Chapter 13
Effects of Phosphodiesterase-5 Inhibitors on Testicular and Sperm Function

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Introduction

Phosphodiesterase-5 inhibitors (PDE-5i) have been proposed as a possible therapeutic option for male infertility. Given the ubiquitous nature of phosphodiesterase (PDE) isoforms throughout the body, several mechanisms of positive PDE-5i effects on male fertility have been purported. Male patients under pressure to ejaculate either for a semen sample or during timed intercourse can often have problems with erections. The majority of published research relating PDE-5i to fertility focuses upon sperm function and spermatogenesis for which results are mixed, but most favor either a benefit or no effect. The process of sperm capacitation and the acrosomal reaction may also be affected by PDE-5i. Contractile cells surrounding seminiferous tubules, efferent ducts, and epididymal ducts play an important role in spermatozoa transport and can be influenced by PDE-5i. PDE-5i use may also affect male fertility via effects on the vas deferens, seminal vesicles (SV), and prostate. Figure 13.1 summarizes current knowledge on the effect of PDE-5i on male fertility. Additional research is required to definitively assess the effects of PDE-5i in these areas, especially in regard to real world clinical application. At the very least, the preponderance of data shows that PDE-5i do not exert a negative effect on fertility.

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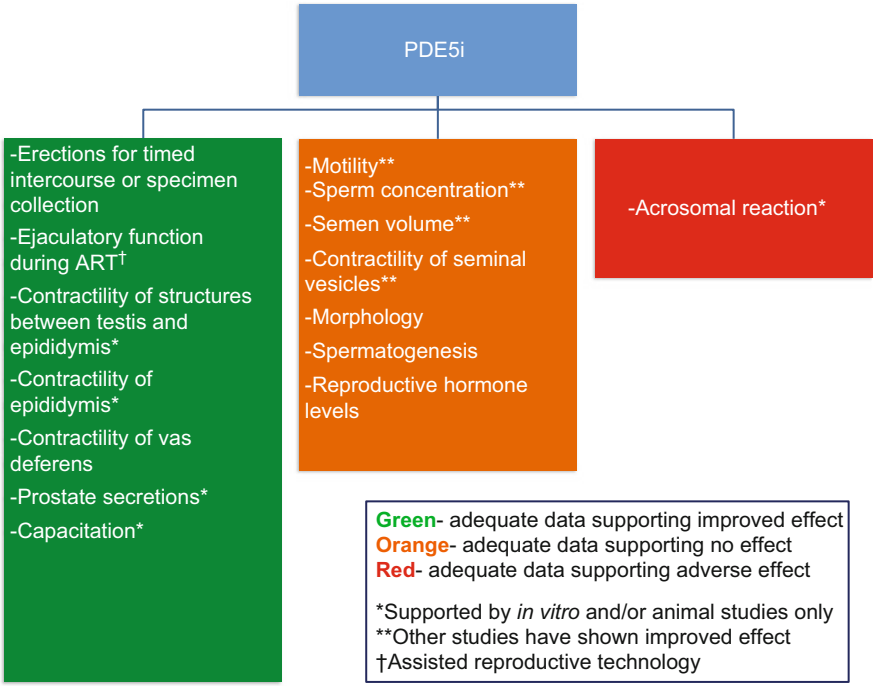

Fig. 13.1 Effects of PDE-5i on testicular and sperm function

Ejaculatory Effects

Involuntary childlessness is a powerful stressor for infertile couples and can result in a high frequency of erectile dysfunction (ED), ejaculatory dysfunction, loss of libido, and decreased frequency of intercourse. These problems are particularly problematic in the context of timed intercourse and multiple specimen collections for assisted reproductive technologies (ART). The use of PDE-5i has been purported to aid in ART compliance [1]. Temporary ED during attempts at ART has been successfully treated with sildenafil [2, 3]. In a study of 20 healthy volunteers not under the stress of ART, 100 mg of sildenafil resulted only in a marked reduction of post-ejaculatory refractory time, but did not affect already normal erectile function or semen characteristics [4]. Ejaculatory dysfunction during infertility treatment has also been successfully treated with PDE-5i [5]. Some investigators have added a 50 mg sertraline dose to PDE-5i to help alleviate temporary ejaculation failure for ART in those who failed PDE-5i doses alone. The utilization of SSRIs in this study is thought to have worked by mitigating associated stresses and anxiety [6].

