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The Role of PDE5 Inhibitors in the Treatment of Testicular Dysfunction

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1. Introduction

The cyclic nucleotide phosphodiesterases (PDEs) play the predominant role in the degradation of the cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). The PDEs function in conjunction with adenylate cyclase (AC) and guanylate cyclase (GC) to regulate the amplitude and duration of intracellular signaling mechanisms (mediated via cAMP and cGMP, respectively). Detailed sequence analyses suggest that there are at least 11 different families of mammalian PDEs. Most of the PDEs families include more than one gene product (Bender & Beavo, 2006). In addition, many of these genes can be alternatively spliced in a tissue specific manner. The overall result is the production of different mRNAs and proteins with altered regulatory properties or subcellular localization. This multiplicity of PDE proteins allows organ- and cell type-specific expression and even a specific intracellular localization of PDEs in the vicinity of various protein effectors inducing fine-tuning of compartmentalized regulation for cAMP and cGMP (Zaccolo & Pozzan, 2002; Bender & Beavo, 2006). For example, cGMP-hydrolyzing PDE5 is highly expressed in smooth muscle cells of the corpus cavernosum penis and vascular smooth muscle cells of the lung allowing the employment of PDE5 inhibitors for the pharmaceutical management of erectile dysfunction (Lue, 2000) and pulmonary hypertension (Ghofrani et al., 2002; Ghofrani et al., 2002; Ghofrani et al., 2003; Schermuly et al., 2004; Galie et al., 2005), respectively.

Phosphodiesterases, which hydrolyze cGMP or cAMP, play a major role in cell signaling by affecting the duration of cyclic nucleotide action (Bender & Beavo, 2006). Phosphodiesterases including PDE1A, PDE1B, PDE5, PDE6, PDE9 and PDE10 preferentially hydrolyze cGMP (Bender & Beavo, 2006). In contrast, PDE1C, PDE2 and PDE11 are dual

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substrate-specific PDEs. In addition, PDE2, PDE5, PDE6 and PDE10 are cGMP-regulated by allosteric binding sites. On the other hand PDE3 represents a cGMP-inhibited PDE (Bender & Beavo, 2006). Exact analyses of tissue- and cell type-specific distribution of PDE gene families in the testis and epididymis are still missing. Published data on PDE expression in testis until now is primarily restricted to cAMP-hydrolyzing PDEs, such as PDE1C, PDE4A, PDE4C, PDE7B and PDE8A and their localization to male germ cells and spermatozoa (Bender & Beavo, 2006). With respect to cGMP pathways, transcripts of the cGMP-hydrolyzing PDE10 have been found in the human testis (Fujishige et al., 1999) and cGMP-hydrolyzing PDE5 has been recently localized to peritubular myoid cells of the rat (Scipioni et al., 2005).

2. Main heading expressions of PDEs in the male reproductive system

Till now, extra-vascular contractile cells of the male reproductive organs have been disregarded. In the testis and epididymis, smooth muscle cells and/or myofibroblasts, are known to surround the epithelium of the seminiferous tubules, the efferent ducts, and the epididymal duct (Setchell et al., 1994). These contractile cells are involved in the transport of immotile spermatozoa from the testis to the cauda epididymis, which ensures sperm maturation, (i.e. spermatozoa acquire their fertilization ability during this epididymal passage) (Setchell et al., 1994). In addition to its role in spermatozoal transport, the peritubular lamina propria of the human testis also affects spermatogenesis. In many cases of idiopathic male infertility the lamina propria is thickened with a reduction of contractile elements and an increase of extracellular components, resembling fibrotic changes in other organs (Davidoff et al., 1990). Moreover, the occurrence of segments of seminiferous tubules displaying pathological dilatations and a thin-walled lamina propria leads in impaired spermatogenesis. Most recently, the physiological significance of peritubular cells for spermatogenesis has been shown in tissue-selective knock-out mice with the androgen receptor gene deleted in peritubular myoid cells resulting in decreased total male germ cell number and oligozoospermia (Zhang et al., 2006).

Detailed analyses of tissue- and cell type-specific distribution of PDE gene families in the testis 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 (Table 1). Additionally it has been shown that PDE3 contributes to the regulation of epididymal contractility (Mewe et al., 2006). At least four genes encoding different isoforms of PDEs are differentially expressed in somatic and germ cells of the testis (Swinnen et al., 1989).

Several studies aiming to the localization of PDE in rat seminiferous tubules indicated that PDE1 and PDE2 are predominantly expressed in germ cells, whereas PDE3 and PDE4 are mainly restricted to Sertoli cells (Geremia et al., 1982; Morena et al., 1995). Three rat PDE3 mRNAs with divergent 5′ untranslated regions are present in Sertoli cells (Sette et al., 1994). The PDE of Sertoli cells may be a testis-specific enzyme because its cDNA only hybridizes to a 3.2-kb mRNA in Sertoli cells and in testicular RNA (Geremia et al., 1982; Swinnen et al., 1989).

Transcripts of the PDE10A has been demonstrated to be present in the human testis (Fujishige et al., 1999). cGMP-hydrolyzing-PDE5 has been localized in peritubular myoid cells of the rat (Scipioni et al., 2005). In another study by Coskran et al., (2006) PDE10A-immunoreactivity was detected in seminiferous tubules and epididymal spermatozoa of

dog and cynomolgus macaque and in the testis of mouse, with no detectable immunoreactivity in testes or epididymes of rat or human (Coskran et al., 2006). Dog testis presented moderate specific PDE10A-immunoreactivity in round and elongated spermatids and to a lesser degree in the more mature spermatocytes (Coskran et al., 2006). In the cynomolgus macaque, testicular and epididymal spermatozoa exhibited the same pattern of PDE10A-immunoreactivity seen in the dog (Coskran et al., 2006). PDE10A-immunoreactivity in the mouse testis was similar to that observed in the cynomolgus macaque but was absent in mouse epididymal spermatozoa (Coskran et al., 2006). In the epididymes of dog and cynomolgus macaque, spermatozoal midsections, their tails, and distal cytoplasmic droplets were positive for PDE10A-immunoreactivity (Coskran et al., 2006). In the head of the epididymis spermatozoa contained PDE10A in their tails and distal droplets, whereas more mature spermatozoa in the tail of the epididymis expressed PDE10A only in the distal droplets (Coskran et al., 2006). Moreover, in other studies, PDE10A mRNA has been reported in the testis and in spermatozoa of humans and rats (Soderling et al., 1998; Fujishige et al., 1999; Fujishige et al., 1999; Baxendale & Fraser, 2005). The role of PDE10A in the spermatogenesis process is not fully elucidated. However, intact reproductive function in PDE10A knockout mice suggests that PDE10A is not essential for sperm fertilizing capacity (Siuciak et al., 2006).

