Effects of phosphodiesterase 5 inhibitors on sperm parameters and fertilizing capacity

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Abstract

The aim of this review study is to elucidate the effects that phosphodiesterase 5 (PDE5) inhibitors exert on spermatozoa motility, capacitation process and on their ability to fertilize the oocyte. Second messenger systems such as the cAMP/adenylate cyclase (AC) system and the cGMP/guanylate cyclase (GC) system appear to regulate sperm functions. Increased levels of intracytosolic cAMP result in an enhancement of sperm motility and viability. The stimulation of GC by low doses of nitric oxide (NO) leads to an improvement or maintenance of sperm motility, whereas higher concentrations have an adverse effect on sperm parameters. Several in vivo and in vitro studies have been carried out in order to examine whether PDE5 inhibitors affect positively or negatively sperm parameters and sperm fertilizing capacity. The results of these studies are controversial. Some of these studies demonstrate no significant effects of PDE5 inhibitors on the motility, viability, and morphology of spermatozoa collected from men that have been treated with PDE5 inhibitors. On the other hand, several studies demonstrate a positive effect of PDE5 inhibitors on sperm motility both in vivo and in vitro. In vitro studies of sildenafil citrate demonstrate a stimulatory effect on sperm motility with an increase in intracellular cAMP suggesting an inhibitory action of sildenafil citrate on a PDE isoform other than the PDE5. On the other hand, tadalafil’s actions appear to be associated with the inhibitory effect of this compound on PDE11. In vivo studies in men treated with vardenafil in a daily basis demonstrated a significantly larger total number of spermatozoa per ejaculate, quantitative sperm motility, and qualitative sperm motility; it has been suggested that vardenafil administration enhances the secretory function of the prostate and subsequently increases the qualitative and quantitative motility of spermatozoa. The effect that PDE5 inhibitors exert on sperm parameters may lead to the improvement of the outcome of assisted reproductive technology (ART) programs. In the future PDE5 inhibitors might serve as adjunct therapeutical agents for the alleviation of male infertility. (Asian J Androl 2008 Jan; 10: 115–133)

Keywords: sperm fertilizing capacity; phosphodiesterase 5 inhibitors; spermatozoa; testis; male infertility

1 Second messenger systems

A second messenger system is a group of intracellular independent but interrelated elements; within this group of molecules an intracellular signal is generated in response to an intercellular first messenger molecule. Hormones or neurotransmitters can serve as primary messengers. Second messenger systems are thought to be intermediate signals in cellular processes such as metabolism, secretion, or cell growth. The primary signal molecule does not enter the cell but utilizes a cascade of molecular events in order to induce a cellular response. Hormones utilize second messenger systems and interestingly it has been shown that a single hormone can

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utilize more than one second messengers.

There are four major classes of second messenger systems: a) the tyrosine kinase system, b) the inositol-1,4,5-trisphosphate (IP3)/diacylglycerol (DAG) system, c) calcium ions (Ca\(^{2+}\)), and d) cyclic nucleotides (e.g., 3',5'-cyclic adenosine monophosphate [cAMP] and 3',5'-cyclic guanosine monophosphate [cGMP]). A second messenger system comprises of several elements including the first messenger, the primary messenger’s receptor, a second receptor called G protein that interacts with the primary messenger’s receptor, an enzyme triggered into action by the interacting pair of receptors, and a second messenger molecule generated by this enzyme. The intracellular signal generated in response to the first messenger is amplified into the cell.

1.1 Tyrosine kinase second messenger system

Insulin is an example of a hormone whose receptor is a tyrosine kinase. This hormone binds to domains exposed on the cell’s surface resulting in a conformational change that activates the kinase domains located in the cytoplasmic regions of the receptor. In several cases, the receptor phosphorylates itself as part of the kinase activation process. The activated receptor phosphorylates a variety of intracellular targets; most of them are enzymes that become activated or are inactivated upon phosphorylation.

1.2 DAG/protein kinase C (PKC) second messenger system

DAG/PKC second messenger system is the second messenger system for primary messengers such as thyroid-stimulating hormone (TSH), angiotensin, or neurotransmitters. The above primary messengers bind to G protein-coupled receptors and subsequently the alpha subunit of the G protein activates an intracellular enzyme called phospholipase C. This enzyme hydrolyzes the phosphatidylinositol-4,5-bisphosphate (PIP2) which is found in the inner layer of the plasma membrane. The products of the hydrolysis are DAG and IP3. After the hydrolysis, DAG remains at the inner layer of the plasma membrane, due to its hydrophobic properties, and recruits PKC (a calcium dependent kinase). The phosphorylation of other proteins by PKC causes several intracellular changes. Calcium ions are required for PKC to be activated; the other second messenger, IP3, renders calcium ions available for PKC. IP3 is a soluble molecule that diffuses through the cytosol and binds to receptors on the smooth endoplasmic reticulum causing the release of Ca\(^{2+}\) into the cytosol. The latter rise of intracellular calcium triggers the cellular response (Figure 1).

1.3 Ca\(^{2+}\)

Ca\(^{2+}\) is the most widely involved and important intra-cellular messenger. As a response to several primary signals the subsequently elevated concentration of Ca\(^{2+}\) triggers many types of events such as muscle contraction, release of neurotransmitters at synapses, secretion of hormones like insulin, activation of T cells and B cells (when they bind antigen with their antigen receptors), and apoptosis.

1.4 Adenylate cyclase (AC)/cAMP second messenger system

cAMP is a nucleotide generated from ATP through the action of the enzyme adenylate cyclase. A variety of hormones can trigger an increase or decrease of the intracellular concentration of cAMP. The elevated concentrations of cAMP can activate a cAMP-dependent protein kinase called protein kinase A (PKA). This protein is at a catalytically-inactive state but becomes active when it binds cAMP.

Cyclic AMP was discovered in 1958 by Rall and Sutherland [1] and since then many biochemical actions were attributed to this molecule such as stimulation of glycogen degradation, gluconeogenesis, lipid degradation, steroid synthesis, inhibition of glycogen synthesis, amino acid uptake, and regulation of ion transport as well as regulation of transcription. It is formed by the action of AC on ATP-Mg\(^{2+}\) complex. Free Mg\(^{2+}\) is a necessary cofactor. Moreover two other proteins are involved in the cAMP-dependent signal transduction mechanisms: (1) a hormone receptor, and (2) a G-protein, a heterodimer. Receptors which associate with G-proteins of the G\(_s\)-type stimulate AC and receptors which associate with

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Figure 1. Phospholipase C hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2). Diacylglycerol (DAG) recruits protein kinase C (PKC), a calcium dependent kinase and inositol-1,4,5-trisphosphate (IP3) binds to receptors on the smooth endoplasmic reticulum causing the release of calcium ions (Ca\(^{2+}\)) and the subsequent activation of PKC.
G-proteins of the G\textsubscript{i}-type inhibit AC. The cAMP that is formed activates “cAMP-dependent protein kinase”, also called PKA, which is involved in several metabolic pathways acting by phosphorylating other proteins (enzymes, transporters, etc.).

Hormones such as adrenaline, glucagon, and luteinizing hormone (LH) exert their effects using cAMP as a second messenger. The above hormones bind to their receptors in the membrane of target cells interacting subsequently with a set of G proteins. This interaction triggers AC which initiates the conversion of ATP to cyclic AMP with an overall result an elevated intracellular concentration of cAMP. The increased levels of cAMP activate PKA as mentioned above. The activated PKA promotes a cascade of events into the cell, adding phosphates to other enzymes, changing their structure and thereby modulating their catalytic activity.

