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Guanylate Cyclase Activity and Sperm Function

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In species with external fertilization, the guanylate cyclase family is responsible for the long-distance interaction between gametes, as its activation allows sperm chemotaxis toward egg-derived substances, gamete encounter, and fertilization. In species with internal fertilization, guanylate cyclase-activating substances, which are secreted by several tissues in the genital tracts of both sexes, deeply affect sperm motility, capacitation, and acrosomal reactivity, stimulating sperm metabolism and promoting the ability of the sperm to approach the oocyte, interact with it, and finally fertilize it. A complex system of intracellular pathways is activated by guanylate cyclase agonists in spermatozoa. Sperm motility appears to be affected mainly through an increase in intracellular cAMP, whereas the acrosome reaction depends more directly on cyclic GMP synthesis. Both cyclic nucleotides activate specific kinases and ion signals. A complex cross-talk between cAMP- and cyclic GMP-generating systems occurs, resulting in an upward shift in sperm function. Excessive amounts of certain guanylate cyclase activators might exert opposite, antireproductive effects, increasing the oxidative stress on sperm membranes. In view of the marked influence exerted by guanylate cyclase-activating substances on sperm function, it seems likely that guanylate cyclase activation or inhibition may represent a new approach for the diagnosis and treatment of male and/or female infertility. (*Endocrine Reviews* 23: 484–494, 2002)

I. Introduction: The Guanylate Cyclase Family

CYCLIC GMP (cGMP) was described as a biological product in 1963, but for many years it was not considered as a potential second messenger. There are several reasons for this, including its relatively low concentration in the tissues (1). It is now clear, however, that cGMP is a key signaling molecule in many tissue functions, such as retinal phototransduction, intestinal secretion, smooth muscle relaxation, platelet activation, and neurotransmission (2). Guanylate cyclases, the ubiquitous enzymes that catalyze the conversion of GTP to cGMP, are expressed in both soluble (sGC) and particulate, membrane-bound (mGC) isoforms (Fig. 1). These isoforms coexist in most cells, where their relative amount depends on the type and physiological state of the tissue (1).

The known guanylate cyclase catalytic domains are homologous to the mammalian adenylate cyclase C1 and C2 catalytic regions (2). The recent resolution of the crystal structure of adenylate cyclases provided important clues to the structure of guanylate cyclases (3). An amphipathic α-helix (hinge region) divides the intracellular part of guanylate cyclase into 1) a protein kinase homology domain in mGC or a putative heme-binding region in sGC, and 2) a cyclase homology domain in both mGC and sGC. In addition, mGC contains an extracellular ligand-binding domain, which recognizes a variety of different peptides (3).

The mGC is a cell surface receptor enzyme that contains an extracellular receptor domain and an intracellular catalytic domain separated by a single transmembrane domain (4). Eight subclasses of mGC have been identified so far in vertebrates: they are homodimeric glycoproteins (3, 5), which may be associated with the plasma membrane, the endoplasmic reticulum, the Golgi bodies, and the nuclear membrane (1). Some mGC subclasses (GC-A, GC-B) appear to represent the receptors for three structurally similar peptides termed natriuretic peptides, which contain 22–32 amino acids and are characterized by a 17-amino acid disulfide ring (6). Atrial natriuretic peptide (ANP) and the B-type natriuretic peptide appear to be synthesized principally in the heart and to circulate in the bloodstream, whereas the C-type natriuretic peptide is distributed in a variety of tissues, is not

Abbreviations: ANP, Atrial natriuretic peptide; AR, acrosome reaction; cGMP, cyclic GMP; mGC, membrane-bound guanylate cyclase; NOS, nitric oxide synthase; PDE, phosphodiesterase; PKC, protein kinase C; PKGI and PKGII, protein kinase GI and GII; SAP-I, sperm-activating peptide I; sGC, soluble guanylate cyclase.

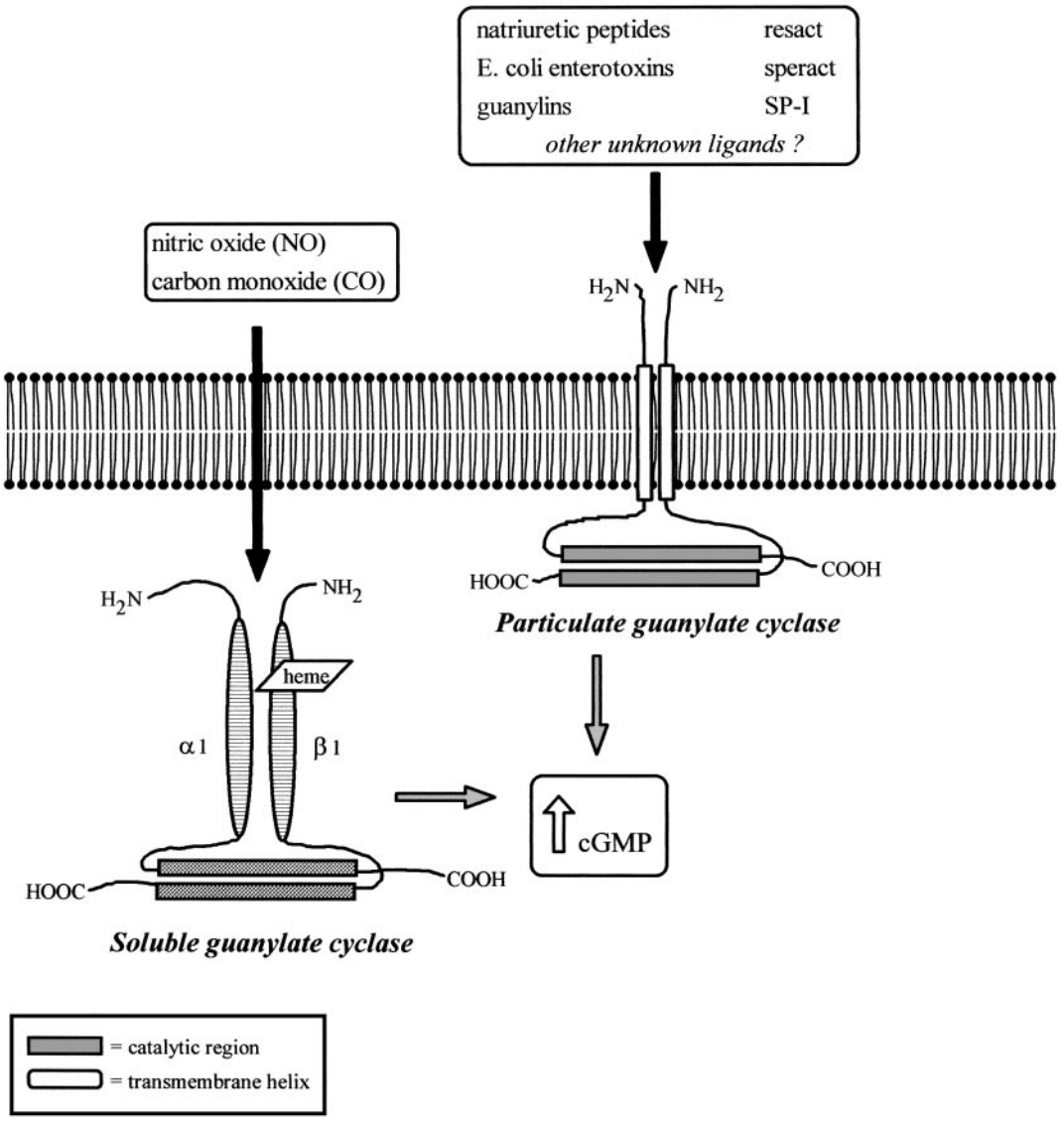

FIG. 1. Schematic representation of the guanylate cyclase isoforms. COOH, Carboxy-terminal end; NH_2, amino-terminal end. [Modified from J. W. Denninger and M. A. Marletta: *Biochim Biophys Acta* 1411:334–350, 1999 (4) with permission from Elsevier Science.]

found in appreciable amounts in the blood, and may therefore act locally (7). The mGC subclass GC-A binds with higher affinity to ANP and the B-type natriuretic peptide, whereas GC-B binds with higher affinity to the C-type natriuretic peptide (3). The mGC subclasses GC-C (cloned from rat intestine) and OK-GC (cloned from opossum kidney) bind the heat-stable enterotoxin of *Escherichia coli* (18–19 amino acids). Guanylin, uroguanylin, and lymphoguanylin, three 15-residue peptides isolated from rat intestinal mucosa, opossum urine, and opossum lymphoid tissues, respectively, were identified as physiological ligands of GC-C and OK-GC (3, 8). Molecular cloning of cDNAs led to the identification of the mGC subclasses GC-D (olfactory epithelium), GC-E, GC-F (retina, pineal gland), and GC-G (lung, intestine, skeletal muscle), but no ligands have been identified for these receptors (termed "orphan" mGCs) (3, 4). The mGC is regulated by phosphorylation and by Ca^{2+}-binding proteins (6).

