Review Article

Nitric Oxide: A Prime Signaling Molecule in Bovine Male Reproduction

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Abstract

Nitric oxide ($NO^{\bullet}$) is an important bioactive molecule with a short half-life. It is synthesized from the enzymatic conversion of L-Arg to L-citrulline in presence of nitric oxide synthases (NOS) and cofactors. The level of $NO^{\bullet}$ is very important as it shows beneficial effects at lower concentration whereas cytotoxic at higher concentration. It acts as protective agent and maintains post thaw motility and viability of spematozoa. $NO^{\bullet}$ plays critical importance in penile erection by relaxing the smooth muscle via inhibiting Rho A/ Rho-kinase pathway. It also participates in spermatogenesis, capacitation and acrosome reaction via cAMP/ cGMP pathways in mammals. Systemic inhibition of NOS caused impaired copulation, decreased erections and alteration in aggressive behaviour. Inhibition of cNOS significantly reduced the invitro fertilization, sperm-zona pellucida binding and proper embryonic development. Therefore, the cross talk and multiple systems induced by $NO^{\bullet}$ act parallelly, safeguard the timely function of the spermatozoa responsible for fertilization.

Key words: Aggression, Capacitation and Acrosome Reaction, Erectile Dysfunction, Mating Behaviour, Nitric Oxide, Signaling Event, Spermatozoa, Protective Action

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Introduction

Nitric oxide is an important signaling molecule in the body of mammals including humans and livestock. The nitric oxide molecule is a free radical, which is relevant to understanding its high reactivity. Despite being a simple molecule, $NO^{\bullet}$ is a fundamental player in the fields of neuroscience, physiology and immunology and was proclaimed "Molecule of the Year" by Science magazine in 1992 and Louis J. Ignerro, Robert F. Furchgott and Ferid Murad were awarded the Nobel Prize for Medicine and Physiology in 1998.

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Furchgott and Zawadski, (1980) recognized the importance of the endothelium in acetylcholine-induced vasorelaxation and later on identified as endothelial-derived relaxing factor (EDRF) as $NO^{\bullet}$ (Ignarro *et al.,* 1987). These scientists identified nitric oxide as an important signaling molecule, opening up a new way of treatment for millions of patients. The endothelium of blood vessels uses nitric oxide to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increasing blood flow. The production of nitric oxide is elevated in populations living at high-altitudes, which helps these people avoid hypoxia. Effects include blood vessel dilatation, neurotransmission, modulation of the hair cycle, penile erections and chemotaxis *in vitro* (Miraglia *et al.,* 2007; Lefievre *et al.,* 2007). Sildenafil, popularly known by the trade name Viagra, stimulates erections primarily by enhancing signaling through the nitric oxide pathway in the penis. Nitric oxide ($NO^{\bullet}$) contributes to vessel homeostasis by inhibiting vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium. In humans, a high-salt intake was demonstrated to attenuate $NO^{\bullet}$ production. Nitric oxide is also generated by macrophages and neutrophils as part of the human immune response. *In vitro* studies have shown that low concentrations of $NO^{\bullet}$ enhance the motility (mouse, hamster, and human), capacitation (Rodriguez *et al.,* 2005), zona pellucida binding and acrosome reaction (AR) of human (Revelli *et al.,* 1999) and bovine spermatozoa (Zamir *et al.,* 1995). Nitric oxide is also having role in diverse physiological and pathological processes such as vitality, penile erection, aggressive behavior, mating behavior, LHRH release, spermatogenesis, steroidogenesis, fertilization and protective role against lipid peroxidation. In this review we have tried to elaborate the effects of nitric oxide in male reproduction.

$NO^{\bullet}$ Synthesis

Nitric oxide is produced by the action of NO synthases (NOS), enzymes responsible for the conversion of L-arginine to $NO^{\bullet}$ and L-citrulline in presence of oxygen. NOSs contain four cofactors: FAD, FMN, tetrahydrobiopterin and haem; the haem center has spectral properties resembling those of cytochrome P450. There are three types of NOSs. Two are constitutive (named cNOS) and one is inducible by cytokines and endotoxins (named iNOS). There are two subtypes of cNOS: one was initially detected in the vascular endothelium and named eNOS or NOS3 and the other is present in the central and peripheral nervous systems and named nNOS (NOS1) or Brain NOS (bNOS) (Moncada and Erusalimsky, 2002). The nNOS and iNOS are predominantly soluble enzymes whereas eNOS is more than 90% particulate (Wallerath *et al.,* 1997). The nNOS and eNOS are Ca^{2+}/calmodulin-dependent enzymes (Lin *et al.,* 2002). The iNOS releases $NO^{\bullet}$ in large quantities (micromolar range) during inflammatory or immunological defense reactions and is involved in host tissue damage. The overexpression of iNOS gene might account for $NO^{\bullet}$ overproduction, as it is reported in rats with portal hypertension (Hsieh *et al.,* 2003). The iNOSs bind

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calmodulin tightly and their activity is essentially Ca^{2+}-independent. The eNOS is expressed constitutively in endothelial cells and synthesizes the $NO^{\bullet}$ needed for regulation of blood pressure. The activation of eNOS is induced by increase in intracellular Ca^{2+} resulting from activation of diverse G-protein-coupled receptors (GPCR) or from mobilization of intracellular Ca^{2+}stores. Liu *et al.* (2003) have demonstrated that Endothelin-1 activates eNOS via heterotrimeric G-protein beta-gamma subunit signaling to protein kinase B/Akt. An increase in the association of heat shock protein 90 (HSP90) with eNOS is well recognized for increasing NO· production. Despite the progress in this field, the mechanisms by which HSP90 modulates eNOS remain unclear. Ou *et al.* (2004) suggested that the tyrosine kinase and HSP90-dependent signaling pathways act in concert to suppress uncoupled eNOS activity. The nNOS is found in a variety of neurons in both the central and peripheral nervous systems and is a constitutionally expressed enzyme, though it can also be induced in neurons by certain treatments. Raines *et al.* (2004) examined the activation of cellular signal transduction pathways in nNOS-transfected cells grown in the presence or absence of L-arginine. NOS isoforms may have roles in reproductive functions and in the developmental processes of the excurrent duct system in the alpaca (*Lama pacos;* Parillo *et al.,* 2017).

$NO^{\bullet}$ Transport

$NO^{\bullet}$ produced from endothelial cells, act on smooth muscles. There is an assumption that diffusion alone moves $NO^{\bullet}$ but does not seem more feasible because of its ultra-short half-life (2-6 seconds) and high reactivity with O_2^{-}, haem and non-haem-iron; the concept of free diffusion of $NO^{\bullet}$ is not acceptable. Since $NO^{\bullet}$ reacts with thiol (-SH) groups of proteins, it can form stable, biologically active, S-nitrosyl compounds. Indeed, $NO^{\bullet}$ has been shown to circulate as an S-nitroso adduct of albumin and possess biological activities identical to those of $NO^{\bullet}$ *in vitro* and *in vivo*. Since iron–nitrosyl compounds are associated with thiol-containing ligands, formation of dinitrosyl iron cysteine has been suggested as a second pathway for $NO^{\bullet}$ transport. Pawloski *et al.* (2001) have suggested that the movement of $NO^{\bullet}$ through erythrocytes occurs by a precisely coordinated series of events. They have shown that the RBCs do not consume $NO^{\bullet}$ irreversibly by combining with Hb. $NO^{\bullet}$ reemerges from RBCs as biologically active S-nitrosothiol, which is protected from reacting with Hb. Some of the $NO^{\bullet}$ captured by Fe^{2+} in Hb can be shuttled intramolecularly to a conserved thiol group, producing S-nitrosohemoglobin. This $NO^{\bullet}$ may be transferred to other thiol-containing molecule, thus enabling $NO^{\bullet}$ activity to leave RBCs. This has been shown to occur preferentially in oxygen-poor tissues where Hb changes conformation.

