation of endocannabinoid synthesis and degradation in the uterus during embryo implantation. *Prostaglandins Other Lipid Mediat* 83, 62–74 (2007)

90. M. Maccarrone, M. De Felici, FG. Klinger, N. Battista, F. Fezza, E. Dainese, G. Siracusa, A. Finazzi Agro': Mouse blastocysts release a lipid which activates anandamide hydrolase in intact uterus. *Mol Hum Reprod* 10, 215-21 (2004)

91. Y. Guo, H. Wang, Y. Okamoto, N. Ueda, P.J. Kingsley, L.J. Marnett, H.H. Schmid, S.K. Das, S.K. Dey: N-acylphosphatidylethanolamine-hydrolyzing phospholipase D is an important determinant of uterine anandamide levels during implantation. *J Biol Chem* 280, 23429-23432 (2005)

92. Y.S. Weems, A.W. Lewis, D.A. Neuendorff, R.D. Randel, C.W. Weems: Endocannabinoid 1 and 2 (CB(1); CB(2)) receptor agonists affect negatively cow luteal function *in vitro*. *Prostaglandins Other Lipid Mediat* 90, 89-93 (2009)

93. M. Maccarrone, H. Valensise, M. Bari, N, Lazzarin, C. Romanini, A. Finazzi Agro': Progesterone up-regulates anandamine hydrolase in human lymphocytes: role of cytokines and implications for fertility. *J Immunol* 166, 7183–7189 (2001)

94. H. Wang, S.K Dey: Lipid signaling in embryo implantation. *Prostaglandins Other Lipid Mediat* 77, 84-102 (2005)

95. M. Maccarrone, T. Bisogno, H. Valensise, N. Lazzarin, F. Fezza, C. Manna, V.Di Marzo, A. Finazzi Agro': 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, 188–195 (2002)

96. E. Trabucco, G. Acone, A. Marenna, R Pierantoni, G. Cacciola, T. Chioccarelli, K. Mackie, S. Fasano, N. Colacurci, R. Meccariello, G. Cobellis, L. Cobellis: Endocannabinoid system in first trimester placenta: low FAAH and high CB1 expression characterize spontaneous miscarriage. *Placenta* 30, 516–522 (2009)

97. G. Acone, E. Trabucco, N. Colacurci, L. Cobellis, K. Mackie, R. Meccariello, G. Cacciola, T. Chioccarelli, S. Fasano, R. Pierantoni, G. Cobellis: Low type I cannabinoid receptor levels characterize placental villous in labouring delivery. *Placenta* 30, 203–205 (2009)

98. M.C. Chang, D. Berkery, R. Schuel, S.G. Laychock, A.M. Zimmerman, S. Zimmerman, H. Schuel: Evidence for a cannabinoid receptor in sea urchin sperm and its role in blockade of the acrosome reaction. *Mol Reprod Dev* 36, 507-516 (1993)

99. M.R. Elphick, Y. Satou, N. Satoh: The invertebrate ancestry of endocannabinoid signalling: an orthologue of vertebrate cannabinoid receptors in the urochordate Ciona intestinalis. *Gene* 302, 95-101 (2003)

100. G. Cobellis, G. Cacciola, D. Scarpa, R. Meccariello, R. Chianese, M.F. Franzoni, K. Mackie, R. Pierantoni, S. Fasano: Endocannabinoid system in frog and rodent testis: type-1 cannabinoid receptor and fatty acid amide hydrolase activity in male germ cells. *Biol Reprod* 75, 82–89 (2006)

101. M. Maccarrone, B. Barboni, A. Paradisi, N. Bernabo, V. Gasperi, M.G. Pistilli, F. FezzA, P. Lucidi, M. Mattioli: Characterization of the endocannabinoid system in boar spermatozoa and implications for sperm capacitation and acrosome reaction. *J Cell Sci* 118, 4393–4404 (2005)

102. M.G. Gervasi, M. Rapanelli, M.L. Ribeiro, M. Farina, S. Billi, A.M. Franchi, S. Perez Martinez: The endocannabinoid system in bull sperm and bovine oviductal epithelium: role of anandamide in sperm-oviduct interaction. *Reproduction* 137, 403-414 (2009)