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 (Francis, 2005). In fact the ejaculated spermatozoa from a knockout mouse model for PDE11 (PDE11-/-) displayed diminished sperm concentration, reduced rate of forward progression, and decreased percentage of a live spermatozoa (Wayman et al., 2005). Moreover, spermatozoa from the male reproductive tract from the same knockout animal model displayed enhanced premature/spontaneous capacitance (Wayman et al., 2005). The above data suggest a role for PDE11 in spermatogenesis and fertilization potential.

3. New horizons for PDE5 inhibitors

Male infertility represents not only a private but also a social problem. Although assisted reproductive technology (ART) represents a popular mode of therapeutic management of couple's infertility due to male factor, a significant subpopulation of infertile men remains childless after employment of intrauterine insemination, in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) techniques. An additional problem is that there are no widely accepted pharmaceutical agents effective for the treatment of the infertile male. Therefore the limitations of the success rate (i.e. live birth rate) of ART and the lack of effective pharmaceutical agents for the treatment of the infertile male represent the two main difficulties to solve the problem of male infertility.

Thus it appears that development of novel pharmaceutical agents with a positive impact on the alleviation of male infertility is of paramount importance. Recently studies have been published tending to justify a role for PDE5 inhibitors as an adjunct tool for male infertility therapeutic management. This review study aims to discuss and comment on the findings of all of the previous studies concerning the effect of PDE5 inhibitors on the male reproductive potential.

4. PDEs and hydrolysis of c-GMP in Leydig cells, Sertoli cells and peritubular cells

4.1 Hydrolysis of c-GMP in tunica albuginea, lamina propria, peritubular cells, and myofibroblasts

In male, the tunica albuginea has been shown to contain abundantly contractile elements (smooth muscle cells and myofibroblasts) as revealed by electron microscopy and immunohistochemical approaches (Middendorff et al., 2002). This tissue is characterized by extraordinarily high concentrations of cGMP-binding proteins including PKG I (Middendorff et al., 2002). In in vitro studies, atrial natriuretic peptide (ANP) and the nitric oxide (NO) donor sodium nitroprusside (SNP) have increased cGMP production in isolated strips of the tunica albuginea, and the underlying mechanism has been demonstrated (Middendorff et al., 2002). In fact, two cGMP-generating enzymes namely GC-A (the receptor for ANP) and soluble guanylate cyclase (sGC), could be identified in the tunica (Middendorff et al., 2002). Both the above enzymes have been found to be functionally active because ANP and SNP strongly enhance the production of cGMP in isolated pieces of the tunica (Middendorff et al., 2002). Contractile cells as well as Leydig cells located in the inner zone of the tunica have been identified as sites of NO synthase expression (Middendorff et al., 2002). Physiological studies have demonstrated spontaneous tunica contractions exclusively in regions close to the rete testis. These contractions could be attenuated by cGMP, SNP and ANP. Noradrenaline-induced contractions, detectable in all parts of the testicular capsule, could be abolished completely by SNP (Middendorff et al., 2002). These data, demonstrating complex contraction and relaxation activities, are indicative of a major physiological role of guanylate cyclase/cGMP pathway in the regulation of tunica albuginea function related to testicular sperm transport (Middendorff et al., 2002). In addition to its role in the tunica albuginea, ANP was found to affect spermatogenesis during postnatal development (Muller et al., 2004). In fact, a male germ specific cGK-anchoring protein, designed as GKAP42 (Yuasa et al., 2000) is expressed in a stage-dependent manner in spermatocytes and round spermatids. The latter protein interacts specifically with PKG Iα regulating germ cell development (Yuasa et al., 2000).

Moreover, recent studies demonstrated that GC-A can act as a regulator of cell size (Kishimoto et al., 2001) and proliferation (Lelievre et al., 2001). Thus growth regulation appears to represent an important aspect of the natriuretic peptide system (Silberbach & Roberts, 2001; Cameron & Ellmers, 2003). Considering that human spermatozoa can respond in a cGMP-dependent manner to ANP (Anderson et al., 1995) and that this peptide is capable of inducing acrosome reactions in spermatozoa (Rotem et al., 1998), there may be a probability for a role of GC-A in the regulation of the spermatozoal function (Muller et al., 2004).

The membrane-bound GC (GC-B) was found in Leydig cells as well as in the peritubular lamina propria (Middendorff et al., 2000) and in the tunica albuginea (Middendorff et al., 2002). The ligand of GC-B, C-type natriuretic peptide (CNP), is exclusively produced by Leydig cells (Middendorff et al., 1996) suggesting paracrine effects in contractile cells.

Middendorff et al., (2000) provided further data about the activation of sGC by carbon monoxide (CO) in the seminiferous tubules of the human testis (Middendorff et al., 2000). The CO-producing enzyme heme oxygenase-1 (HO-1) has been shown to be expressed in

the adluminal compartments of Sertoli cells. Activation of sGC by HO-1-produced CO has resulted in increased cGMP levels (Middendorff et al., 2000). Since sGC has been found to be present in peritubular myofibroblasts, production of CO by HO-1 in the adluminal parts of Sertoli cells may trigger cGMP accumulation in peritubular lamina propria cells suggesting separation of the site of production and action of CO thereby mediating relaxation of myofibroblasts (Middendorff et al., 1997).

Scipioni et al., (2005) have reported that there is expression of PDE5 in Leydig cells and peritubular cells both in prepuberal and in adult rat testis. This gives additional strength to the idea that in mammalian testis cGMP-mediated processes influence not only the vessel dilatation but also the testosterone synthesis by Leydig cells and the transfer of spermatozoa, through the relaxation of peritubular lamina propria cells (Scipioni et al., 2005). In peritubular myoid cells, in particular, besides regulating contractility, cGMP modulation by PDE5 might affect the secretion of a number of substances, including extracellular matrix components (fibronectin, type I and IV collagens, proteoglycans) and growth factors (PModS, TGF beta, IGF-I, activin-A). The latter substances are known to affect the Sertoli cell function and to play a role in retinol processing (Maekawa et al., 1996). Retinol processing is essential for maintenance of the germinal epithelium and the process of spermatogenesis (Eskild & Hansson, 1994). Finally, the demonstration of PDE5 expression in both mature and differentiating rat peritubular cells (Scipioni et al., 2005) allows to suggest that in mammals this enzyme might play key roles in the acquisition and maintenance of the myoid cell contractile phenotype and more generally in the testis maturation.

