Endocannabinoids and Reproductive Events in Health and Disease

Natalia Battista, Monica Bari, and Mauro Maccarrone

Contents

N. Battista
Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy

M. Bari
European Center for Brain Research/IRCCS Santa Lucia Foundation, Rome, Italy

Department of Experimental Medicine and Surgery, Tor Vergata University of Rome, Rome, Italy

M. Maccarrone (✉)
European Center for Brain Research/IRCCS Santa Lucia Foundation, Rome, Italy

School of Medicine and Center of Integrated Research, Campus Bio-Medico University of Rome, Via Alvaro del Portillo 21, 00128 Rome, Italy
e-mail: m.maccarrone@unicampus.it

R.G. Pertwee (ed.), *Endocannabinoids*, Handbook of Experimental Pharmacology 231, DOI 10.1007/978-3-319-20825-1_12

Abstract

The lasting research on the endocannabinoid system (ECS) has now provided solid and convincing evidence that proves the detrimental effects of recreational drug abuse (a growing habit among teenagers) on fertility. Endocannabinoids (eCBs) affect reproductive events from gametogenesis to fertilization, from embryo implantation to the final outcome of pregnancy and, thus, they have been proposed as suitable biomarkers to predict the reproductive potential of male and female gametes in clinical practice. Novel tools for reproductive medicine are highly sought after, and here we report the latest findings on the impact of the ECS on fertility, demonstrating how basic research can be translated into new medical strategies.

Keywords

AEA Anandamide • Biomarkers • Endocannabinoid system • Infertility • Reproduction

Abbreviations

2-AG	2-Arachidonoylglycerol
AEA	*N*-arachidonoylethanolamine (anandamide)
AR	Acrosome reaction
BF	Blastocoelic fluid
CB_1	Type-1 cannabinoid receptor
CB_2	Type-2 cannabinoid receptor
CBR	Cannabinoid receptors
COX	Cyclooxygenase
CRH	Corticotrophic hormone
DAGL	Diacylglycerol lipase
E	Estrogen
eCB	Endocannabinoid
eCBs	Endocannabinoids
ECS	Endocannabinoid system
EMT	Endocannabinoid membrane transporter
ERE	Estrogen-responsive element
FAAH	Fatty acid amide hydrolase
FSH	Follicle-stimulating hormone
ICM	Inner cell mass
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilization
LOX	Lipoxygenase
MAGL	Monoacylglycerol lipase
MAPK	Mitogen-activated protein kinase
NAPE-PLD	*N*-arachidonoyl-phosphatidylethanolamine-specific phospholipase D
OEA	*N*-oleoylethanolamine

P	Progesterone
PEA	*N*-palmitoylethanolamine
PG	Prostaglandin
PPAR	Peroxisome proliferator-activated receptor
TC	Trophoblastic cells
THC	Δ^9-tetrahydrocannabinol
TRPV1	Transient receptor potential vanilloid-1
TS	Trophoblast stem
ZP	Zona pellucida

1 Introduction

The beginning of a new life is the culmination of a series of events finely tuned by endocrine signals, environmental, psychological, and lifestyle factors. Recreational use of marijuana is a habit that is widespread among people of reproductive age, and cannabis consumption is listed among the leading causes of both male and female infertility. The discovery of cannabinoid receptors (CBR), endocannabinoids (eCBs), and their metabolic enzymes and transporters has shed light on the relevance of endocannabinoid (eCB) signaling in the modulation of reproductive events under healthy and pathological conditions (Meccariello et al. 2014). *N*-Arachidonoyl-ethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) are the main eCBs, and their biological actions are controlled by cellular mechanisms that include enzymes responsible for: (i) their synthesis, e.g., the *N*-acyl-phosphatidylethanolamines (NAPE)-specific phospholipase D (NAPE-PLD) and the *sn*-1-specific diacylglycerol lipase (DAGL), respectively and (ii) their degradation, e.g., fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively (Fezza et al. 2014). Besides these pathways, there are other metabolic routes for eCB metabolism (Fezza et al. 2014; Ueda et al. 2013). Thus, AEA and 2-AG are also susceptible to oxidative mechanisms catalyzed by lipoxygenases (LOXs) and cyclooxygenases (COXs) and can be enzymatically transformed into prostaglandin (PG) ethanolamine and PG glyceryl ester, respectively, through the sequential actions of COX-2 and several PG synthases (Rouzer and Marnett 2011). eCBs act principally through type-1 and type-2 cannabinoid (CB_1 and CB_2) receptors. GPR55, that acts like a putative "type-3 cannabinoid (CB_3)" receptor (Moriconi et al. 2010), is also one of their targets. eCBs are also able to interact with non-CBR targets, such as the transient receptor potential vanilloid type 1 (TRPV1) channel, which is activated by both AEA (Di Marzo and De Petrocellis 2010) and 2-AG (Zygmunt et al. 2013), and the peroxisome proliferator-activated receptor (PPAR) α and PPAR γ (Pistis and Melis 2010). The cellular uptake of eCBs from the extracellular to the intracellular space is ascribed to a purported "endocannabinoid membrane transporter (EMT)" that may well take up both AEA and 2-AG. However, the mechanisms through which eCBs are carried across plasma membranes

and transported within the cell are not yet fully understood and remain a matter of debate (Fowler 2013). *N*-oleoylethanolamine (OEA) and *N*-palmitoylethanolamine (PEA) are compounds structurally related to eCBs, known as "eCB-like" substances. They potentiate the effect of genuine eCBs by the so-called "entourage effect", that is by competitively inhibiting eCB degradation, or by allosterically modulating their receptor binding.

To date, the ECS is considered a master system deeply involved in the control of several physiological processes, including fertility. Alterations of eCBs and/or ECS components might affect negatively various reproductive stages from gametogenesis to fertilization, embryo implantation and development, and parturition. Therefore, these bioactive lipids have a huge potential for the diagnosis and/or therapy of female and male defects (Di Blasio et al. 2013; Maccarrone 2013; Meccariello et al. 2014; Rapino et al. 2014).

2 Gametogenesis

2.1 Ovary and Folliculogenesis

Female reproductive cells are produced in the ovary and their survival is regulated by gonadotrophic and sex hormones, which interact with each other to control the ovarian cycle. The first step of oogenesis occurs during intrauterine life, starting in the primary oocyte after the first meiotic division. At puberty meiotic division restarts, leading to the production of follicles that contain secondary oocytes and that will be released at ovulation, in order to be fertilized. The presence of AEA in the human follicular fluid (Schuel et al. 2002), and the observation that alteration of its plasma levels could be related to fertility/infertility in healthy women, identified eCBs as key mediators of reproductive events (Maccarrone et al. 2000, 2002; Lam et al. 2008). AEA, its metabolic enzymes, and its molecular targets have been recently localized in the human ovary (El Talatini et al. 2009a). In particular, it has been shown that both CB_1 and CB_2 are expressed at different time points in follicular cells and oocytes. Indeed, CB_2 is generally more highly expressed than CB_1 in the ovary and is detected in oocytes only at a late stage of development (El-Talatini et al. 2009a). Moreover, NAPE-PLD and FAAH are expressed in secondary and tertiary follicles and in the corpus luteum and albicans (El-Talatini et al. 2009a). Data on the regulation of NAPE-PLD protein expression between the proliferative and secretory phases of the endometrium are conflicting, probably because of differences between studied patients (Taylor et al. 2010; Gebeh et al. 2012; Scotchie et al. 2015). However, published data on increased expression in the middle and late secretory phases are more consistent (Taylor et al. 2010; Gebeh et al. 2012; Scotchie et al. 2015).

Interestingly, AEA measured in the follicular fluid of women undergoing controlled ovarian hyperstimulation for in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) is higher in follicles with mature oocytes than in those with immature oocytes (El-Talatini et al. 2009a) (Fig. 1a, b). These data support the