103. F. Francavilla, N. Battista, A. Barbonetti, M.R. Vassallo, C. Rapino, C. Antonangelo, N.Pasquariello, G. Catanzaro, B. Barboni, M. Maccarrone: Characterization of the endocannabinoid system in human spermatozoa and involvement of transient receptor potential vanilloid 1

receptor in their fertilizing ability. *Endocrinology* 150, 4692-4700 (2009)

104. R. Pierantoni, G. Cobellis, R. Meccariello, G. Cacciola, R. Chianese, T. Chioccarelli, S. Fasano: CB1 activity in male reproduction: mammalian and nonmammalian animal models.*Vitam Horm*. 81, 367-87 (2009)

105. T. Sugiura, S. Kondo, A. Sukagawa, T. Tonegawa, S. Nakane, A. Yamashita, K. Waku: Enzymatic synthesis of anandamide, an endogenous cannabinoid receptor ligand, through N-acylphosphatidylethanolamine pathway in testis: involvement of Ca(2+)-dependent transacylase and phosphodiesterase activities. *Biochem Biophys Res Commun* 218, 113-117 (1996)

106. S. Kondo, T. Sugiura, T. Kodaka, N. Kudo, K. Waku, A. Tokumura: Accumulation of various N-acylethanolamines including N-arachidonoylethanolamine (anandamide) in cadmium chloride-administered rat testis. *Arch Biochem Biophys* 354, 303-310 (1998)

107. E. Cottone, A. Guastalla, K. Mackie, M.F. Franzoni: Endocannabinoids affect the reproductive functions in teleosts and amphibians. *Mol Cell Endocrinol*. 286, S41-S45 (2008)

108. S.R. Sylvester, M.D. Griswold: The testicular iron shuttle: a "nurse" function of the Sertoli cells*. J Androl* 15, 381-385 (1994)

109. M. Maccarrone, S. Cecconi, G. Rossi, N. Battista, R. Pauselli, A. Finazzi Agro': Anandamide activity and degradation are regulated by early postnatal aging and follicle-stimulating hormone in mouse Sertoli cells. *Endocrinology* 144, 20–28 (2003)

110. G. Rossi, V. Gasperi, R. Paro, D. Barsacchi, S. Cecconi, M. Maccarrone: Follicle stimulating hormone activates fatty acid amide hydrolase by protein kinase A and aromatase-dependent pathways in mouse primary Sertoli cells. *Endocrinology* 148, 1431–1439 (2007)

111. P. Grimaldi, P. Orlando, S. Di Siena, F. Lolicato, S. Petrosino, T. Bisogno, R. Geremia, L. De Petrocellis , V. Di Marzo: The endocannabinoid system and piv otal role of the CB2 receptor in mouse spermatogenesis. *Proc Natl Acad Sci USA* 106, 11131-11136 (2009)

112. T. Wenger, C. Ledent, V. Csernus, I. Gerendai: The central cannabinoid receptor inactivation suppresses endocrine reproductive functions. *Biochem Biophys Res Commun* 284, 363-368 (2001)

113. M.C. Gye, H.H. Kang, H.J. Kang: Expression of cannabinoid receptor 1 in mouse testes. *Arch Androl* 51, 247-255 (2005)

114. S.G. Haider: Cell biology of Leydig cells in the testis*. Int Rev Cytol* 233, 181-241 (2004)

115. S.M. Shalet: Normal testicular function and spermatogenesis. *Pediatr Blood Cancer* 53, 285-288 (2009)

116. E.C. Roosen-Runge: Comparative Aspects of Spermatogenesis. *Biol Reprod* 1, 24-39 (1969)

117. M. Rossato: Endocannabinoids, sperm functions and energy metabolism. *Mol Cell Endocrinol* 286: S31-S35 (2008)

118. H. Schuel, M.C. Chang, D. Berkery, R. Schuel, A.M. Zimmerman, S. Zimmerman: Cannabinoids inhibit fertilization in sea urchins by reducing the fertilizing capacity of sperm. *Pharmacol Biochem Behav* 40, 609-615 (1991)