4.2 Hydrolysis of cGMP and cAMP in Leydig cells

A few studies have suggested that administration of PDE5 inhibitors and in particular sildenafil and vardenafil in infertile men has either positive effects or lack of effects on the standard parameters of semen analysis (Dimitriadis et al., 2010). Leydig cells express PDE5 (Scipioni et al., 2005). Dimitriadis et al., (2010) investigated the effects of PDE5 inhibitors on Leydig cellular secretory function and on the standard parameters of semen analysis. The authors evaluated peripheral serum levels of Insl3 in patients with oligoasthenospermia prior to and after PDE5 administration. They reported that either sildenafil or vardenafil enhanced Leydig cell secretory function since serum Insl3 profiles were significantly larger after PDE5 inhibitor administration compared with Insl3 levels prior to administration of PDE5 inhibitors. Moreover the investigators have suggested that an increase in Leydig cell secretory function may contribute to the improvement in sperm parameters observed after administration of PDE5 inhibitors. An enhanced Leydig cell secretory function may result in a more optimal biochemical microenvironment within the seminiferous tubuli of oligoasthenospermic men stimulating spermatogenesis. In addition, an enhanced Leydig cell secretory function increasing probably intraepididymal testosterone concentrations may improve the epididymal sperm maturation process in oligoasthenospermic men increasing sperm motility (Dimitriadis et al., 2010). Additionally, it has been demonstrated that cAMP is the major intracellular messenger for LH action on steroidogenesis and that most, if not all, of the signaling action of cAMP is due to cAMP-dependent protein kinase (PKA)-mediated effects on proteins regulating the steroid biosynthetic pathway (Saez, 1994; Stocco et al., 2005). Substantial evidence for the regulatory function of PDEs in Leydig cells has been reported. A small stimulatory effect of non-selective PDE inhibitors on testosterone release by primary Leydig cells has been reported (Catt & Dufau, 1973; Mendelson et al., 1975). These observations have indicated that one or more PDEs additional to PDE5 might

be active in Leydig cells to modulate the intensity, duration, and perhaps the desensitization of the LH-stimulated hormonal response (Vasta et al., 2006). Moreover, Vasta et al., (2006) have reported that PDE8A is expressed in mouse Leydig cells and have provided evidence that it is one of the PDEs that participates in the regulation of steroid production (Vasta et al., 2006). Although some biochemical, pharmacological, and genetic characteristics of PDE8A have been elucidated, its biological functions still remain largely unknown (Fisher et al., 1998; Soderling et al., 1998). Northern analysis has shown that PDE8A mRNA is highly expressed in testis (Soderling et al., 1998). The expression of PDE8A protein has been also reported for human CD4 T cells (Glavas et al., 2001) and mouse spermatozoa (Baxendale & Fraser, 2005). Vasta et al., (2006) have suggested that this enzyme plays an important role in setting the sensitivity to LH for testosterone production (Vasta et al., 2006).

4.3 Hydrolysis of cGMP in Sertoli cells

PDE5 inhibitors may also have a beneficial effect on Sertoli cell secretory function. Dimitriadis et al., (2011) evaluated the effect of vardenafil on Sertoli cell secretory function in obstructed azoospermic men and in non-obstructed azoospermic men who had had previously negative results in one or more trials of assisted reproduction technology (using frozen/thawed spermatozoa aspirated from the tail of the epididymis). The investigators showed that within the group of the obstructed azoospermic men, androgen-binding protein activity profiles (i.e., a marker of Sertoli cell secretory function) in the testicular cytosols, head epididymal-fluid samples, body epididymal-fluid samples and tail epididymal-fluid samples were significantly larger after vardenafil treatment than prior to vardenafil treatment. Likewise, within the group of the non-obstructed azoospermic men, androgen-binding protein activity profiles in testicular cytosols, epididymal head- fluid samples, epididymal body- fluid samples, and epididymal tail- fluid samples were significantly larger after vardenafil treatment than prior to vardenafil administration. Hence it appears that vardenafil administration enhances Sertoli cell secretory function both in obstructed azoospermic men and non-obstructed azoospermic men. Considering that PDE5 has not been demonstrated in Sertoli cells of several evaluated species, the increase of Sertoli cell secretory function after PDE5 inhibitors may be attributed to a positive action of inhibitors on Leydig cell secretory function or peritubular cells that subsequently enhance Sertoli cell secretory function.

Scipioni et al., (2005) could not detect any PDE5 expression in Sertoli cells in their experiments (Scipioni et al., 2005) suggesting that different PDEs are likely to contribute to the cGMP metabolism. Indeed, in Sertoli cells cGMP hydrolysis has been shown to occur in a calcium-calmodulin stimulated manner (Conti et al., 1982; Rossi et al., 1985; Conti et al., 1995) which identifies this hydrolytic activity as a result of a PDE1 family member. Enzymes of this family, though active towards both cAMP and cGMP, display a substantially higher affinity to the latter nucleotide (Zhao et al., 1997). PDE3 and PDE4 have been localized to Sertoli cells (Geremia et al., 1982; Morena et al., 1995).

5. Hydrolysis of cGMP and effects of PDE5 inhibitors on male accessory genital glands

5.1 Epididymis

Disturbances of the contractile activity in the epididymis have not yet been described (Ricker, 1998; Mewe et al., 2006). Concerning the contractility of the vas deferens, male

infertility has already been described in a mouse model with disturbances in the contractile activity of the vas deferens (Mulryan et al., 2000).

The regulatory mechanisms responsible for the contraction and relaxation processes and thus for the transport of immotile spermatozoa through the epididymis are poorly understood. It has been shown that the messenger molecule cGMP, known to elicit smooth muscle relaxation, plays a crucial role in the regulation of contractility of the epididymal duct (Mewe et al., 2006). As it can be extrapolated from contractility studies and analyses of GC-B-knockouts (Tamura et al., 2004), differentiated cGMP-dependent relaxation processes appear to be fundamental to enable the transport and maturation of spermatozoa in the epididymis. As we have previously mentioned the second messenger cGMP can be generated in the male reproductive tract by different pathways involving either soluble (cytosolic) or particulate (plasma membrane-localized) GC activities (Lucas et al., 2000).

Mewe et al., (2006) using muscular tension recording and patch-clamp techniques analyzed the mechanisms underlying spontaneous phasic contractions (SCs) of the bovine epididymal duct (Glover & Nicander, 1971). SCs were demonstrated in the caput, the corpus and the proximal cauda region and found to be predominantly myogenic in origin. Removal of the luminal fluid induced a burst-like contraction pattern, and removal of the epithelium resulted in a complete loss of SC. Influx of extracellular Ca^{2+} through 'L-type' Ca^{2+} channels, but not Ca^{2+} release from intracellular stores was shown to be crucial for maintaining SCs (Mewe et al., 2006).