1.5. Guanylate cyclase (GC)/cGMP second messenger system

The description of the cyclic nucleotides cAMP and cGMP led to the first formulation of the second messenger concept. Very similar to the cAMP, cGMP is another important second messenger. It was described as a biological product in 1963 but the regulation of its synthesis has remained obscure until very recently. Its concentration in the tissues is relatively low. This was the reason cGMP was not considered as a potential second messenger for several centuries. Subsequently it has become clear that cGMP plays a pivotal role in controlling a wide variety of biological processes such as retinal phototransduction, intestinal secretion, smooth muscle relaxation, platelet activation, and neurotransmission [2]. Cyclic GMP is generated from GTP via a reaction catalyzed by the ubiquitous enzymes GC which are expressed in both soluble (sGC) and particulate, membrane-bound (mGC) isoforms. These isoforms co-exist in most cells in different concentrations depending on the type and the physiological state of the tissue [3]. The mGC is a cell surface receptor enzyme that contains an extracellular receptor domain and an intracellular catalytic domain separated by a single transmembrane domain [4].

Several subclasses of mGC have been identified so far in vertebrates. They are homodimeric glycoproteins [5, 6], and probably are associated with the plasma membrane, the endoplasmic reticulum, the Golgi bodies, and the nuclear membrane [3]. The various subclasses of mGC represent the receptors for three structurally similar peptides (atrial natriuretic peptide, B-type natriuretic peptide, and C-type natriuretic peptide) [7, 8]. Other mGC subclasses bind the heat-stable enterotoxin of Escherichia coli [5, 9].

The soluble isofrom of guanylate cyclase sGC includes a group of heterodimeric hemoproteins composed of α- and β-subunits [6]. It contains also a prosthetic heme group on each heterodimer [4] which can bind diffusible gases such as nitric oxide (NO) and carbon monoxide [5]. The enzyme’s catalytic activity is enhanced after binding from a 5-fold level (with carbon monoxide) to a 400-fold (with NO) [4].

2. cAMP, cGMP and regulation of the erectile function

Penile erection requires an increase in blood flow to the penis as a consequence of cavernous smooth muscle relaxation and restriction of venous outflow from the corpus cavernosum [10]. This process is mediated by parasympathetic cholinergic pregangliotic neurons residing within the sacral spinal cord (S2-4). The cavernous nerves arise from the pelvic nerves that exit the above mentioned S2-4 region of the sacral spinal cord and provide the autonomic input to the penis. These nerves release various neurotransmitters including nitric oxide, acetylcholine (Ach), and vasoactive intestinal peptide (VIP) that are capable of relaxing the cavernous smooth muscle [10]. The release of NO is thought to activate cytosolic GC enzymes increasing the intracellular cGMP level and reducing the cytosolic Ca\textsuperscript{2+} content. Moreover NO appears to reduce norepinephrine release from noradrenergic nerves [11]. The AC/cAMP second messenger system is also implicated in the penile erection. VIP acts through the AC pathway to trigger an increase in intracellular cAMP level [12]. A rise in intracellular cAMP results in a fall in cytosolic Ca\textsuperscript{2+} in cavernous smooth muscle which causes relaxation of cavernous smooth muscle.

3. Regulation of sperm function by second messenger systems

3.1 Sperm function and AC/cAMP second messenger system

Cyclic AMP appears to be involved in the signaling pathways that regulate sperm motility [13, 14] as well as sperm capacitation [15]. In fact increased levels of intracytosolic cAMP have been demonstrated to enhance sperm motility and viability [16, 17] by a) increasing the rate of glycolysis and fructolysis and b) enhancing the oxidation of lactate or pyruvate to CO\textsubscript{2} [18].

Yanagimachi [19] has reported that the asymmetrical, high amplitude beats of the sperm flagellum (referred to as “hyperactivated motility”) and the capacitation process are dependent on the intracellular cAMP levels. These findings are consistent with those of MacLeod et al. [20] who demonstrated that the majority of the cAMP-dependent protein kinases in the rat spermatozoa are located within the flagellum. Furthermore, cAMP seems
to play an important role in the regulation of the capacitation process and the acrosome reaction [21–23].

3.2 Sperm function and GC/cGMP second messenger system

Nitric oxide, this ubiquitous, short-lived, mediator of cell-to-cell interaction is synthesized by nitric oxide synthases (NOS) in many mammalian cell types [24] in response to a large group of stimuli. Spermatozoa themselves express an NOS activity and are able to synthesize nitric oxide [17, 25–28]. Specific chemical stimuli can enhance spermatozoal NO production [26, 28]. The presence of endothelial and neuronal NOS isoforms in human spermatozoa has been demonstrated in several studies [17, 25, 27, 28]. NO is known to affect sperm motility and viability in a concentration-related fashion. At low doses nitric oxide is found to improve or maintain sperm motility probably through the stimulation of cGMP production [17, 29], whereas, at higher concentrations of nitric oxide, sperm motility and viability are adversely affected, most likely due to nitric oxide ability to serve as a free radical and cause direct oxidative damage in the spermatozoal membrane [30].

GC-activating substances (in particular atrial natriuretic peptide and nitric oxide) strongly affect positively sperm motility, capacitation, and acrosomal reactivity. These substances stimulate sperm metabolism and promote the sperm capacity to approach the oocyte, interact with it, and finally fertilize it [31]. Interesting studies have indicated that the sperm acrosome reaction rate is greatly influenced by cGMP synthesis [31]. A complex cross-talk phenomenon between the cAMP- and the cGMP-generating systems regulating the sperm function occurs in human spermatozoa [32]. Spermogenesis, and sperm-egg interaction appears to be positively affected by sperm GC activation, whereas recent experimental observations indicate that excessive amounts of certain GC activators might exert opposite, antireproductive effects through an increase in the oxidative stress and the lipid peroxidation on sperm membranes [30, 33, 34]. In general important final events of the fertilization process (i.e., acrosomal reaction) are regulated by interactions between second messenger systems. Sofikitis et al. [32] have shown an interaction between the AC/cAMP second messenger system and phorbodiester/PKC second messenger system in the regulation of sperm acrosome reaction process.

4 Phosphodiesterases (PDEs): general considerations

The cyclic nucleotide PDEs play the dominant role in the degradation of the cAMP and cGMP. The PDEs function in conjunction with AC and GC to regulate the amplitude and duration of intracellular signaling mechanisms (mediated via cAMP and cGMP, respectively). Sequence analyses suggest that there are at least 11 different families of mammalian PDEs. Most of the families include more than one gene product. In addition, many of these genes can be alternatively spliced in a tissue specific manner. The overall result is the generation of different mRNAs and proteins with altered regulatory properties or subcellular localization.

PDEs are named to precisely identify the isoenzyme being referenced. Thus the first two letters of PDEs describe the species of origin followed by PDE and the arabic numeral of the gene family. The next letter represents the individual gene within the family, and the last arabic numeral identifies the transcript variant. For example, HSPDE1A1 refers to the human PDE1 family, gene A, transcript variant 1. Each PDE family displays different a) substrate specificity, b) kinetic properties, c) allosteric regulation, and d) interaction with specific inhibitors. Thus, some PDEs hydrolyze only cAMP (PDE4, PDE7 and PDE8), some PDEs hydrolyze only cGMP (PDE5, PDE6 and PDE9), while other PDEs demonstrate mixed specificities and hydrolyze both cAMP and cGMP (PDE1, PDE2, PDE3, PDE10 and PDE11). Therefore, the expression profile of PDEs within a given cell may determine the type of cyclic nucleotide hydrolyzed in that cell or subcellular region. The distinct cellular localization and biophysical characteristics of the various PDEs suggest that each PDE transcript variant plays distinct roles in specific physiological processes.