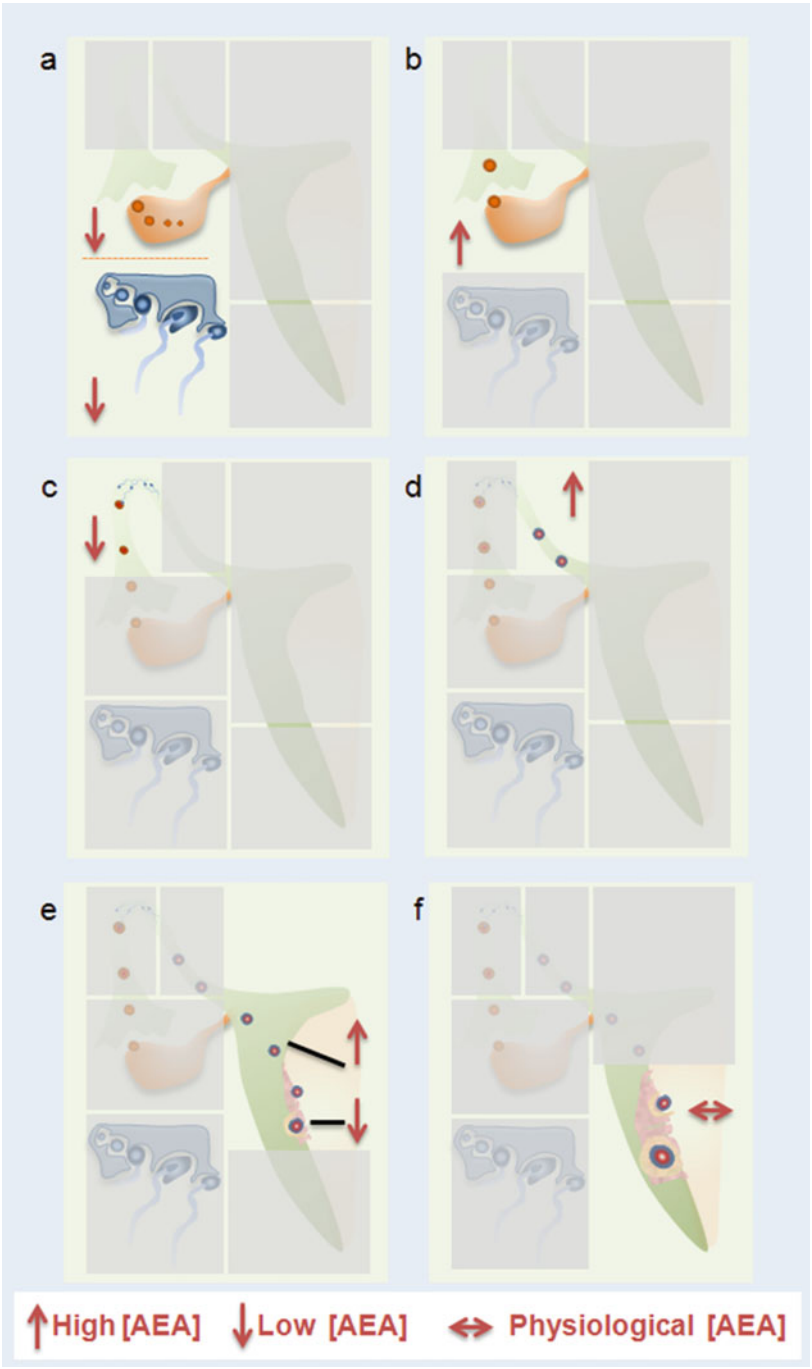

Fig. 1 Role of AEA in the early stages of reproduction: from gametogenesis to decidualization. Schematic representation of fluctuations of AEA levels during early stages of pregnancy. (**a**) *Gametogenesis:* Low AEA levels are required during all phases of this process to warrant

hypothesis that AEA in follicular and oviductal fluids may be involved in oocyte maturation (Schuel et al. 2002; El-Talatini et al. 2009a). On this basis, one can speculate that AEA exerts effects on fertility during the late phase of human folliculogenesis. In addition, these data are in keeping with recent observations that granulosa cells of the rat uterus lack CB receptors (Bagavandoss and Grimshaw 2010). Another recent study has monitored the expression of AEA and 2-AG catabolic enzymes in human endometrium during the menstrual cycle (Scotchie et al. 2015). Maximal FAAH expression was detected in the middle secretory phase, whereas MAGL expression reached its highest level in the early secretory phase (Scotchie et al. 2015). Furthermore, arachidonic acid, generated by the degradation of AEA and 2-AG, serves as a good substrate for COX-2, which is expressed in the cumulus oophorus but not in the egg cell. This leads to an increase in PGE_2 required for the maturation of the egg (Bayne et al. 2009; Feuerstein et al. 2007). In this context, increased mRNA levels of COX-2 were found during the proliferative phase, followed by a reduction in the secretory phases (Scotchie et al. 2015). In summary, regulation of ovarian physiology by eCB signaling is apparent (Cecconi et al. 2014).

2.2 Spermatogenesis and Spermiogenesis

Sertoli cells are supporting cells localized within the seminiferous tubules, where they create a unique protective environment for sperm development during spermatogenesis. Their main role is to provide hormones, growth factors, and other glycoproteins needed for sperm nourishment, as well as to form the blood–testis barrier. The biological function of Sertoli cells is controlled by follicle-stimulating hormone (FSH) that, by binding to its specific receptor on targeted cells, can trigger different signal transduction pathways, thus leading to cell proliferation and differentiation. Some ECS elements have been detected in Sertoli cells at different development stages (Maccarrone et al. 2003; Rossi et al. 2007). In this context, it has been demonstrated that FSH is able to specifically stimulate expression and

Fig. 1 (continued) maturation of both oocytes and sperm cells. (**b**) *Ovulation:* At a late stage of folliculogenesis and at ovulation, follicles and follicular fluid show high AEA levels. (**c**) *Fertilization:* Mature oocytes and sperm cells meet each other in the central portion of the Fallopian tube called the ampulla, where establishment of an increasing AEA gradient favors the journey of the fertilized egg. (**d**) *Oviductal transport:* To reach the implantation site in the uterus, the zygote must cross the isthmus, the end portion of the Fallopian tube, where a gradually increased AEA concentration prevents early extrauterine implantation. (**e**) *Implantation:* Regulated AEA levels are conducive to normal implantation, so that low AEA content must be kept in the uterine epithelium at the implantation site, whereas high AEA content is needed at inter-implantation sites. (**f**) *Decidualization:* The maternal–fetal relationship is definitively established by decidualization, where high AEA levels are often related to ectopic pregnancy, while low AEA levels trigger apoptosis. These findings suggest a dual role of AEA that depends on its concentration. See text for details on the various stages of reproduction

activity of FAAH (the main AEA hydrolase), by triggering protein kinase A- and/or aromatase-dependent pathways (Rossi et al. 2007). Indeed, estrogen (E) activates FAAH transcription by binding to its receptor at two proximal E-responsive element (ERE) sites that trigger demethylation of DNA and of histone H3 (Grimaldi et al. 2009a, 2012, 2013). This process might play a role in finely regulating the endogenous tone of AEA and thus the pro-apoptotic action of this eCB in Sertoli cells. As a consequence, E impacts on the number of spermatids in the adult, that indeed depends on the size of the Sertoli cell population produced during perinatal development (Orth et al. 1988) (Fig. 1a). The crosstalk among androgens, estrogens, and eCBs during murine postnatal testicular development, from puberty to adulthood, has also been documented (Gye et al. 2005; Cacciola et al. 2008a). Indeed, spatiotemporally distinct expression of CB_1 was revealed in Leydig cells, spermatogonia, and spermatocytes, suggesting a functional involvement of CB_1-mediated signaling in steroidogenesis and spermatogenesis. In this context, it has been reported that long-term use of HU-210, a synthetic analogue of Δ^9-tetrahydrocannabinol (THC) and a potent CB_1 agonist, impairs spermatogenesis by decreasing the number of Sertoli cells and by reducing spermatogenic efficiency through a CB_1-mediated mechanism (Lewis et al. 2012a).

Recently, it has been demonstrated that CB_2 also plays a crucial role in the mitotic and meiotic phases of spermatogenesis, but not during the late process of spermiogenesis, that is responsible for the transformation of spermatids into spermatozoa (Grimaldi et al. 2009b). Indeed, CB_2 mRNA and protein expression are detectable in male germ cells from mitotic spermatogonia to haploid spermatids, while they are absent in mature sperm cells (Grimaldi et al. 2009b). Accordingly, the endogenous content of 2-AG, but not of AEA, markedly decreased during the differentiation process and was paralleled by lower or higher mRNA levels of 2-AG synthesizing or degrading enzymes, respectively (Grimaldi et al. 2009b). In addition, it was suggested that a steady AEA tone in testes might activate TRPV1, thus protecting germ cells against testicular hyperthermia (Mizrak et al. 2008). In line with this, experiments performed with *Trpv1*$^{-/-}$ mice demonstrated that this receptor promotes apoptosis of spermatogonia and blocks the progression of germ cell meiosis, as a defensive mechanism against abnormally elevated testicular temperature (Mizrak et al. 2008). Results obtained in a number of studies performed with animal models and humans have further demonstrated that isolated spermatocytes and spermatids, as well as mature spermatozoa, possess both AEA and 2-AG metabolic enzymes, along with cannabinoid and vanilloid receptors (Maccarrone et al. 2005; Rossato et al. 2005; Francavilla et al. 2009; Grimaldi et al. 2009b; Gervasi et al. 2011). Interestingly, a correlation between the expression of some ECS elements, such as CB_1 (Cobellis et al. 2006; Meccariello et al. 2006; Cacciola et al. 2008b), FAAH (Cobellis et al. 2006) and NAPE-PLD (Chianese et al. 2012), and the progression of spermatogenic stages has also been established in non-mammalian vertebrates (Battista et al. 2012).

Altogether, these findings suggest a synchronized biological action of eCBs that, by a timely binding to their specific molecular targets, promote and orchestrate the different stages of spermatogenesis and spermiogenesis (Maccarrone 2009).