The functional role of cGMP-signaling in modulating SC in the bovine epididymal caput and corpus region has been examined by muscular tension recording, and immunological and autoradiographic techniques (Mewe et al., 2006). The cGMP-analogue 8-Br-cGMP, the NO donor SNP and the natriuretic peptides ANP and CNP have displayed distally increasing SC-relaxant effects (Mewe et al., 2006). Consistently a distally increasing epididymal expression of endothelial nitric oxide synthase (eNOS), GC-A, and PKG I has been found. Immunoreactivity for eNOS, sGC and PKG I is localized to the epididymal muscular cells (Mewe et al., 2006). The SC-relevant action of NO, ANP, and CNP is cGMP-dependent, and the action of 8-Br-cGMP, in turn, is modified by epithelial and luminal factors. The nitric oxide synthase (NOS) inhibitor L-NAME causes an increase in SC frequency. The SC-regulatory effect of 8-Br-cGMP was clearly reduced by the PKG inhibitor Rp-8-Br-cGMPS as well as by iberiotoxin, thapsigargin and indomethacin, pointing to PKG as main SC-relevant target of cGMP, and suggesting that large-conductance calcium-activated $K^{(+)}$ channels, the sarcoplasmic-endoplasmic reticulum Ca^{+}-ATPase, and cyclooxygenase-1 are possible targets of PKG (Mewe et al., 2006). These data support an essential role of cGMP signaling in the control of epididymal peristalsis allowing sperm transport and maturation (Mewe et al., 2006). Moreover, in the same study it has been suggested that phosphodiesterases also play a role in the epididymal peristalsis (Mewe et al., 2006) since as contraction-relevant target of cGMP in smooth muscle, the cGMP-inhibited cAMP-degrading PDE3 has been proposed (Lucas et al., 2000). Mewe et al., (2006) have found an SC-modulatory effect of the specific inhibitor of this cGMP-dependent PDE, milrinone, in the corpus of epididymis (Mewe et al., 2006).

On the other hand, Dimitriadis et al., (2011) evaluated the effects of the PDE5 inhibitor vardenafil administration (10 mg daily for at least 45 days) on male epididymal secretory function. Vardenafil has not influenced the secretory function of epididymis (Dimitriadis et

al., 2011). Additionally, Dimitriadis et al., (2011) investigated the effects of sildenafil (50mg) on epididymal secretory function (Dimitriadis et al., 2011). A group of oligozoospermic infertile men provided three semen samples prior to sildenafil administration and three semen samples after sildenafil administration (50mg). The investigators evaluated the semen levels of α-glucosidase as a marker of the epididymal secretory function prior to and after sildenafil treatment and found absence of significant difference (Dimitriadis et al., 2011).

5.2 Vas deferens

Anatomical and physiological findings suggest a role for the nitric oxide/cGMP (NO/cGMP) phosphodiesterase signaling pathway in the modulation of ejaculation. Several studies have suggested that sildenafil could act beneficially in the alleviation of premature ejaculation (Abdel-Hamid, 2004). Chen et al., (2003) have shown that in patients with premature ejaculation, sildenafil plus paroxetine have a significantly higher therapeutic success rate (98%) than paroxetine alone (42%) (Chen et al., 2003). The inhibitory effect of sildenafil on PDE5, the destructive enzyme of cGMP, increases the level of cGMP in the vas deferens muscular fibers achieving the relaxation of the smooth muscle cells in vas deferens. This prolongs the time necessary for the achievement of ejaculation.

Studies with human erythrocytes have shown that the organic anionic pump which transports cGMP to extracellular area is dependent on a) ATP, b) its hydrolysis, and on c) the stimulation of ATPase activity by the cGMP itself (Sundkvist et al., 2002). PDE inhibitors block this transport. Sildenafil inhibiting PDE5 increases cGMP in the vas deferens muscular fibers and additionally blocks the activity of this organic anion pump. The overall result is an increase in the intracellular cGMP by a) prevention of the destruction of cGMP and b) inhibition of cGMP extracellular export (Sundkvist et al., 2002).

It has been suggested that sildenafil activates the opening of prejunctional potassium channels to reduce adrenergic neurotransmission by using NO-independent mechanisms (Medina et al., 2000). If this is true, this sildenafil induced reduction in adrenergic neurotransmission in smooth muscular fibers may alter the adrenergic tone in vas deferens muscular fiber and may reduce its pattern of contraction. Studies have demonstrated the expression of PDE5 in vas deferens (Bilge et al., 2005).

5.3 Seminal vesicles

Dimitriadis et al., (2008) reported the effects of sildenafil on seminal vesicular function. Three semen samples were collected from a group of 13 oligozoospermic infertile men prior to and after sildenafil treatment (50 mg). The authors evaluated seminal fructose as a marker of the seminal vesicular function and they found no significant difference (Dimitriadis et al., 2008) in seminal fructose profiles between samples collected prior to sildenafil and after sildenafil therapy.

In addition the effects of another PDE5 inhibitor namely vardenafil on seminal vesicular function has been evaluated (Dimitriadis et al., 2008). Eighteen infertile men participating in an assisted reproduction program were treated daily with vardenafil (10 mg every day) for at least 45 days. All these men had previously undergone at least one in vitro fertilization trial without success. Prior to and after the vardenafil-treatment six semen samples from each man were collected. The investigators showed that administration of vardenafil

resulted in no significant difference in the secretory function of seminal vesicles (as measured by the fructose concentration in the seminal plasma) (Dimitriadis et al., 2008). This finding is consistent with the observation that there are no reports in the literature supporting the presence of PDE5 in seminal vesicles.

5.4 Prostate

Citrate, the major anion of human seminal fluid is important for maintaining the osmotic equilibrium of the seminal plasma and is secreted by the prostate (Ponchietti et al., 1984). 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 (Fair et al., 1973). Spermine, a substance in seminal fluid, secreted by the prostate, is correlated with the sperm motility. Semen cholesterol content is synthesized in human prostate and is important for stabilizing the sperm membrane against temperature and environmental shock (Meares, 1991). All the above substances having beneficial effects on sperm quality are secreted by the prostate and their secretions are up-regulated by either sildenafil (Dimitriadis et al., 2008) or vardenafil (Dimitriadis et al., 2008).

Nitric oxide synthase has been localized biochemically and immunohistochemically in both the transitional and peripheral zones of the human prostate (Burnett et al., 1995). Specifically NOS has been found in the nerve fibres and ganglia located in the prostatic smooth musculature indicating that NO is important for the prostatic smooth muscular function (Takeda et al., 1995). This is supported by the action of NO donors which have been shown to mediate relaxation of human prostatic smooth muscle in vitro (Takeda et al., 1995).