However, under appropriate conditions, reaction of $NO^{\bullet}$ with O_2^{-} can also result in the generation of peroxynitrite ($ONOO^{-}$), a potent oxidant (Beckman *et al.,* 1990; Herrero *et al.,* 2001). Peroxynitrite decomposes to form the reactive hydroxyl radical HOONO. Moreover, peroxynitrite and its metabolite are

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capable of inducing cytotoxicity by inducing lipid peroxidation, nitrosation of several tyrosine molecules that regulate enzyme function and signal transduction and Na^+ channel inactivation. These findings suggest that the actions of $NO^•$ in a cell depend on its concentration, the cellular redox state, and the abundance of metals, protein thiols and low-molecular weight thiols (glutathione), as well as other nucleophile targets (Mallozzi *et al.,* 1997; Herrero *et al.,* 2001).

Measurement of Nitric Oxide Concentration

Nitric oxide can be measured by many direct and indirect means (e.g. Gas and liquid chromatography, electron paramagnetic resonance, mass spectrometry, spectrophotometry, electrochemistry). But these techniques are having less practability in case of biological samples due to its short half-life and low concentration of nitric oxide. These difficulties may be overcome by measuring its stable metabolites nitrite and nitrate. Plasma, serum and urine predominantly contain nitrite while nitrate is predominant in cerebrospinal fluid, semen and joint fluid. Therefore, measurement of these levels provides a reliable and quantitative estimate of $NO^•$ output *in vivo*. Numerous techniques for detection of these anions have been reported, including spectrophotometric, fluorescent, chemiluminiscent, and chromatographic assays (Feelisch and Stamler, 1996). Griess reaction which was first described in 1879 is the simplest and most frequently used method which employs colorimetric detection with Griess reagents (Griess, 1879). Griess reaction involves the formation of a chromophore from the diazotization of sulfanilamide by acidic nitrite followed by coupling with bicyclic amines such as N-1-(naphthyl) ethylenediamine.

Interactions of Nitric Oxide with other Free Radicals

Free radicals are chemical species possessing an unpaired electron in their outermost orbital. Due to the presence of one or more unpaired electrons, these species are paramagnetic, which makes them highly reactive. A free radical can be formed in a molecule by gaining an additional electron, for example, reduction of molecular oxygen (O_2) to the superoxide anion radical ($O_2^{-·}$) (Tuteja *et al.,* 2001). Some oxides of nitrogen ($NO^•$, $NO_2^·$) are also free radicals. In the absence of L-arginine, nNOS has been shown to generate superoxide ($O_2^{-·}$). Superoxide, either directly or through its self-dismutation to hydrogen peroxide H_2O_2, is likewise believed to be a cell-signaling agent. In oxidative stress condition $O_2{-}·$ is an unusual species, where it can act as a reducing agent by donating its extra electron to $NO^•$ to form peroxynitrite ($ONOO^-$), or it can act as an oxidizing agent; in this case it gets reduced to H_2O_2. Under normal circumstances, the relatively high abundance of superoxide dismutase (SOD) ensures that the later reaction occurs preferentially. However, when $NO^•$ is produced in large quantities, a significant amount of $O_2^{-·}$ reacts with $NO^•$ to produce $ONOO^-$. The $NO^·$ radical can react with peroxyl radical ($RO_{2·}$), hydroxyl radical ($OH^·$), or $NO^•$, (a reactive and short-lived species) to produce alkyl peroxinitrite (ROONO), nitrous acid

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(HNO_2), or nitrous oxide (N_2O), respectively. NO• will react with fluorine, chlorine and bromine to form the XNO species, known as the nitrosyl halides, such as nitrosyl chloride. Nitrosyl iodide can form but is an extremely short-lived species and tends to reform I_2. Nitroxyl halide (HNO) is the reduced form of nitric oxide. Nitric oxide reacts with acetone and an alkoxide to a diazeniumdiolate or nitrosohydroxylamine and methyl acetate. This is a very old reaction but of interest today in NO• prodrug research. Nitric oxide can also react directly with sodium methoxide, forming sodium formate and nitrous oxide. Despite being a free radical nitric oxide can inactivate (Alvarez *et al.,* 1987; McCall *et al.,* 1989) and even inhibit the production (Clancy *et al.,* 1992) of superoxide anions which cause lipid peroxidative damage, particularly in spermatozoa. Nitric oxide is also protected by antioxidants (Vallance and Collier, 1994), suggesting a beneficial role.

Role of NO• in the Male Reproductive System

It plays following diverse roles in male reproduction-

Protective Action of Nitric Oxide on Sperm Functions

NO• play important role in protecting various sperm functions such as motility, vitality and viability by diverse mechanisms. Hellstrom *et al.* (1994) demonstrated that low concentrations of a NO•-releasing compound, sodium nitroprusside (SNP), was beneficial to the maintenance of post-thaw human sperm motility and viability. Siddique and Atreja, 2013 also demonstrated that the lower conc. of spermine NONOate (NO• releasing compound) maintained post-thaw motility, viability, membrane integrity and lipid peroxidation status in the cytopreserved Murrah Buffalo spermatozoa.

Chatterjee and Gagnon (2001) provided evidence for the production of reactive oxygen species (ROS) during cryopreservation of bovine spermatozoa. Sudha *et al.* (2006) observed that nitric oxide inactivated superoxide anions in erythrocytes (Narendra *et al.,* 2008); bioavailability of nitric oxide have rendered tolerance to erythrocyte membrane by protecting the cell from haemolysis and oxidative damage due to its free radical scavenging and antioxidant effects (Narendra *et al.,* 2008). These anions are regularly released by mammalian cells during oxygen consumption and cause peroxidative damage to membrane phospholipids and decreased ability to fuse with the oocyte (Tremellen, 2008). Lipton (1999) observed that sperm are known to be particularly susceptible to such lipid peroxidation. Kisa *et al.* (2004) showed a correlation between sperm motility and the levels of NO• and TBARS present in rat testicular tissue. L-arginine, which provides protection against lipid peroxidation, is less likely to act as an antioxidant. On the other hand, in neutrophils it has been shown that the ratio of NO• to superoxide determines the nature of their interaction. When NO• predominates, it inactivates superoxide; when superoxide predominates, it inactivates NO• (Kausalya and Nath, 1998). Icariin is a weak PDE5 inhibitor *in vitro* and enhances the

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production of nitric oxide. These results collectively suggest that icariin within a certain dose range is beneficial to male reproductive functions; meanwhile, higher doses of icariin may damage reproductive functions by increasing oxidative stress in the testes of rats (Chen *et al.,* 2014). Olive oil also appears to preserve semen quality through enhancing gonadal function, reducing the level of oxidative injury and lipid peroxidation, and improving nitric oxide signaling (Banihani *et al.,* 2017).