3 Fertilization

3.1 Sperm Motility and Interaction With the Oviduct

The fusion of gametes to produce a new organism is the culmination of a multitude of intricate cellular processes. Sperm cells become fertilization-competent after undergoing a maturation process, whereby they become motile and their plasma membrane is reorganized in order to interact with the oocyte. The ability of eCBs to regulate sperm functions required for fertilization is well established in non-mammals and mammals (for a review see Battista et al. 2012). Prior to ejaculation, sperm resides within the epididymis, where it acquires motility upon travelling from caput to cauda. Previous research with the *Rana esculenta* frog (Cobellis et al. 2006), and with *cnr1* (the gene encoding for CB_1) null mice (Ricci et al. 2007), demonstrated that any disruption of CB_1-dependent eCB signaling leads to precocious acquisition of sperm motility along the male reproductive tract. Further evidence has arisen from studies with epididymal spermatozoa of *faah* (the gene encoding for FAAH) null mice that compromised motility and impaired fertilizing ability result from elevated AEA levels in the male reproductive tract (Sun et al. 2009). On the other hand, it has been hypothesized that a decreasing 2-AG gradient from caput to cauda is required to promote sperm start-up and to maintain sperm motility during the journey through the epididymus (Cobellis et al. 2010).

Once sperm cells are released into the vagina, their swimming ability from cervix to oviduct seems to be finely tuned by a differential activation of CB_1 and CB_2. Indeed, binding of selective agonists to CB_1 increases the percentage of immobile cells, whereas activation of CB_2 shifts the sperm population from rapid to sluggish/slow progressing spermatozoa, thus regulating the in vivo proportion of motile sperm (Agirregoitia et al. 2010). In addition, indirect capacitation signals (like heparin) that promote sperm release might remodel the sperm surface and make sperm cells insensitive to AEA (Gervasi et al. 2011).

The involvement of eCB signaling is apparent also in the control of sperm energy homeostasis, which in turn affects critical sperm functions like motility, capacitation, and the acrosome reaction (Rossato 2008). Indeed, AEA negatively affects sperm motility in a CB_1-dependent manner that involves inhibition of mitochondrial activity (Rossato et al. 2005), cellular respiration (Badawy et al. 2009) and glycolysis (Barbonetti et al. 2010).

At physiological concentrations, AEA acts as a capacitating signal in boar (Maccarrone et al. 2005), frog (Cottone et al. 2008), mouse (Catanzaro et al. 2011) and human spermatozoa (Rossato et al. 2005; Francavilla et al. 2009). Consistently, capacitated mouse sperm cells are characterized by the presence of elevated levels of both AEA (due to reduced degradation by FAAH) and 2-AG (due to enhanced synthesis by DAGL). These distinct pathways might allow a differential regulation of the content of these two major eCBs that, through a differential activation of either extracellular CB_1 or intracellular TRPV1, maintain a suitable environment that makes it possible for the sperm to travel along the

uterine tract without activating the acrosome arsenal (Catanzaro et al. 2011). This finding is also consistent with boar (Maccarrone et al. 2005), bull (Gervasi et al. 2011) and human sperm data (Francavilla et al. 2009), showing that increased intracellular AEA levels, by activating TRPV1 receptors at an intracellular binding site during capacitation, promote the fertilizing ability of sperm. In addition, AEA present in both seminal plasma and uterine fluids plays a physiological role, via CB_1 receptors, in maintaining a quiescent, uncapacitated condition before sperm interacts with an oocyte, thus preventing premature capacitation in freshly ejaculated sperm (Battista et al. 2008; Lewis et al. 2012b). Overall, it can be proposed that an increased intracellular content of AEA can lead to the activation of TRPV1, and that by means of its export by a putative EMT, AEA can build up a sufficient extracellular concentration for the activation of CB_1. This regulation facilitates the sperm journey through the uterine tract. Further activation of CB_1 can also be induced by intracellular 2-AG, after this eCB is released from the cell (Catanzaro et al. 2011).

It should be remembered that the presence of an oviductal gradient of AEA might have an impact on the fertilizing properties of sperm cells during their storage within the female reproductive tract. These conditions are required to ensure the presence of enough competent sperm at the time of ovulation (Gervasi et al. 2009; Talevi et al. 2010). Bovine epithelial cells of the oviduct express NAPE-PLD mRNA, and could contribute to release AEA in the isthmus, further enhancing its local concentration (Gervasi et al. 2009). Higher levels of AEA in this region might prolong the fertile life of sperm cells and hence ensure their progression through the oviduct (Gervasi et al. 2009; Talevi et al. 2010).

3.2 Sperm Interaction With the Oocyte

Once capacitation has occurred, the second major stage of fertilization begins. In this second stage, which depends on a sperm cell–egg interaction, only capacitated sperm cells activate the series of processes required for fertilization, among which acrosome reaction (AR) is the key event. The highly specialized gametes begin their interactions by signaling to one another to ensure that fertilization occurs when they meet. The oocyte releases PGs to help to guide sperm cells to the site of fertilization, and these cells secrete a specific protein to trigger oocyte maturation and ovulation. After their encounter, sperm and oocyte fuse in a specific and tightly regulated manner.

The fertilizing ability of sperm and the sperm–oocyte interaction depend on AEA binding to either CB_1 or TRPV1 receptors (Fig. 2). Indeed, experimental data obtained with boar sperm have demonstrated that AEA acts via a CB_1-mediated mechanism to inhibit a physiological AR triggered by zona pellucida (ZP) proteins (Maccarrone et al. 2005). In contrast, activation of TRPV1 reduces the ability of sperm to react with ZP proteins, thus preventing an "out of place" acrosomal exocytosis or spontaneous AR (Maccarrone et al. 2005). This idea is supported by the finding that inhibition of TRPV1 by its selective antagonist, capsazepine,

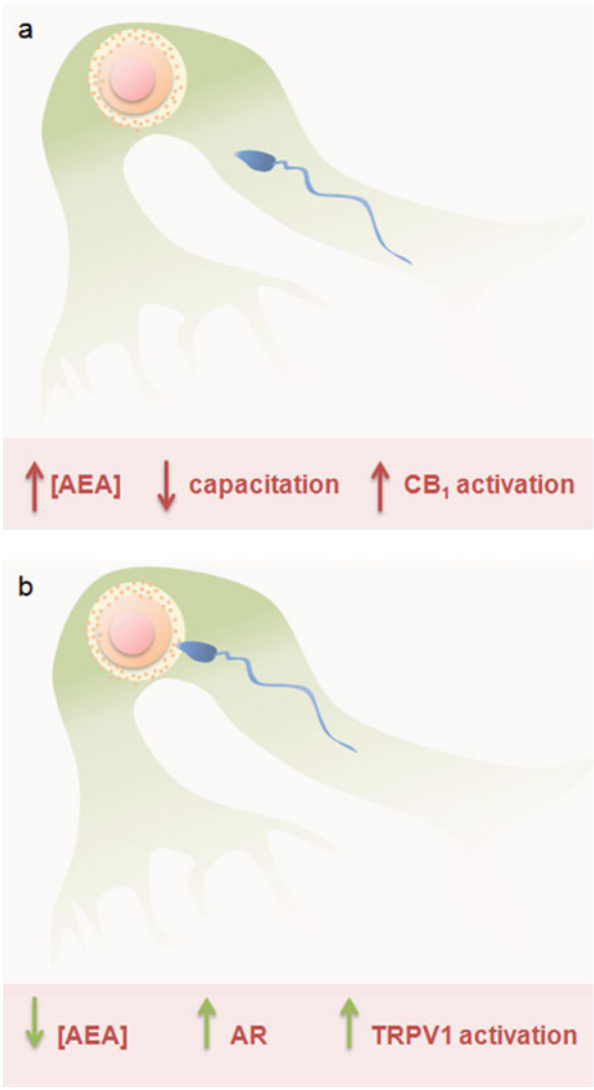

Fig. 2 Dual stage-dependent effect exerted by AEA to regulate sperm fertilizing ability. (**a**) AEA, present in both seminal plasma and uterine fluids, may prevent precocious capacitation in freshly ejaculated sperm via a CB_1-mediated mechanism in the uterine tract. (**b**) Once capacitation is completed, AEA stabilizes acrosome membranes by activating TRPV1 and thus reduces spontaneous acrosome reactions and promotes sperm–oocyte fusion

leads to premature AR in a high percentage of in vitro capacitated boar (Maccarrone et al. 2005) and human (Francavilla et al. 2009) sperm cells. In addition, prolonged exposure of human sperm cells to capsazepine significantly inhibited their ability to fuse with oocytes in response to progesterone (P), an effect prevented by the addition of a specific inhibitor of EMT (Francavilla et al. 2009). Therefore, increased intracellular AEA levels activate TRPV1, and thus maximize sperm responsiveness to physiological inducers of AR, by reducing sperm fusion with the oocyte membrane (Francavilla et al. 2009). Incidentally, in vitro evidence for TRPV1 translocation within sperm after capacitation further suggests a role of this

receptor in the fertilizing ability of sperm (Bernabò et al. 2010). Indeed, this event could be crucial for the completion of spontaneous AR and for preventing premature fusion between the outer acrosome membrane and the plasma membrane (Bernabò et al. 2010). Overall, AEA present in both seminal plasma and uterine fluids prevents premature capacitation in freshly ejaculated sperm via a CB_1-mediated mechanism. Once sperm cells reach the oviduct, they are exposed to a progressively reduced concentration of AEA in the proximal female genital tract (Schuel et al. 2002), and sperm capacitation may finally occur. At this time, increased intracellular AEA content activates TRPV1 and prevents spontaneous AR. Consequently, AR will result only from sperm–egg interactions, thus maximizing sperm fertilizing potential (Fig. 1c).