PDE1 family for example involves three gene products (PDE1A, PDE1B, and PDE1C) [35] which are activated by the binding of calmodulin in the presence of calcium [36] leading to an increase in hydrolysis of both cAMP and cGMP. More specifically, PDE1A and PDE1B enzymes selectively hydrolyze cGMP while the PDE1C variant hydrolyzes both cAMP and cGMP with high affinity [37, 38]. Northern blot analysis and in situ hybridization revealed that PDE1 is expressed in heart, brain, skeletal muscle, smooth muscle, as well as in other peripheral tissues [39, 40]. Direct catalytic site inhibitors such as vinpocetine and 8-methoxy-1-methyl-3-isobutylxanthine (IBMX) inhibit PDE1 activity. However the latter inhibitors demonstrate limited inter-PDE family selectivity [38, 41]. Vinpocetine is actually used in many regions of Europe, Japan and Mexico as pharmaceutical treatments for cerebrovascular and cognitive disorders or as a dietary supplement in the United States. No side effects attributable to this medicine have been observed but the doses of these enzymes required for the pharmacological effect are high.

The cGMP-stimulated PDE2A type hydrolyzes both cAMP and cGMP, although it has a higher affinity for cGMP than for cAMP [42]. There is a single PDE2 gene form.
which encodes three PDE2 splice variants [43, 44]. Human PDE2A is expressed in brain, adrenal cortex [45], and to a lesser extent in heart, liver, skeletal muscle, kidney and pancreas [44]. It has been shown to play a role in regulating aldosterone production in adrenal glomerulosa cells through regulation of cAMP and cGMP intracellular levels. Moreover PDE2A seems to play a role in regulating cGMP-mediated effects in blood platelets [46], cardiomyocyte and vascular endothelial cells [47]. Finally in a recent study it has been demonstrated that the inhibition of this PDE type by a novel PDE inhibitor named Bay-60-7550 seems to improve memory functions by enhancing neuronal plasticity [48]. Furthermore PDE2 is inhibited by erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) a potent adenosine deaminase inhibitor [49].

The PDE3 family members allow cGMP to potentiate a cAMP signal in cells where PDE3 family members are expressed. This family is composed of two genes: PDE3A and PDE3B. PDE3A is involved in the regulation of platelet aggregation while PDE3B mediates the insulin regulation of lipolysis in adipocytes. In addition PDE3B mediates leptin inhibition of insulin secretion in pancreatic beta cells [50]. Furthermore, PDE3B mRNA concentration is highest in adipocytes, hepatocytes, brain, renal collecting duct epithelium, and developing spermatocytes [51]. Some tissues may express both PDE3A and PDE3B but the levels of PDE3B are usually higher [52, 53]. PDE3 variants are activated by PKA or PKB phosphorylation. Consensus sites of phosphorylation for each kinase are located between NHR1 and NHR2 in both PDE3A and PDE3B [53]. On the other hand PDE3A and PDE3B are directly inhibited by cGMP-mediated competition for cAMP binding to the active site. This was the reason why PDE3 was also referred to as cGMP-inhibited cAMP PDE in the earlier literature. PDE3 enzymes were therapeutic targets of great interest in cardiovascular system [53–55]. A few selective inhibitors of PDE3 family exist, including milrinone, amrinone, cilostamide, and cilostazol.

PDE4 family hydrolyses exclusively cAMP. It has been shown that there are four isoforms (A, B, C and D) each coded by a separate gene in both rodents [56] and the human [57]. Each isoform is characterized by a unique N-terminal region. These variants have closely related kinetic properties and requirements for ions. Functional PDE4 isoforms can be divided into three major categories: long, short and super-short [58]. These isoforms are expressed in almost all cell types except blood platelets [37]. PKA-dependent phosphorylation selectively activates many long PDE4 isoforms [59, 60]. It is interesting that PDE4 selective inhibitors demonstrate in animal models potent anti-inflammatory actions. Indeed, there is currently much interest in employing selective PDE4 inhibitors for the treatment of asthma, chronic obstructive pulmonary disease [61], rheumatoid arthritis and cancer. Moreover these inhibitors can also exert antidepressant actions [62–64].

PDE5 specifically hydrolyses cGMP to 5’ GMP. This PDE family consists of a single PDE5 gene (Figure 2). Furthermore, the existence of three alternatively spliced PDE5 isoforms (PDE5A1, 2 and 3) has been demonstrated. These isoforms differ only in the 5’ ends of their corresponding mRNAs and in the corresponding N-termini of sproteins [65, 66]. PDE5A1 and PDE5A2 are co-expressed in a variety of tissues. However, PDE5A3 appears to be expressed in smooth muscle cells only [67, 68]. The success of PDE5 selective inhibitors in the treatment of the erectile dysfunction (ED) has increased the interest to investigate the effects of inhibiting PDE5 in vascular, thrombotic, or pulmonary disorders [69].

Cyclic GMP PDE in retinal photoreceptors, classified as PDE6, is a key enzyme in the vertebrate phototransduction. Indeed phototransduction in cones and rods is mediated primarily through the action of three proteins: the receptor (i.e. rhodopsin), a G-protein (i.e. transducin), and the 3’,5’ cyclic nucleotide PDE6. The two PDE6 isoforms are actually the only PDE family enzymes [69, 70] in the photoreceptor outer segments. PDE6 is a heterotrimeric enzyme functioning to lower cytoplasmic cGMP levels in response to light activation of the receptor rhodopsin [71–73]. It is composed of two homologous catalytic subunits (Pr, β) and two identical inhibitory subunits Py. PDE6 activity is inhibited by selective PDE inhibitors such as zaprinast [74], sildenafil, tadalafil [75], and vardenafil [76].

The PDE7 family includes cAMP-specific PDEs. Two genes of this family have been identified: PDE7A and PDE7B.
PDE7B. PDE7A has three isoforms, generated by alternate splicing, which are found mainly in a) the T cells and the brain (PDE7A1), b) the muscle cells (PDE7A2), and c) the activated T cells (PDE7A3) [77–79]. PDE7B has approximately 70% homology to PDE7A [80, 81]. PDE7 family plays a pivotal role in the regulation of human T cell functions including cytokine production, proliferation, and expression of activation markers. Thus selective PDE7 inhibitors may have role in the treatment of T cell-mediated diseases and disorders of the airways [82]. However, currently there is an absence of a selective PDE7 inhibitor [83]. Promising results are yielded from novel PDE7 inhibitors like iminothiadiazoles [84] which have been proposed on patent literature.

The PDE8 family contains high-affinity cAMP-specific IBMX-insensitive PDEs. They are composed of two isoforms PDE8A and PDE8B and so far, they have been identified in the human and mouse [85–87]. While PDE8A1 is widely distributed in various tissues, such as the testis, spleen, colon, small intestine, ovary, placenta, and kidney [87]. PDE8B is found only in the human thyroid gland.

PDE9 is a cGMP-specific PDE. The encoded protein of this gene plays a role in signal transduction by regulating the intracellular concentration of cGMP. Multiple-tissue Northern blot analyses have revealed high levels of PDE9 in brain, heart, placenta, adult and fetal kidney, spleen, prostate, and colon [88]. Moreover PDE9A was mapped to human chromosomal region 21q22.3, a critical region for two genetic diseases: the nonsyndromic hereditary deafness [89, 90], and the bipolar affective disorder [91–93]. The above observations may suggest a role of disorders in the regulation of the expression of this enzyme in the development of these diseases. The only PDE inhibitor that seems to inactivate PDE9A is zaprinast [94]. The presence of only one inhibitor is a barrier for major research efforts that focus to investigate the role of PDE in the above two pathophysiology.