119. SC. Mizrak, BM. Gadella, H. Erdost, A. Ozer , AM. van Pelt, FM. van Dissel-Emiliani: Spermatogonial stem cell sensitivity to capsaicin: an *in vitro* study. *Reprod Biol Endocrinol*. 6, 52 (2008)

120. V. Pentikaïnen, K. Erkkilä, L. Suomalainen, M. Parvinen, L. Dunkel: Estradiol acts as a germ cell survival factor in the human testis *in vitro*. *J Clin Endocrinol Metab* 85, 2057–2067 (2000)

121. S. Carreau, C. Delalande, D. Silandre, S. Bourguiba, S. Lambard: Aromatase and estrogen receptors in male reproduction. *Mol Cell Endocrinol* 246, 65-68 (2006)

122. H. Krishnamurthy, P.S. Babu, C.R. Morales, M.R. Sairam: Delay in sexual maturity of the follicle-stimulating hormone receptor knockout male mouse. *Biol Reprod*6 5, 522-531 (2001)

123. C.A. McDonald, A.C Millena, S Reddy, S. Finlay, J. Vizcarra, S.A. Khan, J.S. Davis: Follicle-stimulating hormone-induced aromatase in immature rat Sertoli cells requires an active phosphatidylinositol 3-kinase pathway and is inhibited via the mitogenactivated protein kinase signaling pathway. *Mol Endocrinol* 20, 608–618 (2006)

124. R.A. Puffenbarger: Molecular biology of the enzymes that degrade endocannabinoids. *Curr Drug Targets CNS Neurol Disord* 4, 625-631 (2005)

125. C.R. Austin: The “capacitation” of mammalian sperm. *Nature* 170, 326 (1952)

126. R. Yanagimachi: Mammalian fertilization. In. Physiology of Reproduction, second ed. Eds. Knobil, E., Neill, J.D., Raven Press, Ltd., NY (1994)

127. R.P. DeMott, S.S. Suarez: Hyperactivated sperm progression in the mouse oviduct. *Biol Reprod* 46, 779–785 (1993)

128. R. Rathi, B. Colenbrander, M.M. Bevers, B.M. Gadella: Evaluation of *in vitro* capacitation of stallion spermatozoa. *Biol Reprod* 6, 462–470 (2001)

129. J. Harclerode, S.E. Nyquist, B. Nazar, D. Lowe: Effects of cannabis on sex hormones and testicular enzymes of the rodent. *Adv Biosci* 22-23, 395-405 (1978)

130.A.M. Zimmerman, S. Zimmerman, A.Y. Raj: Effects of cannabinoids on spermatogenesis in mice. *Adv Biosci* 22-23, 407-418 (1978)

131. W. Hall, N. Solowij: Adverse effects of cannabis. *Lancet* 352, 1611-1616 (1998)

132. T.G. Cooper: Interactions between epididymal secretions and spermatozoa. *J Reprod Fertil Suppl* 53, 119-136 (1998)

133. G. Ricci, G. Cacciola, L. Altucci, R. Meccariello, R. Pierantoni, S. Fasano, G. Cobellis: Endocannabinoid control of sperm motility: the role of epididymus. *Gen Comp Endocrinol*. 153, 320-322 (2007)

134. S. Aquila, C. Guido, C. Laezza, A. Santoro, V. Pezzi, S. Panza, S. Andò, M. Bifulco: A new role of anandamide in human sperm: focus on metabolism. *J Cell Physiol* 221, 147-153 (2009)

135. G. Cacciola, T. Chioccarelli, K. Mackie, R. Meccariello, C. Ledent, S. Fasano, G.Cacciola, T.Chioccarelli, K. Mackie, R. Meccariello, C. Ledent, S. Fasano, R. Pierantoni, G. Cobellis: Expression of type-1 cannabinoid receptor during rat postnatal testicular development: possible involvement in adult leydig cell differentiation. *Biol Reprod* 79, 758–765 (2008)