A Korean study revealed a 43 % ED and 6 % ejaculatory dysfunction rate in 439 men utilizing timed intercourse [7]. Men affected had significantly lower levels of luteinizing hormone, testosterone, and estradiol. In addition, men who required

high doses of tadalafil in this cohort had not only higher levels of anxiety, but more self-reported aggression. Use of PDE-5i has also been noted to aid in both erectile and ejaculatory dysfunction for artificial intrauterine insemination (IUI) by improving both the patients' ability to have a full erection and their confidence in obtaining and maintaining an erection. This improvement has been effective for transient sexual dysfunction and ultimately helped result in clinical pregnancies [8].

Effects of PDE-5i on Testicular and Sperm Function

There have been multiple clinical trials conducted to establish the role of PDE-5i in testicular and sperm function. Sildenafil (100 mg) has been shown to reach concentrations of 0.1–0.3 μmol/L in the ejaculate [9], with a presumed inhibitory effect on PDE isoforms. Tadalafil, a strong PDE-5 and -11 inhibitor, has been purported to have an effect as well, given the strong expression of PDE-11 in prostate gland, Leydig cells, and developing germ cells in the testes [10–12]. Nonetheless, many studies have been conflicting, some demonstrating improvements in spermatogenesis and sperm function, others showing no unfavorable effect, and few demonstrating reduced function [13]. Table 13.1 summarizes human studies of PDE-5i on testicular and sperm function.

Improved Function

One prospective, randomized, double-blind, crossover study, in which the investigators sought to assess the acute effect of sildenafil (50 mg) and tadalafil (20 mg) on seminal parameters in young, infertile men, demonstrated an increase in sperm progressive motility in these subjects with sildenafil, and a decrease with tadalafil 20 mg [14]. Eighteen subjects were randomized to the individual drugs, and then instructed to collect semen samples 1 h after sildenafil and 2 h after tadalafil administration. Following a 2-week wash-out period, each subject was switched to the other drug. On microscopic evaluation of each semen sample, concentration, motility, agglutination of spermatozoa, and the presence of other cellular elements were assessed; motility was classified as rapid-progressive, slow-progressive, non-progressive, and immotility. A statistically significant increase was demonstrated in the rapid and total progressive motility with sildenafil, compared to baseline [18.5 % vs. 10.5 %, $p = 0.0006$ and 37.0 % vs. 28.5 % $p = 0.009$, respectively]. Tadalafil showed a significant decrease in these motility parameters [6.0 % vs. 10.5 %, $p = 0.006$ and 21.5 % vs. 28.5 %, $p = 0.015$, respectively]. There were no significant differences noted for the other seminal parameters.

Lefievre et al. have shown that sildenafil enhances human sperm motility and capacitation [15]. Semen samples from healthy volunteers were collected after 3 days of abstinence and centrifuged. Samples with at least 70 % motility were

Table 13.1 Human studies of PDE-5i effects on testicular and sperm function

Author and year	Methods	Findings
Improved function		
Pomara (2007) [14]	Prospective, randomized, double-blind, crossover study. $N = 18$	Increased sperm progressive motility in subjects with sildenafil (50 mg); decreased progressive motility with tadalafil (20 mg)
	Assessed acute effect of sildenafil and tadalafil in young, infertile men	Effect appears to be in sperm, the vas ampulla, or seminal components
	Intervention: sildenafil 50 mg or tadalafil 20 mg	
Lefièvre (2000) [15]	PDE activity, motility, sperm capacitation, and the acrosome reaction were measured in the presence of cAMP and cGMP	Dose-dependent increase in sperm cAMP levels, which triggered sperm motility and capacitation
Rago (2012) [16]	Randomized, open-label, parallel group study. $N = 205$	Increased sperm motility after a single dose of vardenafil (10 mg), and after treatment on alternating days
	Intervention: no treatment, 10 mg vardenafil × 1, 10 mg vardenafil qod × 15 days	
Glenn (2007) [17]	Laboratory analysis of sperm motility after exposure to sildenafil using computer-assisted semen analysis; acrosome reaction assessed by fluorescein isothiocyanate-labeled peanut agglutinin staining. $N = 57$	Sildenafil found to significantly increase the number and velocity of progressively motile sperm in the best- and poor-quality sperm of infertile men
		Premature acrosome reaction noted with sildenafil
Ali and Rakkah (2007) [18]	Investigated the role of sildenafil on seminal parameters in diabetics	Significant increase in sperm motility and semen volume with sildenafil (100 mg), in diabetic neuropathics
	Intervention: sildenafil 100 mg given to insulin-dependent and non-insulin-dependent diabetics, and to age-matched controls. $N = 100$	
No change		
du Plessis (2004) [19]	Prospective, double-blind, placebo-controlled, crossover, 2-period clinical study. $N = 20$	In vivo study revealed enhanced sperm binding to the oocyte with a single dose of sildenafil (50 mg); no effect on sperm kinematics
	Intervention: sildenafil 50 mg or placebo; 20 μM 8-Bromo-cGMP added in vitro to semen samples	In vitro study showed no effect of sildenafil or exogenous cGMP on semen parameters; no effect of cGMP on the acrosome reaction
Burger (2000) [21]	Sperm incubated in 125, 250, and 750 ng/mL, as well as pentoxifylline as a positive control and Ham's F10 as a reagent control. $N = 12$	No significant effect of sildenafil on motility, viability, or membrane integrity of normal or infertile sperm at 0, 1, or 3 h incubation
Andrade (2000) [20]	10 μL sildenafil added directly to 90 μL semen sample; computer-assisted assessment of sperm parameters at high	No effect of sildenafil on sperm motility at a concentration of 200 μg/mL