PDE10A has been categorized as cGMP-binding PDE. It is expressed in the putamen and caudate nucleus regions. The latter regions have dopamine receptors and are related to juvenile Parkinsonism. Therefore a genetic relationship between the PDE10A gene and this disease cannot be excluded [95]. PDE10A is moderately inhibited by IBMX a non-specific PDE inhibitor.

By screening a human skeletal muscle cDNA library, Fawcett et al. [96] cloned a human PDE gene family member. The authors denoted the latter PDE genes as PDE11A (in accordance with the standardized nomenclature [97]). This partially purified-recombinant human PDE11A has the ability to hydrolyse both cAMP and cGMP [96]. It is sensitive to the non-selective inhibitor IBMX, to zaprinast, and to pyridamole. In addition, pyridamole inhibits PDE11A with potency approximately equal to that for PDE5 or PDE6. PDE11A is expressed as at least three distinct major transcripts. The latter transcripts can be found at highest levels in skeletal muscles and the human prostate [96]. Northern blot analysis revealed wider expression of these transcripts in kidney liver, pituitary and salivary glands, and testis.

The differential tissue distribution of PDEs makes them attractive targets for the development of cell-specific drugs. Indeed selective inhibitors of PDEs have been widely studied as cardiotonics, vasodilators, smooth-muscle relaxants, antidepressants, antithrombotics, antiasthmatics, and agents for improving cognitive functions such as learning and memory [98–105]. So far a limited number of PDE inhibitors are commercially available. However these compounds display only partial selectivity for specific PDE isoforms. Selective inhibitors for many PDE families are still not available, in particular for the PDE isoforms 8, 9, and 10.

The evaluation and the action of PDE inhibitors in vivo or in vitro is limited by a number of factors including the specific cell permeability, the uncertainty of the actual intracellular concentration of inhibitor, and the profile and subcellular localization of the PDEs in the specific cell type being studied. Several times there is a disparity between the cellular effect of an inhibitor in vitro and the cellular effect of the same inhibitor in vivo. This is the result of the complexity of the in vivo conditions compared with a purified enzyme assay in vitro experiments. For example, trequinsin has been tested successfully in vivo as a PDE2 inhibitor [106]. However, in vitro this inhibitor is actually much more potent for the inhibition of PDE3 [107, 108].

5 PDEs isoforms in the male genital system

Recently, scientists focused their efforts on the understanding of the regulatory mechanisms responsible for the contraction and relaxation of the male genital ducts. These studies may clarify the mechanisms responsible for the transport of spermatozoa from the seminiferous tubuli through the remaining male genital duct. Although there are still many issues to be elucidated, it has been demonstrated that the messenger molecule cGMP is crucial for the regulation of contractility of seminiferous tubules in man [109, 110], the human testicular capsule [111], and the epididymal ducts [112]. In addition, contractility studies and analyses of GC-B-knockouts mice [113] have demonstrated that cGMP-dependent relaxation mechanisms appear to be of paramount importance on the regulation of transport and maturation of spermatozoa in the epididymis.

Detailed analyses of tissue- and cell type-specific distribution of PDE gene families, however, in the testis.

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and epididymis are still lacking. The literature reveals data on PDE expression in testis restricted predominantly to cAMP-hydrolyzing PDEs such as PDE1C, PDE4A, PDE4C, PDE7B, and PDE8A and provides also useful information about PDEs localization in male germ cells and spermatozoa [114]. Transcripts of the PDE10 were found in the human testis [115]. cGMP-hydrolyzing PDE5 was recently localized in peritubular myoid cells of the rat [116]. Moreover the potential functions of PDE11 in the regulation of spermatogenesis process and sperm function has been an issue of major clinical importance since PDE11 serves as a substrate for the commonly used substance tadalafil [117]. Regulation of epididymal duct contractility by PDE3 has been suggested [112].

6 PDE in human spermatozoa

NOS [17] and PDE have also been found in male gametes since 1971 [118]. In fact there is evidence for the presence of more than one isoforms of PDEs in spermatozoa. Measurements of spermatozoal PDE activity in the presence of inhibitors for PDE1 or PDE4 confirmed the presence of PDE1 and PDE4 in human spermatozoa [22]. In the same study the authors concluded that the PDE4 is distributed in sperm flagella, midpiece, and cytoskeletal structure. In contrast, PDE1 activity is more evenly distributed in all the above three sperm regions. Immunocytochemical data has suggested that PDE4 is localized mainly in the sperm midpiece while the PDE1 is found largely in the sperm head [22].

In a recent study Lefèvre et al. [119] identified in ejaculated human spermatozoa two PDE isoforms: PDE1A and PDE3A. Their activities were detected in both the soluble and particulate fractions. The authors also reported that PDE1A is located in the equatorial region of sperm head, midpiece and principal piece of the tail while PDE3A is located in the postacrosomal region of the sperm head. The latter finding may suggest a role of PDE3A in the regulation of sperm membrane alterations which are important for the sperm capacitation process and the acrosomal reaction. Moreover PDE1A location in the midpiece and the principal piece of the flagellum is also consistent for a probable role of the above isoform in the development of sperm motility and sperm capacity to undergo hyperactivation. In earlier studies, however, Cheng and Boettcher [120] using as a method both polyacrilamide gel electrophoresis and DEAE-cellulose column chromatography have proven the presence of at least five isoenzymes of PDEs in human semen. Richter et al. [121] have demonstrated the presence of mRNA for six PDE types/subtypes in ejaculated human spermatozoa. More specifically using the RT-PCR as a method to detect the mRNA transcripts of PDE subtypes, the authors found strongly specific bands for PDE1B, PDE3B, PDE4A, PDE4B, and PDE8 while amplification products of PDE-1A/C, -2, -3A, -4C, and -5 were observed in a part of the samples as weak signals. However it is not clear whether the mRNA transcripts are products of a de novo synthesis in ejaculated spermatozoa or whether they are synthesized at an earlier stage of spermatogenesis and then are stored in ribonucleoprotein particles. Moreover it should be emphasized that the rate of hydrolysis of cyclic nucleotides in spermatozoa is much faster (9 to 600 fold) than the rate of cyclic nucleotides formation suggesting that the PDE have a dominant role in the control of the concentration of cyclic nucleotides in spermatozoa [120].

7 Development of PDE5 inhibitors for the management of ED

Sexual dysfunction represents in many societies, a taboo and scientific research on this field did not expand as it happened in other medical fields. With the introduction in the market of the first effective orally administered medicine for the treatment of ED sildenafil, research efforts for the treatment of ED have become a priority for several pharmaceutical companies. Sildenafil and the substances vardenafil and tadalafl, which have been developed later, are known as PDE5 selective inhibitors. In addition, several other potent PDE5 inhibitors with a variety of scaffolds have been developed. For example quinazoline derivatives [122–124], phthalazine derivatives [125, 126], tetracyclic diketopiperazines [127], indoles [128], pyrido[3,2,1-jk]carbazoles [98] represent the results of research efforts in this field.