Regulation of Penile Erection/ Erectile Dysfunction

Erectile Dysfunction

Erectile dysfunction (ED) is defined as the inability of the male to attain and maintain erection of the penis sufficient to permit satisfactory sexual intercourse (NIH Consensus Conference, 1993). However, sexual complaints are not included in this definition such as loss of rigidity after vaginal intromission and early or premature ejaculation. ED represents a social problem occurring in ~50% of the general male population aged between 40 and 70 years (Feldman *et al.,* 1994) and may be caused by organic and/or psychological disorders, the former being prevalent (65 versus 35%; Benet and Melman, 1995). The incidence of ED increases with age, and the disease frequently occurs in the presence of organic diseases such as chronic renal (20–100%) or hepatic failure (50–70%), diabetes (27.5–60%), hypertension (46%), Peyronie's disease (35%), hypercholesterolaemia/atherosclerosis (33%), ischaemic heart disease (16%) and depression (Schiavi and Rehman, 1995). Almost 30% of ED is due to the presence of systemic disease which affects the blood supply to the penis. Cigarette smoking does not represent a direct cause but may be a risk factor for ED (Feldman *et al.,* 1994). Chronic arsenic exposures leading to ED include the inhibition of the sex hormone level, or reduction of NOS activity to impair the functions of penile smooth muscle and blood vessels (Hsieh *et al.,* 2008). Endocrine disorders have been claimed not to be a cause of ED. However, in some studies, low testosterone concentrations have been shown to be present in >15% of patients complaining of erectile failure (Govier *et al.,* 1996) and hyperprolactinaemia has been indicated to be the cause of ED in 2–3% of impotent men.

Penile Erection

Nitric oxide pathway is of critical importance in the physiologic induction and maintenance of erections (Burnett *et al.,* 1992). The constitutive isoforms (eNOS and nNOS) are tightly regulated and produce physiologically relevant levels of NO• in endothelial cells and autonomic nerve endings of the penis. Both nNOS and eNOS play important role in penile erection. Regulation of eNOS in the penis involves multiple molecular mechanisms that act in concert to both positively and negatively affect the function of this enzyme. Endothelial NO is an important mediator of both physiologic and pathologic responses in the penis. In normal endothelial function, NO• has vasodilatory properties and counterbalances RhoA/Rho-kinase–

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mediated vasoconstriction; thus, regulating vascular tissue homeostasis. Conversely, during pathologic conditions, eNOS uncoupling and formation of peroxynitrite from the reaction of NO• with superoxide anion results in pro-oxidant effects of NO•. The balance between NO• bioavailability, vasoconstrictor function, and vascular generation of reactive oxygen species (ROS) is crucial for maintaining normal erectile ability. Treatments with goji (*Lycium chinense* Mill) extracts increased serum testosterone level, increased the expression of endothelial NO synthase, neuronal NO synthase, and cGMP, improved the oxidative stress marker, and decreased corporal fibrosis and may have a positive effect on erectile dysfunction via its antioxidant effects (Moon *et al.,* 2017).

RhoA/ Rho-Kinase Pathway

Contraction of smooth muscle is primarily mediated by calcium-dependent activation of myosin light chain kinase, resulting in phosphorylation of myosin light chain and actin/myosin assembly. The calcium-independent increase in vascular smooth muscle tone, known as calcium-sensitization, is largely mediated by activation of the small GTPase, RhoA and its downstream effector, Rho-kinase (Ignarro *et al.,* 1990). RhoA may be activated by several signaling pathways, including the binding of G-protein–coupled receptor agonists. RhoA activated Rho-kinase (a and b isoforms) phosphorylates and inhibits regulatory myosin phosphatase target subunit 1 (MYPT1) of myosin light chain phosphatase at Thr-696 and inhibits its activity, promoting smooth muscle contraction (Feng *et al.,* 1999). An inverse functional relationship exists between the NO/cGMP/protein kinase G and RhoA/Rho-kinase signaling pathways within the vasculature. The NO• pathway phosphorylates RhoA at Ser-188, which prevents its translocation to the membrane and activation (Sauzeau *et al.,* 2000). In addition, in human endothelial cells, the RhoA/Rho-kinase pathway inhibits Akt-dependent eNOS activity/phosphorylation at Ser-1177 (Ming *et al.,* 2002), providing an additional means of interaction between eNOS-mediated relaxant and RhoA/Rho-kinase– mediated contractile pathways. The degree of contraction of the corpus cavernosum smooth muscle and the functional state of the penis is determined by the balance between proerectile and antierectile mechanisms that operate physiologically in the penis. Vasoconstriction (evoked by norepinephrine through a-adrenergic receptors, endothelins, angiotensins, and thromboxane A_2), which maintains the penis in the flaccid state, is mediated, in part, by the RhoA/Rho-kinase pathway (Chitaley *et al.,* 2001; Wang *et al.,* 2002; Wingard *et al.,* 2003). During erection, this pathway is inhibited, most likely by NO• (Mills *et al.,* 2002). In addition, RhoA/Rho-kinase suppresses eNOS gene expression and enzyme activity in the penis (Bivalacqua *et al.,* 2004). The selective Rho-kinase inhibitors Y-27632 and H- 1152 (Chitaley *et al.,* 2001; Rees *et al.,* 2001; Teixeira *et al.,* 2005) and adeno-associated viral gene transfer of dominant negative RhoA to the penis (Chitaley *et al.,* 2002) enhance erectile function in rats. Fig.1 schematically depicts regulation of the contractile pathway in smooth muscle and its interaction with the NO• relaxant pathway in the penis.

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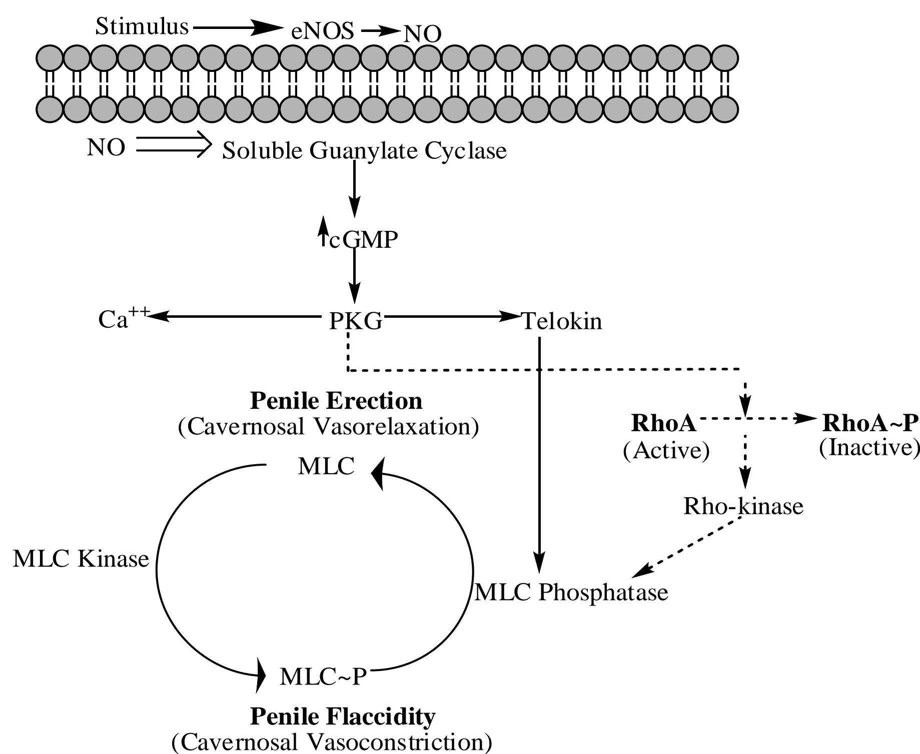