The soluble isoform of guanylate cyclase (sGC) includes a group of heterodimeric hemoproteins composed of α- and β-subunits (5). Four distinct sGC subunits ($\alpha1$, $\alpha2$, $\beta1$, and $\beta2$) have been cloned, but the only sGCs shown to exist as a protein in animal tissues are $\alpha1/\beta1$ and $\alpha2/\beta1$: the existence of other sGCs *in vivo* is still a matter of debate (3, 4). The $\beta2$-subunit, which contains a consensus isoprenylation site, was shown to inhibit the NO-stimulated activity of the $\alpha1/\beta1$ heterodimer (9). The guanylate cyclase sGC isoform contains a prosthetic heme group on each heterodimer at the level of the $\beta1$-subunit (4). The heme moiety can bind diffusible gases such as nitric oxide (NO) and carbon monoxide (3). After such binding, the enzyme's catalytic activity is enhanced from 5-fold (with carbon monoxide) to 400-fold (with NO) (4).

NO is a short-lived, free radical gas synthesized in many mammalian cell types (10) by a class of reduced nicotinamide adenine dinucleotide phosphate-dependent NO synthases (NOSs) (Fig. 2), which catalyze the conversion of L-arginine to L-citrulline and NO with a stoichiometry of 1:1 and are

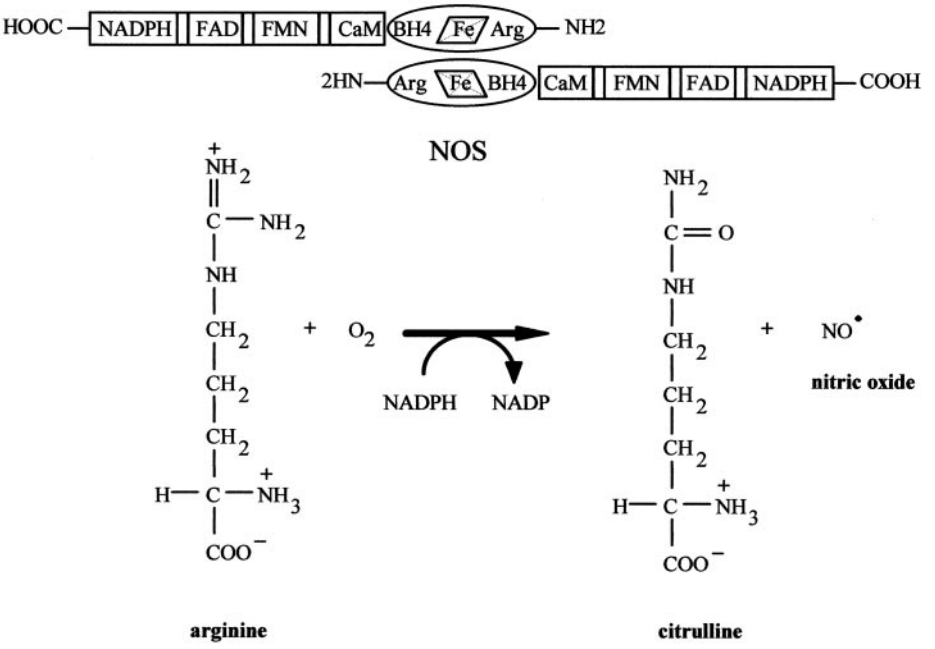

FIG. 2. Schematic representation of the NOS dimer and of NO synthesis. Active NOS is a homodimeric protein: each monomer contains a binding site for reduced nicotinamide adenine dinucleotide phosphate (NADPH), flavine mononucleotide (FMN), flavine adenine dinucleotide (FAD), calmodulin (CaM), tetrahydrobiopterine (BH4), heme (Fe), and the substrate L-arginine (Arg). –COOH, Carboxy-terminal end; NH_2, amino-terminal end. [Modified from D. J. Stuehr: *Annu Rev Pharmacol Toxicol* 37:339–359, 1997 (13). © by Annual Reviews. www.AnnualReviews.org]

competitively inhibited by L-arginine analogs (11). Three different NOS isoenzymes have been characterized to date (12, 13). Two of them are constitutive, Ca^{2+}/calmodulin-activated isoforms (endothelial NOS and neuronal NOS), and one is an inducible, Ca^{2+}-independent isoform detected in macrophages. NO, an ubiquitous mediator of cell-to-cell interaction, is produced in response to a large number of stimuli and displays a wide spectrum of activities, such as smooth muscle relaxation, inhibition of platelet aggregation and adhesion, neurotransmission, and cytotoxicity (14). The activation of sGC and the subsequent induction of cGMP synthesis mediate the effects of NO on vascular smooth muscle cell relaxation and growth, as well as on platelet aggregation and on the adherence of neutrophils to endothelial cells (15).

II. Activators of Guanylate Cyclase in Spermatozoa

The spermatozoon of invertebrate species and of mammals possesses both the soluble and the membrane-bound isoforms of guanylate cyclase (16). The mGC isoform is the most intensively studied in invertebrates, because it participates in the complex mechanisms of gamete recognition over relatively long distances in the open environment and is therefore important for the reproduction of species with extracorporeal fertilization. The mGC isoform acts as a cell-surface receptor on spermatozoa for species-specific chemotactic substances released by the eggs of some species, such as sea urchins. This isoform exists in the sperm cells of echinoderms as two different subclasses (GC-A and GC-B) with similar ligand specificities (17).

The mGC molecule is a large glycoprotein consisting of 986 amino acids, with an oligosaccharidic component containing *N*-acetylglucosamine, mannose, galactose, and 2-amino-erythritol (18). Effector substances acting on sperm mGC in echinoderms are small, species-specific peptides produced by the jelly layer that coats the egg. Overall, 75 sperm-activating peptides have been purified from the solubilized jelly layer of 17 different species of sea urchins. Their biological effects are practically the same in all sea urchin spermatozoa but appear to be species-specific according to the taxonomic ordinal level (19). The best known mGC-activating substances in spermatozoa are the following:

1. Resact. This 14-amino-acid peptide, associated with the eggs of the echinoderm *Arbacia punctulata,* has sperm-specific and species-specific chemotactic properties and stimulates oxygen consumption, motility, and cGMP synthesis in *A. punctulata* spermatozoa (20).

2. Speract. In two species of sea urchins, *Strongylocentrotus purpuratus* and *Lytechinus pictus*, this decapeptide, which is associated with the egg jelly, binds to specific sperm surface receptors inducing an increase in sperm motility and respiration rate (21, 22). The spermatozoa of *L. pictus* possess a surface receptor that responds to picomolar concentrations of the jelly coat-secreted guanylate cyclase activator speract (23). The association rate of speract to the receptor is markedly reduced in seawater that is free of Na^+, rich in K^+, and contains no divalent cations, but is increased by substances that raise the intracellular pH (23). In turn, binding of speract to its receptor elicits an abrupt increase in intracellular pH (23).

3. Sperm-activating peptide-I (SAP-I). This decapeptide has been isolated from the solubilized jelly layer of *Hemicentrotus pulcherrimus*, which stimulates the respiration, motility, and acrosomal rectivity of *H. pulcherrimus* spermatozoa. SAP-I increases cAMP and cGMP and intracellular pH and Ca^{2+} and activates the Na^+/H^+ antiporter in *H. pulcherrimus* spermatozoa (19). It was postulated that two classes of receptors for SAP-I exist on the sperm plasma membrane: high-affinity receptors, which mediate the respiration-stimulating activity

and intracellular pH elevation, and low-affinity receptors that might be responsible for the rise in cGMP and intracellular Ca^{2+} (19).

4. Natriuretic peptides. Specific binding sites for ANP have been detected and localized in viable human spermatozoa (24).

NO is thought to be the main activator of the soluble isoform of guanylate cyclase in spermatozoa. It has significant effects on the function of the male genitourinary system of rodents (25) and humans (26). Because NO-producing tissues have been also identified in the adult human female upper genital tract (27, 28), it is likely that NO affects sperm function as the sperm approaches the oocyte. Spermatozoa themselves express a NOS activity and are able to synthesize NO (29–33): their NO production can be enhanced by specific chemical stimuli (31, 33). Studies have clearly demonstrated the presence of endothelial (29, 32, 33) and neuronal (29, 30) NOS isoforms in human spermatozoa. The NO donor sodium nitroprusside increases the intracellular cGMP levels of the spermatozoa of bulls (34) and humans (35, 36); another NO donor, spermine-NONOate, raises the cGMP concentration in murine spermatozoa (37). A mechanism of negative feedback regulation seems to be active, because NO released from sodium nitroprusside has been shown to desensitize sGC (38), leading to a progressive decrease of cGMP synthesis.