4 Embryogenesis

4.1 Oviductal Transport

At ovulation, the secondary oocyte at its metaphase II stage is released into the oviduct, where it can be fertilized thus completing meiosis. There has been an accumulation of evidence that, in order to allow normal tubal transport, an eCB gradient is established (Sun and Dey 2008). In mouse oviduct, an increasing AEA longitudinal gradient from ampulla to isthmus is achieved by a higher NAPE-PLD/FAAH ratio in the isthmus compared to the ampulla (Guo et al. 2005; Wang et al. 2006), as depicted in Fig. 1c, d. Although most of the studies in this area have been limited to mice for obvious ethical reasons, recently, in a framework of studies on ectopic pregnancies, the ECS has been characterized in the human Fallopian tube (Horne et al. 2008; Gebeh et al. 2012). Higher levels of AEA and its congeners (i.e., PEA and OEA) were found in oviductal tissues of women with ectopic pregnancy, compared to those of nonpregnant women undergoing hysterectomy for medical reasons (Gebeh et al. 2012). NAPE-PLD and FAAH protein expression was found in the epithelium of human Fallopian tubes, and a significant reduction of FAAH mRNA was observed in ectopic pregnancy compared to controls (Gebeh et al. 2012). These data are in keeping with previous observations of a down-regulation of CB_1, but not of CB_2, in Fallopian tubes in ectopic pregnancy (Horne et al. 2008), and of a negative CB_1-mediated effect of AEA on oviduct transport (Wang et al. 2006). It is not clear whether CB_1de-regulation is a cause or an effect of ectopic pregnancy in humans, yet CB_1 clearly plays a key role in pregnancy. Overall, the most relevant outcome of recent studies is that higher AEA levels are associated with: (1) low blastocyst development, (2) oviductal retention of embryos, and (3) implantation failure (Wang et al. 2006; Sun and Dey 2012). Two main processes are involved in normal tubal transport: smooth muscle contraction and cilia beating (Halbert et al. 1976). The former process is under adrenergic control and, since CB_1 was found to co-localize with adrenergic receptors, it has been hypothesized that eCB effects on muscle contraction could be mediated by a regulation of adrenergic signaling (Wang et al. 2004). Additionally,

recent data have suggested that ciliary beat frequency in Fallopian tube epithelial cells is the most critical event in controlling transport of both gametes and fertilized eggs (Shi et al. 2011; Ezzati et al. 2014). Unfortunately, no studies have yet explored the role of eCBs in this process.

4.2 Implantation

Following fertilization, the egg undergoes series of cell divisions to form the blastocyst, which will nestle in the receptive uterus to grow and develop. Implantation is a critical step in pregnancy and starts with the attachment of the blastocyst trophectoderm to the luminal side of the uterus that, by developing protrusions named "pinopodes," promotes the adhesion process. Finally, the blastocyst invades endometrial cells to successfully complete the implantation process. It is clear that blastocyst development, competence, and uterine receptivity are inevitably synchronized events that are needed for successful implantation. Increasing evidence suggests that AEA could play a key role in all of this. Appropriate levels of AEA are ensured by a tight regulation in the NAPE-PLD/FAAH ratio (Paria et al. 1998; Guo et al. 2005). Recently, it has been demonstrated that both too high and too low levels of AEA are deleterious for pregnancy (Sun and Dey 2009). In vitro experiments demonstrated that high AEA levels inhibit the development of the blastocyst and its zona hatching in the mouse. Moreover, they inhibit trophoblast differentiation, whereas low levels of AEA accelerate trophoblast differentiation and outgrowth (Wang et al. 2006). On the maternal side, AEA and 2-AG are present in mouse uterus, and their concentration gradient is finely tuned by NAPE-PLD/FAAH and DAGLα/MAGL ratios, respectively (Wang et al. 2007). As a result, higher AEA levels are found at inter-implantation than at implantation sites (Paria et al. 2001; Guo et al. 2005) (Fig. 1e). These data are paralleled by high expression and activity of NAPE-PLD in inter-implantation sites compared to the implantation site (Guo et al. 2005) and by lower FAAH expression and activity at inter-implantation sites than at the implantation site (Wang et al. 2007). Also the DAGLα/MAGL ratio is higher at inter-implantation than at implantation sites (Wang et al. 2007). In this context, a correlation between AEA uterine content and the autophagic state of the blastocyst has been recently documented (Oh et al. 2013). Overall, these findings have prompted the speculation that the embryo may play a role in the regulation of eCB tone. Indeed, implanting blastocysts decreases the NAPE-PLD/FAAH ratio by releasing a "FAAH activator" that reduces AEA levels at the implantation site (Maccarrone et al. 2004; Guo et al. 2005). Of note, a successful implantation is affected not only by the concentration of eCBs but also by CB_1 activation (Wang et al. 2006). Absence of embryonic and maternal CB_1 causes asynchronous development and oviductal retention of embryos (Wang et al. 2006). Notably, a complex network of hormones, cytokines, and eCBs must be well balanced to guarantee proper folliculogenesis, embryo implantation, and development (Maccarrone 2009; Bambang et al. 2012). Not surprisingly, ovarian hormones influence AEA metabolism during reproductive

events, so that P stimulates FAAH activity, whereas E down-regulates NAPE-PLD activity (Maccarrone et al. 2001; Guo et al. 2005; Battista et al. 2008). Even human plasma AEA levels are regulated by FSH in the follicular phase of the menstrual cycle and by LH and E during ovulation, implantation, and early pregnancy (El-Talatini et al. 2010). Interestingly, circulating lymphocytes also play a critical role in human embryo implantation. The addition of AEA to human lymphocytes in vitro inhibits the release of leukemia inhibitor factor (LIF), further highlighting the tight interaction between the many processes that support early pregnancy (Maccarrone et al. 2001; Melford et al. 2014). Taken together, it is apparent that eCB signaling plays a key role in all the early phases of embryo development, in order to ensure a proper pregnancy outcome.

4.3 Placentation and Parturition

A correct blastocyst implantation is followed by a sequence of events that include uterine decidualization and embryo trophoectodermal invasion of uterine stroma. These events are preliminary to the development of the placenta that will be the vital link between mother and fetus. In humans, decidualization occurs during luteal phases of the uterine cycle, independently of fertilization; in mice, the same event is stimulated after blastocyst implantation (Ramathal et al. 2010). Blastocyst implantation occurs in a very short time frame, that in turn is limited by decidualization triggered by the same blastocyst whose docking prevents implantation at other sites. When decidualization ends, placentation begins, a process in which the blastocyst is organized into outer trophoblastic cells (TCs), inner cell mass (ICM), and a blastocoelic fluid (BF). To work properly, the placenta requires a correct distribution of TCs, that all derive from trophoblast stem (TS) cells. Aberrations in trophoblast differentiation compromises normal placentation (Sun et al. 2010). In this context, it has been demonstrated that AEA controls the fate of TS cells via CB_1 (Sun et al. 2010). In particular, both hyper- and hypo- eCB signaling compromise normal pregnancy at different stages (Sun et al. 2010). Indeed, high AEA influences trophoblast invasion by causing decreased fibronectin-binding activity of the blastocyst; in contrast, low AEA activates mitogen-activated protein kinase (MAPK) signaling, suggestive of an important role in trophoblast behavior (Sun et al. 2010). These observations are in agreement with a recent study showing slower proliferation of CB_1/CB_2 null TS cells compared to wild types (Xie et al. 2012). CB_1 and AEA metabolic enzymes are also expressed during human placental development (Chamley et al. 2008; Taylor et al. 2011), and the whole ECS has been characterized in murine placentas (Sun et al. 2010). CB_1 and FAAH have also been identified in human amniotic epithelial cells, chorionic cytotrophoblasts, and syncytiotrophoblasts (Park et al. 2003; Habayeb et al. 2008). Evidence has emerged that the final outcome of pregnancy correlates with altered levels of CB_1, NAPE-PLD, and FAAH in first trimester placentas and, more generally, with high levels of AEA (Park et al. 2003; Trabucco et al. 2009; Meccariello et al. 2014) (Fig. 1f).

Higher levels of AEA are also detected in the plasma of nonviable than of viable pregnancies (Taylor et al. 2011). In line with this, *cnr1* and *faah* null mice showed pregnancy loss at different stages of midgestation (Sun and Dey 2012).

Interestingly, in the peak phases of rat decidua development, high NAPE-PLD activity elevates AEA levels that are probably involved in decidua remodeling (Fonseca et al. 2013, 2014). In contrast, FAAH activity prevails during placentation, corroborating the sound concept that this AEA-hydrolase is a key regulator of early pregnancy signaling (Fonseca et al. 2014). During endometrial development, other substances such as steroid hormones and cytokines (i.e., interleukin 11) are engaged to guarantee a correct decidual response (Dimitriadis et al. 2002; Paiva et al. 2011) and thus proper and timely pregnancy. All these findings confirm that a tight regulation of eCB signaling is essential for normal trophoblast development and placentation and hence for a healthy pregnancy.