(continued)

Table 13.1 (continued)

Author and year	Methods	Findings
	(2,000 μg/mL) and low (200 μg/mL) sildenafil concentrations	
Aversa (2000) [4]	Double-blind, randomized, placebo-controlled, crossover 2-period study. $N = 20$	No changes in sperm number, percent sperm abnormalities, or motility with sildenafil (100 mg); there was a reduction of post-ejaculatory refractory times
	Intervention: sildenafil 100 mg × 1	
Jarvi (2008) [22]	Randomized, double-blind, placebo-controlled, parallel group, multicenter study. $N = 200$	No effect of vardenafil (20 mg) or sildenafil (100 mg), versus placebo, on sperm concentration or other semen characteristics, when given daily for 6 months
	Intervention: vardenafil 20 mg, sildenafil 100 mg, placebo	Vardenafil daily for 6 months does not impact sperm quality in men with normal baseline semen and reproductive hormone parameters
Purvis (2002) [23]	Double-blind, randomized, 4-period, 2-way crossover study. $N = 17$ Intervention: sildenafil 100 mg × 1	No effect of a single sildenafil (100 mg) dose on sperm motility, count, ejaculate volume, or ejaculate quality in healthy males
Hellstrom (2003) [24]	Parallel studies Intervention: 6 months placebo versus tadalafil 10 mg; 6 months placebo versus tadalafil 20 mg. $N = 421$	No significant adverse effect of tadalafil (10 mg or 20 mg) on spermatogenesis or reproductive hormones in men 45 years or older after 6 months of treatment
Hellstrom (2008) [25]	Double-blind, placebo-controlled study. $N = 253$ Intervention: tadalafil 20 mg versus placebo	No significant adverse effect of tadalafil (20 mg) on sperm production or reproductive hormones in men 45 years or older, after 9 months of treatment
Reduced function		
Andrade (2000) [20]	10 μL sildenafil added directly to 90 μL semen sample, computer-assisted assessment of sperm parameters at high (2,000 μg/mL) and low (200 μg/mL) sildenafil concentrations	50 % reduction in sperm motility at a high sildenafil concentration of 2,000 μg/mL
Ali and Rakkah (2007) [18]	Investigated the role of sildenafil on seminal parameters in diabetics. $N = 100$ Intervention: sildenafil 100 mg given to IDDM, NIDDM, and age-matched controls	Significant decrease in total sperm output and concentration with sildenafil (100 mg), in diabetic neuropathics

used in the investigation. PDE activity in the presence of cAMP and cGMP was measured, as were motility, sperm capacitation, and the acrosome reaction (AR). Sildenafil was found to cause a dose-dependent increase in sperm cAMP levels, which triggered sperm motility and capacitation. The mechanism responsible is

unclear, but it is hypothesized that sildenafil can also act on PDE types other than type 5 to yield this effect.

In a randomized, open-label, parallel group study Rago et al. also showed an increase in sperm motility after a single dose of vardenafil (10 mg) [16]. Two hundred and five male subjects were randomized to no treatment (group A), one dose of vardenafil (group B), and vardenafil every other day for 15 days (group C). Semen analyses were done 1 h after treatment in group B, and at day 15 after treatment in groups A and C. The IIEF-5 questionnaire was also administered to subjects with erectile dysfunction before and after each treatment period. The two groups taking either a single dose or single dose on alternating days for 15 days demonstrated a significant increase in the percentage of spermatozoa with forward motility [$p < 0.001$]. Subjects taking single doses every other day also experienced a significant increase in the mean semen volume and mean total sperm concentration [$p < 0.001$], the former believed possibly due to stimulation of prostatic secretory function by vardenafil. Furthermore, erectile function as subjectively reported on the IIEF-5 questionnaire was improved in groups taking either single dose of vardenafil or single dose of vardenafil on alternating days.