PDE4 and PDE5 have been demonstrated immunohistochemically in the prostatic transitional zone (Uckert et al., 2001). In vitro sildenafil and rolipram, a PDE4 inhibitor, have been shown to reverse tension in prostatic smooth muscle strips from the transitional zone (Uckert et al., 2001). This information could lead to further investigations aiming to recruit PDE4 inhibitors and PDE5 inhibitors to the pharmacologic management of benign prostatic hyperplasia (Uckert et al., 2001). Furthermore PDE5 inhibitors may have some beneficial effects in patients with chronic prostatitis. Grimsley et al., (2007) postulated that PDE5 inhibitors relax prostatic smooth muscular fibers altering the retrograde urinary flow in prostatic ducts allowing greater washout of the ducts and reducing accumulation of irritative urinary contents. The final result may be a reduction of the prostatic inflammation.

Moreover, previous reports have shown that human PDE11 and particularly its splice variant PDE11A4 is abundantly expressed in the prostate, suggesting that the PDE11A gene undergoes tissue-specific alternative splicing that generates structurally distinct gene products (Yuasa et al., 2001; Loughney et al., 2005).

6. The influence of PDE5 inhibitors on standard parameters of semen analysis

6.1 The development of in vivo studies

The influence of PDE inhibitors on the sperm parameters has been investigated by several authors. The positive effect of the PDE inhibition on sperm motility may suggest an

association between the intracellular levels of cAMP or cGMP and the sperm ability to move (Sikka & Hellstrom, 1991; Fisch et al., 1998). However the majority of studies evaluating the effects of PDE inhibitors on spermatozoa have employed non-selective PDE inhibitors. Only few of the above studies have employed a selective PDE5 inhibitor such as sildenafil, vardenafil, or tadalafil.

Many chemical molecules have been studied to evaluate their effects on human sperm functions. Methylxanthines (Tesarik et al., 1992; Tournaye et al., 1995) belong to the first generation of PDE inhibitors and represent a chemical group of drugs derived from xanthine (a purine derivative) including among others: theophylline, caffeine, and pentoxifylline. Their beneficial effects on sperm motility has been recognized since 1970 (Haesungcharern & Chulavatnatol, 1973; Schill, 1975; De Turner et al., 1978; Schill et al., 1979). Jaiswal and Majumder (1996) in an in vitro study investigated the role of theophylline demonstrating that this PDE inhibitor markedly increased (10-fold or greater) the motility of goat spermatozoa derived from proximal corpus, mid-corpus, distal-corpus, and proximal-cauda epididymes. In another in vitro study caffeine has been also shown to increase sperm motility and metabolism when it has been added into the semen (Levin et al., 1981; Rees et al., 1990). However this compound promotes the spontaneous sperm acrosomal reactions. This effect of caffeine on sperm acrosomal cup counteracts the benefits from its role as a motility stimulant (Tesarik et al., 1992). Pentoxifylline (PTX), is the most widely non-selective PDE inhibitor that has been used in assisted reproductive technology programs (Wang et al., 1983; Marrama et al., 1985; Shen et al., 1991; Tesarik et al., 1992; Fuse et al., 1993; Pang et al., 1993; Tournaye et al., 1994). Although its beneficial effect on the outcome of IVF trials in normozoospermic subjects and oligo-asthenozoospermic patients is well documented (Yovich et al., 1988; Yovich et al., 1990; Tasdemir et al., 1993; Yunes et al., 2005) the efficacy of its oral administration to increase sperm fertilizing ability is controversial (Tournaye et al., 1994). PTX has been considered to stimulate flagellar motility by increasing sperm intracellular cAMP (Stefanovich, 1973; Garbers & Kopf, 1980; Tash & Means, 1983; Ward & Clissold, 1987) as well as by reducing sperm intracellular superoxide anion and reactive oxygen species known to damage DNA (Lopes et al., 1998; Twigg et al., 1998). In particular PTX in both in vivo and in vitro studies, appears to increase significantly beat cross frequency, curvilinear velocity, and the percentage of hyperactivated spermatozoa (Rees et al., 1990; Shen et al., 1991; Tesarik et al., 1992; Lewis et al., 1993; Moohan et al., 1993; Pang et al., 1993; Tournaye et al., 1994; Centola et al., 1995; Paul et al., 1996; Nassar et al., 1999).

It should be mentioned that PDE4 inhibitors, as well, increase sperm motility. As it has been demonstrated in an in vitro study (Fisch et al., 1998), 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 (Fisch et al., 1998).

In a double-blinded, four-period, two-way, crossover study encompassing 17 sexually healthy male volunteers, Purvis et al., (2002) examined the effect of sildenafil on sperm motility and morphology parameters. The authors compared an 100-mg dose of sildenafil with placebo. Both sildenafil and placebo were administered as single oral doses for two periods separated by a washout period of at least 5–7 days. The authors reported a lack of influence of sildenafil on sperm motility. 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.

The above study by Purvis et al., (2002) has confirmed earlier findings published by Aversa et al., (2000). The authors have conducted a prospective double-blind, placebo-controlled, cross-over, two-period-investigation study, embracing 20 male subjects, which were treated with sildenafil or placebo. After a washout period of seven days all subjects were crossed over to receive the alternative treatment. The authors found no statistically significant differences in the mean values of sperm number, sperm motility, and percentage of morphologically abnormal spermatozoa between the two groups. In that study the investigators emphasized the potential usage of sildenafil in assisted reproductive programs when a temporary erectile dysfunction may occur due to the stress and the psychological pressure for semen production. The last suggestion had been expressed earlier by Tur-Kaspa et al., (1999) who reported their experience on the usage of sildenafil in men with proven erectile dysfunction during assisted reproductive technologies cycles. It appears that 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 the above study by Aversa et al., (2000) a positive effect of sildenafil on some sperm motility parameters was proven in another study. In a prospective double-blind, placebo-controlled, crossover, two-period-administration, clinical investigation, du Plessis et al., (2004) determined the effect of in vivo sildenafil citrate administration 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. The authors reported no significant differences in the percentage of spermatozoa 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 sildenafil treated group and the placebo group. However borderline statistically 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 spermatozoa after sildenafil administration.