Sildenafil as oral treatment for the ED was approved by the FDA in USA on March 1998. Because of the mechanism of its action, pharmacokinetics, and metabolism, sildenafil is contraindicated in patients receiving organic nitrates or NO donors. Moreover the administration of this medicine should be avoided in patients with hepatic or renal impairment. In vitro studies have indicated that sildenafil is a weak inhibitor of cytochrome P450. Sildenafil administration to hypertensive patients has shown a mean additional reduction of supine blood pressure when sildenafil was administrated with amlodipine. Patients with cardiovascular diseases under treatment with medicines different to nitrates, such as ACE inhibitors, α-adrenoceptor or β-adrenoceptor blockers, calcium channel blockers or diuretics can safely receive sildenafil as well. In fact it has been demonstrated by Kloner et al. [129] that sildenafil does not have a synergic effect on blood pressure with the above antihypertensive agents. Similarly, a significant improvement in satisfaction with their sexual life was reported in patients with spinal cord injury [130] as well as in patients with sexual dysfunction due to treatment with...
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abtidepressants. The most frequently reported side effects of sildenafil are headache (7%–25%), facial flushing (7%–34%), nasal congestion (4%–19%), dyspepsia (1%–11%), and visual effects (1%–6%) [131–134] were also consistent with the control trials [135]. Approximately 0%–10% of men who receive sildenafil discontinue the treatment due to the severity of side effects [131–134].

Vardenafil was the second selective PDE5 inhibitor developed in the market. It received an approval letter from the FDA on September 2001. Its chemical structure is very similar to that of sildenafil. However vardenafil has been proven that it has lower in vitro IC₅₀ value (concentration of the medicine which inhibits 50% of the PDE5 activity) compared with sildenafil. Vardenafil should not be administered in patients who receive treatment with organic nitrates, because vardenafil may potentiate their hypotensive effects. For this reason this compound may be contraindicated in patients receiving β-blockers (FDA approval history). In vitro studies have shown that vardenafil is a weak inhibitor of cytochrome P450. Common side effects are headache, flushing, dyspepsia, and rinitis [136]. No changes in vision have been reported [137, 138]. However the side effects of vardenafil improve to a dose dependent-manner over time and gradually there is an attenuation of the severity of the side effects with continued treatment [139–141].

Tadalafil is the most recently developed selective PDE5 inhibitor which was submitted to the FDA for approval on April 2002. Its molecular structure differs significantly from those of sildenafil and vardenafil. Moreover concentrations of tadalafil which inhibit effectively PDE5 have a lower inhibitory effects in PDE6 compared with the other two approved selective PDE5 inhibitors. Indeed, tadalafil has not been shown to have any visual side effects [142, 143]. In a recent study Weeks et al. [144] showed that tadalafil has a 40-fold selectivity ratio for PDE5 over PDE11A4 whereas sildenafil and vardenafil demonstrate selectivity ratios for PDE5 over PDE11A4 1 000-fold and 9 000-fold, respectively. It appears that PDE11 is inhibited by tadalafil within the therapeutic range of tadalafil [144]. The eventual adverse effects of tadalafil through the inhibition of PDE11 are not yet clearly established [145]. The back and muscle pain reported relatively often by men who receive tadalafil may be mediated through the inhibition of PDE11 [142, 146].

Several investigators have focused their effects to evaluate the pattern of expression of PDE11 in the human. The results of these studies demonstrated the presence of PDE11A4 protein in the prostate, pituitary, heart, and liver [147]. The above findings are partially in agreement with other studies evaluating the distribution of PDE11A mRNA and that of the PDE11A protein [96, 148–150]. Questions have been raised on the effect of tadalafil on testicular function, germ cell viability, and characteristics of prostatic fluid [117].

Tadalafil has a much longer half-life time than sildenafil and vardenafil achieving a period of efficacy of up to 36 h [146]. The onset of the action of tadalafil is rapid. Padma-Nathan et al. [151] have reported effects of tadalafil within a period of 20 min. Similarly with the other PDE5, selective inhibitors, tadalafil should not be administered in patients taking nitrates. Clinical trials investigating the effect of tadalafil in patients under antihypertensive treatment with angiotensin-converting enzyme inhibitors, calcium antagonists, thiazide diuretics, β-blockers, ARBs, loop diuretics, or α-blockers have revealed no statistically significant difference in blood pressure profiles between tadalafil and placebo treatment groups [152]. Headache, dyspepsia, muscle pain and back pain are the typical side effects of this medicine. Moreover adverse events like infection, nasal congestion, and spontaneous erections have also been reported in the literature [142].

8 Effects of non-selective PDE inhibitors on sperm parameters

The in vivo and in vitro influence of PDE inhibitors on the sperm parameters has been the focus of several research efforts. The stimulating effect of the PDE inhibition on sperm motility may suggest an association between the intracellular levels of cytosolic nucleotides and the sperm ability to move [22, 153]. However the majority of studies evaluating the effects of PDE inhibitors on spermatozoa employed non-selective PDE inhibitors which have been used for many years in clinical trials. Only few of the above studies have employed the selective PDE5 inhibitors sildenafil, vardenafil, or tadalafil.

Many chemical molecules have been studied aiming to stimulate human sperm functions in vivo or in vitro. These molecules include poorly defined biologic materials, (e.g., serum, peritoneal fluid, and follicular fluid) as well as defined chemical agents such as adenosine analogues, progesterone, and methylxanthines [154, 155]. Methylxanthines belong to the first generation of PDE inhibitors and represent a chemical group of drugs derived from xanthine (a purine derivative) including those among others: theophylline, caffeine, and pentoxifylline. Their beneficial effect on sperm motility has been recognised since 1970 [156–159]. Jaiswal and Majumder [160] investigating the role of theophylline demonstrated that this PDE inhibitor markedly increased (10-fold or greater) the motility of spermatozoa derived from proximal-corpus, mid-corpus, distal-corpus, and proximal-cauda epididymides. Caffeine has also been shown to increase sperm motility and metabolism when it is added to the semen [18, 161]. However this compound promotes the spontaneous sperm acrosomal reactions. This effect of
caffeine on sperm acrosome counteracts the benefits from its role as a motility stimulant [23]. Pentoxifylline (PTX), is the most widely non-selective PDE inhibitor used [154, 162–168]. Although its beneficial effect on the outcome of in vitro fertilization (IVF) trials in normozoospermic subjects and oligo-asthenozoospermic patients is well documented [169–172] the efficacy of its oral administration to increase sperm fertilizing ability is controversial [168]. PTX has been considered to stimulate flagellar motility by increasing sperm intracellular cAMP [173–176] as well as by reducing sperm intracellular superoxide anion and DNA damaging reactive oxygen species [177, 178]. The improvement of sperm fertilizing ability in vitro may be due to an effect of PTX on sperm motion characteristics and not due to an increase in the number of motile spermatozoa [179]. In particular PTX appears to increase significantly beat cross frequency, curvilinear velocity, and percentage of hyperactivated spermatozoa [18, 154, 164, 166, 180–185]. A beneficial effect of PTX on sperm-oocyte binding assay has been described [186].

It should be mentioned that PDE4 inhibitors, as well, increase sperm motility. PDE4 inhibitors do not have an obvious effect on the sperm acrosome reaction. On the other hand PDE1 inhibitors seem to selectively stimulate the acrosome reaction [22].