Glenn et al. also showed a significant increase in the number and velocity of progressively motile sperm in good- and poor-quality sperm [17]. Fifty-seven infertile male subjects, who had presented for fertility evaluation, produced semen samples 2–5 days after abstaining. The sperm samples were divided into populations of sperm with the best-fertilizing potential as would be used in assisted reproduction treatments (90 % fraction) and poor-fertilizing (45 % fraction) potential as similar to sperm of men with infertility. These were then incubated in the presence or absence of sildenafil and assessed. Sildenafil was found to significantly increase the number and velocity of progressively motile sperm in the best- and poor-quality sperm. More specifically, the PDE-5i demonstrated a sustained enhancement of motility, as well as a premature activation of the AR.

Jannini et al. also studied the effect of sildenafil in infertile men, notably its effect on sexual function and reproductive outcome given the known reduction in sexual function in men undergoing investigation and treatment for infertility [8]. A group of healthy men were evaluated after treatment with 50 mg sildenafil before IUI or planned intercourse for a postcoital test. This group found that sildenafil reduced stress levels, reversing the stress-induced transient ED experienced in some of these men, with more complete ejaculation and more good-quality sperm. Sildenafil also improved the percentage of spermatozoa with linear progressive motility, and the number of spermatozoa that successfully penetrated the cervical mucus. They demonstrated two successful pregnancies after sildenafil treatment.

Ali and Rakkah examined the role of sildenafil on seminal parameters in diabetic males [18]. In this study, 50 insulin-dependent and 50 non-insulin-dependent diabetic males with and without evidence of neuropathy, as well as 50 healthy age-matched males were selected for treatment with sildenafil 100 mg daily for 12 months. Semen was obtained 1 h after intake, and a semen analysis completed. Sildenafil was found to significantly increase sperm motility and semen volume in

the diabetic neuropathics by about 40 % [$p < 0.005$] and 48 % [$p < 0.001$], respectively; there was no significant difference in the non-neuropathics.

No Change

Studies by Hellstrom et al. showed no significant adverse effect of tadalafil after either 6 or 9 months of treatment [24, 25] in men 45 years or older. In the earlier study, tadalafil 10 or 20 mg versus placebo was given daily for 6 months. Semen analyses and reproductive hormones were assessed at baseline and at the end of treatment, following 2–5 days of abstinence. 76–85 % of patients completed this study. Tadalafil was found to be non-inferior to placebo, with no statistically significant difference between placebo and either the 10 or 20 mg dose of tadalafil in subjects that experienced a 50 % or greater decrease in sperm concentration; there was no significant adverse change in sperm morphology or motility.

In the later study, 253 men used either tadalafil 20 mg ($n = 125$) versus placebo ($n = 128$) which was given for 9 months, followed by 6 months of no treatment. Semen analyses and reproductive hormones were evaluated at baseline and every 10–12 weeks, following 2–5 days of abstinence. Seventy-five percent of subjects completed the treatment phase. Tadalafil was again found to be non-inferior to placebo, with no statistically significant difference between placebo and the 20 mg dose of drug among the subjects with a 50 % or greater decrease in sperm concentration. Overall, tadalafil demonstrated no significant adverse effect on sperm production or reproductive hormones in men 45 years or older. Analysis of secondary endpoints in both studies showed no differences in sperm concentration, sperm number per ejaculate, motility and morphology, or serum concentrations of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH).

In a prospective double-blind, placebo-controlled, crossover study, du Plessis et al. demonstrated an enhancement in sperm binding to the oocyte, but no effect on sperm kinetics [19]. The investigators did an in vivo study in which subjects randomly received a single dose of sildenafil (50 mg) or placebo, after having abstained for 3 days prior to treatment. Following a 7-day wash-out period, each patient crossed over to receive the placebo. They also conducted an in vitro study in parallel to explore the effects of increased levels of exogenous cyclic guanosine monophosphate (cGMP) on sperm motility, AR, and sperm–oocyte binding. 8-Bromo-cGMP (8-Br-cGMP), a cGMP analogue, was added to the specimens treated with sildenafil or placebo. Neither sildenafil nor 8-Br-cGMP showed any significant effect on semen parameters, including percentage sperm motility, sperm count, concentration, and morphology. 8-Br-cGMP also did not affect the AR when added to the sildenafil or placebo-treated sperm, although cGMP is known as a signal transducer that mediates the AR. Sildenafil and 8-Br-cGMP did, however, increase sperm–zona pellucida binding. Overall, this study suggests that sildenafil by itself cannot initiate the AR, nor does it produce significant effects on semen parameters.