III. Guanylate Cyclase Activity and Sperm Functions

A. Spermatogenesis and sperm transport

The soluble isoform of guanylate cyclase and its product cGMP have been detected by immunohistochemistry in the peritubular lamina of seminiferous tubules and in the blood vessels of human testis (39). The concentration of nitrites and nitrates, the final stable metabolites of NO, is inversely correlated with the pulsatility index of the transmediastinal artery, a variable that expresses vascular resistance in intratesticular blood vessels (40). The endothelial and neuronal isoforms of NOS were localized in the seminiferous tubules, where NOS activity was demonstrated by decreased cGMP synthesis upon incubation with the NOS inhibitor N^G-nitro-L-arginine methyl ester (39). Endothelial NOS is expressed in Sertoli and Leydig cells in the human (41, 42) and rat (43) testis; in the germ cell line, endothelial NOS is not detectable in viable cells, but only in the cytoplasm of degenerating, apoptotic cells (41, 43). Furthermore, germ cell apoptosis in the rat testis is reduced by administration of NOS inhibitors (44), suggesting a role for NO in spermatogenesis and germ cell apoptosis. Interestingly, the expression of the inducible NOS isoform within the rat testis appears to be enhanced by intratesticular ischemia (45) and inflammation (46), suggesting that the seminiferous epithelium damage caused by some pathological events (*e.g.*, testicular torsion or orchitis) may be linked to a huge local NO production.

In adult rats, NOS immunostaining revealed the presence of nitrinergic nerve fibers along the length of the vas deferens, possibly involved in the regulation of unidirectional sperm transport (47). Even in the human, endothelial NOS was immunohistochemically localized in the epithelium of epididymis and vas deferens (42). According to some authors (40), the concentration of nitrites and nitrates in seminal plasma is higher in normozoospermic subjects than in oligozoospermic or azoospermic patients. This observation suggests that local NO synthesis may be involved in determining the difference in sperm transport between normozoospermic and oligozoospermic men in response to sequential ejaculations within 1–4 h and 24 h (48, 49). Sildenafil, a potent cGMP-dependent phosphodiesterase (type V) blocker that elicits an increase of cGMP levels, may also affect sperm transport, especially in men with erectile disorders (50). Taken together, these data suggest that NO may be part of the regulatory mechanisms of sperm output by human testicles and of sperm transport along the male genital tract.

B. Sperm motility

The motility of human spermatozoa is inhibited by the NOS inhibitor N^G-nitro-L-arginine methyl ester (29) and by the NO scavenger methylene blue (31). These findings suggest that the NO endogenously synthesized by the spermatozoon is necessary to support motility. Even exogenous NO released by sodium nitroprusside can induce an *in vitro* hyperactivated motility of mouse spermatozoa (51) and is able to maintain the motility and viability of frozen-thawed human spermatozoa (52). The cGMP-dependent phosphodiesterase inhibitor sildenafil was reported by some authors (53), but not by others (54–56), to increase the velocity and amplitude of lateral head displacement in human spermatozoa. Low concentrations of NO enhance the *in vitro* motility of hamster (57) and human spermatozoa (29, 35), whereas at higher concentrations NO inhibits the motility of mouse (51) and human spermatozoa (58). High concentrations of NO are also able to affect sperm viability (56, 59, 60), displaying a cytotoxic effect that is probably mediated by oxidative stress and lipid peroxidation of sperm membranes (61).

Seminal plasma concentration of nitrites and nitrates, which reflects the local *in vivo* NO release, was found to be higher in infertile than in fertile men (60) and to correlate with the percentage of immotile spermatozoa (59), leading to the hypothesis that infection and/or flogosis in semen can affect sperm function via induction of excessive NO synthesis by leukocytes or reproductive epithelia. It was even claimed that the dysfunction in sperm motility observed in patients with varicocele depends on excessive local NO synthesis, as nitrite concentration in the dilated varicocele vein is significantly higher than in the peripheral circulation (62), and NO concentration in the seminal plasma of patients with varicocele is higher than that of the controls (63). In other studies, however, the concentration of nitrites and nitrates in seminal plasma was higher in normozoospermic subjects than in oligozoospermic or azoospermic patients (40), and no correlation between nitrites in seminal plasma and the presence of leukocytospermia and/or a positive sperm culture was observed (64). A possible explanation of these contradictory findings is that seminal plasma nitrites could not clearly correspond to the actual NO synthesis in the male genital tract, as nitrogen oxides could be locally transformed into some other compounds not detectable as nitrite/nitrate. The sensitivity of nitrite/nitrate concentration in seminal

plasma as a marker of local NO formation and sperm oxidative stress needs further investigation.

C. Acrosome reaction (AR)

Human follicular fluid contains variable amounts of ANP, with maximal concentrations found in follicles containing oocytes that are subsequently fertilized *in vitro* (65). The ANP from several species is able to induce the AR in humans, but the greatest response is elicited by human ANP (65). The AR-inducing effect of ANP can be observed in both capacitated and noncapacitated spermatozoa. In the latter, it is independent of extracellular Ca^{2+}, is mediated by cGMP synthesis (66), and may be completely inhibited by the addition of 1 μM LY83583, a guanylate cyclase inhibitor (65). The possibility of a complete or partial AR in noncapacitated spermatozoa, observed by some (67) and denied by others (68), may be linked to the activation of alternative pathways that bypass the classical membrane-mediated events leading to acrosomal exocytosis. It was also shown that different AR inducers or the use of different probes to measure AR may explain some of the discrepancies in the findings of different researchers (69). ANP markedly stimulates the AR of capacitated bull spermatozoa in a Ca^{2+}-dependent way. This effect is a result of interaction with GC-A, whose activation elicits cGMP synthesis (34). The AR-inducing effect of ANP in this experimental model may be abolished by the competitive GC-A antagonist anantin and restored by addition of the cGMP analog 8-bromo-cGMP (34).

Uncapacitated human spermatozoa produce low levels of NO, whereas under capacitating conditions, a time-dependent increase in NO synthesis has been observed (70). Studies *in vitro* have shown that low concentrations of NO enhance the AR of mouse (71) and bull (34) spermatozoa, as well as the zona pellucida-binding ability of human spermatozoa (72). Activation of endothelial NOS is implicated in the follicular fluid-induced AR of human spermatozoa (33). Moreover, NO donors such as sodium nitroprusside, 3,3-bis(aminoethyl)-1-hydroxy-2-oxo-1-triazene (DETA-NONOate), and *S*-nitroso-*N*-acetylpenicillamine are able to stimulate the AR of human spermatozoa in a specific and dose-dependent way (33, 73). The AR-inducing effect of sodium nitroprusside is abolished by the guanylate cyclase inhibitors LY83583 and 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ) and can be recovered by the addition of 8-bromo-cGMP (36).

D. Sperm chemotaxis

Sperm chemotaxis is defined as an oriented movement in response to a chemical gradient, resulting in an approach toward the chemical attractant, or in retreat from a chemical repellent. Sperm chemotaxis in humans was demonstrated *in vitro*. Recent studies suggest that its role is to select the fertilizing capacitated spermatozoa and to ensure their continuous availability for an extended period of time (for a comprehensive review see Ref. 74). In some invertebrate species, whose gametes are spawned into water before fertilization, chemotaxis is a key event in reproduction as the spermatozoa must be guided toward the eggs over relatively long distances. Egg-derived, species-specific peptides able to attract spermatozoa have been identified and purified in echinoderms, whose spermatozoa have specific surface receptors for these chemotactic substances (75). Activation of such receptors, which belong to the mGC family, causes an increase in intracellular cGMP (76) and mediates ion fluxes across the sperm membrane (77); in turn, this affects flagellar motion and finally determines the direction of movement (78). In species with internal fertilization, and particularly in mammals, chemotaxis may appear to be less crucial, as millions of spermatozoa are ejaculated directly into the female genital tract, and many of them can be stored in the cervix and/or in the oviduct (79), not far from the fertilization site. It was calculated, however, that only about 1 in 25,000 spermatozoa inseminated into the vagina reaches the fallopian tubes and, considering the overall surface of the tubal epithelium as well as the oocyte's volume, the chance of a chaotic successful collision between gametes is statistically minimal (74, 80). In humans, the existence of some communication at distance between the gametes could positively affect their chance of meeting and, finally, the chance of fertilization. Interestingly, the tubal ampulla in which the human egg resides contains a significantly higher number of sperm cells than the contralateral tube (81).

A chemical attraction to spermatozoa could be caused by a substance (or substances) whose concentration increases from the lower to the upper part of the salpinx. Such substance could be present in the fluid surrounding the oocyte or secreted by cells surrounding the egg. The attention of researchers in this area has been focused mainly on follicular fluid (74, 82). In mammals, sperm chemotaxis and chemokinesis to a follicular factor(s) has been established and has been distinguished from other processes that might cause sperm accumulation (83–85). Human follicular fluid contains ANP (86, 87), and specific receptors for ANP have been identified on the surface of human spermatozoa (24). Experiments accomplished by accumulation (66) and choice assays (88) led to the hypothesis of sperm chemotaxis exerted by ANP toward human spermatozoa. However, the role of ANP as sperm attractant is questionable because no correlation was found between the chemotactic activity of a given follicular fluid and its ANP content (66). Moreover, sperm chemotaxis to ANP at physiological concentrations is observed only when phosphoramidon, a neutral endopeptidase inhibitor probably absent *in vivo*, is added to the system (88). Thus, ANP might simply be a substance capable of activating mGC *in vitro* in a way similar to that caused by the physiological attractant *in vivo*. As to other putative sperm chemoattractants contained in follicular fluid, such as progesterone (89) and N-formylated peptides (90), there is no evidence for the involvement of mGC in their signaling.