9 PDE5 selective inhibitors and sperm parameters: in vivo studies

In a double-blinded, four-period, two-way, crossover study encompassing 16 sexual healthy male volunteers, Purvis et al. [187] examined the effect of sildenafil on sperm motility and morphology parameters. The authors compared a 100-mg dose of sildenafil with placebo. Both sildenafil and placebo administered as single oral doses for two periods separated by a washout period of at least 5–7 days. Sildenafil and sildenafil’s metabolite concentrations were measured in a sample of semen collected 4-h post-administration and in several samples of blood collected during the first hours after sildenafil administration. The authors reported a lack of effect of sildenafil on sperm motility. In fact the authors observed no significant differences between the sildenafil group and the placebo group for the percentage of motile spermatozoa, the percentage of static spermatozoa, the percentage of rapidly moving spermatozoa, and the percentage of progressively moving spermatozoa. Mean values of sperm count, morphology, and viability, as well as seminal plasma volume and viscosity were not significantly different between the placebo group and the control group. Mean semen concentrations of sildenafil were approximately 18% of the mean plasma concentrations at 1.5 h and 4 h after the sildenafil administration. The mean sildenafil metabolite concentrations in the semen at the same periods after sildenafil administration were 5% (of the plasma concentration) and 15% (of the plasma concentration), respectively.

The above study by Purvis et al. [187] has confirmed earlier findings published by Aversa et al. [188]. The authors have conducted a prospective double-blind, placebo-controlled, crossover, two-period-investigation study, embracing 20 male subjects, which were treated with sildenafil or placebo. After a washout period of 7 days all subjects were crossed over to receive the alternative treatment. The authors found no statistically significant variations in the mean values of sperm number, sperm motility, and percentage of abnormal spermatozoa between the two groups. Evaluating the erectile function and the sexual behaviour in the two groups the authors reported that while the penile haemodynamic parameters during erection were not statistically different between the two groups, the post-ejaculatory refractory period was significantly reduced in the sildenafil group. The authors emphasized the potential usage of sildenafil in assisted reproductive programs when a temporary ED may occur due to the stress and the psychological pressure for semen production. The last suggestion has also been expressed earlier by Tur-Kaspa et al. [189] who reported his experience on the usage of sildenafil in men with proven erectile dysfunction during assisted reproductive technologies (ART) cycles. The stress and psychological pressure for semen collection becomes larger if more than one semen samples are necessary during the day of oocyte pick-up.

In contrast to this study by Aversa et al. [188] a positive effect of sildenafil on sperm kinematics was proven. In a prospective double-blind, placebo-controlled, crossover, two-period-administration, clinical investigation du Plessis et al. [190] determined the effect of in vivo sildenafil citrate administration and in vitro 8-Bromo (Br)-cGMP treatment on semen parameters and sperm function. Twenty healthy male subjects randomly were asked to ingest a single dose of 50-mg of sildenafil or placebo. All the subjects were crossed over to receive the alternative treatment after a washout period of seven days. The authors reported no significant differences in the percentage of spermatozoon with progressive motility and in the sperm track velocity, sperm amplitude of lateral head displacement, sperm beat cross frequency, sperm straightness and sperm linearity between the two groups. However borderline statistical significant differences were observed in sperm smoothed path velocity and sperm straight-line velocity. In addition there was a statistically significant increase in the percentage of rapidly moving spermatozoon after sildenafil administration. An increase in the outcome of sperm oocyte binding assay (SOBA) was found after sildenafil
administration. Similar effects on sperm kinetics were noted after 8-Br-cGMP treatment due to elevation in intracellular cGMP levels. An increase of 134% in SOBA outcome was demonstrated after 8-Br-cGMP treatment. The authors speculated that these increases in sperm ability to bind to the oocyte could possibly be explained by the fact that more spermatozoa became rapidly motile after sildenafil administration, and thus the chances for them to bind with the oocyte increase. The authors have concluded that sildenafil may increase some sperm movement parameters as well as the sperm-oocyte binding.

In an open-label pilot study Jannini et al. [191] investigated the effect of 50-mg orally administered sildenafil in a group of sexual healthy men who participated in an intruterine artificial insemination program or planned sexual intercourse to perform a post-coital test (one or two tests). They found no effect of sildenafil administration in sperm motility, in the sperm concentration, or in the total number of spermatozoa ejaculated. Similarly no effect of sildenafil administration was demonstrated in the percentage of nonlinear progressive motile spermatozoa. However, a significant increase was seen in the linear progressive motility due to sildenafil administration. In addition, the administration of sildenafil before the second postcoital test had positive effects on the sperm number and the sperm motility in the cervical mucus. The authors have recommended the administration of sildenafil prior to semen collection and performance of ART in order to reduce the stress that is experienced by the male in the ejaculation room of the infertility clinic. Similar conclusions have been raised by the same group of investigators in an earlier study [192]. However in that earlier study the authors did not demonstrate effects of sildenafil on the linear progressive sperm motility. The authors suggested that sildenafil administration has a role in the reduction of the ejaculation associated stress. According to the authors, sildenafil administration results in an ejaculation with higher sexual satisfaction and a subsequent increased number of good quality spermatozoa in the semen. The importance of the positive effects of sexual satisfaction and orgasm on the semen quality and sperm fertilizing capacity was emphasized in another study comparing masturbation with videotaped sexual images and without videotaped sexual images. Masturbation with videotaped sexual images resulted in recovery of spermatozoa of greater fertilizing potential [193]. In addition in a similar report Sofikitis and Miyagawa [194] demonstrated improved spermatozoal motility in the semen samples collected via sexual intercourse versus masturbation in fertile men. Sofikitis and Miyagawa [194] suggested that the higher the sexual stimulation is, the larger the prostatic secretory function is with an overall result of better sperm motility. In addition Sofikitis and Miyagawa [194] suggested that the higher the sexual stimulation is, the larger the vas deferens loading during ejaculation is. The latter suggestion is supported by a study showing that restraint of bulls or falls mounts before semen collection can increase the number of motile spermatozoa by as much as 50% [195]. Also in bulls, it has been suggested that oxytocin and prostaglandin F2α may be at least partly responsible for the improvement of the ejaculate after sexual stimulation [196, 197]. The effects of sildenafil on semen quality and male accessory genital gland function were the aim of a study conducted by Kanakas et al. [198]. Three semen samples were collected from each of 13 oligozoospermic infertile men without sildenafil treatment and after sildenafil treatment (same men). The authors evaluated the total sperm count, the percentage of motile spermatozoa and, the percentage of morphologically normal spermatozoa in all samples. The first, second, and third semen sample collected from each patient via each method were processed for evaluation of α-glucosidase (marker of epididymal function), fructose (marker of seminal vesicular function), and citrate (marker of prostatic secretory function), respectively. The authors found [198] that the mean values of total sperm count, percentage of motile spermatozoa and seminal plasma citrate levels were significantly larger in semen samples collected after sildenafil administration compared with semen samples collected without prior usage of sildenafil. No significant differences were demonstrated in the markers of the secretory function of seminal vesicles. The authors have suggested that the differences in the markers of prostatic secretions between the two groups of semen samples may be due to the greater sexual stimulation prior to/during ejaculation after sildenafil administration. It appears that sildenafil treatment promoted prostatic secretory function and increased loading of the vas deferens. The authors have also stated that the increase in prostatic secretory function after administration of sildenafil provides an explanation for the enhanced sperm motility. This is consistent with other reports which have demonstrated that secretory dysfunction of the male accessory genital glands due to prostatic infections impairs male fertility potential [199]. The seminal fluid [200] may contain factors that are not absolutely essential to fertilization. However, optimal concentrations of prostatic secretory markers may provide an environment ideal for sperm motility and transport [194]. Citrate, the major anion of human seminal fluid is important for maintaining the osmotic equilibrium of the prostate [201]. A zinc compound (probably a salt) is a potent antibacterial factor which is excreted from the human prostate providing for the high content of zinc in the sperm nucleus and contributes to the stability of the quaternary structure of the sperm nucleus chromatin [202]. Spermine, a substance in seminal fluid, secreted
by the prostate, is also correlated with the sperm count and motility and its concentrations in men with chronic bacterial prostatitis have been shown to be decreased [203]. Semen cholesterol content is synthesized in human prostate and is important for stabilizing the sperm membrane against temperature and environmental shock [203]. Thus enhancement of the concentrations of prostatic secretions in the seminal samples collected after sildenafil administration may explain the higher sperm motility profiles in these samples.