Andrade et al. reported no effect of sildenafil on sperm motility [20]. In this in vitro study, the investigators mixed semen or washed sperm in various doses of sildenafil (or phentolamine), then analyzed the specimens for motility during a 30-min period. Sildenafil demonstrated no effect on sperm motility at a concentration of 200 μg/mL. (Phentolamine demonstrated a dose-related inhibition.)

Burger et al. also showed no effects on motility, viability, or membrane integrity at a range of sildenafil concentrations (125–750 ng/mL) during a 3-h period [21]. After 2 days of abstinence, sperm from six normal, healthy donors and that from six infertile donors was incubated in the presence of sildenafil, as well as pentoxifylline as a positive control and a reagent control. There was no significant change in motility of normal or infertile sperm at 0, 1, and 3 h of incubation with sildenafil. Pentoxifylline, known to improve sperm motion and capacitation, did demonstrate increased percent motility from normal and infertile sperm donors.

Also, in a double-blind, randomized, placebo-controlled, crossover 2-period study, Aversa et al. found no changes in seminal parameters when a group of 20 men were given sildenafil 100 mg orally, with their semen parameters determined 1 h after treatment [4]. They did demonstrate, however, a reduction in the post-ejaculatory refractory times.

The study by Jarvi et al. revealed no clinically significant effect of vardenafil on sperm concentration, compared with sildenafil and placebo, when given daily for 6 months [22]. These investigators obtained semen analyses and levels of reproductive hormones after 2 and 6 months of treatment with vardenafil 20 mg, sildenafil 100 mg, and placebo. The primary variable was the percentage of vardenafil-treated subjects with a $\geq$50 % decrease in mean sperm concentration from baseline compared to placebo-treated subjects. The secondary variables were semen parameters. Neither vardenafil nor sildenafil was found to have any effect versus placebo.

Purvis et al. demonstrated no effect on sperm motility, count, or ejaculate volume, among other semen parameters [23]. Seventeen healthy male volunteers received a single dose of sildenafil 100 mg for two periods and a single dose of placebo for two periods, each period separated by 5–7 days. The study concluded that sildenafil had not adverse effect on sperm function or ejaculate quality.

Reduced Function

In the aforementioned studies, Andrade et al. revealed a 50 % reduction in sperm motility at high sildenafil concentrations, i.e., 2,000 μg/mL [20] and Ali and Rakkah demonstrated a significant decrease in the total sperm output (63 %) and sperm concentration (45 %) [$p < 0.001$] with chronic sildenafil treatment [18].

In addition, of interest is a study conducted by Khalaf et al., in which they demonstrated overall poor effects of tadalafil on the structure and function of rat testes [26]. The investigators studied the effects of chronic tadalafil administered in 60 old albino rats, divided into three groups: (1) saline orally for 90 days, (2) tadalafil 1.8 mg/kg (equivalent to the 20 mg/day human dose) for 3 months,

and (3) tadalafil 1.8 mg/kg for 6 months. The animals were closely monitored daily, with their body weight and food consumption recorded weekly. They were then sacrificed at the end of the treatment period. The testes were examined grossly in situ; the epididymis was processed for assessment of sperm parameters. The animals in group 3 were found to have a significantly lower average testicular weight. Sperm count was found to exhibit a significant dose-dependent decrease. Sperm motility also decreased significantly in groups 2 and 3, with a worse effect in group 3. Furthermore, there were increased abnormal forms in groups 2 and 3.

Effects of PDE-5i on Spermatogenesis

Along with the numerous effects that PDE-5i may have on sperm characteristics, studies have also reported the effects of these medications on parameters of spermatogenesis including Leydig cell secretory function (LCSF), Sertoli cell secretory function (SCSF), and reproductive hormones including FSH, LH, and testosterone. In the therapeutic use for erectile dysfunction, PDE-5i have had both positive and non-detrimental effects in regard to SCSF, LCSF, spermatogenesis, and reproductive hormone levels.