In an open-label pilot study, Jannini et al., (2004) investigated the effect of 50-mg orally administered sildenafil in a group of sexually healthy men who participated in an intrauterine artificial insemination program or planned sexual intercourses to perform a post-coital test. The authors found no effect of sildenafil administration on sperm motility, on the sperm concentration, or on the total number of spermatozoa ejaculated. Similarly no effect of sildenafil administration was demonstrated on the percentage of non-linear progressive motile spermatozoa. However, a significant increase was seen in the linear progressive motility after sildenafil administration. The authors have additionally suggested that the administration of sildenafil prior to semen collection in ART programs reduces the stress that is experienced by the male in the ejaculation room. Similar conclusions have been raised by the same group of authors in an earlier study (Lenzi et al., 2003). In that earlier study the authors noted that sildenafil is effective in increasing compliance of male patients facing infertile couple management procedures, and also in improving cervical mucus sperm penetration assay (Lenzi et al., 2003).

Evaluation of the effects of sildenafil on semen quality has been the aim of a study conducted by Dimitriadis et al., (2008). The authors found 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 prior to usage of sildenafil (Dimitriadis et al., 2008). The authors have suggested 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 that have demonstrated that secretory dysfunction of the male accessory genital glands due to prostatic infections impairs male fertility potential (Sofikitis & Miyagawa, 1991). The seminal fluid may contain factors that are not absolutely essential to fertilization (Portnoy, 1946). However, optimal concentrations of prostatic secretory markers may provide an environment ideal for sperm motility and transport (Sofikitis & Miyagawa, 1993). The increase in the prostate secretory function, sperm motility, and total sperm count in the study by Dimitriadis and co-workers (Dimitriadis et al., 2008) may be attributable to the higher sexual satisfaction observed in samples collected after sildenafil treatment. The importance of the positive effects of sexual satisfaction and orgasm on the semen quality and sperm fertilizing capacity have been emphasized in another study comparing masturbation with videotaped sexual images and masturbation without videotaped sexual images. Masturbation with videotaped sexual images resulted in recovery of spermatozoa of greater fertilizing potential (Yamamoto et al., 2000). In addition in a similar report Sofikitis and Miyagawa (1993) demonstrated improved spermatozoal motility in the semen samples collected via sexual intercourse versus masturbation in infertile men. Sofikitis and Miyagawa (1993) have suggested that the higher the sexual stimulation is, the larger the prostatic secretory function is and the larger the vas deferens loading during ejaculation is. The latter suggestion is supported by a study showing that restraint of bulls or false mounts prior to semen collection increases the number of motile spermatozoa by as much as 50% (Salisbury & Vandermark, 1961). Furthermore in bulls, it has been suggested that oxytocin and prostaglandin F2a may be at least partly responsible for the improvement of the ejaculate after sexual stimulation (Hafs et al., 1962; Sharma & Hays, 1973).

Few other studies support the findings of the above investigation by Dimitriadis et al., (2008). Ali et al., (2007) 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 have proposed that sildenafil administration is associated with an improvement in the entire smooth musculature architecture of the male reproductive and urinary tract which has been altered due to neuropathy. Sildenafil administration resulted in reduction in the excessive accumulation of interstitial collagen and calcification in the smooth muscles which leaded to bladder atonia in the diabetic men. On the other hand the authors have noticed that long time sildenafil treatment is associated with a significant decrease in total sperm output and sperm concentration.

Pomara et al., (2007) 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 PDE-5 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 effect of sildenafil on sperm motility may be due to a direct action of sildenafil on sperm mitochondria and calcium channels. Another report (Nomura & Vacquier, 2006) has demonstrated that PDE5A is localized mainly on sea urchin sperm flagella regulating intracellular cGMP levels. Thus a direct effect of sildenafil on sperm flagella cannot be ruled out (Nomura & Vacquier, 2006). Interestingly, the study by Pomara et al., (2007) revealed a significant decrease in the sperm motility after a single dose of tadalafil (Pomara et al., 2007). These latter findings are inconsistent with an earlier study conducted by Hellstrom and colleagues (2003) who investigated the effects of tadalafil on semen characteristics and serum concentrations of reproductive hormones in healthy men and men with mild erectile dysfunction. Hellstrom et al., (2003) 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, luteinizing hormone, 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. In addition, the authors demonstrated that there were no significant alterations in the serum levels of reproductive hormones after tadalafil administration concluding that administration of tadalafil at doses of 10 mg and 20 mg for six months did not adversely affect testicular spermatogenesis process or serum levels of reproductive hormones. However other investigators have emphasized 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 adequate (Pomara et al., 2007). Taking in consideration the above dilemma Hellstrom et al., (2008) expanded their investigation efforts in a double-blind, placebo-controlled, noninferiority study, assessing the effects of tadalafil (20mg) on spermatogenesis over three spermatogenesis cycles in men elder than 45-year-old. The investigators demonstrated no deleterious effects of 9-month daily tadalafil (20mg) administration on spermatogenesis.

Bauer et al., (2002) performed a randomized, placebo control, double-blind, crossover study to determine the effects of a single dose of vardenafil (20 mg) on sperm parameters. Sixteen healthy males participated in this study. The scientists found no statistically significant effects of vardenafil on sperm motility, sperm concentration, sperm viability, and sperm morphology.

In another study, Dimitriadis et al., (2008) evaluated the effects of vardenafil administration (10 mg) on semen quality. The investigators noted that semen samples from infertile men treated with 10 mg of vardenafil presented a significantly larger total number of spermatozoa, quantitative sperm motility, qualitative sperm motility, and percentage of morphologically normal spermatozoa compared with semen samples collected prior to vardenafil administration from the same individuals. The authors suggested that vardenafil stimulated the prostatic secretory function due to an enhanced sexual stimulation increasing the quantitative and qualitative motility of spermatozoa and decreasing sperm abnormalities.

Jarvi et al., (2008) performed a randomized, double-blind, placebo controlled, parallel group, multicenter study investigating the effect of vardenafil and sildenafil on sperm characteristics. A total of 200 men with or without erectile dysfunction, able to produce semen samples without therapy for erectile dysfunction, 25 to 64 years old, were randomized to daily treatment with vardenafil, sildenafil or placebo for 6 months. Vardenafil or sildenafil had no effect vs. placebo on the percentage of patients with 50% or greater decrease in sperm concentration (Jarvi et al., 2008). Additionally, vardenafil or sildenafil did not affect any sperm parameters or peripheral serum gonadotropin profiles (Jarvi et al., 2008).

6.2 The development of 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., (2000) in an ex vivo study investigated the effect of sildenafil on the motility and viability of human spermatozoa. The above spermatozoal parameters were evaluated in sperm samples of both healthy donors (n=6) and clinically infertile men (n=6). Separate sperm 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.