Few recent studies support the findings of the above investigation by Kanakas et al. [198]. Ali et al. [204] administered 100-mg sildenafil citrate in diabetic neuropathic patients. The authors found that sperm motility and semen volume were increased in men treated with sildenafil. On the other hand sperm morphology remained unaffected. In addition the authors proposed that sildenafil administration is associated with an improvement in the entire smooth musculature of the male reproductive tract which has been altered due to neuropathy. Sildenafil administration resulted in reduction in the excessive accumulation of interstitial collagen and calciumification in the smooth muscles which had resulted in bladder atonia in the diabetic men. The overall result in diabetic men was partial or total retrograde ejaculation associated with decreased sperm motility. In this study sildenafil administration improved sperm motility. On the other hand the authors noticed that long time sildenafil treatment was associated with a significant decrease in total sperm output and sperm concentration.

Pomara et al. [205] performed a prospective, double-blind, randomized, crossover study describing the acute effect of both sildenafil (50 mg) and tadalafil (20 mg) in young infertile men. Eighteen young infertile men were asked to ingest a single dose of either sildenafil or tadalafil in a blind, randomized order. Semen samples were collected one or two hours after the administration of each PDE5 inhibitor. The authors reported a significant increase in sperm progressive motility in semen samples collected after sildenafil administration compared with semen samples collected prior to sildenafil administration. The authors have suggested that the stimulatory result of sildenafil on sperm motility may be due to a direct action of sildenafil on sperm mitochondria and calcium channels. Another report demonstrated that PDE5A is localized mainly to sea urchin sperm flagella regulating intracellular cGMP levels [206]. Thus a direct effect of sildenafil on sperm flagella cannot be ruled out [206]. Interestingly, the study by Pomara et al. [205] revealed a significant decrease in the sperm motility after a single dose of tadalafil [205]. These latter findings are inconsistent with an earlier study conducted by Hellstrom and colleagues [142] who investigated the effects of tadalafil on semen characteristics and serum concentrations of reproductive hormones of healthy men and men with mild erectile dysfunction. Hellstrom et al. [142] performed two randomized, double-blind, placebo controlled, parallel group studies (one study for a 10-mg dose tadalafil and one study for a 20-mg dose tadalafil) enrolling 204 subjects in the 10-mg tadalafil study and 217 subjects in the 20-mg tadalafil study. The investigators assessed the effect of daily tadalafil or placebo administration for six months on semen samples and serum levels of reproductive hormones (testosterone, LH and follicle-stimulating hormone). The investigators demonstrated that in each study the proportion of participants with a 50% or greater decrease in sperm concentration was relatively small and similar for the placebo group and the 10 mg-tadalafil group or the 20-mg tadalafil group. Similarly there were no significant alterations in sperm morphology or sperm motility after treatment with 10 mg or 20 mg tadalafil. The authors demonstrated that there were no significant alterations in the semen levels of reproductive hormones after tadalafil administration concluding that administration of tadalafil at doses of 10 mg and 20 mg for 6 months did not adversely affect testicular spermatogenesis process or serum levels of reproductive hormones. However other investigators emphasise their dilemma concerning the administration of tadalafil on a daily basis, as they believe that up today the available data confirming the safety of tadalafil administered on a daily basis are not yet sufficient, particularly in high-risk patients [205, 207].

Bauer et al. [208] performed a randomized, placebo control, double-blind, crossover study to determine the effects of a single dose of vardenafil 20 mg on indices of testicular function. Sixteen healthy males participated in this study. The scientists found no statistically significant effects of tadalafil on sperm motility, sperm concentration, sperm viability, and sperm morphology.

In another study, Grammenitakis et al. [209] evaluated the effects of vardenafil administration (10 mg) on male accessory genital gland function. Vardenafil administration increased the secretory function of prostate. In contrast, there were no effects of vardenafil administration on the secretory function of seminal vesicles and epididymis. In addition, the investigators noted that semen samples from infertile men treated with 10 mg of vardenafil in a daily basis for at least 45 days presented a significantly larger total number of spermatozoa, quantitative sperm motility, qualitative sperm motility, percentage of morphologically normal spermatozoa, semen citrate concentration, and semen acid phosphatase concentration compared with semen samples from the same individuals collected prior to vardenafil administration. The authors suggested that vardenafil stimulated the prostatic secretory function increasing the quantitative and qualitative motility of spermatozoa. Moreover the en-
hanced sexual satisfanction during ejaculation due to vardenafil administration has been thought to be the reason for an increased loading of the vas deferens and the subsequent significant increase in the total number of spermatozoa per ejaculate. The significant increase in the percentage of morphologically normal spermatozoa may be attributable to the enhancement of prostatic secretory function due to vardenafil administration. Since it is known that optimal prostatic secretory function regulates the osmotic equilibrium of the seminal plasma decreasing the percentage of spermatozoa that undergo osmotic shock and morphological abnormalities.

10 PDE5 selective inhibitors and sperm parameters: in vitro studies

After the introduction of sildenafil in the market, several studies have evaluated the in vitro effects of this compound on sperm parameters. Burger et al. [210] in an ex vivo study investigated the effect of sildenafil on the motility, viability, membrane integrity, and functional capacity of human spermatozoa. The above spermatozoal parameters were evaluated on the spermatozoa of both healthy donors (n = 6) and clinically infertile men (n = 6). Separate aliquots were incubated for 0 h, 1 h and 3 h in the absence or presence of sildenafil (125 ng/mL, 250 ng/mL, and 750 ng/mL), PTX (as a positive control), or Ham’s medium (as a reagent control). The authors have reported no statistically significant effect of sildenafil on sperm viability, sperm motility, and sperm forward progression after incubation of spermatozoa with various doses of sildenafil. However, the authors noted a marked decrease of sperm membrane integrity in spermatozoa of infertile patients treated with sildenafil. This should be taken into consideration when treatment with sildenafil is planned in subfertile couples with a male factor infertility. Finally, in this study, sperm penetration assay data suggested that there is neither a beneficial nor a detrimental effect of sildenafil on its outcome.

Similarly in another study the group of Andrade and colleagues [33] attempted to evaluate a direct effect of sildenafil and phentolamine on sperm motility. Using samples of either unwashed or washed spermatozoa of 10 men, the investigators added directly to the samples sildenafil at a concentration of 20 mg/mL or phentolamine in various doses and incubated the samples for 10 and 30 min. The authors demonstrated a dose-related inhibition of sperm motility in sperm samples treated with phentolamine whereas sildenafil (at a concentration of 200 µg/mL) did not adversely affect sperm motility either in unwashed or washed sperm. In contrast the highest dose of sildenafil (2,000 µg/mL) reduced the sperm motility approximately 50%. However, it should be emphasized that at this concentration sildenafil caused a marked acidification of the medium which may be the reason for the reduced sperm motility [211]. Thus a direct effect of a high dose of sildenafil on sperm motility cannot be strongly supported.

In an experimental study Su and Vacquier [212] determined the motility, chemotaxis, and the acrosome reaction of sea urchin sperm. By cloning and characterizing a sea urchin sperm PDE (suPDE5) which is an ortholog of human PDE5 the authors found that phosphosuPDE5 localizes mainly on sperm flagella and the PDE5 phosphorylation increases when spermatozoa contact the jelly layer that surrounds the eggs. Since the in vitro dephosphorylation of suPDE5 decreased its activity the authors suggested that PDE5 inhibitors such as sildenafil affect sperm motility on sperm motility may inhibit the activity of suPDE5 and increase sperm motility.

