

## **Endocannabinoids and Regulation of Female and Male Fertility**

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**Running head:** Endocannabinoids and reproduction

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**Abstract**

Anandamide (*N*-arachidonylethanolamine, AEA) is known to impair mouse pregnancy and embryo development and to induce apoptosis in blastocysts. In humans, high levels of AEA in maternal blood coincide with pregnancy failure, attributable to dysregulation of the lymphocyte-mediated signalling at the feto-maternal interface. Here, I shall briefly review the roles of AEA, of the AEA-binding cannabinoid (CB) receptors, of the selective AEA membrane transporter (AMT), of the AEA-synthesizing enzyme NAPE-specific phospholipase D (NAPE-PLD), and of the AEA-hydrolyzing enzyme fatty acid amide hydrolase (FAAH) in fertility. In particular, I shall discuss the role of AEA degradation in human reproduction, showing that FAAH in maternal lymphocytes has a critical role for successful pregnancy, and that key-modulators of fertility like follicle-stimulating hormone, progesterone and leptin regulate FAAH activity and/or *FAAH* gene expression at specific sites of the promoter region. I shall also discuss new findings on the role of the endocannabinoid system in regulating sperm functions critical for male reproduction. Taken together, available evidence points towards a key-role for endocannabinoids in the hormone-cytokine networks that regulate human reproduction, at both female and male sides.

**Key words:** Anandamide – Blastocyst - FAAH - Lymphocytes – Sertoli cells - Sperm

### **The endocannabinoid system**

Anandamide (arachidonylethanolamide, AEA) belongs to a group of endogenous lipids, which include amides, esters and ethers of long chain polyunsaturated fatty acids, collectively termed “endocannabinoids” (1). It binds to type-1 (CB1) and type-2 (CB2) cannabinoid receptors (2), thus having many actions in the central nervous system (3) and in the periphery (4). These activities of AEA are terminated by cellular uptake through an AEA membrane transporter (AMT) (5), followed by degradation to ethanolamine and arachidonic acid by the fatty acid amide hydrolase (FAAH) (6, 7). The checkpoint in AEA synthesis is the *N*-acyl-phosphatidylethanolamines (NAPE)-hydrolyzing phospholipase D (NAPE-PLD), which releases on demand AEA from membrane NAPEs (8). Together with AEA and congeners like 2-arachidonoylglycerol (2-AG), *N*-arachidonoyldopamine, noladin ether and virodhamine, these proteins form the “endocannabinoid system” (1). In addition, type 1 vanilloid receptors (now called transient receptor potential channel vanilloid receptor subunit 1, TRPV1), that are six trans-membrane spanning proteins with intracellular N- and C-terminals (9), have been shown to be activated by AEA (10). Therefore, this lipid is also a true “endovanilloid” (11). Overall, the endocannabinoid system has been implicated in various aspects of the regulation of mammalian reproduction, as briefly summarized in the next two sections.

### **Endocannabinoids and female fertility**

Among the peripheral activities of AEA, regulation of female fertility has attracted growing interest and has been the subject of extensive reviews (for a recent one, see ref. 12). As a matter of fact, this activity of AEA extends to the endogenous ligands of CB receptors the well-known effects of exogenous cannabinoids (reviewed in ref. 13). For example, low FAAH in circulating maternal lymphocytes has been shown to be an early (< 8 weeks of gestation) predictor of spontaneous abortion in humans (12), and consistently FAAH expression is under control of fertility signals like progesterone and leptin (14). Conversely CBR, NAPE-PLD and AMT were not affected, and remained the same in healthy subjects and women who miscarried (12). Interestingly, the lack of leptin in knockout (*ob/ob*) mice is paralleled by

increased uterine levels of AEA, and of the other major endocannabinoid, 2-AG (15). In this line, mouse uterus contains the highest amounts of AEA as yet measured in any tissue (16), and uterine AEA can activate CB1R in this organ, thus allowing epithelial changes needed for reproduction (12). Additionally, within a very narrow concentration range AEA regulates blastocyst function and implantation by differentially modulating mitogen-activated protein kinase signaling and calcium channel activity via CB1R (17). Of interest is the fact that the embryo-uterine interactions are further complicated by the recent finding that mouse blastocysts rapidly (within 30 min of culture) release a soluble compound, that increases by ~2.5-fold the activity of FAAH present in the mouse uterus, without affecting gene expression at the translational level (Maccarrone et al., 2004). This “FAAH activator” was not present in uterine fluid, was not released by dead blastocysts nor by mouse embryonic fibroblasts, and was produced by trophoblast and inner cell mass cells. Though its identity has not been identified with any factor known to be released by blastocysts, the “FAAH activator” seems to be critical for the protection of blastocysts against the toxic effects of AEA, thus facilitating the implantation process (12).

### **Endocannabinoids and male fertility**

On the male side of reproduction, it has been demonstrated that rat testis is able to synthesize AEA (18), that has been detected also in human seminal plasma at nanomolar concentrations (19). The presence of CB1R in Leydig cells and their involvement in testosterone secretion have been demonstrated also in mice (20), and Sertoli cells of these animals have been shown to possess CB2 receptors, AMT and FAAH (21). Overall, these data have led to the suggestion that the endocannabinoid network may play a key-role in the regulation of male fertility too (21). In this line, AEA-signaling has been proposed to regulate sperm functions required for fertilization in humans (reviewed in ref. 22), and consistently human spermatozoa have been recently shown to express CB1R at the protein and mRNA level (23). Yet, the molecular basis of the involvement of the endocannabinoid system in controlling sperm function and male fertility in mammals remains unclear.

AEA reduces human sperm motility by reducing mitochondrial activity, and it also inhibits capacitation-induced acrosome reaction (23). These effects of AEA were prevented by the CB1R antagonist SR141716, leading to the suggestion that they required CB1R activation (23). However, the functionality of CB1R in human sperm was not ascertained, neither was the ability of these cells to bind, synthesize, transport and degrade AEA. It should be recalled that the analysis of these components of the endocannabinoid system is of utmost importance, because converging evidence suggests that AEA signaling and biological activity is tightly regulated via a “metabolic control” (6, 7, 12, 13). On this background, we have recently started an investigation, aimed at ascertaining whether sperm cells of boar (*Sus scropha*) are able to bind and metabolize AEA, and whether this endocannabinoid might modulate their function. It is noteworthy that boar closely resembles the physiology of humans (24), and its spermatozoa are largely used as a model system for experiments on reproductive physiology of mammals (25). Preliminary evidence seems to suggest that boar sperm have indeed the biochemical machinery to bind and degrade AEA, and that the non-hydrolyzable AEA analogue methanandamide reduces sperm capacitation (M.M., unpublished data). The eventual demonstration that sperm cells have a complete and efficient endocannabinoid system, and that this system controls sperm functions required for fertilization, may open new perspectives to the understanding and treatment of male fertility problems.

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