Relationship between seminal plasma levels of anandamide congeners palmitoylethanolamide and oleoylethanolamide and semen quality

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Objective: To determine whether changes in seminal plasma concentrations of the endogenous lipid signaling molecules palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) have significant effects on sperm quality.
Design: Biochemical and physiological studies of human seminal plasma and spermatozoa.
Setting: Academic tertiary care medical center.
Patient(s): Ninety men attending an infertility clinic for semen analysis.
Intervention(s): Palmitoylethanolamide and OEA extracted from seminal plasma were quantified by ultra high-performance liquid chromatography (HPLC)-tandem mass spectrometry. Patient sperm from semen with normal parameters were exposed in vitro to PEA or OEA to determine effects on sperm motility, viability, and mitochondrial activity.
Main Outcome Measure(s): The relationship between seminal plasma concentrations of PEA and OEA and sperm quality and the effect of these compounds on sperm motility, viability, and mitochondria activity in vitro.
Result(s): Palmitoylethanolamide and OEA concentrations in seminal plasma were lower in men with asthenozoospermia and oligoasthenoteratozospermia compared with men with normal semen parameters. Palmitoylethanolamide and OEA rapidly and significantly improved sperm motility and maintained viability without affecting mitochondria activity in vitro.
Conclusion(s): Maintenance of normal PEA and OEA tone in human seminal plasma may be necessary for the preservation of normal sperm function and male fertility. Exocannabinoids found in *Cannabis*, such as delta-9-tetrahydrocannabinol and cannabidiol, could compete with these endocannabinoids upsetting their finely balanced, normal functioning and resulting in male reproductive failure. (Fertil Steril® 2014;102:1260–7. ©2014 by American Society for Reproductive Medicine.)
Key Words: Palmitoylethanolamide, oleoylethanolamide, anandamide, endocannabinoid system, seminal plasma

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Received January 20, 2014; revised and accepted July 9, 2014; published online September 8, 2014.
A.A.A. has nothing to disclose. T.H.M. has nothing to disclose. J.E. has nothing to disclose. A.H.T. has nothing to disclose. J.M.W. has nothing to disclose. J.C.K. has nothing to disclose.
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Supported in part by miscellaneous educational funds from the University Hospitals of Leicester National Health Services Trust to support the Endocannabinoid Research Laboratory of University of Leicester.
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Fertility and Sterility® Vol. 102, No. 5, November 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.07.767

Infertility affects one in six (15%–20%) couples trying to achieving pregnancy (1–4). Female factors are responsible for half of cases of infertility, whereas male factors pure or in association with female factors account for the remaining 50% (3, 5). Male factor infertility is a complex and growing problem (6) with multifactorial causes and usually present as qualitative or quantitative

sperm defects in the form of either deficiency of sperm production, transportation, and/or morphology (7). Semen analysis remains the standard method of evaluation of male factor infertility but fails to elucidate the causes of sperm dysfunction. Recent studies have focused on a number of molecules with predictive significance of male reproductive potential. Among these are a group of lipid mediators, the endocannabinoids that are emerging as potential biomarkers of male reproductive health (8).

Endocannabinoid are endogenous bioactive lipid mediators that bind to cannabinoid receptors and mimic the adverse reproductive effects of *Cannabis*. The endocannabinoids, their molecular targets, synthetic and degradation enzymes, and protein transporters constitute the endocannabinoid system. The cannabinoid receptors were first identified as molecular targets for Δ^9-tetrahydrocannabinol, the active principle of marijuana and later shown to be two well-characterized G-protein–coupled cannabinoid receptors, CB1 and CB2 (9, 10), which also bind endocannabinoids.

N-arachidonoylethanolamide (Anandamide, AEA) is the most extensively studied member of this group of ligands and is widely distributed in most central and peripheral tissues including the reproductive system (11–13). The AEA and its congeners oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), commonly referred to as *N*-acylethanolamides, are hydrophobic molecules present at the low nanomolar range in many mammalian tissues and cells and produced from cell membrane phospholipid precursors in response to depolarizing agents, neurotr ansmitters, and hormones (14, 15). They seem to always occur together in tissues and biofluids, suggesting a common production pathway.