Fig. 1 Signaling Pathway of Penile Erection

NO• in Capacitation and Acrosome Reaction

Many studies have suggested that ROS such as O_2^- and H_2O_2 are involved in the regulation of mammalian sperm capacitation (Roy and Atreja, 2008a; Roy and Atreja, 2008b), hyperactivation, acrosome reaction and associated protein tyrosine phosphorylation (Aitken *et al.,* 1995, 1998; Herrero *et al.,* 1999, 2000, 2001; Rivlin *et al.,* 2004; de Lamirande and O' Flaherty, 2008). Capacitation also depends on NADPH oxidase and the shuttle creatine-creatine phosphate, both sensitive to diphenyleneiodonium (Cordoba *et al.,* 2008). The first experimental evidence for the involvement of NO• in capacitation came from the work of Zini *et al.* (1995). They observed that incubation of human spermatozoa with low concentration (0.1mM) of NO•-releasing compounds such as diethylamine-NONOate (da) or spermine-NONOate resulted in a significant increase in sperm capacitation (24% ± 4%, as assessed by lysophosphatidyl choline-induced AR) when compared to control spermatozoa (Ham's F-10 alone, 12% ± 2%) without affecting viability and motility. Further, catalase, but not SOD, blocked this NO•-induced capacitation and it suggested a complex mechanism of action of NO• involving H_2O_2 (Zini *et al.*, 1995). The presence of NO•-releasing compounds (0.1 mM SNP or 0.1mM or 1 mM da) accelerated the capacitation process and thus ability of spermatozoa to undergo human follicular fluid (hFF)-induced AR (Herrero *et al.,* 1999).

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NO• also appears to be involved in sperm physiology, such as hyperactivation, capacitation and acrosome reaction (de Lamirande *et al.,* 1997; Yeoman *et al.,* 1998; Roy and Atreja, 2008a). Hyperactivation may be modulated by chemotactic signals to turn sperm towards the oocyte (Suarez, 2008). The presence of NOS has been observed in the acrosome and tail of mouse (Herrero *et al.,* 1997) and human spermatozoa (Lewis *et al.,* 1996). The NO•-releasing compounds induce acrosome reaction in human (Herrero *et al.,* 1999) and rabbit spermatozoa (Guzman-Grenfell *et al.,* 1999). Relatively high concentrations (0.01–1.0 mM) of sodium nitroprusside, a NO• donor, inhibit motility and viability of human spermatozoa, but low concentrations (10–100 nM) result in increased capacitation without an effect on motility (Sengoku *et al.,* 1998). Joo *et al.* (1999) demonstrated that sodium nitroprusside reduces both sperm motility and hyperactivation at 0.1–1.0 mM, but increases the percentage of acrosome-reacted human spermatozoa at 0.01–1.0 mM. NOS activity stimulated by follicular fluid proteins also increases the percentage of acrosome-reacted human spermatozoa (Revelli *et al.,* 1999). L-arginine also acts as a resource of NO• may induce capacitation and acrosome reaction through the NO• signal pathway.

The ability to accelerate the capacitation process varies among NO• releasing compounds. The difference in potency was related to the kinetics of NO• formation, because SNP generated NO• instantaneously (3-10 s) whereas da and spermine-NONOate released NO• over a longer period of time. The half-lives of da and spermine-NONOate were 18 ± 3 and 80 ± 15 min, respectively (Zini *et al.,* 1995). Hence, it seems, NO• requires a short period of time to exert its action on the capacitation process, perhaps initiating a cascade of events that will lead to capacitation. Moreover, the presence of NOS inhibitors (0.1-1 mM L-NAME; 10 or 100 μM 7-nitro indazole) at the onset of incubation period decreased the percentage of AR (measured by hFF or A23187-induced AR) and indicated that endogenous NO• was necessary for spermatozoa to display their full fertilizing ability (Herrero *et al.,* 1999). O'Flaherty *et al.* (2004) showed that addition of L-arginine to the incubation media promoted both capacitation and acrosome reaction in cryopreserved bovine spermatozoa.

Signal Transduction Pathways Induced by NO• During Capacitation and Acrosome Reaction

In mammalian spermatozoa, studies suggested the role of NO• during capacitation and acrosome reaction. In bovine spermatozoa, SNP caused a 50% increase in cGMP levels during AR (Zamir *et al.,* 1995). However, Herrero *et al.* (2000) demonstrated that human spermatozoa when incubated in Tyrode-BSA medium with NO• releasing compounds, intracellular cAMP concentrations increased to levels higher than those of spermatozoa incubated in Tyrode-BSA alone. In contrast, incubation with NOS inhibitor, L-NAME decreased intracellular sperm cAMP concentrations. Further, the inhibitory effect observed with 1 mM L-NAME on capacitation and tyrosine phosphorylation of two sperm proteins (105, 85 kDa) was partially

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over come by the presence of 1mM db-cAMP + 0.5 mM IBMX (db-cAMP: N^6, 2′-O-dibutyryl cAMP, a cell permeable cAMP analog; IBMX: 3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor). The results suggested that $NO^•$ modulates the capacitation and protein tyrosine phosphorylation at some point upstream of cAMP formation. The partial reversal of L-NAME inhibition by db-cAMP + IBMX may again indicate that $NO^•$ is also modulating enzymes that are downstream cAMP formation or processes other than those involved in the cAMP/PKA pathway.

Roy and Atreja (2008c) also reported that ejaculated buffalo spermatozoa were capacitated in the absence or presence of heparin, or L-arginine or L-NAME for 6 h. Capacitating spermatozoa generated NO• both spontaneously and following stimulation with L-arginine and L-NAME quenched such L-arginine-induced NO• production. Immunolocalization of NOS suggested for existence of constitutive NOS in buffalo spermatozoa. L-Arginine (10 mm) was found to be a potent capacitating agent and addition of L-NAME to the incubation media attenuated both L-arginine and heparin-induced capacitation and suggested that NO• is involved in the capacitation of buffalo spermatozoa. Two sperm proteins of Mr 38 000 (p38) and 20 000 (p20) were tyrosine phosphorylated extensively by both heparin and L-arginine. The tyrosine phosphorylation of p38 was insensitive to both inductions by cAMP agonists as well as inhibition by a protein kinase A (PKA) inhibitor. Further, most of these L-arginine-induced tyrosine phosphorylated proteins were localized to the midpiece and principal piece regions of flagellum of capacitated spermatozoa and suggested that sperm flagellum takes active part during capacitation. These results indicated that L-arginine induces capacitation of buffalo spermatozoa through NO• synthesis and tyrosine phosphorylation of specific sperm proteins involving a pathway independent of cAMP/PKA (Roy and Atreja, 2008c). The tyrosine phosphorylation of p95 was induced extensively by both $O_2^{•-}$ as well as exogenous source of H_2O_2 and using specific activators and inhibitors of signaling pathways, it was found that the induction was regulated through a cAMP-dependent PKA pathway (Roy and Atreja, 2008a). Siddique and Atreja, 2013 reported recently that the ejaculated buffalo spermatozoa were capacitated in the presence of spermine-NONOate (100µM) and inhibited by L-NAME. It also caused the increase in motility and hyperactivation of buffalo spermatozoa.