In a recent study, an accumulation assay was used to examine the possible role of NO in sperm chemotaxis (91). Mouse sperm was allowed to migrate in an experimental plate to medium containing sodium nitroprusside (50 nM) or to a control medium: after 3 or 5 h of incubation, sperm concentration was significantly higher in the medium containing the NO donor. This finding, however, could not be attributed unequivocally to chemotaxis, as in accumulation assays a "trapping" phenomenon caused by the onset of nonlinear, hyperactivated sperm motility cannot be ex-

cluded. The role of NO in mammalian sperm chemotaxis still awaits clarification.

E. Sperm-egg interaction

NO is claimed to play a role in sperm-egg interaction (zona pellucida binding, sperm-oocyte fusion), as well as in the egg activation after sperm penetration. Incubation of human spermatozoa with the NOS inhibitor N^G-nitro-L-arginine methyl ester was found to inhibit their ability to penetrate zona-free hamster eggs *in vitro*, but not their ability to bind to the human zona pellucida in the hemizona assay (92).

The freshly laid oocytes of the fathead minnow *Pimephelas promelas* express NOS protein near the site of sperm entry and transiently produce NO a few seconds before fertilization (93). An abrupt increase in nitrosylation within oocytes was recorded a few seconds after insemination, just before the onset of intracellular Ca^{2+} oscillations, a phenomenon that represents a typical egg response to fertilization in several species and is known to influence early embryo development (94). Microinjection of NO donors or recombinant NOS can mimic egg activation after fertilization, whereas an oocyte preload with oxyhemoglobin, a physiological NO scavenger, prevents Ca^{2+} pulses in fertilized eggs (94). On the contrary, in chordate eggs, the sperm-induced Ca^{2+} rise is not associated with any change in intracellular NO and is not affected by the presence of the NOS inhibitor N^G-nitro-L-arginine methyl ester (95). In conclusion, the role played by NO in egg activation and fertilization is still unclear, and further studies are needed to clarify it.

IV. Signaling Pathways Following Guanylate Cyclase Activation in Spermatozoa

A. Changes in guanylate cyclase phosphorylation state

After the binding of chemotactic peptides, the phosphorylation level of mGC changes, affecting the extent of its activation (16, 76). In sperm cells of the sea urchin *A. punctulata*, mGC is a major glycoprotein of the flagellar plasma membrane; when the jelly layer peptide resact binds to mGC, it triggers dephosphorylation of the enzyme. Each mGC molecule loses approximately 15 phosphate groups, resulting in a sudden decrease (within 1 min) in the enzyme activity (96). Similarly, when intact *S. purpuratus* spermatozoa are incubated with the egg-derived peptide speract, the activity of mGC decreases, its apparent molecular weight shifts, and there is a detectable loss of ^{32}P label from the enzyme (97).

The phosphorylation state of mGC modulates the absolute activity of the enzyme and the extent of interaction between the catalytic site and the GTP-binding site (98). If a preparation containing protein phosphatase from *E. coli* is used to catalyze the dephosphorylation of the enzyme, there is a sudden decrease in mGC activity, together with a rapid decrease in the molecular mass (from 160 to 150 kDa) (98). The regulatory site for phosphorylation is likely to be the mGC carboxy-terminal 95-amino acid portion, which contains 20% serine (98, 99).

Extracellular pH is an important factor in determining the mGC phosphorylation state. Sea urchin spermatozoa incubated in seawater containing ammonia (pH 8.8) undergo sudden mGC dephosphorylation, with accompanying changes in molecular mass and enzymatic activity. Transfer of the cells into ammonia-free seawater (pH 7.4) results in rephosphorylation, reconversion to a 160-kDa mass, and recovery of the initial enzymatic activity (100).

B. Increase in cGMP and cAMP

Early biochemical responses of spermatozoa to resact (*A. punctulata*) or speract (*S. purpuratus*) include H^+ efflux and an increase in cAMP (other than cGMP) concentration (20). Human spermatozoa subjected to capacitating conditions increase their endogenous NO synthesis and their intracellular cAMP content; the latter is further increased by NO-releasing compounds and conversely decreased by incubation with NOS inhibitors (70). Furthermore, the cGMP-specific phosphodiesterase (type V) inhibitor sildenafil causes a dose-dependent cAMP increase in human spermatozoa (53), and human spermatozoa incubated with NO releasers under capacitating conditions show a marked increase in intracellular cAMP concentrations (70). Activation of adenylate cyclase requires an increase in intracellular pH, which appears to be critical in determining whether cGMP-mediated or cAMP-mediated pathways predominate in speract signal transduction (101). Speract-induced accumulation of cGMP and cAMP is enhanced by phosphodiesterase inhibitors (53). In addition, the sperm-activating peptide SAP-V, isolated from the egg jelly of *Brissus agassizii*, stimulates sperm respiration and thereby increases intracellular cAMP and cGMP levels in a concentration-dependent manner (102). The concomitant increase of both cyclic nucleotides is suggestive of a cross-talk between the cAMP and cGMP signaling pathways, a phenomenon that is facilitated in many tissues by the presence of cGMP-regulated phosphodiesterase isoforms. Of the seven known phosphodiesterase isoforms, three (PDE-II, PDE-V, and PDE-VI) are allosterically regulated by cGMP, and one (PDE-III) is inhibited by the binding of cGMP to its active site (4). Thus, an increase in cGMP could evoke a concomitant increase in cAMP by inhibiting its PDE-III-catalyzed hydrolysis to AMP, as observed in human platelets and vascular smooth muscle cells (103, 104) (Fig. 3). An alternative way of increasing intracellular cAMP levels occurs in rat liver and other tissues, where sGC, stimulated by NO, undergoes striking modifications in its activity, becoming able to synthesize cAMP too (105).

Speract and resact markedly stimulate the incorporation of ^{32}P into various proteins of isolated sperm membranes in the presence, but not in the absence, of GTP, the substrate of guanylate cyclases. The addition of cAMP and cGMP further stimulates protein phosphorylation in the same domains as those phosphorylated by egg peptides plus GTP, indicating that a peptide-induced increase in intracellular cyclic nucleotides is responsible for the observed changes in the phosphorylation state of proteins on the plasma membrane (106). In human spermatozoa, the potent cGMP-dependent phosphodiesterase blocker sildenafil markedly increases the tyrosine phosphorylation of two proteins belonging to the fibrous sheet (p105 and p81), increasing sperm velocity, lateral head displacement, and sperm capacitation (53). When ca-

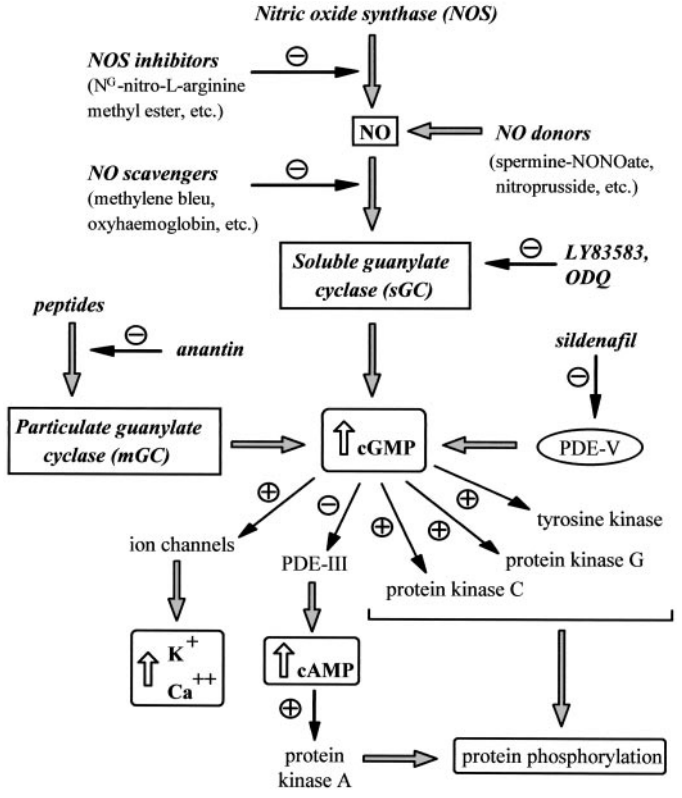

FIG. 3. Schematic representation of the NO/cGMP pathway and its inhibitors and activators. PDE, Phosphodiesterase; protein kinase G, cGMP-dependent protein kinases; protein kinase A, cAMP-dependent protein kinase.

pacitation is stimulated in the presence of NO-releasing compounds, the overall tyrosine phosphorylation level in human spermatozoa significantly increases (70).

C. Changes in intracellular ions concentration

Experimental data indicate that a specific Ca^{2+}-activated K^+ channel participates in an early phase of speract signal transduction in sea urchin spermatozoa (107). This hyperpolarizing effect of speract on the membrane potential involves intracellular GTP (108). Activation of K^+ channels is indeed mediated by cGMP; the speract-induced increase in cGMP content seems to directly mediate K^+ channel opening, which in turn causes hyperpolarization of the sperm membrane (109) (Fig. 3). The resulting increase in intracellular pH leads to mGC inactivation and K^+ channel closure; however, K^+ permeability may be restored upon subsequent increase in intracellular Ca^{2+} (107).