Oleoylethanolamide and PEA do not activate the classic cannabinoid receptors CB1 and CB2 and their exact biological roles remain elusive. They are purported to act as "entourage compounds," whereby they enhance the biological activity of AEA by inhibiting its degradation by the enzyme fatty acid amide hydrolase, acting as alternative substrates. Concentrations of AEA, PEA, and OEA have been detected at nanomolar levels in human reproductive fluids and tissues, such as midcycle oviductal fluid, follicular fluid (FF), and seminal plasma (16–18), and it has been shown that spermatozoa are sequentially exposed to declining levels of AEA as they swim from the ejaculate deposited in the vagina to the fertilization site in the oviductal ampulla (18).

Several in vitro studies have shown a dose-dependent inhibition of mammalian sperm functions by AEA mediated by CB1 receptor activation (19–21). In mature human spermatozoa AEA reduces sperm motility, capacitation, and acrosomal exocytosis (21). Similarly AEA exerts an inhibitory role on sperm functions in several species (18, 19, 21, 22) through inhibition of mitochondrial activity that preserves energy and ensures gradual acquisition of sperm fertilizing capacity during ascent through the female reproductive tract (23). Similarly, Δ^9-tetrahydrocannabinol interferes with downstream endogenous signaling pathways, modulating sperm functions, and male reproduction in invertebrates and mammals (18, 19, 21, 24). It is thought that a critical "endocannabinoid tone" is required for the maintenance of mammalian spermatozoa in the normal state and loss of these endocannabinoid protective functions results in sperm dysfunction and loss of fertilizing potential. Support for this comes from the observations that down-regulation of AEA tone and CB1 expression in seminal plasma and spermatozoa, respectively, occurs in men with abnormal semen parameters, indicating that aberrant endocannabinoid signaling in human spermatozoa plays a role relevant in the etiopathogenesis of human sperm dysfunction (25, 26).

Palmitoylethanolamide and OEA concentrations in most human reproductive tracts and fluids are higher than those of AEA, with emerging evidence suggesting that PEA and OEA possess antioxidant, anti-inflammatory, and antimicrobial activities that could protect sperm cells from oxidative damage, inflammation, and microbial activity. For example, PEA and OEA supplementation enhances sperm antioxidant activity, improves sperm kinematic parameters and hyperactivation, and protects cells from oxidative damage in some cases of idiopathic infertility (27–29).

We proposed that seminal plasma levels of PEA and OEA may reflect sperm quality. The aims of this study were therefore to explore possible relationships between PEA and OEA seminal plasma concentrations and semen quality and to determine the effects of these compounds on human spermatozoa in vitro.

MATERIALS AND METHODS

Chemicals

Dimethyl sulfoxide (DMSO) and formic acid were from Sigma Aldrich. Palmitoylethanolamide, OEA, and their deuterated equivalents (PEA-d4 and OEA-d2), each of >98% purity (and >99% deuterated content), and 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolyl carbocyanine iodide (JC-1) were purchased from Cayman Chemicals. The high-performance liquid chromatography (HPLC) grade acetonitrile, chloroform, methanol, and ammonium acetate were purchased from Fisher Scientific and HPLC grade water was obtained using a water purification system (Maxima ELGA, ELGA). Mobile phases were filtered through 0.2-μm, 47-mm diameter polytetrafluoroethylene filters (Waters UK Ltd.) before use. Oasis hydrophilic-Lipophilic-Balanced (HLB) solid phase extraction cartridges (1 mL, 30 mg) were purchased from Waters. Live/dead sperm viability kit was obtained from Invitrogen.

Study Design

This prospective study was approved and conducted according to the guidelines of the Leicestershire and Rutland local research ethics committee and all participants signed informed consent to take part. Semen was obtained from men who attended the Andrology Unit of the Leicester Royal Infirmary, an affiliated hospital of University of Leicester School of Medicine for routine semen analysis. Semen were collected from 90 consecutive patients by masturbation into a sterile plastic container after 2–5 days of sexual abstinence. Samples were allowed to liquefy at room temperature for