In humans, successful full pregnancy occurs after ~40 weeks of gestation and pregnancy, whereas less than 37 weeks of pregnancy is considered preterm. Preterm birth is the leading cause of infant death in developed countries, and its causes remain unclear. However, many factors seem to affect this complication: genetic and environmental factors, viral infections, drug abuse, and alcohol consumption (Kourtis et al. 2014; Sowell et al. 2014). Normally, labor is the last stage of pregnancy where maternal inflammatory signals and fetal hormones cooperate to allow childbirth. P and corticotrophin hormone (CRH) are the main mediators in labour. P is essential for maintenance of myometrial quiescence, and its release induces labour to such an extent that it has been proposed for the prevention of preterm birth (Dodd and Crowther 2010; Tan et al. 2012; Areia et al. 2013). In agreement with this, a decreased P/E ratio has been reported to set the timing of parturition in rodents (Mesiano and Welsh 2007). Also placental CRH plays a critical role during pregnancy, by adjusting length of gestation and the time frame of delivery (Grammatopoulos 2007; Iliodromiti et al. 2012).

It should be recalled that in mice, genetic or pharmacological ablation of CB_1, but not of CB_2, causes preterm birth (Wang et al. 2008). Incidentally, *cnr1*$^{-/-}$ mice have altered P and E levels, leading to a decreased P/E ratio that impairs normal parturition. Premature birth in mice lacking CB_1 can be restored by subcutaneous injection of P just before they give birth (on day 18), further supporting the involvement of CB_1 in P and E regulation of pregnancy (Wang et al. 2008). Another interesting finding is the regulation of fetal PGE_2 production by eCBs via CB_1 activation (Mitchell et al. 2008). PGE_2 is involved in the early stage of labour, and its main source is the amnion. Since PGs are products of eCB hydrolysis (that releases arachidonic acid) and subsequent oxidative metabolism by COX-2, once again regulation of the eCB content impacts on late human pregnancy with implications for timing and progression of labour (Mitchell et al. 2008). In line with this, increased plasma AEA levels and low placental CB_1 expression have been observed in women in labour compared to women not in labour (Acone et al. 2009; Nallendran et al. 2010). Overall, alterations of eCB signaling are related to defects in various stages of reproduction that are clinically diagnosed as subfertile conditions. Therefore, eCBs must be kept at physiological levels from

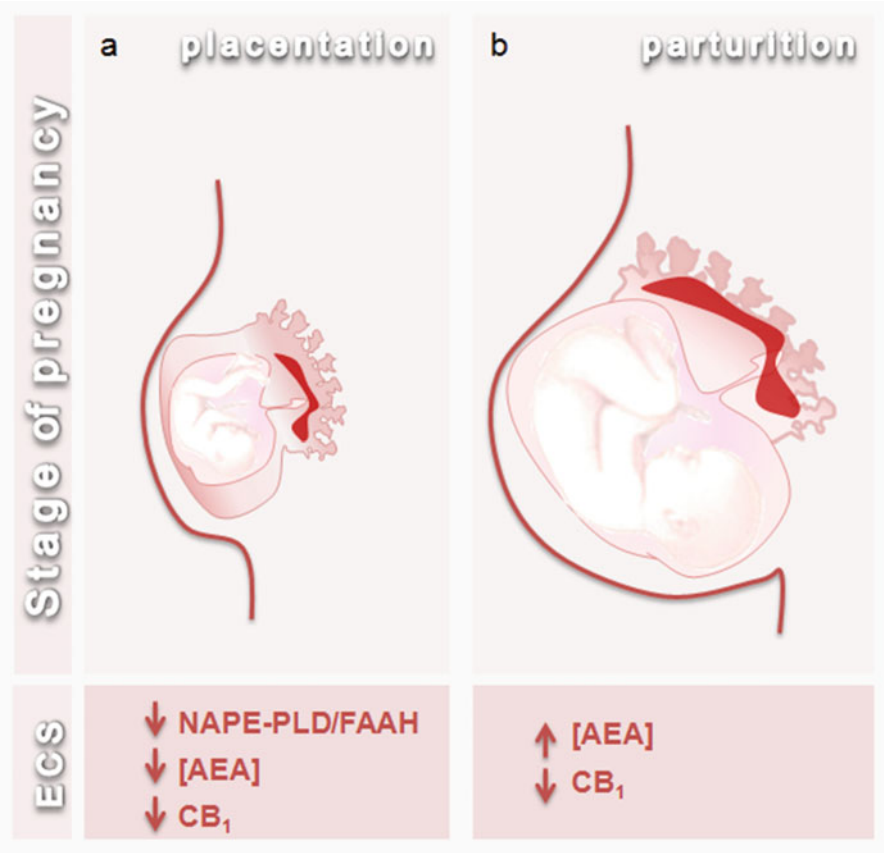

Fig. 3 Role of ECS elements in pregnancy. (**a**) *Placentation:* In the first trimester of pregnancy, a decreased NAPE-PLD/FAAH ratio and high CB_1 expression are required to ensure a successful pregnancy. (**b**) *Parturition:* In the late stage of pregnancy, high AEA levels and low CB_1 expression are essential for a timely onset of labour

the early phases of pregnancy to gestation and parturition, in order to warrant healthy pregnancy and successful delivery. The main alterations of ECS elements, that negatively affect placentation and parturition, are shown in Fig. 3.

5 The Endocannabinoid System as a Biomarker of Infertility

Data described in the previous sections highlight that alterations of distinct ECS components, and/or dysregulation of eCB tone in various stages of reproduction, may impair fertilization and the beginning of a new life. Therefore, they point to ECS elements/eCBs as biomarkers for the diagnosis of reproductive defects and/or

as therapeutic targets for the treatment of human infertility in clinical practice (Maccarrone 2009; Di Blasio et al. 2013; Rapino et al. 2014).

On the female side, follicular fluid withdrawn from women undergoing IVF/ICSI treatment contains higher AEA concentrations when obtained from follicles with mature oocytes than when obtained from follicles with immature oocytes (El-Talatini et al. 2009a), suggesting that higher AEA levels are required to guarantee proper follicular maturation and development (Bagavandoss and Grimshaw 2010). During early pregnancy, low levels of AEA, without differences between first and second trimesters with respect to the levels measured in the luteal phase of the menstrual cycle, are needed to promote uterine receptivity and pregnancy maintenance (El-Talatini et al. 2009b). Consistent with these data, elevated levels of AEA in blood, due to lower FAAH activity, were found in T lymphocytes of women who spontaneously miscarried in the first 8 weeks of pregnancy, compared to healthy controls (Maccarrone et al. 2000, 2001, 2002). Similarly, women who failed to achieve an ongoing pregnancy after IVF treatment, through either embryo transfer (Maccarrone et al. 2002) or ICSI (El-Talatini et al. 2009b), had higher plasma AEA concentration than women who became pregnant. Moreover, elevated AEA levels and reduced FAAH activity might also be predictive of early pregnancy complications due to incorrect attachment of the fertilized egg outside the womb, as in the case of ectopic pregnancy. Since other ECS elements, and notably the AEA biosynthetizing enzyme NAPE-PLD, are not affected in these pathological conditions, it can be suggested that AEA levels and/or FAAH activity could be a suitable biomarker to distinguish viable from nonviable pregnancies (Kuc et al. 2011; Kagan et al. 2012; Daponte et al. 2013; Nicolaides et al. 2013). Interestingly, it has been recently reported that, despite the involvement of the ECS in the control of nausea and emesis (Mechoulam and Parker 2013), plasma eCBs remain at physiological levels in women suffering from hyperemesis gravidarum (Gebeh et al. 2014). As a final note, a recent study by Abán and colleagues has shown differential expression of NAPE-PLD and FAAH in the syncytiotrophoblasts of normal and pre-eclamptic human placentas (Abán et al. 2013). Increased AEA could, via CB_1, affect nitric oxide synthase (NOS) activity, and thus NO production (Abán et al. 2013), as observed also in rat uterus during the peri-implantation period (Sordelli et al. 2011). Since NO is fundamental for implantation, and for maintaining low vascular resistance in the fetoplacental circulation, CB_1 could represent a novel target for the treatment of implantation deficiencies and preeclampsia.