136. G. Cobellis, G. Ricci, G. Cacciola, P. Orlando, S. Petrosino, M.G. Cascio, T. Bisogno, L. De Petrocellis, T. Chioccarelli, L. Altucci, S. Fasano, R. Meccariello, R. Pierantoni, C. Ledent, V. Di Marzo: A gradient of 2-arachidonoylglycerol regulates mouse epididymal sperm cell start-up. *Biol Reprod* 82, 451-458 (2010)

137.P.E. Visconti, J.L. Bailey, G.D. Moore, D. Pan, P. Olds-Clark, G.S. Kopf: Capacitation of mouse spermatozoa. Correlation between the capacitation state and protein tyrosine phosphorylation. *Development* 121, 1129-1137 (1995)

138. H. Schuel, L.J. Burkman, J. Lippes, K. Crickard, M.C. Mahony, A. Giuffrida, R.P. Picone, A. Makriyannis: Evidence that anandamide-signaling regulates human sperm functions required for fertilization. *Mol Reprod Dev* 63, 376-387 (2002)

139. J.C. Kirkman-Brown, E.L. Punt, C.L.R. Barratt, S.J: Publicover Zona Pellucida and Progesterone-Induced Ca2+ Signalling and Acrosome Reaction in Human Spermatozoa. *J Androl* 23, 306-315 (2002)

140. H. Breitbart, B. Spungin: The biochemistry of the acrosome reaction. *Mol Hum Reprod*. 3, 195-202 (1997)

141. D.R.P. Tulsiani, H. Yoshida-Komiya, Y. Araki: Mammalian fertilization: a carbohydrate-mediated event. *Biol Reprod* 57, 487–494 (1997)

142. D.R.P. Tulsiani, A. Abou-Haila: Mammalian sperm molecules that are potentially important in interaction with female genital tract and egg vestments. *Zygote* 9, 51-69 (2001)

143. T.S. Witte, S. Schäfer-Somi: Involvement of cholesterol, calcium and progesterone in the induction of capacitation and acrosome reaction of mammalian spermatozoa. *Anim Reprod Science* 102, 181-193 (2007)

144. A. Abou-haila, D.R.P. Tulsiani: Signal transduction pathways that regulate sperm capacitation and the acrosome reaction. *Arch of Biochem and Biophys* 485, 72-81 (2009)

145. D.N. Neild, B.M. Gadella, A. Agüero, T.A.E. Stout, B. Colenbrander: Capacitation, acrosome function and chromatin structure in stallion sperm. *Anim Reprod Sci* 89, 47–56 (2005)

146. H. Schuel, E. Goldstein, R. Mechoulam , A.M. Zimmerman, S. Zimmerman: Anandamide (arachidonylethanolamide), a brain cannabinoid receptor agonist, reduces sperm fertilizing capacity in sea urchins by inhibiting the acrosome reaction. *Proc Natl Acad Sci USA* 91, 7678–7682 (1994)

147. E. Agirregoitia, A. Carracedo, N. Subirán, A. Valdivia, N. Agirregoitia, L. Peralta, G. Velasco, J. Irazusta: The CB(2) cannabinoid receptor regulates human sperm cell motility. *Fertil Steril* 15;93:1378-1387 (2010)

148. A. Ambrosini, G. Zolese, S. Ambrosi, E. Bertoli, F. Mantero, M. Boscaro, G. Balercia: Idiopathic infertility: effect of palmitoylethanolamide (a homologue of anandamide) on hyperactivated sperm cell motility and Ca2+ influx. *J Androl* 26, 429-436 (2005)

149. A. Ambrosini, E. Bertoli, P. Mariani, F. Tanfani, M. Wozniak, G. Zolese: N-acylethanolamines as membrane topological stress compromising agents. *Biochim Biophys Acta* 1148, 351-3555 (1993)

150. A. Ambrosini, G. Zolese, M. Wozniak, D. Genga, M. Boscaro, F. Mantero, G. Balercia: Idiopathic infertility: susceptibility of spermatozoa to in-vitro capacitation, in the presence and the absence of palmitylethanolamide (a homologue of anandamide), is strongly correlated with membrane polarity studied by Laurdan fluorescence. *Mol Hum Reprod* 9, 381-388 (2003)