An increase in intracellular cGMP precedes the major increase in cytoplasmic free Ca^{2+} (78, 110) (Fig. 3). In sperm, this increase appears to depend on the influx of extracellular Ca^{2+}, as no detectable Ca^{2+} response to 8-bromo-cGMP is observed in Ca^{2+}-free medium or in the presence of the Ca^{2+} channel blocker pimozide (111). The need for extracellular Ca^{2+} in mammalian sperm AR has often been reported (reviewed in Ref. 112). Ca^{2+}-dependence was observed during the induction of human sperm AR by ANP (113), although it should be mentioned that high concentrations of ANP elicit the AR even in noncapacitated human spermatozoa and/or in the absence of Ca^{2+} (65). The NO donor sodium nitroprusside appears to be ineffective in inducing the AR when spermatozoa are incubated in a Ca^{2+}-free, EDTA-containing medium (36).

The role of cyclic ADP-ribose in mammalian spermatozoa activation still awaits clarification. It is now known that sea urchin sperm cells can synthesize cyclic ADP-ribose and that they contain this compound in amounts comparable to those measured in other tissues (114). In several cell types (gut interstitial cells, macrophages, pancreatic β-cells) NO increases intracellular Ca^{2+} via activation of a ryanodine receptor, which allows ion efflux from inositol-1,4,5-trisphosphate-insensitive Ca^{2+} pools (115). In sea urchin eggs, NO and cGMP seem to mobilize Ca^{2+} from intracellular stores by inducing synthesis of cyclic ADP-ribose, a putative agonist of the ryanodine receptor (115). In mammalian spermatozoa, the induction of AR is accomplished by an influx of external Ca^{2+} via opening of voltage-dependent calcium channels (116), possibly as a result of NO-induced activation of plasma membrane Ca^{2+} channels or inhibition of a Ca^{2+} pump. NO and cGMP are known to inhibit voltage-dependent Ca^{2+} channels in other cell types (117), but cyclic nucleotide-gated channels were found to be expressed in mammalian sperm (118), where they can regulate a Ca^{2+} entry pathway that responds more sensitively to cGMP than to cAMP (119). On the other hand, NO inhibits the sarcoplasmic reticulum Ca^{2+}-ATPase (120, 121), and the sarcoplasmic reticulum Ca^{2+}-ATPase antagonist thapsigargin is able to induce a rise in intracellular Ca^{2+} and AR in human spermatozoa (122), and to induce the AR in mouse spermatozoa (123). Interestingly, the increase in intracellular Ca^{2+} in human sperm by the use of the internal Ca^{2+}-ATPase inhibitor 2,5-di(tert-butyl) hydroquinone initiates the AR, but only in the presence of extracellular calcium (124). Because it has been shown that human spermatozoa, despite their requirement for extracellular Ca^{2+}, require also the movement of Ca^{2+} through internal stores before the AR occurs (122), it is conceivable that the NO/cGMP-elicited AR needs extracellular Ca^{2+} to restore the Ca^{2+} content of intracellular pools after a stimulus has caused their emptying.

D. Activation of cGMP-dependent protein kinases

Among the molecular targets of cGMP are cGMP-dependent protein kinases (Fig. 3). Two different cGMP-dependent protein kinases [protein kinase GI (PKGI) and protein kinase GII (PKGII)] have been identified in mammals (125, 126). The PKG inhibitors 8-bromoguanosine-3′,5′-monophosphorothioate Rp-isomer (Rp-8-Br-cGMPS) and 8-(4-chlorophenylthio)guanosine-3′,5′-monophosphorothioate Rp-isomer (Rp-8-pCPT-cGMPS) are able to block the sodium nitroprusside-induced AR in human sperm, suggesting that the NO/cGMP pathway, which is activated by sodium nitroprusside stimulation, needs the activation of a PKG to trigger the AR in human spermatozoa (36). On the other hand, in mice lacking PKGI, spermatozoa can normally undergo the AR and fertilize oocytes (127).

E. Protein kinase C (PKC) activation

The PKC inhibitor calphostin can block the AR-inducing effect of sodium nitroprusside, pointing to a role for PKC in the transduction mechanism of NO-dependent AR (36). Studies have demonstrated the involvement of PKC in the AR of human spermatozoa (112), as well as the effectiveness of calphostin in blocking the PKC-mediated AR of these cells (128). Both the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate and the diacylglycerol analog 1-oleoyl-2-acetylglycerol are powerful inducers of the AR in capacitated human spermatozoa (129). A recent study showed that the ANP-induced AR of human spermatozoa involves PKC, because it can be inhibited by the PKC inhibitors staurosporine and GF-109203X (113). The mechanism by which cGMP (the natural product of ANP receptor activation) can lead to an increase in PKC activity is still unknown.

V. Conclusions

A large body of experimental evidence indicates that the activation of guanylate cyclase strongly influences sperm function both in species with external fertilization and in mammals. In the former, the guanylate cyclase enzyme family appears to be mainly responsible for the long-distance interaction between gametes, as sperm chemotaxis toward egg-derived substances is crucial for reproduction, allowing sperm-egg encounter in open spaces. Echinoderms, fishes, and amphibians have served as useful models for studying the effects of egg-secreted, species-specific peptides on sperm movement. The intracellular mechanisms elicited by guanylate cyclase activation, leading ultimately to an oriented flagellar movement, have been thoroughly elucidated in these species.

In species with internal fertilization, the need for such precise chemotactic mechanisms seems to be less vital, as gamete encounter is apparently easier in a "closed" system such as the female internal genital apparatus. However, sperm chemotaxis evidently exists in mammals, and the acquisition of chemotactic responsiveness is likely to represent a part of the activation process that the spermatozoa of mammals undergo in their trip through the female genital tract. The concept of chemotaxis as part of the capacitation process in mammals is supported by experimental findings, and although the substance(s) responsible for sperm chemotaxis in mammals has not yet been identified, it seems likely that guanylate cyclase activation is an essential part of the process.

Guanylate cyclase-activating substances (in particular ANP and NO) strongly affect sperm motility, capacitation, and acrosomal reactivity. They therefore stimulate sperm metabolism and promote the ability of the sperm to approach the oocyte, interact with it, and finally fertilize it. The guanylate cyclase-activating system seems to be an important regulatory feature in mammalian reproduction. Several tissues in the genital tract of both sexes may produce guanylate cyclase agonists capable of interacting with gametes, and furthermore, the spermatozoon itself can produce the powerful sGC activator NO in response to substances physiologically present in the female genital tract. Thus, NO may influence sperm function via both endocrine and autocrine mechanisms.

Important sperm characteristics are affected by guanylate cyclase activation, and a complex system of intracellular pathways is activated by its agonists. Sperm motility appears to be affected by guanylate cyclase activation mainly through an increase in intracellular cAMP, whereas the acrosome reaction depends more directly on cGMP synthesis. Both cyclic nucleotides activate specific kinases and ion signals. A complex cross-talk between cAMP- and cGMP-generating systems occurs in response to guanylate cyclase activation, resulting in an upward shift in sperm function. Recent data suggest that guanylate cyclase activation could also affect the two extremes of sperm existence, spermatogenesis, on the one hand, and sperm-egg interaction. In addition, experimental observations indicate that excessive amounts of certain guanylate cyclase activators might exert opposite, antireproductive effects, increasing the oxidative stress on sperm membranes.

In view of the marked influence exerted by guanylate cyclase-activating substances on sperm function, it seems likely that sperm production and transport *in vivo*, and sperm motility, capacitation, and acrosomal reactivity *in vitro* will be amenable to pharmacological modulation by interaction with the sperm guanylate cyclases. Future exploration of the therapeutic potential of such a tool will require a more complete knowledge of the effects of guanylate cyclase-modulating drugs on human spermatozoa. Such drugs may serve as a new contraception modality. On the other hand, faulty precontact sperm-egg communication may be one of the causes of infertility, and activation or inhibition of guanylate cyclase may represent an exciting new approach for the diagnosis and treatment of male and/or female infertility.