A concentration-dependent stimulatory effect of sildenafil on sperm motility was also demonstrated recently by Mostafa [213] when 85 semen specimens from asthenozoospermic patients were exposed to different five concentrations of sildenafil (4.0 mg/mL, 2.0 mg/mL, 1.0 mg/mL, 0.5 mg/mL, 0.1 mg/mL). However, the evaluation of sperm motility in this study was only 3 hours after the spermatozoa exposure to the medicine.

Lefèvre et al. [214] investigated whether PDE5 is present in human spermatozoa and whether sildenafil affects sperm function. The authors showed that this PDE5 inhibitor stimulates human sperm motility with an increase in intracellular cAMP suggesting an inhibitory action on a PDE that is different to PDE5. The authors attempted to inhibit the enzyme activity in washed spermatozoa using increasing concentrations of sildenafil or dipyridamole. The latter substances are selective inhibitors of cGMP-specific PDE5. Both compounds suspended the enzyme activity successfully. However sildenafil exhibited an inhibition potential that was four times higher. In semen samples incubated with increasing concentrations of sildenafil, the authors noted a dose-dependent increase in intracellular cAMP levels. Sildenafil at 30 µmol/L, 100 µmol/L, and 200 µmol/L triggered capacitation of washed spermatozoa. Capacitated spermatozoa underwent an acrosome reaction when challenged with lysophosphatidylcholine (LPC) alone or LPC plus PDE inhibitors. However capacitated spermatozoa did not undergo acrosomal reaction when they were challenged with sildenafil or another PDE inhibitor alone. The investigators have suggested that sildenafil might act on PDEs other than type 5. They have also suggested that sildenafil in high concentrations as high as 30, 100 and 200 µmol/L acts no longer as type-5 specific and probably partially inhibits other PDEs present in spermatozoa such as PDE1 and PDE4 which have high affinity for cAMP [215]. This may explain the intracellular increase in cAMP in spermatozoa incubated with high doses.
of sildenafil.

In another study conducted by Cuadra and colleagues [216] the effect of sildenafil on sperm motility and on acrosomal reaction was determined. Spermatozoa were exposed to sildenafil at either 0 nmol/L, 0.4 nmol/L, 4.0 nmol/L, or 40 nmol/L, simulating in this way the post-administration concentrations of sildenafil in the semen and plasma. The scientists observed increased sperm motility parameters in the presence of 0.4 nmol/L sildenafil compared with the control sample four hours after the exposure to sildenafil. However, the motility parameters decreased 48 h after the exposure to sildenafil. Spermatozoa exposed to higher concentration of sildenafil (40 mol/L) showed decreased motility parameters.

In this study, sildenafil affected the sperm acrosome reaction with an increase of almost 50% compared to the control samples. It is known that cGMP directly opens cyclic nucleotide-gated channels for calcium entry into the spermatozoa, initiating the acrosome reaction. In the same way cGMP regulates calcium entry into microdomains along the sperm flagellum affecting sperm motility. Since PDE5 hydrolyzes cGMP, inhibition of PDE5 by sildenafil citrate enhances the effects of cGMP on sperm motility and sperm acrosome reaction. The data provided by the authors suggest a dual mechanism for PDE5 inhibition with a stimulatory effect on sperm motility when PDE5 is moderately inhibited; however, extensive inhibition of PDE5 leads to decreased sperm motility.

Another group of researchers [217] attempted to determine the influence of sildenafil on sperm motility or acrosome reaction. Semen samples from fifty-seven unselected men with asthenozoospermic profiles were prepared and then exposed to 0.67 μmol/L of sildenafil which is equivalent to the plasma concentration of sildenafil, one hour after oral ingestion of 100 mg of sildenafil. The authors found that both the number and the velocity of progressively motile spermatozoa were significantly increased. They also noticed that sildenafil caused a significant increase in the proportion of acrosome-reacted spermatozoa suggesting that sildenafil may adversely affect male fertility. The scientists suggested that the raised levels of cGMP as a result of the inhibitory effect of sildenafil affect many sperm functions such as calcium transport into spermatozoa. Altered levels of intracellular calcium may potentially affect sperm motion and an energy-dependent influx of calcium into the sperm cell which may be responsible for initiation of the acrosome reaction. In a case report of sildenafil administration for semen collection for human-assisted reproduction the investigators failed to fertilize oocytes despite the intracytoplasmic injection of the sperm [189]. Although this fertilization failure was attributed to the advanced age of oocytes due to the delay in obtaining the semen sample, a deleterious effect of sildenafil on sperm function can not be excluded.

The effects of tadalafil on human sperm motility in vitro have been investigated. Mostafa [218] assessed the ability of tadalafil on human sperm motility in 70 asthenozoospermic semen specimens. The semen samples were exposed to three different concentrations of tadalafil (4.0, 1.0, 0.5 mg/mL) and it was found sperm samples treated with 4 mg/mL tadalafil solution demonstrated a significant decrease in sperm motility compared with the controls samples whereas sperm samples treated with 1.0 or 0.5 mg/mL tadalafil solution demonstrated a significant increase in sperm progressive forward motility. The authors suggested that the concentration of tadalafil plays an important role in the degree of sperm enhancement. The normal mammalian sperm motility seems to be governed predominantly by the cAMP/PKA pathway and calcium signalling pathway, whereas mechanisms involving heterotrimeric and small G-protein have also been entailed the regulation of sperm motility [19, 219, 220]. It should be emphasized that cAMP may also act through PKA independent pathways. In fact, Burton et al. [221] speculated that cAMP may activate a cyclic nucleotide-gated ion channel in spermatozoa and/or cAMP-mediated guanine nucleotide exchange factors in testes, providing these ways as alternative pathways for the PKA-mediated regulation of flagellar motility. The dual effect of in vitro usage of tadalafil on sperm motility in regard of its concentration in the semen could be explained by one or more of these pathways.

Alternatively, the effect of tadalafil on sperm motility may be related also to the inhibitory effect of this compound on PDE11. In fact, PDE11 is highly expressed in the testis, prostate, and developing spermatozoa even if its physiological role is not known. Wayman et al. [222] in an effort to investigate the role of PDE11 in spermatozoa physiology, retrieved spermatozoa from PDE11 knockout mice (PDE11−/−). The authors found a reduced sperm concentration, decreased forward motility, and lower percentage of alive spermatozoa. In addition spermatozoa from PDE11−/− animals demonstrated increased premature/spontaneous capacitance. These data suggest a role for PDE11 in spermatogenesis and fertilization potential.

Fisch et al. [22] showed that PDE4 inhibitors enhanced in vitro sperm motility over controls without affecting the acrosome reaction. On the other hand PDE inhibitors selectively stimulated the acrosome reaction.

Gathering the results of the above ex vivo studies we may discern a dose dependent effect of sildenafil and tadalafil on sperm motility. In fact this effect seems to be enhanced at low doses but it may be reduced at high concentrations. Moreover sildenafil appears to exhibit a stimulatory effect on sperm capacitation, acrosome
reaction, and sperm-oocyte binding. Doubtless, further investigations are required to evaluate the mechanisms of the effects of PDE5 selective inhibitors on sperm motility and sperm fertilization capacity. To elucidate whether or not PDE inhibitors one day will be used as an adjunct tool for male infertility treatment, more studies are necessary.

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