Similarly, in another study, the group of Andrade et al., (2000) attempted to evaluate a direct effect of sildenafil or phentolamine on sperm motility. Using samples of either unwashed or washed spermatozoa, the investigators added directly to the sperm samples sildenafil at a concentration of 20 mg/mL or phentolamine in various doses and incubated the samples for 30 minutes. 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 (2000 μg/mL) reduced the sperm motility approximately at a level of 50% of the original value. 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 (Su & Vacquier, 2006).

In an experimental study Su and Vacquier (2006) cloned and characterized a sea urchin spermatozoal PDE (suPDE5) which is an ortholog of human PDE5. The authors have found that phospho-suPDE5 localizes mainly on sperm flagella and that the PDE5 phosphorylation increases when spermatozoa contact the jelly layer that surrounds the eggs. Since the in vitro dephosphorylation of suPDE5 decreases its activity, the authors have suggested that PDE5 inhibitors such as sildenafil may inhibit the activity of suPDE5 and increase sperm motility.

A concentration-dependent stimulatory effect of sildenafil on sperm motility was also demonstrated by Mostafa (2007) when 85 semen specimens from asthenozoospermic patients were exposed to different concentrations of sildenafil (4.0 mg/mL, 2.0 mg/mL, 1.0 mg/mL, 0.5 mg/mL, and 0.1 mg/mL). However, the evaluation of sperm motility in that study was performed 3 hours only after the spermatozoal exposure to sildenafil.

Lefièvre et al., (2000) have investigated whether PDE5 is present in human spermatozoa and whether sildenafil affects sperm function. The authors have demonstrated 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.

Cuadra et al., (2000) evaluated the effect of sildenafil on sperm motility. Spermatozoa were exposed to sildenafil at either 0 nmol/L, 0.4 nmol/L, 4.0 nmol/L, or 40 nmol/L. The investigators 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 hours after the exposure to sildenafil. Spermatozoa exposed to higher concentration of sildenafil (40 nmol/L) showed decreased sperm motility parameters. cGMP regulates calcium entry into microdomains along the sperm flagellum affecting sperm motility. Since PDE5 hydrolyzes cGMP, the authors have suggested that inhibition of PDE5 by sildenafil citrate enhances the effects of cGMP on sperm motility. The data provided by the authors (Cuadra et al., 2000) suggest that there is a stimulatory effect on sperm motility when PDE5 is moderately inhibited; however, extensive inhibition of PDE5 appears to lead to decreased sperm motility.

Another group of researchers (Glenn et al., 2007) attempted to determine the influence of sildenafil on sperm motility. Semen samples from 57 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 (Glenn et al., 2007). The authors found that both the number and the velocity of progressively motile spermatozoa were significantly increased. The investigators have suggested that the elevated levels of cGMP, attributable to the inhibitory effect of sildenafil, may affect calcium transport into spermatozoa which potentially affects the sperm motion.

The effects of tadalafil on human sperm motility in vitro have been investigated. Mostafa (2007) assessed the effects 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 that sperm samples treated with 4 mg/mL tadalafil solution demonstrated a significant decrease in sperm motility compared with the control samples. On the other hand, sperm samples treated with 1.0 or 0.5 mg/mL tadalafil solution demonstrated a significant increase in sperm progressive forward motility. The authors have suggested that the concentration of tadalafil is an important factor in such studies.

Alternatively, the effect of tadalafil on sperm motility may be related additionally to the inhibitory effect of this compound on PDE11. In fact, PDE11 is highly expressed in the testis, prostate, and developing sperm cells even if its physiological role is not known (Table 1). Wayman et al., (2005) 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 the latter animals which suggest a role for PDE11 in spermatogenesis and fertilization potential.

Taken together the results of the above ex vivo studies, we may postulate a dose dependent effect of sildenafil and tadalafil on sperm motility. In fact this effect seems to be enhanced at

low doses of PDE5 inhibitors but it may be reduced at high concentrations. Definitely further investigations are required to evaluate the mechanisms mediating the effects of PDE5 selective inhibitors on sperm motility.

7. The effect of PDE5 inhibitors on sperm capacitation and acrosome reaction process

Lefièvre et al., (2000) investigated the potential effect of PDE5 inhibitor sildenafil on spermatozoal ability to undergo capacitation process. The authors showed that sildenafil at 30 µmol/L, 100 µmol/L, and 200 µmol/L triggered the capacitation process of washed spermatozoa. Capacitated spermatozoa underwent an acrosome reaction when challenged with lysophosphatidylcholine (LPC) alone or LPC plus IBMX (a non selective PDE inhibitor), but not with sildenafil alone. The authors have suggested that PDE inhibitors by themselves cannot initiate the acrosome reaction nor can they potentiate the acrosome reaction of capacitated spermatozoa.

Cuadra et al., (2000) investigated the effect of sildenafil on sperm acrosomal reaction. Spermatozoa were exposed to different doses of sildenafil (from 0 nmol/L to 40 nmol/L). The authors reported that sildenafil affected the sperm acrosomal reaction with an increase in the percentage of acrosomally reacted spermatozoa 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 (Biel et al., 1998). Since PDE5 hydrolyzes cGMP, inhibition of PDE5 by sildenafil citrate enhances the effects of cGMP on sperm acrosome reaction.

Glenn et al., (2007) evaluated the influence of sildenafil on sperm acrosomal reaction. The study included fifty-seven unselected men with asthenozoospermic profiles who provided semen samples which were further processed for sperm isolation. Then spermatozoa were exposed to 0.67 µmol/L of sildenafil. The investigators (Glenn et al., 2007) noticed that sildenafil caused a significant increase in the proportion of acrosome-reacted spermatozoa. In a case report, sildenafil was administered for semen collection for ART purposes. The investigators failed to fertilize oocytes despite the intracytoplasmic injection of the sperm (Tur-Kaspa et al., 1999). 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 spermatozoal function can not be excluded.

8. The effects of PDE5 inhibitors on sperm functional assays

Several studies have evaluated the in vitro effects of PDE5 inhibitors on sperm functional assays. Burger et al., (2000) performed an in vitro study investigating and comparing the effects of sildenafil on the membrane integrity, and functional sperm capacity between healthy donors and clinically infertile men. The authors noted a marked decrease of sperm membrane integrity in spermatozoa of infertile patients treated with sildenafil (Burger et al., 2000). This observation may be taken into consideration when treatment with sildenafil is planed in subfertile couples with a male factor infertility (Burger et al., 2000). However, sperm penetration assay data has suggested that there is neither a beneficial nor a detrimental effect of sildenafil on its outcome (Burger et al., 2000).