Acknowledgments

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References

1. **Waldman SA, Murad F** 1987 Cyclic GMP synthesis and function. Pharmacol Rev 39:163–196
2. **Hurley JH** 1998 The adenylyl and guanylyl cyclase superfamily. Curr Opin Struct Biol 8:770–777
3. **Wedel BJ, Garbers DL** 2001 The guanylate cyclase family at Y2K. Annu Rev Physiol 63:215–233
4. **Denninger JW, Marletta MA** 1999 Guanylate cyclase and the NO/cGMP signaling pathway. Biochim Biophys Acta 1411:334–350
5. **Liu Y, Ruoho AE, Rao VD, Hurley JH** 1997 Catalytic mechanism of the adenylyl and guanylyl cyclases: modeling and mutational analysis. Proc Natl Acad Sci USA 94:13414–13419
6. **Garbers DL, Lowe DG** 1994 Guanylyl cyclase receptors. J Biol Chem 269:30741–30744
7. **Barr CS, Rhodes P, Struthers AD** 1996 C-type natriuretic peptide. Peptides 17:1243–1251
8. **Forte LR, London RM, Freeman RH, Krause WJ** 2000 Guanylin peptides: renal actions mediated by cyclic GMP. Am J Physiol 278:F180–F191
9. **Gupta G, Azam M, Yang L, Danziger RS** 1997 The β2 subunit inhibits stimulation of the α1/β1 form of soluble guanylyl cyclase

by nitric oxide. Potential relevance to regulation of blood pressure. J Clin Invest 100:1488–1492
10. **Nathan C** 1992 Nitric oxide as a secretory product of mammalian cells. FASEB J 6:3051–3064
11. **Palmer RMJ, Moncada S** 1989 A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. Biochem Biophys Res Commun 158:348–352
12. **Knowles RG, Moncada S** 1994 Nitric oxide synthases in mammals. Biochem J 298:249–258
13. **Stuehr DJ** 1997 Structure-function aspects in the nitric oxide synthases. Annu Rev Pharmacol Toxicol 37:339–359
14. **Gross SS, Wolin MS** 1995 Nitric oxide: pathophysiological mechanisms. Annu Rev Physiol 57:737–769
15. **Murad F** 1994 Regulation of cytosolic guanylyl cyclase by nitric oxide: the NO-cyclic GMP signal transduction system. Adv Pharmacol 26:19–33
16. **Bentley JK, Tubb DJ, Garbers DL** 1986 Receptor-mediated activation of spermatozoan guanylate cyclase. J Biol Chem 261:14859–14862
17. **Garbers DL** 1990 Guanylate cyclase receptor family. Recent Prog Horm Res 46:85–96
18. **Radany EW, Gerzer R, Garbers DL** 1983 Purification and characterization of particulate guanylate cyclase from sea urchin spermatozoa. J Biol Chem 258:8346–8351
19. **Suzuki N** 1995 Structure, function and biosynthesis of sperm-activating peptides and fucose sulphate glycoconjugate in the extracellular coat of sea urchin eggs. Zool Sci 12:13–27
20. **Garbers DL, Bentley JK, Dangott LJ, Ramarao CS, Shimomura H, Suzuki N, Thorpe D** 1986 Peptides associated with eggs: mechanisms of interaction with spermatozoa. Adv Exp Med Biol 207: 315–357
21. **Cardullo RA, Herrick SB, Peterson MJ, Dangott LJ** 1994 Speract receptors are localized on sea urchin sperm flagella using a fluorescent peptide analog. Dev Biol 162:2600–2607
22. **Suzuki N, Shimomura HL, Radany EW, Ramarao CS, Ward GE, Bentley JK, Garbers DL** 1984 A peptide associated with eggs causes a mobility shift in a major plasma membrane protein of spermatozoa. J Biol Chem 259:14874–14879
23. **Nishigaki T, Darszon A** 2000 Real-time measurements of the interactions between fluorescent speract and its sperm receptor. Dev Biol 223:17–26
24. **Silvestroni L, Palleschi S, Guglielmi R, Tosti Croce C** 1992 Identification and localization of atrial natriuretic factor receptors in human spermatozoa. Arch Androl 28:2 75–282
25. **Burnett AL, Ricker DD, Chamness SL, Maguire MP, Crone JK, Bredt DS, Snyder SH, Chang TSK** 1995 Localization of nitric oxide synthase in the reproductive organs of the male rat. Biol Reprod 52:1–7
26. **Burnett AL, Lowenstein CJ, Bredt DS, Chang TSK, Snyder SH** 1992 Nitric oxide: a physiologic mediator of penile erection. Science 257:401–403
27. **Rosselli M, Dubey RK, Rosselli MA, Makas E, Fink D, Lauper U, Keller PJ, Imthurn B** 1996 Identification of nitric oxide synthase in human and bovine oviduct. Mol Hum Reprod 2:607–612
28. **Rosselli M, Keller PJ, Dubey RK** 1998 Role of nitric oxide in the biology, physiology and pathophysiology of reproduction. Hum Reprod Update 4:3–24
29. **Lewis SEM, Donnelly ET, Sterling ESL, Kennedy MS, Thompson W, Chakravarthy U** 1996 Nitric oxide synthase and nitrite production in human spermatozoa: evidence that endogenous nitric oxide is beneficial to sperm motility. Mol Hum Reprod 2:873–878
30. **Herrero MB, Perez-Martinez S, Viggiano JM, Polak JM, Gimeno MF** 1996 Localization by indirect immunofluorescence of nitric oxide synthase in mouse and human spermatozoa. Reprod Fertil Dev 8:931–934
31. **Donnelly ET, Lewis SEM, Thompson W, Chakravarthy U** 1997 Sperm nitric oxide and motility: the effects of nitric oxide synthase stimulation and inhibition. Mol Hum Reprod 3:755–762
32. **O'Bryan MK, Zini A, Cheng CY, Schlegel PN** 1998 Human sperm endothelial nitric oxide synthase expression: correlation with sperm motility. Fertil Steril 70:1143–1147
33. **Revelli A, Soldati G, Costamagna C, Pellerey O, Aldieri E, Massobrio M, Bosia A, Ghigo D** 1999 Follicular fluid proteins stimulate nitric oxide (NO) synthesis in human sperm: a possible role for NO in acrosomal reaction. J Cell Physiol 178:85–92
34. **Zamir N, Barkan D, Keynan N, Naor Z, Breitbart H** 1995 Atrial natriuretic peptide induces acrosomal exocytosis in bovine spermatozoa. Am J Physiol 269:E216–E221
35. **Zhang H, Zheng RL** 1996 Possible role of nitric oxide on fertile and asthenozoospermic infertile human sperm functions. Free Radic Res 25:347–354
36. **Revelli A, Costamagna C, Moffa F, Aldieri E, Ochetti S, Bosia A, Massobrio M, Lindblom B, Ghigo D** 2001 Signaling pathways of nitric oxide-induced acrosome reaction in human sperm. Biol Reprod 64:1708–1712
37. **Herrero MB, Cebral E, Franchi A, Motta A, Gimeno MF** 1998 Progesterone enhances prostaglandin E_2 production via interaction with nitric oxide in the mouse acrosome reaction. Biochem Biophys Res Commun 252:324–328
38. **Tsuchida S, Sudo M, Muramatsu I** 1996 Stimulatory and inhibitory effects of sodium nitroprusside on soluble guanylate cyclase. Life Sci 58:829–832
39. **Middendorff R, Muller D, Wichers S, Holstein AF, Davidoff MS** 1997 Evidence for production and functional activity of nitric oxide in seminiferous tubules and blood vessels of the human testis. J Clin Endocrinol Metab 82:4154–4161
40. **Battaglia C, Giulini S, Regnani G, Di Girolamo R, Paganelli S, Facchinetti F, Volpe A** 2000 Seminal plasma nitrite/nitrate and intratesticular Doppler flow in fertile and infertile subjects. Hum Reprod 15:2554–2558
41. **Fujisawa M, Yamanaka K, Tanaka H, Okada H, Arakawa S, Kamidone S** 2001 Expression of endothelial nitric oxide synthase in the Sertoli cells of men with infertility of various causes. Br J Urol 87:85–88
42. **Zini A, O'Bryan MK, Magid MS, Schlegel PN** 1996 Immunohistochemical localization of endothelial nitric oxide synthase in human testis, epididymis, and vas deferens suggests a possible role for nitric oxide in spermatogenesis, sperm maturation, and programmed cell death. Biol Reprod 55:935–941
43. **Zini A, Abitbol J, Schulsinger D, Goldstein M, Schlegel PN** 1999 Restoration of spermatogenesis after scrotal replacement of experimentally cryptorchid rat testis: assessment of germ cell apoptosis and eNOS expression. Urology 53:223–227
44. **El-Gohary M, Awara WM, Nassar S, Hawas S** 1999 Deltamethrin-induced testicular apoptosis in rats: the protective effect of nitric oxide synthase inhibitor. Toxicology 132:1–8
45. **Shiraishi K, Naito K, Yoshida K** 2001 Nitric oxide promotes germ cell necrosis in the delayed phase after experimental testicular torsion of rat. Biol Reprod 65:514–521
46. **O'Bryan MK, Schlatt S, Gerdprasert O, Phillips DJ, de Kretser DM, Hedger MP** 2000 Inducible nitric oxide synthase in the rat testis: evidence for potential roles in both normal function and inflammation-mediated infertility. Biol Reprod 63:1285–1293
47. **Ventura S, Burnstock G** 1996 Variation in nitric oxide synthase-immunoreactive nerve fibres with age and along the length of the vas deferens in the rat. Cell Tissue Res 285:427–434
48. **Tur-Kaspa I, Maor Y, Levran D, Yonish M, Mashiach S, Dor J** 1994 How often should infertile men have intercourse to achieve conception? Fertil Steril 62:370–375
49. **Tur-Kaspa I, Dudkiewicz AB, Confino E, Gleicher N** 1990 Pooled sequential ejaculates: a way to increase total number of motile spermatozoa from oligozoospermic males. Fertil Steril 54:906–909
50. **Tur-Kaspa I, Segal S, Moffa F, Massobrio M, Meltzer S** 1999 Viagra for temporary erectile dysfunction during treatments with assisted reproductive technologies. Hum Reprod 14:1783–1784
51. **Herrero MB, Cebral E, Boquet M, Viggiano JM, Vitullo A, Gimeno MA** 1994 Effect of nitric oxide on mouse sperm hyperactivation. Acta Physiol Pharmacol Ther Latinoam 44:65–69
52. **Hellstrom WJG, Bell M, Wang R, Sikka SC** 1994 Effects of sodium nitroprusside on sperm motility, viability, and lipid peroxidation. Fertil Steril 61:1117–1122
53. **Lefievre L, DeLamirande E, Gagnon C** 2000 The cyclic GMP-specific phosphodiesterase inhibitor, sildenafil, stimulates human sperm motility and capacitation but not acrosome reaction. J Androl 21:929–937
54. **Burger M, Sikka SC, Bivalacqua TJ, Lamb DJ, Hellstrom WJ** 2000