Sivram (2008) reported that Ang-II increases the Ca^{2+} during buffalo sperm capacitation and is mediated via the phosphoinositide pathway. This pathway is central to Ang-II signaling in buffalo spermatozoa, which is responsible for the initiation of cAMP and nitric oxide signaling. It stimulated cAMP production during as well as acrosome reaction indirectly via the $Ca^{2+}$and calmodulin. Both insulin and leptin enhance $NO^•$ production in human and this increase is possibly through the PI3K signaling pathway, as evidenced by reduction of $NO^•$ production when the PI3K inhibitor, wortmannin, was given. They play role in enhancing the fertilizing capacity by increasing motility, AR and $NO^•$ production (Lampiao *et al.,* 2008).

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It is interesting that the activity of cyclooxygenase (COX), another heme-containing enzyme, appeared to be modulated by NO• during the progesterone induced acrosome reaction, because the presence of the NOS inhibitor, L-NAME completely blocked the increase in prostaglandin E_2 (PGE_2) synthesis induced by progesterone. Conversely, treatment of mouse spermatozoa with SNP stimulated both COX and lipoxygenase (LOX) activities during capacitation (Herrero *et al.,* 1995). Thus, it is possible that such a NO•-induced conformational change in GC (Guanylate cyclase), COX and LOX occurs in spermatozoa during capacitation, the acrosome reaction, or both. During the progesterone-induced acrosome reaction, mouse sperm NOS activity is increased by about 70% (Herrero *et al.,* 1998), raising the question of how this steroid can modulate sperm NO• production. It is tempting to speculate that progesterone, which promotes the influx of extracellular calcium, activates a calcium-dependent isoform of sperm NOS. This would lead to an increase in NO• synthesis, which in turn would activate enzymes (i.e. COX) involved in different signal transduction pathways that finally result in the acrosome reaction.

In human spermatozoa, during capacitation, double phosphorylation of specific threonine-glutamine-tyrosine (Thr-Glu-Tyr) motifs in two proteins (80 and 105 kDa) occurs (Thundathil *et al.,* 2002; Thundathil *et al.,* 2003). L-NAME (a competitive inhibitor of L-arginine for NOS) prevented, and spermine-NONOate, a NO• donor promoted the increase in double phosphorylation of Thr-Glu-Tyr motif during sperm capacitation. In addition, L-arginine reversed the inhibitory effect of L-NAME on capacitation and the associated increase in double phosphorylation of this motif. Therefore, NO• regulates the double phosphorylation of the Thr-Glu-Tyr motif in proteins of human spermatozoa during capacitation. Further, it was found that NO•-mediated increase of P-Thr-Glu-Tyr-P involves protein tyrosine kinase (PTK), MEK or MEK-like kinase and protein kinase C (PKC) but not PKA (Thundathil *et al.,* 2003). Previously, de Lamirande and Gagnon (2002) had demonstrated that the double phosphorylation of Thr-Glu-Tyr motif of human sperm proteins (80 and 105 kDa) during capacitation activates the component of extracellular signal-regulated protein kinase (ERK) pathway. Elements of the ERK pathway are present in human spermatozoa and involved in capacitation (Luconi *et al.,* 1998, de Lamirande *et al.,* 2002). The basic assembly of all MAPK pathways is a module in which three kinases are sequentially activated; the ERK module includes Raf [MAPK kinase kinase, for serine/threonine (Ser/Thr)], MEK (MAPK2K, dual specificity for Ser/Thr and Tyr), as well as ERK 1 (MAPK3) and ERK2 (MAPK1, for Ser/Thr) (Kolch *et al.,* 2000; Windmann *et al.,* 1999). The MEK phosphorylates the Thr and Tyr residues within the Thr-Glu-Tyr motif, which is present at the active site not only of ERK1 and ERK2 but also that of ERK 5 (big MAPK) (Zhou *et al.,* 1995), ERK7 (Yan *et al.,* 2001), and other important signal transduction elements, such as MOK (Miyata *et al.,* 1999). Inhibitors of MEK and MEK-like kinases (PD98059 and U126) block capacitation and the associated phosphorylation of Thr-Glu-Tyr in sperm proteins, indicating that such kinases are present in

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spermatozoa and are active during capacitation (Thundathill *et al.,* 2002; de Lamirande *et al.,* 2002; Thundathill *et al.,* 2003). Immunoblotting indicated the presence of Shc, Grb2, Ras^{p21}, Raf and ERK1 and ERk2 during capacitation in human spermatozoa. Therefore, $NO^{\bullet}$appears to be involved in capacitation through two mechanisms, one dependent on cAMP/PKA (Herrero *et al.,* 2000) and another dependent on the ERK pathway (Thundathil *et al.,* 2003). These two pathways are essential for capacitation, because inhibition of PKA or elements of the ERK pathway prevent capacitation and tyrosine phosphorylation in human spermatozoa (Leclerc *et al.,* 1996; Herrero *et al.,* 2000; de Lamirande and Gagnon, 2002). Probably, in human spermatozoa, the PKA and ERK pathways act in parallel but, ultimately, lead to tyrosine phosphorylation of proteins of 80 and 105 kDa during capacitation.

Roy and Atreja (2009) studied the extent of sperm capacitation after various periods of incubations by lysophosphatidyl choline-induced acrosome reaction. In both cattle and buffalo spermatozoa, at 6h, four proteins of molecular weight 49, 45, 32, and 20 kDa (designated as p49, p45, p32, and p20) were tyrosine phosphorylated. However, in buffalo, two additional proteins of 38 and 30 kDa were also tyrosine phosphorylated. In a time-course study, p20 appeared as early as at 0 h in capacitated buffalo spermatozoa as compared to 4h in cattle. Further, in heparin-treated buffalo spermatozoa, with a time-dependent increase in tyrosine phosphorylation of some proteins, there was time-dependent dephosphorylation of some other proteins that was never seen in heparin-treated cattle sperm. And they revealed that buffalo sperm took more time than cattle for capacitation but its associated protein tyrosine phosphorylation event started very early as compared to cattle.

The role of cGMP was reported on capacitation in human (Zhang and Zheng, 1996), bull (Zamir *et al.,* 1995) and buffalo bull (Siddique and Atreja*, unpublished data) spermatozoa. Revelli *et al.* (2001) suggested that the AR-inducing effect of exogenous $NO^{\bullet}$ (added in the form of 100 μM SNP) on capacitated human spermatozoa is accomplished via stimulation of an $NO^{\bullet}$-sensitive soluble guanylate cyclase (sGC), cGMP(3′, 5′-cyclic guanosine monophosphate) synthesis, and PKG (cGMP-dependent protein kinase G) activation (Lohman *et al.,* 1997). In this effect, the activation of protein kinase C (PKC; Benoff, 1998) is also involved, and the presence of extracellular Ca^{2+} is required (Benoff, 1998). In Sertoli cells, when the cellular concentration $NO^{\bullet}$ is less than 1μM, then it interacts directly with soluble guanylate cyclase (sGC) to induce synthesis of cGMP, which further activates cGMP-regulated phosphodiesterase (PDE), protein kinase G (PKG) and cyclic nucleotide-gated channels (Lee and Cheng, 2004).