Among the various roles of the Leydig cells is maintaining an optimal biochemical environment in seminiferous tubules for sperm growth and development. In one study by Dimitriadis et al., LCSF improvement was seen in males with oligoasthenospermia using vardenafil or sildenafil for 12 weeks [27]. The investigators used insulin-like-3 protein (INSL3) as a marker for increased LCSF and found that INSL3 levels were significantly elevated in patients that used vardenafil 10 mg daily or sildenafil 50 mg for 12 weeks compared to patients with no treatment. Testosterone was also elevated in patients using PDE-5i, though the difference was not statistically significant. The mechanism of this increased LCSF in response to PDE-5i is unclear, but it may be related to the presence of PDE-5 receptors on peritubular cells that regulate testosterone synthesis.

This group also used androgen-binding protein (ABP) as a marker for SCSF in a study of 87 males with azoospermia using vardenafil for 14 weeks. They assessed ABP levels, fertilization rates, and the maintenance of foci of advanced spermatogenesis. Again ABP, which is normally secreted by Sertoli cells to concentrate androgens in the seminiferous tubules, was used as a marker for SCSF. Decreased levels of ABP have been shown to represent decreased SCSF. This study assessed the response to vardenafil treatment in 19 patients with obstructive azoospermia, and 68 men with non-obstructive azoospermia. After ABP levels were initially assessed in the whole sample, biopsy was done to detect the presence of spermatozoa. Intracytoplasmic sperm injection cycles (ICSI) were done on these samples, and those unable to achieve pregnancy were extracted into a subset. Treatment with vardenafil was then begun on patients in this subset. At the end of the 14-week trial, increased levels of ABP in these azoospermic males was noted, though fertilization rate via ICSI was not affected. This study also found that in both obstructive and

non-obstructive azoospermic patients, no detrimental effects were observed in small foci of advanced spermatogenesis.

Tadalafil has been tested in men over the age of 45 years with moderate ED to determine whether or not unfavorable adverse effects would arise [24]. A trial of tadalafil in a parallel study which compared 101 patients receiving placebo versus 103 receiving 10 mg daily and 106 patients receiving placebo versus 111 patients receiving 20 mg daily provided similar results. Parameters monitored during the study included semen analysis, testosterone, FSH, and LH at baseline before treatment, 3 months into treatment, and at the end of the 6-month treatment period. Results from the study showed that spermatogenesis and reproductive hormone levels were statistically non-inferior in men receiving treatment compared to those receiving placebo.

Thus, patients using PDE-5i for reasons such as ED may be reassured by these studies that use will, at the very least, not have a detrimental effect on spermatogenesis and reproductive hormone levels.

Capacitation and Acrosomal Reaction

Capacitation

Sperm's ability to prepare for and achieve oocyte penetration via capacitation and the AR may also be affected by PDE-5i. Capacitation involves a modification of proteins and cholesterol derivatives on the exterior sperm membrane after ejaculation in the female genital tract. This allows the sperm to attach to the zona pellucida and begin the AR and dispensing of paternal DNA. Second messenger systems involving cGMP synthesis and cAMP/Protein kinase A interactions have been implicated in capacitation and the AR.

Phosphodiesterase is a universal protein involved in the breakdown of cyclic nucleotides, with various subtypes in different locations. Baxendale et al. used PCR analysis and antibodies to isolate PDE subtypes 1, 4, 6, 8, 10, and 11 from sperm [28]. In regard to specific locations, PDE-1A was found in the flagellum and PDE-4D and PDE-10A both were isolated in the acrosomal region and the flagellum. Inhibition of PDE-4 with rolipram led to increased capacitation and in-vitro fertilization ability. Fournier et al. performed a study on bovine sperm testing the role of PDE inhibition [29]. Since subtypes 1 and 4 have been linked to sperm function and capacitation, this study attempted to assess the effect of PDE-1 and PDE-4 inhibition on capacitation. Sperm were incubated along with either 15 μg/mL heparin as the control group, or the PDE-1 inhibitor vinpocetine and PDE-3 inhibitor rolipram. Capacitation and motility were assessed at 0, 3, and 5 h. Initially at 3 h, increased sperm parameters including capacitation were higher in the vinpocetine group compared to control, though by 5 h there was no difference between the vinpocetine group and control group. Rolipram was ineffective.

Other effects included the fact that vinpocetine reduced sperm mortality more than control. Unfortunately, vinpocetine did not aid in oocyte penetration.