The effect of sildenafil on human sperm motion and function from normal and infertile men. Int J Impot Res 12:229–234
55. **Aversa A, Mazzilli F, Rossi T, Delfino M, Isidori AM, Fabbri A** 2000 Effects of sildenafil (Viagra) administration on seminal parameters and post-ejaculatory refractory time in normal males. Hum Reprod 15:131–134
56. **Andrade JR, Traboulsi A, Hussain A, Dubin NH** 2000 *In vitro* effects of sildenafil and phentolamine, drugs used for erectile dysfunction, on human sperm motility. Am J Obstet Gynecol 182: 1093–1095
57. **Yeoman RR, Jones WD, Rizk BM** 1998 Evidence for nitric oxide regulation of hamster sperm hyperactivation. J Androl 19:58–64
58. **Weinberg JB, Doty E, Bavaventena J, Haney JF** 1995 Nitric oxide inhibition of sperm motility. Fertil Steril 64:408–413
59. **Rosselli M, Dubey RK, Imthurn B, Macas E, Keller PJ** 1995 Effects of nitric oxide on human spermatozoa: evidence that nitric oxide decreases sperm motility and induces sperm toxicity. Hum Reprod 10:1786–1790
60. **Nobunaga T, Tokugawa Y, Hashimoto K, Kubota Y, Sawai K, Kimura T, Shimoya K, Takemura M, Matsuzaki N, Azuma C, Saji F** 1996 Elevated nitric oxide concentration in the seminal plasma of infertile males: nitric oxide inhibits sperm motility. Am J Reprod Immunol 36:193–197
61. **Sikka SC** 2001 Relative impact of oxidative stress on male reproductive function. Curr Med Chem 8:851–862
62. **Ozbek E, Turkoz Y, Gokdeniz R, Davarci M, Ozugurlu F** 2000 Increased nitric oxide production in the spermatic vein of patients with varicocele. Eur Urol 37:172–175
63. **Aksoy H, Aksoy Y, OzbeyI, Altuntas I, Akcay F** 2000 The relationship between varicocele and semen nitric oxide concentrations. Urol Res 28:357–359
64. **Revelli A, Bergandi L, Massobrio M, Lindblom B, Bosia A, Ghigo D** 2001 The concentration of nitrite in seminal plasma does not correlate with sperm concentration, sperm motility, leukocytospermia, or sperm culture. Fertil Steril 76:496–500
65. **Anderson RA, Feathergill KA, Drisdel RC, Rawlins RG, Mack SR, Zaneveld LJ** 1994 Atrial natriuretic peptide (ANP) as a stimulus of the human acrosome reaction and a component of ovarian follicular fluid: correlation of follicular ANP content with *in vitro* fertilization outcome. J Androl 15:61–70
66. **Anderson RA, Feathergill KA, Rawlins RG, Mack SR, Zaneveld LJ** 1995 Atrial natriuretic peptide: a chemoattractant of human spermatozoa by a guanylate cyclase-dependent pathway. Mol Reprod Dev 40:371–378
67. **Bielfeld P, Anderson RA, Mack SR, De Jonge CJ, Zaneveld LJ** 1994 Are capacitation or calcium ion influx required for the human sperm acrosome reaction? Fertil Steril 62:1255–1261
68. **Jaiswal BS, Cohen-Dayag A, Tur-Kaspa I, Eisenbach M** 1998 Sperm capacitation is, after all, a prerequisite for both partial and complete acrosome reaction. FEBS Lett 427:309–313
69. **Jaiswal BS, Eisenbach M, Tur-Kaspa I** 1999 Detection of partial and complete acrosome reaction in human sperm: which inducers and probes to use? Mol Hum Reprod 5:214–219
70. **Belen Herrero M, Chatterjee S, Lefievre L, de Lamirande E, Gagnon C** 2000 Nitric oxide interacts with the cAMP pathway to modulate capacitation of human spermatozoa. Free Radic Biol Med 15:522–536
71. **Herrero MB, Viggiano JM, Perez-Martinez S, Gimeno MF** 1997 Evidence that nitric oxide synthase is involved in progesterone-induced acrosomal exocytosis in mouse spermatozoa. Reprod Fertil Dev 9: 433–439
72. **Sengoku K, Tamate K, Yoshida T, Takaoka Y, Miyamoto T, Ishikawa M** 1998 Effects of low concentrations of nitric oxide on the zona pellucida binding ability of human spermatozoa. Fertil Steril 69:522–527
73. **Joo BS, Park SH, Park SJ, Kang HS, Moon HS, Kim HD** 1999 The effect of nitric oxide on sperm cell function and embryo development. Am J Reprod Immunol 42:327–334
74. **Eisenbach M, Tur-Kaspa I** 1999 Do human eggs attract spermatozoa? Bioessays 21:203–210
75. **Shimomura HL, Dangott LJ, Garbers DL** 1986 Covalent coupling of a resact analogue to guanylate cyclase. J Biol Chem 261:15778–15782
76. **Ward GE, Garbers DL, Vacquier VD** 1985 Effects of extracellular egg factors on sperm guanylate cyclase. Science 227:768–770
77. **Repaske DR, Garbers DL** 1983 A hydrogen ion flux mediates stimulation of respiratory activity by speract in sea urchin spermatozoa. J Biol Chem 258:1524–1529
78. **Shapiro BM, Cook S, Quest AFG, Oberdof J, Wothe D** 1990 Molecular mechanisms of sea-urchin sperm activation before fertilization. J Reprod Fertil 42(Suppl):3–8
79. **Pacey AA, Hill CJ, Scudamore IW, Warren MA, Barratt CLR, Cooke ID** 1995 The interaction *in vitro* of human spermatozoa with epithelial cells from the human uterine (Fallopian) tube. Hum Reprod 10:360–366
80. **Tur-Kaspa I** 1992 Pathophysiology of the fallopian tube. In: Gleicher N, ed. Tubal catheterization. New York: Wiley-Liss, Inc.; 5–14
81. **Williams M, Hill CJ, Scudamore I, Dunphy B, Cooke ID, Barratt CLR** 1993 Sperm numbers and distribution within the human fallopian tube around ovulation. Hum Reprod 8:2019–2026
82. **Eisenbach M, Tur-Kaspa I** 1994 Human sperm chemotaxis is not enigmatic anymore. Fertil Steril 62:233–235
83. **Ralt D, Manor M, Cohen-Dayag A, Tur-Kaspa I, Ben-Shlomo I, Makler A, Yuli I, Dor J, Blumberg S, Mashiach S, Eisembach M** 1994 Chemotaxis and chemokinesis of human spermatozoa to follicular factors. Biol Reprod 50:774–785
84. **Cohen-Dayag A, Tur-Kaspa I, Dor J, Mashiach S, Eisenbach M** 1995 Sperm capacitation in humans is transient and correlates with chemotactic responsiveness to follicular factors. Proc Natl Acad Sci USA 92:11039–11043
85. **Cohen-Dayag A, Ralt D, Tur-Kaspa I, Manor M, Makler A, Dor J, Mashiach S, Eisenbach M** 1994 Sequential acquisition of chemotactic responsiveness by human spermatozoa. Biol Reprod 50: 786–790
86. **Sunfjord JA, Forsdahl F, Thibault G** 1989 Physiological levels of immunoreactive ANH-like peptides in human follicular fluid. Acta Endocrinol (Copenh) 121:578–580
87. **Steegers EAP, Hollanders JMG, Jongsma HW, Hein PR** 1990 Atrial natriuretic peptide and progesterone in ovarian follicular fluid. Gynecol Obstet Invest 29:185–187
88. **Zamir N, Riven-Kreitman R, Manor M, Makler A, Blumberg S, Ralt D, Eisenbach M** 1993 Atrial natriuretic peptide attracts human spermatozoa *in vitro*. Biochem Biophys Res Commun 197:116–122
89. **Jaiswal BJ, Tur-Kaspa I, Dor J, Mashiach S, Eisenbach M** 1999 Human sperm chemotaxis: is progesterone a chemoattractant? Biol Reprod 60:1314–1319
90. **Gnessi L, Fabbri A, Silvestroni L, Moretti C, Fraioli F, Pert CB, Isidori A** 1986 Evidence for the presence of specific receptors for N-formyl chemotactic peptides on human spermatozoa. J Clin Endocrinol Metab 63:841–846
91. **Sliwa L, Stochmal E** 2000 Effect of sodium nitroprusside on mouse sperm migration *in vitro*. Arch Androl 45:29–33
92. **Francavilla F, Santucci R, Macerola B, Ruvolo G, Romano R** 2000 Nitric oxide synthase inhibition in human sperm affects sperm-oocyte fusion but not zona pellucida binding. Biol Reprod 63: 425–429
93. **Creech MM, Arnold EV, Boyle B, Muzinich MC, Montville C, Bohle DS, Atherton RW** 1998 Sperm motility enhancement by nitric oxide produced by the oocytes of fathead minnows, *Pimephelas promelas*. J Androl 19:667–674
94. **Kuo RC, Baxter GT, Thompson SH, Stricker SA, Patton C, Bonaventura J, Epel D** 2000 NO is necessary and sufficient for egg activation at fertilization. Nature 406:633–636
95. **Hyslop LA, Carroll M, Nixon VL, McDougall A, Jones KT** 2001 Simultaneous measurement of intracellular nitric oxide and free calcium levels in chordate eggs demonstrates that nitric oxide has no role at fertilization. Dev Biol 234:216–230
96. **Vacquier VD, Moy GW** 1986 Stoichiometry of phosphate loss from sea urchin sperm guanylate cyclase during fertilization. Biochem Biophys Res Commun 137:1148–1152
97. **Ramarao CS, Garbers DL** 1985 Receptor-mediated regulation of guanylate cyclase activity in spermatozoa. J Biol Chem 260:8390–8396
98. **Ramarao CS, Garbers DL** 1988 Purification and properties of the