Hence, $NO^{\bullet}$ can activate multiple signaling pathways such as cAMP/ PKA pathway, cGMP/PKG pathway, PI3K/ Akt pathway and the MAPK pathway to induce the protein tyrosine phosphorylation during capacitation in mammalian spermatozoa (Fig. 2). And it also regulates the phosphorylation of the Thr-Glu-Tyr motif during human sperm capacitation (Thundathill *et al.,* 2003).

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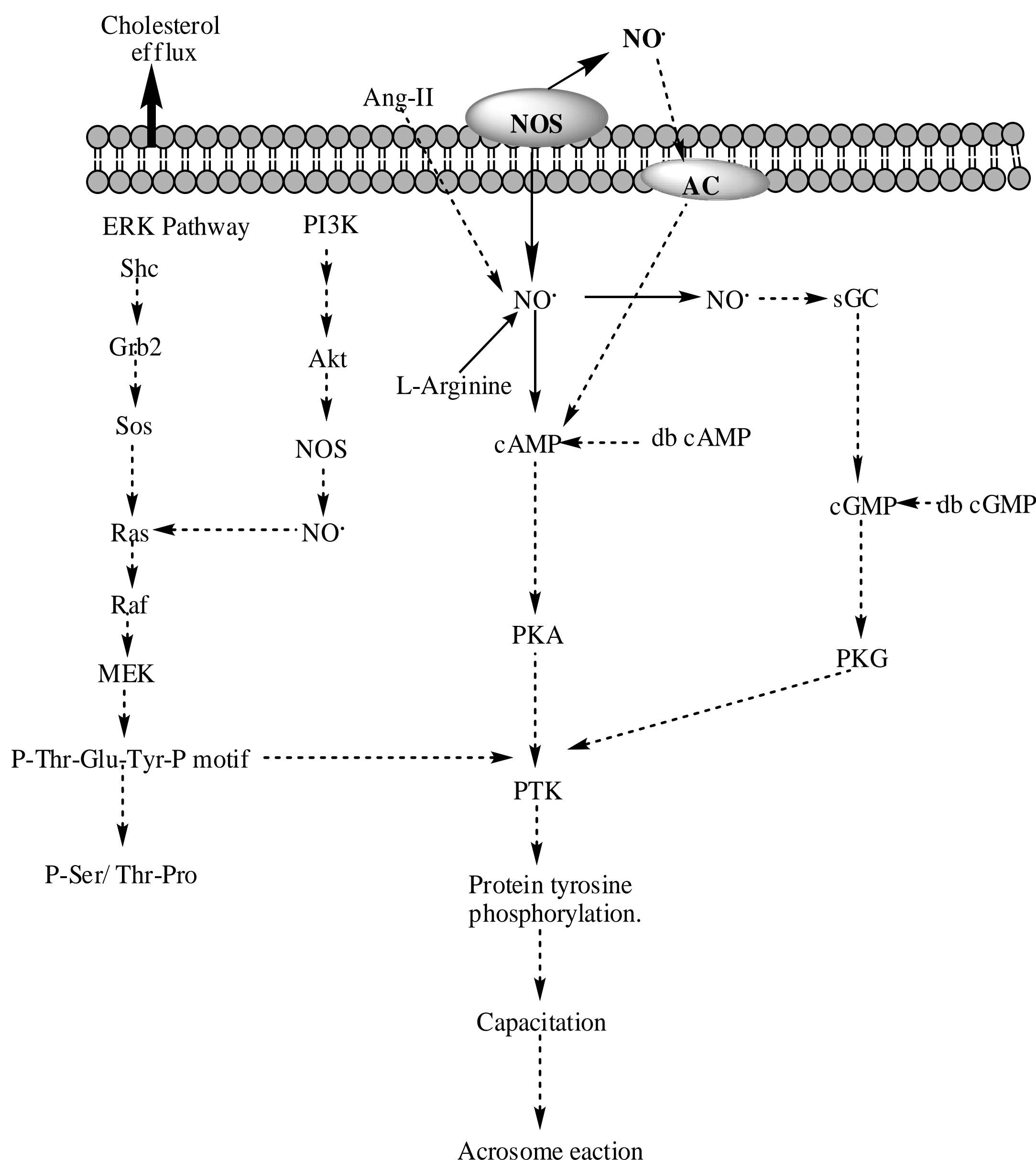

Fig.2 Signaling Pathway during Capacitation and Acrosome Reaction in mammals

Sperm Motility

Two conflicting opinions are there for the effects of NO• on sperm motility. Hellstrom *et al.* (1994) found that NO• improves sperm motility, whereas Rosselli *et al.* (1995) and Weinberg *et al.* (1995) observed a NO•-induced inhibition of motility. Bahmanzadeh *et al.* (2008) found that nitric oxide synthase inhibitor (L-NAME) may improve sperm count and morphology that are associated with infertility in varicocelized rat. Moran *et al.* (2008) also reported that NO• is a major free radical involved in boar sperm damage during

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cryopreservation and showed significant caspase activity. Similarly, NO is an important mediator in the pathogenesis of infertility with nicotine treatment. NOS inhibitor and perhaps L-NAME could be useful in prevention of nicotine induced infertility in smokers (Oyeyipo *et al.,* 2015). But most of the results support the improvement in motility. Schachter *et al.* (1973) have reported that oral administration of amino acid L-arginine to oligospermic men results in an improvement in both sperm count and motility in the majority of individuals treated. Krampitz and Doepfmer, (1962) and Keller and Polakoski (1975) added L-arginine to ejaculated human spermatozoa in *vitro* and reported that its addition stimulated the motility of human spermatozoa in specimens having an initially low motility. $NO^{\bullet}$ also regulates sperm motility, with low concentrations of $NO^{\bullet}$ enhancing (Hellstrom *et al.,* 1994) and medium/high concentrations of $NO^{\bullet}$ decreasing sperm motility (Rosselli *et al.,* 1995). Under physiological conditions, small amounts of $NO^{\bullet}$ are generated and neutralize free radicals which inhibit sperm motility. Thereby low concentrations of $NO^{\bullet}$ may protect against O_2^- mediated reduction of sperm motility. In contrast, excessive generation of $NO^{\bullet}$ under pathological conditions such as infection or endometriosis can cause sperm toxicity as well as reduce sperm motility by contributing to the formation of peroxynitrite, a highly toxic anion of peroxidation. Normozoospermic samples showed significantly greater concentrations of nitrite than those from as then ozoospermic individuals, implying that $NO^{\bullet}$ can improve or maintain sperm motility. These results are in accord with the observations of Hellstrom *et al.* (1994). Hellstrom *et al.* (1994) also provided the direct evidence for a positive effect of $NO^{\bullet}$ on human sperm viability and motility. Using sodium nitroprusside (SNP, at 50 nM and 100 nM concentrations), an instant $NO^{\bullet}$ donor, they could improve the post-thaw human sperm motility and viability. Percent viability was significantly reduced from 0 min (60.06% ± 3.5%) to 6 h post-thaw in controls (38.0% ± 5.1%) but not in 50 nM (46.8% ± 10.4%) or 100 nM (48.8% ± 6.5%) SNP-treated samples. Compared with controls (18.3% ± 3.4%), maintenance of percent motility at 3h post-thaw was significantly improved in 50 nM (24.5% ± 2.9%) and in 100 nM (26.3% ± 3.2%) SNP-treated samples. Addition of NOS inhibitors such as N^{ω}-nitro-L-arginine, N^{ω}-nitro-L-arginine-methyl ester (L-NAME) to the incubation medium depressed motility and hyperactivation in hamster and human spermatozoa (Yeoman 1994; Donnelly *et al.,* 1997; Yeoman *et al.,* 1998; Kameshwari *et al.,* 2003). This suggested that endogenous $NO^{\bullet}$ produced by spermatozoa regulates the sperm motility and hyperactivation. Siddique and Atreja, 2013, also reported similar results while treating the buffalo spermatozoa using 100 M spermine-NONOate. Lewis *et al.* (1996) provided evidence for the presence of eNOS and bNOS in the human spermatozoon that regulates (increases) sperm motility in an autocrine fashion. Similarly, resveratrol led to improved sperm parameters (motility, morphology and viability) and histological characteristic of testis as well as increased level of testosterone in the morphine-treated mice (Jalili *et al.,* 2017). The viability of spermatozoa remained unaffected at all concentrations of L-arginine (0-50 mM) compared to control and