On the male side, semen analysis, in which the concentration, motility, and morphology of sperm is monitored, has long represented the standard test for evaluating male fertility. The evaluation of the eCB content of human sperm cells and/or of seminal plasma could be predictive of sperm anomalies in humans, and thus could be used as a diagnostic tool in the field of reproductive medicine. In this context, a few studies have demonstrated that seminal plasma levels of eCBs and their cogeners decrease in infertile men with normozoospermia who were diagnosed with idiopathic infertility, asthenozoospermia, oligoasthenoteratozoospermia, and teratozoospermia (Lewis et al. 2012b; Amoako et al. 2013,

2014). Usually poor semen quality, such as a decreased sperm cell count and abnormal sperm motility, is directly associated with pathological semen subtypes (Lewis et al. 2012b; Amoako et al. 2013, 2014). However, these differences were not detected in these studies (Lewis et al. 2012b), pinpointing seminal plasma eCB content as a new biomarker of male reproductive defects. Interestingly, TRPV1 is the only eCB target receptor whose activity was markedly less in infertile than in fertile human sperm (Lewis et al. 2012b). These dysfunctions could lead to reduced fertilizing capacity of sperm in infertile subjects, and could be at least partly responsible for their oligospermia, as a consequence of TRPV1-triggered apoptosis (Mizrak et al. 2008). On this basis, one might speculate that reduction of AEA causes infertile sperm cells to lose their quiescent state and consequentially their ability to prevent premature capacitation. This condition could then precipitate a premature AR, making sperm infertile because of a reduced ability to penetrate the oocyte in vivo, as well as in assisted conceptions like IVF.

Incidentally, it should be recalled that semen analysis is not yet a perfect test, as it fails to accurately predict fertility status in certain situations (Guzick et al. 2001; Jequier 2010) nor does it take into account functional aspects of sperm, like the ability to fertilize the oocyte. Over the past few years, a number of tests have been developed to evaluate more specifically sperm DNA quality, which may be more informative and clinically relevant than semen analysis alone (Vasan 2011). In this context, recent studies have explored the possible involvement of CB_1 signaling in chromatin remodeling of mouse spermatids (Cacciola et al. 2013a, b). Genetic inactivation of *cnr1* appears to reduce both histone displacement and transient protein 2 (*Tnp*2) gene expression that have a direct role in maintaining DNA integrity (Chioccarelli et al. 2010). Against the concept that histone retention and poor chromatin quality in spermatids might negatively affect nuclear size elongation of mature sperm (Dadoune 2003; Johnson et al. 2011), it was reported that in $cnr1^{-/-}$, sperm nuclear length may become a valuable biomarker to identify morphologically normal sperm with good chromatin quality (Cacciola et al. 2013a). As yet, evaluation of sperm DNA integrity does not represent a useful criterion, in clinical practice of reproductive medicine, for predicting the final outcome of pregnancy through natural or artificial conception (Bartoov et al. 2002; Practice Committee of American Reproductive Society 2013).

On a final note, the AEA congener PEA also seems to play a role in the development of sperm hyperactivation during capacitation. Sperm from men with idiopathic infertility are more susceptible to PEA treatment and acquire a very energetic motility compared to controls. PEA-induced changes might help to switch sperm motility, from progressive to hyperactivated, and to provide sperm with more strength for penetrating the extracellular matrix of the egg (Ambrosini et al. 2005). These effects could be due to perturbations in the physicochemical properties of the lipid bilayer of membranes (Ambrosini et al. 2003) or to a direct interaction between PEA and membrane-bound enzymes.

Altogether, these data point to eCBs as new biomarkers of male and female defects and suggest ways in which they could help in the management of infertility

and in combating reproductive defects in humans, through the development of new ECS-oriented drugs.

6 Conclusions and Future Directions

In conclusion, endogenous levels of eCBs and activity/expression of distinct ECS elements (like FAAH, CB_1, and TRPV1) have the potential to provide useful diagnostic biomarkers and therapeutic targets of fertility defects (Maccarrone 2013; Rapino et al. 2014). Since some alterations of the ECS in blood cells mirror defects observed in reproductive organs, assays of FAAH activity/expression and of AEA content in easily accessible cells could be used in combination with other conventional diagnostic tests (e.g., progesterone or human chorionic gonadotropin assays) to track the progress of a normal pregnancy, to determine complications related to this process and to evaluate semen quality.

New perspectives on the therapeutic exploitation of PEA have recently emerged from studies with dietary supplements that contain this eCB-like compound in different amounts and are commercially available under different brand names (i.e., PeaVera or Normast). These medications are mainly painkillers for the treatment of neuropathies, but recently a PEA-containing cream appeared on the market as a useful treatment also for chronic vulvodynia (Keppel Hesselink et al. 2014). Indeed, some clinical cases of infertility *sine causa* (approximately 1 in 8 couples) might be due to unconsummated sexual relationships, caused by involuntary contractions of the pelvic floor muscles that provoke pain, burning, and stinging during intercourse. Therefore, the use of these PEA-containing products could help in the care of a problem (like infertility) that could be actually secondary to a central one (like vulvodynia/vaginismus).

Funding This investigation was partly supported by Ministero dell'Istruzione, dell'Università e della Ricerca (PRIN 2010–2011 grant) to M.M.

References

Abán C, Leguizamón GF, Cella M, Damiano A, Franchi AM, Farina MG (2013) Differential expression of endocannabinoid system in normal and preeclamptic placentas: effects on nitric oxide synthesis. Placenta 34:67–74

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

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

Ambrosini A, Zolese G, Wozniak M, Genga D, Boscaro M, Mantero F, Balercia G (2003) 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

Ambrosini A, Zolese G, Ambrosi S, Bertoli E, Mantero F, Boscaro M, Balercia G (2005) Idiopathic infertility: effect of palmitoylethanolamide (a homologue of anandamide) on hyperactivated sperm cell motility and Ca^{2+} influx. J Androl 26:429–436

Amoako AA, Marczylo TH, Marczylo EL, Elson J, Willets JM, Taylor AH, Konje JC (2013) Anandamide modulates human sperm motility: implications for men with asthenozoospermia and oligoasthenoteratozoospermia. Hum Reprod 28:2058–2066

Amoako AA, Marczylo TH, Elson J, Taylor AH, Willets JM, Konje JC (2014) Relationship between seminal plasma levels of anandamide congeners palmitoylethanolamide and oleoylethanolamide and semen quality. Fertil Steril 102:1260–7

Areia A, Fonseca E, Moura P (2013) Progesterone use after successful treatment of threatened pre-term delivery. J Obstet Gynaecol 33:678–681

Badawy ZS, Chohan KR, Whyte DA, Penefsky HS, Brown OM, Souid AK (2009) Cannabinoids inhibit the respiration of human sperm. Fertil Steril 91:2471–2476

Bagavandoss P, Grimshaw S (2010) Temporal and spatial distribution of the cannabinoid receptors (CB1, CB2) and fatty acid amide hydroxylase in the rat ovary. Anat Rec 293:1425–1432

Bambang KN, Lambert DG, Lam PMW, Quenby S, Maccarrone M, Konje JC (2012) Immunity and early pregnancy events: are endocannabinoids the missing link? J Reprod Immunol 96:8–18

Barbonetti A, Vassallo MR, Fortunato D, Francavilla S, Maccarrone M, Francavilla F (2010) Energetic metabolism and human sperm motility: impact of CB1 receptor activation. Endocrinology 151:5882–5892

Bartoov B, Berkovitz A, Eltes F, Kogosowski A, Menezo Y, Barak Y (2002) Real-time fine morphology of motile human sperm cells is associated with IVF-ICSI outcome. J Androl 23:1–8

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

Battista N, Meccariello R, Cobellis G, Fasano S, Di Tommaso M, Pirazzi V, Konje JC, Pierantoni R, Maccarrone M (2012) The role of endocannabinoids in gonadal function and fertility along the evolutionary axis. Mol Cell Endocrinol 355:1–14

Bayne RA, Eddie SL, Collins CS, Childs AJ, Jabbour HN, Anderson RA (2009) Prostaglandin E2 as a regulator of germ cells during ovarian development. J Clin Endocrinol Metab 94:4053–4060

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

Cacciola G, Chioccarelli T, Ricci G, Meccariello R, Fasano S, Pierantoni R, Cobellis G (2008a) The endocannabinoid system in vertebrate male reproduction: a comparative overview. Mol Cell Endocrinol 286:S24–S30

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

Cacciola G, Chioccarelli T, Altucci L, Viggiano A, Fasano S, Pierantoni R, Cobellis G (2013a) Nuclear size as estrogen-responsive chromatin quality parameter of mouse spermatozoa. Gen Comp Endocrinol 193:201–209

Cacciola G, Chioccarelli T, Fasano S, Pierantoni R, Cobellis G (2013b) Estrogens and spermiogenesis: new insights from type 1 cannabinoid receptor knockout mice. Int J Endocrinol 201:5013–5050

Catanzaro G, Battista N, Rossi G, Di Tommaso M, Pucci M, Pirazzi V, Cecconi S, Maccarrone M (2011) Effect of capacitation on the endocannabinoid system of mouse sperm. Mol Cell Endocrinol 343:88–92

Cecconi S, Rossi G, Castellucci A, D'Andrea G, Maccarrone M (2014) Endocannabinoid signaling in mammalian ovary. Eur J Obstet Gynecol Reprod Biol 178:6–11

Chamley LW, Bhalla A, Stone PR, Liddell H, O'Carroll S, Kearn C, Glass M (2008) Nuclear localisation of the endocannabinoid metabolizing enzyme fatty acid amide hydrolase (FAAH) in invasive trophoblasts and an association with recurrent miscarriage. Placenta 29:970–975