phosphorylated form of guanylate cyclase. J Biol Chem 263:1524–1529
99. **Singh S, Lowe DG, Thorpe DS, Rodriguez H, Kuang WJ, Dangott LJ, Chinkers M, Goeddel DV, Garbers DL** 1988 Membrane guanylate cyclase is a cell-surface receptor with homology to protein kinases. Nature 334:708–712
100. **Ward GE, Moy GW, Vacquier VD** 1986 Phosphorylation of membrane-bound guanylate cyclase of sea urchin spermatozoa. J Cell Biol 103:95–101
101. **Cook SP, Babcock DF** 1993 Activation of Ca^{2+} permeability by cAMP is coordinated through the pHi increase induced by speract. J Biol Chem 268:22408–22413
102. **Yoshino K, Takao T, Shimonishi Y, Suzuki N** 1992 Sperm-activating peptide type-V (SAP-V), a fifth member of the sperm-activating peptide family, purified from the egg-conditioned media of the heart urchin *Brissus agassizii*. Comp Biochem Physiol B 102: 691–700
103. **Maurice DH, Haslam RJ** 1990 Molecular basis of the synergistic inhibition of platelet function by nitrovasodilators and activators of adenylate cyclase: inhibition of cyclic AMP breakdown by cyclic GMP. Mol Pharmacol 37:671–681
104. **Trovati M, Massucco P, Mattiello L, Costamagna C, Aldieri E, Cavalot F, Anfossi G, Bosia A, Ghigo D** 1999 Human vascular smooth muscle cells express a constitutive nitric oxide synthase that insulin rapidly activates, thus increasing guanosine 3′:5′-cyclic monophosphate and adenosine 3′,5′-cyclic monophosphate concentrations. Diabetologia 42:831–839
105. **Mittal KC, Murad F** 1977 Formation of adenosine 3′,5′-monophosphate by preparations of guanylate cyclase from rat liver and other tissues. J Biol Chem 252:3136–3140
106. **Bentley JK, Khatra AS, Garbers DL** 1987 Receptor-mediated phosphorylation of spermatozoan proteins. J Biol Chem 262:15708–15713
107. **Cook SP, Babcock DF** 1993 Selective modulation by cGMP of the K^+ channel activated by speract. J Biol Chem 268:22402–22407
108. **Lee HC** 1988 Internal GTP stimulates the speract receptor mediated voltage changes in sea urchin spermatozoa membrane vesicles. Dev Biol 126:91–97
109. **Galindo BE, Beltrán C, Cragoe Jr EJ, Darszon A** 2000 Participation of a K^+ channel modulated directly by cGMP in the speract-induced signaling cascade of *Strongylocentrotus purpuratus* sea urchin sperm. Dev Biol 221:2285–2294
110. **Ciapa B, Epel D** 1996 An early increase in cGMP follows fertilization of sea urchin eggs. Biochem Biophys Res Commun 223: 633–636
111. **Kobori H, Miyazaki S, Kuwabara Y** 2000 Characterization of intracellular Ca^{2+} increase in response to progesterone and cyclic nucleotides in mouse spermatozoa. Biol Reprod 63:113–120
112. **Benoff S** 1998 Modelling human sperm-egg interactions *in vitro*: signal transduction pathways regulating the acrosome reaction. Mol Hum Reprod 4:453–471
113. **Rotem R, Zamir N, Keynan N, Barkan D, Breitbart H, Naor Z** 1998 Atrial natriuretic peptide induces acrosomal exocytosis of human spermatozoa. Am J Physiol 274:E218–E223
114. **Chini EN, Thompson MA, Chini CC, Dousa TP** 1997 Cyclic ADP-ribose signaling in sea urchin gametes: metabolism in spermatozoa. Am J Physiol 272:C416–C420
115. **Willmott N, Sethi JK, Walseth TF, Lee HC, White AM, Galione A** 1996 Nitric oxide-induced mobilization of intracellular calcium via the cyclic ADP-ribose signaling pathway. J Biol Chem 271: 3699–3705
116. **Linares-Hernández L, Guzmán-Grenfell AM, Hicks-Gomez JJ, González-Martínez MT** 1998 Voltage-dependent calcium influx in human sperm assessed by simultaneous optical detection of intracellular calcium and membrane potential. Biochim Biophys Acta 1372:1–12
117. **Quignard JF, Frapier JM, Harricane MC, Albat B, Nargeot J, Richard S** 1997 Voltage-gated calcium channel currents in human coronary myocytes. Regulation by cyclic GMP and nitric oxide. J Clin Invest 99:185–193
118. **Weyand I, Godde M, Frings S, Weiner J, Müller F, Altenhofen W, Hatt H, Kaupp UB** 1994 Cloning and functional expression of a cyclic-nucleotide-gated channel from mammalian sperm. Nature 368:859–863
119. **Wiesner B, Weiner J, Middendorff R, Hagen V, Kaupp UB, Weyand I** 1998 Cyclic nucleotide-gated channels on the flagellum control Ca^{2+} entry into sperm. J Cell Biol 142:473–484
120. **Adachi T, Matsui R, Weisbrod RM, Najibi S, Cohen RA** 2001 Reduced sarco/endoplasmic reticulum Ca^{2+} uptake activity can account for the reduced response to NO, but not sodium nitroprusside in hypercholesterolemic rabbit aorta. Circulation 104:1040–1045
121. **Ishii T, Sunami O, Saitoh N, Nishio H, Takeuchi T, Hata F** 1998 Inhibition of skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPase by nitric oxide. FEBS Lett 440:218–222
122. **Rossato M, Di Virgilio F, Rizzuto R, Galeazzi C, Foresta C** 2001 Intracellular calcium store depletion and acrosome reaction in human spermatozoa: role of calcium and plasma membrane potential. Mol Hum Reprod 7:119–128
123. **O'Toole CM, Arnoult C, Darszon A, Steinhardt RA, Florman HM** 2000 Ca^{2+} entry through store-operated channels in mouse sperm is initiated by egg ZP3 and drives the acrosome reaction. Mol Biol Cell 11:1571–1584
124. **Perry RL, Barratt CL, Warren MA, Cooke ID** 1997 Elevating intracellular calcium levels in human sperm using an internal calcium ATPase inhibitor, 2,5-di(tert-butyl) hydroquinone (TBQ), initiates capacitation and the acrosome reaction but only in the presence of extracellular calcium. J Exp Zool 279:291–300
125. **Lohmann SM, Vaandrager AB, Smolenski A, Walter U, De Jonge R** 1997 Distinct and specific functions of cGMP-dependent protein kinases. Trends Biochem Sci 22:307–312
126. **Pfeifer A, Ruth P, Dostmann W, Sausbier M, Klatt P, Hofmann F** 1999 Structure and function of cGMP-dependent protein kinases. Rev Physiol Biochem Pharmacol 150:105–149
127. **Hedlund P, Aszòdi A, Pfeifer A, Alm P, Hofmann F, Ahmad M, Fässler R, Andersson KE** 2000 Erectile dysfunction in cyclic GMP-dependent kinase I-deficient mice. Proc Natl Acad Sci USA 97: 2349–2354
128. **Bielfeld P, Faridi A, Zaneveld LJ, De Jonge CJ** 1994 The zona pellucida-induced acrosome reaction of human spermatozoa is mediated by protein kinases. Fertil Steril 61:536–541
129. **De Jonge C, Han HL, Mack SR, Zaneveld JD** 1991 Effect of phorbol diesters, synthetic diacyl glycerols, and a protein kinase C inhibitor on the human sperm acrosome reaction. J Androl 12:62–70