In 2000, Lefievre et al. showed that sildenafil led to a dose-dependent increase in sperm cAMP via inhibition of PDE, which may trigger sperm capacitation [30]. Doses used for treatment included 30, 100, and 200 μmol/L. Sildenafil's ability to improve the AR in this study will be described in the following section. Importantly, this study alludes to the fact that PDE-5i may have a role in influencing PDE subtypes other than 5.

Acrosome Reaction

The process of the AR involves the release of sperm products such as hyaluronidase and acrosin to penetrate the oocyte. In a review done by Biel et al., tricyclic nucleotide cGMP synthesis was implicated in the sperm AR [31]. Sildenafil itself leads to a decrease in cGMP degradation.

Sildenafil's effect on the acrosome reaction was evaluated by Cuadra et al. in 2000 [32]. In this study, sperm were isolated, washed, and incubated with concentrations of Sildenafil ranging from 0 to 40 nmol/L. Fractions of sperm were removed at 0, 4, 24, and 48 h and both sperm motility and the AR were analyzed. A dose-dependent relationship was not found between sildenafil and the AR, though an increase of 50 % over control was found in the percentage of sperm that had undergone the AR. Sperm motility had increased by hour 4, but decreased in the ensuing hours. Conversely, the study done by Lefievre previously mentioned, with improvement in capacitation, found that there was no improvement in the AR in sperm that did and did not undergo acrosomal activity.

Glen et al. studied the effects of sildenafil on premature acrosome activation in 57 males via fluorescein isothiocyanate-labeled peanut agglutinin staining [16]. The sample population was divided into a study group of sperm with the best fertilizing potential and a control group of a poorer population, with 90 and 45 % fractions respectively. Both groups were incubated at 37 °C for 180 min. Sildenafil caused a significant increase in the proportion of acrosome-reacted sperm in both groups. Thus, it was concluded that the use of sildenafil might lead to an increase in premature acrosome activation and a harmful effect on male fertility.

Contractility

Contractile cells surrounding seminiferous tubules, efferent ducts, and epididymal ducts play an important role in spermatozoa transport from the testis to the epididymis [33]. The ability of PDE inhibitors such as sildenafil and tadalafil to have an impact on contractility might depend on their ability to affect the various subtypes of PDE gene families that have been studied in regard to location

and function in the reproductive tract. Specifically, PDE-3 has been shown to contribute to epididymal contractility [34], as well as have PDE-5 in myoid cells of rats [33]. PDE-11 is an important subtype, as it is used as a target for drug therapy by tadalafil. One study in mice showed reduced sperm count in a PDE-11 gene knockout [35, 36].

Middendorff et al. showed on electron microscopy and immunohistochemistry that smooth muscle cells and myofibroblasts were present in the tunica albuginea [37]. Other findings in their study suggested an importance of cGMP regulation in contraction and relaxation in this tissue allowing sperm transport through the tunica albuginea. The contractile tissue is highly regulated by cGMP-generating enzymes and showed significant response to agonists such as atrial natriuretic peptide (ANP) on guanylate cyclase A (GCa) and sodium nitroprusside (SNP) on soluble guanylate cyclase (sGC). Along with this, nitric oxide (NO) synthase was identified in the inner zone of the tunica albuginea where contractile cells are located. Increases in spontaneous contractions of these cells near the rete testis had also been revealed physiologically. These responses were opposed by the addition of cGMP, SNP, and ANP. SNP reduced the amplitude of these spontaneous contractions, while ANP reduced both contraction amplitude and frequency of contractions. This was also seen in noradrenaline-induced contractions, where SNP was able to inhibit contractions throughout the testicular capsule [38]. Thus, with evidence revealing an important responsibility of cGMP in regulating contractions in the tunica albuginea, PDE-5i may play an important role in improving fertility in patients with pathology involving decreased sperm transport.

In addition to decreasing cGMP breakdown, one study by Sundkvist et al. also showed that inhibition of PDE resulted in improvements in concentrating cGMP intracellularly [39]. After isolating inside-out vesicles from fresh blood, they were incubated with [3H]-cGMP with or without cGMP efflux inhibitors for 120 min at 37 °C. One inhibitor, sildenafil, showed a high affinity for the efflux pump responsible for actively transporting cGMP extracellularly. Thus, PDE-5i may be useful not only in decreasing breakdown of cGMP, but also in prohibiting cellular efflux of cGMP.