Chianese R, Ciaramella V, Scarpa D, Fasano S, Pierantoni R, Meccariello R (2012) Anandamide regulates the expression of GnRH1, GnRH2, and GnRH-Rs in frog testis. Am J Physiol Endocrinol Metab 303:E475–E487

Chioccarelli T, Cacciola G, Altucci L, Lewis SE, Simon L, Ricci G, Ledent C, Meccariello R, Fasano S, Pierantoni R, Cobellis G (2010) Cannabinoid receptor 1 influences chromatin remodeling in mouse spermatids by affecting content of transition protein 2 mRNA and histone displacement. Endocrinology 151:5017–5029

Cobellis G, Cacciola G, Scarpa D, Meccariello R, Chianese R, Franzoni MF, Mackie K, Pierantoni R, Fasano S (2006) 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

Cobellis G, Ricci G, Cacciola G, Orlando P, Petrosino S, Cascio MG, Bisogno T, De Petrocellis L, Chioccarelli T, Altucci L et al (2010) A gradient of 2-arachidonoylglycerol regulates mouse epididymal sperm cell start-up. Biol Reprod 82:451–458

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

Dadoune JP (2003) Expression of mammalian spermatozoal nucleoproteins. Microsc Res Tech 61:56–75

Daponte A, Deligeoroglou E, Garas A, Pournaras S, Hadjichristodoulou C, Messinis IE (2013) Activin a and follistatin as biomarkers for ectopic pregnancy and missed abortion. Dis Markers 35:497–503

Di Blasio AM, Vignali M, Gentilini D (2013) The endocannabinoid pathway and the female reproductive organs. J Mol Endocrinol 50:1–9

Di Marzo V, De Petrocellis L (2010) Endocannabinoids as regulators of transient receptor potential (TRP) channels: a further opportunity to develop new endocannabinoid-based therapeutic drugs. Curr Med Chem 17:1430–1449

Dimitriadis E, Robb L, Salamonsen LA (2002) Interleukin 11 advances progesterone-induced decidualization of human endometrial stromal cells. Mol Hum Reprod 8:636–643

Dodd JM, Crowther CA (2010) The role of progesterone in prevention of preterm birth. Int J Womens Health 1:73–84

El-Talatini MR, Taylor AH, Elson JC, Brown L, Davidson AC, Konje JC (2009a) Localisation and function of the endocannabinoid system in the human ovary. PLoS One 4, e4579

El-Talatini MR, Taylor AH, Konje JC (2009b) 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

El-Talatini MR, Taylor AH, Konje JC (2010) The relationship between plasma levels of the endocannabinoid, anandamide, sex steroids, and gonadotrophins during the menstrual cycle. Fertil Steril 93:1989–1996

Ezzati M, Djahanbakhch O, Arian S, Carr BR (2014) Tubal transport of gametes and embryos: a review of physiology and pathophysiology. J Assist Reprod Genet 31:1337–1347

Feuerstein P, Cadoret V, Dalbies-Tran R, Guerif F, Bidault R, Royere D (2007) Gene expression in human cumulus cells: one approach to oocyte competence. Hum Reprod 22:3069–3077

Fezza F, Bari M, Florio R, Talamonti E, Feole M, Maccarrone M (2014) Endocannabinoids, related compounds and their metabolic routes. Molecules 19:17078–17106

Fonseca BM, Correia-da-Silva G, Almada M, Costa MA, Teixeira NA (2013) The endocannabinoid system in the postimplantation period: a role during decidualization and placentation. Int J Endocrinol 2013:510540

Fonseca BM, Battista N, Correia-da-Silva G, Rapino C, Maccarrone M, Teixeira NA (2014) Activity of anandamide (AEA) metabolic enzymes in rat placental bed. Reprod Toxicol 49C:74–77

Fowler CJ (2013) Transport of endocannabinoids across the plasma membrane and within the cell. FEBS J 280:1895–1904

Francavilla F, Battista N, Barbonetti A, Vassallo MR, Rapino C, Antonangelo C, Pasquariello N, Catanzaro G, Barboni B, Maccarrone M (2009) 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

Gebeh AK, Willets JM, Marczylo EL, Taylor AH, Konje JC (2012) Ectopic pregnancy is associated with high anandamide levels and aberrant expression of FAAH and CB1 in fallopian tubes. J Clin Endocrinol Metab 97:2827–2835

Gebeh AK, Willets JM, Marczylo TH, Konje JC (2014) Plasma anandamide and related n-acylethanolamide levels are not elevated in pregnancies complicated by hyperemesis gravidarum. J Matern Fetal Neonatal Med 27:954–959

Gervasi MG, Rapanelli M, Ribeiro ML, Farina M, Billi S, Franchi AM, Perez MS (2009) The endocannabinoid system in bull sperm and bovine oviductal epithelium: role of anandamide in sperm-oviduct interaction. Reproduction 137:403–414

Gervasi MG, Osycka-Salut C, Caballero J, Vazquez-Levin M, Pereyra E, Billi S, Franchi A, Perez-Martinez S (2011) Anandamide capacitates bull spermatozoa through CB1 and TRPV1 activation. PLoS One 6, e16993

Grammatopoulos DK (2007) The role of CRH receptors and their agonists in myometrial contractility and quiescence during pregnancy and labour. Front Biosci 12:561–571

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

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

Grimaldi P, Pucci M, Di Siena S, Di Giacomo D, Pirazzi V, Geremia R, Maccarrone M (2012) The faah gene is the first direct target of estrogen in the testis: role of histone demethylase LSD1. Cell Mol Life Sci 69:4177–4190

Grimaldi P, Di Giacomo D, Geremia R (2013) The endocannabinoid system and spermatogenesis. Front Endocrinol 4:192

Guo Y, Wang H, Okamoto Y, Ueda N, Kingsley PJ, Marnett LJ, Schmid HH, Das SK, Dey SK (2005) N-acylphosphatidylethanolamine-hydrolyzing phospholipase D is an important determinant of uterine anandamide levels during implantation. J Biol Chem 280:23429–23432

Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C et al (2001) Sperm morphology, motility, and concentration in infertile and fertile men. N Engl J Med 345:1388–1393

Gye MC, Kang HH, Kang HJ (2005) Expression of cannabinoid receptor 1 in mouse testes. Arch Androl 51:247–255

Habayeb OM, Taylor AH, Bell SC, Taylor DJ, Konje JC (2008) Expression of the endocannabinoid system in human first trimester placenta and its role in trophoblast proliferation. Endocrinology 149:5052–5060

Halbert SA, Tam PY, Blandau RJ (1976) Egg transport in the rabbit oviduct: the roles of cilia and muscle. Science 191:1052–1053

Horne AW, Phillips JA 3rd, Kane N, Lourenco PC, McDonald SE, Williams AR, Simon C, Dey SK, Critchley HO (2008) CB1 expression is attenuated in Fallopian tube and decidua of women with ectopic pregnancy. PLoS One 3, e3969

Iliodromiti Z, Antonakopoulos N, Sifakis S, Tsikouras P, Daniilidis A, Dafopoulos K, Botsis D, Vrachnis N (2012) Endocrine, paracrine, and autocrine placental mediators in labor. Hormones (Athens) 11:397–409

Jequier AM (2010) Semen analysis: a new manual and its application to the understanding of semen and its pathology. Asian J Androl 12:11–13

Johnson GD, Lalancette C, Linnemann AK, Leduc F, Boissonneault G, Krawetz SA (2011) The sperm nucleus: chromatin, RNA, and the nuclear matrix. Reproduction 141:21–36

Kagan KO, Hoopmann M, Abele H, Alkier R, Lüthgens K (2012) First-trimester combined screening for trisomy 21 with different combinations of placental growth factor, free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol 40:530–535

Keppel Hesselink JM, Kopsky DJ, Sajben NL (2014) Vulvodynia and proctodynia treated with topical baclofen 5 % and palmitoylethanolamide. Arch Gynecol Obstet 290:389–393

Kourtis AP, Read JS, Jamieson DJ (2014) Pregnancy and infection. N Engl J Med 370:2211–2218

Kuc S, Wortelboer EJ, van Rijn BB, Franx A, Visser GH, Schielen PC (2011) Evaluation of 7 serum biomarkers and uterine artery Doppler ultrasound for first-trimester prediction of preeclampsia: a systematic review. Obstet Gynecol Surv 66:225–239

Lam PM, Marczylo TH, El-Talatini M, Finney M, Nallendran V, Taylor AH, Konje JC (2008) Ultra performance liquid chromatography tandem mass spectrometry method for the measurement of anandamide in human plasma. Anal Biochem 380:195–201

Lewis SE, Paro R, Borriello L, Simon L, Robinson L, Dincer Z, Riedel G, Battista N, Maccarrone M (2012a) Long-term use of HU210 adversely affects spermatogenesis in rats by modulating the endocannabinoid system. Int J Androl 35:731–740

Lewis LS, Rapino C, Di Tommaso M, Pucci M, Battista N, Paro R, Simon L, Lutton D, Maccarrone M (2012b) Differences in the endocannabinoid system of sperm from fertile and infertile men. PLoS One 7, e47704