9. Conclusions

The expression of PDEs has been documented in several regions of the male reproductive tract. Increasing evidence based on several studies tend to suggest that PDE5 inhibitors may have a therapeutic effect in some disorders of semen parameters. Moreover, PDE5 inhibitors may promote sperm capacitation process. In addition PDE5 inhibitors may affect sperm transfer through the male reproductive tract by affecting the contractility of the tunica albuginea and the epididymis. However, the current evidence needs further confirmation by additional studies that are necessary to suggest unequivocally a therapeutic role of PDE5 inhibitors in the management of defects in testicular or epididymal function.

Type of cell/ Tissue		PDE	Species	Relative References
Testis		PDE5 PDE8A PDE8B PDE1A PDE11A PDE11A2 PDE11A3	Human	Foresta et al., (2008) Baxendale et al., (2005) Dousa et al., (1999) Michibata et al., (2001) Fawcett et al., (2000) Kotera et al., (1999) Yuasa et al., (2001) D'Andrea et al., (2005) Horvath et al., (2006)
		PDE1A PDE1B PDE1C PDE3B PDE4A PDE4B PDE4C PDE4D PDE5A PDE6γ′ PDE7A PDE8A PDE10A	Mouse	Baxendale et al., (2005)
		PDE1C	Rat	Dousa (1999)
Germ cells		PDE11A	Human	D'Andrea et al., (2005)
		PDE1 PDE2	Rat	Swinnen et al., (1989)
	Spermatogonia	PDE11	Human	Baxendale et al., (2001)
		PDE11	Mouse	Baxendale et al., (2001)

Germ cells	**Spermatocytes**		PDE11	Human	Baxendale et al., (2001)
	Developing spermatocytes		PDE3B	Rat	Degerman et al.,(1997)
	Pachytene spermatocytes		PDE4D PDE4A PDE1C	Rat	Salanova et al., (1999) Yan et al., (2001)
	Spermatids		PDE11	Human	Baxendale et al., (2001)
			PDE11	Mouse	Baxendale et al., (2001)
			PDE4A	Rat	Farooqui et al., (2001)
		Round	PDE4A PDE4D	Rat	Salanova et al., (1999) Farooqui et al., (2001)
		Round~ Elongating	PDE1A PDE1C	Mouse	Yan et al., (2001)
		Elongating	PDE4D	Rat	Salanova et al., (1999)
	Spermatozoa		PDE1 PDE1A PDE3A PDE4 PDE5	Human	Fisch et al., (1998) Aversa et al., (2000) Lefievre et al., (2002)
			PDE6 PDE3B	Mouse	Baxendale et al., (2005)
		Acrosomal region/ head	PDE4D	Mouse	Baxendale et al., (2005)
		Flagellum	PDE1A PDE4D PDE10A	Mouse	Baxendale et al., (2005)
	Sertoli cells		PDE3 PDE4	Rat	Swinnen et al., (1989)
			PDE4B	Rat	Farooqui et al., (2001)
			PDE4D	Rat	Levallet et al., (2007)
	Peritubular myoid cells		PDE5	Rat	Scipioni et al., (2005)

Leydig cells	PDE11A	Human	D'Andrea et al., (2005)
	PDE4B	Rat	Farooqui et al., (2001)
	PDE5	Rat	Scipioni et al., (2005)
	PDE8A PDE11	Mouse	Vasta et al., (2006) Baxendale et al., (2001)
Vascular myocytes	PDE11	Human	Baxendale et al., (2001)
	PDE5	Rat	Scipioni et al., (2005)
	PDE11	Mouse	Baxendale et al., (2001)
Epididymis	PDE3	Bull	Mewe et al., (2006)
Vas Deferens	PDE5	Human Rabbit	Mancina et al., (2005)

Prostate		PDE4 PDE5A PDE11A4 PDE11A1	Human	Kotera et al., (1999) Yuasa et al., (2001) Fawcett et al., (2000) Uckert et al., (2001)
	Epithelial cells	PDE11A	Human	Loughney et al., (2005) D'Andrea et al., (2005)
	Endothelial cells	PDE11A	Human	D'Andrea et al., (2005)
	Smooth muscle cells	PDE11A	Human	D'Andrea et al., (2005)
Penis		PDE2 PDE3 PDE4 PDE5	Human	Milhoua et al., (2007)
	Corpus cavernosum	PDE1A PDE1B PDE1C PDE2A	Human	D'Andrea et al., (2005) Kuthe et al., (2001) Foresta et al., (2008)

Penis	**Corpus cavernosum**	PDE3A PDE4A PDE4B PDE4C PDE4D PDE5A PDE6 PDE7A PDE8A PDE9A PDE11A	Human	
	Endothelial cells	PDE11A	Human	D'Andrea et al., (2005) Kuthe et al., (2001) Foresta et al., (2008)
	Tunica Albuginea	PDE4 PDE5	Rat	Valente et al., (2003)

Table 1. Expression of phosphodiesterases PDEs in the male reproductive tract.

10. Abbreviations

AC = adenylate cyclase
ANP = atrial natriuretic peptide
ART = assisted reproductive technology
cAMP = cyclic adenosine monophosphate
cGMP = cyclic guanosine monophosphate
CNP = C-type natriuretic peptide
CO = carbon monoxide
eNOS = endothelial nitric oxide synthase
GC = guanylate cyclase
GC-B = membrane-bound GC
HO-1= heme oxygenase-1
ICSI = intracytoplasmic sperm injection
IVF = in vitro fertilization
LPC = lysophosphatidylcholine
NO = nitric oxide
NOS = nitric oxide synthase
PDEs = phosphodiesterases
PKA = protein kinase
PTX = pentoxifylline
SCs = spontaneous phasic contractions
sGC = soluble guanylate cyclase
SNP = sodium nitroprusside
suPDE5= sea urchin spermatozoal PDE

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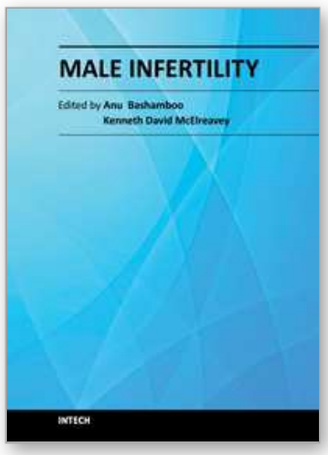

Male Infertility

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Male infertility is a multifaceted disease where genetic, epigenetic and environmental factors all contribute to the development of the phenotype. In recent years, there has been an increasing concern about a decline in reproductive health, paralleled by an increase in demand for infertility treatments. This calls for a detailed and thorough understanding of normal and aberrant testicular function and the environmental influences on the establishment and integrity of the male germ cell. This is crucial for understanding the complex pathophysiology of male infertility and eventual success of Assisted Reproductive Technologies.

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