Epididymis

A study by Mewe et al. showed that cGMP regulates contractility in the epididymis by causing smooth muscle relaxation [40]. In this study, evaluation of the role of cGMP was conducted by using cGMP analogs and subsequently performing muscle tension recordings, immunological techniques, and autoradiographic techniques. Protein kinase G (PKG), GCs, and endothelial nitric oxide synthase (eNOS) were localized to epididymal muscle cells. Contractions were found to be dependent on cGMP and inhibition of eNOS and PKG led to increased frequencies of smooth muscle contractions. These data emphasize the importance of cGMP signaling in

the control of epididymal peristalsis and aiding in sperm transport and maturation. Translation of these findings to medical therapy, however, has been lacking.

Vas Deferens

PDE-5i may have a more promising role in contractility of the vas deferens. PDE-5i have been studied in regard to their effects on premature ejaculation. Chen et al. showed an improvement in rates of premature ejaculation in patients using sildenafil with paroxetine as opposed to paroxetine alone [41]. This study involved 138 men with moderate primary premature ejaculation as determined by frequency of premature ejaculation on a scale of 0–8 and intravaginal ejaculatory latency time on a scale of 0–3. These scales were also used to measure improvement throughout the study. Treatment was done in a stepwise manner. Initial treatment included topical 5 % lidocaine ointment. Dissatisfied patients took one tablet of paroxetine 20 mg for 30 days as well as one tablet 7 h before intercourse. Those that were dissatisfied with that treatment began sildenafil. The end result showed that 56 of the 58 patients taking sildenafil reported significant improvement in frequency of premature ejaculation and intravaginal ejaculatory latency time.

In a study by Bilge et al., the role of PDE inhibition was examined in relation to contractility [42]. Epididymal and prostatic portions of isolated vas deferens were exposed to noradrenaline, adenosine triphosphate (ATP), alpha, beta-methylene ATP and electrical field stimulation (EFS). Contractions were measured after sildenafil was added, and it was found that contractions caused by ATP and EFS were inhibited, while those caused by noradrenaline and alpha, beta-methylene ATP were unaffected.

Medina et al. studied the effects of sildenafil on the activation of prejunctional potassium channels in tissue from the vas deferens [43]. They found that sildenafil was able to inhibit electrically induced contractions of ring segments of human vas deferens tissue from 34 vasectomies. An inhibitor of guanylate cyclase did not affect this result, but its inhibitory effect was stopped by a potassium channel blocker. This means that sildenafil also works in a manner independent of the cGMP second messenger system and attenuates adrenergic neurotransmission in human vas deferens. This is more likely due to the activation of prejunctional potassium channels.

Seminal Vesicle Effects

Previous studies of the secretory function of the SV utilizing fructose concentrations in seminal plasma have revealed no differences in men treated with PDE-5i [9]. However, several studies have noted functional features in the SV that may be affected by PDE-5i [44–47]. In a study evaluating infertile men determined to have

infection induced hypertrophic congestive SV on ultrasound, daily 5 mg tadalafil resulted in improved ultrasound appearance and improvement in total sperm count progressive motility, seminal levels of fructose, and ejaculate volume [48].

Prostate

PDE-5i have been shown to upregulate several prostatic secretions that enhance sperm quality including citrate, zinc, spermine, and semen cholesterol [49]. Citrate helps maintain the osmotic equilibrium of prostate. Zinc stabilizes the sperm nucleus and has antibiotic properties. Spermine correlates with sperm motility. Semen cholesterol stabilizes sperm against environmental stressors and temperature.

Conclusion

PDE-5i are well known for their therapeutic role in the treatment of ED. They are also understood to affect male infertility in varying capacities, including an overall non-detrimental effect on sperm and testicular function as demonstrated in multiple studies. Notably in infertile patients, there are no clear negative effects of the PDE-5i on spermatogenesis or reproductive hormone levels, which is altogether encouraging as these medications are often utilized, as male patients prepare for their role in IUI and in vitro fertilization cycles. In therapeutic doses, PDE-5i have indeed been shown to ultimately improve both the erectile and ejaculatory dysfunction during infertility treatments, and to sometimes improve progressive sperm motility, semen volume, total sperm concentration, capacitation, and contractility. The AR has not been shown to improve with PDE-5i, rather PDE-5i may contribute to a premature AR, although not significantly. These agents have primarily positive or no effect on the vas deferens, SV, and prostate tissues.

Overall, there is a demand for additional research to more definitively identify a consistent role for PDE-5i in testicular and sperm function, given the few studies that refute, and the multiple that collectively demonstrate a positive effect.

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