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heparin-treated cells. At lower concentrations (10 mM or lower), L-arginine maintained the sperm motility compared to the control. However, at higher concentrations of L-arginine (> 10 mM), sperm motility was significantly decreased (Roy and Atreja, 2008c).

Role of NO• in Aggression

Reduced calcium- dependent constitutive NOS enzymatic activity was found in the prefrontal cortex of postmortem brains of patients with schizophrenia and depression (Xing *et al.,* 2002). A specific role for nNOS-derived NO in aggression was first addressed in mice in which the nNOS gene was deleted by homologous recombination (nNOS–/–), thus inhibiting NOS production in neurons (Huang *et al.,* 1995). In all test situations, male nNOS–/– mice were significantly more aggressive and rarely displayed submissive behaviors (Nelson *et al.,* 1995).

Male reproductive and aggressive behaviors are both generally regulated by androgens. However, nongonadal mechanisms may have evolved to regulate aggression in animals living in habitats that require competition outside of the breeding season (Soma and Wingfield, 2001). Plasma androgen concentrations directly influence aggression. nNOS–/– and WT mice do not differ in blood testosterone concentrations either before or after agonistic encounters (Nelson *et al.,* 1995). Data on castrated nNOS–/– males, however, suggest that testosterone is necessary, if not sufficient, to promote increased aggression in these mutants (Kriegsfeld *et al.,* 1997). Castrated nNOS–/– mice displayed low levels of aggression that were equivalent to the reduced aggression observed among castrated WT males. Androgen replacement therapy restored the elevated levels of aggression in nNOS–/– mice. Additional studies using perinatal castration on males and androgen treatment on females are required to sort out the organizational effects of androgens on NO•-related aggression in males.

Numerous studies have implicated serotonin (5-hydroxytryptamine, or 5-HT) as a key neurotransmitter involved in aggression and impulsivity. Gene targeting strategies in mice that either directly or indirectly affect the functional integrity of the 5-HT system have generally strengthened the influence of 5-HT on aggression (Miczek *et al.,* 2001; Nelson and Chiavegatto, 2001). Depending on whether it is synthesized from nNOS or eNOS, NO• can have opposite effects on 5-HT and therefore opposite effects on male aggressive behavior. It seems that differences in the localization of the source of NO• and/or subcellular sites may account for the distinctive alterations in the brain 5-HT system. Additionally, regarding the putative role of NO• in the aggression related to mental disabilities, a possible link with the 5-HT system may also be envisaged, because 5-HT dysfunction has been reported in Down Syndrome (Gulesserian *et al.,* 2000; Mann *et al.,* 1985; Seidl *et al.,* 1999; Whitaker-Azmitia, 2001), autism (Chugani, 2002; Posey and McDougle, 2001), depression and schizophrenia (Lee and Meltzer, 2001; Meltzer *et al.,* 2003). Isolation-induced aggression is correlated to changes with 5-HT turnover (Garattini *et al.,* 1967). Several

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5-HT drugs, such as 5-HT1A, 1B agonists or 5-HT uptake blockers, ameliorate isolation-induced aggression in mice (Olivier *et al.,* 1989).

Role of NO• in Mating Behaviour

Medial preoptic area (MPOA) in CNS is important for the expression and sensitization of male sexual behaviour, as evidenced by reports that increasing NO• in the MPOA increased mounting rate (Sato *et al.,* 1998), whereas inhibiting NOS impaired mating (Sato *et al.,* 1998). Dominguez *et al.* (2006) reported the repeated sexual experience with increased levels of nitric oxide synthase (NOS) in the MPOA of male rats. And experience-induced increase in NOS in the MPOA may be one mechanism through which sexual experience facilitates sexual behavior in male rats (Dominguez *et al.,* 2006).

Systemic inhibition of NOS caused impaired copulation and decreased erections in rats (Hull *et al.,* 1994). One way by which NO• in the MPOA might facilitate behavior is by increasing extracellular dopamine (Dominguez *et al.,* 2004; Lorrain *et al.,* 1996), which in turn facilitates male sexual behavior (Hull *et al.,* 2004). Dopamine release may be affected by influences on axon terminals, as well as changes in the firing rate of dopamine neurons. Since nitric oxide mediates the release of dopamine and it has been reported to enhance catecholamine release (Prast and Philippu, 2001; West *et al.,* 2002) as well as inhibit the reuptake, possibly by reversing the transporter (Kiss *et al.,* 1999). Administration of the NO• precursor L-Arginine through a microdialysis probe increased the mount rate of sexually experienced male rats, whereas administration of a NOS inhibitor reduced mount rate (Sato *et al.,* 1998). The role of nitric oxide in libido and fertility of male rats was also investigated by administration of the L-NAME (25 or 50 mg/kg/day). L-NAME caused marked reduction of precoital sexual behaviour, and a failure of most rats to mount or ejaculate during the test interval (Ratnasooriya *et al.,* 2000). Gonadal hormone activation of nitric oxide-cyclic guanosine-monophosphate pathway is important for lordosis behavior, as well as that this system is activated during mating behavior (Panzica *et al.,* 2006). Laura *et al.,* 2002, reported that Ginseng enhances libido and copulatory performance and this action is due to release of nitric oxide from endothelial cells and perivascular nerves. LHRH controls lordosis behavior in the female rat (McCann *et al.,* 1999) and is also involved in mediating male sex behavior. *In vivo* studies have shown that NO• stimulates the release of LHRH that induces sex behavior. This behavior can be stimulated by injection of sodium nitroprusside and is blocked by inhibitors of NOS. Apparently, there are two LHRH neuronal systems: one, with axons terminating on the hypophyseal portal vessels, the other with axons terminating on neurons which mediate sex behavior (Mani *et al.,* 1994).