Maccarrone M (2009) Endocannabinoids: friends and foes of reproduction. Prog Lipid Res 48:344–354

Maccarrone M (2013) Endocannabinoids as markers of sperm quality: hot spots. Front Endocrinol 4:169

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

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

Maccarrone M, Bisogno T, Valensise H, Lazzarin N, Fezza F, Manna C, Di Marzo V, Finazzi-Agrò A (2002) 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

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

Maccarrone M, De Felici M, Klinger FG, Battista N, Fezza F, Dainese E, Siracusa G, Finazzi-Agrò A (2004) Mouse blastocysts release a lipid which activates anandamide hydrolase in intact uterus. Mol Hum Reprod 10:215–221

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

Meccariello R, Chianese R, Cacciola G, Cobellis G, Pierantoni R, Fasano S (2006) Type-1 cannabinoid receptor expression in the frog, Rana esculenta, tissues: a possible involvement in the regulation of testicular activity. Mol Reprod Dev 73:551–558

Meccariello R, Battista N, Bradshaw HB, Wang H (2014) Endocannabinoids and reproduction. Int J Endocrinol 2014:378069

Mechoulam R, Parker LA (2013) The endocannabinoid system and the brain. Annu Rev Psychol 64:21–47

Melford SE, Taylor AH, Konje JC (2014) Of mice and (wo)men: factors influencing successful implantation including endocannabinoids. Hum Reprod Update 20:415–428

Mesiano S, Welsh TN (2007) Steroid hormone control of myometrial contractility and parturition. Semin Cell Dev Biol 18:321–331

Mitchell MD, Sato TA, Wang A, Keelan JA, Ponnampalam AP, Glass M (2008) Cannabinoids stimulate prostaglandin production by human gestational tissues through a tissue- and CB1-receptor-specific mechanism. Am J Physiol Endocrinol Metab 294:E352–E356

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

Moriconi A, Cerbara I, Maccarrone M, Topai A (2010) GPR55: current knowledge and future perspectives of a purported "Type-3" cannabinoid receptor. Curr Med Chem 17:1411–1429

Nallendran V, Lam PM, Marczylo TH, Bankart MJ, Taylor AH, Taylor DJ, Konje JC (2010) The plasma levels of the endocannabinoid, anandamide, increase with the induction of labour. BJOG 117:863–869

Nicolaides KH, Wright D, Poon LC, Syngelaki A, Gil MM (2013) First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing. Ultrasound Obstet Gynecol 42:41–50

Oh HA, Kwon S, Choi S, Shin H, Yoon KH, Kim WJ, Lim HJ (2013) Uncovering a role for endocannabinoid signalling in autophagy in preimplantation mouse embryos. Mol Hum Reprod 19:93–101

Orth JM, Gunsalus GL, Lamperti AA (1988) Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. Endocrinology 122:787–794

Paiva P, Hannan NJ, Hincks C, Meehan KL, Pruysers E, Dimitriadis E, Salamonsen LA (2011) Human chorionic gonadotrophin regulates FGF2 and other cytokines produced by human endometrial epithelial cells, providing a mechanism for enhancing endometrial receptivity. Hum Reprod 26:115311–115362

Paria BC, Ma W, Andrenyak DM, Schmid PC, Schmid HH, Moody DE, Deng H, Makriyannis A, Dey SK (1998) Effects of cannabinoids on preimplantation mouse embryo development and implantation are mediated by brain-type cannabinoid receptors. Biol Reprod 58:1490–1495

Paria BC, Song H, Wang X, Schmid PC, Krebsbach RJ, Schmid HH, Bonner TI, Zimmer A, Dey SK (2001) Dysregulated cannabinoid signaling disrupts uterine receptivity for embryo implantation. J Biol Chem 276:20523–20528

Park B, Gibbons HM, Mitchell MD, Glass M (2003) Identification of the CB1 cannabinoid receptor and fatty acid amide hydrolase (FAAH) in the human placenta. Placenta 24:990–995

Pistis M, Melis M (2010) From surface to nuclear receptors: the endocannabinoid family extends its assets. Curr Med Chem 1:1450–1467

Practice Committee of the American Society for Reproductive Medicine (2013) The clinical utility of sperm DNA integrity testing: a guideline. Fertil Steril 99:673–677

Ramathal CY, Bagchi IC, Taylor RN, Bagchi MK (2010) Endometrial decidualization: of mice and men. Semin Reprod Med 28:17–26

Rapino C, Battista N, Bari M, Maccarrone M (2014) Endocannabinoids as biomarkers of human reproduction. Hum Reprod Update 20:501–516

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

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

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

Rossi G, Gasperi V, Paro R, Barsacchi D, Cecconi S, Maccarrone M (2007) 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

Rouzer CA, Marnett LJ (2011) Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways. Chem Rev 111:5899–5821

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

Scotchie JG, Savaris RF, Martin CE, Young SL (2015) Endocannabinoid regulation in human endometrium across the menstrual cycle. Reprod Sci 22:113–23

Shi D, Komatsu K, Uemura T, Fujimori T (2011) Analysis of ciliary beat frequency and ovum transport ability in the mouse oviduct. Genes Cells 16:282–290

Sordelli MS, Beltrame JS, Burdet J, Zotta E, Pardo R, Cella M, Franchi AM, Ribeiro ML (2011) The effect of anandamide on uterine nitric oxide synthase activity depends on the presence of the blastocyst. PLoS One 6, e18368

Sowell ER, Charness ME, Riley EP (2014) Pregnancy: no safe level of alcohol. Nature 513:172

Sun X, Dey SK (2008) Aspects of endocannabinoid signaling in periimplantation biology. Mol Cell Endocrinol 286:S3–S11

Sun X, Dey SK (2009) Cannabinoid/endocannabinoid signaling impact on early pregnancy events. Curr Top Behav Neurosci 1:255–273

Sun X, Dey SK (2012) Endocannabinoid signaling in female reproduction. ACS Chem Neurosci 3:349–355

Sun X, Wang H, Okabe M, Mackie K, Kingsley PJ, Marnett LJ, Cravatt BF, Dey SK (2009) Genetic loss of faah compromises male fertility in mice. Biol Reprod 80:235–242

Sun X, Xie H, Yang J, Wang H, Bradshaw HB, Dey SK (2010) Endocannabinoid signaling directs differentiation of trophoblast cell lineages and placentation. Proc Natl Acad Sci USA 107:16887–16892

Talevi R, Barbato V, De Iorio S, Mollo V, Capriglione T, Ricchiari L, Samo A, Gualtieri R (2010) Is there a role for endocannabinoids in sperm-oviduct interaction? Reproduction 140:247–257

Tan H, Yi L, Rote NS, Hurd WW, Mesiano S (2012) Progesterone receptor-A and -B have opposite effects on proinflammatory gene expression in human myometrial cells: implications for progesterone actions in human pregnancy and parturition. J Clin Endocrinol Metab 97: E719–E730

Taylor A, Abbas M, Habiba M, Konje J (2010) Histomorphometric evaluation of cannabinoid receptor and anandamide modulating enzyme expression in the human endometrium through the menstrual cycle. Histochem Cell Biol 133:557–565

Taylor H, Finney M, Lam PM, Konje JC (2011) Modulation of the endocannabinoid system in viable and non-viable first trimester pregnancies by pregnancy-related hormones. Reprod Biol Endocrinol 29:152

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

Ueda N, Tsuboi K, Uyama T (2013) Metabolism of endocannabinoids and related N-acylethanolamines: canonical and alternative pathways. FEBS J 280:1874–1894

Vasan SS (2011) Semen analysis and sperm function tests: How much to test? Indian J Urol 27:41–48

Wang H, Guo Y, Wang D, Kingsley PJ, Marnett LJ, Das SK, DuBois RN, Dey SK (2004) Aberrant cannabinoid signaling impairs oviductal transport of embryos. Nat Med 10:1074–1080

Wang H, Dey SK, Maccarrone M (2006) Jekyll and Hyde: two faces of cannabinoid signaling in male and female fertility. Endocr Rev 27:427–448

Wang H, Xie H, Sun X, Kingsley PJ, Marnett LJ, Cravatt BF, Dey SK (2007) Differential regulation of endocannabinoid synthesis and degradation in the uterus during embryo implantation. Prostaglandins Other Lipid Mediat 83:62–74

Wang H, Xie H, Dey SK (2008) Loss of cannabinoid receptor CB1 induces preterm birth. PLoS One 3, e3320

Xie H, Sun X, Piao Y, Jegga AG, Handwerger S, Ko MS, Dey SK (2012) Silencing or amplification of endocannabinoid signaling in blastocysts via CB1 compromises trophoblast cell migration. J Biol Chem 287:32288–32297

Zygmunt PM, Ermund A, Movahed P, Andersson DA, Simonsen C, Jönsson BA, Blomgren A, Birnir B, Bevan S, Eschalier A, Mallet C, Gomis A, Högestätt ED (2013) Monoacylglycerols activate TRPV1–a link between phospholipase C and TRPV1. PLoS One 8, e81618