151. X. Sun, H. Wang, M. Okabe, K. Mackie, P.J. Kingsley, L.J. Marnett, B.F. Cravatt, S.K. Dey: Genetic loss of Faah compromises male fertility in mice. *Biol Reprod* 80, 235-242 (2009)

152. U. Pagotto, G. Marsicano, D. Cota, B. Lutz, R. Pasquali: The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr Rev* 27, 73-100 (2006)

153. M. Maccarrone: Anandamide as a marker of human disease. *JAMA* 300, 281-282 (2008)

154. V. Nallendran, P.M. Lam, T.H. Marczylo, M.J. Bankart, A.H. Taylor, D.J. Taylor, J.C. Konje: The plasma levels of the endocannabinoid, anandamide, increase with the induction of labour. *BJOG* In Press (2010)

155. M. Maccarrone, H. Wang, S.K. Dey: Antagonism of type-1 cannabinoid receptors: Good for obesity, but is it safe for fertility?. *Clin Endocrinol* 66, 456-457 (2007)

156. R. Talevi, V. Barbato, S. De Iorio, V. Mollo, T. Capriglione, L. Ricchiari, A. Samo, R. Gualtieri: Is there a role for endocannabinoids in sperm-oviduct interaction? *Reproduction* In Press (2010)

157. N. Bernabò, M.G. Pistilli, M. Mattioli, B. Barboni: Role of TRPV1 channels in boar spermatozoa acquisition of fertilizing ability. *Mol Cell Endocrinol* 323, 224-231 (2010)

Abbreviations: 17-beta-HSD: 17-beta-hydroxysteroid dehydrogenase; 2-AG: 2- arachidonoylglycerol; Abh4: alpha-/beta-hydrolase; AEA: *N*-arachidonoylethanolamine; AR: acrosome reaction; cAMP: cyclic adenosine monophosphate; CB_1: type-1 cannabinoid receptor; CB_2: type-2 cannabinoid receptor; CBD: cannabidiol; COX: cyclooxygenase; DAG: diacylglycerol; DAGL: diacylglycerol lipase; ECS: endocannabinoid system; eCBs: endocannabinoids; EMT: endocannabinoid membrane transporter; ERK: extracellular regulated kinase; $EtNH_2$: ethanolamine; FAAH: fatty acid amide hydrolase; FAK: focal adhesion kinase; FSH: follicle stimulating hormone; G6PDH: glucose-6-phosphate dehydrogenase; HAEA: hydroxy-anandamide; HETE-G: hydroxy-eicosatetraenoic-glycerol; ICSI: intracytoplasmatic sperm injection; iNOS: inducible nitric oxide synthase; IP_3: inositol triphosphate; IVF: *in vitro* fertilization; KO: knockout; LH: luteinizining hormone; LOX: lipoxygenase; MAGL: monoacylglycerol lipase; MAPK: mitogen-activated protein kinase; Met-AEA: methanandamide; NAAA: *N*-acylethanolamine-hydrolyzing acid amidase; NAPE-PLD: *N*-arachidonoyl-phosphatidylethanolamine phospholipase D; NArPE*:* *N*-arachidonoyl-phosphatidylethanolamine; NAT: *N*-acyltransferase; OEA: *N*-oleoylethanolamine; PA: phosphatidic acid; PC: 1,2-*sn*-di-arachidonoylphosphatidylcholine; PE: phosphatidylethanolamine; PEA: *N*-palmitoylethanolamine; PG-GE: prostaglandin glyceryl ester; PG-EA: prostaglandin ethanolamide; PKA: protein kinase A; PI: phosphatidylinositol; PLA: phospholipase A; PLC: phospholipase C; PLD: phospholipase D; PPAR: peroxisome proliferator-activated receptors; THC: delta-9-tetrahydrocannabinol; TRPV1: transient receptor potential vanilloid-1; ZP: zona pellucida

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