Effect of NO• on Spermatogenesis

Presence of low levels of cNOS activity in the testes and high levels in other male urogenital organs (urethra, bladder neck, vas deferens, prostate, seminal vesicle) was first reported by Ehren *et al.* (1994).

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The important role of NO• within the testis was also evident from the findings that the testis contains: (i) GTP-cyclohydrolase I, the initial enzyme for THB synthesis; (ii) arginosuccinate synthetase as well as argininosuccinate lyase, the enzymes responsible for L-arginine generation, and they are abundant in the testis and (iii) haem oxygenase isozymes HO-1 and HO-2, which oxidatively cleave the haem molecule to produce NOS. cNOS activity was identified in the Leydig cells, Sertoli cells and endothelial cells of the human testis. Interestingly, soluble guanylate cyclase and cGMP were found to co-localize within the cytoplasm of the Leydig cells, some apically situated spermatids and residual bodies of seminiferous tubules. Testosterone, calmodulin, aspartate, glutamate and $Ca^{2+/}$calmodulin- dependent protein kinase II has been shown to co-localize with NOS-I in Leydig cells. The testicular cells are well equipped with a NO•–cGMP pathway, which may function, such as spermatogenesis and steroidogenesis. Zini *et al.* (1996) demonstrated the expression of eNOS in both Sertoli and Leydig cells at all stages of spermatogenesis. Additionally, intense eNOS expression was also observed in prematurely shed spermatocytes and spermatids, suggesting a role for eNOS in spermatogenesis and germ cell degeneration. Interestingly, eNOS activity is differentially expressed in Leydig cells, suggesting that concentrations of steroids in the cells may be regulating this activity and NO• subsequently acts in an autocrine/paracrine fashion. Within the testis, NO• has also been shown to regulate blood flow, cell permeability and contractile function of myofibroblasts, which in turn regulate steroid synthesis and transport. Wang *et al.* (1997) have identified a testis-specific variant of nNOS (TnNOS) in the testis.

In Sperm-Oocyte Fusion

Exogenous NO• has been shown to enhance human sperm binding to zona pellucida (Sengoku *et al.,* 1998). Francavilla *et al.,* 2000 investigated whether the inhibition of human sperm cNOS could affect sperm-oocyte fusion and sperm binding to the zona pellucida. L-NAME was used as cNOS inhibitor. Sperm-oocyte fusion was evaluated using the hamster egg penetration test (HEPT). The ZP binding was evaluated using the hemizona assay. L-NAME added from the onset of capacitation strongly inhibited sperm-oocyte fusion. This inhibitory effect was dose dependent, stereo specific, and suppressed by L-arginine in a dose-dependent manner. L-NAME also inhibited sperm-oocyte fusion in the HEPT enhanced with progesterone (P), where P (5 mM) was added for 15 min to capacitated sperm. A lesser but significant inhibition was also observed when sperm suspensions were exposed to L-NAME following capacitation in both versions of HEPT. On the contrary, L-NAME did not affect ZP binding. That study provided the evidence that cNOS played a role in the human sperm's capacity to fuse with oocyte but not in the ZP binding. L-NAME did not exert any significant effect on the zona pellucida binding at the dose affecting sperm-oocyte fusion. Exogenous NO• has been shown to enhance human sperm binding to the zona pellucida (Sengoku *et al.,* 1998). Herrero *et al.* (1997) reported that in mouse sperm, the inhibition of constitutive NOS significantly

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reduced the in vitro fertilization rate without impairing sperm-zona pellucida binding. It is not surprising that the L-NAME inhibition of capacitation events required for the sperm-oocyte fusion was not associated with the inhibition of sperm ability to bind to the zona pellucida. L-NAME likely inhibits capacitation events not required for ZP binding.

NO• also plays important roles in many events during embryonic development, such as regulation of egg activation at fertilization in sea urchin oocytes (Kuo *et al.,* 2000; Kim *et al.,* 2004) and regulation of the balance between cell proliferation and differentiation in Drosophila embryo development (Kuzin *et al.,* 1996). In addition, several studies have reported the activities of the NO•-related enzyme, NOS, in preimplantation development (Gouge *et al.,* 1998) and implantation in mammals (Novaro *et al.,* 1997; Biswas *et al.,* 1998; Gagioti *et al.,* 2000). Although it has been reported that eNOS and iNOS are expressed in blastocysts collected from the uterus of delayed-implanting mice, and preovulatory and ovulatory oocytes (Jablonka- Shariff and Olson, 1997, 1998; Gouge *et al.,* 1998), expression and activities of NOS in preimplantation embryos and the role of NO in preimplantation development has not been fully elucidated. NO• is a well-recognized activator of guanylate cyclase that induces an increase in cyclic guanosine monophosphate (cGMP) levels in target cells (Moncada *et al.,* 1991; Nathan., 1992). The importance of the ratio of cyclic adenosine monophosphate (cAMP) to cGMP in the regulation of preimplantation embryonic development and differentiation has been previously proposed. Chen *et al.* (2001) reported an interesting data showing the importance of NO• in the early stages of mouse embryonic development. However, the precise mechanism of NO• in the embryonic development still remains unclear. Using a specific inhibitor and precursor of NO•, the study examined whether NO• could regulate the rates of fertilization and early embryonic development in mice in order to demonstrate the essential role of NO• in the regulation of fertilization and early embryonic development. When mouse oocytes were treated with L-NAME or L-arginine, *in vitro* fertilization rate and early embryonic development were significantly reduced by L-NAME, but not by L-arginine. It is likely that the physiological level of NO• is involved in the process of oocytes maturation and fertilization. These results are well consistent with the previous results reported by Kuo *et al.* (2000), which demonstrated that NOS and nitric oxide related bioactivity satisfy the primary criteria of an egg activator: they were present in an appropriate place, active at an appropriate time and sufficient for successful fertilization in sea urchin.

Conclusion

Nitric oxide has an indispensable role in male reproduction. Despite being a free radical it acts as an antioxidant and have protective role in sperm motility, viability and vitality. It regulates the most of the discussed functions by modulating the cAMP and cGMP pathways. L-Arginine/ spermine NONOate participates in increasing motility, viability and decreases lipid peroxidation in spermatozoa. It mediates

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penile erection by cNOS and there is an inverse functional relationship exists between the NO• cGMP/ protein kinase G and Rho A/ Rho-kinase signaling pathway within vasculature. The balance between NO• bioavailability, vasoconstrictor function and vascular generation of ROS is crucial for erectile function. It is well established that cyclic nucleotides and alteration in protein tyrosine phosphorylation play pivotal role in capacitation in AR. Both the nNOS-/- and eNOS-/- have effects on aggressive behavior, eNOS-/- act to increase and nNOS-/- decreases the male aggressive behavior. NO also induces sex behavior in male by the release of LHRH. Testicular cells are well equipped with NO-cGMP pathway responsible for spermatogenesis and steroidogenesis. Pleiotropic signaling provided by NO• through cGMP/ cADPR formation and S-nitrosylation account for the diverse modes of Ca^{2+} release, which play role during fertilization.

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